University of Nottingham
UK | CHINA | MALAYSIA

GENOME DIVERSITY AND ENVIRONMENTAL ADAPTATION OF NIGERIAN INDIGENOUS CHICKEN

Submitted by

Mifta Pratiwi Rachman, B.Sc., M.Biotech

A thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

April 2022

Declaration

I hereby declare that this thesis has not been previously presented or submitted for examination to this University or any other one. The work described herein is entirely by me and references to other people's work have been acknowledged.

This thesis may be made available for consultation within the University library and may be photocopied or loaned to other libraries for consultation.

Mifta Pratiwi Rachman

April 2022

Conferences and Workshop

Genome Science Conference, University of Nottingham, UK. 4-6th September 2018. (Attended).

Population Genomic Workshop, University of Sheffield, UK. 26-28th February 2019. (Attended).

School of Biosciences Postgraduate Research Symposium, University of Nottingham, UK. 26-27th June 2019. Oral presentation on "Genomic diversity of Nigerian indigenous chicken".

38th *International Society for Animal Genetics* Conference. Held virtually during July 26-30, 2021. Poster title: Genetic basis of thermo-tolerance in African indigenous chickens.

12th World Congress on Genetics Applied to Livestock Production (WCGALP) to be held during 3-8th July 2022. Abstract accepted for both oral and poster presentations. Abstract title: Whole-genome sequencing reveals genetic diversity and heat-stress adaptation in Nigerian indigenous chickens.

Awards

The Lamming Postgraduate Travel Awards to attend "Fundamentals of populations genetics using R" online workshop.

The Sam Burton Memorial Award (small grant for non-HEU International students studying at the School of Biosciences).

Acknowledgement

In the name of Allah, the Most Gracious, the Most Merciful.

Firstly, All the praises and thanks be to Allah, the Lord of the universe for His countless blessings and help to have come this far to submit one of His orders to pursue the good knowledge.

My special thank goes to my Supervisors, Professor Olivier Hanotte and Dr Almas Gheyas for their guidance and for sharing with me an abundance of knowledge and research experiences, Dr Ravinder Anand-Ivell and Professor Richard-Ivell for their countless help, advice, and support during my research experience in the School of Biosciences. I appreciate all your scientific insight and the motivation that helped me during my PhD years at the University of Nottingham. Thanks to Dr Sarah Blott and Dr Jacqueline Smith (Roslin Institute, The University of Edinburgh) for several inputs to make this thesis much more meaningful.

Thanks to the Indonesia Endowment Funds for Education (LPDP) for making one of my dreams come into reality through the 4 years full scholarship. And I wish to acknowledge the support of the staff of the School of Biosciences, UoN and my colleagues in Professor Olivier Hanottes's and Ivell's lab for their support.

My deepest appreciation goes to my family for their massive support in many ways. To my beloved husband, Hanief and our adorable sons, Algoritma and Avicenna, I appreciate you all for your abundance of love, patience, and for your understanding. Special thanks to my mother, mother-in-law, my father and my father-in-law also my

brothers for the prayers and support. Thank you very much and may Allah the Almighty rewards all your kindness!

Finally, this thesis has been inspired by two chapters of the Holy Qur'an,

"He is the One Who produces ***gardens-both cultivated and wild****, and palm trees,* ***crops of different flavours****, olives, and pomegranates-****similar 'in shape', but dissimilar 'in taste'****..."*

(Surah Al-An'am / Livestock: chapter 141)

"And on the earth, there are ***'different'*** *neighbouring tracts, gardens of grapevines,* ***'various' crops, palm trees-some stemming from the same root, others standing alone****.* ***They are all irrigated with the same water, yet We make some taste better than others****. Surely these are signs for those who understand"*

(Surah Ar-Ra'd / the Thunder: chapter 4)

Abstract

Indigenous breeds develop their genomic characteristics by adapting to local environments over a long period of time. Most of them have abilities to survive in harsh environments or tolerate particular diseases but are not productive in commercial terms. Moreover, climate changes, particularly those that contribute to weather extremes like a long dry season, excessive humidity, and high precipitation, may result in reduced performance and reproduction and could compromise the organism's immune function. Their adaptive characteristics could be in response to natural selection and artificial selection for production traits that over time may leave selection signatures in the genome. The goal of this study is to analyse genome sequence data of Nigerian indigenous chickens from diverse ecotypes to assess their genetic diversity and to identify selection signatures that may be involved in their adaptation to tropical environments.

The study started by unravelling genomic diversity, which is presented in **Chapter 2**. I analysed Whole-Genome Sequencing (WGS) data from 120 Nigerian indigenous chickens from 14 populations and detected ~17 million SNP variants after applying some filtration steps. About 24% of these SNPs are novel from the catalogue present in public databases. Genomic sequence data generated under this study have been submitted to the European Nucleotide Archive (ENA) under the Accession: PRJEB39536 (publicly released in 2023) for wider research use. In Chapter 2, several analyses were performed on autosomal SNP data to assess genetic diversity within and among the Nigerian indigenous chicken populations, including assessing nucleotide diversity and inbreeding co-efficients of individual populations, investigating population structure and ancestry based on Principal Component Analysis (PCA), pairwise population differentiation (*FST* and Mantel test), genetic admixture, and

assessing linkage disequilibrium decay (LD decay). The result at the autosomal genome level indicates high genomic diversity but a low population structure across the Nigerian indigenous chicken populations.

In Chapter 3, I conducted a selection signature analysis from the same individuals genotyped in Chapter 2. The aim here was to identify candidate genomic regions related to climatic heat stress and rainfall pattern based on temperature and precipitation data from the WorldClim database. Eighty-seven samples from 12 regions, which were not significantly different in terms of average annual temperature, were analysed together to unravel the adaptation for heat stress using a pooled heterozygosity (*Hp*) method, whereas two extreme groups based on precipitation data (high and low) - with ten samples per group - were analysed using an *FST* approach to identify genomic regions of differential selection. The result of *Hp* analysis uncovered seventeen candidate genes for heat stress adaptation that are either overlapping or residing close to the putative selection signature regions. These genes have either direct involvement in thermal or heat tolerance or have other stress-response related functions such as important roles in the oxidoreductase pathway (oxidative stress), hypoxia and angiogenesis, immune system, and thermogenesis, e.g., *HSF1*, *CDC37*, *SFTPB*, *HIF3A*, and *ILF3* genes. Meanwhile, thirty-three genes were found overlapping the candidate regions from the *FST* analysis between high *versus* low precipitation groups and many of them are related to immune response (e.g., *DGKB, REL, EGFR*) while the rest were involved in heat stress and oxidative stress (*AHSA2*) and reproductive performance (e.g., *LRP8, GRM3*).

In Chapter 4 I investigated the same WGS dataset in a different way; this time the analyses were done based on Nigeria's agro-ecological zones which were considered distinct ecotypes. Eight ecotypes from the northern part to the southern part of Nigeria

were classified as: Sudan savanna, Northern Guinea savanna, Southern Guinea savanna, Derived savanna, Mid-Altitude, Rainforest, Freshwater swamp, and Mangrove Swamp. The first two ecotypes were living under warm-arid conditions, the next two ecotypes were from warm semi-arid regions, while the last three ecotypes were living in a warm-humid zone. Only the Mid-Altitude ecotype was located in a cool region. Analyses of the selective sweeps within ecotypes (using the *Hp* approach) and among ecotypes (using the *FST* approach) found several genes (differently) linked to oxidative stress and immune response. Besides, heat response-related genes dominated in the chicken ecotypes living in warm-arid, warm semi-arid, and warm-humid zones, while cold response, hypoxia response and osmotic stress contributed to the adaptation in cool and warm-humid zones, respectively.

To sum up, my project identified a number of important candidates for adaptation to be under selection in Nigerian indigenous chickens, especially in relation to thermotolerance and immune response as a consequence of living under tropical conditions. Furthermore, this study, for the first time, has performed high coverage WGS on a large number of Nigerian indigenous chickens from diverse ecotypes and has unravelled significant genomic diversity in them. The results of this study offer valuable insight into the genetic diversity of Nigerian indigenous chickens and their population structure, which will guide their improvement by helping design specific breeding programs as well as poultry management strategies to minimise heat stress and enhance disease resistance and productive performance. Put together, the findings of this thesis introduce the fact that Nigerian indigenous chickens are a group of unique birds whose genetic resources could be employed for the genetic improvement of livestock productivity. I hope that our results will be the baseline for further investigation into the genomic landscape of the Nigerian chicken.

Table of Contents

List of Figures

List of Tables

Abbreviations

AEZ	agro-ecological zones
BAM	binary alignment and map
bp	base pairs
BQSR	base quality score recalibration
BWA	Burrows-wheeler aligner
FST	fixation index
GATK	Genome analysis toolkits
GO	Gene ontology
GWAS	Genome-wide association study
Hp	Pool heterozygosity
HWE	HardyWeinberg-equilibrium
LD	Linkage disequilibrium
PCA	Principal component analysis
QTL	Quantitative trait loci
SAM	sequence alignment and map
SNP	Single nucleotide polymorphism
VCF	Variant calling format
VQSR	Variant quality score recalibration
WGS	Whole-genome sequencing
ZFST	Z-transformation of the fixation index
ZHp	Z-transformation of the pooled heterozygosity

CHAPTER 1:

General Introduction

1.1 Background

Chickens (*Gallus gallus domesticus*) are the most ubiquitous of all livestock species. They may be found in nearly all agro-ecologies, where they are particularly valued for their short-generation time and efficient feed conversion (FAO, 2010). Thus, they provide the first response of the agricultural sector to the growing demand for livestock products. In 2020 has reported that the global chicken population was over 33 billion, with more than 1,600 different chicken breeds as the result of centuries of natural selection, crossbreeding and breeding within flocks (FAO, 2022). It comprises world poultry meat production (soared from 9 to 133 million tonnes between 1961 and 2020), and egg production (shot up from 15 to 93 million tonnes). To meet the the current worldwide demand, poultry meat represented almost 40 percent of global meat production, while in the last three decades, world egg production has increased by 150 percent. Interestingly, poultry is raised by approximately 80 percent of rural households in developing countries (FAO, 2022).

The Nigerian poultry industry comprises about 180 million birds, and Nigeria has the second-largest chicken population in Africa after South Africa (SAHEL, 2015). The poultry production systems in Nigeria have been classified into three types: free-range (extensive), semi-intensive, and commercial (intensive). Free-range or extensive types are where farmers keep indigenous chicken flocks and leave them to roam around to scavenge for food and water. Production from these types of farming is subsistence-oriented, mainly for family consumption, and these types are present mainly in the northern parts of Nigeria. The semi-intensive types constitute keeping flocks of about 50 to 2,000 birds, including both improved and unimproved breeds, and the farmers tend to sell live birds through informal marketing channels; these farming practices are mainly located in the southern regions of the countries. The commercial system

(intensive) is where the producers keep more than 2000 exotic birds of one breed, producing either meat or eggs for the consumer market. This production system is the dominant one in the southern parts of Nigeria (FAO, 2018). About 80 million chickens (44%) are raised in extensive systems, 60 million (33%) in semi-intensive systems and the remaining 40 million (22%) in intensive systems (FAO, 2018).

The local breeds of chicken, also referred to as indigenous chickens, are genetically adapted to the harsh conditions prevalent in their origin environments, such as surviving on limited feed resources, resistance to severe climatic weather, and evading predators without much loss in production (Mwacharo et al., 2007, Dessie et al., 2011, Orunmuyi et al., 2020) as a consequence of natural selection under scavenging conditions (Pym et al., 2006). Moreover, the study by Bett et al. (2013) showed that most consumers (98%) prefer to consume the indigenous chicken product compared with exotic chicken as they look for particular qualities such as fat content, colour, and flavour in meat as well as shell colour, size, and yolk colour in the eggs.

Advances in genomic technology or tools, along with the availability of chicken genome references, provide us with the opportunity to perform studies aimed at unravelling the genetic variations of Nigerian chickens as well as characterizing their functional genomes (Okpeku et al., 2019; Perini et al., 2021) through, for instance, Genome-Wide Association Studies (GWAS) and positive selection signature analyses for the understanding of the genetic control of production and adaptive traits (Perini et al., 2021). These studies mostly require the identification of a substantial number of genome-wide Single Nucleotide Polymorphisms (SNPs) as genetic markers. The availability of full genome DNA sequencing (2^{nd} and 3^{rd} generations) technologies and their relative affordability is providing an increasing amount of genetic information for all livestock species (Okpeku et al., 2019; Perini et al., 2021). Second-generation

sequencers generate massive amounts of short reads, which differ in throughput and length from reads produced by Sanger sequencers while third-generation generates long reads with a mean length of 4.5 kbp and with randomly distributed sequencing errors (Miyamoto et al., 2014). Allelic variants linked to traits of interest – as determined based on genomic analyses - may be then selected or introgressed by selective breeding programmes and/or crossbreeding schemes (Yadav A. K., 2017; Salisu et al., 2018).

The SNPs are more commonly used in functional and neutral genetic diversity studies compared to other different types of genetic markers (e.g., structural variants, copy number variants (CNV)). Extremely common and with a low mutation rate, they remain the markers of choice for diversity and full genome association studies (Amorim and Pereira, 2005; Bush and Haines, 2010). Moreover, SNP markers are compatible with high-throughput genotyping platforms. They are also valuable for a variety of genetic and genomic applications such as the construction of genetic maps and they have now been used widely in chicken studies (Jalving et al., 2004, Wong et al., 2004).

Investigation of the genomic variations and the identification of mutation(s) that are responsible for the adaptation of Nigerian chickens to their environments is a multi-step process. Many studies using chicken genome sequences - mapped against the chicken genome reference - typically analysed signatures of positive natural or artificial selection (Rubin et al., 2010, Walugembe et al., 2019, Seo et al., 2018). However, only a few genomic studies on African chickens are available. They include the studies from Fleming et al. (2017) on Ugandan and Rwandan chicken, Elbeltagy et al. (2019) and Walugembe et al. (2019) on Northern African chicken, and the studies of Lawal et al. (2018), and Gheyas et al. (2021) on Ethiopian indigenous chickens.

Selection signature analyses have been performed in both human and domestic species (Qanbari and Simianer, 2014). Three different types of natural selection may occur in a population: positive, purifying, and balancing selections. However, positive selection is easier to detect in a population as a result of an increase in the prevalence of advantageous alleles, which is noticed as a unique pattern of genetic variation in the genome of the population. This type of selection increases the number of alleles observed at high frequencies and favours alleles that increase the fitness of individuals (López et al., 2015). Within (intra) or between (inter) population analysis may be performed. Methods have been developed based on allele frequency spectrum analysis, local variability, and haplotype structure, which are all suitable to detect selective sweeps (Akey et al., 2002, Biswas and Akey, 2006, de Simoni Gouveia et al., 2014, Qanbari and Simianer, 2014).

1.2 The origin and domestication of African chicken

The modern chicken (*Gallus gallus domesticus*) was initially reported to be domesticated from a single wild ancestor, the Red Jungle Fowl (RJF) (Darwin, 1868), with Southeast Asia as the centre of origin (Fumihito et al., 1994, Fumihito et al., 1996). However, a recent study using 863 chicken genomes suggests that domestic chickens derived from the RJF subspecies *Gallus gallus spadiceus* around 9,500 ± 3,300 years ago, and this subspecies is currently distributed predominantly in south-western China, northern Thailand, and Myanmar (Wang et al., 2020). Subsequently, following its dispersion, domestic chicken hybridized with local RJF subspecies as well as their jungle fowl species (Wang et al., 2020). Indeed, the study of introgression in chicken reveals a polyphyletic origin of domestic chicken diversity with the RJF as the main ancestor and subsequent introgression from the Grey, Ceylon, and Green junglefowl (Lawal et al., 2020).

Small-scale contact and trade along ancient land and sea routes ultimately led to the chicken spreading and its exploitation across Asia and most regions of the world, including Africa (Perry-Gal et al., 2015). It is a multipurpose bird being exploited as food (egg, meat), for ornamental (e.g. fancy breeds) or for recreational (e.g. cockfighting) purposes (Gifford-Gonzalez and Hanotte, 2011, Perry-Gal et al., 2015). The ancient bones and linguistic sources, as well as molecular genetic evidence, suggest that domestic chickens were introduced to Africa at least three times through two separate routes: from North Africa through the Sahara to West Africa and from the East African coast to Central Africa (Chaix, 2001, Mwacharo et al., 2013). The earliest written reference to chickens in West Africa comes from Ibnu Battuta, a Muslim Moroccan scholar, who noted the presence of chickens "for sale" along his route from Iwalatan (Walata) to the capital of Mali in 1352 AD (Lewicki, 1974). It is known that the chicken first arrived in sub-Saharan Africa sometime before 850 AD (MacDonald, 1992).

The genetic evidence of the West African chicken, including in Nigeria, suggests a single maternal origin (from the Indian subcontinent). The chicken could have reached today's Nigeria from the North of the African continent through trans-Saharan trading; from East Africa through migrations along the Sahelian belt and/or they could have also reached West Africa through trading along its coast (Adebambo et al., 2010, Ajibike et al., 2017). Several studies have now investigated the maternal origin and diversity of African indigenous chickens including in East Africa (Mobegi, 2006), Zimbabwe (Muchadeyi et al., 2008), South Africa (Mtileni et al., 2011), Tanzania (Lyimo et al., 2014), Sudan and Southern Sudan (Wani et al., 2014), Chad and Central Africas, and Algeria and Libya (Al-Jumaili et al., 2020).

Over the years, these domesticated chickens introduced to different localities have acquired diverse genetic characteristics and adapted to different environmental challenging conditions (Hall and Bradley, 1995), such as heat stress, humidity, and diseases presence (Tirawattanawanich et al., 2011, Bobbo et al., 2013, Lawal et al., 2018, Walugembe et al., 2019).

1.3 Nigerian chicken types and their adaptation to the tropical environments and diseases

1.3.1 The agro-ecological zones of Nigeria

Nigeria is located mainly within the lowland tropics, characterised by high temperatures of up to 32°C in the coastal south and as high as 41°C in the North. The climate varies from the very wet conditions, typical in the coastal areas with an annual rainfall greater than 3500 mm, to substantially dry conditions in the Sahel region in the North-West and North-Eastern parts, with an annual rainfall below 600 mm. Nigeria also has two main altitudinal regions: the high plateau, ranging between 300 m and more than 900 m above sea level, and the lowlands, which are generally less than 300 m (Federal Department of Forestry, 2019).

The ecological zones in Nigeria followed in this study, are defined from South to North: Mangrove Swamp and Coastal Vegetation, Freshwater Swamp Forest, Lowland Rainforest, Derived Savanna, Guinea Savanna, Sudan Savanna and, Sahel Savanna (Keay, 1949; Federal Department of Forestry, 2019). A few mountainous areas are found in the Jos Plateau, Adamawa, Taraba, and the Northern part of Cross River State (Figure 1.1).

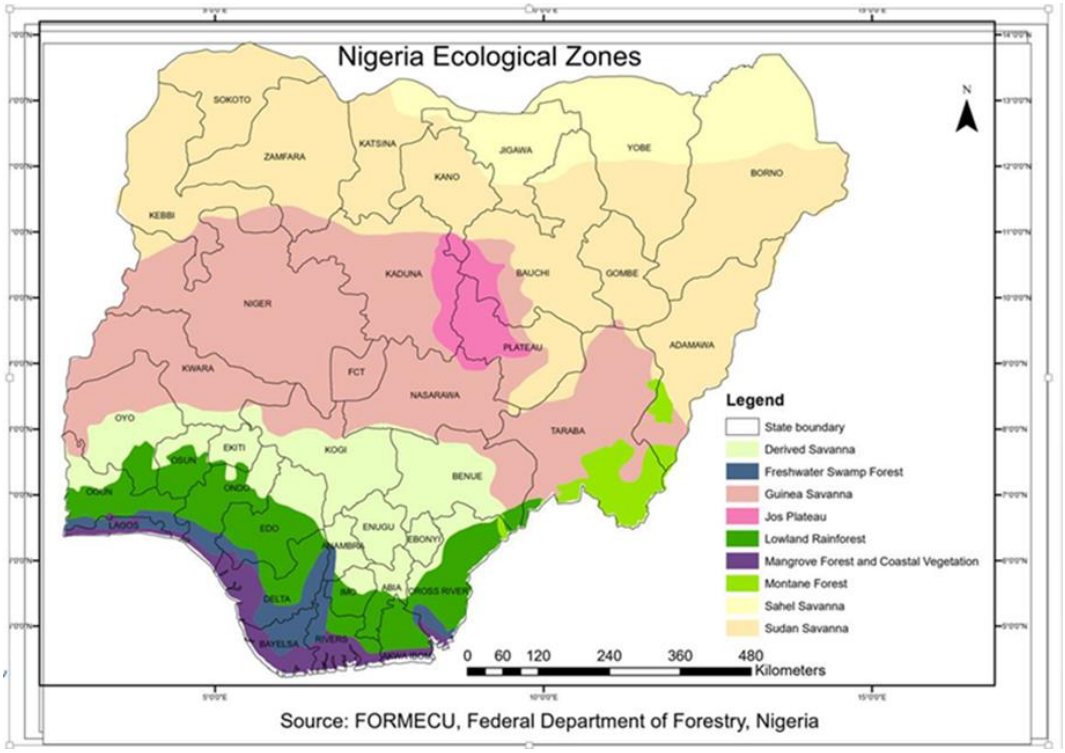


Figure 1.1. Ecological zones of Nigeria.

Source: Federal Department of Forestry (2019).

1.3.2 Breed types, characteristics, and distribution of Nigerian indigenous chicken

The term indigenous or local chicken is used interchangeably to denote unimproved, unpedigreed and unselected population of random breeding native chickens (Ikpeme et al., 2020). To avoid such discrepancies and limit further variations, I adopted the naming referred to by Halima et al. (2007), which uses the term ‘indigenous’ instead of native or local chickens. These chickens are spread throughout the rural areas of Nigeria in different agro-ecological zones (Adetayo, 2001, Ajayi, 2010), and they are commonly called the Nigerian Indigenous Chicken (NIC). Classification of the NICs has been done based on the agro-ecological zones (Adetayo, 2001) and some studies denoted the chicken found in different agricultural zones as ecotypes (Momoh et al.,

2010). They have been classified into two breeds based on their geographic location namely, the Fulani and the Yoruba. The Fulani chicken is found in some parts of Nigeria mainly in the Sahel/Sudan and Guinea savanna, the cattle Kraals and Montane parts of northern Nigeria. On the other hand, the Yoruba is located around the Rainforest, Swamp, and Derived savanna areas (Nwogwugwu et al., 2019).

Nigerian chickens have also been characterized along genetic lines of feather types (e.g. normal or frizzled feathered), comb styles, body structure (e.g. naked neck, dwarf types), and plumage colour (e.g. black, white, brown, mottled, etc.) (Ajayi, 2010, Ajayi et al., 2010, Adekoya et al., 2013; Rotimi et al., 2016). The normal feathered birds are the most common phenotypes in comparison to the frizzled feathered and naked neck ones (Ajayi, 2010). The naked neck (Na) and frizzle (F) loci are associated with heat stress tolerance, and their presence in indigenous chickens in Nigeria may be considered as adaptative phenotypes (Fathi et al., 2013), thus the high performance of naked neck chickens is associated with less stress induced by heat load (Desta, 2021). The resilience of indigenous chickens is manifested in their ability to thrive well under harsh conditions and their tolerance to many disease pathogens in the tropical hot environment (Sonaiya, 1998). Hot climates are characterized by a diurnal cycle of ambient temperature, which varies between geographical locations and seasons. Under high-temperature conditions, chicken body heat increases. To control its body temperature, the bird may dilate the blood vessels of the skin, wattles, and comb, bringing the internal body heat to the skin surface favouring heat loss (Fathi et al., 2013).

1.3.3 Genetic diversity and potential

Genetic diversity is core to genetic improvement as well as to a species' ability to adapt to changing environments. With large variations within populations, it is more likely that some individuals will possess variations of alleles that are suited to changing environment challenges (Nonić and Šijačić-Nikolić, 2019). Previously, I mentioned that Nigerian indigenous chickens display a lot of phenotypic diversity-supporting presence of genetic variation among the chicken populations. They are characterized by a large variation in their body size, plumage colour, comb type, and feather appearance, which makes them morphologically different from each other. Ojoh et al. (2010) reported that although Nigerian indigenous chickens are low producer type of birds, they do possess a good genetic potential for laying traits.

1.3.4 Genetic approaches to improve performance of Nigerian indigenous chickens

Some of the approaches that have been applied to improve the productivity of Nigerian indigenous chickens are crossbreeding with exotic commercial lines, and the development of new improved indigenous lines selected within the indigenous populations (Adeleke et al., 2011, Sola-Ojo and Ayorinde, 2011, Adedokun and Sonaiya, 2002, Ogbu, 2011, Alabi et al., 2020).

Crossbreeding is mating different breeds to introgressed desirable traits from one parental line to the others or to combine the crossbred traits of interest from both parental breeds. Nwosu (1985 and 1990) in Peter et al., (2018) mentioned that crossbreeding experiments in Nigeria between exotic breeds and indigenous chickens, led to genetic improvement following hybrid vigour. One example is the significant improvement in daily weight gain, the number of eggs, and egg weight following crossbreeding between Nigerian indigenous chicken with the Dahlem Red exotic

chicken (German breed) (Adedokun and Sonaiya, 2002). Higher performance was also observed among crossbred Fulani ecotype and exotic egg-type compared to the native Fulani ecotype (Sola-Ojo and Ayorinde, 2011, Adeleke et al., 2011). Padhi (2016) indicated that body weight, egg weight, egg production, and age at sexual maturity were found to be better in crosses compared to indigenous chickens.

An example of the development of new, improved indigenous lines is presented by Osinbowale (2017) as reported by Nwogwugwu et al. (2019). Indigenous naked neck and frizzled feathered birds were utilized to develop broiler lines due to their quality of carcass traits. Following different crosses, they developed two lines of improved indigenous commercial breeds, with 37.5% to 62.5% indigenous blood. Furthermore, these lines were then distributed to the rural farmers in Nigeria to evaluate their performance (Nwogwugwu et al., 2019).

1.4 Tools for chicken genome characterisation

Molecular genetic characterization aimed at assessing the genetic diversity within and between indigenous and improved Nigerian chickens is an essential step towards conserving the genetic resources of Nigerian indigenous chicken breeds and improving their productivity. Analyses of DNA marker polymorphisms may provide information about diversity and selection. Polymorphic DNA markers typically include microsatellites, Singe Nucleotide Polymorphism (SNP), insertion/deletion (indels), Copy Number Variations (CNV), etc (Emara and Kim, 2003, Wang and Byers, 2014). DNA markers have been used in livestock for different genetic population studies including diversity studies, quantitative trait loci (QTL) mapping, and for assessing population conservation genetic parameters such as effective population size, the identification of past bottlenecks, inbreeding status and gene flow (Montaldo and Meza-Herrera, 1998, Gholizadeh et al., 2008, Wilkinson et al., 2011).

Moreover, DNA markers have been used in livestock breeding programmes for the determination of kinship, marker-assisted selection and genomic selection.

Some studies on the genetic characterization of African chicken such as genetic diversity studies, introgression, relationships among different breeds as well as the evolution of African chicken have previously utilized microsatellites (Muchadeyi et al., 2007, Mtileni et al., 2011, Lyimo et al., 2014), and mitochondrial (mtDNA) DNA sequences (Liu et al., 2006, Adebambo et al., 2010, Miao et al., 2013, Ajibike et al., 2017, Al-Jumaili et al., 2020). SNPs are now popular as the molecular marker of choice for chicken diversity studies with the possibility of genome analysis via high-throughput sequencing and genotyping platforms (Fan et al., 2010, Pértille et al., 2016).

Genome-wide characterisation of SNP polymorphism in chicken is facilitated by the availability of low to high-density SNP genotyping arrays (e.g. 3K genotyping array with 3,072 SNPs (Muir et al., 2008), a 60K bead chip (Groenen et al., 2011) and a high density genotyping chip (600K Affymetrix® Axiom® array). These facilitate a wide range of potential applications, e.g. genomic selection within breeding programs, Genome-Wide Association Studies (GWAS) analysis and investigation of the diversity of different chicken breeds (Kranis et al., 2013). Furthermore, these tools have been applied in several African chicken studies such as in southern African chickens using the Illumina chicken iSelect SNP60K (Khanyile et al., 2015), and using the 600K Affymetrix® Array in East African chickens (Rwanda and Uganda) (Fleming et al., 2017), North African chicken (Baladi) and Egyptian chicken (Dandarawi and Fayoumi) (Elbeltagy et al., 2019), and Tanzanian chicken (Walugembe et al., 2019). However, compared to SNP chip-based genome information, genome-wide sequencing is more informative as it allows for

identification of all genetic variants present in a species or population. Several recent studies have now reported genome-wide sequence analysis of African chicken (Lawal et al., 2018, Banos et al., 2020, Gheyas et al., 2021).

1.5 Sequencing of the chicken genome

The development and advances in genomic technologies have enabled the possibility of generating the entire genome sequence of an organism. The chicken (*Gallus gallus*), an important livestock species and a model organism that bridges the evolutionary gap between mammals and other vertebrates, was the first agricultural animal to have its genome sequenced (Hillier et al., 2004).

The chicken genome is composed of haploid content of 1.1 x 10^9 base pairs of DNA and is divided into thirty-nine chromosomes, consisting of nine cytologically distinct macrochromosomes (MACs) and thirty microchromosomes (MICs) which are much smaller than the typical mammalian genomes whose sizes are in the order of 2,500-3,000 Mb (Ladjali-Mohammedi et al., 1999). The sex chromosomes are denominated Z and W and unlike in mammals, the males are homogametic (Z/Z), while the females are heterogametic (Z/W) (Burt and White, 2007). Further distinctions have been made among MACs and MICs, with chromosomes 1 through 5 and Z include within the MAC group, while chromosomes 6 through 10 can be assigned within an intermediate size chromosome group, and the remaining ones included in the MIC group (International Chicken Genome Sequencing, 2004). The thirty chicken MICs contain about one-third of the genomic DNA, They are gene-rich, with estimates suggesting that MICs contain at least twice as many genes as the MACs (Smith et al., 2000), also with differences in GC content and proportion of repetitive sequences (International Chicken Genome Sequencing, 2004).

At the start of the 21st century, there was a proposal to sequence the chicken genome, and the first draft was assembled using a whole-genome sequencing (WGS) strategy in 2004. This initial genome sequence study using a single, partially inbred RJF had a 6.6-fold coverage of the genome with a total genome size of 1.2×10^9 base pairs (one-third of a typical mammal). The main characteristic of this genome was the low content of repetitive DNA (~11% compared to ~50% in mammals (Burt and White, 2007). Furthermore, the first versions of GGAZ and GGAW were sequenced only to ~3.3X due to their hemizygous state in the female bird used (Warren et al., 2017). Over the years, advanced improvements were made to the chicken genome reference assembly (Schmid et al., 2015). A second build (Gallus_gallus 2.1; GCA_000002315.1) of the original genome generated in 2004, had a total genome size of 1.09 Gb, ~95% of which was anchored to autosomes 1-28 and 32, along with the GGAZ and GGAW sex chromosomes. This genome version included information from the targeted sequencing of BACs and fosmids (Warren et al., 2017). The third build of the genome (Gallus_gallus-4.0; GCA_000002315.2) increased the N50 contig and scaffold size to 252 kb and 12,4 Mb, respectively, with the total amount of sequence mapped to the chromosomes increased by 15 Mb. In addition, nine additional MICs were reported to be gene-rich and either not assembled or assigned, due to mostly the lack of genetic linkage group markers to anchor the unplaced sequences (Rubin et al., 2010, Qanbari et al., 2015, Warren et al., 2017).

The next version of the chicken genome assembly was Gallus_gallus-5.0 (GCA_000002315.3, Galgal 5), with deep coverage of ~51X. Long-range single-molecule sequencing technology and BAC (Bacterial Artificial Chromosomes) sequencing (International Chicken Genome Sequencing, 2004) lead to an improved physical map of the genome with a gain of 183 Mb, including 16.4 Mb in already

placed chromosomes (Warren et al., 2017). Moreover, in the 1.21 Gb genome, three previously missing autosomes, GGA30, 31, and 33 were now included. Gallus_gallus-5.0 also had an improved sequence contig length 10-fold over the previous Gallus_gallus-4.0 reference. However, 138 Mb of the sequence was still not yet assigned to any chromosomes. Gallus_gallus-5.0 includes 4,679 more annotated genes (2,768 noncoding and 1,911 protein-coding) in comparison to Gallus_gallus-4.0 (Warren et al., 2017).

The Gallus_gallus-6.0 (GRCg6a) chicken assembly is 1,065 Mb in length. Six microchromosomes (GGA29 and GGA34-38) are still, however, missing. GRCg6a includes an 82X coverage of PacBio (Pacific Biosciences) long-reads sequence. It shows a dramatic increase of both contig and scaffold N50, together with a shorter overall assembly length, meaning that the number of gaps in the sequence is much lower (Table 1.1) (Kraus, 2019), then, this chicken genome reference was being used in this work.

The latest chicken assemblies (GRCg7b and GRCg7w) came from an F1 individual from a broiler mother and a layer father, generated as part of the Vertebrate Genomes Project. This provides the foundation for a substantially improved genome assembly and annotation that leverages a diverse set of short (Illumina) and long-read (IsoSeq, PacBio) sequencing data along with high resolution scaffolding data. These reference genomes provide information lacking in the previous reference, such as missing micro-chromosomes, and have corrected misassembled chr31, chr32 and chr33 of GRCg6a. The broiler and layer references represent assembled pseudo-haplotype genomes (VGP, 2022).

Table 1.1. Assembly contiguity metrics of chicken genomes by version (in base pairs)

Assembly statistics	**Gallus_gallus-4.0**	**Gallus_gallus-5.0**	**GRCg6a**	**GRCg7b**
Total sequence length	1,046,915,324	1,230,241,782	1,065,348,650	1,053,332,251
Total ungapped length	1,032,841,023	1,218,476,783	1,055,564,190	1,049,948,333
Gaps between scaffolds	915	397	68	0
Number of scaffolds	16,846	23,869	524	214
Scaffold N50	12,877,381	6,379,610	20,785,086	90,861,225
Scaffold L50	23	47	12	4
Number of contigs	27,040	24,692	1,402	677
Contig N50	279,750	2,894,815	17,655,422	18,834,961
Contig L50	950	110	19	18
Total number of chromosomes	33	35	34	42

Sources: *https://www.ncbi.nlm.nih.gov/assembly*

1.6 Full genome sequence analysis

In population genetic terms, genetic diversity reflects the interplay of mutation, genetic drift, selection, recombination and gene flow on DNA sequence variation (Dutoit et al., 2017). Full genome sequence analysis allows the discovery of various types of genomic variants and provides the opportunity to assess the extent of genetic diversity within and among populations. Genotype information on genome-wide variants (e.g., SNPs) can also be used for many other purposes for livestock species like chicken, such as identifying loci associated with production and performance traits, detecting genomic regions under positive selection (either from artificial selection or natural adaptive selection), and improving breeding performance through the application of genomic selection.

1.6.1 Estimation of genomic diversity

Changes in genetic diversity may be identified using SNPs and measured by nucleotide diversity, heterozygosity, genomic inbreeding coefficient and/or using estimates derived from runs of homozygosity (ROH) (Zhang et al., 2018). Another parameter that is important to be considered in population genetics is linkage disequilibrium (LD) which refers to the nonrandom association of alleles at different loci (Amaral et al., 2008). This method has been used to determine the association between variants and traits and efforts to understand selection pressures (Weir, 2008), whereas natural populations are affected by many factors such as genetic drift, population structure, migration, admixture, selection, mutation and recombination (Hedrick, 2009).

Nucleotide diversity (π) can determine the DNA sequence diversity in a population. It describes the average proportion of nucleotide differences between all possible pairs of sequences obtained for that population (Marks, 1988). Allelic diversity determines a population's ability to respond to long-term selection over many generations; accordingly, it may be linked to the long-term survival of a population. Genome-wide heterozygosity may also act as a proxy for an individual inbreeding coefficient, meaning that a decrease in heterozygosity would indicate the expression of deleterious, recessive alleles with an associated fitness cost (Hansson and Westerberg, 2002). Also, genetic variation associated with specific loci may influence fitness (e.g., heterozygote advantage) (Jingade et al., 2011).

The inbreeding coefficient (*F*) of an individual is defined as the probability of two randomly chosen alleles at the same locus from two gametes to be identical by descent (IBD) from a common ancestor (Crow and Kimura, 1970). Linkage disequilibrium (LD) is the non-random assortment of alleles at different loci, and the pattern of LD is

a powerful indicator of the genetic forces shaping a population. The decay and extent of LD at a pair-wise distance can be used to determine the evolutionary history of populations (Andreescu et al., 2007). For example, indigenous chicken breeds generally exhibit a shorter extent of LD (Khanyile et al., 2015) compared to commercial breeds. Among commercial populations, broiler lines generally show faster decay of LD than layer layers (Seo et al., 2018). This is a consequence of a more intensive selection scheme over many generations in layer lines resulting in a lower population haplotype diversity and a smaller effective population size (*Ne*) (Qanbari, 2020).

1.6.2 Estimation of population structure

The two most widely used approaches to model population structure using genomic data are admixture-based models, such as those implemented in the software packages STRUCTURE (Pritchard and Donnelly, 2001, Falush et al., 2003), FRAPPE (Tang et al., 2005), SABER (Tang et al., 2006), and ADMIXTURE (Alexander et al., 2009) and Principal Component Analysis (PCA) as implemented in the programmes like SmartPCA (Price et al., 2006). In admixture-based models, every individual is assumed to have inherited some proportion of their ancestry from an inferred number of parental populations. The proportions are known as the admixture proportions of each individual (Engelhardt and Stephens, 2010). While PCA analysis will result in the projection of the individuals into a low-dimensional subspace, with the locations of individuals in the projected space showing the genetic similarities among them (Engelhardt and Stephens, 2010).

1.7 Signatures of selection

The diversity that exists within various species is observed as variations in their phenotypes such as physical appearance (morphology), production traits, environmental adaptations, and disease resistance or susceptibility. It is often challenging to understand the underlying genetic variations and mechanisms contributing to phenotypic diversity (Biswas and Akey, 2006). Identification of genomic loci under selection also called “selection signature analysis”, is one of the mainstream approaches to gaining insight into the underlying genetics associated with a phenotypic trait. The ability to identify the molecular signatures of natural selection(s) provides a powerful tool for identifying loci that have contributed to adaptation.

Regions of the genome with selection signature signals also referred to as selective sweep regions, are expected to harbour functionally important sequence variants and therefore to have been under either natural or artificial selection (Qanbari and Simianer, 2014; Koropoulis et al., 2020). In terms of molecular evolution, three different forms of selection are recognized: positive selection or directional selection, negative or purifying selection or background selection, and balancing selection. Each of them will impact the diversity of the genome in particular ways by changing allele frequency at the selected loci as well as surrounding loci by preferring certain genotypes over others. Balancing selection is defined as a class of selective regimes that maintains polymorphism above what is expected under neutrality (Charlesworth, 2006). Negative selection acts upon new deleterious mutation for removing it and maintaining the functional integrity of the DNA sequence (Charlesworth, 2006, Barreiro et al., 2008). Positive selection, on the other hand, is defined as any type of selection where mutations are advantageous (have positive selection coefficients). It

may be associated with adaptation and the evolution of a new form or function (Nielsen, 2005). This type of selection is also important in explaining the pattern of variability within and between species. When the frequency of the favoured allele increases in the population due to positive selection, it causes linked alleles in nearby regions to increase in frequency too, thereby reducing the variation at the selected locus as well as its surrounding regions following genetic hitchhiking (Maynard and Haigh, 2007). Even if only a single allele is being selected, it may result in a local selective sweep (Qanbari and Simianer, 2014). The two main models of selective sweeps are referred to as hard sweep and soft sweep. Hard sweep refers to the phenomenon where an allele with a strong selective advantage increases quickly in frequency in the population until eventually reaching fixation. In contrast, soft sweeps e.g. from standing variation, or sweeps in which multiple mutations start to sweep simultaneously at a single locus (if the favoured mutations are roughly equivalent, then no single allele sweeps rapidly to fixation). Simulations have shown that soft sweeps typically have weaker effects on linked sites and, therefore, may be more difficult to detect than hard sweeps (Pritchard et al., 2010).

1.7.1 Approaches for detecting the signature of positive selection

Several approaches have been designed for the detection of selection and most of the methods were developed and initially applied to human genomic data, with subsequent applications in livestock species including chicken. The advances in high-throughput sequencing and SNP genotyping technology have greatly expanded the ability to identify the signatures of selection based on site frequency spectrum (SFS), linkage disequilibrium, and reduced local variability. The majority of the tests are designed to detect loci under selection either within the population or by comparison between populations. The tests are performed either on individual variants or over genomic

windows covering multiple variants and look for outlier regions (i.e. variants of windows showing extreme scores) compared to the rest of the genome (Qanbari et al., 2019).

1.7.1. a. Tests based on linkage disequilibrium pattern

An excess of allelic association between loci is termed linkage disequilibrium (LD) (Kim and Nielsen, 2004). LD may arise from genetic drift, population admixture, and selection, but the extent of LD will be reduced by recombination at each generation. LD is, therefore, higher between close loci, and it will decay with increasing physical distance (Slatkin, 2008). LD tests include LD Decay (LDD), Extended Haplotype Homozygosity (EHH), Long Range Haplotype (LRH), integrated Haplotype Score (*iHS*), Relative Extended Haplotype Homozygosity (REHH) and Cross-Population Extended Haplotype Homozygosity (XP-EHH) calculations (Sabeti et al., 2002, Biswas and Akey, 2006, Voight et al., 2006, Qanbari and Simianer, 2014).

Most of the LD-based methods focus on long homozygous regions with high frequencies of specific haplotypes generated by hard sweeps (Sabeti et al., 2002, Garud et al., 2015). Variants of the tests incorporate different modifications, i.e. correcting for variation in the recombination rate (Sabeti et al., 2002) or using two populations instead of one (Sabeti et al., 2007). The selective sweep also generates a specific spatial pattern of LD owing to the independent recombination events on both sides of the beneficial mutation (Kim and Nielsen, 2004, Weigand and Leese, 2018).

EHH is defined as the probability that two randomly chosen chromosomes carrying the tested core haplotype are homozygous at all SNPs for the entire interval from the core region to the distance x (Sabeti et al., 2002a). LRH is calculated by dividing EHH on a core haplotype by EHH among all the samples not containing the core haplotype (Huff et al., 2010).

The recombination maps are required for some methods; in particular, *iHS* and XP-EHH. As the recombination rate across a genome can vary strongly, this can influence genetic diversity patterns (e.g. reduced recombination rate increases LD locally). Additionally, genetic variation is reduced more rapidly by selective sweeps in regions with intermediate or high recombination rates (Weigand and Leese, 2018).

1.7.1. b. Test based on reduced local variability

Runs of Homozygosity (ROH) and pooled heterozygosity (*Hp*) tests have been widely used to detect regions of the genome where there are reduced variations (e.g. nucleotide diversity or heterozygosity) compared to the average across the genome (Rubin et al., 2010).

ROH test searches persistent regions of the genome with homozygosity in the diploid state. ROH is used on a genome-wide scale to detect signals of past selection (Lencz et al., 2007), while *Hp* statistics are designed to estimate variability by looking for the regions of the genome with low heterozygosity.

The *Hp* test specifically involves counting the most and least abundantly observed alleles at every detected SNP position across sliding windows. Following hitchhiking, a reduction of local variability compared to genome average will be observed at the selective sweeps (Braverman et al., 1995).

1.7.1. c. Tests based on alleles frequency changes

These tests rely on the assumption that selective sweeps affect the frequency of variants (increased proportion of low and high-frequency variants and a reduced proportion of intermediate frequency variants). Some widely used tests are Tajima's D (Tajima, 1989) and Fay and Wu's H test (Fay and Wu, 2000), while a more recent test is the composite likelihood ratio (CLR) (Nielsen et al., 2005).

Tajima's D is the standardised difference between two commonly used measures of variability; θw, which is based on the total number of segregating sites and is influenced most by low-frequency variation (Watterson, 1975), and $\theta\pi$, which is based on average heterozygosity and is influenced most by intermediate-frequency variants (Tajima, 1989). A negative value of Tajima's D is a deviation from the allele frequency spectrum. It is caused by an excess of low-frequency alleles, which indicate positive selection whereas a value of zero is expected in a standard neutral model.

Fay and Wu's H test (2000), estimates the scaled population mutation rate in two different ways (average homozygosity and average heterozygosity of the derived allele) and then the estimates are compared. For the selective sweep case, high-frequency-derived allele excess is expected and thus leading to a higher homozygosity estimate relative to the heterozygosity and negative H values (Weigand and Leese, 2018).

The CLR test is comparing the likelihood of a model including selection with a neutral model for a specific genomic region (Weigand and Leese, 2018).

1.7.1. d. Tests based on population differentiation

Genetic differentiation between multiple populations can be determined from the estimation of Wright's fixation index *FST* (Wright, 1949). A more novel statistic based on single-site differentiation is FLK with the haplotype-based extension of the FLK statistic called hapFLK (Bonhomme et al., 2010, Fariello et al., 2013). FLK is a parametric statistical test for the detection of selection signatures in complex population trees. It is a quick and powerful tool for large data sets in the context of genomic scans (Bonhomme et al., 2010). While the hapFLK statistic takes into account both hierarchical population structure and haplotype information, it has the power to

detect soft sweeps, incomplete sweeps and sweeps occurring in several populations (Fariello et al., 2013).

Signature of positive selection particularly between populations can be detected by comparing the multi-locus values of the metric with the values in a neutral model. It is expected that the *FST* value estimated for regions of the genome under selection should exhibit larger divergence (i.e. high *FST*) among populations than neutral loci, while regions under uniform balancing selection in all populations should be less differentiated (low *FST*) (Qanbari and Simianer, 2014). This approach has been employed to identify selection signature and population structure in several chicken populations including African chicken (Fleming et al., 2017, Elbeltagy et al., 2019).

1.7.2 Signatures of selection in African chickens

Since their domestication, and in line with their dispersion, chickens have been subjected to both human selection (selective breeding) and natural selection (environmental adaptation) leading to the development of different breeds and ecotypes. Therefore, the investigation of selection signatures in indigenous African chickens may provide insight into regions targeted by selection during domestication, breed formation as well as adaptation to the tropics. Several studies on African chickens have applied different selection scan approaches and have analysed genome-wide SNP information from low density (60K) chips, high density (600K) chips to full genome sequence data (Khanyile et al., 2015, Fleming et al., 2017, Elbeltagy et al., 2019, Gheyas et al., 2021). The focus of these studies has been to identify selection signatures related to tropical environmental adaptation (e.g., disease resistance and heat tolerance) and production traits (e.g., egg and meat production) and with the majority of them utilising 60K chip information instead of whole-genome sequence data.

Several studies have now reported candidate signature of selection in the genome is possibly linked to environmental selection in African chickens. These include studies on three indigenous chicken ecotypes from East Africa, North Africa and Egyptian local breeds (Walugembe et al., 2019, Elbeltagy et al., 2019), village chickens from Zimbabwe, Malawi, and South Africa (Hadebe et al., 2019, Khanyile et al., 2015, Fleming et al., 2017), and indigenous Ethiopian chicken (Gheyas et al., 2020, Lawal et al., 2018). These analyses have revealed genomic regions under selection pressure including genes with functions that may be linked to environmental challenges.

More specifically, using several analyses Fleming et al. (2017) identified a commonly detected *FST*, *iHS* and *Rsb* candidate region on chromosome 27 of ~1–4 Mb. This region includes two genes *HSP25* and *DNAJC7*, both of which are directly related to temperature variation stress tolerance responses (Jenuth, 2000, Consortium, 2015). The study from Elbeltagy et al. (2019), based on ROH analysis in Egyptian chickens, showed selection footprints in candidate regions with genes for adaptation to heat, solar radiation, ion transport and immunity. The high-altitude-adapted East-African chicken populations showed a selection signature with genes for angiogenesis, oxygen-heme binding and transport (Elbeltagy et al., 2019). In the meantime, two candidate genes, *HRH1* and *AGTR1* have been found in Ethiopian village chicken, which are associated with "vasoconstriction regulation" and have been linked to a reduction in peripheral blood flow leading to an increase in internal body temperature (Lawal et al., 2018). These genes may also play important roles in thermoregulation. Meanwhile, strong selective signals concerning high-altitude or cold temperature and drought in Ethiopian chickens have been reported by Gheyas et al. (2020). The results of these signature selection studies in African chickens may enhance the

understanding of the roles of the natural selection forces in shaping African chicken genomes for adaptation to the stressful tropical environment.

1.8 Aims and objectives of this thesis

This thesis analyses whole-genome sequence data from Nigerian indigenous chicken populations representing diverse agro-ecological zones to investigate genetic diversity and landscape of genomic selection signatures in relation to environmental adaptation. As a farm animal, an understanding of the chicken genome can lead to genes or genomic regions which are associated with advantageous traits. The study of genomic characterization could give valuable insight into the variant information that can help improve applications of genomic selection and access to population structure and genetic diversity among the Nigerian chicken.

This thesis includes six chapters:

Chapter 1 (this chapter) is the general introduction.

Chapter 2 reports the genomic diversity and the population structure of Nigerian indigenous chickens based on whole-genome sequence data.

Chapter 3 applies *Hp* and *FST* analysis for selection signature detection in Nigerian chicken in relation to specific climatic parameters viz. high temperature and rainfall/precipitations variations.

Chapter 4 investigates the genomic landscape of selection signatures in different ecotypes of Nigerian indigenous chickens based on their agro-ecological zones. The *Hp* method is used to identify selective sweep regions within each ecotype and *FST* analysis is used to perform comparisons between ecotypes.

Chapter 5 is a general conclusion, highlighting key contributions of the thesis and discussing the limitation of the study and the ways forward.

CHAPTER 2:

Genomic diversity and population structure of Nigerian indigenous chickens

2.1 Introduction

The term indigenous chicken, also referred to as “native”, “scavenging”, “village”, “local”, “traditional”, or “free-range”, is used interchangeably to denote a group of scavenging or semi-scavenging birds largely unimproved, unpedigreed and unselected (human selection) native chickens. They are found in environmental areas either unsuitable for exotic or commercial breeds or when farmers have not been able to afford the high input requirement of maintaining these breeds (Kitalyi, 1998, Ogbu, 2010). Indigenous chickens are usually reared under household production systems (Conan et al., 2012). The household production system is commonly related to poor biosecurity conditions, small size (under 100 heads per flock), and little or no veterinary intervention (Mapiye et al., 2008). Based on the Food and Agricultural Organisation (FAO, 2017), farmers keep indigenous chickens in flocks, which are left to roam around and scavenge for food and water, and the flocks may contain birds of different species (e.g. chicken, ducks) and varying ages. Such farming practice is referred to as a free-range system (extensive).

Nigeria has many indigenous chickens, which are able to better survive in low input systems, are more tolerant and/or resistant to disease challenges, and can tolerate variable production and climate conditions better than commercial birds (Ajibike et al., 2017). Overall, it may be expected that the diversity in agro-ecological and climatic conditions across the country has contributed to the current diversity in the adaptation of livestock genetic resources in Nigeria, including chicken. Interestingly, Ajayi (2010) compared the bodyweight of different chicken ecotypes. The heavier ecotypes (often referred to as Fulani, defined by a chicken body weight of about 0.9 - 2.5 kg at maturity) are found in the dry savanna (Guinea and Sahel savanna) and the mountainous regions of the North of the country, while the lighter ecotypes

(bodyweight between 0.68 - 1.5 kg at maturity) are found in the rainforest and derived humid savanna agro-ecological zones.

Genetic studies on Nigerian indigenous chicken remain few. Adebambo et al. (2010) were the first to study the mitochondrial DNA (mtDNA) D-loop diversity of Nigerian indigenous chickens. Examining 232 village chickens from Southern and Northern Nigeria, they reported that all mitochondrial DNA sequences belong to a single clade or haplogroup; a haplogroup predominantly found today in South Asia (Indian subcontinent) within the wild distribution of the red junglefowl. Their study also suggested extensive genetic intermixing within the country, resulting in a lack of phylogeographic structure among the Nigerian village chicken populations (Adeleke et al., 2011). Furthermore, they examined the mtDNA diversity of the three major phenotypes of Nigerian chicken (naked neck, normal-feathered, and frizzle-feathered). The results show that frizzle-and normal-feathered chickens belong to the same genetic cluster, which was found to be distantly related to the naked neck chickens.

The recent study on the genetic diversity of Nigerian indigenous and commercial chickens was conducted by Ajibike et al. (2017). This study supports the results from the previous studies with the identification of a single main mtDNA maternal lineage, likely originating from the Indian subcontinent and with no clear mtDNA substructuring between and within populations. So far, there have been no previous studies that have assessed the genetic diversity of indigenous Nigerian chickens at the whole-genome level.

In this chapter, I analysed whole-genome sequence (WGS) data from a large number of Nigerian indigenous chickens representing different agro-ecological zones. I examine and report for the first time, the genetic diversity and population structure of

Nigerian indigenous chicken populations at the genome-wide level using the WGS data. The SNP polymorphisms identified here will then be further analysed for the identification of candidate signatures of selection for environmental adaptation (Chapters 3 and 4).

2.2 Materials and methods

2.2.1 Chicken populations

A total of 120 chicken samples representing fourteen populations were studied. These populations represent the eight agro-ecological zones of Nigeria (Figure 2.1): Sudan Savanna (n = 10), Northern Guinea Savanna (n = 10), Southern Guinea Savanna (n = 20), Plateau Mid-Altitude (n = 20), Derived Savanna (n = 20), Humid Rainforest (n = 20), Humid Savanna Freshwater Swamp (n = 10), and Humid Savanna Mangrove (n = 10). Further details regarding the populations from each agro-ecological zones are provided in Table 2.1

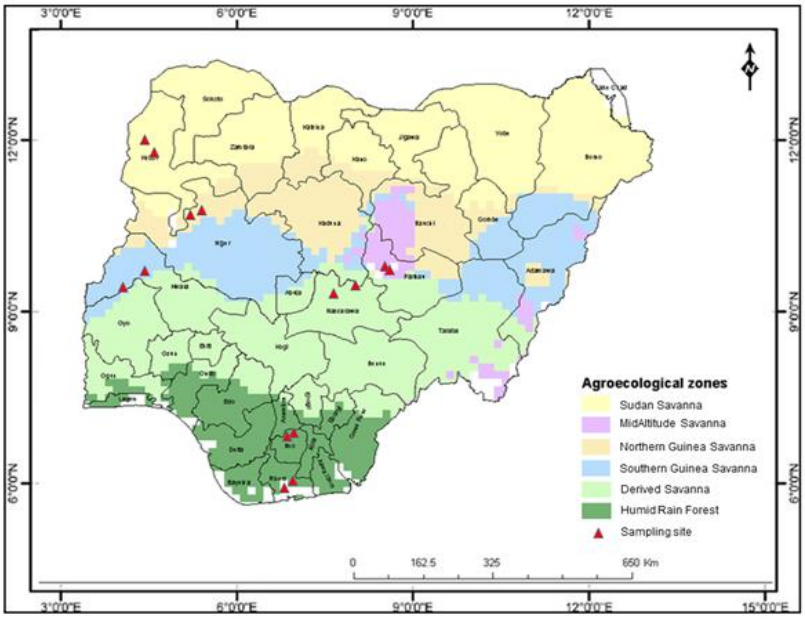


Figure 2.1. Sampling site of the studied population against the Nigerian agro-ecological zones.

Figure modified from https://redd.unfccc.int/files/2019_submission_frel_nigeria.pdf.

Table 2.1. Details of chicken populations included in the study

No	Population/Village	Sample (n)	State	Agro-ecological zones	Longitude	Latitude	Bodyweight (Mean ± SD in kg)
1	Sabiyal	6	Kebbi	Sudan Savanna	E $4^0$26'33"	N $12^0$23'9"	1.06 ± 0.12
2	Jiga	4	Kebbi	Sudan Savanna	E $4^0$32'5"	N $12^0$17'1"	1.10 ± 0.15
3	Senchi	4	Kebbi	Northern Guinea Savanna	E $5^0$13'49"	N $11^0$18'15"	1.14 ± 0.29
4	Kwendo/Doro/Tadurga	6	Kebbi	Northern Guinea Savanna	E $5^0$21'47"	N $11^0$32'32"	1.09 ± 0.21
5	Ogbondoroko	10	Kwara	Southern Guinea Savanna	E $4^0$36'13"	N 8^0 23'49"	0.96 ± 0.21
6	Sanchitagi	10	Kwara	Southern Guinea Savanna	E $5^0$7'3"	N 8^0 56'48"	1.06 ± 0.10
7	Wat-Karu	10	Plateau	Plateau Mid-Altitude	E $8^0$50'0"	N $9^0$43'16"	1.26 ± 0.25
8	Dakan-Karu	10	Plateau	Plateau Mid-Altitude	E $8^0$50'52"	N $9^0$43'43"	1.06 ± 0.26
9	Gitta Mbasha	10	Nasarawa	Derived Savanna	E $8^0$36'380"	N $9^0$00'610"	1.31 ± 0.31
10	Karu	10	Nasarawa	Derived Savanna	E $7^0$38'037"	N $9^0$00'117"	1.09 ± 0.27
11	Okpofe	10	Imo	Humid Rainforest	E $7^0$17'50"	N $5^0$29'38"	1.13 ± 0.20
12	Odenkume	10	Imo	Humid Rainforest	E $7^0$19'17"	N $5^0$36'10"	0.95 ± 0.20
13	Degema	10	Rivers	Humid Savanna Mangrove	E $6^0$46'2"	N $4^0$45'38"	0.88 ± 0.10
14	Isiokpo	10	Rivers	Humid Savanna Freshwater Swamp	E $6^0$52'29"	N $4^0$59'11"	0.95 ± 0.12

2.2.2 Blood samples and DNA extraction

Blood samples from 120 adult chickens (older than six months of age) were collected from the wing vein in approximately 50 – 250 µl amounts using cryotubes filled with 1.5 ml absolute ethanol (100%) following the procedure described at https://www.sheffield.ac.uk/nbaf-s/protocols_list (Accessed in 2017). Total DNA was extracted using the Qiagen DNeasy Blood and tissue kit protocol and resuspended at a final concentration of 50 ng/µl. The DNA was then sent to Edinburgh Genomics (http://genomics.ed.ac.uk/) in the UK for whole-genome sequencing.

2.2.3 DNA quality checking and library preparation

The quality control, library preparation, and sequencing were performed at Edinburgh Genomics as part of their whole-genome sequencing services. Genomic DNA (gDNA) samples were evaluated for both quantity and quality using AATI Fragment Analyzer and the DNF-487 Standard Sensitivity Genomic DNA Analysis Kit. Based on the quantification results, gDNA samples were pre-normalised to fall within the acceptable range (quality score > 7) of the Illumina SeqLab TruSeq Nano library preparation method using the Hamilton MicroLab STAR.

Next-Generation Sequencing libraries were prepared for paired-end sequencing using Illumina SeqLab specific TruSeqNano High Throughput library preparation kits in conjunction with the Hamilton MicroLab STAR and Clarity LIMS X Edition. The gDNA samples were normalised to the concentration and volume required for the Illumina TruSeq Nano library preparation kits, and then sheared to a 450 bp mean insert size using a Covaris LE220 focused-ultrasonicator. The inserts were then ligated with blunt-ended, A-tailed size selected TruSeq adapters and enriched using eight

cycles of PCR amplification, followed by library-quality checking and sequencing process.

2.2.4 Sequence mapping

The quality of the sequence data was checked using the FASTQC package (Babraham Bioinformatics, 2016) to ensure there was no adapter contamination or major issues in quality. All the 120 whole-genome sequences were aligned to the chicken reference genome GRCg6a, also known as Galgal6.0 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000002315.6) using the Burrows-Wheeler Aligner (BWA) version 0.7.15 (Li and Durbin, 2010) with the option "bwa-mem". Following the mapping, the alignment files in SAM format were converted to BAM files using SAMtools version 1.4.0 (Li et al., 2009). I then followed the Broad institute recommended Genome Analysis Toolkit (GATK) Best Practices pre-processing workflow (https://software.broadinstitute.org/gatk/best-practices/) before variant discovery. The pre-processing steps included sorting the BAM files into coordinate order, marking the duplicate reads, and indexing the BAM files. All these steps were performed using Picard tools version 2.9.0 (http://broadinstitute.github.io/picard/). GATK version 3.8.0 (McKenna et al., 2010) (Van der Auwera et al., 2013) was used for recalibrating the base quality scores (BQSR) with the default setting to improve the base quality score estimation. About 20 million known chicken SNPs from the dbSNP (version 150) database were also used as known polymorphic sites in the BQSR step to assess the accuracy of the SNPs calling pipeline.

2.2.5 Variant discovery, and quality control

To discover variants, I ran the "HaplotypeCaller" function from GATK on each BAM file to create a gVCF file for each sample using the "-emitRefConfidence GVC" option. Then, I performed joint genotyping by taking all gVCF files. The variant quality score recalibration (VQSR) step was applied to increase sensitivity and minimise false positives. The flowchart of the variant discovery analysis is described in Figure 2.2.

About 1 M validated SNPs (Kranis et al., 2013) were used as known 'true SNPs' and as a training set for the machine-learning algorithm of VQSR. The VQSR step was the first step of filtration of variants. I used the following seven annotations or context statistics for applying VQSR filtration: DP (Depth of coverage), QD (Quality by Depth) to normalise the variant quality to avoid inflation caused when there is deep coverage, MQ (root mean square of Mapping Quality), MQRankSum (Mapping Quality Rank Sum test statistic), ReadPosRankSum (read position rank sum test statistic), FS (Fisher Strand bias) which is the Phred-scaled probability that can tell us the strand bias (if the alternate allele was seen more or less often on the forward or reverse strand than the reference allele), and SOR (Strand Odds Ratio), which is also used to estimate strand bias. Based on these annotations, I specified the tranche sensitivity threshold to 99%, with only variants above the threshold passing the filter. After the VQSR filtration, I selected only the bi-allelic SNPs for downstream analysis using the GATK's "SelectVariants" function.

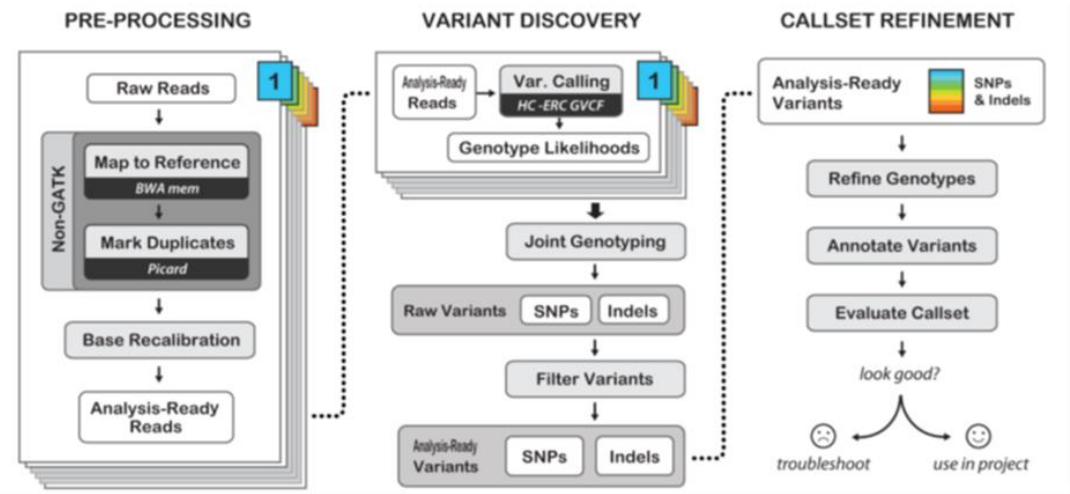


Figure 2.2. Data analysis pipeline using BWA, PICARD, and GATK.
Source: GATK website (https://gatk.broadinstitute.org/hc/en-us).

For downstream analysis, further filtration was applied using the VCFtools version 0.1.13 (https://vcftools.github.io/man_latest.html) to select a set of high confidence SNPs: Hardy-Weinberg-Equilibrium (HWE) probability > 0.00001, genotype quality > 15, depth of coverage > 3 and maximum missing genotype rate < 20% . Further quality checking on samples was performed by estimating the proportion of missing genotypes per individual sample using the option "--missing-indv" in VCFtools version 0.1.14 (Danecek et al., 2011).

For analyses of population structure and relatedness between samples, I also removed SNPs in linkage disequilibrium (LD) by applying LD pruning on SNPs with PLINK version 1.9 (https://www.cog-genomics.org/plink2) using the parameters "indep-pairwise 50 5 0.5" (calculating LD between SNP pairs in a window of 50 SNPs, then shifting the window by 5 SNPs forward to repeat the same procedure and removing one SNP from each pair showing LD > 0.5). Relatedness among all individuals to exclude closely related individuals was calculated using the option "--relatedness" in

VCFtools (I filtered out any pairwise individuals with more than 60% relatedness). For admixture analysis, further thinning of SNP data was performed following LD pruning using the "--thin 0.3" option in PLINK (causing 30% thinning of the data).

2.2.6 Estimation of genetic diversity and population structure

For genetic diversity analysis at the genome level, VCFtools version 0.1.14 (Danecek et al., 2011) was used to calculate the inbreeding coefficient for individual chickens, and nucleotide diversity (π) for the individual chicken population, and pairwise population-differentiation (F_{ST}) between populations. The average genome nucleotide diversity (π) or "pi" and F_{ST} were estimated in 20 kb windows over 10 kb sliding step using the option "*--window-pi*" and "*--window-pi-step*", while the inbreeding coefficient was estimated using the "*--het*" option in VCFtools. SNP-based inbreeding coefficient (*F*) in the VCFtools was calculated per individual using the equation F = (O – E) / (N – E), where O is the observed number of homozygotes, E is the expected number of homozygotes (given population allele frequency), and N is the total number of genotyped loci (Mooney et al., 2018).

Population structure among the fourteen populations was inferred with principal component analysis (PCA) and estimation of proportion of ancestry (admixture) using SNPs that passed the quality control. For the PCA, I ran the "smartpca" program in "eigenstrat" version 6.0.1 (Price et al., 2006) using the pruned data. The proportion of variance explained by the eigenvectors (or principal components) was calculated by dividing the corresponding eigenvalue by the sum of all the eigenvalues. I then assessed the structure of each population using ADMIXTURE version 1.3.0 (Alexander and Lange, 2011, Alexander et al., 2009) for up to five inferred ancestral

clusters (*K*). The lowest cross-validation error was considered to be the optimal *K* value. Population structure and admixture plots were plotted using R version 3.4.3.

2.2.7 Mantel Test

The most popular approach to evaluate spatial processes driving population structure is usually performed using a Mantel test. It compares genetic distances, estimated here by pairwise *FST*, with geographical distances (Diniz-Filho et al., 2013). The Mantel test was proposed to test the association between two matrices and was first applied in population genetics by Sokal (1979). The Mantel test formulation is given by:

$$Zm = \sum_{i=1}^{n} \sum_{j=1}^{n} g_{ij} \times d_{ij}$$

Here, g_{ij} and d_{ij} are the genetic and geographical distances between populations i and j, considering n populations and Zm are given by the sum of products of the two distances. In our case, *P*-value was computed using 10000 Monte Carlo simulations. The Mantel test was performed in this study using the R packages *vegan* (Oksanenm, 2017).

2.2.8 Linkage disequilibrium (LD) decay

The availability of high-density SNP from genotyping or sequencing platforms makes it possible to investigate LD at an unprecedented resolution. LD is affected by the recombination rate and the number of generations of recombination. In this work, I characterised LD decay by combining closely related populations (based on PCA) into groups. A pairwise r^2 estimation was used to measure LD between pairs of SNPs within a chromosome using the PopLDdecay (v3.40) program (Zhang et al., 2018). SNPs on both autosomal and sex chromosomes that had passed the quality control using options "-MAF 0.05" (minimum minor allele frequency of 0.05) and "-MaxDist

15" (maximum window bin 15kb) were used. The decay of LD was plotted using R version 3.4.3.

2.2.9 Annotation and enrichment analysis of detected SNPs

SNPs were classified as either 'known' if their position and non-reference allele was found in the dbSNP database (version 150) or 'novel' if a novel polymorphism. ANNOVAR version 2.1.1 (Wang et al., 2010) was used to ascertain genomic locations of the SNPs relative to known genes in the Ensembl database (Version 98) and their potential effects. The annotation of the SNPs categorises them as (1) intergenic when the SNP does not overlap any genes but is located in between two genes; (2) upstream or downstream when the SNP is either within 1 kb of the transcription start or end sites of a gene; (3) UTR3 or UTR5, when the SNP is located in the untranslated regions of a gene (either 3' or 5'); (4) intronic, when the SNP is located in the intronic (non-coding) regions of a gene; (5) exonic, when the SNP is located in the exonic (coding) region of a gene, and (6) splicing, when a variant is within two bp of a splice junction. Exonic variants are further classified as non-synonymous (missense), synonymous or stop/gain/loss. 'Non-synonymous' (missense) SNPs are of particular interest because they may lead to amino acid changes in proteins, and such polymorphisms thereby may be associated with the phenotypic variations. 'Stop gain' refers to a non-synonymous SNP that leads to the creation of a stop codon at the variant site, while 'stop-loss' leads to the elimination of a stop codon at the variant site. Stop gain/loss types of non-synonymous SNPs are expected to have a functional impact.

The effects of non-synonymous SNPs on protein function were predicted using the Sorting Intolerant from the Tolerant (SIFT) prediction algorithm, which depends on the degree of conservation at individual amino acid (AA) positions (Sim et al., 2012).

Using multiple alignments of homologous but distantly related peptide sequences, SIFT calculates normalised probabilities (SIFT score) of observing all possible AA residues at a position (Gheyas et al., 2015, Sim et al., 2012, Vaser et al., 2015). If the SIFT score is greater or equal to 0.05 the variant is considered evolutionary tolerant (TOL), whereas variants with a score less than 0.05 are regarded as intolerant (INTOL) and potentially deleterious (Kumar et al., 2009, Vaser et al., 2015).

The web-based PANTHER Classification System (Thomas et al., 2003) was used to establish the biological significance of a list of genes with non-synonymous SNPs - either tolerated or deleterious ones - by performing over-representation tests with Gene Ontology (GO) including biological processes, molecular functions and pathways. I applied the Binomial-Bonferroni correction P-value < 0.05 as the default threshold for significance.

2.3 Results

2.3.1 Sequencing and variant discovery

The total number of paired sequences reads generated from each population ranged between 1.5 billion and six billion depending on the total number of individuals analysed per population (Table 2.2). For each sample, the mapping rate (MR) against the chicken reference genome was very high (99%), and the average genome coverage (X) after mapping was between 50X and 80X. The percentage of both mates of a read pair (PMR) that mapped to the same chromosome and in the correct orientation was also very high, ranging from 96% to 98% (Table 2.2). The number of SNPs detected in each population ranged from 8.9 million to 11.8 million depending on the number of samples analysed per population. From the combined analysis of all populations and samples, the total number of SNPs detected before any filtration step was 17,888,119. After further filtration (HWE probability > 0.00001, genotype quality > 15, depth of coverage > 3 and maximum missing genotype rate < 20%), only a small number of variants were removed, retaining 17,302,272 SNPs. This supports the high quality of the sequencing data. The proportions of known and novel SNPs in this filtered set were 76% and 24%, respectively.

The average SNP density across the genome is about 16 SNPs per kb or 1 SNP every 62 bases. While the number of SNPs detected from each chromosome generally depended on the size of the chromosome, the SNP density per kb, however, varied drastically in different categories of chromosomes (Table 2.3 and Figure 2.3). The macro and intermediate chromosomes show a higher average SNP density compared to the micro-chromosomes in line with the study of Ethiopian indigenous chickens by Kebede (2018). There is also a large variation in SNP density between autosomes and

sex chromosomes. The density in the sex chromosome is very low (average 3 SNPs/kb) compared to 17 SNPs/kb for autosomes. This result is supported by a previous study in Ethiopian chicken which also reported significantly lower SNP density on chromosome W (Kebede, 2018). Two autosomes also have very low SNP density, viz, chr16 and chr31. Chromosome 16 is interesting as it contains the chicken MHC (Major histocompatibility complex) region which is known to contribute significantly to genetic resistance/tolerance to infectious diseases (Miller and Taylor, 2016), and some chicken studies have found high SNP density in this region (about 25 SNPs/kb (Schmid et al., 2015). However, for the entire chromosome 16, I have found a much lower SNPs density, with around 3 SNPs per kb in our study (Figure 2.3).

Table 2.2. Summary of sequencing, mapping reads, and variant discovery for Nigerian chicken populations

Population	No. of Samples	Total QC Passed Reads	MR (%)	PMR (%)	Genome coverage (X)	Total number of SNPs
Dakan-Karu	10	3,897,069,737	99%	97%	55	10,902,651
Degema	10	4,676,902,102	99%	97%	66	10,686,758
Gitta Mbasha	10	4,418,425,814	99%	97%	62	11,781,070
Isiokpo	10	5,192,093,501	99%	97%	73	11,791,901
Jiga	4	1,900,574,868	99%	97%	67	8,904,888
Karu	10	4,212,152,802	99%	98%	59	11,686,153
Kwendo/Doro /Tadurga	6	2,140,521,870	99%	96%	50	9,991,722
Odenkume	10	3,838,193,741	99%	98%	60	10,553,277
Ogbondoroko	10	5,978,336,143	99%	98%	84	11,690,468
Okpofe	10	5,698,975,563	99%	98%	80	11,258,930
Sabiyal	6	2,962,004,533	99%	97%	70	9,833,868
Sanchitagi	10	4,039,142,060	99%	98%	57	11,894,082
Senchi	4	1,499,013,204	99%	97%	53	9,248,477
Wat-Karu	10	4,810,439,051	99%	97%	68	11,321,073

MR (%): Percentage of paired mapped reads, PMR (%): Percentage of properly paired mapped reads. Genome coverage (X) was calculated as: Length of read (150 bp) x Number of Reads / Haploid genome size (1,065,365,425).

Table 2.3. The average SNPs densities across chromosome categories based on ~17 million filtered SNPs.

Chromosomes	Average SNP density (per kb)
Macrochromosomes (Chr 1-5)	18.15 ± 0.29
Intermediate (Chr 6–10)	18.86 ± 1.31
Microchromosomes (Chr 11–28, Chr 30-33)	14.05 ± 6.52
Sex chromosomes (W = 0.02, Z = 7.97)	1.01 ± 1.42
All autosomes	17.02 ± 2.60

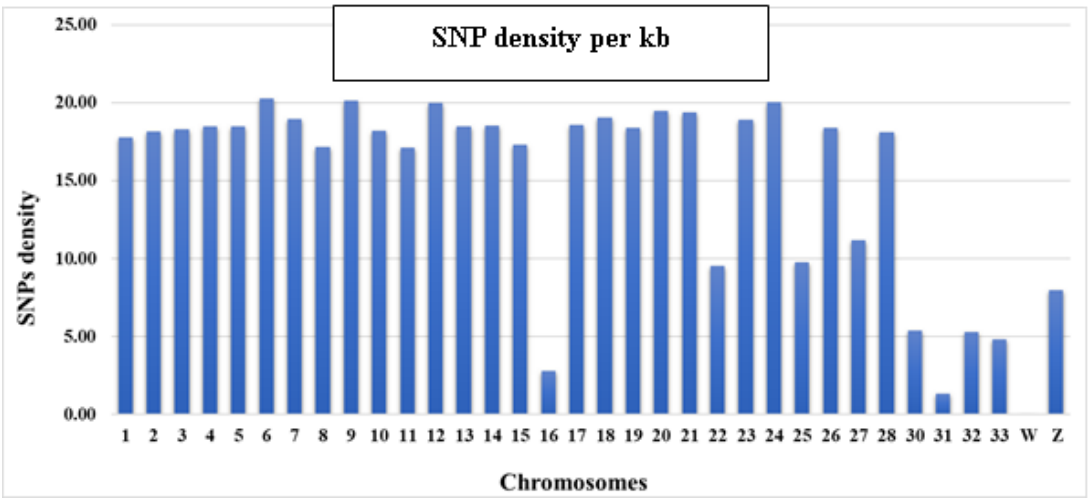


Figure 2.3. The chromosome-wise SNP density for the 17 million SNPs.

2.3.2. Genetic diversity of the Nigerian chicken populations

Genetic parameters, including inbreeding coefficient (F), nucleotide diversity (π), and the percentage of heterozygous and homozygous SNPs were estimated to examine the genetic diversity among and within the different chicken populations. Inbreeding level was estimated as the probability that any two alleles at any given locus are identical by descent (IBD) (Leutenegger et al., 2003). As inbreeding increases, the frequency of alleles being homozygous at a particular locus also increases, hence inbreeding reduces the amount of variation in a population. The average inbreeding coefficients

for Nigerian chicken populations are relatively low and range from 0% to 4.6% (Table 2.4), with the lowest value observed in Sanchitagi and the highest in Gitta Mbasha.

Table 2.4. Estimates of genetic diversity parameters among the 14 chicken populations

Population	**Inbreeding coefficient (F)**	**Nucleotide diversity/kb (π)**	**% of Heterozygous SNPs**	**% of Homozygous SNPs**
Sabiyal	0.034	0.0031	29.96	70.04
Jiga	0.010	0.0032	33.90	66.10
Senchi	0.011	0.0034	35.17	64.83
Kwendo/Doro /Tadurga	0.038	0.0032	30.57	69.43
Ogbondoroko	0.017	0.0033	27.78	72.22
Sanchitagi	-0.001	0.0033	28.18	71.82
WatKaru	0.044	0.0034	28.25	71.75
Dakankaru	0.019	0.0034	29.76	70.24
GittaMbasha	0.046	0.0034	27.33	72.67
Karu	0.016	0.0033	27.71	72.29
Okpofe	0.0003	0.0035	30.46	69.54
Odenkume	-0.017	0.0031	29.82	70.18
Degema	0.019	0.0031	28.24	71.76
Isiokpo	0.042	0.0035	27.87	72.13

The average nucleotide diversity (π) for the Nigerian chicken populations is comparable across populations (in most cases the π value is about 3.0×10^{-3}). Okpofe and Isiokpo show slightly higher values ($\pi = 0.00346$ and $\pi = 0.00345$, respectively) compared to other populations (Table 2.4), whereas the lowest nucleotide diversity is observed in the Degema population ($\pi = 0.00312$). The percentage of homozygous SNPs is higher for all populations than the percentage of heterozygous SNPs (Table 2.4), with Senchi having the highest percentage of heterozygous SNPs followed by Jiga (35% and 33%, respectively), for the remaining populations it ranged from 27% to 30%, Table 2.4).

2.3.3. Population Structure as revealed by PCA, Admixture and Mantel Test

Population structures at the autosomal level were examined using PCA and admixture analyses. PCA was performed using a total of 4,468,866 SNPs following the LD pruning of the 17 million variants. The largest two principal components (PC1 and PC2) jointly explained about 25% of the total genetic variance (Figure 2.4). Most of the population samples cluster together but in three populations (Degema, Dakan-Karu and Okpofe), I observe some outlier individuals separated from the other populations. When the populations are plotted with PC1 and PC3, which together explain about 24% of the total genetic variance, three populations (Degema, Dakan-Karu, and Odenkume) show outlier individuals.

Investigation using the Mantel test did not find any correlation between population genetic distance (F_{ST}) and geographic distance (*P-value* > 0.05) (See Figure 2.5 and 2.6, respectively, and Supplementary Table 1 (S2.1)).

Admixture analysis was performed using 583,700 SNPs, which were obtained by thinning (retaining 30%) of the LD pruned data. The thinning approach was applied to increase the computational speed of the admixture analysis. I then used the cross-validation (CV) procedure to determine the optimum K with the lowest CV error (Alexander and Lange, 2011, Alexander et al., 2009). The result of the analysis supports K = 4 as the most likely number of ancestral populations for this study (Figure 2.7).

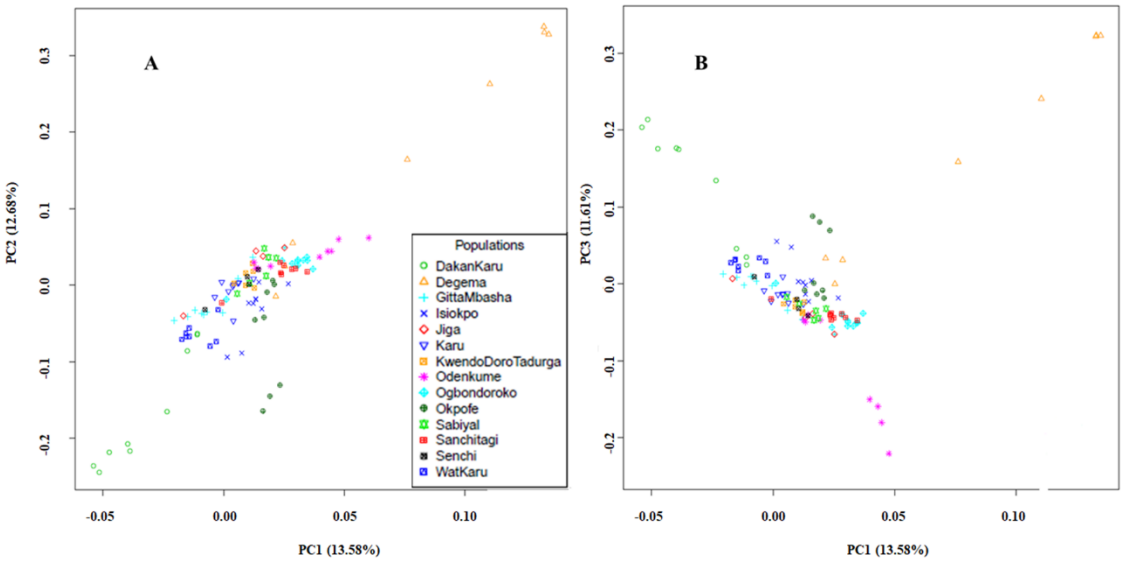


Figure 2.4. Principal component analysis of Nigerian indigenous chicken populations

(A) Principal component (PC 1 versus PC 2) analysis, (B) Principal component (PC 1 versus PC 3).

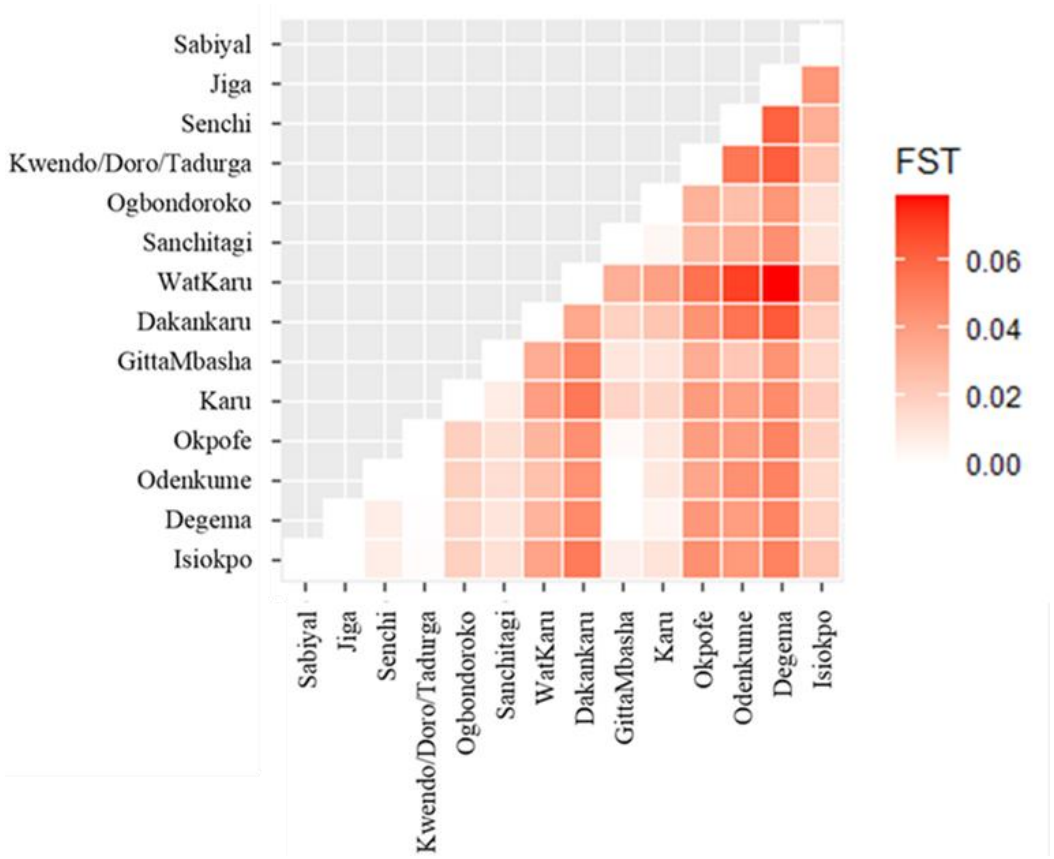


Figure 2.5. The pairwise weighted *FST* heatmap between populations.

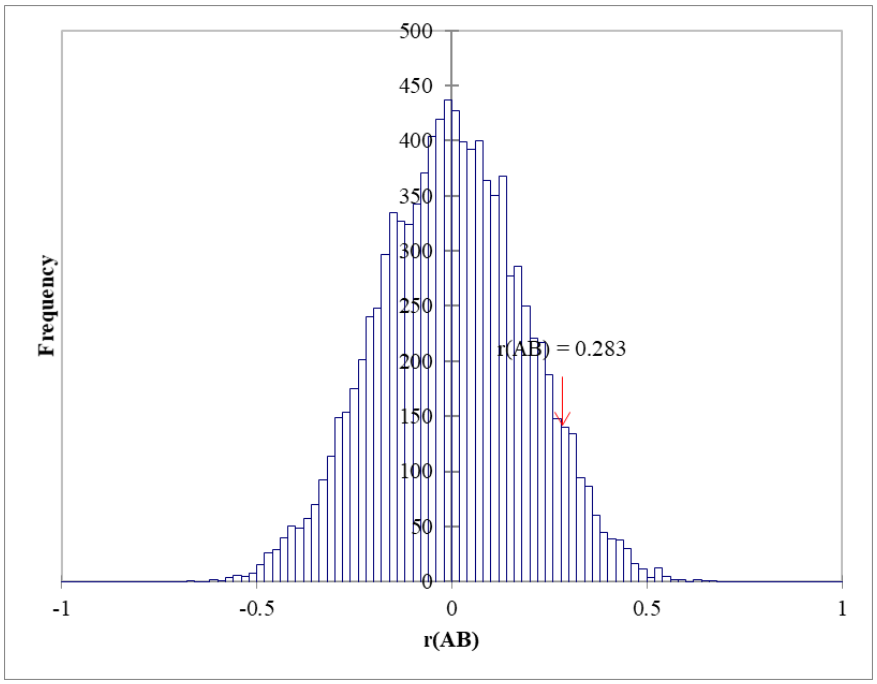


Figure 2.6. Histogram of Mantel test correlation coefficients calculated over 10000 replicates.

*The position of the significance threshold ($p < 0.05$) for the correlation coefficient (rAB) is indicated by the red arrow.

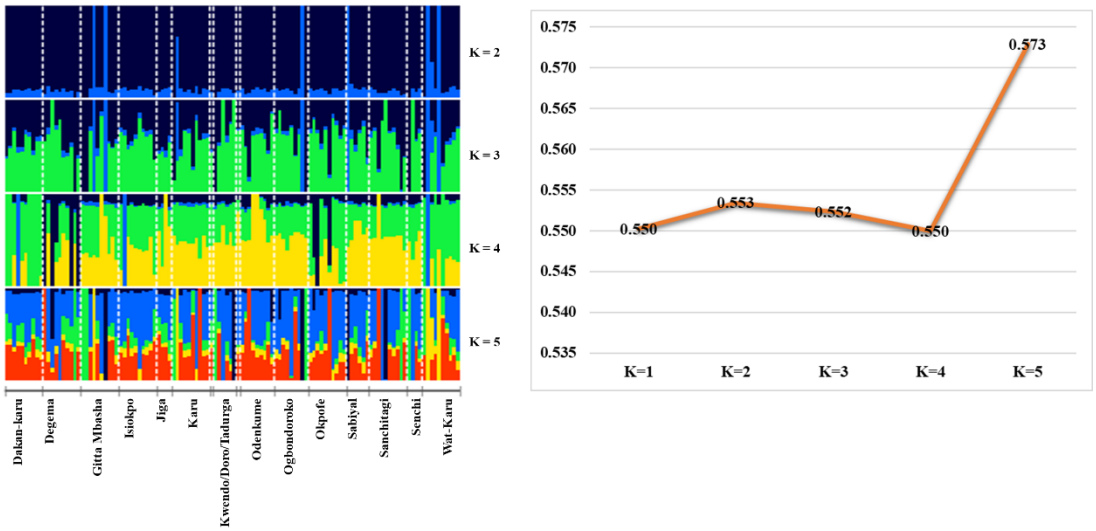


Figure 2.7. Admixture analysis of Nigerian chicken populations (left is the admixture plot for 120 samples, right is the cross-validation score value).

2.3.4. Linkage disequilibrium (LD) decay

Linkage disequilibrium maps are fundamental tools for exploring the genetic basis of economic traits in chickens (Seo et al., 2018). The decay of LD at a pairwise distance can also be used to measure the evolutionary history of populations (Wragg et al., 2012). LD decay measures are useful for determining the resolution of association mapping or assessing the desired number of SNPs for association analysis genome-wide. The result of LD decay analysis for autosomes shows that LD (r^2) drops very rapidly with distance. LD decay was estimated in four groups following the results of the PCA (Group 1 = Dakankaru / 10 samples, Group 2 = Degema / 10 samples, Group 3 = Odenkume / 10 samples, Group 4 = mix population / 90 samples) (Figure 2.8). The plot of LD decay against genome distance indicates a LD average (r^2) ranging from 0.15 – 0.30 at a distance of about 10 to 15 kb with Groups 1, 2, 3 showing higher average of LD while Group 4, with the largest number of samples, showed the lowest average LD.

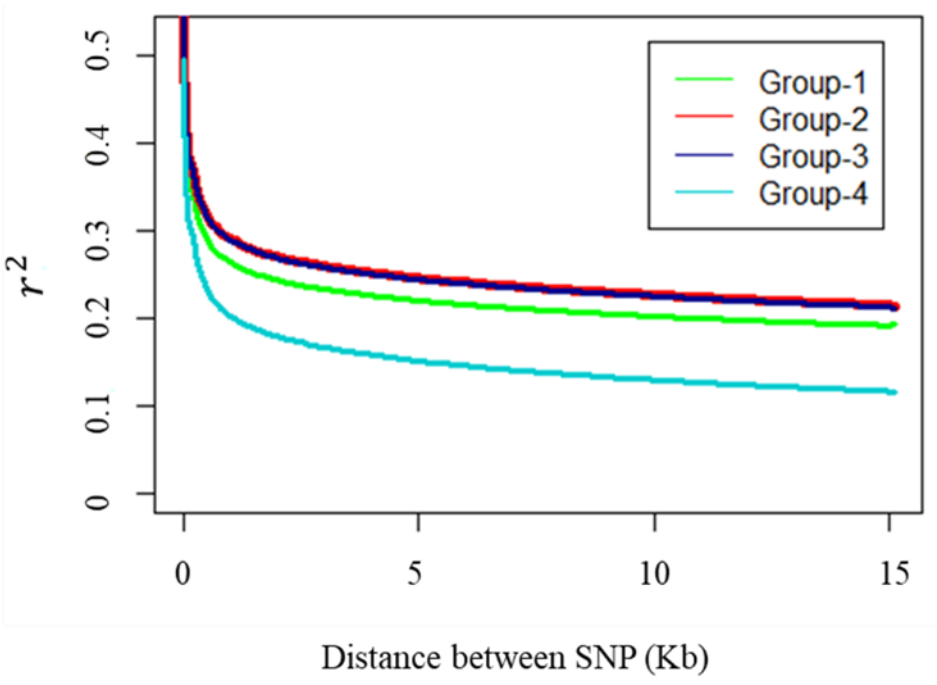


Figure 2.8. LD decay result (r2 values of correlation between the closest markers).

2.3.5. Annotation of chicken genetic variants

Annotation of the 17 million detected variants against the Ensembl gene annotation database (version 98) reveals that 59.42% of SNPs are located within genes (intronic, exonic, UTRs, and splicing), while 40.58% are located outside the gene (intergenic and up/downstream locations) (Table 2.5). However, only 1.5% of the SNPs are found in the protein-coding region (exonic) of the gene (65.1% synonymous, 34.5% non-synonymous and 0.4% stop gain/loss). Among the non-genic SNPs, 92% were located in the intergenic region, while the remainder were located in the downstream and upstream regions (around 4% for each region). SNPs within gene regions were mostly located in the introns (92%), then exons (4% respectively), while the splicing and untranslated regions (UTR) included 1,816 and 191,999 SNPs, respectively. The variants in the exonic region from protein-coding genes were further classified as synonymous (66% of the variants), non-synonymous (34%) and stop gain or loss variant polymorphisms (Figure 2.9). In terms of number, I detected about 90,000 non-synonymous and over 1,000 stop/gain-loss SNPs. Although non-synonymous SNPs change the amino acid sequence within a protein, the effects are not always harmful or radical to protein function. Using SIFT, 19.44% of the non-synonymous variants (n = 26,884) were predicted as ‘deleterious’ or ‘intolerant’ (INTOL) with possible radical effects, 60.64% (83,871) were predicted ‘tolerated’ (TOL), whereas the prediction for other variants had low confidence level (Figure 2.10). The synonymous and non-synonymous (AA-altering) number of SNPs are 169,980 (0.98%) and 89,964 (0.51%) respectively. Whereas, in the other AA altering variant, the number of stop gain/loss accounts for about 0.006% (n = 1,048). Even though non-synonymous SNPs change the amino acid sequence within a protein, the effects are not always harmful or radical to protein function.

Table 2.5. Annotation of the detected SNPs based on Ensembl gene database (Version 98).

Annotation category	No. of variants (%)	Mean AAF ± SD	No. of variants with AAF > 0.9 (%)
Exonic:	259,878 (1.5 %)	0.21 ± 0.28	11,619 (0.07)
Synonymous		0.22 ± 0.28	8,018 (0.05)
Non-synonymous		0.17 ± 0.27	3,580 (0.02)
Stop loss; stop gain		0.15 ± 0.24*	21 (0.00)
Intergenic	6,437,529 (37 %)	0.24 ± 0.29	32,1237 (1.86)
Splicing	1,816 (0.04 %)	0.16 ± 0.24	15 (0.00)
Intronic	7,986,543 (46 %)	0.24 ± 0.29	475,759 (2.75)
Upstream;downstream	582,153 (3.36 %)	0.23 ± 0.29	27,737 (0.16)
ncRNA	1,838,247(10.63%)	0.24 ± 0.29	94,982 (0.55)
UTR3;UTR5	191,999 (1.1 %)	0.22 ± 0.28	8,814 (0.05)
Total	**17,297,019**		

*Total mean ± SD of stop gain and stop loss.

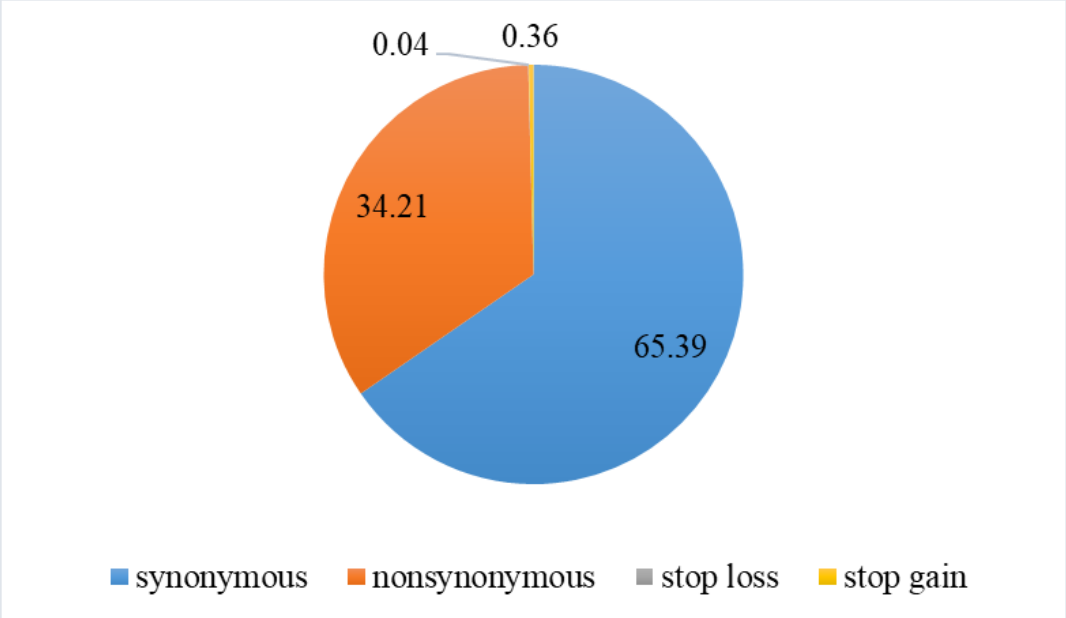


Figure 2.9. Proportion (%) of different categories of exonic SNPs annotated by ANNOVAR.

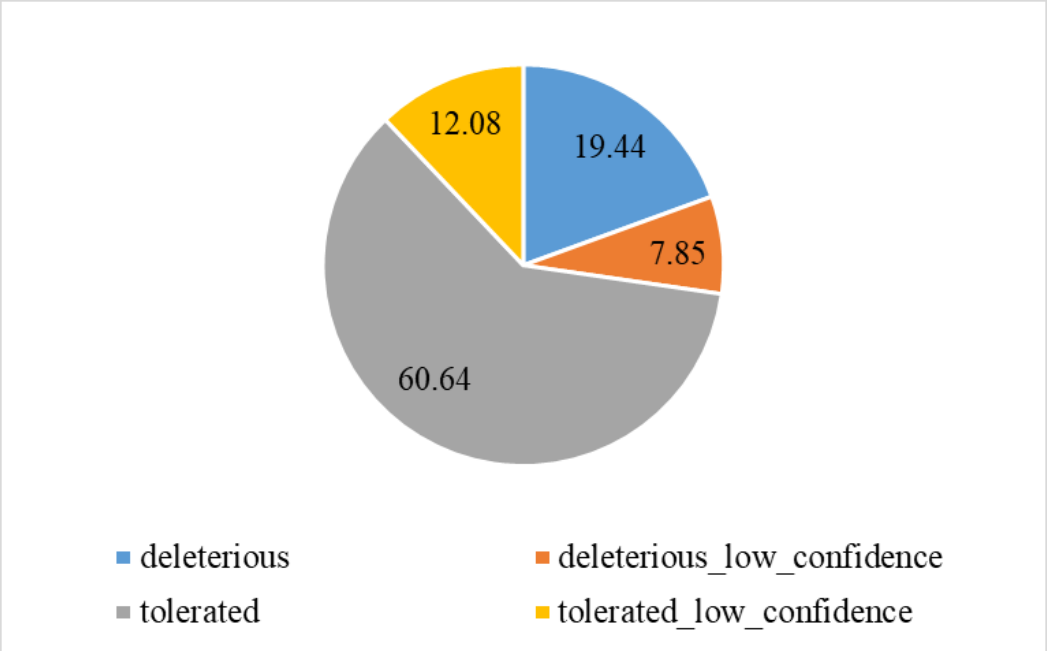


Figure 2.10. VEP based SIFT analysis for non-synonymous and stop gain/loss SNPs (%).

2.3.6. Allele frequency spectrum of SNPs in different annotation categories

The frequency spectrum of alternative alleles (AAF) or non-reference alleles of variants from different annotation categories was compared (Figure 2.11 and Figure 2.12). It indicates little difference in allele frequencie between them. The allele frequency distribution of different annotation categories shows that the largest proportion of variants (more than 50%) fell within an AAF bin of ≤ 0.1 (or 10%), while in other frequency bins only 3-10% SNPs are distributed. Only 3-4% of SNPs from different annotation categories show very high frequency (> 0.9 -1) of non-ref alleles. Figure 2.12 shows that a much larger proportion of nonsynonymous and stopgain/loss SNPs (67-68%) belong to low frequency bin (≤0.1) compared to synonymous SNPs (55%).

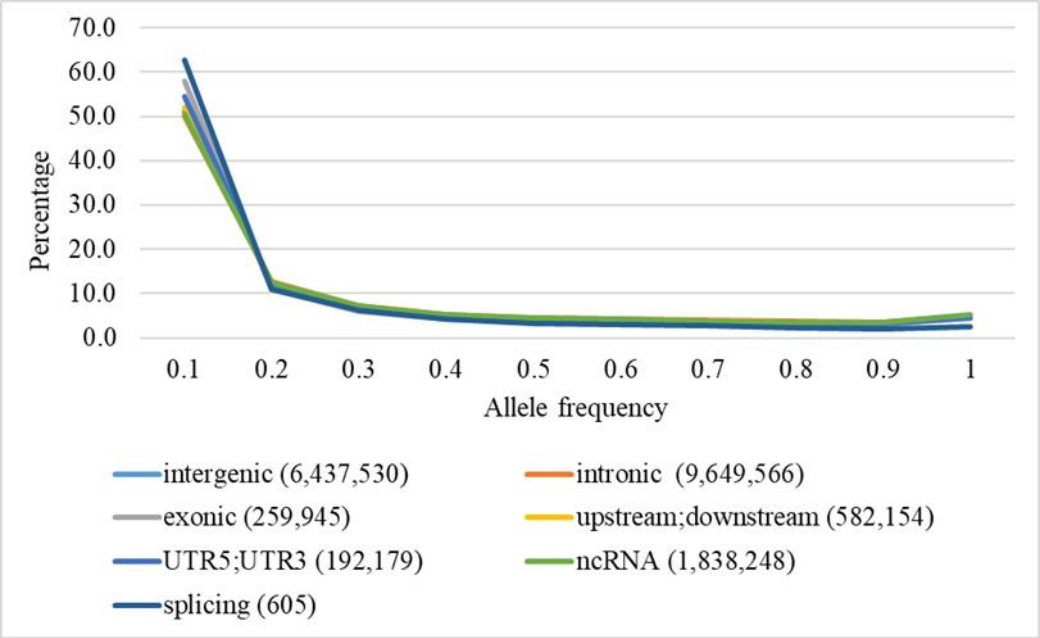


Figure 2.11. Frequency distribution of non-reference alleles of SNPs for different annotation categories.

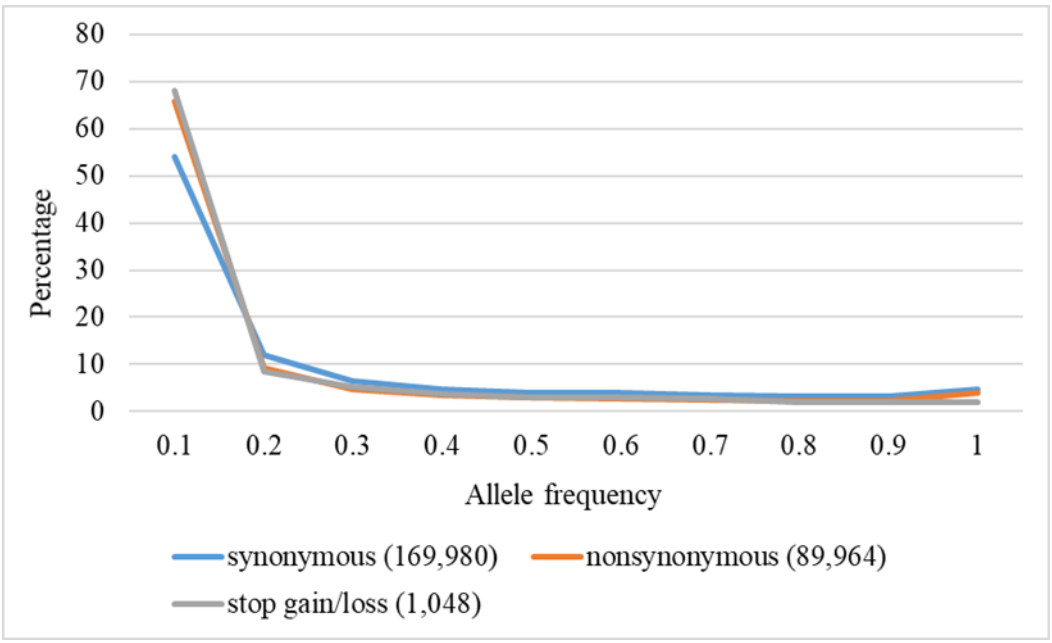


Figure 2.12. The frequency spectrum of non-reference alleles of different categories of exonic SNPs.

2.3.7. Functional annotation and enrichment analyses

I identified 3,600 functional coding variants (nonsynonymous and stop loss and stop gain) (Table 2.5) overlapping with 2,286 unique genes in high frequencies for the non-reference allele (AAF > 0.9) (*see* Supplementary Table S2.2 – S2.3). Moreover, I identified 158 predicted deleterious alleles that were fixed and nearly fixed (23 and 135, respectively). To gain insight into the functions of these potential variants, I performed functional annotation and enrichment analyses and then checked the biological processes (GO term) for these genes using the Panther classification system. The high frequency of these potential function altering variants indicates a possible adaptive advantage.

The summaries of all GO terms for the genes harbouring the functional coding SNPs in high frequency, using Fisher's exact test with FDR correction of significance ($P < 0.05$) are shown in Supplementary Tables S2.4 – S2.7. Investigation of the individual genes from these ubiquitous pathways shows involvement in a myriad of biological processes, but prominently in processes which are expected to be necessary for the survival of scavenging chickens. The main significant GO terms for the biological process include DNA replication, cillium organisation, DNA repair, B cell receptor signalling pathway, and positive regulation of protein kinase activity, while the molecular function includes serine-type endopeptidase activity, protein kinase activity, and olfactory receptor activity. Moreover, the basement membrane, centriole, and nucleus are annotated for the cellular component, while gonadotropin-releasing hormone receptor and angiogenesis were found to be related pathways of the overlapped genes.

The annotation terms for genes overlapped with deleterious missense mutations in high allele frequency are involved in apoptosis, cytokine receptor interaction, phagosome, response to virus, Salmonella infection, oxidation-reduction process, etc. (*see* Supplementary Table S2.8 – S2.10). In addition, some genes that contained fixed deleterious variants were involved in protein kinase activity (*TWF1, STK39*), cellular stress response (*STK39, TTI1*), and ion channel activity (*TMEM266*), Phosphatidylinositol 3/4-kinase (*INPP5J*) and immune system (*TLR, RPN1*). Furthermore, gene-set enrichment analysis showed that the corresponding genes are involved in immune cell development (e.g. Phosphatidylinositol 3/4-kinase, protein glycosylation, methyltransferase activity) (*see* Supplementary Table S2.11).

2.4 Discussion

In this chapter, I report the discovery of genome-wide SNP variants in 120 Nigerian indigenous chickens following the comparison of their genomes with the GRCg6a chicken genome reference. About 17 million high-quality SNPs were identified from the analysis of 14 populations. Lawal et al. (2018) detected 13, 10, and 14 million SNPs in domestic chickens from Ethiopia, Saudi Arabia, and Sri Lanka, respectively. Also, a recent study detected 19.5 M SNPs in 245 chickens from 25 Ethiopian indigenous chicken populations (Gheyas et al., 2020). Factors that may contribute to such differences in SNP number between datasets include the number of samples analysed, the difference in filtration criteria, chicken reference genome version, and sequence coverage.

The mean SNP density reported in this study (16 SNPs per kb or 1 SNP every 62 bases) was slightly higher than that reported by Gheyas et al. (2015), who found a

density of 15 SNPs/kb from the analysis of diverse commercial and experimental chicken lines. It emphasises the diversity of indigenous chickens compared to commercial ones. However, chromosome 16, which hosts the chicken's major histocompatibility complex shows a lower SNP density in Nigerian chickens compared to Ethiopian chickens (Kebede, 2018), possibly the consequence of the different number of samples examined in the two studies. Previous studies by Yan et al. (2014) and Stainton et al. (2017) also found that Red Jungle Fowl and some inbred lines as well as in Broiler have a low density in chromosome 16.

Genetic diversity among the different chicken populations was assessed using genetic parameters such as inbreeding coefficient (F), nucleotide diversity (π), percentage of heterozygous and homozygous SNPs, and population differentiation (F_{ST}). Inbreeding level is generally low (F between -0.017 and 0.046) among the studied chicken populations, even lower compared to a previous study in Southern African indigenous chickens from Malawi and Zimbabwe (0.15 and 0.12, respectively) (Khanyile et al., 2015). Muir et al. (2008) observed that the lowest percentage of inbreeding coefficient for some commercial chicken lines was 10%; whilst Ameli et al. (1991) investigated cumulative inbreeding in commercial White Leghorn lines under long-term reciprocal recurrent selection and observed that the inbreeding coefficient increased on average 0.7% per year. Higher inbreeding in a population will result in the loss of genetic variation and thereby may lead to the loss of favourable alleles within the population (Santana et al., 2012). Compared to these values, the inbreeding level in the Nigerian indigenous village population is indeed very low, indicating high genetic diversity. F_{ST} values between population pairs were generally low in most cases (<0.05) indicating no or little differentiation between populations (Hartl and Clark, 1997, Balloux and Moulin, 2002). Only for a few pairs (mostly involving Degama), I see

slightly elevated *FST* values (up to > 0.05 to 0.08) indicating some (but low) level of population differentiation. As a general rule of thumb, *FST* < 0.05 between populations would indicate little genetic differentiation, 0.05-0.15 as moderate genetic differentiation and > 0.15 as large differentiation (Hartl and Clark, 1997, Balloux and Moulin, 2002).

The average nucleotide diversity for all 14 populations is about 0.0030. This result is lower compared to the nucleotide diversity observed in the different village chicken populations investigated by Lawal et al. (2018), e.g. red junglefowl ($\pi = 0.0052$), Sri Lankan domestic chicken ($\pi = 0.0046$), Ethiopian Horro ($\pi = 0.0040$), Saudi Arabian domestic chicken ($\pi = 0.0039$) and Ethiopian Jarso chicken population ($\pi = 0.0036$). Furthermore, the average percentage of homozygous SNPs is higher than the heterozygous SNPs for all chicken populations examined (around 70% and 30%, respectively). A previous study, using the Affymetrix 600K SNP array on Brazilian commercial chicken (broiler and egg-layers) showed rather a lower proportion of homozygous SNPs (50.6% - 57.3%) compared to heterozygous ones (Boschiero et al., 2018). The high ratio of homozygous SNPs in Nigerian indigenous chickens is due to many SNPs being very low-frequency, and the lower nucleotide diversity value compared to other African indigenous chicken populations may be indicative of past bottleneck events for these chicken populations following their arrival in Nigeria (Kim et al., 2017, Bortoluzzi et al., 2019).

The result of principal component (PC1 *versus* PC2 and PC1 *versus* PC3) and admixture analyses (K = 2 to 4) indicated that the fourteen chicken populations are weakly differentiated as the majority of chicken samples clustered together. However, only a few individuals in 9 out of the 14 populations (Gitta Mbasha, Karu, Ogbondoroko, Sabiyal, Watkaru, Dakankaru, Degema, Odenkume, Okpofe) are

separated from this main cluster. Furthermore, the result of the Mantel test showed no correlation between genetic divergence and geographic distance between populations. These results support a common ancestry for the Nigerian chicken populations examined. Outlier individuals within the population may indicate the presence within these indigenous populations of exotic birds and/or crossbreed exotic x indigenous. These outlier birds will be removed for the selection signatures analysis (Chapters 3 and 4).

Annotation of the 17 M SNPs against the Ensembl gene annotation database shows that 59.42% of SNPs are located within genes (intronic + exonic + UTRs + splicing), with the remaining ones found outside genes (intergenic and up/downstream) regions. Only 1.5% (n = 259,878) of the SNPs are present in protein-coding regions (e.g.. exon). In a previous study, Wong et al. (2014) reported that only ~37% of the variants were found within genes, with only 1.2% of these within the coding regions. These lower values may be explained by the use of a previous less complete chicken reference genome and a previous version of the annotation database. The majority of SNPs in coding regions are synonymous which do not cause any change in amino acids, while the majority of non-synonymous variants including, stop gain/loss, are present at low frequency. For most SNP annotation categories, I found no evidence of distinct allele frequency patterns in each categories, either in coding or non-coding region, except between synonymous and non-synonymous (including stopgain/loss) categories at the low-frequency bin (i.e. <0.01).

About 3,400 functional coding variants were present at high frequency in selected genes and their effect was determined using VEP (Variants Effect Predictor). These variants were predicted to be either tolerated or deleterious. They overlap with more than 260 genes, with the majority of these variants being missense variants rather than

stop gain or loss variants (Supplementary Table S.2.2 – S.2.3). They are found in genes belonging to major avian physiological pathways, and, accordingly, it may be expected that such variants may impact the individual fitness of the bird (Makino et al., 2018). The missense mutations that are present at high frequencies (AF > 90%) may be regulated by selection with the overlapped genes mentioned in the result section.

Some interesting genes were involved in several pathways, such as those associated with the immune response (toll-like receptor signalling, apoptosis, and MAPK signalling), e.g. *CASP1*, *STK39*, and *TLR21* as mentioned by a previous study (Hasenstein et al., 2007) or *ATR*, *TTI1*, and *MSH2* involved in the related pathways of DNA damage response and cellular response in heat stress in chicken, mice, and humans (Ciccia and Elledge, 2010, Lian et al., 2019). Furthermore, it may be expected that high-frequency missense mutation may be associated with improved bird fitness. Missense mutations present at low frequency may be either neutral or weakly deleterious or advantageous (Eyre-Walker and Keightley, 2007; Charlesworth, 2009, Lynch, 2010). Another possibility is the presence of genetic hitchhiking that can lead to an increase in the prevalence of deleterious variants, possibly because a missense variant is not too harmful or may only be lowly or moderately harmful, and it is in genetic linkage with nearby variants under positive selection (Maynard and Haigh, 2007).

2.5 Conclusion

The results presented here confirm the existence of significant genomic diversity within and across the indigenous chicken populations of Nigeria. This chapter presents, for the first time the entire genome diversity of Nigerian indigenous chickens (autosomes and sex chromosome) using high coverage whole-genome sequencing information on a large dataset (120 samples). Seventeen million SNPs were found after stringent filtration. Our findings on the genome diversity of Nigerian chicken, estimated by assimilating within and between population variance, may inform conservation and breeding improvement of these indigenous poultry populations. No or low population differentiation suggested a common ancestry for all the Nigerian chickens examined. Nonsynonymous variants at low-frequency show much larger proportion compared with synonymous variants (67% and 55%, respectively), possibly can lead to an increase in the prevalence of deleterious variants, possibly due to the presence of genetic hitchhiking that can lead to an increase in the prevalence of deleterious variants whis is not too harmful or weakly deleterious.

CHAPTER 3:

Candidate signatures of positive selection in response to extreme temperature and rainfall patterns in Nigerian indigenous chickens

3.1 Introduction

Nigeria is located in the tropical zone of West Africa which and lies within latitudes 4°N and 14°N and longitudes 3°E and 14°E. The country has a land area of about 923,769 km^2, a North-South length of about 1,450 km and a West-East breadth of about 800 km, located mainly within the lowland humid tropics (FRN, 2019). The climate of Nigeria is characterized by strong latitudinal zones which become progressively drier from the coast (in the South) to the hinterland (in the North) caused mainly by having two seasons: wet and dry, with rainfall as the key climatic variable. Usually, it rains for seven to eight months per year with the total annual rainfall ranging from 3,800 mm at the coast to less than 650 mm at the extreme North-East. The Nigerian climate is characterized by relatively high temperatures throughout the year. The average annual maximum temperature varies from 35°C to 31°C (in the North and South, respectively), whereas the average annual minimum temperature ranges from 23°C to 18°C (in the South and North, respectively) (Akinwumiju et al., 2020). A summary of the temperature and precipitation in Nigeria is shown in Table 3.1 and Figures 3.1 - 3.2.

Nigeria is seriously threatened by climate change with a significant proportion of its terrestrial ecosystem frequently affected by desertification, sheet erosion, and droughts, while the coastal and mangrove agroecological zones in the South are also prone to incessant flooding because of their proximity to the Atlantic Ocean, the riverine nature of the setting, the very low altitude, and all-year-round high volume of rainfall. Moreover, variations in climatic conditions have resulted in undesirable effects on food production and nutritional security. Unfortunately, the country has very weak adaptive strategies and capacity to mitigate the effects of a changing climate and the impacts of rising temperatures and rainfall variability on farming

including chickens, are being felt across major agroecological zones in Nigeria (Ayanlade et al., 2018b, Fadairo et al., 2020).

The climate projections for Nigeria predict a rise in temperature of 1.1°C to 2.5°C by 2060 and an increase in the number of extreme heat days to 260 by 2100 compared with 10 days in 1990 (USAID, 2019). Several studies have also documented increased variability in rainfall, dry spells during the rainy seasons, and increased irregularity in the amount of precipitation once the rainy season sets in (Adejuwon and Odekunle, 2006, Ayanlade et al., 2017, Abatan et al., 2018). Drought is one of the extreme climatic events which is a natural hazard, leading to water shortage, with notable adverse impacts on crops, livestock and income of rural farmers as it leads to crop loss and death of livestock in arid and semi-arid areas (Ayanlade et al., 2018).

Studies have shown that livestock farmers across Africa have associated changes in temperature and rainfall patterns with reduced feed sources, increased mortality, lower herd sizes, reduced water sources in the dry season, decreased animal productivity, and the appearance of new animal diseases (Liverpool-Tasie et al., 2019, Ayanlade et al., 2018). Liverpool-Tasie et al. (2019) in addition, although still largely undocumented, increased dry spells and temperatures in the region which can affect poultry production directly and indirectly. Climate change affects poultry (and other livestock) production indirectly because of its effect on maize yields, the main ingredient in poultry feed, with lower maize yields affecting the availability and price of feed and the profitability of the poultry enterprise (Liverpool-Tasie et al., 2019). Furthermore, chickens reduce their feed intake when faced with heat stress with the priority of regulating their internal temperature which, in turn, affects their growth and productivity (Nyoni et al., 2019). Additionally, increased temperature and heat stress have been linked to losses in poultry production from death, low egg production

(quantity and quality), and reduced growth rate in intensive poultry farming systems commonly found in Africa and Asia (Bhadauria et al., 2014).

Table 3.1. The temperature, rainfall, and length of the wet season of seven Nigerian agro-ecological regions.

Zones	Mean Annual Temperature (°C)	Mean Annual Rainfall (mm)	Type of Rainfall Distribution	Length of Wet Season (days)
Mangrove Swampy Forest	26 - 26.3	1453 – 3553.6	Bimodal	300 - 360
Rainforest	25.8 – 27.3	1071.7 – 1906.5	Bimodal	200 - 250
Montane Forest / Grassland	20.3 – 22.7	108 – 1141.7	Bimodal	200 - 300
Derived Savanna	25.4 – 27.6	448.3 – 1314	Extended bimodal	200 - 250
Guinea Savanna	26.4 – 29.2	586 – 1517.5	Unimodal	150 - 200
Sudan Savanna	25.3 – 27.3	262.3 – 1052.3	Unimodal	90 - 150
Sahel Savanna	20.6 – 31.6	232 – 84 2.6	Unimodal	< or equal to 90

Source: Sowunmi, (2010)

This chapter aims to identify environmentally selected candidate regions and associated candidate genes in Nigerian indigenous chickens in response to extreme temperature and rainfall patterns. To achieve this, two selection signature analysis methods were applied for a genome-wide selection scan: Pooled heterozygosity (*Hp*) and *FST*. It is expected that the outcomes of this study will help to improve understanding of the genetic basis of adaptation in response to these environmental pressures. The candidate genes and variants could then be used in breeding programmes for the development of climate-resilient breeds, e.g. selecting those adapted to heat stress and water scarcity and which are also highly productive.

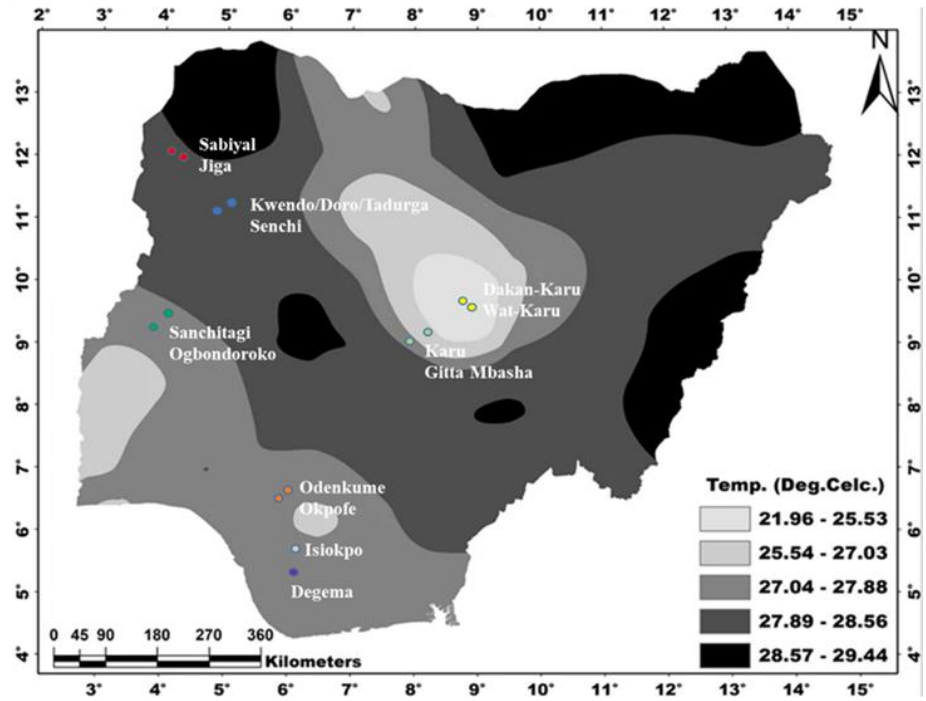


Figure 3.1. The daily average temperature in Nigeria (1981–2017).

Maps modified from Akinwumiju et al. (2020).

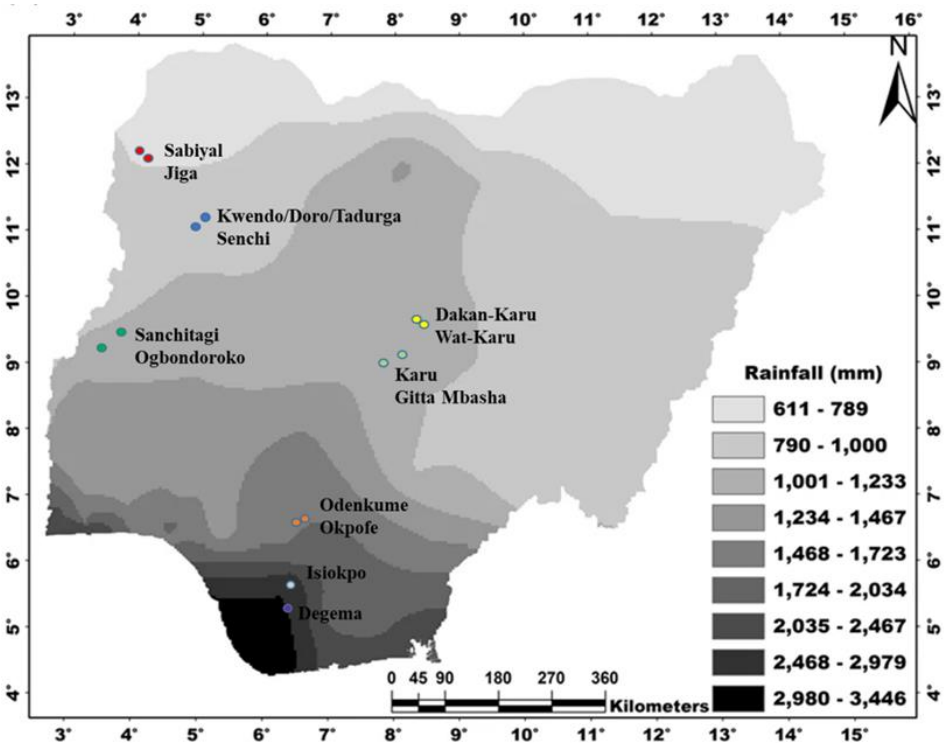


Figure 3.2. Annual rainfall amount in Nigeria (1981–2017).

Maps modified from Akinwumiju et al. (2020).

3.2 Materials and methods

3.2.1 Study populations and genome-wide SNP data

Some of the samples described in Chapter 2 were analysed in this chapter for the detection of selection signatures in relation to heat stress and extreme rainfall patterns. Samples found in the Admixture analysis that show no admixture pattern were not included in this analysis (to removed possible exotic and or crossbreed based in the population). SNP data generated and described in Chapter 2 were used in this analysis.

3.2.2 Environmental contribution to genomic selection

Bioclimatic variables from a 30-year period (1970 – 2000) were available from the WordClim database (version 2) (Fick and Hijmans, 2017) using GPS coordinates (latitude and longitude) at each district or state level for each individual (data available in Chapter 2). Temperature (°C) and precipitation (mm) variables included Annual Mean Temperature (BIO1), Mean Temperature of the Warmest Month (BIO5), Annual Precipitation (BIO12), and Precipitation of the Wettest Month (BIO13).

3.2.3 Selective sweep detection

Autosomal SNPs generated from the analysis of 120 chicken samples from fourteen populations (as described in Chapter 2) have been used for the selection signature analyses in this chapter (with the criteria as mentioned in part 3.2.1).

Selection sweep detection was carried out using *Hp* and F_{ST} analyses in overlapping 20 kb windows with a step size of 10 kb, incrementally advancing along the genome, a few SNPs at a time, to ensure that every possible window region was considered (Beissinger et al., 2015). The pool heterozygosity (*Hp*) method by (Rubin et al., 2010) was applied by measuring the level of heterozygosity in the autosomal genome

(chromosome 1-28, and 30-33). The *Hp* values were calculated using the following equation:

$$Hp = \frac{2 \sum n_{MAJ} \sum n_{MIN}}{(\sum n_{MAJ} + \sum n_{MIN})^2}$$

Where $\sum n_{MAJ} \sum n_{MIN}$ are the sums of major and minor allele frequencies respectively for all SNPs in the 20 kb windows. The value for *Hp* calculated for each window size was subsequently Z-transformed using this equation:

$$Z(Hp) = \frac{Hp - \overline{\mathrm{X}}(Hp)}{\sigma(Hp)}$$

Where $\overline{\mathrm{X}}$ is the mean and σ is the standard deviation of *Hp*. Only windows with at least 10 SNPs were employed for the downstream analysis. Based on Rubin et al. (2010), a genome-wide threshold score for Z(*Hp*) ≤ - 4.0 was considered significant.

Wright's fixation index, F_{ST}, is a useful index of genetic differentiation between populations (Wright, 1949). If $\bar{p}$ is the average of an allele frequency in the total population, σ_s^2 is the variance in the frequency of alleles in different sub-populations, and σ_T^2 is the variance of allele frequencies in the total population, the formula for the F_{ST} is defined as:

$$F_{ST} = \frac{\sigma_s^2}{\sigma_T^2} = \frac{\sigma_s^2}{\bar{\mathrm{p}}(1 - \bar{\mathrm{p}})}$$

Other estimators of F_{ST} have been proposed as well, including a modern analogue for multi-allele loci known as Weir & Cockerham's F_{ST} estimator (Weir and Cockerham, 1984), and also another F_{ST} estimator with a Bayesian model (Gianola et al., 2010). In this study I were using Cockerham's F_{ST} estimator as it is common to use the fixation index (*FST*) statistic measuring genetic differentiation based on variations in

allelic frequencies among populations (Qanbari and Simianer, 2014). The loci in the tails of the empirical distribution of F_{ST} are the candidate targets of selection (Akey, 2002).

F_{ST} is defined as the degree of differentiation between populations at any given locus, ranging in value from 0 (no differentiation) to 1 (fixed difference between populations). Negative or balancing selection tends to decrease F_{ST}, whereas local positive selection tends to increase F_{ST} (Barreiro et al., 2008). Based on Myles et al. (2008), genes responsible for phenotypic differences between populations are expected to show large allele frequency differences and theoretically reveal the actual genetic variants under selection. It is better to compute F_{ST} estimates for entire regions. Selection is expected to not only affect a single SNP but also other SNPs in linkage disequilibrium, and the power to detect a selective sweep is thus higher for genomic regions. The evolution of new functions and adaptation to new environments occurs by positive selection, whereby beneficial mutations increase in frequency and eventually become fixed in a population (Tang et al., 2007). Local environmental adaptation and artificial selection can change the allele frequencies at specific loci, leading to a higher level of population differentiation (F_{ST}) (Yang et al., 2014). Adaptation or positive natural selection leaves an imprint on the pattern of genetic variation found in a population near the site of selection (Xue et al., 2009). In this study, putatively selected regions were detected based on windows that showed the top 0.1% of F_{ST} values.

3.2.4 Functional annotation of candidate genes

Genes that overlap the candidate sweep regions were identified based on Ensembl Genes 104 database using the Ensembl BioMart online tool (http://www.ensembl.org/biomart). Candidate gene sets were processed using the Panther classification system (http://www.pantherdb.org/) for the functional annotation and identification of over-represented genes involved in biological processes (GO) and pathways. Further characterization of candidate selected regions was performed by finding regions overlapping with the Quantitative Trait Loci (QTL) (https://www.animalgenome.org/cgi-bin/QTLdb/GG/ontrait?class_ID=1) database.

3.3 Results

The first step toward analysing selective sweep regions in relation to extreme heat and extreme rainfalls was to select suitable populations for analysis. Therefore, the 14 available populations were first investigated for their local temperature and rainfall patterns.

3.3.1 Climatic parameters driving adaptations

The average of the bioclimatic variables Annual Mean Temperature (annualTemp, BIO1), Mean Temperature of the Warmest Month (tempWM, BIO5), Annual Precipitation (annualPrecip, BIO12), and Precipitation of the Wettest Month (precipWM, BIO13) over the 30 years (1970 to 2000) for the 14 Nigerian chicken populations are presented in the figures below (Figure 3.3). The distribution of bioclimatic variables for Mean Annual Temperature (BIO1) and Mean Temperature of the Driest Quarter (BIO5) range from 21°C to 28°C and 30°C to 40°C respectively, with Sabiyal and Jiga from Kebbi state showing the highest temperature (Figures 3.3

A-B). In contrast, Wat-Karu and Dakan-Karu from Plateau state have the lowest temperatures.

The distribution for both annualPrecip and precipWM show that Degema and Isiokpo from Rivers state have the highest annual precipitation (Figures 3.3 C-D). The annual rainfall amounts for these two populations are 1,690 mm and 2,479 mm respectively, while the rainfall amounts during the wettest month are 1,107 mm and 1,081 mm respectively. Jiga and Sabiyal from Kebbi state show the lowest precipitation. These are 775 mm and 798 mm for annual precipitation, and 508 mm to 527 mm respectively for the precipitation of the wettest month.

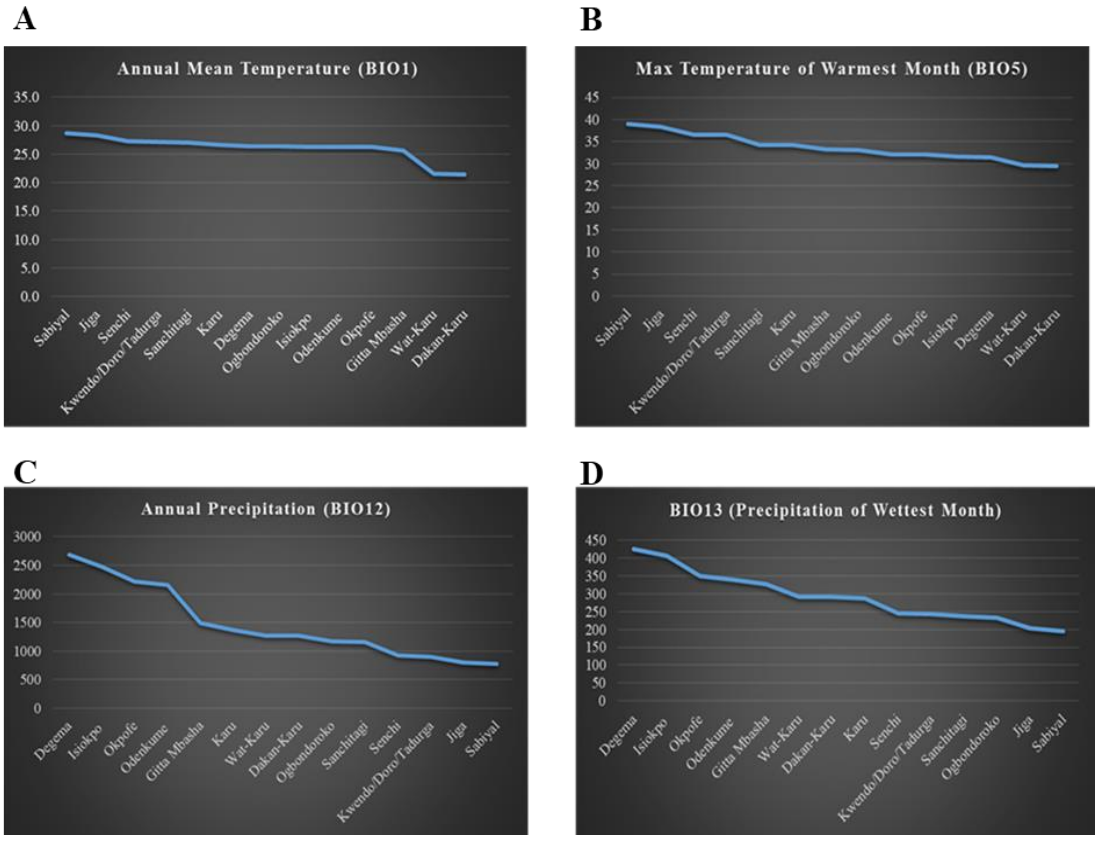


Figure 3.3. The average of the bioclimatic variables for the 14 Nigerian chicken populations.

A.) Annual Mean Temperature / BIO1, B.) Maximum Temperature of Warmest Month / BIO5, C.) Annual Precipitation / BIO12, D.) Precipitation of Wettest Month / BIO13.

The results for all bioclimatic variables likely driving chicken adaptation in Nigeria are presented in Figure 3.3. The climatic elements' impact may be expressed singly or in combinations. For example, the low temperature plus high precipitation or high temperature plus low precipitation will have a different impact, either beneficial or detrimental, depending on the extent of their variations.

Based on annualTemp and tempWM, I see little variation in temperature data among the agroecological zones of Nigerian chicken populations except for the two populations from the Plateau state (Wat-Karu and Dakan-Karu), a colder region. Therefore, the majority of the Nigerian indigenous chicken populations are predominantly subjected to the effects of heat stress with a range of annual temperature between 25°C to 29°C (excluding cold regions), while the optimum ambient temperature range for poultry is 12 - 26°C (Ayo et al., 2011). It has been suggested that high ambient temperatures sharply reduce the chicken's physiological potential (Ayo et al., 2011). Hence, based on the bioclimatic variables only twelve populations with eighty-seven samples (except chicken populations from Plateau state or cold region) were used for downstream analysis to detect the putative signatures of selection for adaptation to heat stress.

On the other hand, the pattern of annualPrecip and precipWM variables suggest that rainfall patterns likely drive adaptations for two types of stresses: (i) water deprivation from low rainfall in some populations and (ii) adaptation to cope with excess rainfall which provides conducive conditions for pathogens and parasites, in some other populations. Southern Nigeria is known to have much greater rainfall compared to the northern part of the country (Onyekuru and Marchant, 2014) and this is also reflected in the patterns observed in Figure 3.3 C-D. In this study, I use populations from two extreme ends of precipitation distribution in Figures 3.3 C-D for detecting selection

signatures in relation to water scarcity or excess rainfall. F_{ST} analysis is used to compare the high precipitation population group (Degama and Isiokpo) from Southern Nigeria with the low precipitation group (Sabiyal and Jiga) from Northern Nigeria.

3.3.2 Putative selective sweeps in relation to heat stress adaptation based on *Hp* analysis

A combined analysis of SNP data from 12 Nigerian populations (87 samples; excluding samples from the Plateau region) was performed with the *Hp* method to identify candidate regions of selection for heat tolerance. The combined analysis of populations allowed reduction of the confounding effect of demography or any other selection pressures on selective sweep regions in relation to heat stress. A total of 92,935 windows were analysed, and 92,299 windows with more than 10 SNPs were finally retained. Only 10 windows (0.02%) passed the genome-wide significance threshold of $ZHp \leq -4$, which overlapped with seventeen genes (two of them are genes void or deserted gene) and located on chromosomes 1, 2, 5, 14, 22, 30, and 32 (Table 3.2, Supplementary Table S3.1 and Figure 3.4).

The detected genes overlapping and/or nearby the candidate selection genomic regions across the Nigerian indigenous chickens, shown in Table 3.2 and Supplementary Table S3.3 – S3.4, are involved in different functions which appear to be highly relevant for thermal/heat tolerance; for example, thermogenesis (*TSHR, HSF1, CDC37*), oxidoreductase or oxidative stress (cytochrome P450 like genes), hypoxia and angiogenesis (*HIF3A*), and immune system (*SLC44A2, LIPE, ILF3*). Three candidate sweep regions are also overlapping with the chicken QTL (https://www.animalgenome.org/cgi-bin/QTLdb/GG/ontrait?class_ID=1) for residual feed intake, wattle weight, and egg number (Supplementary Table S3.4). However,

none of the associated biological processes, molecular functions or biological pathways are overrepresented (not significant / *p-value* > 0.05) based on the Panther classification system test.

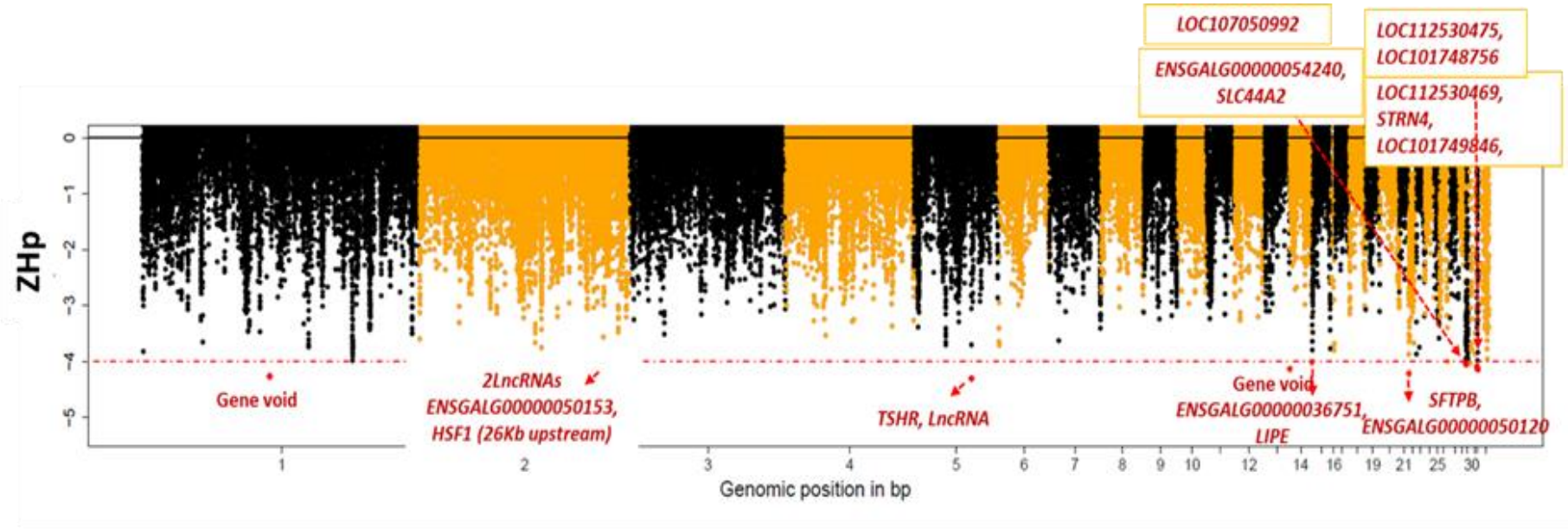


Figure 3.4. Manhattan plot for warm region Nigerian indigenous chicken group (eighty-seven samples) based on *ZHp* analysis.

Table 3.2. Candidate regions and genes under positive selection signatures in Nigerian indigenous chickens in relation to heat stress adaptation based on *Hp* analysis.

Sweep region	Candidate genes and functions	References
Chr 5:41000000-41020000	*TSHR*: Role in thermogenesis;	Uniprot, Xie et al. (2018), (Guo et al., 2022)
Chr 22:40000-60000	*SFTPB*: Pulmonary surfactant protein; role in respiratory gaseous exchange	Uniprot, Hughes (2007), To et al. (2014), Lin et al. (2018).
Chr 2:131020000-131040000	Contains multiple LncRNAs with possible cis-regulatory function on a nearby gene.	Ensembl BioMart, Shamovsky et al. (2006) et al, Zhan (2013).
Chr 32:590000-610000	*LOC112530469* & *LOC101749846*: Both are cytochrome P450 like genes; oxidoreductase activity and heme-binding. *STRN4*: Calmodulin binding & calcium channel activities	Biomart, Genecards
Chr 32:0-20000	*LOC112530475* & *LOC101748756*: both RYR1 like genes; RYR1 is involved in calcium channel activity and calmodulin-binding. Mutations in RYR1 cause malignant hyperthermia in humans and mice	Biomart, Genecards
Chr 30:200000-220000	*LOC107050992*: iron-sulfer binding and electron transfer activity.	Biomart, Genecards
Chr 30:10000-30000	*SLC44A2*: Choline transport (important for the nervous system); involved in positive regulation of I-kappaB kinase.	Biomart, Genecards
Chr 14:16010000-16030000	*LIPE*: Hormone-sensitive lipase activity	Genecards, Kong et al. (2017)
Chr 1: 90830000-90850000, Chr 14: 210000- 230000	Gene voids	

The heat shock transcription factor 1 (*HFS1*) gene is involved in the biological process of cellular response to heat (GO:0034605) and also has a molecular function of being a heat shock protein binding (GO:0031072). This gene has a function as a stress-inducible and DNA-binding transcription factor that plays a role in the transcriptional activation of the heat shock response (HSR), leading to the expression of a large class of molecular chaperones of heat shock proteins (HSPs) that protect cells from cellular damage in the chicken (Nakai and Morimoto, 1993, Fujimoto and Nakai, 2010). Meanwhile, a gene in the nearest candidate region (Chr 2:131020000- 131040000), which is, Cell Division Cycle 37 (*CDC37*) (Supplementary Table S3.4) has the molecular function as a heat shock protein binding (GO:0031072) and probably becomes a co-chaperone of *HSP90* or non-client protein binding partners that also assist in repairing denatured proteins or promoting their degradation caused by heat stress (Whitley et al., 1999, Dayalan Naidu and Dinkova-Kostova, 2017). In addition to HSP, HSFs' gene expression has been studied and could be used as a marker during acute heat stress in chickens (Xie et al., 2014).

The Surfactant Protein B (*SFTPB*) gene, also known as *SF-B,* is involved in the biological process of gaseous exchange between an organism and its environment (GO:0007585). The respiratory system of birds exposed to heat stress operates both for gaseous exchange and as the evaporative cooling system (Powell, 2015). In humans, surfactant proteins play a key role in alveolar stability and single nucleotide polymorphisms (SNPs) related to the surfactant protein B genes are associated with severe influenza (To et al., 2014).

In chickens, extreme environmental temperatures lead to the generation of reactive oxygen species (ROS), causing oxidative stress and lipid peroxidation (Altan et al., 2010). Oxidative stress that occurs with heat exposure can be manifest in all parts of the body; but mitochondrial dysfunction underlies oxidative stress. In the initial acute heat stress phase, mitochondrial substrate oxidation and electron transport chain activity are increased, resulting in excessive superoxide production. It was reported that a protein named avian uncoupling protein (UCP) could play a role in the mediation of thermogenesis and in controlling the production of ROS and protecting against the deleterious effect of ROS (Mujahid et al., 2007). As shown in Figure 3.5, it should be noted that mild mitochondrial uncoupling via the action of A nucleotide translocator (ANT), as well as UCP, in skeletal muscle may play a role in alleviating the generation of harmful ROS for which an increased mitochondrial flux may occur on exposure to acute heat stress in white leghorn chicken (Mujahid et al., 2007). During the later stage of acute heat stress, down-regulation of avian uncoupling protein worsens the oxidative stress situation causing mitochondrial dysfunction and tissue damage (Akbarian et al., 2016). I have found some candidate genes on chromosomes 30 and 32 (*LOC107050992*, *cytochrome P450 2B4-like: LOC112530469* & *LOC101749846*) which are associated with oxidative stress. Those genes are involved in several GO terms associated with ion sulfur cluster binding (GO:0051536), mitochondrion (GO:0005739), oxidoreductase activity (GO:0016705), and oxidation-reduction process (GO:0055114).

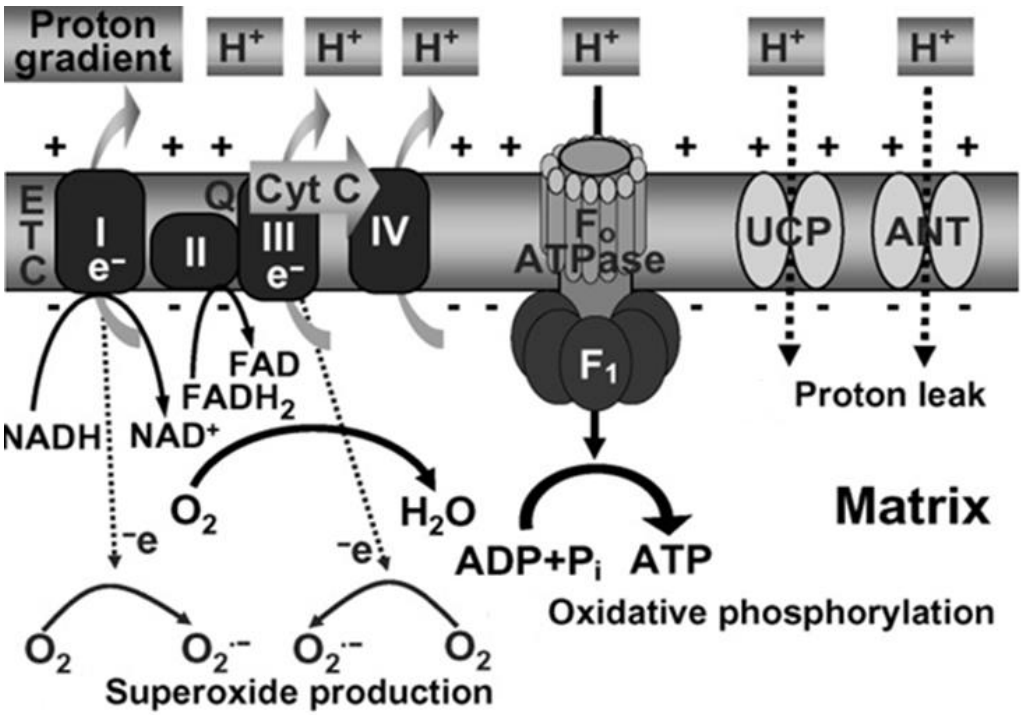


Figure 3.5. Possible role of uncoupling protein (UCP) and A nucleotide translocator (ANT) in control of superoxide production in mitochondria.

Image modified from Mujahid et al. (2007).

Under high ambient temperature, birds' respiratory rates are enhanced to dissipate heat, which decreases blood CO_2 levels and changes the acid-base balance, resulting in respiratory alkalosis at the initial phase of heat stress, which can alter many responses to hypoxia (Borges et al., 2003). A study by Varasteh et al. (2015) reported that the chicken's critical adaptive response to heat stress increases the peripheral blood flow, resulting in a reduced blood supply in the intestines and a hypoxia-induced oxidative stress response. Two genes, *LOC101749846* and Hypoxia-inducible factor 3 subunit alpha (*HIF3A*) overlap the sweep on chromosome 32, thus being candidate selection regions in this population. They are involved in the epoxygenase P450 pathway (GO:0019373), response to hypoxia (GO:0001666) as well as angiogenesis (GO:0001525) in this chicken population. This finding is in line with Zahoor et al.

(2017), which reported that some angiogenic pathways are involved in hypoxia-induced angiogenesis in the chicken.

For the immunity-relevant genes, the Interleukin Enhancer Binding Factor 3 (*ILF3*), and Solute Carrier Family 44 Member 2 (*SLC44A2*) contribute to the negative regulation of viral genome replication (GO:0045071) and are involved in innate immune system pathway and positive regulation of I-kappa B kinase/IκKβ (GO:0043123). As described by May and Ghosh (1999) when IκKβ is activated, and in turn activates NF-κβ in response to proinflammatory cytokines such as TNF-α (tumor necrosis factor-alpha). Furthermore, insults to the immune system and various forms of cellular stress activate the transcription factor NF-κβ, regulating the expression of multiple genes involved in the control of cell growth, division, and survival. A variety of stimuli can activate NF-κβ-mediated gene transcription, including tumor necrosis factor- (TNF), interleukin-1, T and B cell mitogens, bacterial products, viral proteins, double-stranded RNA, as well as physical and chemical stress (Kray et al., 2005). In addition, previous reports have suggested that immunity is suppressed under high ambient temperature (El-Kassas et al., 2018, Quinteiro-Filho et al., 2017) which has an important impact in poultry becauseof its direct relationship with production performance (Figure 3.6).

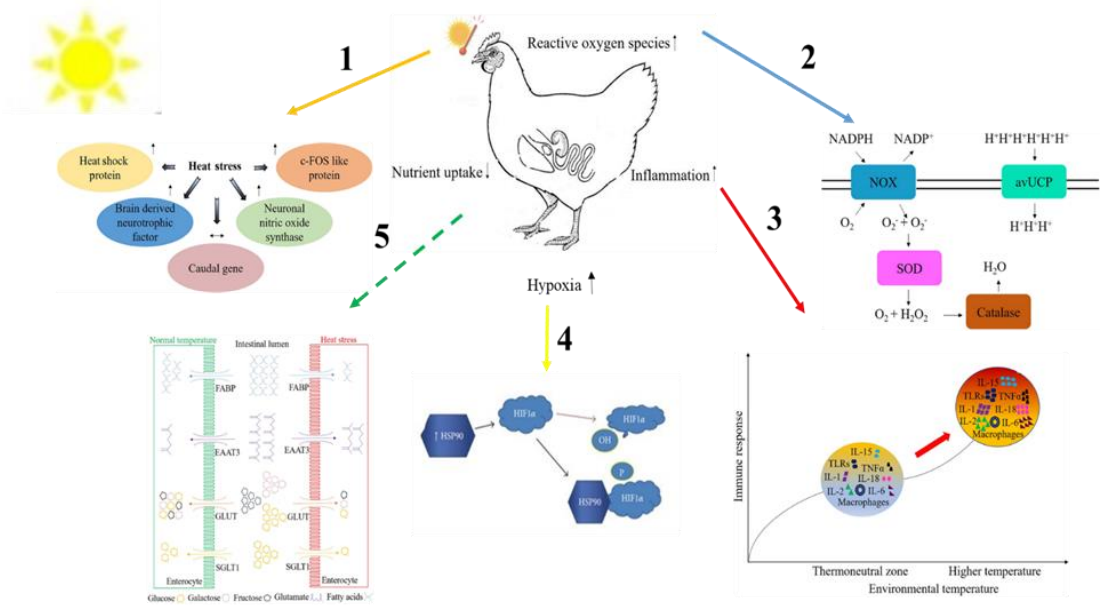


Figure modified from Goel *et al*., 2021
https://doi.org/10.1186/s40104-020-00523-5

Figure 3.6. The effect of high temperature on several cellular responses in the chicken.

1. Heat shock protein activation, 2. Oxidative stress-related mechanism, 3. Immune response-related, 4. Hypoxia related, 5. Nutrient uptake.

In this study, I have found that the strongest peak (*ZHp* = - 4.3) is located on chromosome five where it overlaps with the Thyroid Hormone Stimulating Receptor (*TSHR*) gene, which is involved in multicellular organism growth, especially reproduction (GO: 0040018; GO: 0004996) and transcription factor expression (GO: 0006366). A selective sweep in this region is found in most domestic chicken populations (Rubin et al., 2010). Recently, many studies have demonstrated that *TSHR* may be involved in regulating energy balance, metabolism, and thermoregulation (Warner et al., 2013, Jiang et al., 2015, Zhang et al., 2020). In addition, energy metabolism-related genes may contribute to the chicken response and adaptation to hot temperature environments and other possible mechanisms, such as epigenetic regulation, may also be involved in the tropical adaptation of chicken (Karlsson et al., 2015). This cannot be detected by genetic screening and requires other

approaches e.g. methylation analysis. A recent study from Guo et al. (2022) also reported that missense mutations in the *TSHR* gene (41020238:G>A) can facilitate heat tolerance and adaptation to higher ambient temperature conditions for chickens in tropical climates.

Furthermore, I have identified three selected candidate regions overlapping with chicken QTL for residual feed intake, wattle weight, and egg number in chromosomes 1, 2, and 5 respectively.

LncRNAs are not only cis-acting molecules that regulate the expression of target genes that are located at or near the same genomic locus but can also be trans-acting lncRNAs that can either inhibit or activate gene transcription at independent chromosomal loci (Fatica and Bozzoni, 2014). Several lncRNAs under selection were found in this study as well as in a recent study in the chicken population from tropical climates by Guo et al. (2022). A novel gene (*ENSGALG00000050153*) on chromosome 2 overlapped with the wattle weight QTL (Sun et al. (2015). However, the link between the three QTL and the variations within the overlapped genes is not yet known, while the role of these noncoding RNAs needs further investigation.

Based on *Hp* analysis, I have found that within the candidate selected regions 7,829 out of 92,094 SNPs have high alternative allele frequencies (AAF > 0.9). The proportion of the variant categories (all variants and exonic variants) are shown in Supplementary Table S3.2 and Supplementary Figure S3.1, while the list of candidate genes harbouring non-synonymous SNPs at high AAF is given in Supplementary Tables S3.5 and S3.6. I have checked the effect of twenty-two annotated exonic missense variants on protein structure (Supplementary Table S3.6), while the function and biological process of the genes were checked using Ensembl-Biomart (Table 3.3).

Table 3.3. Gene ontology (GO) term for genes with non-synonymous SNPs present at high frequency (twenty-two SNPs)

Related environmental stressor(s) / adaptation	**GO ID**	**Gene ontology term**	**Genes under GO term**	**References**
Heat stress	GO:0022610	Biological adhesion	*CDH9*	(Joutsen et al., 2020)
	GO:0017128	Phospholipid scramblase activity	*ANO3*	(Frasch et al., 2000, Zininga et al., 2018)
	GO:0006970	Response to osmotic stress	*TSC22D2*	(McKechnie and Wolf, 2019)
	GO:0015718	Monocarboxylic acid transport	*SLC16A5*	(Khan and Iqbal, 2016)
	GO:0005829	Cytosol	*TTI2*	(Hurov et al., 2010)
	GO:0006874	Cellular calcium ion homeostasis	*SYPL2*	(Li et al., 2017)
	GO:0022610	Biological adhesion	*CELSR2*	(Vihervaara et al., 2013)
Immunity and disease	GO:0005576	Extracellular region	*HHLA1*	(Pettersson and Jern, 2019, Pham-Dinh et al., 1993)
	GO:0050896	Response to stimulus	*EFR3A*	(Hui and Leung, 2015, Pettersson and Jern, 2019)
	GO:0042025	Host cell nucleus	*BAZ2B*	Uniprot(Hui and Leung, 2015)
	GO:0016020	Membrane	*TMEM94*	(Stephen et al., 2018)
	GO:0004222	Metalloendopeptidase activity	*MEP1B, ADAM9*	(Lin et al., 2008, Stephen et al., 2018)
Multicellular growth	GO:0120162	positive regulation of cold-induced thermogenesis	*TSHR*	(Rubin et al., 2010)

3.3.3 Putative selective sweeps in relation to rainfall patterns

FST genetic differentiation was performed between population groups for high precipitation (Degema and Isiokpo) *versus* low precipitation (Sabiyal and Jiga). From the total number of 92,828 windows, 92,107 windows had more than 10 SNPs and were analysed (the histogram are shown in Supplementary Figure S3.3 – S3.6). I considered 105 windows (top 0.1%), which have weighted $FST \geq 0.36$ and or $ZFST \geq 6.0$ to be candidate signatures of selection regions. The candidate regions are on chromosomes 1, 2, 3, 5, 8, and 11 and these regions overlap with thirty-three genes (Supplementary Table S3.7 - S3.8, Figure 3.7, Supplementary Figure S3.5 and S3.7)

I then characterised these candidate genes for their functional annotation using Ensembl Biomart and DAVID annotation web tools (Supplementary Table S3.9). These genes have roles in various biological processes such as immune response to viral and bacterial infections, heat stress, reproduction and production.

The associated molecular functions, biological processes and pathways can be grouped under three major categories: defence response against pathogens/diseases, heat and oxidative stress response, and reproductive or productive performance. The GO terms and genes associated with pathogen/disease defence include an innate immune response (GO:0045087; *REL*), positive regulation of toll-like receptor-9 signalling pathway (GO:0034165, *XPO1*), positive regulation of B-cell receptor signalling pathway (GO: 0050861, *CMTM3*), positive regulation of defence response to virus by the host (GO:0002230, *PEX3*), inflammatory response (GO:0006954, *CHID1*, *REL*), defence from pathogens such as cysteine-type endopeptidase activity (GO:0004197, *USP34*). Other genes were associated with the stress response (GO:0006950, *AHSA2*) and calcium ion binding (GO:0005509, *LRP8),* and positive

regulation of canonical Wnt signalling pathway (GO:0090263, *USP34*) and for reproductive and productive performance, the GO terms included endocytosis (GO:0006897, *LRP8*).

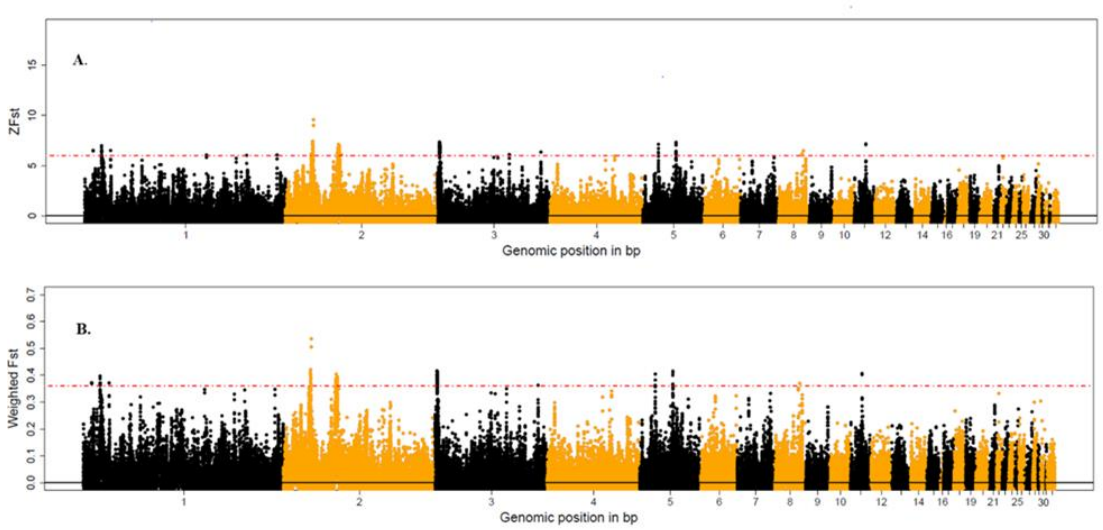


Figure 3.7. Manhattan plot for *ZFST* and weighted *FST.*

A) *ZFST* and B) weighted *FST* based on the differences between the chicken population in the high (Degema and Isiokpo) and low precipitation (Sabiyal and Jiga) group.

I further ran *Hp* analyses on both the high and low precipitation groups to determine in which group the candidate *FST*-sweep regions are showing lower heterozygosity, as that would indicate the group where the selection is occurring (Supplementary Table S3.10 – S3.14). The results show that the *FST*-candidate regions in relation to immunity or pathogen defence are predominantly selected in the high precipitation group. This is expected as a hot-humid climate is highly conducive for pathogens and parasites to thrive and spread. Moreover, the candidate region overlapping *AHSA* (Z*Hp* = -1.172 and -0.532 in high precipitation and low precipitation groups, respectively) is reported to have a contribution to heat stress or thermal adaptation with the biological process for Hsp90 (heat shock protein-90) protein binding (GO: 0051879) based on annotation from Ensembl Biomart.

3.4 Discussion

Poultry and other livestock farmers are already experiencing the adverse effect of climate change. Several studies have demonstrated a link between shrinkage of livestock food sources and the emergence of new diseases with changes in temperature and rainfall patterns across Africa and the emergence of new animal diseases (Ayanlade et al., 2017, Liverpool-Tasie et al., 2019, Goel et al., 2021). More than half of the poultry farmers reported by Liverpool-Tasie et al. (2019) have observed an increase in the length of heat stress in the two major poultry producing states in Northern (Kaduna state) and Southern (Oyo) Nigeria. Heatwaves and acute heat stress have been reported to cause considerable mortality in chickens (Anna et al., 2013). However, indigenous breeds of livestock in tropical areas can cope with and survive in harsh environments due to physiological and genetic adaptations (Rojas-Downing et al., 2017). Our study, for the first time, dissects the genetic basis of local adaptation in the Nigerian indigenous chicken population, especially in relation to heat stress based on whole-genome sequence data and taking into account a large number of samples and populations. Selection signature analyses presented in this study, dissect heat stress adaptation in two different ways. The *Hp* analysis identifies candidate genes and regions in relation to heat tolerance, irrespective of humid or dry conditions, by grouping populations with similar temperature stress independently of precipitations. The *FST* analysis on the other hand identifies candidate regions showing differential selection in relation to precipitation contrasting humid and arid conditions.

Candidate genes for thermal adaptation in Nigerian indigenous chicken populations

Heat stress results from the imbalance (or negative balance) between the amount of energy flowing from the chicken's body to its surrounding environment and the amount of heat energy produced by the bird (Lara and Rostagno, 2013b). This imbalance may be caused by various combinations of environmental factors (e.g., sunlight, thermal irradiation, air temperature, humidity, movement), and the characteristics of the chicken (e.g., species, metabolic rate, thermoregulatory mechanisms) (Nienaber and Hahn., 2007, Nardone et al., 2010, Lara and Rostagno, 2013).

In this study, I have performed *Hp* analysis on 87 chickens from different populations, which all belong to high-temperature regions. The rationale for the combined analysis of diverse populations is that it will reduce spurious signals (e.g. those originating from demographic effects) and identify regions showing low heterozygosity or fixation across all these populations. This study has detected only a handful of putative sweep regions and candidate genes for thermal adaptation because of this combined analysis. The genes overlapping the putative selection signature windows appeared highly relevant for heat stress adaptation, showing involvement in biological processes and pathways related to oxidative stress, cellular responses to heat and hypoxia, transcriptional regulation, immune response, and metabolic activities (e.g. lipid metabolism) important for the thermal adaptation.

Our study identified the *TSHR* gene as a strong candidate for selection (*ZHp* = -4.3). The *THSR* is involved in the metabolic regulation and reproductive process and appears to be under strong positive selection in most domestic chickens populations

(Rubin et al., 2010, (Gheyas et al., 2015)). Because of its ubiquitous presence in sweep regions across all or most chicken populations, the gene is considered related to chicken domestication (Rubin et al. 2010). We have found the relevant variant for *TSHR* gene (rs13587540 / Gly558Arg missense variant) which also reported by Rubin et al., (2010). The variant is located on chromosome 5:41020238; forward strand using Ensembl databases and web tools.

Moreover, the *TSHR* gene is also known to have a role in thermogenesis (Karlsson et al., 2015), and therefore it is possible that in the case of chickens its function in heat-stress adaptation is regulated by an epigenetic mechanism.

Heat stress adversely affects the growth and reproduction of animals, causes cell-level responses and changes the expression of the genes involved (Cai et al., 2005). When the body receives a stress stimulus (e.g. high temperature), it stresses proteins which have crucial roles in folding/unfolding of other proteins, assembly of multiprotein complexes, transport/sorting of proteins into correct subcellular compartments, cell-cycle control and signalling, and protection of cells against stress/apoptosis or play an important role in cell survival and homeostasis (Li and Srivastava, 2003, Testori et al., 2008). Figure 3.6 shows an overview of the heat-stress induced physiological responses in chickens. In the first mechanism, heat is the foremost inducer of HSP-related genes, as an early response system in the chicken. Heat shock proteins (HSPs) are an important family of highly conserved proteins and are also known as stress proteins. Thus, the expression of *HSP90* has been studied extensively and used as a marker for heat stress in chickens. This gene interacts with client proteins during the later stages of folding and modifies their configuration (Li and Srivastava, 2003, Cheng et al., 2020). *HSF1* and *CDC37* genes – detected as candidates in our study

have important regulatory roles in the expression of HSPs (Anckar and Sistonen, 2011).

Previous studies have also shown that *HSF1* regulates many genes, including HSPs, during neurodevelopment (Miller and Fort, 2018), which should also play an important role in stress adaptation. Moreover, *HSF1* also plays a role in the cellular response to gamma radiation (GO:0071480), and light stimulus (GO:0009416), and is annotated for the molecular function called 'heat shock binding protein' (GO:0031072). This gene is a transcription factor that is quickly induced after temperature stress and binds heat shock promoter elements (HSE), and expression of this gene is repressed by phosphorylation, which promotes binding by heat shock protein 90 (Wang et al., 2009). Several studies have now shown that these genes are involved in heat stress regulation (Wang et al., 2009; Tatebe and Shiozaki, 2003).

Since the chicken's body attempts to maintain its thermal homeostasis at elevated heat, increased levels of free radicals from reactive oxygen species (ROS) are formed, causing oxidative stress (Goel et al., 2021). Oxidative stress (Figure 3.5) is a significant detrimental consequence of most common chicken stressors, including heat stress, as revealed by studies on some indigenous poultry production systems in arid and tropical regions (e.g. Egypt, Sri Lanka, and Brazil) (Walugembe et al., 2019).

Our study has found four genes with GO terms related to oxidative stress: *ENSGALG00000050634*, *Cytochrome P450 2B4-like*, *ENSGALG00000050608*, and *ENSGALG00000036831*. These genes play a part in the oxidation-reduction process, epoxygenase P450 pathway, metal ion binding which has a possible association with oxidative stress (Abasht et al., 2016) and these have also been annotated to have a molecular function in iron ion binding (GO:0005506), which plays a crucial role in

cellular adaptation to oxidative stress, as this element is involved in the synthesis and activation of oxidoreductase enzymes (Li and Yang, 2018). Besides, iron can catalyse the formation of free radicals from ROS via the Fenton reaction (Imam et al., 2017), and is also used primarily as a component of heme in red blood cells for oxygen transport. In this study, I have also found the *SFTBP* gene related to the respiratory gaseous exchange that may be involved in thermoregulation.

Much information has been published on the effects of heat stress on productivity and how stress affects poultry's immune response, such as reduced antibody response, macrophage phagocytic ability, and induced oxidative burst (Goel et al., 2021). Upon contact with a phagocyte, the pathogens are engulfed, trapped within an intracellular vesicle, and targeted for destruction by a complex set of digestive enzymes or reactive oxygen species (ROS, such as free radicals) produced within the cell in the chicken, thus oxidative stress and inflammatory reactions are part of normal defence mechanisms against pathogens (Lauridsen, 2019). In the case of viral infections, ROS triggers a different pathway to kill or spread viruses, including the apoptosis pathway (Goel et al., 2021). Innate immune cells are activated in all viral infections, causing ROS and prooxidant cytokines and enhancing the iron uptake of a mononuclear phagocytic system (Imam et al., 2017). Moreover, ROS also interfere with antigen presentation by innate immune cells and adaptive immune responses (Paiva and Bozza, 2013). In our result, I detected several genes (*LOC107050992*, *cytochrome P450 2B4-like: LOC112530469* & *LOC101749846*) related to oxidative stress or ROS.

Hypoxia occurs when the oxygen tension drops below what is required for normal cellular function in a specific tissue and can occur in response to the lack of blood flow into the tissue or reduced oxygen transport capacity (Höckel and Vaupel, 2001). Heat

stress causes an increase in the expression of HIF1A (hypoxia-inducible factor 1 alpha) mRNA expression and translocation of HIF1A protein to the cell nucleus, consistent with hypoxic stress (Paul et al., 2009). Hypoxia-inducible factor (HIF) is composed of α and β (ARNT) subunits, which dimerise under hypoxic conditions (Wang and Semenza, 1995). In this study, a *HIF* gene (*HIF3A*) was detected 16 kb downstream from the sweep region on chromosome 32. This gene has an adaptive response to low oxygen tension or hypoxia (GO: 0001666). This gene was also demonstrated to induce increased production of ROS in the brain by altering the activity of oxidative phosphorylation which results in a decrease in ATP synthesis (Coimbra-Costa et al., 2017). Moreover, activation of HIF during hypoxic conditions leads to *HIF1A* binding to specific response elements in target genes involved in angiogenesis (formation of new blood vessels), vasodilation, and glycolysis (Shweiki et al., 1992).

The Nigerian indigenous chickens are mostly village chickens, reared in a rural, scavenger/forage type of farming system where they are exposed to diverse environmental conditions (i.e., desert, plateau, forest, and savanna). The results of our study support the suggestion from previous experimental studies on other African chickens (Walugembe et al., 2019, Elbeltagy et al., 2019) that indigenous chickens are capable of living under heat stress and harsh environmental conditions.

Candidate genes in relation to hot-humid and hot-arid climatic adaptation in Nigerian chicken populations

Poultry birds and poultry production are generally affected by seasonal climatic or weather changes. In most cases, when the hot conditions are extreme in both humid

and arid areas, the birds become stressed, and this affects their production (e.g. reduction in egg production) and ability to withstand diseases. Furthermore, the cold and wet seasons trigger various pathogenic and parasitic diseases causing heavy losses (Sharma and Tripathi, 2015).

Full genome sequencing of high precipitation and low precipitation chicken populations was analysed in this study to unravel the molecular mechanisms of adaptation to the condition based on high or low rainfall patterns in the hot climate. According to our findings, the most striking genes regulate defence response following viral infection. However, genes that play roles in oxidative stress and heat stress also stand out. These results are in line with the results from Olanrewaju et al. (2016) who found in Kwara state, Nigeria the prevalence of different poultry diseases during the wet and dry seasons. Newcastle, Gumboro diseases and heat stress characterised the weather types of the dry season, while coccidiosis and Gumboro diseases prevailed during the wet season (during harmattan).

The disease pressure from pathogen infection drives chickens to acquire strong disease resistance such as that observed in Fayoumi chicken (Weng et al., 2020). Furthermore, the avian immune response is divided into two arms, the innate immune response and the acquired immune response (Kaiser, 2012; Ferreira Júnior et al., 2018). The initial stage of infection involves the quick activation of innate immune mechanisms, such as acute inflammatory reactions. On the contrary, the acquired immune response is delayed and characterised by antibody production and immune memory (Guo et al., 2008; Singh et al., 2010).

The biological process and molecular function of genes under the putative sweeps showed positive regulation of innate and acquired immune responses. Relevant

annotated genes overlapping the sweep regions include *REL, CHID1, PUS10, BCL11A* and *CMTM3* for both immune responses, *RTN4* and *XPO1* for toll-like receptor nine signalling pathway (innate immune system), and *SCIN, HS1BP3, VOPP1,* and *AP2A2* for the apoptotic process.

The innate immune response begins once a cell traps antigens (non-self-compounds) by recognising the pathogen-associated molecular patterns by recognition receptor such as the Toll-like receptors (Ferreira Júnior et al., 2018). In this study, our results support the fact that the biological process for the innate response mechanism is positively regulated by *REL, RTN4* and *XPO1*. They trigger an acute inflammatory reaction by the production of pro-inflammatory cytokines and chemokines and defensin molecules. I found that *CHID1, PUS10,* and *CMTM3* are also involved in these regulations. *CHID1* has been reported to regulate cytokine production (innate inflammation response) (Lee et al., 2011), while *PUS10* has been linked to RNA activities such as RNA modification and RNA binding which regulate mediators of inflammation (Stumpo et al., 2010, Festen et al., 2011). The *CMTM3* is a protein family linking chemokines, that are widely expressed in the immune system as well as associated with an autoimmune disease in humans (Duan et al., 2020, Zhong et al., 2006), through positive regulation of the B cell receptor signalling pathway (GO: 0050861).

The avian and mammalian Rel/NF-κB family members are c-Rel, RelA, NF-κB1 (p50), NF-κB2 (p52), and RelB (Nehyba et al., 2002). v-REL rapidly induces an invariably fatal lymphoma in avian species then transforms cells by inappropriately activating or repressing genes coding for cytokines, transcription factors, chaperons, receptors, inhibitors of apoptosis, and adhesion molecules, which are generally regulated by c-Rel and other Rel/NF-κB family members (Bose, 1992).

Apoptosis or programmed cell death is an important defence mechanism of multicellular organisms in pathological events. This is a multi-pathway biological process that regularly takes place and is recognised by morphological and biochemical changes such as the formation of apoptotic bodies, cell shrinkage, caspase activation, and DNA fragmentation at the cellular and molecular levels (Zimmermann et al., 2001). In this study, I have found several genes (*SCIN, AP2A2, VOPP1*) involved in apoptotic process regulation (GO: 0043065, GO: 0042981, GO: 0030122, and GO: 0030659) within the candidate sweep regions.

Besides the annotated genes for pathogen and immune response, the result from the *FST* analysis also identified a few genes involved in heat stress adaptation (such as *EGFR, AHSA2, and PEX13*). These genes are involved in the biological process and cellular response to reactive oxygen species (GO: 0034614), Hsp90 protein binding (GO: 0051879), calcium ion binding (GO: 0005509), eyelid development in the camera-type eye (GO: 0061029) and response to UV-A (GO: 0070141) to prevent the negative effect of heat stress.

A few genes involved in reproductive and growth traits have also been detected from the sweep regions. The *STXBP6* gene has a potential pleiotropic effect on bone tissue and fecundity traits in chickens (Johnsson et al., 2014, Fu et al., 2016), while the *LRP8* gene has a possible role in egg development as it has a molecular function in very-low-density lipoprotein particle receptor activity (GO: 0030229). During the accelerated final stage of growth, chicken oocytes take up enormous amounts of plasma components and convert them to the yolk. The oocyte expresses a receptor that binds both major yolk lipoprotein precursors, vitellogenin and very-low-density lipoprotein (Shen et al., 1993). *STXBP6* has been found to have a lower *ZHp* value in the high precipitation group compared to the low precipitation one. This finding may

indicate that chicken in the high precipitation region, though facing the disease challenges, has adapted to remain productive and reproduce, while in low precipitation areas characterized by water scarcity chicken may be facing more challenging physiological conditions.

3.5 Conclusions

Overall, the results of this study support the hypothesis that the Nigerian indigenous chicken genomes have been shaped by environmental stresses (selection) with strong evidence for several positively selected regions. Intra-population *ZHp* and inter-population *FST* mapping revealed selection footprints for heat stress and pathogen and disease challenges. The *ZHp* of the 87 chicken genomes examined here show selection footprints with genes involved in the reduction of oxidative stress, heat stress adaptation, hypoxia, reproduction and growth performance.

FST analysis supports distinct genetic mechanisms that impart resistance to endemic diseases and heat tolerances. These results enhance our understanding of the role of natural selection in shaping genomic variation, and genes contributing to the adaptation under the stressful Nigerian environmental conditions.

CHAPTER 4:

Genomic landscape of selection signatures in Nigerian indigenous chickens from different agro-ecological zones

4.1 Introduction

Indigenous chickens are widely distributed throughout Nigeria under diverse geographical and agro-ecological conditions. Geographically isolated chicken populations are subjected to local climatic conditions, and therefore it may be expected that over time they have acquired genetic adaptation to their unique local agro-ecological and climatic conditions (Ngeno, 2015, Mpenda et al., 2019). These locally adapted populations may be called ecotypes.

An ecotype refers to a chicken population from one agro-ecological zone or area. The ecotype name is usually derived from the ecological zone where the population lives but, in some areas, regional names have been used (Sonaiya, 1998, Kebede, 2018). Ecotype names have usually been given to distinct geographical populations, breeds, strains, or races within a species adapted to distinct and specific environmental conditions (Begon et al., 2006). The geographic delimitations of the agro-ecological zones in Nigeria are presented in Figure 4.1.

Some efforts have been made to identify and characterize the indigenous ecotypes of chickens in Africa (Msoffe et al., 2001). Distinct ecotypes have been reported in Tanzania (Msoffe et al., 2001), Ethiopia (Tadelle and Ogle, 2001), Zimbabwe (Mcainsh et al., 2004), and Botswana (Badubi et al., 2006) and Kenya (Ngeno, 2011). These populations present a high between-and within-ecotype variation in body weight, egg weight, reproduction performance, plumage colour, comb type and skin colour. It is assumed that chicken ecotypes have special characteristics to survive in specific habitats (ecological zones). Due to different climatic and physical factors, farmers from different agro-ecological zones experience different production challenges and economic needs. The type of enterprises farmers engage in are influenced by environmental factors, such as climate, leading to a variation across

ecological zones. Therefore, the resulting ecotypes are defined by the breeding strategies set up to achieve the village chicken production goals under specific environmental conditions (Muchadeyi et al., 2009).

Nigerian indigenous chicken ecotypes are the product of mutation, genetic drift, adaptation and evolution. The different selection pressures imposed on these chickens include diet, variation in climate and endemic parasites and diseases (Agbaje and Alabi, 2018). However, the differences in how chickens respond to stressors may depend upon their evolutionary course and the intensity of selection pressures for survival. Constant selection of these survival traits can lead to the presence of genomic signatures. In particular, selection for survival traits can reduce the variability around genomic regions associated with these traits. This reduction in variability, referred to as a selection signature or selective sweep, can be detected and examined for its biological importance (Fleming et al., 2016).

On the other hand, characterisation studies of Nigerian indigenous chickens from different ecological zones (Oluyemi, 1979, Sonaiya, 1998, Okpeku, 2003) have also reported many similarities among the birds within and across the zones. Oluyemi (1979) characterised indigenous chickens from southwestern Nigeria and found no significant variation in body weight, egg weight, egg production, or other physical characteristics. Another study by Sonaiya, (1998) did not find any significant genetic variation in either immunological competence or egg production traits among local chicken populations assembled from Kaduna and Jos (Guinea savanna), Makurdi, Ogun, Ilorin (Derived savanna), Nsukka and Osun (Rainforest) Okpeku (2003) investigated the phenotypic and genetic variation among the local chickens of Edo State of Nigeria (Rainforest zone) and found no significant variation in body weight

or other biometrical body measurements. Ogbu (2010) also concluded that the birds could not be classified as separate strains.

The chicken ecotypes in this study were sampled from eight agro-ecological zones (AEZ); namely, from South to North: Mangrove swamp, Freshwater swamp, Rainforest, Mid-Altitude (Plateau), Derived Savanna, Guinea Savanna (two zones, Southern and Northern), and Sudan Savanna (Table 4.1). Climatic, e.g. temperature and rainfall patterns delineate these zones (Udoh et al. (2000), Atehnkeng et al. (2008), Yakubu et al. (2019)). The availability of diverse AEZ, climatic conditions, and variation in chicken rearing purposes in the tropics have contributed to the existence of high chicken genetic diversity (Padhi, 2016). Apart from the classification of the AEZs based on climatic conditions, the AEZs have also been characterised by their vegetation types, which in combination with local climatic conditions create unique agro-ecological conditions that may have equally shaped the genomes of local indigenous chicken populations. The main features of the AEZ in this study are described in Table 4.1.

It is important to know the types of vegetation in an AEZ as it plays a crucial role in the exchange of carbon, water, and energy on the land surface of the regional ecosystem (Duo et al., 2016). Besides, human alteration of the vegetation structure in the rearing environment has been reported to influence early chick survival through increased predation risk by influencing predator densities, lack of cover and the speed at which chicks detect predators (Whittingham and Evans, 2004, Chikumba and Chimonyo, 2021). Vegetation cover also influences the spatial and temporal distribution of food and the foraging efficiency of chicks and adults (Manzer and Hannon, 2008, Davis, 2009). Categorical land vegetation cover types (sparse, dense)

are commonly used to describe the habitat suitability and potential to support livestock production, including free-range indigenous chicken (Strohbach, 2017).

Following Keay (1949), as described in FRN (2019), the types of vegetation of each ecotype in Nigeria from North to South are as follows: (i) The Sudan Savanna has less vegetation than the Guinea Savanna. Existing vegetation consists mainly of short grasses, about 1-2 m high, and some stunted tree species, such as Acacia species. (ii) The Derived Savanna and Guinea Savanna are much the same with the most dense vegetation in the middle belt of Nigeria where it consists of a mixture of trees and grass. The typical vegetation is open woodland with tall grasses (1 to 3 m high) in open areas, and trees (up to 15 m high), usually with short boles and broad leaves. (iii) Plateau or Mid-Altitude zone is characterized by grassland vegetation at the base, forest vegetation on the windward slope and grassland vegetation on the Plateaux. Hills are covered with forest vegetation, while the upper slopes and the Plateau surfaces have grassland vegetation, which usually supports the cattle population. (iv) The Rainforest is a dense evergreen forest of tall trees with thick undergrowth consisting of three layers of trees: the emergent layer with trees more than 36 m high; the middle layer between 15-30 m; while the lowest layer is generally below 15 m. (v) The Freshwater swamp has a more open canopy, which may reach 45 m in height, densely tangled, and almost impenetrable undergrowth, while (vi) the Mangrove swamp is dominated by *Rhizophora sp* that can attain heights of up to 40 meters. These last two agro-ecological zones are usually flooded during the wet season and dry out during the dry season, leaving portions of the dry forest floor interspersed with permanent pools of water. However, much of this vegetation type has been converted to agricultural and urban lands, and the original swamp forest remains mostly on alluvial sites along the major rivers.

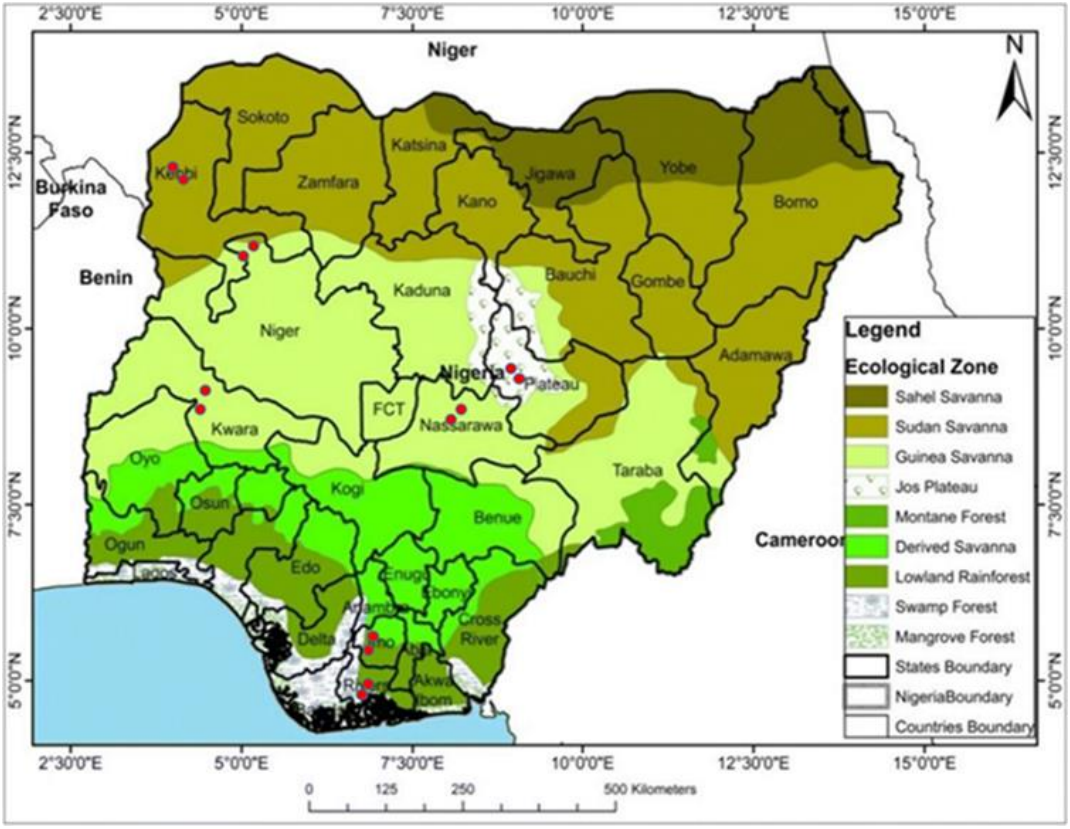


Figure 4.1. The different agro-ecological zones in Nigeria.
Modified from Akinwumiju et al. (2020).

Considering the diverse agroecology in Nigeria and the important link between adaptation and the AEZ for the indigenous chicken, in this chapter, I aim to identify candidate genome regions and associated candidate genes that show strong evidence of positive selection in relation to the different ecotypes of the Nigerian indigenous chicken. For this purpose, two signature selection approaches were applied at the genome-wide scale: pooled heterozygosity (*Hp*) to identify within-ecotype candidate sweep regions and F_{ST} to identify regions showing differential selection among the eight ecotypes studied viz. Mangrove swamp, Freshwater swamp, Rainforest, Plateau or Mid-Altitude, Derived Savanna, Southern Guinea Savanna and Northern Savanna, Sudan Savanna. While Chapter 3 specifically dissected the heat stress adaptation and

adaptation to hot-humid and hot-arid conditions merging multiple populations, this chapter reports candidate sweep regions within and between each ecotype. It is expected that the outcomes of this study will help to improve our understanding of the genetic basis of local adaptation of Nigerian indigenous chicken under varied agro-ecological challenges.

Table 4.1. Main features and differences between the agro-ecological zones.

Features	**Zones**							
	Sudan Savanna	Northern Guinea Savanna	Southern Guinea Savanna	Derived Savanna	Mid-Altitude	Rainforest	Freshwater swamp	Mangrove swamp
Geographic coordinates	Latitude 4° 45′ N and longitude 6° 50′ E	Latitude 4° 45′ N and longitude 6° 50′ E	Between latitudes 8° 30′ N and 8° 50′ N and longitudes 4° E 20′ and 4° 35′ E	Between latitudes 7° 52′ N and 8° 56′ N and longitudes 7° 25′ E and 9° 37′ E	Latitude 9° 43' N and longitude 8° 50' E	Between latitudes 4° 45′ N and 7° 15′ N and longitudes 6° 50′ E and 7° 25′ E	Between Latitude 4° 45′ N and longitude 6° 50′ E	Between Latitude 4° 45′ N and longitude 6° 50′ E
AEZ type	Tropic-warm / arid	Tropic-warm / arid	Tropic-warm / semiarid	Tropic-warm / semiarid	Tropic-cool	Tropic-warm / humid	Tropic-warm / humid	Tropic-warm / humid
Temperature (°C)	28.5	27.2	26.8	26.2	21.5	26.3	26.3	26.4
Relative humidity (%)	47.4	47.4	74.4	74.0	15.6	80.0	83.4	83.4
Rainfall (mm, per annum)	807	807	1217	1169	1233	2219	2708	2708

Sources: Sowunmi (2010), Eludoyin et al. (2014), Esiobu and Onubuogu (2014), Zitta and Madaki (2020), Yakubu et al. (2020).

4.2 Materials and Methods

4.2.1 Study populations and genome-wide SNP data

A total of 80 chicken samples representing eight different chicken ecotypes were included in the analysis. From the northern part to the southern part of Nigeria, namely: Sudan Savanna, Northern Guinea Savanna, Southern Guinea Savanna, Derived Savanna, Mid-Altitude (Plateau), Humid Rainforest, Humid Savanna Freshwater Swamp, and Humid Savanna Mangrove.

I sub-sampled our genome data (*see* Chapters 2 and 3) analysing an equal number of samples per ecotype. Then, the genome-wide sequence data went through several steps: whole-genome sequencing, reads mapping, and variant discovery followed by downstream analysis for SNP quality control (*see* Chapter 2).

4.2.2 Selective sweep analyses and functional annotation of candidate genes

The *Hp* and F_{ST} analyses were performed using the same protocol as described in Chapter 3. The $ZHp \leq -4$ and top 0.1% values ($ZF_{ST} \geq 7.0$) were considered as candidate selection signature signals in all tests as to those described in Chapter 3. Bedtools version 2.25.0 (Quinlan and Hall, 2010) was used to merge the overlapping selected windows. Chicken genes that overlapped genomic windows passing the significant selective sweep threshold were retrieved from the *Ensembl Genes 98 database* using the Biomart online tool (http://www.ensembl.org/biomart). The candidate genes were then processed in a web-based PANTHER Classification System (Thomas et al., 2003) to map the candidate genes to known biological processes, molecular function, cellular processes, and molecular pathways.

4.3 Results and Discussion

4.3.1. Signatures of selection detected by *ZHp* analysis

To identify the selective sweep regions within each of the Nigerian chicken ecotypes, I performed a genome-wide scan of chicken autosomes using the *Hp* test. Results of *Hp* analysis on the eight ecotypes are summarized in Table 4.2 and Figure 4.2. Around 92,000 windows with at least 10 SNPs per window were analysed in each chicken ecotype, with 19 to 168 candidate per selected windows. After merging adjacent candidate windows within ecotypes, the sweep regions ranged from eight (Sudan Savanna) to thirty-four (Mid-Altitude). The Humid Freshwater swamp and Mid-Altitude (Plateau) ecotypes showed the strongest signal for *ZHp* values (-5.7 and -5.6, respectively), followed by the Humid Rainforest and Southern Guinea savanna (*ZHp* = -5.2). Meanwhile, the numbers of candidate genes varied from eleven in the Sudan Savanna to fifty in the Mid-Altitude (Plateau) zones. The number of candidates sweep regions and their overlapped genes are detailed in Supplementary Tables S4.1 to S4.8.

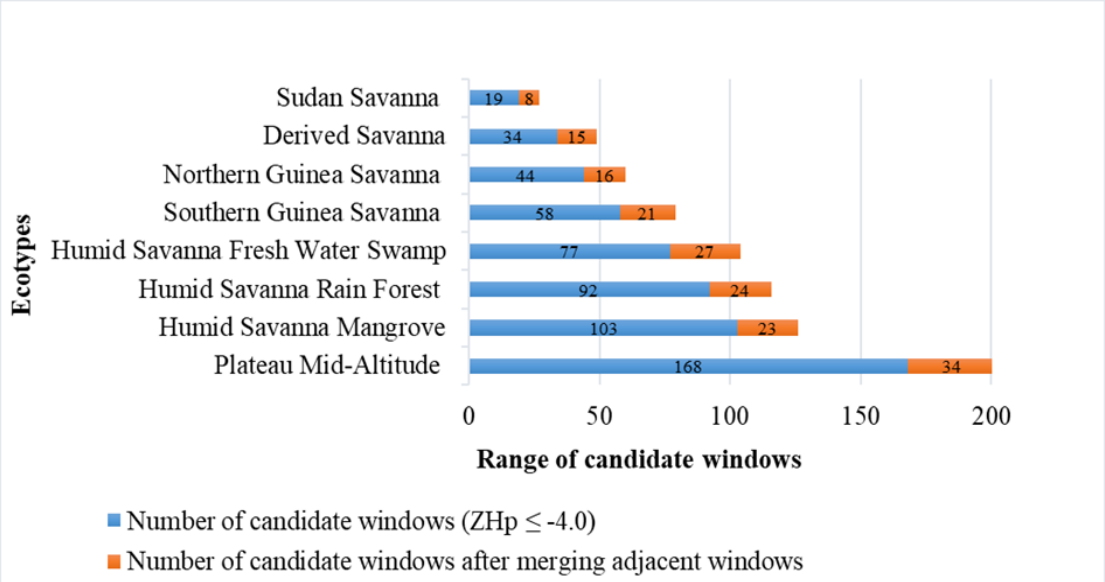


Figure 4.2. Distribution of the number of candidate windows and regions in each ecotype

According to the results, the main environmental stressors are oxidative and immune responses stressors. Selection for heat response was present in chicken ecotypes living in warm-arid, warm semi-arid, and warm-humid zones, while cold response and osmotic stressors contributed to the adaptation in cool and warm-humid zones.

I observed that between three to thirteen genes were shared between ecotypes (Supplementary Tables S4.9-S4.13 and Supplementary Figures S4.1-S4.6) with *TSHR* (thyroid-stimulating hormone receptor) and *ENSGALG00000047413* (long non-coding RNA) in chromosome 5 overlapping sweep regions in all ecotypes. Several other genes, like *OVSLT* (ovostatin-like), *LCORL* (ligands dependent nuclear receptor corepressors), *NCAGP* (non-SMC condensin I complex subunit G), and the novel genes (*ENSGALG00000054958, ENSGALG00000026901, ENSGALG00000052351*) were shared frequently among ecotypes (at least in three ecotypes). Sudan Savanna ecotype (warm-arid zone) and Mangrove swamp ecotype (warm-humid zone) have the least number of shared candidate regions and genes compared to the other ecotypes.

Table 4.2. Summary of the pooled heterozygosity (*Hp*) test for the eight ecotypes

Ecotypes	Number of samples	Total SNPs	Pool heterozygosity (*Hp*) statistics				
			Total analysed windows (SNPs > 10)	Sweep regions (*ZHp* ≤ -4)	Range of *Hp*	*ZHp* value	Number of genes overlapping the sweep regions
Sudan Savanna	10	11,047,403	91,943	8	0.001 to 0.017	-4.323 to -4.087	11
Northern Guinea Savanna	10	11,205,205	91,937	16	0.001 to 0.054	-4.956 to -4.100	22
Southern Guinea Savanna	10	11,816,923	92,013	21	0.002 to 0.071	-5.247 to -4.000	25
Derived Savanna	10	11,645,212	91,989	15	0.004 to 0.064	-5.029 to -4.019	22
Plateau Mid-Altitude	10	11,374,240	91,952	34	0.001 to 0.093	-5.636 to -4.019	50
Humid Savanna Rainforest	10	11,409,440	91,983	24	0.001 to 0.071	-5.251 to -4.022	33
Humid Savanna Freshwater Swamp	10	11,731,625	92,006	27	0.001 to 0.096	-5.719 to -4.003	38
Humid Savanna Mangrove	10	10,632,424	91,906	23	0.002 to 0.028	-4.433 to -4.007	42

a. Selection signatures in ecotypes from the warm-arid zones: Sudan Savanna and Northern Guinea Savanna ecotypes

The Sudan Savanna is known for the coexistence of trees and grasses (Amoako et al., 2018). The annual precipitation is very low compared to other ecological zones with annual precipitation of about 700 - 1100 mm with a prolonged dry season of about 6–9 months (Aremu et al., 2017, Ayanlade et al., 2021), while the Northern Guinea Savanna has an average annual rainfall between 900–1,200 mm. Grasses with scattered trees and shrubs are the dominant vegetation in this zone (Tambo and Abdoulaye, 2013).

Sudan Savanna ecotype

The eight candidates sweep regions detected in the Sudan Savanna ecotype overlap with eleven genes. The sweep regions are from chromosomes 1, 2, 3, 5, 6, and 7 (Supplementary Table S4.1). The annotation of these genes indicates involvement in reproduction e.g. *TSHR, ENSGALG00000047413* (Lawal et al., 2018) and *BMPR2* (Divya and Bhattacharya, 2021), stress response including heat stress or thermal response e.g *CERK* (Chen and Narum, 2021), *SGMS1* (Nagai et al., 2011), *MINPP1* (Humburg et al., 2016) and immune response e.g *HHLA1, ASB1* (Jern and Coffin, 2008, Albooshoke and Bakhtiarizadeh, 2019).

Solute Carrier Family 24 Member 3 (*SLC24A13*), Sphingomyelin Synthase 1 (*SGMS1*), Ceramide Kinase (*CERK*), Multiple Inositol-Polyphosphate Phosphatase 1 (*MINPP1*), are associated with heat responses. Previous studies have shown that the intracellular concentration of free calcium increases in several types of cells during stress, such as high temperatures (Feske, 2007). Thus the *SLC24A13* gene (GO:0006874) may play an essential role in this imbalanced condition due to its role in cellular calcium ion homeostasis. Sphingolipids' biosynthetic process

(GO:0030148) also plays a crucial role in cell response to heat stress (Chen et al., 2013); *SGMS1* and *CERK* genes have a molecular function in this process.

Chicken has various immune responses, including lymphocyte proliferation when exposed to high ambient temperature (Han et al., 2010); hence, lymphocytes play essential roles in cellular and humoral immunity (Arora et al., 1981). In this stage, calcium ions have an essential function in the activation and maturation of lymphocytes. The potential role of the *MINPP1* gene in response to a variety of cellular stress conditions has also been reported (Kilaparty et al., 2016). Meanwhile, *HHLA1* and *ASB1* genes are likely also involved in the immune response. Exposure to heat stress suppresses chicken immune responses, which can increase susceptibility to infectious diseases, therefore with negative effects on chicken performance and welfare (Monson et al., 2018).

It is likely that the Sudan Savanna ecotype is probably suffering from heat stress following hot temperatures during the prolonged dry season. The recent study from Ayanlade et al. (2021) also revealed that in the Sudan Savanna ecological zone, there is always a great decline in vegetation greenness that lasts nearly five months, until it experiences a short wet season. This finding is in line with Liverpool-Tasie et al. (2019), who reported that about 68% of poultry farmers in the Sudan Savanna region stated that the temperature had increased significantly in recent years with almost 50% of the farmers affected by a production loss, likely related to heat stress. This study also mentioned that farmers who have personal experience of poultry production loss due to extreme heat were more likely to adopt multiple adaptation strategies including giving their poultry medicines and vitamins with the expectation of reducing the mortality rate.

Northern Guinea Savanna ecotype

Sixteen sweep regions overlapping with 22 genes were detected in the Northern Guinea Savanna ecotype (Supplementary Table S4.2). Six regions overlapped with seven genes that have biological functions that are involved in heat stress and tropical adaptation (e.g., *CTNNA3, DRD3*), immune response (e.g., *HHLA1, CCDC93, DOCK2, EIF4A3*), growth traits (e.g., *LCORL, NCAPG*) and reproduction (e.g., *OVSTL, TSHR, CCDC93*) (Supplementary Table S4.2).

The GO term associated with Catenin alpha 3 (*CTNNA3*) is cell-cell adhesion (GO:0098609). This gene is linked to acute heat stress in the testes of a broiler-type strain of Taiwanese country chicken (Wang et al., 2015). The dopamine receptor (*DRD3*), with GO term for cellular calcium ion homeostasis (GO:0006874), is involved in the regulation of systemic arterial blood pressure. It is a candidate gene for tropical adaptation (adaptation in tropics climate) in chickens (Tian et al., 2020), Bactrian camel (Wang et al., 2012) and sheep (Yang et al., 2016).

HHLA1 (HERV–H LTR-associating protein 1) is an essential regulator of stem cell differentiation. It is strongly upregulated during early embryogenesis. It is also co-localised with a provirus (Vargiu et al., 2016). The *CCDC93* (coiled-coil domain containing 93) gene is responsible for early endosome (GO:0032456) and chemokine signalling or immunological synapse formation (GO:0001771) as a response to infection by several microorganisms (e.g. virus) in chicken (Goraya, 2017). *DOCK2 (dedicator of cytokinesis 2)* was found to have many roles in the immune response through its involvement in activating B-cells (Tanigaki et al., 2002, Wang et al., 2018). This gene has been found to improve broilers' humoral immune function and enhance the NDV (Newcastle Disease Virus) vaccine (Li et al., 2021). *EIF4A3* (Eukaryotic Translation Initiation Factor 4A3) has been implicated in cellular response involving

alteration of RNA secondary structure (GO:0006366). It has been revealed as a key mediator of Avian Influenza polymerase activity (Ren et al., 2019).

Some genes associated with reproduction (*TSHR, GTF2A1*) and growth (*NCAPG* and *LCORL*) were also identified. They are involved in embryogenesis and spermatogenesis (Karlsson et al., 2015, Lawal et al., 2018). Non-SMC condensin I complex subunit G (*NCAPG*), and ligand-dependant nuclear receptor corepressor-like protein (*LCROL*) genes have been implicated in several QTL and genome-wide association studies for human height, swine body length, equine height, cattle growth, and chicken carcass phenotypes (Lindholm-Perry et al., 2013, Liu et al., 2013).

Similarly, to the Sudan Savanna ecotype, the sweep regions in Northern Guinea savanna ecotypes emphasize the selection pressure from heat stress in domestic chickens and the impact of such stress on reproduction and immunity. Several other studies on Northern Guinea Savanna chicken also revealed that heat stress adversely affects well-being, fertility, and hatchability (Oladele et al., 2003, Ayo et al., 2011, Sinkalu et al., 2015).

I have found two common sweeps in chromosomes 2 and 5 in these two ecotypes from the warm-arid zone, which overlap with the *HHLA1* and *TSRH* and *LncRNA* genes. *HHLA1* is a non-enveloped viral sequence that has integrated into the human genome and may regulate the immune response (Balada et al., 2010). However, little is known about the function of *HHLA1* in chickens. In comparison, the *TSHR* locus is involved in metabolic regulation and the reproduction process (Yoshimura et al., 2003, Rubin et al., 2010).

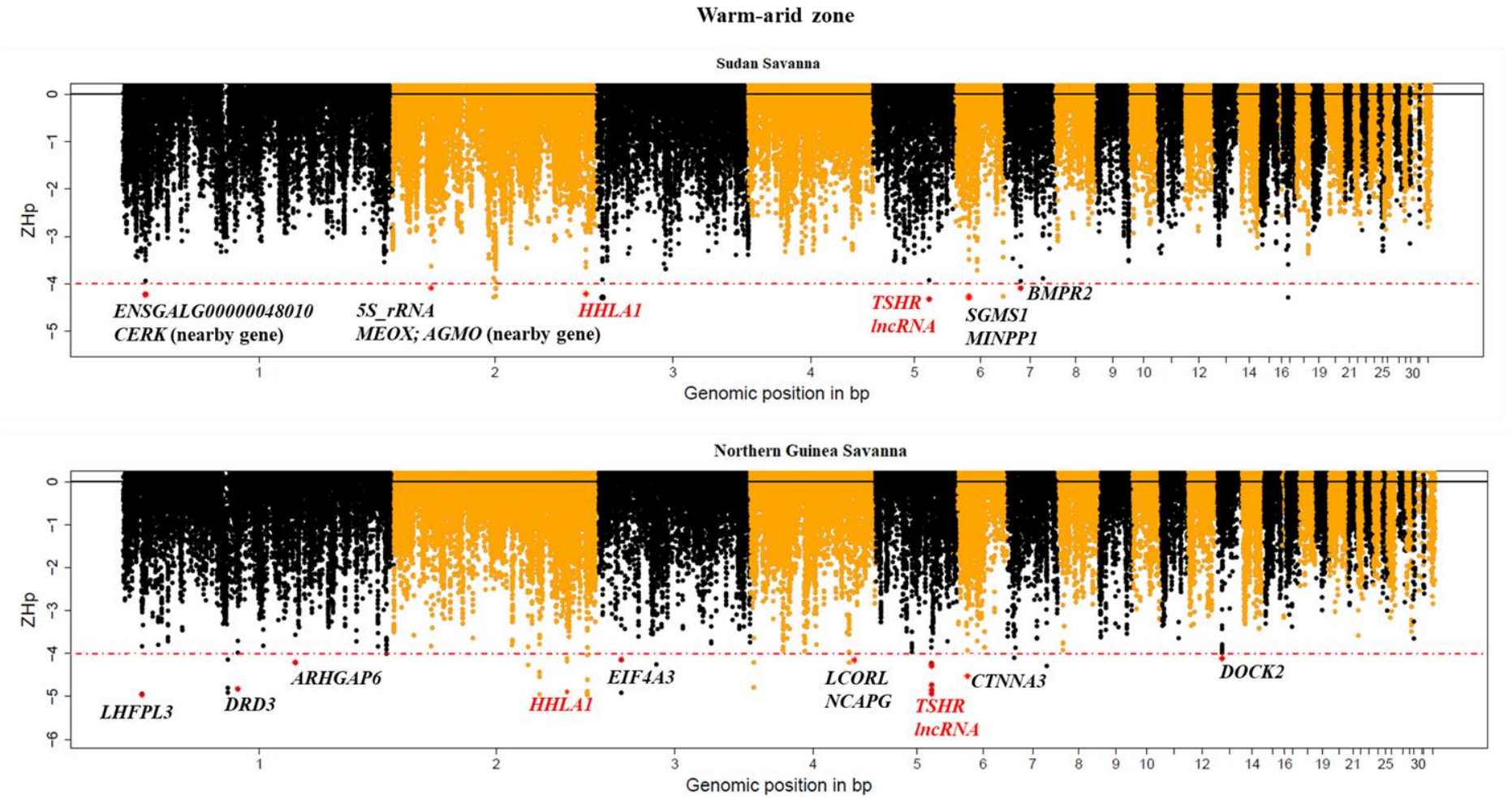


Figure 4.3. Manhattan plot and important candidate genes under selective sweeps in Sudan Savanna and Northern Guinea Savanna ecotypes. *Common genes among ecotypes are in red font.

b. Selection signatures in ecotypes from the warm semi-arid zones: Southern Guinea Savanna and Derived Savanna

The Southern Guinea Savanna and Derived Savanna regions have a typical savanna climate with distinct wet and dry seasons. These classifications reflect environmental characteristics such as the length of the growing period, which for instance, is 181-210 days for the Southern Guinea Savanna and 211-270 days for the Derived Savanna (Salako, 2004). Typical vegetation in the Southern Guinea Savanna and Derived Savannas are dry woodlands and moist woodland, respectively. Moreover, the Nigerian Environmental Study/Action Team (1991) defined the Derived Savanna as the transitional forest-savanna mosaic immediately North of the lowland rainforest belt.

The mean annual temperature and rainfall in Southern Guinea Savanna are 27°C and 1,165 mm, respectively, with the wet season stretching from April to October, while the dry season is from November to March (Amao, 2017, Anoh et al., 2021). Meanwhile, the mean temperature and annual mean rainfall of the Derived Savanna are about 27°C and 1,247 mm, respectively. This zone has two major seasons: wet (April-September) and dry (October–March) (Amao et al., 2011, Adedeji et al., 2015).

Twenty putative sweep regions overlapping twenty-five genes were detected in the Southern Guniea Savanna ecotype, whereas fifteen sweep regions and twenty-two candidate genes were detected in that of the Derived Savanna. The genes in each ecotypes were mostly linked to the immune response, behaviour and reproduction of the chicken (Supplementary Table S4.3 and S4.4). The Manhattan plot and interesting genes under putative selective sweep regions are shown in Figure 4.4.

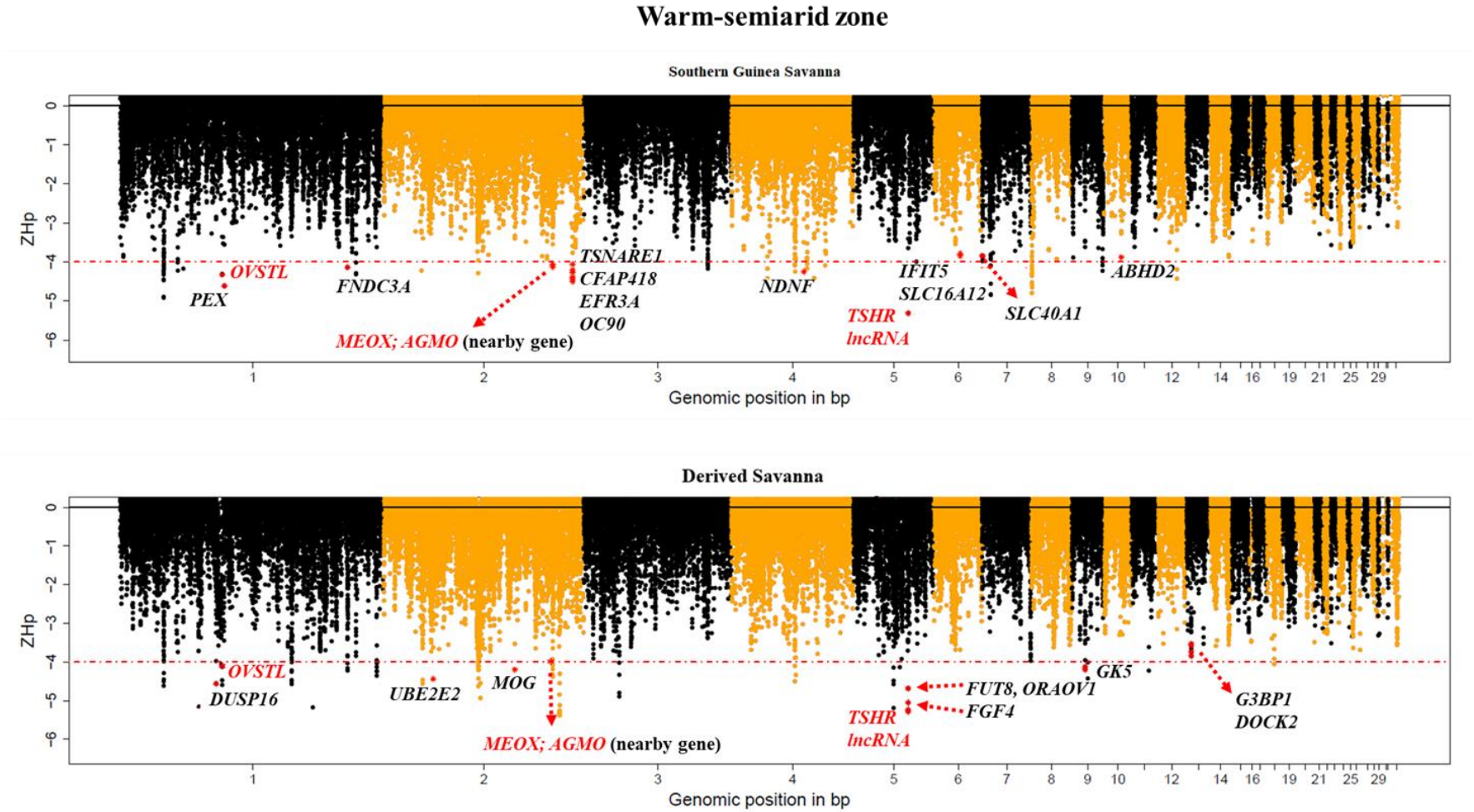


Figure 4.4. Manhattan plot and important candidate genes under selective sweeps regions in Southern Guinea Savanna and Derived Savanna ecotypes.

*Common genes among ecotypes are in red.

Southern Guinea Savanna ecotype

Disease challenge is a common threat to poultry production in the Southern Guinea Savanna zone (Okaeme, 1988, Atehmengo Ngongeh et al., 2017, Shittu et al., 2016), and is a major threat to both the large and small scale poultry industries. Coccidiosis and Newcastle disease (ND) have been ranked as the most economically important diseases in this region, with a high level of morbidity and mortality (Ngongeh et al., 2017). Several genes (*IFIT5, SLC16A12, SLC40A1)* associated with disease challenges were found to be under strong positive selection in this ecotype. The annotation terms associated with these genes are 'defence response to virus' (GO:0051607), monocarboxylic acid transport (GO:001571), and iron ion homeostasis (GO:0055072), respectively.

Another candidate gene, *ABHD2* (abhydrolase domain containing 2), has been suggested to play a role in adaptation to environmental temperature changes by modifying vascularization properties to facilitate heat dissipation (Loyau et al., 2016). Also, *OC90* (otoconia 90), involved in arachidonic acid metabolism, has been reported to be under selection following tropical climatic challenges in birds and mammals (Tian et al., 2020).

The above finding is of relevance to the study of Olanrewaju et al. (2016) on indigenous chicken from the Southern Guinea Savanna zone reporting several disease outbreaks during the harmattan period, a dust-laden north-easterly wind in the dry season that blows from December to the middle of March). The season is different from other 'winter' seasons as it is not just cold, but also extremely dry, windy and dusty with wide diurnal ambient temperatures variation; from as low as 12 °C in the morning to up to 34°C in the afternoon hours (Habibu et al., 2021). A strong positive relationship has been reported between the chicken mortality rate and the amount of

rainfall and a negative correlation with temperature. For the period after harmattan (March-April) it is the reverse with a strong negative correlation between rainfall, relative humidity and mortality rate, and a strong positive correlation between the temperature (maximum or minimum) and the mortality rate of chicken in the Kwara state in the Southern Guinea Savanna zone (Olanrewaju et al., 2016).

For efficient poultry development, Scott (1999) emphasized that the temperature of a chicken pen must not go above 35°C for any prolonged time. The increased vulnerability of chickens during the dry season may be attributed to the physiological imbalance created by various weather types. For instance, harmattan chill may make chickens more susceptible to diseases with the observation that many chickens died of heat stress and Newcastle disease in the Southern Guinea Savanna zone following harmattan (Olanrewaju et al., 2016).

Derived Savanna ecotype

The candidate genes from the sweep regions in the Derived Savanna ecotype related to the immune response are *DUSP16*, *MOG*, *ORAOV1*, *FGF19*, *BIN1*, *GK5*, *G3BP1*, *FUT8*, and *DOCK2* (Supplementary Table S4.4). Some of these genes (e.g., *DUSP16* and *BIN1*) overlapped the strongest selection signals ($ZHp < -5.00$), indicating great pressure on the immune system from environmental stresses. The roles of some of the above genes in the chicken immune responses have been reported in previous studies.

For example, the *ORAOV1* (oral cancer overexpressed 1) gene was found to be associated with resistance to infectious bursal disease (IBD) caused by the IBD virus in the chicken, which triggered cell apoptosis (Qin et al., 2017). Over-expression of *ORAOV1* leads to the inhibition of VP2 (one part of IBD virus segments) or IBDV-induced apoptosis, accompanied by decreased viral release ($p < 0.05$) (Qin et al.,

2017). Apoptosis is a defence mechanism of host cells in response to virus infection to limit viral propagation, and it is well-known that apoptosis is responsible for the rapid depletion of lymphocytes during IBDV infection, which is important for IBDV-induced immune suppression and its pathogenesis (Qin et al., 2017).

Another gene, *G3BP1* (G3BP stress granule assembly factor 1) has been reported in response to the Newcastle disease virus (NDV) in chickens (Sun et al., 2017). Hence, it was used as a marker to verify the formation of stress granules (cells' response to various environmental stressors, e.g. virus) in the course of NDV infection in the chicken cells. This gene also contributes to stress granule formation that is caused by exposure to various stresses such as arsenite, hypoxia, and heat shock, where cells inhibit their translation and apoptosis (Matsuki et al., 2013)

FGF19 (fibroblast growth factor 19) has a role in the activation of the cytokine pathway, which has enormous potential in the control of infectious diseases in poultry (Wigley and Kaiser, 2003); while *GK5* (glycerol kinase 5) was found to play a pivotal role in the variation of blood metabolites in Iranian chicken (Javanrouh-Aliabad et al., 2018). Blood parameters are considered good indicators of health status. They are not only helpful in the diagnosis of specific poultry diseases but also provide basic knowledge for studies in immunology and comparative avian pathology (Rehman et al., 2017). For example, serum total protein is used to determine the quality of dietary protein (Alikwe, 2011), and serum biochemical parameters are used to check the immune status of animals (Kral and Suchý, 2000). Similarly, glucose and triglycerides fulfil the energy demand for the maintenance of the physiological and biochemical functions in the body (Klasing, 1999).

Other genes, like *DOCK2* (Dedicator Of Cytokinesis 2) are involved in cytoskeletal rearrangements required for lymphocyte migration in the response of chemokines (Wang et al., 2017), *DUSP16* (Dual Specificity Phosphatase 16) is involved in T helper cell differentiation in mice through dephosphorylation of JNK in the cytosol, the *FUT8* (Fucosyltransferase 8) gene is associated with not only an immune response but also several other roles such as multicellular organism development, DNA integration, melanin biosynthetic process, muscle organ development and oxidation-reduction in the chicken (Claire D'Andre et al., 2013), *BIN1* (bridging integrator 1) and *MOG* (myelin oligodendrocyte glycoprotein) were previously reported to be involved in apoptosis/programmed death cell (DuHadaway et al., 2001) and are known as a cellular receptor for rubella virus in human (Haralambieva et al., 2014).

Similar conditions regarding disease incidence as observed in the Southern Guinea ecotype were also recorded in the Derived Savanna ecotype, e.g the prevalence or outbreak of Newcastle disease was observed during the cold and harsh harmattan period (from November to March) rather than during the wet season (Anene and Onuoha, 1999, Okwor and Eze, 2011). Meanwhile, the presence of selection candidates such as *ABHD2* in the Southern Guinea Savanna and *G3BP1* gene in the Derived Savanna are likely involved with thermal stress or environmental stress adaptation. Furthermore, several overlapped genes are common among these ecotypes, such as *TSHR* and a *LncRNA* (*ENSGALG00000047413*), *OVSTL* (involved in reproduction), and nearby genes *MEOX* and *AGMO* (both of them are located 20-40 Kb upstream of the sweep region).

c. Selection signatures in ecotypes from the cool zone (Mid-Altitude/Plateau ecotype)

The Mid-Altitude zone or Plateau region, which lies at 1,280 m above sea level, has a mean minimum and maximum temperature of 16°C and 26°C, respectively. It is controlled by two wind systems that affect the Nigerian climate, the moist south-westerly winds during the rainy season and the dry northeasterly winds during the dry season (Chinda and Danladi, 2019). The south-westerly winds are responsible for much of the rains occurring between April and October, while the north-easterly winds are responsible for the dry season lasting from November to March. The mean annual rainfall on the Jos Plateau is around 1245mm with a standard deviation of 139 mm (Binbol et al., 2020).

Most selection signature regions identified from *Hp* analysis in this ecotype show stronger signals compared to other ecotypes. A total of thirty-four regions (after merging the adjacent windows) were identified as candidate regions under selection, and these overlap with fifty genes (Supplementary Table S4.5). The functions of the annotated candidate genes under some of the strongly supported selected regions are related to cold stress response, immune response, oxidative stress, hypoxic protection, production and reproduction traits.

In the Mid-Altitude ecotype, I identified a strong positive selection signal for *IL12B* (Interleukin 12B) which is involved in the cellular response to cold stress (GO:0070417) in addition to its immune response role (bacterial and viral infection). The *IL12B* candidate encodes a subunit of interleukin 12, a cytokine that acts on T-cells and natural killer cells. This indicates that cold stress stimulates both the innate and parts of the adaptive cellular immune systems. Also, the effect of cold stress could enhance the expression in peripheral blood leukocytes (PBL) of mRNA of the

interleukin-1beta (one of them is *IL12B*) cytokine gene family (Hangalapura et al., 2006).

Other genes present within candidate selected regions are *MDH1B* (malate dehydrogenase 1B) and *KCNMA1* (potassium calcium-activated channel subfamily M alpha 1), both likely involved in high altitude adaptation. In particular, the *MDH1B* gene (oxidation-reduction process; GO:0055114) is found within the strongest selection signal (*ZHp* = -5.636). It has previously been reported to be under positive selection in the Tibetan yak, Tibetan ground tit, and in human populations living at high altitudes in Tibet, the Andes and Ethiopia, indicating its probable role in the adaptation to high altitudes (Simonson et al., 2010, Qiu et al., 2012, Brugniaux et al., 2007, Qu et al., 2013). *KCNMA1* has a function in the voltage-gated ion channel activity (GO:0005244). It is likely linked to the hypoxia response challenge, being involved in the regulation of the smooth muscle contraction through the activation of calcium ions and an increase in calcium ions stimulated by the hypoxia-inducible factor-1 gene (a gene that regulates adaptation to the hypoxic condition in the high altitude region) (Williams et al., 2004, Hui et al., 2006, Lawal et al., 2018).

Several candidate genes (e.g. *OVSLT, RAB30, CYSLTR2, MOG, SGMS1, DYTN,* and *FBXO38*) have GO annotation terms associated with an immune response, while the others are associated with production and reproduction traits (see Supplementary Table S4.5 for details). Several studies have reported the prevalence of various chicken diseases in the Mid-Altitude zone, such as Coccidioidomycosis (fatal fungal infection) in chicken pullets (Jambalang et al., 2010), Newcastle disease (ND) (Musa et al., 2009, Abraham-Oyiguh et al., 2014), infectious bursal disease (IBD) and Marek's disease (Adedeji et al., 2019, Sani et al., 2021), and the occurrence and manifestation of *Samonella*, *H5N1*, and fowlpox (Cecchi et al., 2008, Adebajo et al., 2012, Fagbamila

et al., 2017). Further findings by Musa et al. (2009) in their work on village chickens in the Plateau zone, detected antibodies to ND throughout the year but with high prevalence or epizootics only during the harmattan period of November-March due to cold weather inducing physiological stress on village chickens and subsequently decreases in their immunity to ND.

The above findings indicate that hypoxic conditions at high altitudes have direct implications for the immune status of chickens. Beyond this, the role of the *ILI2B* gene, detected as one of the candidate genes of the Plateau ecotype, was discussed in relation to NK T-cell differentiation. In a previous study, McNamee et al. (2013) indicated that the differentiation of T-cells could be stimulated by hypoxia and high altitude conditions. Also, Zhang et al. (2016) showed that there is an association between the high altitude condition and the inflammatory responses. Therefore, the results of our study corroborate the findings of these previous studies. The detection of candidate genes in this study may provide new insights into chicken's adaptation in the Mid-Altitude zone. These adaptive genes are related to important functions including cold response, hypoxia, and immune response. There were also genes commonly found with other ecotypes such as *HHLA1* (also found in the semi-arid ecotypes), and *NCAPG*, *LCORL*, *OVSTL*, *FNDC3A*, *TSANARE1*, and *LDB2* found in the Freshwater swamp ecotype from the warm-humid zone.

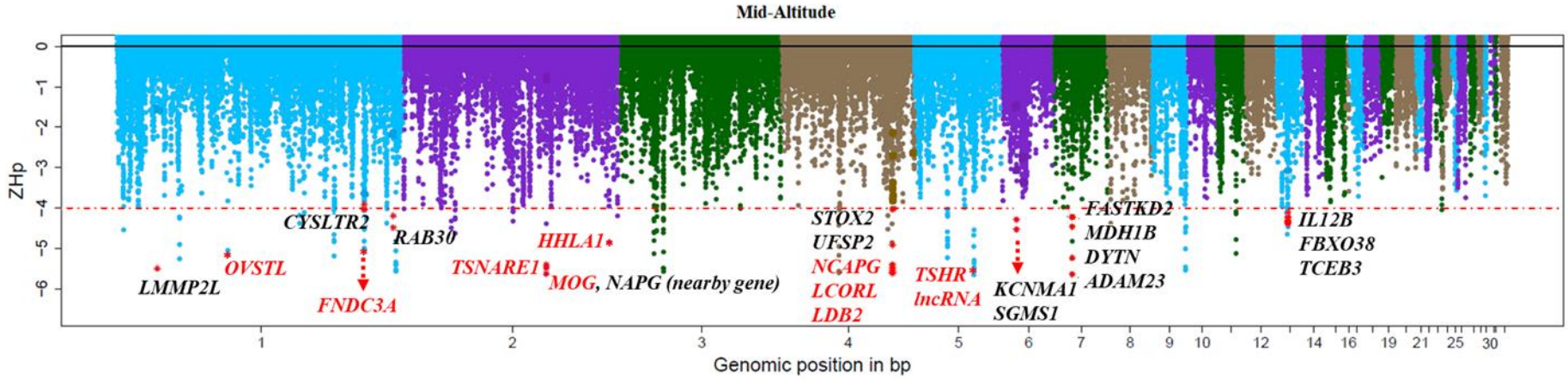


Figure 4.5. Manhattan plot and important candidate genes under candidate selective sweep region in the Mid-Altitude ecotypes.

*Common genes with other ecotypes from different zones are in red.

d. Selection signatures in ecotypes from the warm-humid zone (Rainforest, Freshwater swamp, and Mangrove swamp)

The warm-humid zone lies adjacent to the low-lying coastal area. It includes three ecological zones namely., Rainforest, Freshwater swamps and Mangrove swamps. Essentially, the Rainforest lies on the upper level, the Freshwater zones is in the middle and the Mangroves swamp on the outer ridge.

The Rainforest zone is tropical in nature with two distinct seasons: the rainy (March - October) and the dry (November - February) seasons. The dry season is characterized by the harmattan dust brought by cool-dry winds from the northern deserts into the southern region (Otitoju and Enete, 2014). The temperature ranges between 21°C and 34°C, while the annual rainfall ranges between 1500 mm and 3000 mm (Falade, 2017).

The Nigerian Freshwater swamp forests are found between the Lowland Rainforest and the Mangrove swamp forests. The region is generally a low lying vast sedimentary basin and generally swampy with mainly medium to coarse unconsolidated sands, silt, clay, shale and peat (Igu and Marchant, 2017). The zone is characterized by a short dry season (normally between December and February) and a long rainy season, which mainly lasts from March to October. Average monthly maximum and minimum temperatures vary between 28°C to 33°C and 21°C to 23°C, respectively (Ighedosa, 2019). Annual rainfall is 2500 mm. The flood regime of the region begins toward the end of the rainy season in August, with peaks in October and tapering off in December (Merem et al., 2019).

The Mangrove swamps support a complex and sensitive ecosystem, which is vital to the fishing industry and the local economy of the Niger Delta people. It is thus the most economically rich region among the zones. It accommodates the most important

flora and fauna (Boyle and Akujuru, 2021). However, most of the oil-spill incidents reported in Nigeria occurred in the Mangrove swamp forest of the Niger Delta region (Sakib, 2021). The annual rainfall in this zone is about 2,022 mm, and the mean annual temperature is about 26°C, with the warmest month on average in March (29°C) and the coolest month on average in July (25.5°C) (Akonjom et al., 2021). The rainfall pattern shows two identifiable seasons, the rainy season (April to October) and the relatively short dry (November to March) season (Rim-Rukeh, 2018).

Rainforest ecotype

Hp analysis of the Rainforest ecotype identified twenty-five regions that passed the genome-wide significance threshold. These overlap with thirty-three genes (Supplementary Table S4.6). In this ecotype, I found several genes related to disease challenges/immune response, apoptotic response, and ROS such as *FASTK, GBF1, LRBA, RNF7, and DOCK2* (genes also found in Northern and Derived Savanna ecotypes). The remaining genes are involved in the response to a a xenobiotic stimulus, or they are related to reproduction and growth traits.

Parasites and pathogens pose major immunological challenges in the rainforest zone. Chickens in the rainforest zone in Nigeria are under the challenges of several diseases with direct implications on productivity and reproduction (Opara et al., 2014, Ajayi et al., 2011, Ajayi and Agaviezor, 2016). Particularly predominant are parasitic diseases and among them, haemoparasite infections are the most prevalent. The haemoparasites generally found in poultry in tropical areas include the following genera: *Plasmodium sp*., *Leucytozoon sp*., *Haemoproteus sp*., *Aegytinella sp*., *Trypanosoma sp*. and microfilariasis of nematodes belonging to the suborder filaria (Permin, 1998). Enteric bacteria in the family *Enterobacteriaceae*, including *Escherichia coli, Salmonella spp*. and *Klebsiella spp.* are also major pathogens or secondary invaders, hampering the

realization of the full potential of free-range poultry production in Nigeria. A higher prevalence of avian coccidiosis has been found in the rainforest zone of Nigeria during the period of rainfall (Bachaya et al., 2012), primarily because it positively influences the warm and humid environmental conditions needed for oocysts sporulation. Other studies by Awais et al. (2012) and Grema et al. (2014) have also shown that avian coccidiosis is more prevalent during the rainy season compared to the dry season.

The candidate genes associated with immune response from this ecotype include *FASTK* (Fas activated serine/threonine kinase), which becomes activated during Fas-mediated apoptosis via phosphorylation of intracellular repressor T-cells, suggesting its immunological significance in various immune-regulatory diseases in humans (Srivastava et al., 2017). In chicken, it may have a role in the response to avian influenza virus and infectious bursal disease (Rauf et al., 2012, Xing et al., 2009). Another candidate gene is *GBF1* (Golgi brefeldin A resistant guanine nucleotide exchange factor 1), which is also involved in the replication of the infectious bursal disease virus (Gimenez et al., 2021).

LRBA (LPS responsive beige-like anchor protein) was previously reported as the most promising gene involved in the resistance to *Salmonella pullorum* (a gram-negative bacterium infection that caused acute infectious disease in chickens (Li et al., 2019). *Salmonella pullorum* (SP) infection generally leads to three disease outcomes: the most susceptible birds die when showing typical SP infection symptoms such as white diarrhoea and cecal cores (Li et al., 2018); some chicks survive by clearing the pathogen through a series of immune responses, and other chicks develop a carrier state with SP present in their splenic macrophages for a long period (Chappell et al., 2009). Deleterious mutations in *LRBA* cause defects in B cell activation and autophagy and can increase susceptibility to apoptosis. This gene might be also linked to the NF-

κB (nuclear factor kappa beta) immune pathway (Lopez-Herrera et al., 2012, Wang et al., 2014). Another candidate gene, *RNF7* (ring finger protein 7) encodes a protein that inhibits apoptosis induced by reactive oxygen species in response to viral infection (O'Brien et al., 2019).

Freshwater swamp ecotype

The Freshwater swamp ecotype is situated between the lowland rainforest in the North and the Mangrove swamp ecotype in the South, hence, providing a transition zone between the two ecosystems and a passageway for biodiversity migration (Igu and Marchant, 2017). The areas with freshwater resources are often referred to as freshwater swamps. Within the freshwater swamps, other habitats such as riparian and arable farmland are common (Izzah, 2018). However, several human activities including oil and gas exploration, invasive plant infestation and wetland reclamation have led to an increased case of water pollution or contamination (Adekola and Mitchell, 2011). Thirty-eight candidate genes overlapping twenty-seven candidate selection signature regions (Supplementary Table S4.7) were detected in this ecotype. These include several interesting genes such as *GUK1*, *TSC22D2*, *GJC2*, *CHCHD3*, *RNF13*, *SERP1*, *KCNK5*, *WWTR1*, *CAMK2D*, *ARAP2*, *PACC1*, *CCDC94*, and *CCL1*, all of which are involved in environmental stress responses such as xenobiotic metabolic process (GO:0006805), osmotic stress (GO:0006970), stress-activated protein kinase signalling cascade (GO:0070304), voltage-gated potassium channel activity (GO:0005249), calmodulin-dependent protein kinase activity (GO:0004683), tissue homeostasis (GO:0001894), the toxic substrate (GO:0009636), and immune response (GO:0006955). These GO terms are associated with the stressful environment of southern Nigeria, including the increasingly hot and humid weather following climatic changes (Okpara et al., 2013). Similar climatic variations in Nepal

indicate that chickens living in such tropical areas with high humidity and rainfall pattern experienced more severe stress compared to the chickens in the sub-tropical hills. Hence, the tropical zone (warm-humid) is relatively less favourable for broiler farming, as shown by their high blood corticosteroid levels and H/L blood ratios and worse growth performance (Osti et al., 2017). Moreover, poultry responds physiologically and behaviourally when encountering heat stress, attempting to return the body to homeostasis. The body cells are subjected to osmotic stress, since hot weather may cause water imbalances and osmotic changes in the cells through dehydration (Ratriyanto and Mosenthin, 2018). The candidate genes detected in this ecotype represent functions related to these responses.

Mangrove ecotype

In the Mangrove swamp ecotype, thirty-three candidate sweeps regions, and forty-two overlapping genes have been detected (Supplementary Table S4.8).

ARAP2 (ArfGAP with RhoGAP Domain, Ankyrin Repeat and PH Domain 2) overlaps with the strongest signal observed in the Mangrove ecotype. One of the molecular functions of this gene is phosphatidylinositol-3,4,5-trisphosphate binding, which indicates its involvement in osmotic stress regulation, as reported by Dove et al., (1997). A crucial factor in stress tolerance is the dynamic relationship between cations and anions to maintain body fluid and cell homeostasis (Mongin, 1980). Some genes (*SELENOT* and *WWTR1*) are involved in cell redox (GO:0045454) and tissue homeostasis (GO:0001894).

Additionally, between the three ecotypes in the warm-humid zone (i.e. Rainforest, Freshwater swamp and Mangrove), I have found genes with similar functions related to xenobiotic response (in all three ecotypes) and osmotic stress (in the Freshwater

swamp and Mangrove ecotypes only). The genes related to xenobiotic response are *AHRR* (aryl-hydrocarbon receptor repressor) in the Rainforest ecotype, *GUK1* (guanylate kinase 1) in the Freshwater swamp ecotype, and *AADAC* (arylacetamide deacetylase) in the Mangrove ecotype, while the gene, *TSC22D2* (TSC22 domain family member 2), related to osmotic stress is found in both the Freshwater swamp and Mangrove swamp ecotypes.

Nigeria is a country with diverse vegetative zones, and animals interact directly or indirectly with the environment (Abatan, 2012). The vegetative and non-vegetative part of the ecosystem possesses various sources of xenobiotics (NRC, 2001). Some biological sources include phytotoxins, zootoxins, mycotoxins and marine toxins (Garg, 2008, Biobaku et al., 2016), while non-biologic sources include edaphic exposure to xenobiotics such as in extraction companies, refineries, drug companies, mining firms, and insecticide production. Access to heavy metal toxicity also compromises the immunity of the animals (ATSDR, 2000, Al-Forkan et al., 2016). Animals that are exposed to xenobiotics are therefore more vulnerable to diseases compared to those animals that are not. Xenobiotic exposure during the prenatal period may result in susceptibility to infectious diseases, allergic reactions or autoimmune diseases (Clarke et al., 2019). There could be disruption of T-cell maturation in the thymus resulting in immunosuppression. Exogenous chemicals may also interfere with receptor ligands binding at the cell surface (Clarke et al., 2019). Antigen-antibody (IgG) complexes that accumulate in tissues or are in the circulation, activate macrophages and the complement systems and trigger the influx of granulocytes and lymphocytes (inflammation) (Biobaku and Amid, 2018).

Arad et al. (1985) and Sharma et al. (2009) suggested that the osmotic changes in blood during combined dehydration and water deprivation and heat exposure (mainly

osmolality, sodium concentration and sodium-to-calcium ratio) might alter the hypothalamic thermoregulatory set-point together with activation of the osmoregulatory system. This suggestion is based on experiments in mammals in which ventricular or hypothalamic perfusions with excess sodium or calcium in the perfusion fluid resulted in hyper- or hypothermy, respectively.

Similarly, to the finding for the Freshwater swamp zone, in the Mangrove ecotype I found, within candidate regions under selection gene ontology terms for xenobiotic metabolism process, response to osmotic stress, oxidation-reduction process, tissue homeostasis, calmodulin-dependent protein kinase activity, positive regulation of stress-activated protein kinase signalling. Also, ontology terms related to the immune response have been found in this group.

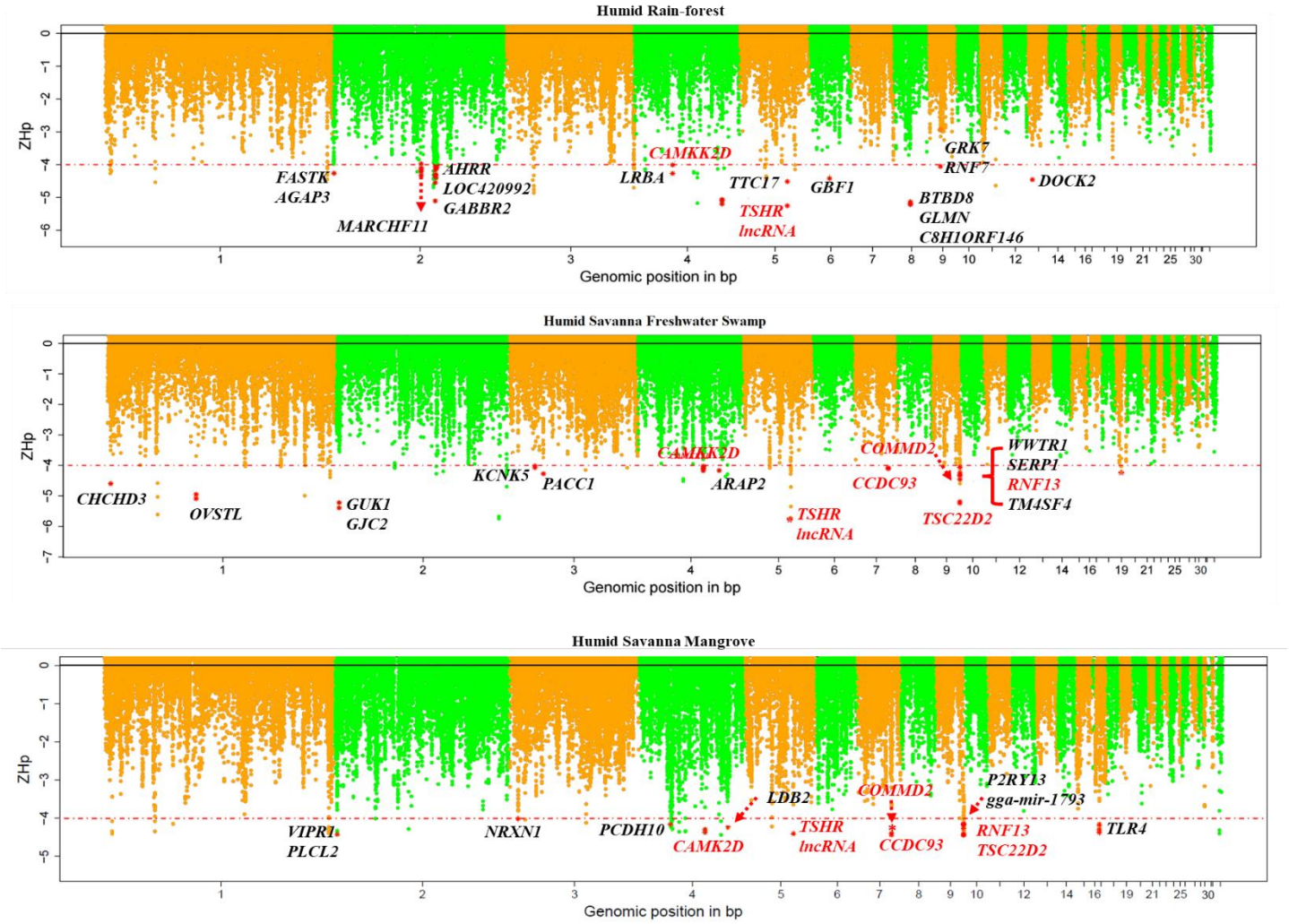


Figure 4.6. Manhattan plot and important candidate genes within candidate selected regions in the Warm-humid region.

*Common genes among ecotypes are labelled in red

4.3.2. Functional classification of genes overlapping sweep regions in different ecotypes

Candidate sweeps are related to diverse biological functions and phenotypes. Genes overlapping the sweep regions from different ecotypes were checked for their functional classification according to Panther Pathways (Figure 4.8). Only 179 genes (~0.14% of all chicken genes) intersected sweep regions from different ecotypes. These genes showed twenty-six of the Panther pathways, indicating their involvement in several physiological processes, however, none of the pathways is represented by genes from all or most ecotypes. Inflammation mediated by chemokine and cytokine signalling pathway (P00031) was found in three ecotypes (Southern Guinea savanna, Freshwater swamp, and Mangrove swamp), while the other pathways were mostly only found in one ecotype. For instance, the Gonadotropin-releasing hormone receptor pathway (P06664) in the Sudan savanna, Nicotine pharmacodynamics pathway (P06587) in the Northern Guinea savanna, Oxidative stress response (P00046) in the Derived savanna, Fructose galactose metabolism (P02744) in Mid-Altitude, GABA-B receptor II signalling (P05731) in Rainforest, Toll receptor signalling pathway (P00054) in Mangrove swamp.

Meanwhile, regulatory relationship among 179 shared genes were investigated using protein-protein interaction network analysis with STRING database ver. 11.5 (Szklarczyk et al., 2016). At high confidence level (0.700), the protein-protein interaction networks include 14 out of 179 genes (Figures 4.7). Several interesting networks of protein-protein interactions were showed between *CCDC93--COMMD2*, *ORAOV1--FGF19* (immune response related genes), *EIF4A3--LOC422214* (related to nucleic acid binding and mRNA binding); *LCORL* and *NCAPG* (growth trait related).

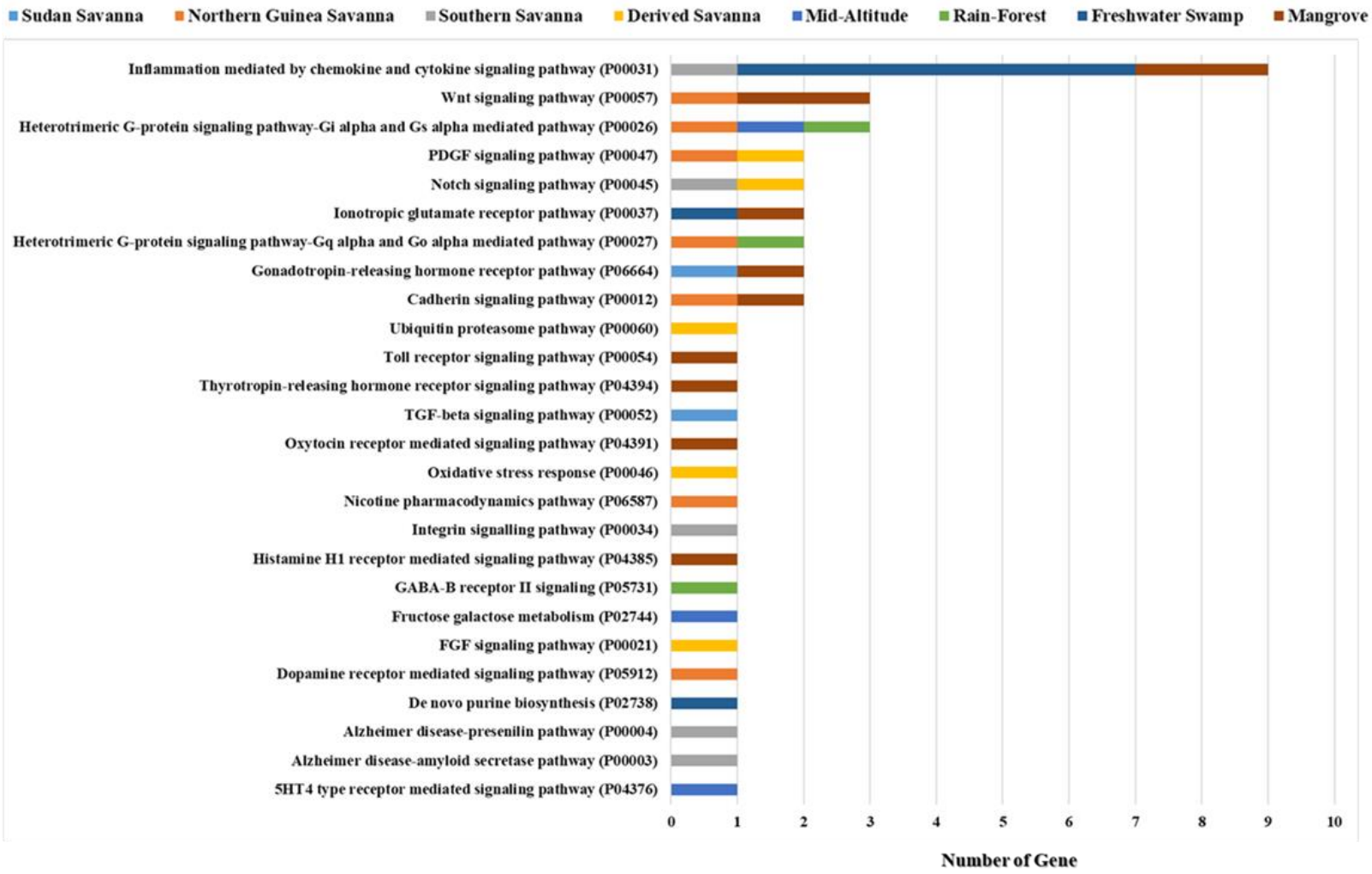


Figure 4.7. Functional classification of genes overlapping sweep regions in different ecotypes according to Panther biological pathways.

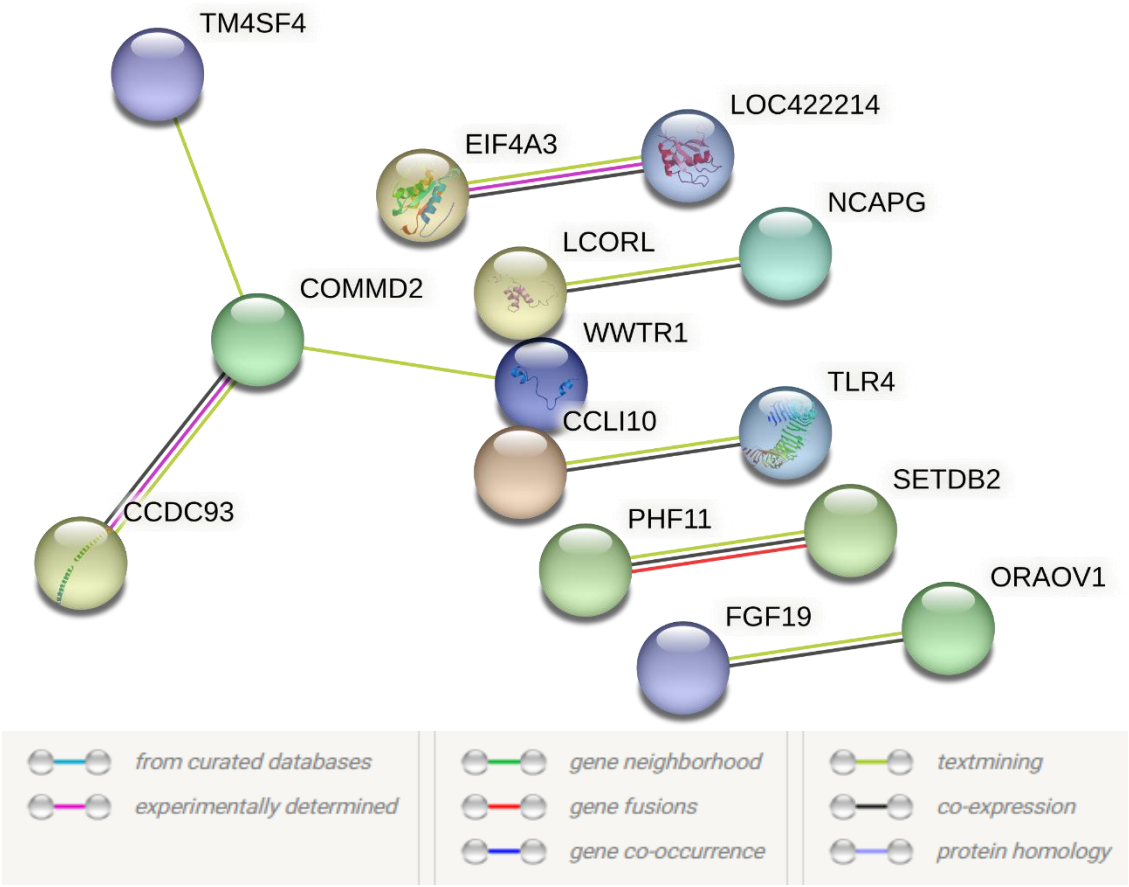


Figure 4.8. STRING protein network view of strong regulatory relationships between protein-protein interaction at a high confidence level (0.700).

4.3.3. Differential selection between ecotypes

To identify regions showing differential selection, I performed pairwise comparisons among ecotypes using *FST* statistics. This method is the most commonly used metric for measuring genetic differentiation between populations (Holsinger and Weir 2009). It compares the variance in allele frequencies among populations with that of the within populations. A larger *FST* value means that the allele frequencies are different; therefore, populations are different. When it is small, populations are considered to have similar allele frequencies. In this study, I used the *FST* statistics to compare the results from each pairwise analysis (Figure 4.8), with the overall *FST* values showing no or low differentiation between ecotypes (*FST* < 0.05), and in a few cases, moderate differentiation (*FST* > 0.05-0.15).

The Z-transformed *FST* to detect the selection signature region was then applied. The weighted *FST* values were standardized (Z*FST*) to allow the same threshold to be set across analyses, and then the top 0.01% transformed *FST* values (≥ 7) were considered as candidate selected regions. The highest *FST* and Z*FST* values were 0.621 and 10, respectively. Traditionally, studies have often taken the top 1% *FST* windows as outliers, henceforth putative selection signature regions. Applying this threshold, there were ~ 920 windows in our dataset (Supplementary Table S4.25-S4.53) with Z*FST* generally > 3.00. Here, I applied a much more stringent criterion with threshold Z*FST* score ≥ 7.0 to get stronger signal and preventing the bias result for detecting candidate selection signature regions (i.e. top 0.01%). The summary of *FST* and *ZFST* values and the comparison between ecotypes are presented in Supplementary Tables S4.15-S4.24 and the Manhattan plots for all comparisons in Supplementary Figures S4.5-S4.12.

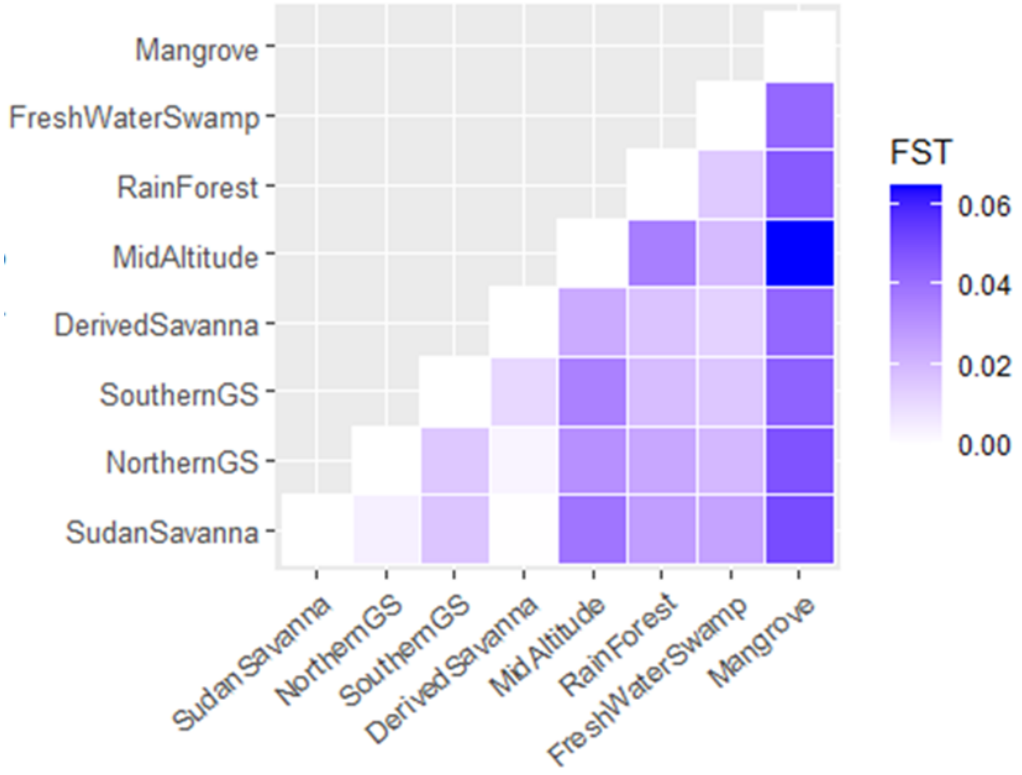


Figure 4.9 Heatmap of average weighted *FST* value for each ecotypes comparison

The genes that overlapped within and around these candidate regions were then used for the downstream analysis. Like the results in the within-ecotype *Hp* analyses, the candidate genes from the *FST* analyses were also found to play roles in the immune response, response to heat stress, cold response, body growth, reproduction and behaviour (Supplementary Tables S4.18 – S4.23).

The highest *FST* value was found in the Mid-Altitude *versus* Mangrove comparison, and the corresponding region overlapped with *KCNMA1* (chr6_14620000_14660000), while the strongest signals for Z*FST* values were found in several comparisons (e.g Southern Guinea savanna *versus* Derived Savanna, Northern Guinea Savanna *versus* Derived Savanna, Derived Savanna *versus* Rainforest). They overlap with several genes such as *GRM5* (chr1_189240000_189260000), *ENSGALG00000054642* (chr3_93900000_93920000), *FGF4*

(chr5_17810000_17850000). I further annotated the thirteen regions with the highest Z*FST* values corresponding to the lowest *ZHp* scores (Supplementary Table S4.25).

Candidate genes under differential selection in warm-arid ecotypes compared to other ecotypes

Most of the genes that overlapped with candidate regions in the Sudan savanna ecotype have GO terms associated with immune response (Supplementary Table S4.18). *GRM5* (glutamate receptor, metabotropic 5) overlaps with the strongest selection signal region (Z*FST* = 10; and *ZHp* = -2.905). *GRM5* has been reported to be strongly associated with egg production and found to be potentially involved in dermatological diseases/conditions in chickens (Li et al., 2020), while another study hypothesized that variants of the *GRM5* gene could be related to plumage colour in chicken (Mastrangelo et al., 2020).

Meanwhile, for the comparison with Northern Guinea Savanna, I detected genes with functions linked to heat stress (*ANO1*, *HAAO,* and *CLCN1*) immune response and behaviour (Supplementary Table S4.19).

CLCN1 (chloride voltage-gated channel 1) and *HAAO* (3-Hydroxyanthranilate 3,4-Dioxygenase) were detected in the comparison of Northern Guinea Savanna *versus* Southern Guinea savanna ecotypes. They belong to the GO terms voltage-gated chloride channel activity (GO:0005247) and oxidation-reduction process (GO:0055114), respectively. The family of chloride intracellular channel proteins is highly conserved in vertebrates, and that exist both in a soluble, globular form in the cytoplasm and as integral membrane proteins. Furthermore, they have functions as intracellular chloride channels, with pH-dependent and redox-regulated channel activity (Korte et al., 2013). Meanwhile, a previous study on chicken liver showed that oxidation-reduction balance was associated with stress response, resulting in a high ROS level, with a number of oxidation-reduction-related proteins/enzymes found among both up-

regulated and down-regulated proteins in chicken (Tang et al., 2015). In comparison to the Derived savanna ecotype, *ANO1* (anoctamin 1) associated with the cellular response to heat stress (GO:0034605), was found here within a candidate selected region. This gene has been shown to activate the Cl^- channel in mice (Palkar et al., 2015). The sweep region (chr5_17810000_18070000) harbouring this gene overlaps with other candidate genes (*ORAOV1*, *FGF19*, *FGF4*, and *FADD*) which are involved in the immune response.

Candidate genes under differential selection in warm semi-arid ecotypes compared to other ecotypes

Three interesting candidate genes (*SLC40A1*, *FGF4* and *G3BP1*) are present within the candidate selected region under differential selection in warm semi-arid ecotypes compared to the other ecotypes. *SLC40A1* (chr7_370000_390000, *ZHp* = -4.776) has a role in bacterial defence resistance to *E. coli* infection (Pacht et al., 2021). *FGF4* (*ZHp* = -4.239) function has been linked to the feathered-leg phenotype in chickens (Yang et al., 2019). The evidence that FGF (fibroblast growth factor) signaling acts to promote feather development comes from studies in which FGF induces feather formation in the chicken scaleless mutant that does not form most feathers, and in wild-type chick skin (Song et al., 1996, Widelitz et al., 1996). *G3BP1* is also likely involved in the response to thermal stress. These two genes were previously identified in the Derived Savanna ecotype (*ZHp*).

Candidate genes under differential selection in cool region (Mid-Altitude) ecotypes compared to other ecotypes

Two genes previously identified (*KCNMA1* and *NCAPG*) in Mid-Altitude ecotypes (*ZHp* = -4.151) also present a highly significant *FST* region in the comparison Mid-Altitude *versus* Mangrove ecotype (Supplementary Table S4.25). *KCNMA1* (Potassium Calcium-Activated

Channel Subfamily M Alpha 1) may be linked with hypoxia response challenge (Lawal et al., 2018). This gene was reported to be associated with the regulation of smooth muscle contraction through the activation of calcium ions, thus stimulating hypoxia-inducible factor-1 (Williams et al., 2004, Hui et al., 2006).

The environmental conditions in the Plateau region with an altitude of around 1,200 meters above sea level likely may have triggered hypoxia adaptation, thus explaining the selection pressure on this gene in the Mid-Altitude ecotype. However, the *KCNMA1* gene was found also in a candidate selected region with a strong *FST* signal in the comparison of the Mangrove swamp ecotype with other ecotypes (e.g., Southern Savanna *versus* Mangrove swamp, Freshwater swamp *versus* Mangrove swamp, Sudan Savanna *versus* Mangrove swamp, etc) with *ZHp* score no lower than the one obtained for the Mid-Altitude ecotype. Meanwhile, the *NCAPG* gene (*ZHp* = -5.418) has been found, in several species, in QTL and genome-wide association studies related to body growth (Lindholm-Perry et al., 2013, Liu et al., 2013).

Candidate genes under differential selection between ecotypes from the warm-humid region

Several interesting genes were found in the *FST* analysis between ecotypes in the warm-humid region (Supplementary Tables S4.23 - S4.24), e.g. *GABBR2* (Gamma-Aminobutyric Acid Type B Receptor Subunit 2), *GJC2* (Gap Junction Protein Gamma 1), *MARCH11, AhRR* (aryl-hydrocarbon receptor repressor), and *TSPAN9*. The GO categories for these genes are toxic substance (GO:0009636), gamma-aminobutyric acid signalling pathway (GO:0004965), zinc ion binding (GO:0008270), response to xenobiotic stimulus (GO:0009410), an integral component of membrane (GO:0016021), respectively (Shen et al., 2022; Morukuma et al. 2007, Protty et al., 2009). Interestingly, *ZHp* results have also indicated the presence of genes with functions linked to the xenobiotic and toxicity responses (ATSDR, 2000, Al-Forkan et al.

2016) that could be a result of being exposed from some biological sources such as phytotoxins, zootoxins, mycotoxins and marine toxins and non-biologic sources include edaphic exposure such as in extraction companies, refineries, drug companies, mining firms, and insecticide production in the region.

4.4 Conclusion

This chapter investigates the genomic signatures of positive selection in different Nigerian chicken ecotypes following within ecotype analyses (*Hp*) and between-ecotype comparisons (*FST*). The results support the hypothesis that environmental stressors have played major roles in shaping the genome of these Nigerian ecotypes from different agro-climatic regions. Similar selection patterns were observed in the Northern (Sudan and Northern Guinea Savanna or Warm-arid zone) and the Southern (Rainforest, Freshwater swamp, and Mangrove or Warm-Humid zone) parts of Nigeria with genes in candidate regions under selection which may be linked to heat, osmotic and xenobiotics stresses. Meanwhile, for the region in the centre (Southern Guinea Savanna and Derived Savanna or Warm semi-arid zone as well as Mid-Altitude zones) of the country selection signature regions includes genes linked to disease challenges. Cold response and high-altitude related stress regions were specifically found in the Mid-Altitude zone. Candidate selected genes linked to the immune response were detected in all ecotypes, indicating their importance in the adaptive response to pathogens and parasite challenges.

In addition, indigenous chickens, raised in scavenging/semi-scavenging conditions often suffer from poor nutrition and malnourished animals are more predisposed to diseases. Poor nutrition stress compromises both innate and adaptive immunity and could generate oxidative radicals that are detrimental to homeostasis in the body, which invariably will compromise immunity in the long run (Biobaku and Amid, 2018). Overall, the results described in this chapter enhance our understanding of the role of natural selection in the shaping of the genome structure of

indigenous chickens living both in the warm-arid and warm-humid tropical conditions of Nigeria.

Finally, we closely inspected the strongest signalling sweep region from each ecotype to see if a functional relevance can be drawn between the overlapping genes and the ecotype's environment (Supplementary Tables 4.26). In most cases, a strong relevance is observed through the involvement of the genes in various stress responses, immune responses, body growth and egg production. The environmental association of the gene functions is more prominent for ecotypes where only a few environmental variables act as major driving forces. Mid-Altitude perhaps offers the most striking example where this ecotype has the most selective sweeps with the lowest temperature environment due to its high-altitude location (1,280 m).

CHAPTER 5:

Conclusion and Future direction

Conclusion and Future direction

This chapter concludes the results of this PhD study, describes its significant achievements, discusses the limitations of the study, and proposes future research directions to expand and improve the current results.

This PhD thesis has explored, for the first time, the genomic characterisation of Nigerian indigenous chicken by performing high coverage whole genome sequencing of 120 chickens collected from diverse Nigerian agro-ecologies. The genomic characterisation was accomplished by studying the genetic diversity within and among populations and identifying putative genomic signatures of adaptive selection in response to tropical environmental challenges, including heat stress-response, adaptation to high and low precipitation regions, immune response, and xenobiotic responses. Genomic sequence data generated under this study have been submitted to the European Nucleotide Archive (ENA) under the Accession: PRJEB39536 (to be publicly released in 2023). This will provide an important resource for future research on chicken in general and on Nigerian indigenous chickens specifically. This study has also generated and characterised over 17 million good quality genome-wide SNPs of which 24% are novel variants. These large volumes of SNPs provide an additional resource for future applications and characterisation of Nigerian chickens, e.g. in SNP-genotyping arrays for genetic studies or genomic selection. Furthermore, the identification of candidate genes/genomic regions under selection will help towards understanding their evolution and functional roles in relation to environmental challenges.

The diversity analysis (performed in Chapter 2) shows that the inbreeding level in the studied Nigerian indigenous village population is very low, supporting high genetic diversity. However, The number of SNPs from different populations varied between 9M and 12M; variation largely reflecting the number of samples analysed. Nucleotide diversity (pi) – calculated in overlapping windows of 20 kb size and 10 kb steps - was similar across all the

populations and the value was lower compared to other African indigenous chicken populations. Also, differentiation between chicken populations – as observed from PCA and admixture analyses - may be indicative of common ancestry and past bottleneck events for these chicken populations following their arrival in Nigeria. In addition, there was no correlation between genetic divergence and geographic distance between populations. However, even though there was no overall genetic differentiation among populations, selection signature analyses described in Chapters 3 and 4 identified localised genomic regions of divergence selection using the *FST* approach between ecotypes from different agro-ecologies.

This thesis also made an attempt to dissect genes related specifically to heat stress by the combined analysis of 87 chicken samples (Chapter 3) from multiple populations living in hot climate regions, which otherwise varied in other agro-ecological and climatic features like rainfall, vegetation, and agro-chemistry. This approach not only allowed the number of samples to be increased to improve the power of the analysis to improve the power of the analysis but also increased resolution by minimizing spurious signals from any demographic effects or selection signals from non-heat stress challenges. A small number (fifteen genes) of highly plausible candidate genes for hot climate adaptation were identified, which have involvement in highly relevant biological processes and pathways related to oxidative stress, cellular responses to heat and hypoxia, transcriptional regulation, immune response, and metabolic activities – all of which are important for thermal adaptation. Two genes, in particular, *HSF1* and *CDC37* were detected as candidates in our study which have important regulatory roles in the expression of heat shock proteins that are also known as stress proteins (Whitley et al., 1999, Dayalan Naidu and Dinkova-Kostova, 2017).

Moreover, this study allowed the dissection of candidate genes involved in response to hot-arid and hot-humid climates (Chapter 3, *FST* analysis). A comparison of chicken populations from

high and low precipitation regions found predominantly immune response genes to overlap candidate sweep regions showing differential selection. This indicates that variation in rainfall patterns affects the pathogen/parasitic predisposition and thereby disease resistance in hot tropical climates. Important genes identified in this regard include *REL, CHID1, PUS10, BCL11A* and *CMTM3* for immune responses, *RTN4* and *XPO1* for *TLR9* signalling pathway (innate immune system), and *SCIN, HS1BP3, VOPP1*, and *AP2A2* for the apoptotic process, all having involvement in the biological processes and molecular functions in the regulation of innate and acquired immune responses. Besides these immune response genes, low-*versus*-high rainfall analysis also identified a few genes under differential selection involved in heat stress adaptation (such as *EGFR, AHSA2,* and *PEX13*).

Finally, I also performed signature selection analyses in relation to specific agro-ecologies in Nigeria (i.e. ecotypes) and attempted to find an important link between the mechanism of genetic adaptation to the unique conditions of the agro-ecological zones (AEZ) of Nigeria (Chapter 4). The results indicate that environmental stresses played major roles in shaping the genome of these Nigerian ecotypes from different types of agro-climatic regions. A similar pattern was observed among the populations from the Northern (Sudan and Northern Guinea savanna or Warm-arid zone) and the Southern (Rainforest, Freshwater swamp, and Mangrove or Warm-Humid zone) Nigeria for the overlapping candidate genes likely involved in the response to environmental stresses (e.g. heat stress, osmotic stress, xenobiotics). Meanwhile, the regions in the centre of the country (Southern Guinea savanna and Derived savanna or Warm semi-arid zone as well as the Mid-altitude zone) were dominated by selection signature to disease challenge. Cold response and high-altitude related stress responses at the genome level were specific to the Mid-Altitude zone.

The candidate genes related to the immune response were detected in all ecotypes indicating their importance as an adaptive response to the manifestation of climatic and other

environmental stresses like high heat and/or high humidity as well as existing pathogens and parasites. In addition, indigenous chickens, raised in scavenging/semi-scavenging conditions, often suffer from poor nutrition and malnourished animals are more predisposed to diseases. Poor nutrition stress compromises both the inherent and adaptive immunity and could generate oxidative radicals that are detrimental to homeostasis in the body, which invariably compromises immunity in the long run. This explains why in most ecotypes – both from warm-arid or warm-humid conditions - the sweep regions predominantly show immune-related genes.

The candidate genes and genomic regions identified in this PhD study, enhance our understanding of the role of natural selection in shaping the genome of indigenous Nigerian chickens for adaptation to both warm-arid and warm-humid tropical conditions.

Understanding genetic diversity is a prerequisite in setting up an effective breeding program and selection of population to use. The study showed the level of genetic diversity between and within the Nigerian indigenous chicken population as well as the genetic adaptation, which can be exploited for their genetic improvement. As the study showed, Nigerian chickens are adapted to locally available feeds, disease challenges, and local scavenging conditions. Thus, the opportunity to develop improved locally adapted chicken is quite promising in Nigeria.

In spite of its important achievements, this PhD study also has a number of limitations due to time and resources.

First, our results (candidate genes and regions) still require further experimental validation, such as confirming those in other similar climate populations, and gene-expression analysis using, e.g. Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) as well RNA-seq (RNA-sequencing) and gene-editing approaches.

Second, this study did not identify any candidate variants. For this, GWAS analysis or association analysis (e.g. genotype-environment association or genotype-phenotype

association) will be needed. This would require a larger number of samples, phenotypic and/or environmental data.

Third, this study only investigated SNP variations. It is important to explore other types of variants like Indels (Insertion and Deletion) and structural variants (SVs). However, our high coverage genome data is a good resource for identifying to some extent such variants, although large SVs will also require long-read sequences.

Although my study has several limitations, our catalogue of genome characteristics of eight chicken ecotypes will provide the basis for establishing various appropriate breeding strategies. Also, our findings on the genomic and adaptive characteristics of the Nigerian indigenous chicken will help set the direction for biodiversity conservation programs, crossbreeding and grading-up programs for improving chicken ecotypes. While I was able to identify the genomic diversity, population structure, several candidate regions, and associated candidate genes, the exact genetic control underlining these adaptations remain unknown. Moreover, the relative importance of different climatic influences and social practices (management) are difficult to disentangle. Molecular studies bring complementary information to social surveys and phenotypic data allowing the set up of an integrated program of characterization and conservation of indigenous populations. Future research such as further studies employing mitochondrial DNA to track the maternal lineage of Nigerian indigenous chicken, introgression degree across the chicken population using the genome sequence data, and research involving phenotypic and poultry management structure conditions characterisation are necessary to further interpret our findings.

References

ALIKWE, P.C.N. 2011. Biochemical Evaluation of Serum Metabolites, Enzymes and Haematological Indices of Broiler Chicks Fed with Varying Levels of Rumen Epithelial Scraps in Place of Fish Meal Protein. *Pak. J. Sci. Ind*. Res, 54(2).

ABASHT, B., Mutryn, M.F., Michalek, R.D. and Lee, W.R., 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. *PloS one*, *11*(4), p.e0153750.

ABATAN, A. A., OSAYOMI, T., AKANDE, S. O., ABIODUN, B. J. & GUTOWSKI, W. J. 2018. Trends in mean and extreme temperatures over Ibadan, Southwest Nigeria. *Theoretical and Applied Climatology*, 131, 1261-1272.

ABATAN, M. O. 2012. Poisons and Poisoning the Nightmarish of Society Without Diagnostic Tools: An Inaugural Lecture Delivered at the University of Ibadan, University of Ibadan.

ABRAHAM-OYIGUH, J., SULAIMAN, L., MESEKO, C., ISMAIL, S., SULEIMAN, I., AHMED, S. & ONATE, E. 2014. Prevalence of Newcastle disease antibodies in local chicken in federal capital territory, Abuja, Nigeria. *International scholarly research notices*, 2014.

ADEBAJO, M. C., ADEMOLA, S. I. & OLUWASEUN, A. 2012. Seroprevalence of fowl pox antibody in indigenous chickens in Jos North and South council areas of Plateau State, Nigeria: Implication for vector vaccine. *International Scholarly Research Notices*, 2012.

ADEBAMBO, A., V.A, M., MWACHARO, J., B.M, O., R.A, A., L.O, I., MAKANJUOLA, B., AFOLAYAN, O., BJØRNSTAD, G., JIANLIN, H. & HANOTTE, O. 2010. Lack of Phylogeographic Structure in Nigerian Village Chickens Revealed by Mitochondrial DNA D-loop Sequence Analysis. *International Journal of Poultry Science*, 9(5), 503-507.

ADEDEJI, A. J., AKANBI, O. B., LUKA, P. D. & ABDU, P. 2019. Natural outbreak of Marek's disease in indigenous chicken and Japanese quail (Coturnix coturnix japonica) in Jos, Plateau State, Nigeria. *Open veterinary journal*, 9, 151-156.

ADEDEJI, T., AMAO, S., POPOOLA, A. & OGUNDIPE, R. 2015. Fertility, hatchability and eggs quality traits of Nigerian locally adapted chickens in the derived savanna environment of Nigeria. *Journal of Biology, Agriculture and Healthcare*, 5, 36-42.

ADEDOKUN, S. & SONAIYA, E. 2002. Crossbreeding Nigeria Indigenous with the Dahlem Red chickens for improved productivity and adaptability. *Archives Animal Breeding*, *45*(3), 297-305.

ADEJUWON, J. O. & ODEKUNLE, T. O. 2006. Variability and the Severity of the "Little Dry Season" in Southwestern Nigeria. *Journal of Climate*, 19, 483-493.

ADEKOLA, O. & MITCHELL, G. 2011. The Niger Delta wetlands: threats to ecosystem services, their importance to dependent communities and possible management measures. *International Journal of Biodiversity Science, Ecosystem Services & Management*, 7, 50-68.

ADEKOYA, K., OBOH, B., ADEFENWA, M. & OGUNKANMI, A. 2013. Morphological Characterisation of Five Nigerian Indigenous Chicken Types. *Journal of Sci Res. Dev*, 14, 55-66.

ADELEKE, M. A., PETERS, S. O., OZOJE, M. O., IKEOBI, C. O. N., ADEBAMBO, A. O., OLOWOFESO, O., BAMGBOSE, A. M. & ADEBAMBO, O. A. 2011. A preliminary screening of genetic lineage of Nigerian local chickens based on

blood protein polymorphisms. *Animal Genetic Resources/Ressources Génétiques Animales/Recursos Genéticos Animales* , 48, 23-28.

ADELEKE, M., PETERS, S., OZOJE, M., IKEOBI, C., BAMGBOSE, A. & ADEBAMBO, O. A. 2011. Growth performance of Nigerian local chickens in crosses involving an exotic broiler breeder. *Tropical Animal Health and Production*, 43, 643-650.

ADENAIKE, S., OLUSOLA, S., FAFIOLU, A. O., ADELEKE, M., TAKEET, M., MATHEW, W., ADEBAMBO, O. & IKEOBI, C. 2019. Genetic Diversity of zyxin and TNFRSF1A genes in Nigerian Local Chickens and Nera Black Chickens. *Agriculturae Conspectus Scientificus*, 84, 305-311.

ADETAYO, A. S., & BABAFUNSO, S.E. 2001. Comparison of the performance of Nigerian indigenous chickens from three agro-ecological zones. *Livestock Research for Rural Development* 13 (2).

AGBAJE, H. & ALABI, O. 2018. Application of GIS for biodiversity conservation of indigenous chickens in Ile-Ife, Nigeria. *IOSR Journal of Agriculture and Veterinary Science*, 11, 18-38.

AJAYI, F. & AGAVIEZOR, B. 2016. International Journal of Livestock Production Fertility and Hatchability Performance of Pure and Crossbred Indigenous Chicken Strains in the High Rainforest Zone of Nigeria. *International Journal of Livestock Production*, 7, 141-144.

AJAYI, F. O. 2010. Nigerian Indigenous Chicken: A Valuable Genetic Resource for Meat and Egg Production. *Asian Journal of Poultry Science*, 4, 164-172.

AJAYI, F., AGAVIEZOR, B. & AJUOGU, P. 2011. Semen Characteristics of Three Strains of Local Cocks in the Humid Tropical Environment of Nigeria. *Journal of Animal and Veterinary Advances*, 3, 125-127.

AJAYI, F., AGAVIEZOR, B. & IRONKWE, M. 2010. Phenotypic Characteristics of Local Chickens in Bayelsa State, South-South, Nigeria. *Animal Production Research Advances*, *6*(1), 73-75.

AJIBIKE, A. B., ADELEYE, O. O., ILORI, B. M., OSINBOWALE, D. A., ADENIYI, O. A., DUROSARO, S. O., SANDA, A. J., ADEBAMBO, O. A. & ADEBAMBO, A. O. 2017. Genetic diversity, phylogeographic structure and effect of selection at the mitochondrial hypervariable region of Nigerian chicken populations. *Journal of Genetics*, 96, 959-968.

AKBARIAN, A., MICHIELS, J., DEGROOTE, J., MAJDEDDIN, M., GOLIAN, A. & DE SMET, S. 2016. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. *Journal of Animal Science and Biotechnology*, 7, 37.

AKEY, J., ZHANG, G., ZHANG, K., JIN, L. & SHRIVER, M. 2002. Interrogating a high-density SNP map for signatures of natural selection. *Genome research*, *12*(12), 1805-1814.

AKINWUMIJU, A. S., ADELODUN, A. A. & ORIMOOGUNJE, O. I. 2020. Agro-Climato-Edaphic Zonation of Nigeria for a Cassava Cultivar using GIS-Based Analysis of Data from 1961 to 2017. *Scientific Reports*, 10, 1259.

AKONJOM, N. A., UMUJI, J. I. & EKAH, U. J. 2021. Performance evaluation of polycrystalline photovoltaic modules in a Guinea Savanna and Mangrove swamp. *World Journal of Engineering and Technology*, 4, 11-21.

ALABI, O., AJAYI, F., BAMIDELE, O., YAKUBU, A., UDUAK, O., SONAIYA, E., OJO, M., HASSAN, W. & ADEBAMBO, O. 2020. Impact assessment of improved chicken genetics on livelihoods and food security of smallholder poultry farmers in Nigeria. *Livestock Research for Rural Development*, *32*, 77.

ALBOOSHOKE, S. & BAKHTIARIZADEH, M. 2019. Divergent gene expression through PI3K/akt signalling pathway cause different models of hypertrophy growth in chicken. *Italian Journal of Animal Science*, 18, 1161-1173.

ALDERS, R. G., DUMAS, S. E., RUKAMBILE, E., MAGOKE, G., MAULAGA, W., JONG, J. & COSTA, R. 2018. Family poultry: Multiple roles, systems, challenges, and options for sustainable contributions to household nutrition security through a planetary health lens. *Maternal & child nutrition, 14, p.e12668*.

ALEXANDER, D. H. & LANGE, K. 2011. Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC bioinformatics, 12(1), 1-6*.

ALEXANDER, D. H., NOVEMBRE, J. & LANGE, K. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome research, 19(9), 1655-1664.*

AL-FORKAN, M., ISLAM, S., AKTER, R., SHAMEEN ALAM, S., KHALEDA, L., RAHMAN, Z. & SALMA CHOWDHURY, D. 2016. A sub-chronic exposure study of arsenic on hematological parameters, liver enzyme activities, histological studies and accumulation pattern of arsenic in organs of Wistar albino rats. *J Cytol Histol S, 5(2).*

AL-JUMAILI, A. S., BOUDALI, S. F., KEBEDE, A., AL-BAYATTI, S. A., ESSA, A. A., AHBARA, A., ALJUMAAH, R. S., ALATIYAT, R. M., MWACHARO, J. M., BJØRNSTAD, G., NAQVI, A. N., GAOUAR, S. B. S. & HANOTTE, O. 2020. The maternal origin of indigenous domestic chicken from the Middle East, the north and the horn of Africa. *BMC genetics, 21(1*), 1-16.

ALTAN, O., PABUÇCUOĞLU A FAU - ALTAN, A., ALTAN A FAU - KONYALIOĞLU, S., KONYALIOĞLU S FAU - BAYRAKTAR, H. & BAYRAKTAR, H. 2010. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British poultry science, 44*(4), 545-550.

AMAO, S. 2017. Egg production and growth performance of naked-neck and Rhode Island Red chickens crosses under southern guinea savanna condition of Nigeria. *International Journal of Agriculture and Earth Science, 3,* 1-10.

AMAO, S. R., OJEDAPO, L. O. & SOSINA, O. A. 2011. Evaluation of growth performance traits in three strains of broiler chickens reared in derived savanna environment of Nigeria. *World Journal Young Research, 1*, 28-31.

AMARAL, A. J., MEGENS, H.-J., CROOIJMANS, R. P. M. A., HEUVEN, H. C. M. & GROENEN, M. A. M. 2008. Linkage Disequilibrium Decay and Haplotype Block Structure in the Pig. *Genetics, 179,* 569-579.

AMOAKO, E., MISANA, S., KRANJAC-BERISAVLJEVIC, G., ZIZINGA, A. & BALLU DUWIEJUAH, A. 2018. Effect of the seasonal burning on tree species in the Guinea savanna woodland, Ghana: Implications for climate change mitigation. *Appl. Ecol. Environ. Res, 16,* 1935-1949.

AMORIM, A. & PEREIRA, L. 2005. Pros and cons in the use of SNPs in forensic kinship investigation: a comparative analysis with STRs. *Forensic Science International, 150*, 17-21.

ANCKAR, J. & SISTONEN, L. 2011. Regulation of HSF1 function in the heat stress response: implications in aging and disease. *Annu Rev Biochem, 80*, 1089-115.

ANDREESCU, C., AVENDANO, S., BROWN, S. R., HASSEN, A., LAMONT, S. J. & DEKKERS, J. C. M. 2007. Linkage disequilibrium in related breeding lines of chickens. *Genetics, 177*, 2161-2169.

ANENE, L. & ONUOHA, E. 1999. Seroprevalence of Newcastle disease in local chickens from Southeast derived savannah zone of Nigeria. *PATHOLOGIE INFECTIEUSE, 52*, 185-188.

ANNA, W., JESUS, A., TOMASZ, J., PETEK, S., JANET, E. F., NEIL, P. O. S., ROHAN, F., DORIAN, J. G. & JACK, C. M. D. 2013. Genome-Wide Association Study for Marek's Disease Mortality in Layer Chickens. *Avian Diseases, 57*, 395-400.

ANOH, K., ABDULAHI, I. & AL-HABIB, I. 2021. Influence of season on herd size and birth rate of small-holder goats in the southern guinea savanna. *Sokoto Journal of Veterinary Sciences, 19*, 182-187.

ARAD, Z., ARNASON, S. S., CHADWICK, A. & SKADHAUGE, E. 1985. Osmotic and hormonal responses to heat and dehydration in the fowl. *Journal of Comparative Physiology B, 155*, 227-234.

AREMU, O., BELLO, E., AGANBI, B. & FESTUS, O. 2017. Trend analysis and change point detection of rainfall across the Agro-ecological zones for sustainability development in Nigeria. *Environ Risk Assess Remediation, 1*, 36-46.

ARORA, P., MILLER, H. & ARONSON, L. 1981. Protease inhibitor regulation of B-cell differentiation. *Cellular immunology, 60*, 155-167.

ATEHNKENG, J., OJIAMBO, P. S., DONNER, M., IKOTUN, T., SIKORA, R. A., COTTY, P. J. & BANDYOPADHYAY, R. 2008. Distribution and toxigenicity of Aspergillus species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of Food Microbiology, 122*, 74-84.

ATSDR, T. 2000. ATSDR (Agency for toxic substances and disease registry). Prepared by clement international corp., under contract, *205*, 88-0608.

AWAIS, M. M., AKHTAR, M., IQBAL, Z., MUHAMMAD, F. & ANWAR, M. I. 2012. Seasonal prevalence of coccidiosis in industrial broiler chickens in Faisalabad, Punjab, Pakistan. *Tropical animal health and production, 44*, 323-328.

AYANLADE, A., JEJE, O. D., NWAEZEIGWE, J. O., ORIMOOGUNJE, O. O. & OLOKEOGUN, O. S. 2021. Rainfall seasonality effects on vegetation greenness in different ecological zones. *Environmental Challenges, 4*, 100144.

AYANLADE, A., RADENY, M. & AKIN-ONIGBINDE, A. I. 2018a. Climate variability/change and attitude to adaptation technologies: a pilot study among selected rural farmers' communities in Nigeria. *GeoJournal, 83*, 319-331.

AYANLADE, A., RADENY, M. & MORTON, J. F. 2017. Comparing smallholder farmers' perception of climate change with meteorological data: A case study from southwestern Nigeria. *Weather and Climate Extremes, 15*, 24-33.

AYO, J. O., OBIDI, J. A. & REKWOT, P. I. 2011. Effects of Heat Stress on the Well-Being, Fertility, and Hatchability of Chickens in the Northern Guinea Savanna Zone of Nigeria: A Review. *ISRN Veterinary Science*, 2011, 838606.

AYO, J. O., OBIDI, J. A. & REKWOT, P. I. 2011. Effects of Heat Stress on the Well-Being, Fertility, and Hatchability of Chickens in the Northern Guinea Savannah Zone of Nigeria: A Review. ISRN Veterinary Science, 2011, 838606.

BABRAHAM BIOINFORMATICS. 2016. Fastqc a quality control tool for high throughput sequence data, Accessed v0.11.5 on 2017-06-08.

BACHAYA, H., RAZA, M., KHAN, M., IQBAL, Z., ABBAS, R., MURTAZA, S. & BADAR, N. 2012. Predominance and detection of different Eimeria species causing coccidiosis in layer chickens. *J Anim Plant Sci, 22*, 597-600.

BALADA, E., VILARDELL-TARRÉS, M. & ORDI-ROS, J. 2010. Implication of human endogenous retroviruses in the development of autoimmune diseases. *International reviews of immunology, 29*, 351-370.

BAMIDELE, O., SONAIYA, E. B., ADEBAMBO, O. A. & DESSIE, T. 2020. On-station performance evaluation of improved tropically adapted chicken breeds for smallholder poultry production systems in Nigeria. *Tropical Animal Health and Production, 52*, 1541-1548.

BANOS, G., LINDSAY, V., DESTA, T. T., BETTRIDGE, J., SANCHEZ-MOLANO, E., VALLEJO-TRUJILLO, A., MATIKA, O., DESSIE, T., WIGLEY, P., CHRISTLEY, R. M., KAISER, P., HANOTTE, O. & PSIFIDI, A. 2020. Integrating Genetic and Genomic Analyses of Combined Health Data Across Ecotypes to Improve Disease Resistance in Indigenous African Chickens. *Frontiers in genetics*, *11*, 543890-543890.

BARREIRO, L. B., LAVAL, G., QUACH, H., PATIN, E. & QUINTANA-MURCI, L. 2008. Natural selection has driven population differentiation in modern humans. *Nature genetics*, *40*, 340.

BEISSINGER, T. M., ROSA, G. J., KAEPPLER, S. M., GIANOLA, D. & DE LEON, N. 2015. Defining window-boundaries for genomic analyses using smoothing spline techniques. *Genetics Selection Evolution*, *47*, 1-9.

BETT, H. K., PETERS, K. J., NWANKWO, U. M. & BOKELMANN, W. 2013. Estimating consumer preferences and willingness to pay for the underutilized indigenous chicken products. *Food Policy*, *41*, 218-225.

BHADAURIA, P., KATARIA, J., MAJUMDAR, S. & BHANJA, S. 2014. Impact of hot climate on poultry production system-a review. *Journal of Poultry Science and Technology*, *12*, 56-63.

BINBOL, N., LABIRU, M., MATON, S., SADIKU, Y. & YAROSON, A. 2020. A temporal analysis of rainfall characteristics on the Jos Plateau, Nigeria. *Journal of Meteorology and Climate Science*, *18*, 7-13.

BIOBAKU, K. & AMID, S. 2018. Predisposing factors associated with diseases in animals in Nigeria and possible botanical immunostimulants and immunomodulators: a review. *Bangladesh Journal of Veterinary Medicine*, *16*, 87-101.

BIOBAKU, K. T., ODETOKUN, I. A., RAJI, L. O., OLURODE, S. O. & AMEEN, S. A. 2016. A case report of abortion induced by Aspergillus mycotoxicosis in Sokoto Red goat. *Alexandria Journal of Veterinary Sciences*, *49*, 91-94.

BISWAS, S. & AKEY, J. 2006. Biswas, S. & Akey, J. Genomic insights into positive selection. Trends Genet. 22, 437-446. *Trends in genetics* : TIG, *22*, 437-46.

BOBBO, A., YAHAYA, M. & BABA, S. 2013. Comparative assessment of fertility and hatchability traits of three phenotypes of local chickens in Adamawa State. IOSR *J. Agric. Vet. Sci*, *4*, 22-28.

BOIVIN, N. & FULLER, D. Q. 2009. Shell Middens, Ships and Seeds: Exploring Coastal Subsistence, Maritime Trade and the Dispersal of Domesticates in and Around the Ancient Arabian Peninsula. *Journal of World Prehistory*, *22*, 113-180.

BONHOMME, M., CHEVALET, C., SERVIN, B., BOITARD, S., ABDALLAH, J., BLOTT, S. & SANCRISTOBAL, M. 2010. Detecting selection in population trees: the Lewontin and Krakauer test extended. *Genetic*s, *186*, 241-262.

BOOKER, T. R., JACKSON, B. C. & KEIGHTLEY, P. D. 2017. Detecting positive selection in the genome. *BMC Biology*, *15*, 98.

BORGES, S. A., FISCHER DA SILVA AV FAU - ARIKI, J., ARIKI J FAU - HOOGE, D. M., HOOGE DM FAU - CUMMINGS, K. R. & CUMMINGS, K. R. 2003. Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat-stress environments. *Poultry Science*, *82(3)*, 428-435.

BORTOLUZZI, C., BOSSE, M., DERKS, M. F. L., CROOIJMANS, R. P. M. A., GROENEN, M. A. M. & MEGENS, H.-J. 2019. The type of bottleneck matters: Insights into the deleterious variation landscape of small managed populations. *Evolutionary applications*, *13*, 330-341.

BOYLE, S. H. & AKUJURU, V. A. 2021. Incorporating Environmental Restoration in Compensation Assessment for Contamination of Land in the Niger Delta of

Nigeria. *American Journal of Humanities and Social Sciences Research (AJHSSR), 5*, 297-306.

BRAVERMAN, J. M., HUDSON, R. R., KAPLAN, N. L., LANGLEY, C. H. & STEPHAN, W. 1995. The hitchhiking effect on the site frequency spectrum of DNA polymorphisms. *Genetics*, *140*, 783-96.

BRUGNIAUX, J. V., HODGES, A. N., HANLY, P. J. & POULIN, M. J. 2007. Cerebrovascular responses to altitude. *Respiratory physiology & neurobiology*, *158*, 212-223.

BURT, D. W. & WHITE, S. J. 2007. Avian genomics in the 21st century. Cytogenetic and *Genome Research*, *117*, 6-13.

BUSH, W. S. & HAINES, J. 2010. Overview of Linkage Analysis in Complex Traits. Current Protocols in Human Genetics, *64*, 1.9.1-1.9.18.

CAI, Y., LIU, Q., XING, G., ZHOU, L., YANG, Y., ZHANG, L., LI, J. & WANG, G. 2005. Polymorphism of the Promoter Region of Hsp70 Gene and Its Relationship with the Expression of HSP70mRNA, HSF1mRNA, Bcl-2mrna and Bax-AMrna in Lymphocytes in Peripheral Blood of Heat Shocked Dairy Cows. *Asian-Australas J Anim Sci*, *18*, 734-740.

CECCHI, G., ILEMOBADE, A., LE BRUN, Y., HOGERWERF, L. & SLINGENBERGH, J. 2008. Agro-ecological features of the introduction and spread of the highly pathogenic avian influenza (HPAI) H5N1 in northern Nigeria. *Geospatial health*, *3*, 7-16.

CHAIX, L. 2001. The Origins and Development of African Livestock. Archaeology, Genetics, Linguistics and Ethnography. RM Blench and KC Macdonald (Eds), University College London Press, Taylor & Francis Group, 2000. 546 pp.

CHAPPELL, L., KAISER, P., BARROW, P., JONES, M. A., JOHNSTON, C. & WIGLEY, P. 2009. The immunobiology of avian systemic salmonellosis. *Veterinary immunology and immunopathology*, *128*, 53-59.

CHARLESWORTH, B. 1998. Principles of Population Genetics. 3rd edition (1993-re-issued 1997). By Daniel L. Hartl and Andrew G. Clark. Sunderland, MA: Sinauer 519 pp. *BioEssays*, *20*, 1055-1055.

CHARLESWORTH, B. 2009. Effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics*, *10*, 195-205.

CHARLESWORTH, D. 2006. Balancing Selection and Its Effects on Sequences in Nearby Genome Regions. *PLoS genetics, 2(4), e64*.

CHEN, P.-W., FONSECA, L. L., HANNUN, Y. A. & VOIT, E. O. 2013. Coordination of Rapid Sphingolipid Responses to Heat Stress in Yeast. *PLOS Computational Biology*, *9*, e1003078.

CHEN, Z. & NARUM, S. R. 2021. Whole-genome resequencing reveals genomic regions associated with thermal adaptation in redband trout. *Molecular Ecology*, *30*, 162-174.

CHENG, S.-Z., GUANG-XIN, E., LIU, C.-L., BASANG, W.-D., ZHU, Y.-B., NA, R.-S., HAN, Y.-G., ZENG, Y., WANG, X., NI, W.-W., YANG, B.-G., DUAN, X.-H., GUO, Z.-H., SONG, M. & HUANG, Y.-F. 2020. SNP of AHSA2 gene in three cattle breeds using snapshot technology. *Journal of Genetics*, *99*, 54.

CHIKUMBA, N. & CHIMONYO, M. 2021. Effect of vegetation density on survival of South African free-ranging indigenous chicken broods. *Tropical Animal Health and Production*, *53*, 1-10.

CHINDA, P. A. & DANLADI, T. E. 2019. Evidence of Climate Change on Derived Precipitation Effectiveness Indices and Anomalous Precipitaion Pattern of Jos South Local Government Area of Plateau State, Nigeria.

CICCIA, A. & ELLEDGE, S. J. 2010. The DNA Damage Response: Making It Safe to Play with Knives. *Molecular Cell*, *40*, 179-204.

CLAIRE D'ANDRE, H., PAUL, W., SHEN, X., JIA, X., ZHANG, R., SUN, L. & ZHANG, X. 2013. Identification and characterization of genes that control fat deposition in chickens. *Journal of Animal Science and Biotechnology*, *4*, 43.

CLARKE, G., SANDHU, K. V., GRIFFIN, B. T., DINAN, T. G., CRYAN, J. F. & HYLAND, N. P. 2019. Gut reactions: breaking down xenobiotic–microbiome interactions. *Pharmacological reviews*, *71*, 198-224.

COIMBRA-COSTA, D., ALVA, N., DURAN, M., CARBONELL, T. & RAMA, R. 2017. Oxidative stress and apoptosis after acute respiratory hypoxia and reoxygenation in rat brain. *Redox biology*, *12*, 216-225.

CONAN, A., GOUTARD, F. L., SORN, S. & VONG, S. 2012. Biosecurity measures for backyard poultry in developing countries: a systematic review. *BMC Veterinary Research*, *8*, 240.

CONSORTIUM, U. 2015. UniProt: a hub for protein information. *Nucleic Acids Res*, 43, D204-D212.

CROW, J. F. & KIMURA, M. 1970. An introduction to population genetics theory. An introduction to population genetics theory.

DANA, N., DESSIE, T., VAN DER WAAIJ, L. & ARENDONK, J. 2010. Morphological features of indigenous chicken populations of Ethiopia. *Animal Genetic Resources © Food and Agriculture Organization of the United Nations*, *46*, 11-23.

DANECEK, P., AUTON, A., ABECASIS, G., ALBERS, C. A., BANKS, E., DEPRISTO, M. A., HANDSAKER, R. E., LUNTER, G., MARTH, G. T., SHERRY, S. T., MCVEAN, G., DURBIN, R. & GENOMES PROJECT ANALYSIS, G. 2011. The variant call format and VCFtools. *Bioinformatics*, *27*, 2156-8.

DARWIN, C. 1868. The variation of animals and plants under domestication, London, John Murray. C.

DAVIS, D. M. 2009. Nesting ecology and reproductive success of Lesser Prairie-Chickens in shinnery oak-dominated rangelands. *The Wilson Journal of Ornithology*, *121*, 322-327.

DAYALAN NAIDU, S. & DINKOVA-KOSTOVA, A. T. 2017. Regulation of the mammalian heat shock factor 1. *The FEBS Journal*, *284*, 1606-1627.

DE SIMONI GOUVEIA, J. J., DA SILVA, M. V. G. B., PAIVA, S. R. & DE OLIVEIRA, S. M. P. 2014. Identification of selection signatures in livestock species. *Genetics and molecular biology*, *37*, 330-342.

DESSIE, T., TADESSE, M., YAMI, A. & PETERS, K. J. 2003. Village chicken production systems in Ethiopia: 1. Flock characteristics and performance. *Livestock Research for Rural Development*.

DESSIE, T., TAYE, T., DANA, N., AYALEW, W. & HANOTTE, O. 2011. Current state of knowledge on phenotypic characteristics of indigenous chickens in the tropics. *World's Poultry Science Journal*, *67*, 507-516.

DESTA, T. T. 2021. The genetic basis and robustness of naked neck mutation in chicken. *Tropical Animal Health and Production*, *53*, 95.

DINIZ-FILHO, J. A., SOARES, T. N., LIMA, J. S., DOBROVOLSKI, R., LANDEIRO, V. L., DE CAMPOS TELLES, M. P., RANGEL, T. F. & BINI, L. M. 2013. Mantel test in population genetics. *Genet Mol Biol*, *36*, 475-85.

DIVYA, D. & BHATTACHARYA, T. K. 2021. Bone morphogenetic proteins (BMPs) and their role in poultry. *World's Poultry Science Journal*, *77*, 539-564.

DOVE, S. K., COOKE, F. T., DOUGLAS, M. R., SAYERS, L. G., PARKER, P. J. & MICHELL, R. H. 1997. Osmotic stress activates phosphatidylinositol-3,5-bisphosphate synthesis. *Nature*, *390*, 187-192.

DUAN, H.-J., LI, X.-Y., LIU, C. & DENG, X.-L. 2020. Chemokine-like factor-like MARVEL transmembrane domain-containing family in autoimmune diseases. *Chinese medical journal*, *133*, 951-958.

DUHADAWAY, J. B., SAKAMURO, D., EWERT, D. L. & PRENDERGAST, G. C. 2001. Bin1 Mediates Apoptosis by c-Myc in Transformed Primary Cells. *Cancer Research*, *61*, 3151-3156.

DUO, A., ZHAO, W., QU, X., JING, R. & XIONG, K. 2016. Spatio-temporal variation of vegetation coverage and its response to climate change in North China plain in the last 33 years. *International Journal of Applied Earth Observation and Geoinformation*, *53*, 103-117.

DUTOIT, L., BURRI, R., NATER, A., MUGAL, C. F. & ELLEGREN, H. 2017. Genomic distribution and estimation of nucleotide diversity in natural populations: perspectives from the collared flycatcher (Ficedula albicollis) genome. *Molecular Ecology Resources*, *17*, 586-597.

ELBELTAGY, A. R., BERTOLINI, F., FLEMING, D. S., VAN GOOR, A., ASHWELL, C. M., SCHMIDT, C. J., KUGONZA, D. R., LAMONT, S. J. & ROTHSCHILD, M. F. 2019. Natural Selection Footprints Among African Chicken Breeds and Village Ecotypes. *Frontiers in genetics*, *10*, 376-376.

EL-KASSAS, S., ABDO, S. E., EL-NAGGAR, K., ABDO, W., KIRRELLA, A. A. K. & NASHAR, T. O. 2018. Ameliorative effect of dietary supplementation of copper oxide nanoparticles on inflammatory and immune reponses in commercial broiler under normal and heat-stress housing conditions. *Journal of thermal biology*, *78*, 235-246.

ELNAGAR, S. A., SCHEIDELER, S. E. & BECK, M. M. 2010. Reproductive hormones, hepatic deiodinase messenger ribonucleic acid, and vasoactive intestinal polypeptide-immunoreactive cells in hypothalamus in the heat stress-induced or chemically induced hypothyroid laying hen12. *Poultry Science*, *89*, 2001-2009.

ELUDOYIN, O. M., ADELEKAN, I. O., WEBSTER, R. & ELUDOYIN, A. O. 2014. Air temperature, relative humidity, climate regionalization and thermal comfort of Nigeria. *International Journal of Climatology*, *34*, 2000-2018.

EMARA, M. G. & KIM, H. 2003. Genetic markers and their application in poultry breeding. *Poultry Science*, *82*, 952-957.

ENGELHARDT, B. E. & STEPHENS, M. 2010. Analysis of Population Structure: A Unifying Framework and Novel Methods Based on Sparse Factor Analysis. *PLOS Genetics*, *6*, e1001117.

ESIOBU, N. & ONUBUOGU, G. 2014. Trends, perceptions and adaptation options of livestock farmers to climate change in imo state, Nigeria: a multinomial logit model approach. *Journal of Economics and Sustainable Development*, *5*, 21-36.

FADAIRO, O., OLAJUYIGBE, S., OSAYOMI, T., ADELAKUN, O., OLANIYAN, O., OLUTEGBE, S. & ADELEKE, O. 2020. Climate Change, Rural Livelihoods and Ecosystem Nexus: Forest Communities in Agro-ecological zones of Nigeria. In: LEAL FILHO, W., OGUGE, N., AYAL, D., ADELAKE, L. & DA SILVA, I. (eds.) *African Handbook of Climate Change Adaptation*. Cham: Springer International Publishing.

FAGBAMILA, I. O., BARCO, L., MANCIN, M., KWAGA, J., NGULUKUN, S. S., ZAVAGNIN, P., LETTINI, A. A., LORENZETTO, M., ABDU, P. A. & KABIR, J. 2017. Salmonella serovars and their distribution in Nigerian commercial chicken layer farms. *PLoS One*, *12*, e0173097.

FALADE, M. 2017. In vitro evaluation of antifungal activities of six plant extracts against Colletotrichum lindemuthianum Sensu-lato. *America Journal of Plant Biology*, *2*, 61-65.

FALUSH, D., STEPHENS, M. & PRITCHARD, J. K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, *164*, 1567-1587.

FAN, B., DU, Z.-Q., GORBACH, D. M. & ROTHSCHILD, M. F. 2010. Development and Application of High-density SNP Arrays in Genomic Studies of Domestic Animals. *Asian-Australas J Anim Sci*, *23*, 833-847.

FAN, H., WU, Y., QI, X., ZHANG, J., LI, J., GAO, X., ZHANG, L., LI, J. & GAO, H. 2014. Genome-wide detection of selective signatures in Simmental cattle. *Journal of applied genetics*, *55*, 343-351.

AQUASTAT, F. (2005). Irrigation in Africa in figures: AQUASTAT Survey. *FAO Water Report*.

FAO. 2014. Decision tools for family poultry development. *FAO Animal Production and Health Guidelines* No. 16, Rome, Italy.

FAO. 2010. Global Livestock Production Systems [Online]. Available: http://www.fao.org/livestock-systems/production-systems/chicken/en/.

FAO. 2017. Livestock and environment spotlight NIGERIA: Cattle and poultry sectors. Rome, Italy

FAO. 2018. Livestock and Livelihoods Spotlight Nigeria Cattle and Poultry Sectors.

FAOSTAT. 2013. FAOSTAT Database.

FAO. 2018. Livestock and Livelihoods Spotlight Nigeria Cattle and Poultry Sectors.

FAO. 2022. Gateway to poultry production and products.

FARIELLO, M. I., BOITARD, S., NAYA, H., SANCRISTOBAL, M. & SERVIN, B. 2013. Detecting signatures of selection through haplotype differentiation among hierarchically structured populations. *Genetics*, *193*, 929-941.

FATICA, A. & BOZZONI, I. 2014. Long non-coding RNAs: new players in cell differentiation and development. *Nature Reviews Genetics*, *15*, 7-21.

FAY, J. C. & WU, C.-I. 2000. Hitchhiking under positive Darwinian selection. *Genetics*, *155*, 1405-1413.

FEDDE, M. R. 1998. Relationship of structure and function of the avian respiratory system to disease susceptibility. *Poultry Science*, *77*, 1130-1138.

FEDERAL DEPARTMENT OF FORESTRY, F. M. O. E. 2019. National Forest Reference Emission Level (FREL) for the Federal Republic of Nigeria. Abuja, Nigeria.

FENG, X., CAO, R., ZHOU, B., LIU, Q., LIU, K., LIU, X., ZHANG, Y., GU, J., MIAO, D. & CHEN, P. 2013. The potential mechanism of Bursal-derived BPP-II on the antibody production and avian pre-B cell. *Vaccine*, *31*, 1535-1539.

FERREIRA JÚNIOR, Á., SANTOS, J. P. D., SOUSA, I. D. O., MARTIN, I., ALVES, E. G. L. & ROSADO, I. R. 2018. Gallus gallus domesticus: immune system and its potential for generationof immunobiologics. *Ciência Rural*, *48*.

FESKE, S. 2007. Calcium signalling in lymphocyte activation and disease. *Nature Reviews Immunology*, *7*, 690-702.

FESTEN, E. A. M., GOYETTE, P., GREEN, T., BOUCHER, G., BEAUCHAMP, C., TRYNKA, G., DUBOIS, P. C., LAGACÉ, C., STOKKERS, P. C. F., HOMMES, D. W., BARISANI, D., PALMIERI, O., ANNESE, V., VAN HEEL, D. A., WEERSMA, R. K., DALY, M. J., WIJMENGA, C. & RIOUX, J. D. 2011. A Meta-Analysis of Genome-Wide Association Scans Identifies IL18RAP, PTPN2,

TAGAP, and PUS10 As Shared Risk Loci for Crohn's Disease and Celiac Disease. *PLoS genetics, 7(1), e1001283*.

FICK, S. E. & HIJMANS, R. J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*, 4302-4315.

FISHWICK, K. J., KIM, E. & BRONNER, M. E. 2012. ILF-3 is a regulator of the neural plate border marker Zic1 in chick embryos. Developmental dynamics: an official publication of the American Association of Anatomists, *241*, 1325-1332.

FLEMING, D. S., KOLTES, J. E., MARKEY, A. D., SCHMIDT, C. J., ASHWELL, C. M., ROTHSCHILD, M. F., PERSIA, M. E., REECY, J. M. & LAMONT, S. J. 2016. Genomic analysis of Ugandan and Rwandan chicken ecotypes using a 600 k genotyping array. *BMC Genomics*, *17*, 407.

FLEMING, D. S., WEIGEND, S., SIMIANER, H., WEIGEND, A., ROTHSCHILD, M., SCHMIDT, C., ASHWELL, C., PERSIA, M., REECY, J. & LAMONT, S. J. 2017. Genomic Comparison of Indigenous African and Northern European Chickens Reveals Putative Mechanisms of Stress Tolerance Related to Environmental Selection Pressure. *G3* (Bethesda), *7*, 1525-1537.

FRASCH, C., HENSON, P., KAILEY, J., RICHTER, D., JANES, M., FADOK, V. & BRATTON, D. 2000. Regulation of Phospholipid Scramblase Activity during Apoptosis and Cell Activation by Protein Kinase C. *The Journal of biological chemistry*, *275*, 23065-73.

FRN. 2019. National Forest Reference Emission Level (FREL) for the Federal Republic of Nigeria. Abuja, Nigeria.

FU, W., LEE, W. R. & ABASHT, B. 2016. Detection of genomic signatures of recent selection in commercial broiler chickens. *BMC Genetics*, *17*, 122.

FUJIMOTO, M. & NAKAI, A. 2010. The heat shock factor family and adaptation to proteotoxic stress. *The FEBS Journal*, *277*, 4112-4125.

FULLER, DORIAN Q., BOIVIN, N., HOOGERVORST, T. & ALLABY, R. 2011. Across the Indian Ocean: the prehistoric movement of plants and animals. *Antiquity*, *85*, 544-558.

FUMIHITO, A., MIYAKE T FAU - TAKADA, M., TAKADA M FAU - SHINGU, R., SHINGU R FAU - ENDO, T., ENDO T FAU - GOJOBORI, T., GOJOBORI T FAU - KONDO, N., KONDO N FAU - OHNO, S. & OHNO, S. 1996. Monophyletic origin and unique dispersal patterns of domestic fowls. *Proc Natl Acad Sci* USA, *93(13)*, 6792-6795.

FUMIHITO, A., MIYAKE, T., SUMI, S., TAKADA, M., OHNO, S. & KONDO, N. 1994. One subspecies of the red junglefowl (Gallus gallus gallus) suffices as the matriarchic ancestor of all domestic breeds. *Proc Natl Acad Sci* U S A, *91*, 12505-9.

GARG. 2008. Veterinary Toxicology, CBS publishers and Distributors New Delhi.

GARUD, N. R., MESSER, P. W., BUZBAS, E. O. & PETROV, D. A. 2015. Recent selective sweeps in North American Drosophila melanogaster show signatures of soft sweeps. *PLoS genetics*, *11*, e1005004.

GHEYAS, A. A., BOSCHIERO, C., EORY, L., RALPH, H., KUO, R., WOOLLIAMS, J. A. & BURT, D. W. 2015. Functional classification of 15 million SNPs detected from diverse chicken populations. *DNA Res*, *22*, 205-17.

GHEYAS, A. A., TRUJILLO, A. V., KEBEDE, A., LOZANO-JARAMILLO, M., DESSIE, T., SMITH, J. & HANOTTE, O. 2020. Integrated environmental and genomic

analysis reveals the drivers of local adaptation in African indigenous chickens. bioRxiv, 2020.08.19.257212.

GHOLIZADEH, M., RAHIMI-MIANJI, G. & ZADEH, H. 2008. Potential Use of Molecular Markers in the Genetic Improvement of Livestock. Asian Journal of Animal and Veterinary Advances - *ASIAN J ANIM VET ADV*, *3*, 120-128.

GIANOLA, D., SIMIANER, H. & QANBARI, S. 2010. A two-step method for detecting selection signatures using genetic markers. *Genet Res (Camb)*, *92*, 141-55.

GIFFORD-GONZALEZ, D. & HANOTTE, O. 2011. Domesticating Animals in Africa: Implications of Genetic and Archaeological Findings. *Journal of World Prehistory*, *24*, 1-23.

GIMENEZ, M. C., FRONTINI-LOPEZ, Y. R., POCOGNONI, C. A., ROLDÁN, J. S., SAMARTINO, C. G., UHART, M., COLOMBO, M. I., TEREBIZNIK, M. R. & DELGUI, L. R. 2021. Rab1b-GBF1-ARF1 secretory pathway axis is required for Birnavirus replication. *Journal of Virology*, JVI. 02005-21.

GOEL, A., NCHO, C. M. & CHOI, Y.-H. 2021. Regulation of gene expression in chickens by heat stress. *Journal of Animal Science and Biotechnology*, *12*, 11.

GORAYA, M. U., ALI, L. AND YOUNIS, I 2017. Innate Immune Responses Against Avian Respiratory Viruses. *Hosts and Viruses*, *4(5)*, 78.

GORDON, L., YANG, S., TRAN-GYAMFI, M., BAGGOTT, D., CHRISTENSEN, M., HAMILTON, A., CROOIJMANS, R., GROENEN, M., LUCAS, S., OVCHARENKO, I. & STUBBS, L. 2007. Comparative analysis of chicken chromosome 28 provides new clues to the evolutionary fragility of gene-rich vertebrate regions. *Genome research*, *17*, 1603-1613.

GREMA, H., SULEIMAN, A., RABANA, J. & GEIDAM, Y. 2014. A six year (2005-2010) retrospective study of avian coccidiosis diagnosed in Gombe veterinary clinic, Nigeria. Sokoto Journal of Veterinary Sciences, *12*, 8-13.

GROENEN, M. A., MEGENS, H.-J., ZARE, Y., WARREN, W. C., HILLIER, L. W., CROOIJMANS, R. P., VEREIJKEN, A., OKIMOTO, R., MUIR, W. M. & CHENG, H. H. 2011. The development and characterization of a 60K SNP chip for chicken. *BMC genomics*, *12*, 1-9.

GU, X. H., HAO, Y. & WANG, X. L. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress1. *Poultry Science*, *91*, 790-799.

GUO, X., XIN, C.-H., WEI, W., ZHANG, X.-F., WEI, Z.-Y., REN, L.-L., JANG, J.-J., LI, M., WANG, J.-X., HE, X.-X., WANG, M.-S. & JIANG, R.-S. 2022. Genome-wide scan for selection signatures and genes related to heat tolerance in domestic chickens in the tropical and temperate regions in Asia. Poultry Science, 101821.

HABIBU, B., UMAR, K. M., YAQUB, L. S., SALIFU, S. & MAKUN, H. J. 2021. Thermoregulation in humid climate-adapted and Savannah breeds of goats exposed to West African cold (harmattan) season. *Agricultura Tropica et Subtropica*, *54*, 192-200.

HADEBE, K., DZOMBA, E. F. & MUCHADEYI, F. C. 2019. The population genomic signature of environmental selection in chickens from Malawi, South Africa and Zimbabwe. Armidale: Association for the Advancement of Animal Breeding and Genetics.

HALIMA, H., NESER, F. W. C., VAN MARLE-KOSTER, E. & DE KOCK, A. 2007. Phenotypic variation of native chicken populations in northwest Ethiopia. *Tropical Animal Health and Production*, *39*, 507-513.

HALL, S. J. G. & BRADLEY, D. G. 1995. Conserving livestock breed biodiversity. *Trends in Ecology & Evolution, 10*, 267-270.

HAN, A. Y., ZHANG, M. H., ZUO, X. L., ZHENG, S. S., ZHAO, C. F., FENG, J. H. & CHENG, C. 2010. Effect of acute heat stress on calcium concentration, proliferation, cell cycle, and interleukin-2 production in splenic lymphocytes from broiler chickens. *Poultry Science, 89*, 2063-2070.

HANSSON, B. & WESTERBERG, L. 2002. On the correlation between heterozygosity and fitness in natural populations. *Molecular Ecology, 11*, 2467-2474.

HARALAMBIEVA, I. H., LAMBERT, N. D., OYANNIKOVA, I. G., KENNEDY, R. B., LARRABEE, B. R., PANKRATZ, V. S. & POLAND, G. A. 2014. Associations between Single Nucleotide Polymorphisms in Cellular Viral Receptors and Attachment Factor-Related Genes and Humoral Immunity to Rubella Vaccination. *PloS one, 9(6), e99997*.

HILLIER, L. W., MILLER, W., BIRNEY, E., WARREN, W., HARDISON, R. C., PONTING, C. P., BORK, P., BURT, D. W., GROENEN, M. A. M., DELANY, M. E., DODGSON, J. B., CHINWALLA, A. T., CLIFTEN, P. F., CLIFTON, S. W., DELEHAUNTY, K. D., FRONICK, C., FULTON, R. S., GRAVES, T. A., KREMITZKI, C., LAYMAN, D., MAGRINI, V., MCPHERSON, J. D., MINER, T. L., MINX, P., NASH, W. E., NHAN, M. N., NELSON, J. O., ODDY, L. G., POHL, C. S., RANDALL-MAHER, J., SMITH, S. M., WALLIS, J. W., YANG, S.-P., ROMANOV, M. N., RONDELLI, C. M., PATON, B., SMITH, J., MORRICE, D., DANIELS, L., TEMPEST, H. G., ROBERTSON, L., MASABANDA, J. S., GRIFFIN, D. K., VIGNAL, A., FILLON, V., JACOBBSON, L., KERJE, S., ANDERSSON, L., CROOIJMANS, R. P. M., AERTS, J., VAN DER POEL, J. J., ELLEGREN, H., CALDWELL, R. B., HUBBARD, S. J., GRAFHAM, D. V., KIERZEK, A. M., MCLAREN, S. R., OVERTON, I. M., ARAKAWA, H., BEATTIE, K. J., BEZZUBOV, Y., BOARDMAN, P. E., BONFIELD, J. K., CRONING, M. D. R., DAVIES, R. M., FRANCIS, M. D., HUMPHRAY, S. J., SCOTT, C. E., TAYLOR, R. G., TICKLE, C., BROWN, W. R. A., ROGERS, J., BUERSTEDDE, J.-M., WILSON, S. A., STUBBS, L., OVCHARENKO, I., GORDON, L., LUCAS, S., MILLER, M. M., INOKO, H., SHIINA, T., KAUFMAN, J., SALOMONSEN, J., SKJOEDT, K., WONG, G. K.-S., WANG, J., LIU, B., WANG, J., YU, J., YANG, H., NEFEDOV, M., KORIABINE, M., DEJONG, P. J., GOODSTADT, L., WEBBER, C., DICKENS, N. J., LETUNIC, I., SUYAMA, M., TORRENTS, D., VON MERING, C., et al. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature, 432*, 695-716.

HINCKE, M. T., DA SILVA, M., GUYOT, N., GAUTRON, J., MCKEE, M. D., GUABIRABA-BRITO, R. & RÉHAULT-GODBERT, S. 2019. Dynamics of Structural Barriers and Innate Immune Components during Incubation of the Avian Egg: Critical Interplay between Autonomous Embryonic Development and Maternal Anticipation. *Journal of Innate Immunity, 11*, 111-124.

HÖCKEL, M. & VAUPEL, P. 2001. Tumor Hypoxia: Definitions and Current Clinical, Biologic, and Molecular Aspects. *JNCI: Journal of the National Cancer Institute, 93*, 266-276.

HOFFMANN, I. 2005. Research and investment in poultry genetic resources – challenges and options for sustainable use. *World's Poultry Science Journal, 61*, 57-70.

HOWARD, J. T., PRYCE, J. E., BAES, C. & MALTECCA, C. 2017. Invited review: Inbreeding in the genomics era: Inbreeding, inbreeding depression, and management of genomic variability. *Journal of Dairy Science, 100*, 6009-6024.

HUANG, Y.-W., JANSEN, R. A., FABBRI, E., POTTER, D., LIYANARACHCHI, S., CHAN, M. W. Y., LIU, J. C., CRIJNS, A. P. G., BROWN, R., NEPHEW, K. P., VAN DER ZEE, A. G. J., COHN, D. E., YAN, P. S., HUANG, T. H. M. & LIN, H.-J. L. 2009. Identification of candidate epigenetic biomarkers for ovarian cancer detection. *Oncology reports*, *22*, 853-861.

HUFF, C. D., HARPENDING, H. C. & ROGERS, A. R. 2010. Detecting positive selection from genome scans of linkage disequilibrium. *BMC genomics*, *11*, 8-8.

HUGHES, A. L. 2007. Evolution of the lung surfactant proteins in birds and mammals. *Immunogenetics*, *59*, 565-572.

HUI, A. S., BAUER, A. L., STRIET, J. B., SCHNELL, P. O. & CZYZYK-KRZESKA, M. F. 2006. Calcium signaling stimulates translation of HIF-α during hypoxia. *The FASEB Journal*, *20*, 466-475.

HUI, R. K. & LEUNG, F. C. 2015. Differential Expression Profile of Chicken Embryo Fibroblast DF-1 Cells Infected with Cell-Adapted Infectious Bursal Disease Virus. *PloS one, 10(6), e0111771*

HUMBURG, P., MAUGERI, N., LEE, W., MOHR, B. & KNIGHT, J. C. 2016. Characterisation of the global transcriptional response to heat shock and the impact of individual genetic variation. *Genome Medicine*, 8, 87.

HUROV, K. E., COTTA-RAMUSINO C FAU - ELLEDGE, S. J. & ELLEDGE, S. J. 2010. A genetic screen identifies the Triple T complex required for DNA damage signaling and ATM and ATR stability. *Genes & development*, *24*(17), 1939-1950.

IGHEDOSA, S. U. 2019. Climate Change: Vulnerability of the Niger Delta Region, in Nigeria. *generations*, *1*(2), 4-5.

IGU, N. I. & MARCHANT, R. 2017. Freshwater swamp forest use in the Niger Delta: perception and insights. *Journal of Forest Research*, *22*, 44-52.

IKPEME, E., EKERETTE, E., JOB, I. E., UMOYEN, A., OSIM, P. B. & OZOJE, M. O. 2020. Genetic Relationship among Three Nigerian Chicken (Gallus gallus) Genotypes Based on Cytochrome b of Mitochondrial DNA. *Asian Journal of Animal Sciences*, *15*, 35-42.

IMAM, M. U., ZHANG, S., MA, J., WANG, H. & WANG, F. 2017. Antioxidants Mediate Both Iron Homeostasis and Oxidative Stress. *Nutrients*, *9*, 671.

INTERNATIONAL CHICKEN GENOME SEQUENCING, C. 2004. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature*, *432*, 695-716.

JAKOBSSON, M., EDGE, M. D. & ROSENBERG, N. A. 2013. The relationship between F ST and the frequency of the most frequent allele. *Genetics*, *193*, 515-528.

JALVING, R., VAŃT SLOT, R. & VAN OOST, B. A. 2004. Chicken single nucleotide polymorphism identification and selection for genetic mapping1. *Poultry Science*, *83*, 1925-1931.

JAMBALANG, A., OGO, I., IBU, J., GISILANBE, M., BERTU, W., JWANDER, L., BENJAMIN, B., CHUKWUKERE, S., NASIR, Y. & SANDA, N. 2010. Coccidioidomycosis in chicken pullets in Jos, Plateau State, Nigeria: A case report. *Nigerian Veterinary Journal*, *31*(3).

JANKOWSKI, J., OGNIK, K., STĘPNIOWSKA, A., ZDUŃCZYK, Z. & KOZŁOWSKI, K. 2018. The effect of manganese nanoparticles on apoptosis and on redox and immune status in the tissues of young turkeys. *PloS one*, 13, e0201487-e0201487.

JAVANROUH-ALIABAD, A., VAEZ TORSHIZI, R., MASOUDI, A. A. & EHSANI, A. 2018. Identification of candidate genes for blood metabolites in Iranian chickens using a genome-wide association study. *British Poultry Science*, *59*, 381-388.

JENUTH, J. P. 2000. The NCBI Bioinformatics methods and protocols. *Springer*.

JERN, P. & COFFIN, J. M. 2008. Effects of retroviruses on host genome function. *Annual review of genetics*, *42*, 709-732.

JI, B., MIDDLETON JL FAU - ERNEST, B., ERNEST B FAU - SAXTON, A. M., SAXTON AM FAU - LAMONT, S. J., LAMONT SJ FAU - CAMPAGNA, S. R., CAMPAGNA SR FAU - VOY, B. H. & VOY, B. H. Molecular and metabolic profiles suggest that increased lipid catabolism in adipose tissue contributes to leanness in domestic chickens. *Physiological Genomics*, *46*(9), 315-327.

JIANG, D., MA, S., JING, F., XU, C., YAN, F., WANG, A. & ZHAO, J. 2015. Thyroid-stimulating hormone inhibits adipose triglyceride lipase in 3T3-L1 adipocytes through the PKA pathway. *PLoS One*, *10*, e0116439.

JINGADE, A. H., VIJAYAN, K., SOMASUNDARAM, P., SRIVASABABU, G. K. & KAMBLE, C. K. 2011. A review of the implications of heterozygosity and inbreeding on germplasm biodiversity and its conservation in the silkworm, Bombyx mori. *Journal of insect science (Online)*, *11*, 8-8.

JOHNSSON, M., RUBIN, C. J., HÖGLUND, A., SAHLQVIST, A. S., JONSSON, K. B., KERJE, S., EKWALL, O., KÄMPE, O., ANDERSSON, L., JENSEN, P. & WRIGHT, D. 2014. The role of pleiotropy and linkage in genes affecting a sexual ornament and bone allocation in the chicken. *Molecular Ecology*, *23*, 2275-2286.

JOUTSEN, J., DA SILVA, A. J., LUOTO, J. C., BUDZYNSKI, M. A., NYLUND, A. S., DE THONEL, A., CONCORDET, J.-P., MEZGER, V., SABÉRAN-DJONEIDI, D., HENRIKSSON, E. & SISTONEN, L. 2020. Heat Shock Factor 2 Protects against Proteotoxicity by Maintaining Cell-Cell Adhesion. *Cell Reports*, *30*, 583-597.e6.

KAISER, P. 2012. The long view: a bright past, a brighter future? Forty years of chicken immunology pre- and post-genome. *Avian Pathology*, *41*, 511-518.

KARLSSON, A.-C., SVEMER, F., ERIKSSON, J., DARRAS, V. M., ANDERSSON, L. & JENSEN, P. 2015. The Effect of a Mutation in the Thyroid Stimulating Hormone Receptor (TSHR) on Development, Behaviour and TH Levels in Domesticated Chickens. *PloS one*, *10*, e0129040-e0129040.

KEBEDE, A. 2018. Whole Genome Based Characterisation of Indigenous Chicken Populations in Ethiopia (Doctoral dissertation, Addis Ababa University).

KHAN, S. H. & IQBAL, J. 2016. Recent advances in the role of organic acids in poultry nutrition. *Journal of Applied Animal Research*, *44*, 359-369.

KHANYILE, K. S., DZOMBA, E. F. & MUCHADEYI, F. C. 2015. Population genetic structure, linkage disequilibrium and effective population size of conserved and extensively raised village chicken populations of Southern Africa. *Front Genet*, *6*, 13.

KILAPARTY, S. P., AGARWAL, R., SINGH, P., KANNAN, K. & ALI, N. 2016. Endoplasmic reticulum stress-induced apoptosis accompanies enhanced expression of multiple inositol polyphosphate phosphatase 1 (Minpp1): a possible role for Minpp1 in cellular stress response. *Cell stress & chaperones*, *21*, 593-608.

KIM, J.-M., LIM, K.-S., BYUN, M., LEE, K.-T., YANG, Y.-R., PARK, M., LIM, D., CHAI, H.-H., BANG, H.-T., HWANGBO, J., CHOI, Y.-H., CHO, Y.-M. & PARK, J.-E. 2017. Identification of the acclimation genes in transcriptomic responses to heat stress of White Pekin duck. *Cell stress & chaperones*, 22, 787-797.

KIM, Y. & NIELSEN, R. 2004. Linkage Disequilibrium as a Signature of Selective Sweeps. *Genetics*, *167*, 1513.

KITALYI, A. J. 1998. Village Chicken Production Systems in Rural Africa: Household Food Security and Gender Issues (No. 142). Food & Agriculture Org.

KLASING, K. C. 1999. Comparative avian nutrition. *CABI Publishing*, Walingford, United Kingdom.

KONG, B.-W., HUDSON, N., SEO, D., LEE, S., KHATRI, B., LASSITER, K., COOK, D., PIEKARSKI, A., DRIDI, S., ANTHONY, N. & BOTTJE, W. 2017. RNA sequencing for global gene expression associated with muscle growth in a single male modern broiler line compared to a foundational Barred Plymouth Rock chicken line. *BMC genomics*, *18*, 82-82.

KOPITAR-JERALA, N. 2012. The role of cysteine proteinases and their inhibitors in the host-pathogen cross talk. *Current protein & peptide science*, *13*, 767-775.

KOROPOULIS, A., ALACHIOTIS, N. & PAVLIDIS, P. 2020. Detecting Positive Selection in Populations Using Genetic Data. In: DUTHEIL, J. Y. (ed.) *Statistical Population Genomics*. New York, NY: Springer US.

KORTE, J., FRÖHLICH, T., KOHN, M., KASPERS, B., ARNOLD, G. J. & HÄRTLE, S. 2013. 2D DIGE analysis of the bursa of Fabricius reveals characteristic proteome profiles for different stages of chicken B-cell development. *Proteomics, 13(1), 119-133.*

KRAL, I. & SUCHÝ, P. 2000. Haematological studies in adolescent breeding cocks. *Acta Veterinaria Brno*, *69*, 189-194.

KRANIS, A., GHEYAS, A. A., BOSCHIERO, C., TURNER, F., YU, L., SMITH, S., TALBOT, R., PIRANI, A., BREW, F., KAISER, P., HOCKING, P. M., FIFE, M., SALMON, N., FULTON, J., STROM, T. M., HABERER, G., WEIGEND, S., PREISINGER, R., GHOLAMI, M., QANBARI, S., SIMIANER, H., WATSON, K. A., WOOLLIAMS, J. A. & BURT, D. W. 2013. Development of a high density 600K SNP genotyping array for chicken. *BMC Genomics*, *14*, 59.

KRANIS, A., GHEYAS, A.A., BOSCHIERO, C. et al. Development of a high density 600K SNP genotyping array for chicken. 2013. *BMC genomics*, *14*(1), 1-13.

KRAUS, R. 2019. Avian Genomics in Ecology and Evolution From the Lab into the Wild: From the Lab into the Wild. In *Avian Genomics in Ecology and Evolution* (pp. 1-6). Springer, Cham.

KRAY, A. E., CARTER, R. S., PENNINGTON, K. N., GOMEZ, R. J., SANDERS, L. E., LLANES, J. M., KHAN, W. N., BALLARD, D. W. & WADZINSKI, B. E. 2005. Positive regulation of IkappaB kinase signaling by protein serine/threonine phosphatase 2A. *J Biol Chem*, *280*, 35974-82.

KRISTENSEN, T. N. & SØRENSEN, A. C. 2005. Inbreeding–lessons from animal breeding, evolutionary biology and conservation genetics. *Animal Science*, *80*, 121-133.

KUMAR, P., HENIKOFF, S. & NG, P. C. 2009. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc*, *4*, 1073-81.

LADJALI-MOHAMMEDI, K., BITGOOD, J. J., TIXIER-BOICHARD, M. & PONCE DE LEON, F. A. 1999. International System for Standardized Avian Karyotypes (ISSAK): sstandardized banded karyotypes of the domestic fowl (Gallus domesticus). *Cytogenetic and Genome Research*, *86*, 271-276.

LARA, L. J. & ROSTAGNO, M. H. 2013. Impact of Heat Stress on Poultry Production. *Animals*, *3*, 356-369.

LAURIDSEN, C. 2019. From oxidative stress to inflammation: redox balance and immune system. *Poultry Science*, *98*, 4240-4246.

LAWAL, R. A., AL-ATIYAT, R. M., ALJUMAAH, R. S., SILVA, P., MWACHARO, J. M. & HANOTTE, O. 2018. Whole-Genome Resequencing of Red Junglefowl and Indigenous Village Chicken Reveal New Insights on the Genome Dynamics of the Species. *Frontiers in genetics, 9*, 264-264.

LAWAL, R. A., MARTIN, S. H., VANMECHELEN, K., VEREIJKEN, A., SILVA, P., AL-ATIYAT, R. M., ALJUMAAH, R. S., MWACHARO, J. M., WU, D.-D., ZHANG, Y.-P., HOCKING, P. M., SMITH, J., WRAGG, D. & HANOTTE, O. 2020. The wild species genome ancestry of domestic chickens. *BMC Biology, 18*, 13.

LEE, C. G., DA SILVA, C. A., DELA CRUZ, C. S., AHANGARI, F., MA, B., KANG, M.-J., HE, C.-H., TAKYAR, S. & ELIAS, J. A. 2011. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. *Annual review of physiology, 73*, 479-501.

LENCZ, T., LAMBERT, C., DEROSSE, P., BURDICK, K. E., MORGAN, T. V., KANE, J. M., KUCHERLAPATI, R. & MALHOTRA, A. K. 2007. Runs of homozygosity reveal highly penetrant recessive loci in schizophrenia. *Proceedings of the National Academy of Sciences, 104*, 19942-19947.

LEUTENEGGER, A. L., PRUM, B., GENIN, E., VERNY, C., LEMAINQUE, A., CLERGET-DARPOUX, F. & THOMPSON, E. A. 2003. Estimation of the inbreeding coefficient through use of genomic data. *Am J Hum Genet, 73*, 516-23.

LEWICKI, T. 1974. Meat and fish. West African Food in the Middle Ages: According to Arabic Sources. Cambridge: Cambridge University Press.

LI, D., TIAN, S., ZHU, W., SU, Y., WANG, T., MA, T., MISHRA, S. K., SHI, L., WEI, R. & XU, Z. 2020. Influence of the digestive/reproductive tract microbiota on chicken egg production beyond host genetics. Research Square Publising.

LI, H., HANDSAKER, B., WYSOKER, A., FENNELL, T., RUAN, J., HOMER, N., MARTH, G., ABECASIS, G. & DURBIN, R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics, 25*, 2078-9.

LI, L. & YANG, X. 2018. The Essential Element Manganese, Oxidative Stress, and Metabolic Diseases: Links and Interactions. *Oxidative medicine and cellular longevity, 2018*.

LI, W., LI, J., HE, N., DAI, X., WANG, Z., WANG, Y., NI, X., ZENG, D., ZHANG, D. & ZENG, Y. 2021. Molecular mechanism of enhancing the immune effect of the Newcastle disease virus vaccine in broilers fed with Bacillus cereus PAS38. *Food & Function, 12*, 10903-10916.

LI, X., NIE, C., LIU, Y., CHEN, Y., LV, X., WANG, L., ZHANG, J., LI, K., JIA, Y., BAN, L., NING, Z. & QU, L. 2019. A genome-wide association study explores the genetic determinism of host resistance to Salmonella pullorum infection in chickens. *Genetics Selection Evolution, 51*(1), 1-12.

LI, X., NIE, C., LIU, Y., CHEN, Y., LV, X., WANG, L., ZHANG, J., YANG, W., LI, K., ZHENG, C., JIA, Y., NING, Z. & QU, L. 2020. The Genetic Architecture of Early Body Temperature and Its Correlation With Salmonella Pullorum Resistance in Three Chicken Breeds. *Frontiers in genetics, 1287*.

LI, X., NIE, C., ZHANG, Z., WANG, Q., SHAO, P., ZHAO, Q., CHEN, Y., WANG, D., LI, Y. & JIAO, W. 2018. Evaluation of genetic resistance to Salmonella Pullorum in three chicken lines. *Poultry science, 97*, 764-769.

LI, Z. & SRIVASTAVA, P. 2003. Heat-Shock Proteins. *Current Protocols in Immunology, 58(1)*, A-1T.

LI, Z.-Y., LIN, J., SUN, F., LI, H., XIA, J., LI, X.-N., GE, J., ZHANG, C. & LI, J.-L. 2017. Transport stress induces weight loss and heart injury in chicks: disruption of ionic homeostasis via modulating ion transporting ATPases. *Oncotarget, 8*, 24142-24153.

LIAN, X., BAO, C., LI, X., ZHANG, X., CHEN, H., JUNG, Y.-S. & QIAN, Y. 2019. Marek's disease virus disables the ATR-Chk1 pathway by activating STAT3. *Journal of virology, 93*, e02290-18.

LIN, J., LUO, J. & REDIES, C. 2008. Differential expression of five members of the ADAM family in the developing chicken brain. *Neuroscience, 157*, 360-375.

LIN, Z., THORENOOR, N., WU, R., DIANGELO, S. L., YE, M., THOMAS, N. J., LIAO, X., LIN, T. R., WARREN, S. & FLOROS, J. 2018. Genetic association of pulmonary surfactant protein genes, SFTPA1, SFTPA2, SFTPB, SFTPC, and SFTPD with cystic fibrosis. *Frontiers in immunology, 9*, 2256.

LINDHOLM-PERRY, A. K., KUEHN, L. A., OLIVER, W. T., SEXTEN, A. K., MILES, J. R., REMPEL, L. A., CUSHMAN, R. A. & FREETLY, H. C. 2013. Adipose and muscle tissue gene expression of two genes (NCAPG and LCORL) located in a chromosomal region associated with cattle feed intake and gain. *PloS one, 8*, e80882-e80882.

LIU, R., SUN, Y., ZHAO, G., WANG, F., WU, D., ZHENG, M., CHEN, J., ZHANG, L., HU, Y. & WEN, J. 2013. Genome-wide association study identifies Loci and candidate genes for body composition and meat quality traits in Beijing-You chickens. *PloS one, 8*, e61172-e61172.

LIU, Y.-P., WU, G.-S., YAO, Y.-G., MIAO, Y.-W., LUIKART, G., BAIG, M., BEJA-PEREIRA, A., DING, Z.-L., PALANICHAMY, M. G. & ZHANG, Y.-P. 2006. Multiple maternal origins of chickens: Out of the Asian jungles. *Molecular Phylogenetics and Evolution, 38*, 12-19.

LIVERPOOL-TASIE, L. S. O., SANOU, A. & TAMBO, J. A. 2019. Climate change adaptation among poultry farmers: evidence from Nigeria. *Climatic Change, 157*, 527-544.

LÓPEZ, M. E., NEIRA, R. & YÁÑEZ, J. M. 2015. Applications in the search for genomic selection signatures in fish. *Frontiers in genetics, 5, 458*.

LOPEZ-HERRERA, G., TAMPELLA, G., PAN-HAMMARSTRÖM, Q., HERHOLZ, P., TRUJILLO-VARGAS, C. M., PHADWAL, K., SIMON, A. K., MOUTSCHEN, M., ETZIONI, A. & MORY, A. 2012. Deleterious mutations in LRBA are associated with a syndrome of immune deficiency and autoimmunity. *The American Journal of Human Genetics, 90*, 986-1001.

LOYAU, T., HENNEQUET-ANTIER, C., COUSTHAM, V., BERRI, C., LEDUC, M., CROCHET, S., SANNIER, M., DUCLOS, M. J., MIGNON-GRASTEAU, S., TESSERAUD, S., BRIONNE, A., MÉTAYER-COUSTARD, S., MOROLDO, M., LECARDONNEL, J., MARTIN, P., LAGARRIGUE, S., YAHAV, S. & COLLIN, A. 2016. Thermal manipulation of the chicken embryo triggers differential gene expression in response to a later heat challenge. *BMC Genomics, 17*, 329.

LU, Z., HE, X., MA, B., ZHANG, L., LI, J., JIANG, Y., ZHOU, G. & GAO, F. 2017. Chronic Heat Stress Impairs the Quality of Breast-Muscle Meat in Broilers by Affecting Redox Status and Energy-Substance Metabolism. *Journal of Agricultural and Food Chemistry, 65*, 11251-11258.

LYIMO, C. M., WEIGEND, A., MSOFFE, P. L., EDING, H., SIMIANER, H. & WEIGEND, S. 2014. Global diversity and genetic contributions of chicken populations from African, Asian and European regions. *Anim Genet, 45*, 836-48.

LYNCH, M. 2010. Evolution of the mutation rate. *TRENDS in Genetics, 26,* 345-352.

MACCIOTTA, N. P. P., GASPA, G., BOMBA, L., VICARIO, D., DIMAURO, C., CELLESI, M. & AJMONE-MARSAN, P. 2015. Genome-wide association analysis in Italian Simmental cows for lactation curve traits using a low-density (7K) SNP panel. *Journal of Dairy Science, 98,* 8175-8185.

MACDONALD, K. C. & EDWARDS, D. N. 1993. Chickens in Africa: the importance of Qasr Ibrim. *Antiquity, 67,* 584-590.

MACDONALD, K. C. 1992. The domestic chicken (*Gallus gallus*) in sub-Saharan Africa: A background to its introduction and its osteological differentiation from indigenous fowls (Numidinae and Francolinus *sp*.). *Journal of Archaeological Science, 19,* 303-318.

MAKINO, T., RUBIN, C.-J., CARNEIRO, M., AXELSSON, E., ANDERSSON, L. & WEBSTER, M. T. 2018. Elevated Proportions of Deleterious Genetic Variation in Domestic Animals and Plants. *Genome Biology and Evolution, 10,* 276-290.

MANZER, D. L. & HANNON, S. J. 2008. Survival of sharp-tailed grouse Tympanuchus phasianellus chicks and hens in a fragmented prairie landscape. *Wildlife Biology, 14,* 16-25.

MAPIYE, C., MWALE, M., MUPANGWA, J. F., CHIMONYO, M., FOTI, R. & MUTENJE, M. J. 2008. A Research Review of Village Chicken Production Constraints and Opportunities in Zimbabwe. *Asian-Australas J Anim Sci, 21,* 1680-1688.

MARDER, J. & ARAD, Z. 1975. The acid base balance of Abdim's stork (*Sphenorhynchus abdimii*) during thermal panting. *Comparative Biochemistry and Physiology Part A: Physiology, 51,* 887-889.

MARKS, J. 1988. Molecular evolutionary genetics. By M. Nei. New York: Columbia University Press. *American Journal of Physical Anthropology, 75,* 428-429.

MASTRANGELO, S., CENDRON, F., SOTTILE, G., NIERO, G., PORTOLANO, B., BISCARINI, F. & CASSANDRO, M. 2020. Genome-wide analyses identifies known and new markers responsible of chicken plumage color. *Animals, 10,* 493.

MATSUKI, H., TAKAHASHI, M., HIGUCHI, M., MAKOKHA, G. N., OIE, M. & FUJII, M. 2013. Both G3BP1 and G3BP2 contribute to stress granule formation. *Genes to Cells, 18,* 135-146.

MAY, M. J. & GHOSH, S. 1999. IkappaB kinases: kinsmen with different crafts. *Science, 284,* 271-3.

MAYNARD, J. & HAIGH, J. 2007. The hitchhiking effect of a favourable gene. *Genetics Research, 89,* 391-403.

MCDANIEL, C. D., BRAMWELL, R. K., WILSON, J. L. & HOWARTH, B. 1995. Fertility of Male and Female Broiler Breeders Following Exposure to Elevated Ambient Temperatures1. *Poultry Science, 74,* 1029-1038.

MCDANIEL, C. D., HOOD, J. E. & PARKER, H. M. 2004. An Attempt at Alleviating Heat Stress Infertility in Male Broiler Breeder Chickens with Dietary Ascorbic Acid. *International Journal of Poultry Science, 3,* 593-602.

MCKECHNIE, A. E. & WOLF, B. O. 2019. The Physiology of Heat Tolerance in Small Endotherms. *Physiology, 34,* 302-313.

MCKENNA, A., HANNA, M., BANKS, E., SIVACHENKO, A., CIBULSKIS, K., KERNYTSKY, A., GARIMELLA, K., ALTSHULER, D., GABRIEL, S., DALY, M. & DEPRISTO, M. A. 2010. The Genome Analysis Toolkit: a MapReduce framework for analysing next-generation DNA sequencing data. *Genome Res, 20,* 1297-303.

MCNAMEE, E. N., JOHNSON, D. K., HOMANN, D. & CLAMBEY, E. T. 2013. Hypoxia and hypoxia-inducible factors as regulators of T cell development, differentiation, and function. *Immunologic research*, *55*, 58-70.

MEREM, E., TWUMASI, Y., WESLEY, J., ALSARARI, M., FAGEIR, S., CRISLER, M., ROMORNO, C., OLAGBEGI, D., HINES, A. & OCHAI, G. 2019. Regional assessment of climate change hazards in Southern Nigeria with GIS. *Journal of safety engineering*, *8*, 9-27.

MIAO, Y. W., PENG, M. S., WU, G. S., OUYANG, Y. N., YANG, Z. Y., YU, N., LIANG, J. P., PIANCHOU, G., BEJA-PEREIRA, A., MITRA, B., PALANICHAMY, M. G., BAIG, M., CHAUDHURI, T. K., SHEN, Y. Y., KONG, Q. P., MURPHY, R. W., YAO, Y. G. & ZHANG, Y. P. 2013. Chicken domestication: an updated perspective based on mitochondrial genomes. *Heredity (Edinb)*, *110*, 277-82.

MILLER, D. J. & FORT, P. E. 2018. Heat shock proteins regulatory role in neurodevelopment. *Frontiers in Neuroscience*, 12.

MILLER, M. M. & TAYLOR, R. L., JR. 2016. Brief review of the chicken Major Histocompatibility Complex: the genes, their distribution on chromosome 16, and their contributions to disease resistance. *Poultry science*, *95*, 375-392.

MIYAMOTO, M., MOTOOKA, D., GOTOH, K. et al. 2014. Performance comparison of second- and third-generation sequencers using a bacterial genome with two chromosomes. *BMC Genomics*, 15, 699.

MOBEGI, A. V. 2006. Mitochondrial DNA D-loop sequences reveal the genetic diversity of African chicken. In The role of biotechnology in animal agriculture to address poverty in Africa: opportunities and challenges (eds. J. E. O. Rege, A.M. Nyamu and D. Sendalo). Nairobi: ILRI Publications.

MONSON, M. S., VAN GOOR, A. G., ASHWELL, C. M., PERSIA, M. E., ROTHSCHILD, M. F., SCHMIDT, C. J. & LAMONT, S. J. 2018. Immunomodulatory effects of heat stress and lipopolysaccharide on the bursal transcriptome in two distinct chicken lines. *BMC genomics*, *19*, 643-643.

MONTALDO, H. & MEZA-HERRERA, C. A. 1998. Use of molecular markers and major genes in the genetic improvement of livestock. *Electronic Journal of Biotechnology*, *1*, 1-9.

MOONEY, J. A., HUBER, C. D., SERVICE, S., SUL, J. H., MARSDEN, C. D., ZHANG, Z., SABATTI, C., RUIZ-LINARES, A., BEDOYA, G., COSTA RICA/COLOMBIA CONSORTIUM FOR GENETIC INVESTIGATION OF BIPOLAR, E., FREIMER, N. & LOHMUELLER, K. E. 2018. Understanding the Hidden Complexity of Latin American Population Isolates. *American journal of human genetics*, *103*, 707-726.

MOROKUMA, Y., NAKAMURA, N., KATO, A., NOTOYA, M., YAMAMOTO, Y., SAKAI, Y., FUKUDA, H., YAMASHINA, S., HIRATA, Y. & HIROSE, S. 2007. MARCH-XI, a novel transmembrane ubiquitin ligase implicated in ubiquitin-dependent protein sorting in developing spermatids. *Journal of Biological Chemistry*, *282*, 24806-24815.

MPENDA, F. N., SCHILLING, M. A., CAMPBELL, Z., MNGUMI, E. B. & BUZA, J. 2019. The genetic diversity of local african chickens: A potential for selection of chickens resistant to viral infections. *Journal of Applied Poultry Research*, *28*, 1-12.

MSOFFE, P. L., MINGA, U. M., OLSEN, J., YONGOLO, M., JUUL-MADSEN, H. R., GWAKISA, P. S. & MTAMBO, M. 2001. Phenotypes including immunocompetence in scavenging local chicken ecotypes in Tanzania. *Tropical Animal Health and Production*, *33*, 341-354.

MTILENI, B. J., MUCHADEYI, F. C., MAIWASHE, A., GROENEVELD, E., GROENEVELD, L. F., DZAMA, K. & WEIGEND, S. 2011. Genetic diversity and conservation of South African indigenous chicken populations. *Journal of Animal Breeding and Genetics, 128,* 209-218.

MUCHADEYI, F. C., EDING, H., SIMIANER, H., WOLLNY, C. B. A., GROENEVELD, E. & WEIGEND, S. 2008. Mitochondrial DNA D-loop sequences suggest a Southeast Asian and Indian origin of Zimbabwean village chickens. *Animal genetics, 39,* 615-622.

MUCHADEYI, F. C., EDING, H., WOLLNY, C. B. A., GROENEVELD, E., MAKUZA, S. M., SHAMSELDIN, R., SIMIANER, H. & WEIGEND, S. 2007. Absence of population substructuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis. *Animal Genetics, 38,* 332-339.

MUCHADEYI, F. C., WOLLNY, C. B. A., EDING, H., WEIGEND, S. & SIMIANER, H. 2009. Choice of breeding stock, preference of production traits and culling criteria of village chickens among Zimbabwe agro-ecological zones. *Tropical Animal Health and Production, 41,* 403-412.

MUIR, W., WONG, G., ZHANG, Y., WANG, J., GROENEN, M., CROOIJMANS, R., MEGENS, H.-J., ZHANG, H., MCKAY, J. & MCLEOD, S. 2008. Review of the initial validation and characterization of a 3K chicken SNP array. *World's Poultry Science Journal, 64,* 219-226.

MUJAHID, A., AKIBA, Y. & TOYOMIZU, M. 2007. Acute Heat Stress Induces Oxidative Stress and Decreases Adaptation in Young White Leghorn Cockerels by Downregulation of Avian Uncoupling Protein. *Poultry Science, 86,* 364-371.

MUSA, U., ABDU, P., DAFWANG, I., UMOH, J., SA'IDU, L., MERA, U. & EDACHE, J. 2009. Seroprevalence, seasonal occurrence and clinical manifestation of Newcastle disease in rural household chickens in Plateau State, Nigeria. *International Journal of Poultry Science, 8,* 200-204.

MWACHARO, J. M., BJØRNSTAD, G., HAN, J. L. & HANOTTE, O. 2013. The History of African Village Chickens: an Archaeological and Molecular Perspective. *African Archaeological Review,* 30, 97-114.

MWACHARO, J. M., NOMURA, K., HANADA, H., HAN, J. L., AMANO, T. & HANOTTE, O. 2013. Reconstructing the origin and dispersal patterns of village chickens across East Africa: insights from autosomal markers. *Molecular Ecology, 22,* 2683-2697.

MWACHARO, J. M., NOMURA, K., HANADA, H., JIANLIN, H., HANOTTE, O. & AMANO, T. 2007. Genetic relationships among Kenyan and other East African indigenous chickens. *Anim Genet, 38,* 485-90.

MYLES, S., TANG, K., SOMEL, M., GREEN, R. E., KELSO, J. & STONEKING, M. 2008. Identification and analysis of genomic regions with large between-population differentiation in humans. *Ann Hum Genet, 72,* 99-110.

NAGAI, K.-I., TAKAHASHI, N., MOUE, T. & NIIMURA, Y. 2011. Alteration of fatty acid molecular species in ceramide and glucosylceramide under heat stress and expression of sphingolipid-related genes. *Advances in Biological Chemistry, 1(03), 35.*

NAKAI, A. & MORIMOTO, R. I. 1993. Characterization of a novel chicken heat shock transcription factor, heat shock factor 3, suggests a new regulatory pathway. *Molecular and Cellular Biology, 13*(4), 1983-1997.

NARDONE, A., RONCHI, B., LACETERA, N., RANIERI, M. S. & BERNABUCCI, U. 2010. Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science, 130,* 57-69.

NEST. 1991. Nigeria's threatened environment: a national profile. Environmental Study/Action Team, NEST, Ibadan.

NGENO, K. 2015. Breeding Program for Indigenous Chicken in Kenya: Analysis of Diversity in Indigenous Chicken Populations. *(Doctoral dissertation, Wageningen University and Research).*

NGONGEH, L., ONYEABOR, A., NZENWATA, E. & SAMSON, G. K. 2017. Comparative Response of the Nigerian Indigenous and Broiler Chickens to a Field Caecal Isolate of Eimeria Oocysts. *Journal of Pathogens*, 2017.

NIELSEN, R. 2005. Molecular Signatures of Natural Selection. *Annual Review of Genetics*, *39*, 197-218.

NIELSEN, R., WILLIAMSON, S., KIM, Y., HUBISZ, M. J., CLARK, A. G. & BUSTAMANTE, C. 2005. Genomic scans for selective sweeps using SNP data. *Genome Res*, *15*, 1566-75.

NIENABER, J. A. & HAHN, G. L. 2007. Livestock production system management responses to thermal challenges. *International Journal of Biometeorology*, *52*, 149-157.

NONIĆ, M. & ŠIJAČIĆ-NIKOLIĆ, M. 2019. Genetic Diversity: Sources, Threats, and Conservation. In: LEAL FILHO, W., AZUL, A. M., BRANDLI, L., ÖZUYAR, P. G. & WALL, T. (eds.) *Life on Land*. Cham: Springer International Publishing.

NOVEMBRE, J., JOHNSON, T., BRYC, K., KUTALIK, Z., BOYKO, A. R., AUTON, A., INDAP, A., KING, K. S., BERGMANN, S. & NELSON, M. R. 2008. Genes mirror geography within Europe. *Nature*, *456*, 98-101.

NRC. 2001. Arsenic in the Drinking Water (Update). pp. 1–225.

NWOGWUGWU, C. P., LEE, J., FREEDOM, E. C. & LEE, S. 2019. Review on the genetic potential of Nigerian local chickens. *Journal of Animal Breeding and Genomics*, *2*(2).

NYONI, N., GRAB, S. & ARCHER, E. R. 2019. Heat stress and chickens: climate risk effects on rural poultry farming in low-income countries. *Climate and Development*, *11*, 83-90.

O'BRIEN, T. R., YANG, H.-I., GROOVER, S. & JENG, W.-J. 2019. Genetic Factors That Affect Spontaneous Clearance of Hepatitis C or B Virus, Response to Treatment, and Disease Progression. *Gastroenterology*, *156*, 400-417.

OGBU, C. S. 2010. Genetic Change in the Nigerian Heavy Local Chicken Ecotype Through Selection for Body Weight and Egg Production Traits. PhD Thesis, University of Nigeria.

OGBU. 2011. Within population variation in performance traits in the Nigerian indigenous chicken (NIC). *International Journal of Science and nature*, *2*(2), 192-197.

ADEYINKA, I. 2010. Comparative evaluation of two Nigerian local chicken ecotypes and their crosses for growth traits. *International Journal of Poultry Science*, *9*(8), pp.738-743.

OKAEME, A. N. 1988. Ectoparasites of guinea fowl (Numida meleagris galeata Pallas) and local domestic chicken (Gallus gallus) in southern guinea savanna, Nigeria. *Veterinary Research Communications*, *12*, 277-280.

OKPARA, J. N., A.TARHULE, A. & PERUMAL, M. Study of climate change in Niger River Basin, West Africa: Reality not a myth. *Climate Change: Realities, Impacts Over Ice Cap, Sea Level and Risks*, *1*.

OKPEKU, M., OGAH, D. M. & ADELEKE, M. A. 2019. A review of challenges to genetic improvement of indigenous livestock for improved food production in Nigeria. *African Journal of Food, Agriculture, Nutrition and Development*, *19*, 13959-13978.

OKPEKU, M., ORHERUATA, M., AND IMUMORIN, I.G. Phenotypic and genetic variation among local chicken in Edo State Nigeria. *Proc. 25th Annual conf. Nig. Soc. Anim. Prod, 2003*. 119-121.

OKSANENM, J., BLANCHET, F.G., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P.R., O'HARA, R.B., SIMPSON, G.L., SOLYMOS, P., ET AL. 2017. Vegan: Community ecology package (*R package version* 2.4-3).

OKWOR, E. & EZE, D. 2011. Epizootic Newcastle disease in local chickens reared in South East Savannah zone of Nigeria. *Int J Poult Sci, 10,* 212-215.

OLADELE, S., AYO, J., OGUNDIPE, S. & ESIEVO, K. 2003. Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern Guinea Savannah zone of Nigeria. *Bulletin of Animal Health and Production in Africa, 51*, 204-214.

OLANREWAJU, R. M., TILAKASIRI, S. L. & ADELEKE, E. A. 2016. Effects of Climate on Chicken Production in Ilorin, Kwara State, Nigeria. *Journal of Agricultural Sciences 11(2)*, 88-96.

OLEFORUH-OKOLEH, V., NWOSU, C., ADEOLU, A., UDEH, I., UBERU, N. & HARRIET NDOFOR, D. F. 2012. Egg Production Performance in a Nigerian Local Chicken Ecotype Subjected to Selection. *Journal of Agricultural Science*, 4.

OLEKSYK, T. K., SMITH, M. W. & O'BRIEN, S. J. 2010. Genome-wide scans for footprints of natural selection. Philosophical Transactions of the Royal Society B: *Biological Sciences, 365,* 185-205.

OLUYEMI, J. A., AND ROBERTS, F.A 1979. Poultry Production in Warm Wet Climates, London, The Macmillan Press Ltd.

ONYEKURU, A. N. & MARCHANT, R. 2014. Climate change impact and adaptation pathways for forest dependent livelihood systems in Nigeria. *African Journal of Agricultural Research, 9,* 1819-1832.

OPARA, M. N., OSOWA, D. K. & MAXWELL, J. A. 2014. Blood and Gastrointestinal Parasites of Chickens and Turkeys Reared in the Tropical Rainforest Zone of Southeastern Nigeria. *Open Journal of Veterinary Medicine,* Vol.04No.12, 6.

ORUNMUYI, M., MUHAMMAD, H., MUSA, A., OLUTUNMOGUN, A. & NWAGU, B. 2020. Effect of hemoglobin polymorphism on performance traits in indigenous chicken genotypes in Nigeria. *Tropical animal health and production*, 1-9.

OSTI, R., BHATTARAI, D. & ZHOU, D. 2017. Climatic Variation: Effects on Stress Levels, Feed Intake, and Bodyweight of Broilers. *Brazilian Journal of Poultry Science, 19*, 489-496.

OTITOJU, M. & ENETE, A. 2014. Climate Change Adaptation Strategies and Farm-level Efficiency in Food Crop Production in Southwestern, Nigeria. *Tropicultura*, 32.

PACHT, E., MURDOCH, B. & MCKAY, S. 2021. Examining the extent of environmental contributions toward DNA methylation and phenotypic variation. *Animal Frontiers, 11*, 83-89.

PADHI, M. K. 2016. Importance of Indigenous Breeds of Chicken for Rural Economy and Their Improvements for Higher Production Performance. *Scientifica, 2016.*

PAIVA, C. N. & BOZZA, M. T. 2013. Are Reactive Oxygen Species Always Detrimental to Pathogens? *Antioxidants & Redox Signaling, 20*, 1000-1037.

PALKAR, R., LIPPOLDT, E. K. & MCKEMY, D. D. 2015. The molecular and cellular basis of thermosensation in mammals. *Current Opinion in Neurobiology, 34*, 14-19.

PAUL, C., TENG, S. & SAUNDERS, P. T. K. 2009. A Single, Mild, Transient Scrotal Heat Stress Causes Hypoxia and Oxidative Stress in Mouse Testes, Which Induces Germ Cell Death1. *Biology of Reproduction, 80,* 913-919.

PENG, M., HAN, J., LI, L. & MA, H. 2016. Suppression of fat deposition in broiler chickens by (-)-hydroxycitric acid supplementation: A proteomics perspective. *Scientific Reports*, *6*, 32580.

PERINI, F., CENDRON, F., ROVELLI, G., CASTELLINI, C., CASSANDRO, M. & LASAGNA, E. 2021. Emerging Genetic Tools to Investigate Molecular Pathways Related to Heat Stress in Chickens: A Review. *Animals*, 11.

PERMIN, A. A. H., J.W. FAO ANIMAL HEALTH 1998. *Epidemiology, diagnosis and control of poultry parasites* (No. 636.08947 F32 v. 4). FAO.

PERRY-GAL, L., ERLICH, A., GILBOA, A. & BAR-OZ, G. 2015. Earliest economic exploitation of chicken outside East Asia: Evidence from the Hellenistic Southern Levant. *Proceedings of the National Academy of Sciences*, *112*, 9849-9854.

PÉRTILLE, F., GUERRERO-BOSAGNA, C., SILVA, V. H. D., BOSCHIERO, C., NUNES, J. D. R. D. S., LEDUR, M. C., JENSEN, P. & COUTINHO, L. L. 2016. High-throughput and Cost-effective Chicken Genotyping Using Next-Generation Sequencing. *Scientific Reports*, *6*, 26929.

PERWITASARI, O., JOHNSON, S., YAN, X., HOWERTH, E., SHACHAM, S., LANDESMAN, Y., BALOGLU, E., MCCAULEY, D., TAMIR, S., TOMPKINS, S. M. & TRIPP, R. A. 2014. Verdinexor, a novel selective inhibitor of nuclear export, reduces influenza a virus replication in vitro and in vivo. *Journal of virology*, *88*, 10228-10243.

PETTERSSON, M. E. & JERN, P. 2019. Whole-Genome Analysis of Domestic Chicken Selection Lines Suggests Segregating Variation in ERV Makeups. *Genes* [Online], 10. Available:https://doi.org/10.3390/genes10020162.

PHAM-DINH, D., MATTEI, M. G., NUSSBAUM, J. L., ROUSSEL, G., PONTAROTTI, P., ROECKEL, N., MATHER, I. H., ARTZT, K., LINDAHL, K. F. & DAUTIGNY, A. 1993. Myelin/oligodendrocyte glycoprotein is a member of a subset of the immunoglobulin superfamily encoded within the major histocompatibility complex. *Proceedings of the National Academy of Sciences of the United States of America*, 90, 7990-7994.

POWELL, F. L. 2015. Chapter 13 - Respiration. In: SCANES, C. G. (ed.) Sturkie's Avian Physiology (Sixth Edition). San Diego: Academic Press.

PRICE, A. L., PATTERSON, N. J., PLENGE, R. M., WEINBLATT, M. E., SHADICK, N. A. & REICH, D. 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*, *38*, 904-9.

PRITCHARD, J. K. & DONNELLY, P. 2001. Case–control studies of association in structured or admixed populations. *Theoretical population biology*, *60*, 227-237.

PROTTY, M. B., WATKINS, N. A., COLOMBO, D., THOMAS, S. G., HEATH, V. L., HERBERT, J. M. J., BICKNELL, R., SENIS, Y. A., ASHMAN, L. K., BERDITCHEKI, F., OUWEHAND, W. H., WATSON, S. P. & TOMLINSON, M. G. 2009. Identification of Tspan9 as a novel platelet tetraspanin and the collagen receptor GPVI as a component of tetraspanin microdomains. *The Biochemical journal*, *417*, 391-400.

PYM, R., GUERNEBLEICH, E. & HOFFMANN, I. 2006. The relative contribution of indigenous chicken breeds to poultry meat and egg production and consumption in the developing countries of Africa and Asia. In *Proceedings of the XII European Poultry Conference* (Vol. 1014).

QANBARI, S. & SIMIANER, H. 2014. Mapping signatures of positive selection in the genome of livestock. *Livestock Science*, *166*, 133-143.

QANBARI, S. 2020. On the Extent of Linkage Disequilibrium in the Genome of Farm Animals. *Frontiers in Genetics, 10, 1304.*

QANBARI, S., RUBIN, C. J., MAQBOOL, K., WEIGEND, S., WEIGEND, A., GEIBEL, J., KERJE, S., WURMSER, C., PETERSON, A. T., BRISBIN, I. L., JR., PREISINGER, R., FRIES, R., SIMIANER, H. & ANDERSSON, L. 2019. Genetics of adaptation in modern chicken. *PLoS Genet*, *15*, e1007989.

QANBARI, S., SEIDEL, M., STROM, T.-M., MAYER, K. F. X., PREISINGER, R. & SIMIANER, H. 2015. Parallel Selection Revealed by Population Sequencing in Chicken. *Genome biology and evolution*, *7*, 3299-3306.

QIN, Y., XU, Z., WANG, Y., LI, X., CAO, H. & ZHENG, S. J. 2017. VP2 of Infectious Bursal Disease Virus Induces Apoptosis via Triggering Oral Cancer Overexpressed 1 (ORAOV1) Protein Degradation. *Frontiers in Microbiology*, *8*, 1351.

QIU, Q., ZHANG, G., MA, T., QIAN, W., WANG, J., YE, Z., CAO, C., HU, Q., KIM, J. & LARKIN, D. M. 2012. The yak genome and adaptation to life at high altitude. *Nature genetics*, *44*, 946-949.

QU, Y., ZHAO, H., HAN, N., ZHOU, G., SONG, G., GAO, B., TIAN, S., ZHANG, J., ZHANG, R. & MENG, X. 2013. Ground tit genome reveals avian adaptation to living at high altitudes in the Tibetan plateau. *Nature communications*, 4, 1-9.

QUINLAN, A. R. & HALL, I. M. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* (Oxford, England), 26, 841-842.

QUINTEIRO-FILHO, W. M., CALEFI, A. S., CRUZ, D. S. G., ALOIA, T. P. A., ZAGER, A., ASTOLFI-FERREIRA, C. S., PIANTINO FERREIRA, J. A., SHARIF, S. & PALERMO-NETO, J. 2017. Heat stress decreases expression of the cytokines, avian β-defensins 4 and 6 and Toll-like receptor 2 in broiler chickens infected with Salmonella Enteritidis. *Veterinary Immunology and Immunopathology*, 186, 19-28.

RATRIYANTO, A. & MOSENTHIN, R. 2018. Osmoregulatory function of betaine in alleviating heat stress in poultry. *Journal of Animal Physiology and Animal Nutrition*, *102*, 1634-1650.

RAUF, A., KHATRI, M., MURGIA, M. V. & SAIF, Y. M. 2012. Fas/FasL and perforin–granzyme pathways mediated T cell cytotoxic responses in infectious bursal disease virus infected chickens. *Results in Immunology*, *2*, 112-119.

REHMAN, M. S., MAHMUD, A., MEHMOOD, S., PASHA, T. N., HUSSAIN, J. & KHAN, M. T. 2017. Blood biochemistry and immune response in Aseel chicken under free range, semi-intensive, and confinement rearing systems. *Poultry Science*, *96*, 226-233.

REN, X., YU, Y., LI, H., HUANG, J., ZHOU, A., LIU, S., HU, P., LI, B., QI, W. & LIAO, M. 2019. Avian Influenza A Virus Polymerase Recruits Cellular RNA Helicase eIF4A3 to Promote Viral mRNA Splicing and Spliced mRNA Nuclear Export. *Frontiers in Microbiology*, 1625.

RICAÑO-PONCE, I. & WIJMENGA, C. 2013. Mapping of Immune-Mediated Disease Genes. *Annual Review of Genomics and Human Genetics*, *14*, 325-353.

RIM-RUKEH, A. 2018. Acidification Status of Surface Waters in the Niger Delta, Nigeria: A Review of Results and Predictions for the Future *Equatorial Journal of Agriculture and Earth Sciences*, *2*(1).

ROJAS-DOWNING, M. M., NEJADHASHEMI, A. P., HARRIGAN, T. & WOZNICKI, S. A. 2017. Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management*, *16*, 145-163.

ROMANOV, M., SAZANOV, A. & SMIRNOV, A. 2004. First century of chicken gene study and mapping - A look back and forward. *World s Poultry Science Journal*, *60*, 19-41.

ROTIMI, E., EGAHI, J. & ABAYOMI, A. 2016. Phenotypic scharacterization of indigenous chicken population in Gwer-West, Benue State, Nigeria. *world scientific news*, *53*, 343-353.

ROWLAND, K., SAELAO, P., WANG, Y., FULTON, J. E., LIEBE, G. N., MCCARRON, A. M., WOLC, A., GALLARDO, R. A., KELLY, T., ZHOU, H., DEKKERS, J. C. M. & LAMONT, S. J. 2018. Association of Candidate Genes with Response to Heat and Newcastle Disease Virus. *Genes*, *9*, 560.

ROZENBOIM, I., TAKO, E., GAL-GARBER, O., PROUDMAN, J. A. & UNI, Z. 2007. The Effect of Heat Stress on Ovarian Function of Laying Hens. *Poultry Science*, *86*, 1760-1765.

RUBIN, C. J., ZODY, M. C., ERIKSSON, J., MEADOWS, J. R., SHERWOOD, E., WEBSTER, M. T., JIANG, L., INGMAN, M., SHARPE, T., KA, S., HALLBOOK, F., BESNIER, F., CARLBORG, O., BED'HOM, B., TIXIER-BOICHARD, M., JENSEN, P., SIEGEL, P., LINDBLAD-TOH, K. & ANDERSSON, L. 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*, *464*, 587-91.

SABETI, P. C., REICH, D. E., HIGGINS, J. M., LEVINE, H. Z., RICHTER, D. J., SCHAFFNER, S. F., GABRIEL, S. B., PLATKO, J. V., PATTERSON, N. J. & MCDONALD, G. J. 2002a. Detecting recent positive selection in the human genome from haplotype structure. *Nature*, *419*, 832-837.

SABETI, P. C., REICH, D. E., HIGGINS, J. M., LEVINE, H. Z., RICHTER, D. J., SCHAFFNER, S. F., GABRIEL, S. B., PLATKO, J. V., PATTERSON, N. J., MCDONALD, G. J., ACKERMAN, H. C., CAMPBELL, S. J., ALTSHULER, D., COOPER, R., KWIATKOWSKI, D., WARD, R. & LANDER, E. S. 2002b. Detecting recent positive selection in the human genome from haplotype structure. Nature, *419*, 832-7.

SABETI, P. C., VARILLY, P., FRY, B., LOHMUELLER, J., HOSTETTER, E., COTSAPAS, C., XIE, X., BYRNE, E. H., MCCARROLL, S. A., GAUDET, R., SCHAFFNER, S. F., LANDER, E. S., FRAZER, K. A., BALLINGER, D. G., COX, D. R., HINDS, D. A., STUVE, L. L., GIBBS, R. A., BELMONT, J. W., BOUDREAU, A., HARDENBOL, P., LEAL, S. M., PASTERNAK, S., WHEELER, D. A., WILLIS, T. D., YU, F., YANG, H., ZENG, C., GAO, Y., HU, H., HU, W., LI, C., LIN, W., LIU, S., PAN, H., TANG, X., WANG, J., WANG, W., YU, J., ZHANG, B., ZHANG, Q., ZHAO, H., ZHAO, H., ZHOU, J., GABRIEL, S. B., BARRY, R., BLUMENSTIEL, B., CAMARGO, A., DEFELICE, M., FAGGART, M., GOYETTE, M., GUPTA, S., MOORE, J., NGUYEN, H., ONOFRIO, R. C., PARKIN, M., ROY, J., STAHL, E., WINCHESTER, E., ZIAUGRA, L., ALTSHULER, D., SHEN, Y., YAO, Z., HUANG, W., CHU, X., HE, Y., JIN, L., LIU, Y., SHEN, Y., SUN, W., WANG, H., WANG, Y., WANG, Y., XIONG, X., XU, L., WAYE, M. M., TSUI, S. K., XUE, H., WONG, J. T., GALVER, L. M., FAN, J. B., GUNDERSON, K., MURRAY, S. S., OLIPHANT, A. R., CHEE, M. S., MONTPETIT, A., CHAGNON, F., FERRETTI, V., LEBOEUF, M., OLIVIER, J. F., PHILLIPS, M. S., ROUMY, S., SALLEE, C., VERNER, A., HUDSON, T. J., KWOK, P. Y., CAI, D., KOBOLDT, D. C., MILLER, R. D., PAWLIKOWSKA, L., et al. 2007. Genome-wide detection and characterization of positive selection in human populations. Nature, *449*, 913-8.

CAPITAL, S., 2015. An assessment of the Nigerian poultry sector. *Sahel Capital Newsletter*, *11*(2).

SAKIB, S. 2021. The Impact of Oil and Gas Development on the Landscape and Surface in Nigeria. *Asian Pacific Journal of Environment and Cancer*.

SALAKO, F. K. (2004). *Soil physical conditions in Nigerian savannas and biomass production* (No. INIS-XA--989).

SALISU, I. B., OlAWAWE, A. S., JABBAR, B., KOLOKO, B. L., ABDURRAHMAN, S. L., AMIN, A. B., & ALI, Q. (2018). Molecular markers and their Potentials in Animal Breeding and Genetics. *Nigerian Journal of Animal Science*, *20*(3), 29-48.

SANI, N. A., UGOCHUKWU, I. C., SALEH, A., ABALAKA, S. E., MUHAMMED, M. S., IDOKO, I. S., OLADELE, S. B., ABDU, P. A. & NJOKU, C. 2021. Antibody profiles of avian leukosis virus subgroups A/B and J In layer flocks suspected to have Marek's disease in Nigeria. *Acta Veterinaria*, *71*, 451-461.

IZAH, S. 2018. Ecosystem of the Niger Delta region of Nigeria: Potentials and Threats. *Biodiversity Int J, 2(4),* 338-345.

SCHMID, M., SMITH, J., BURT, D. W., AKEN, B. L., ANTIN, P. B., ARCHIBALD, A. L., ASHWELL, C., BLACKSHEAR, P. J., BOSCHIERO, C., BROWN, C. T., BURGESS, S. C., CHENG, H. H., CHOW, W., COBLE, D. J., COOKSEY, A., CROOIJMANS, R. P., DAMAS, J., DAVIS, R. V., DE KONING, D. J., DELANY, M. E., DERRIEN, T., DESTA, T. T., DUNN, I. C., DUNN, M., ELLEGREN, H., EORY, L., ERB, I., FARRE, M., FASOLD, M., FLEMING, D., FLICEK, P., FOWLER, K. E., FRESARD, L., FROMAN, D. P., GARCEAU, V., GARDNER, P. P., GHEYAS, A. A., GRIFFIN, D. K., GROENEN, M. A., HAAF, T., HANOTTE, O., HART, A., HASLER, J., HEDGES, S. B., HERTEL, J., HOWE, K., HUBBARD, A., HUME, D. A., KAISER, P., KEDRA, D., KEMP, S. J., KLOPP, C., KNIEL, K. E., KUO, R., LAGARRIGUE, S., LAMONT, S. J., LARKIN, D. M., LAWAL, R. A., MARKLAND, S. M., MCCARTHY, F., MCCORMACK, H. A., MCPHERSON, M. C., MOTEGI, A., MULJO, S. A., MUNSTERBERG, A., NAG, R., NANDA, I., NEUBERGER, M., NITSCHE, A., NOTREDAME, C., NOYES, H., O'CONNOR, R., O'HARE, E. A., OLER, A. J., OMMEH, S. C., PAIS, H., PERSIA, M., PITEL, F., PREEYANON, L., PRIETO BARJA, P., PRITCHETT, E. M., RHOADS, D. D., ROBINSON, C. M., ROMANOV, M. N., ROTHSCHILD, M., ROUX, P. F., SCHMIDT, C. J., SCHNEIDER, A. S., SCHWARTZ, M. G., SEARLE, S. M., SKINNER, M. A., SMITH, C. A., STADLER, P. F., STEEVES, T. E., STEINLEIN, C., SUN, L., TAKATA, M., ULITSKY, I., WANG, Q., WANG, Y., et al. 2015. Third Report on Chicken Genes and Chromosomes 2015. *Cytogenet Genome Res*, *145*, 78-179.

SEO, D., LEE, D. H., CHOI, N., SUDRAJAD, P., LEE, S.-H. & LEE, J.-H. 2018. Estimation of linkage disequilibrium and analysis of genetic diversity in Korean chicken lines. *PLoS One*, *13*(2), e0192063..

SHAMOVERKY, I., IVANNIKOV, M., KANDEL, E. S., GERSHON, D. & NUDLER, E. 2006. RNA-mediated response to heat shock in mammalian cells. Nature, *440*, 556-560.

SHARMA, A. & TRIPATHI, P. 2015. Seasonal Prevalence of Poultry Diseases and their interaction with IBD and Mycotoxicosis in Chitwan, Nepal. *The Research Gate*, 1-9.

SHARMA, D., CORNETT, L. E. & CHATURVEDI, C. M. 2009. Osmotic stress-induced alteration in the expression of arginine vasotocin receptor VT2 in the pituitary gland and adrenal function of domestic fowl. *General and Comparative Endocrinology*, *160*, 216-222.

SHEN, C., ZHAO, X., HE, C. & ZUO, Z. 2022. Developmental toxicity and neurotoxicity assessment of R-, S-, and RS-propylene glycol enantiomers in zebrafish (Danio rerio) larvae. *Environmental Science and Pollution Research*, 1-11.

SHEN, X., STEYRER, E., RETZEK, H., SANDERS, E. J. & SCHNEIDER, W. J. 1993. Chicken oocyte growth: receptor-mediated yolk deposition. *Cell and Tissue Research*, *272*, 459-471.

SHITTU, I., JOANNIS, T. M., ODAIBO, G. N. & OLALEYE, O. D. 2016. Newcastle disease in Nigeria: epizootiology and current knowledge of circulating genotypes. *Virusdisease*, *27*, 329-339.

SHWEIKI, D., ITIN, A., SOFFER, D. & KESHET, E. 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*, *359*, 843-845.

SIM, N.-L., KUMAR, P., HU, J., HENIKOFF, S., SCHNEIDER, G. & NG, P. C. 2012. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic acids research*, *40*, W452-W457.

SIMONSON, T. S., YANG, Y., HUFF, C. D., YUN, H., QIN, G., WITHERSPOON, D. J., BAI, Z., LORENZO, F. R., XING, J. & JORDE, L. B. 2010. Genetic evidence for high-altitude adaptation in Tibet. *Science*, *329*, 72-75.

SINKALU, V. O., AYO, J. O., ADELAIYE, A. B. & HAMBOLU, J. O. 2015. Ameliorative effects of melatonin administration and photoperiods on diurnal fluctuations in cloacal temperature of Marshall broiler chickens during the hot dry season. *International journal of biometeorology*, *59*, 79-87.

SLATKIN, M. 2008. Linkage disequilibrium understanding the evolutionary past and mapping the medical future. *Nature Reviews Genetics*, 9, 477-485.

SMITH, J., BRULEY, C. K., PATON, I. R., DUNN, I., JONES, C. T., WINDSOR, D., MORRICE, D. R., LAW, A. S., MASABANDA, J., SAZANOV, A., WADDINGTON, D., FRIES, R. & BURT, D. W. 2000. Differences in gene density on chicken macrochromosomes and microchromosomes. *Anim Genet*, *31*, 96-103.

SOLA-OJO, F. & AYORINDE, K. 2011. Evaluation of reproductive performance and egg quality traits in progenies of dominant black strain crossed with Fulani Ecotype chicken. *Journal of Agricultural Science*, *3*, 258-265.

SONAIYA, E. B., OTUBOLE, I.K. BAELMAN, R., DEMEY, F., WIMMERS, K., VALLE ZARATE, A., AND HORST 1998. Evaluation of Nigerian local poultry ecotypes for genetic variation, disease resistance and productivity. Proc. Silver Aniv. Conf. Nig. Soc. *Anim. Prod*, 316.

SOWUNMI, F. A. A. A., J.O 2010. Effect of Climatic Variability on Maize Production in Nigeria. *Research Journal of Environmental and Earth Sciences 2(1*): 19-30.

SRIVASTAVA, S., SYED, S. B., KUMAR, V., ISLAM, A., AHMAD, F. & HASSAN, M. I. 2017. Fas-activated serine/threonine kinase: Structure and function. *Gene Reports*, *8*, 117-127.

STAINTON, J.J., CHARLESWORTH, B., HALEY, C.S., KRANIS, A., WATSON, K. and WIENER, P. 2017). Use of high-density SNP data to identify patterns of diversity and signatures of selection in broiler chickens. *J. Anim. Breed. Genet*., *134*: 87-97.

STEPHEN, J., MADDIREVULA, S., NAMPOOTHIRI, S., BURKE, J. D., HERZOG, M., SHUKLA, A., STEINDL, K., ESKIN, A., PATIL, S. J., JOSET, P., LEE, H., GARRETT, L. J., YOKOYAMA, T., BALANDA, N., BODINE, S. P., TOLMAN, N. J., ZERFAS, P. M., ZHENG, A., RAMANTANI, G., GIRISHA, K. M., RIVAS, C., SURESH, P. V., ELKAHLOUN, A., ALSAIF, H. S., WAKIL, S. M., MAHMOUD, L., ALI, R., PROCHAZKOVA, M., KULKARNI, A. B., BEN-OMRAN, T., COLAK, D., MORRIS, H. D., RAUCH, A., MARTINEZ-AGOSTO, J. A., NELSON, S. F., ALKURAYA, F. S., GAHL, W. A. &

MALICDAN, M. C. V. 2018. Bi-allelic TMEM94 Truncating Variants Are Associated with Neurodevelopmental Delay, Congenital Heart Defects, and Distinct Facial Dysmorphism. *The American Journal of Human Genetics*, *103*, 948-967.

STUMPO, D. J., LAI, W. S. & BLACKSHEAR, P. J. 2010. Inflammation: cytokines and RNA-based regulation. Wiley interdisciplinary reviews. *RNA*, *1*, 60-80.

SUN, Y., DONG, L., YU, S., WANG, X., ZHENG, H., ZHANG, P., MENG, C., ZHAN, Y., TAN, L., SONG, C., QIU, X., WANG, G., LIAO, Y. & DING, C. 2017. Newcastle disease virus induces stable formation of bona fide stress granules to facilitate viral replication through manipulating host protein translation. *The FASEB Journal*, *31*, 1482-1493.

SUN, Y., LIU, R., ZHAO, G., ZHENG, M., SUN, Y., YU, X., LI, P. & WEN, J. 2015. Genome-Wide Linkage Analysis Identifies Loci for Physical Appearance Traits in Chickens. *G3* (Bethesda, Md.), *5*, 2037-2041.

SURAI, P. F., KOCHISH, I. I., FISININ, V. I. & KIDD, M. T. 2019. Antioxidant Defence Systems and Oxidative Stress in Poultry Biology: An Update. *Antioxidants*, *8*(7), 235.

TAJIMA, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, *123*, 585-595.

TAMBO, J. A. & ABDOULAYE, T. 2013. Smallholder farmers' perceptions of and adaptations to climate change in the Nigerian savanna. *Regional Environmental Change*, *13*, 375-388.

TANG, H., CORAM, M., WANG, P., ZHU, X. & RISCH, N. 2006. Reconstructing genetic ancestry blocks in admixed individuals. *The American Journal of Human Genetics*, *79*, 1-12.

TANG, H., PENG, J., WANG, P. & RISCH, N. J. 2005. Estimation of individual admixture: analytical and study design considerations. Genetic Epidemiology: *The Official Publication of the International Genetic Epidemiology Society*, *28*, 289-301.

TANG, X., MENG, Q., GAO, J., ZHANG, S., ZHANG, H. & ZHANG, M. 2015. Label-free Quantitative Analysis of Changes in Broiler Liver Proteins under Heat Stress using SWATH-MS Technology. *Scientific Reports*, *5*, 15119.

TANIGAKI, K., HAN, H., YAMAMOTO, N., TASHIRO, K., IKEGAWA, M., KURODA, K., SUZUKI, A., NAKANO, T. & HONJO, T. 2002. Notch–RBP-J signaling is involved in cell fate determination of marginal zone B cells. *Nature Immunology*, *3*, 443-450.

TATEBE, H. & SHIOZAKI, K. 2003. Identification of Cdc37 as a novel regulator of the stress-responsive mitogen-activated protein kinase. *Molecular and cellular biology*, *23*, 5132-5142.

TESTORI, A., RICHARDS, J., WHITMAN, E., MANN, G. B., LUTZKY, J., CAMACHO, L., PARMIANI, G., TOSTI, G., KIRKWOOD, J. M., HOOS, A., YUH, L., GUPTA, R. & SRIVASTAVA, P. K. 2008. Phase III Comparison of Vitespen, an Autologous Tumor-Derived Heat Shock Protein gp96 Peptide Complex Vaccine, With Physician's Choice of Treatment for Stage IV Melanoma: The C-100-21 Study Group. *Journal of Clinical Oncology*, *26*, 955-962.

THOMAS, P. D., CAMPBELL, M. J., KEJARIWAL, A., MI, H., KARLAK, B., DAVERMAN, R., DIEMER, K., MURUGANUJAN, A. & NARECHANIA, A. 2003. PANTHER: a library of protein families and subfamilies indexed by function. *Genome research*, *13*, 2129-2141.

THURESSON, A.-C. 2002. Regulation of Mammalian Poly(A) Polymerase Activity. Doctoral thesis, comprehensive summary, Acta Universitatis Upsaliensis.

TIAN, S., ZHOU, X., PHUNTSOK, T., ZHAO, N., ZHANG, D., NING, C., LI, D. & ZHAO, H. 2020. Genomic Analyses Reveal Genetic Adaptations to Tropical Climates in Chickens. *iScience*, *23*, 101644.

TIRAWATTANAWANICH, C., CHANTAKRU, S., NIMITSANTIWONG, W. & TONGYAI, S. 2011. The effects of tropical environmental conditions on the stress and immune responses of commercial broilers, Thai indigenous chickens, and crossbred chickens. *Journal of Applied Poultry Research*, *20*, 409-420.

TIXIER-BOICHARD, M., BED'HOM, B. & ROGNON, X. 2011. Chicken domestication: from archeology to genomics. *C R Biol*, *334*, 197-204.

TO, K. K., ZHOU, J., SONG, Y.-Q., HUNG, I. F., IP, W. C., CHENG, Z.-S., CHAN, A. S., KAO, R. Y., WU, A. K. & CHAU, S. 2014. Surfactant protein B gene polymorphism is associated with severe influenza. *Chest*, *145*, 1237-1243.

TOSTI, G., KIRKWOOD, J. M., HOOS, A., YUH, L., GUPTA, R. & SRIVASTAVA, P. K. 2008. Phase III Comparison of Vitespen, an Autologous Tumor-Derived Heat Shock Protein gp96 Peptide Complex Vaccine, With Physician's Choice of Treatment for Stage IV Melanoma: The C-100-21 Study Group. *Journal of Clinical Oncology*, *26*, 955-962.

TYLICKI, A., ŁOTOWSKI, Z., SIEMIENIUK, M. & RATKIEWICZ, A. 2018. Thiamine and selected thiamine antivitamins - biological activity and methods of synthesis. *Bioscience Reports, 38(1).*

UDOH, J., CARDWELL, K. & IKOTUN, T. 2000. Storage structures and aflatoxin content of maize in five agroecological zones of Nigeria. *Journal of Stored Products Research*, *36*, 187-201.

USAID. 2019. Climate risk profile Nigeria. Available at: https://www.climatelinks.org/sites/default/files/asset/document/2019_USAID-ATLAS-Nigeria-Climate-Risk-Profile.pdf.

VAN DER AUWERA, G. A., CARNEIRO, M. O., HARTL, C., POPLIN, R., DEL ANGEL, G., LEVY-MOONSHINE, A., JORDAN, T., SHAKIR, K., ROAZEN, D., THIBAULT, J., BANKS, E., GARIMELLA, K. V., ALTSHULER, D., GABRIEL, S. & DEPRISTO, M. A. 2013. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Current protocols in bioinformatics, 43(1), 11-10.*

VARASTEH, S., BRABER, S., AKBARI, P., GARSSEN, J. & FINK-GREMMELS, J. 2015. Differences in Susceptibility to Heat Stress along the Chicken Intestine and the Protective Effects of Galacto-Oligosaccharides. *PloS one*, *10*(9), e0138975.

VARGIU, L., RODRIGUEZ-TOMÉ, P., SPERBER, G. O., CADEDDU, M., GRANDI, N., BLIKSTAD, V., TRAMONTANO, E. & BLOMBERG, J. 2016. Classification and characterization of human endogenous retroviruses; mosaic forms are common. *Retrovirology,* 13, 7.

VASER, R., ADUSUMALLI, S. A.-O. H. O. O., LENG, S. N., SIKIC, M. A.-O. H. O. O. & NG, P. C. 2015. SIFT missense predictions for genomes. *Nature protocols*, *11*(1), 1-9.

VGP. 2022. NCBI Gallus gallus Annotation Release 106. https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Gallus_gallus/106/.

VIHERVAARA, A., SERGELIUS, C., VASARA, J., BLOM, M. A. H., ELSING, A. N., ROOS-MATTJUS, P. & SISTONEN, L. 2013. Transcriptional response to stress in the dynamic chromatin environment of cycling and mitotic cells. *Proceedings of the National Academy of Sciences*, *110*, E3388.

VOIGHT, B. F., KUDARAVALLI, S., WEN, X. & PRITCHARD, J. K. 2006. A Map of Recent Positive Selection in the Human Genome. *PLoS biology, 4(3), e72.*

WALUGEMBE, M., BERTOLINI, F., DEMATAWEWA, C. M. B., REIS, M. P., ELBELTAGY, A. R., SCHMIDT, C. J., LAMONT, S. J. & ROTHSCHILD, M. F. 2019. Detection of Selection Signatures Among Brazilian, Sri Lankan, and Egyptian Chicken Populations Under Different Environmental Conditions. *Frontiers in Genetics, 9,* 737.

WALUGEMBE, M., MUSHI, J. R., AMUZU-AWEH, E. N., CHIWANGA, G. H., MSOFFE, P. L., WANG, Y., SAELAO, P., KELLY, T., GALLARDO, R. A., ZHOU, H., LAMONT, S. J., MUHAIRWA, A. P. & DEKKERS, J. C. M. 2019. Genetic Analyses of Tanzanian Local Chicken Ecotypes Challenged with Newcastle Disease Virus. *Genes, 10,* 546.

WANG, M.-S., THAKUR, M., PENG, M.-S., JIANG, Y., FRANTZ, L. A. F., LI, M., ZHANG, J.-J., WANG, S., PETERS, J., OTECKO, N. O., SUWANNAPOOM, C., GUO, X., ZHENG, Z.-Q., ESMAILIZADEH, A., HIRIMUTHUGODA, N. Y., ASHARI, H., SULADARI, S., ZEIN, M. S. A., KUSZA, S., SOHRABI, S., KHARRATI-KOOPAEE, H., SHEN, Q.-K., ZENG, L., YANG, M.-M., WU, Y.-J., YANG, X.-Y., LU, X.-M., JIA, X.-Z., NIE, Q.-H., LAMONT, S. J., LASAGNA, E., CECCOBELLI, S., GUNWARDANA, H. G. T. N., SENASIGE, T. M., FENG, S.-H., SI, J.-F., ZHANG, H., JIN, J.-Q., LI, M.-L., LIU, Y.-H., CHEN, H.-M., MA, C., DAI, S.-S., BHUIYAN, A. K. F. H., KHAN, M. S., SILVA, G. L. L. P., LE, T.-T., MWAI, O. A., IBRAHIM, M. N. M., SUPPLE, M., SHAPIRO, B., HANOTTE, O., ZHANG, G., LARSON, G., HAN, J.-L., WU, D.-D. & ZHANG, Y.-P. 2020. 863 genomes reveal the origin and domestication of chicken. *Cell Research, 30,* 693-701.

WANG, C., LIU, Y., ZHANG, J., WANG, H. Y., WU, H. L., GONG, S. M. & HE, D. Q. 2014. Molecular cloning, expression and polymorphism of goose LRP8 gene. *British Poultry Science, 55,* 284-290.

WANG, G. & SEMENZA, G. L. 1995. Purification and Characterisation of Hypoxia-inducible Factor 1. *The Journal of Biological Chemistry, 270,* 1230 - 1237.

WANG, J., XU, L., SHAHEEN, S., LIU, S. & LIU, W. 2018. PI3K-and PTEN-Governed PIP 2 and PIP 3 Equilibrium Regulates the Activation and Function of B Cells Through Dock2 Controlled Growth of Antigen Receptor Microclusters. *Available at SSRN 3155621*.

WANG, J., XU, L., SHAHEEN, S., LIU, S., ZHENG, W., SUN, X., LI, Z. & LIU, W. 2017. Growth of B Cell Receptor Microclusters Is Regulated by PIP2 and PIP3 Equilibrium and Dock2 Recruitment and Activation. *Cell Reports, 21,* 2541-2557.

WANG, J.-W., REISER, M. A., LI, K., RIFKIN, E., WANG, B., KOLLIPUTI, N. & LOCKEY, R. F. 2014. LRBA causes immunodeficiency and autoimmunity by deregulating NFkB-mediated multiple immune effectors critical for B cell activation. *Journal of Allergy and Clinical Immunology, 133,* AB251.

WANG, M.-S., THAKUR, M., PENG, M.-S., JIANG, Y., FRANTZ, L. A. F., LI, M., ZHANG, J.-J., WANG, S., PETERS, J., OTECKO, N. O., SUWANNAPOOM, C., GUO, X., ZHENG, Z.-Q., ESMAILIZADEH, A., HIRIMUTHUGODA, N. Y., ASHARI, H., SULADARI, S., ZEIN, M. S. A., KUSZA, S., SOHRABI, S., KHARRATI-KOOPAEE, H., SHEN, Q.-K., ZENG, L., YANG, M.-M., WU, Y.-J., YANG, X.-Y., LU, X.-M., JIA, X.-Z., NIE, Q.-H., LAMONT, S. J., LASAGNA, E., CECCOBELLI, S., GUNWARDANA, H. G. T. N., SENASIGE, T. M., FENG, S.-H., SI, J.-F., ZHANG, H., JIN, J.-Q., LI, M.-L., LIU, Y.-H., CHEN, H.-M., MA, C., DAI, S.-S., BHUIYAN, A. K. F. H., KHAN, M. S.,

SILVA, G. L. L. P., LE, T.-T., MWAI, O. A., IBRAHIM, M. N. M., SUPPLE, M., SHAPIRO, B., HANOTTE, O., ZHANG, G., LARSON, G., HAN, J.-L., WU, D.-D. & ZHANG, Y.-P. 2020. 863 genomes reveal the origin and domestication of chicken. *Cell Research, 30*, 693-701.

WANG, R. E., KAO, J. L. F., HILLIARD, C. A., PANDITA, R. K., ROTI ROTI, J. L., HUNT, C. R. & TAYLOR, J.-S. 2009. Inhibition of heat shock induction of heat shock protein 70 and enhancement of heat shock protein 27 phosphorylation by quercetin derivatives. *Journal of medicinal chemistry, 52*, 1912-1921.

WANG, S.-H., CHENG, C.-Y., TANG, P.-C., CHEN, C.-F., CHEN, H.-H., LEE, Y.-P. & HUANG, S.-Y. 2015. Acute Heat Stress Induces Differential Gene Expressions in the Testes of a Broiler-Type Strain of Taiwan Country Chickens. *PLoS One, 10(5), e0125816.*

WANG, X. & BYERS, S. 2014. Copy Number Variation in Chickens: A Review and Future Prospects. *Microarrays, 3(1), 24-38.*

WANG, Z., DING, G., CHEN, G., SUN, Y., SUN, Z., ZHANG, H., WANG, L., HASI, S., ZHANG, Y. & LI, J. 2012. Genome sequences of wild and domestic bactrian camels. *Nature Communications*, 3, 1202.

WANI, C. E., YOUSIF, I. A., IBRAHIM, M. E. & MUSA, H. H. 2014. Molecular Characterisation of Sudanese and Southern Sudanese Chicken Breeds Using mtDNA D-Loop. *Genetics research international, 2014.*

WARNER, A., RAHMAN, A., SOLSJÖ, P., GOTTSCHLING, K., DAVIS, B., VENNSTRÖM, B., ARNER, A. & MITTAG, J. 2013. Inappropriate heat dissipation ignites brown fat thermogenesis in mice with a mutant thyroid hormone receptor α1. *Proceedings of the National Academy of Sciences, 110*, 16241-16246.

WARREN, W. C., HILLIER, L. W., TOMLINSON, C., MINX, P., KREMITZKI, M., GRAVES, T., MARKOVIC, C., BOUK, N., PRUITT, K. D., THIBAUD-NISSEN, F., SCHNEIDER, V., MANSOUR, T. A., BROWN, C. T., ZIMIN, A., HAWKEN, R., ABRAHAMSEN, M., PYRKOSZ, A. B., MORISSON, M., FILLON, V., VIGNAL, A., CHOW, W., HOWE, K., FULTON, J. E., MILLER, M. M., LOVELL, P., MELLO, C. V., WIRTHLIN, M., MASON, A. S., KUO, R., BURT, D. W., DODGSON, J. B. & CHENG, H. H. 2017. A New Chicken Genome Assembly Provides Insight into Avian Genome Structure. *G3: Genes, Genomes, Genetics, 7(1), 109-117*.

WASHINGTON PHILIPSON, C., HELTZEL, C., EDEN, K., HONTECILLAS, R. & BASSAGANYA-RIERA, J. 2015. Immune modulatory mechanisms of lanthionine synthetase C-like protein 2 (IRM12P.656). *The Journal of Immunology, 194*, 133.15-133.15.

WEIGAND, H. & LEESE, F. 2018. Detecting signatures of positive selection in non-model species using genomic data. *Zoological Journal of the Linnean Society*, *184*, 528-583.

WEIR, B. S. & COCKERHAM, C. C. 1984. Estimating F-Statistics for the Analysis of Population Structure. *Evolution, 38*, 1358-1370.

WENG, Z., XU, Y., LI, W., CHEN, J., ZHONG, M., ZHONG, F., DU, B., ZHANG, B. & HUANG, X. 2020. Genomic variations and signatures of selection in Wuhua yellow chicken. *PLoS One, 15(10), e0241137.*

WHITLEY, D., GOLDBERG, S. P. & JORDAN, W. D. 1999. Heat shock proteins: A review of the molecular chaperones. *Journal of Vascular Surgery, 29*, 748-751.

WHITTINGHAM, M. J. & EVANS, K. L. 2004. The effects of habitat structure on predation risk of birds in agricultural landscapes. *Ibis, 146*, 210-220.

WIGLEY, P. & KAISER, P. 2003. Avian cytokines in health and disease. *Brazilian Journal of Poultry Science, 5*, 1-14.

WILKINSON, S., HALEY, C., ALDERSON, L. & WIENER, P. 2011. An empirical assessment of individual-based population genetic statistical techniques: application to British pig breeds. *Heredity, 106*, 261-269.

WILLIAMS, S. E., WOOTTON, P., MASON, H. S., BOULD, J., ILES, D. E., RICCARDI, D., PEERS, C. & KEMP, P. J. 2004. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. *Science, 306*, 2093-2097.

WOLLENBERG VALERO, K. C., PATHAK, R., PRAJAPATI, I., BANKSTON, S., THOMPSON, A., USHER, J. & ISOKPEHI, R. D. 2014. A candidate multimodal functional genetic network for thermal adaptation. *PeerJ, 2*, e578.

WONG, G. K., LIU, B., WANG, J., ZHANG, Y., YANG, X., ZHANG, Z., MENG, Q., ZHOU, J., LI, D., ZHANG, J., NI, P., LI, S., RAN, L., LI, H., ZHANG, J., LI, R., LI, S., ZHENG, H., LIN, W., LI, G., WANG, X., ZHAO, W., LI, J., YE, C., DAI, M., RUAN, J., ZHOU, Y., LI, Y., HE, X., ZHANG, Y., WANG, J., HUANG, X., TONG, W., CHEN, J., YE, J., CHEN, C., WEI, N., LI, G., DONG, L., LAN, F., SUN, Y., ZHANG, Z., YANG, Z., YU, Y., HUANG, Y., HE, D., XI, Y., WEI, D., QI, Q., LI, W., SHI, J., WANG, M., XIE, F., WANG, J., ZHANG, X., WANG, P., ZHAO, Y., LI, N., YANG, N., DONG, W., HU, S., ZENG, C., ZHENG, W., HAO, B., HILLIER, L. W., YANG, S. P., WARREN, W. C., WILSON, R. K., BRANDSTROM, M., ELLEGREN, H., CROOIJMANS, R. P., VAN DER POEL, J. J., BOVENHUIS, H., GROENEN, M. A., OVCHARENKO, I., GORDON, L., STUBBS, L., LUCAS, S., GLAVINA, T., AERTS, A., KAISER, P., ROTHWELL, L., YOUNG, J. R., ROGERS, S., WALKER, B. A., VAN HATEREN, A., KAUFMAN, J., BUMSTEAD, N., LAMONT, S. J., ZHOU, H., HOCKING, P. M., MORRICE, D., DE KONING, D. J., LAW, A., BARTLEY, N., BURT, D. W., HUNT, H., CHENG, H. H., GUNNARSSON, U., WAHLBERG, P., et al. 2004. A genetic variation map for chicken with 2.8 million single-nucleotide polymorphisms. *Nature, 432*, 717-22.

WRAGG, D., MWACHARO, J. M., ALCALDE, J. A., HOCKING, P. M. & HANOTTE, O. 2012. Analysis of genome-wide structure, diversity and fine mapping of Mendelian traits in traditional and village chickens. *Heredity (Edinb), 109*, 6-18.

WRIGHT, S. 1949. The Genetical Structure Of Populations. *Annals of Eugenics, 15*, 323-354.

XIE, J., TANG, L., LU, L., ZHANG, L., XI, L., LIU, H. C., ODLE, J. & LUO, X. 2014. Differential expression of heat shock transcription factors and heat shock proteins after acute and chronic heat stress in laying chickens (Gallus gallus). *PloS one, 9*(7), e102204.

XIE, S., YANG, X., WANG, D., ZHU, F., YANG, N., HOU, Z. & NING, Z. 2018. Thyroid transcriptome analysis reveals different adaptive responses to cold environmental conditions between two chicken breeds. *PloS one, 13*, e0191096.

XING, Z., CARDONA, C. J., ADAMS, S., YANG, Z., LI, J., PEREZ, D. & WOOLCOCK, P. R. 2009. Differential regulation of antiviral and proinflammatory cytokines and suppression of Fas-mediated apoptosis by NS1 of H9N2 avian influenza virus in chicken macrophages. *Journal of general virology, 90*, 1109-1118.

YADAV A. K., T. S. S., JHA A.K. AND SINGH J. 2017. Importance of Molecular Markers in Livestock Improvement: A Review. *International Journal of Agriculture Innovations and Research, 5*, 614-621.

YAKUBU, A., BAMIDELE, O., HASSAN, W. A., AJAYI, F. O., OGUNDU, U. E., ALABI, O., SONAIYA, E. B. & ADEBAMBO, O. A. 2020. Farmers' choice of genotypes and trait preferences in tropically adapted chickens in five agro-ecological zones in Nigeria. *Tropical Animal Health and Production, 52,* 95-107.

YAKUBU, A., DAHLOUM, L. & GIMBA, E. G. 2019. Smallholder cattle farmers' breeding practices and trait preferences in a tropical Guinea savanna agro-ecological zone. *Tropical Animal Health and Production, 51,* 1497-1506.

YANG, J., LI, W.-R., LV, F.-H., HE, S.-G., TIAN, S.-L., PENG, W.-F., SUN, Y.-W., ZHAO, Y.-X., TU, X.-L. & ZHANG, M. 2016. Whole-genome sequencing of native sheep provides insights into rapid adaptations to extreme environments. *Molecular biology and evolution, 33,* 2576-2592.

YANG, S., SHI, Z., OU, X. & LIU, G. 2019. Whole-genome resequencing reveals genetic indels of feathered-leg traits in domestic chickens. *Journal of Genetics, 98,* 1-8.

YAN Y, YI G, SUN C, QU L, YANG N. 2014. Genome-Wide Characterization of Insertion and Deletion Variation in Chicken Using Next Generation Sequencing. *PLOS ONE 9(8*): e104652

YOSHIMURA, T., YASUO, S., WATANABE, M., IIGO, M., YAMAMURA, T., HIRUNAGI, K. & EBIHARA, S. 2003. Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. *Nature, 426,* 178-181.

YOU, Z., ZHANG, Q., LIU, C., SONG, J., YANG, N. & LIAN, L. 2019. Integrated analysis of lncRNA and mRNA repertoires in Marek's disease infected spleens identifies genes relevant to resistance. *BMC Genomics, 20,* 245.

YUAN, J., SUN, C., DOU, T., YI, G., QU, L., QU, L., WANG, K. & YANG, N. 2015. Identification of Promising Mutants Associated with Egg Production Traits Revealed by Genome-Wide Association Study. *PLoS One, 10(10), e0140615.*

ZHAN, F. 2013. Identification and characterization of long intergenic noncoding RNAs associated with Marek's disease resistance in chicken (Doctoral dissertation).

ZHANG, C., DONG, S.-S., XU, J.-Y., HE, W.-M. & YANG, T.-L. 2018. PopLDdecay: a fast and effective tool for linkage disequilibrium decay analysis based on variant call format files. *Bioinformatics, 35,* 1786-1788.

ZHANG, M., HAN, W., TANG, H., LI, G., ZHANG, M., XU, R., LIU, Y., YANG, T., LI, W., ZOU, J. & WU, K. 2018. Genomic diversity dynamics in conserved chicken populations are revealed by genome-wide SNPs. *BMC Genomics, 19,* 598.

ZHANG, Q., GOU, W., WANG, X., ZHANG, Y., MA, J., ZHANG, H., ZHANG, Y. & ZHANG, H. 2016. Genome resequencing identifies unique adaptations of Tibetan chickens to hypoxia and high-dose ultraviolet radiation in high-altitude environments. *Genome biology and evolution, 8,* 765-776.

ZHONG, J., WANG, Y., QIU, X., MO, X., LIU, Y., LI, T., SONG, Q., MA, D. & HAN, W. 2006. Characterization and expression profile of CMTM3/CKLFSF3. *Journal of biochemistry and molecular biology, 39,* 537-545.

ZHUANG, Z.-X., CHEN, S.-E., CHEN, C.-F., LIN, E.-C. & HUANG, S.-Y. 2020. Genomic regions and pathways associated with thermotolerance in layer-type strain Taiwan indigenous chickens. *Journal of Thermal Biology, 88,* 102486.

ZIMMERMANN, K. C., BONZON, C. & GREEN, D. R. 2001. The machinery of programmed cell death. *Pharmacology & Therapeutics, 92,* 57-70.

ZININGA, T., RAMATSUI, L. & SHONHAI, A. 2018. Heat Shock Proteins as Immunomodulants. *Molecules, 23*(11), 2846.

ZITTA, W. S. & MADAKI, D. H. 2020. Research Article Climate Change, Rainfall Trends and Variability in Jos Plateau. *Journal of Applied Sciences, 20,* 76-82.

Supplementary Tables

Chapter 2

Table S2.1 | Pairwise Population fixation (*FST*) values among 14 chicken populations (**file is provided in an electronic format**).

Table S2.2 | List of missense variants present in high frequency (**file is provided in an electronic format**).

Table S2.3 | List of putatively functional variants with overlapped genes (unique) that present in high frequency (**file is provided in an electronic format**).

Table S2.4 | Functional annotation for the biological process of genes overlapped with missense (present in high allele frequency) **(file is provided in an electronic format**).

Table S2.5 | Functional annotation for the molecular function of genes overlapped with missense (present in high allele frequency) (**file is provided in an electronic format**).

Table S2.6 | Functional annotation for the cellular response of genes overlapped with missense (present in high allele frequency) (**file is provided in an electronic format**).

Table S2.7 | Functional annotation for the pathway of genes overlapped with missense (present in high allele frequency) (**file is provided in an electronic format**).

Table S2.7 | Functional annotation for the pathway of genes overlapped with missense (present in high allele frequency) (**file is provided in an electronic format**).

Table S2.8 | Functional annotation for the pathway of genes overlapped with missense (present in high allele frequency) **(file is provided in an electronic format**).

Table S2.9 | GO for the biological process of overlapped genes with missense deleterious that present in high AF based on DAVID annotation web tool **(file is provided in an electronic format**).

Table S2.10 | KEGG pathway of overlapped genes with missense deleterious that present in high AF based on DAVID annotation web tool **(file is provided in an electronic format**).

Table S2.11 | Enrichment analysis of genes overlap with missense deleterious that present in high AF based on DAVID functional annotation web tool **(file is provided in an electronic format**).

Chapter 3

Table S3.1 | Genome-wide pool heterozygosity statistics for this study (**file is provided as an electronic format**).

Table S3.2 | SNPs annotation of hot region, high and low precipitation population (**file is provided as an electronic format**).

Table S3.3 | List of selected genome regions and related candidate genes in eighty-seven Nigerian indigenous chickens based on *ZHp* test (**file is provided as an electronic format**).

Table S3.4 | List of nearest genes related to heat stress and overlapped chicken QTL to the candidate selected region in eighty-seven Nigerian indigenous chickens based on *ZHp* test (**file is provided as an electronic format**).

Table S3.5 | Annotation of detected high allelic non-synonymous SNPs based on Ensembl gene database (based on *Hp* analysis of eighty-seven Nigeria indigenous chicken (**file is provided as an electronic format**).

Table S3.6 | List of genes overlapped with non-synonymous variants at high frequency in 87 chickens (**file is provided as an electronic format**).

Table S3.7 | Top 0.1% windows based on Weir and Crockman Fst analysis between High *versus* Low precipitation groups (**file is provided as an electronic format**).

Table S3.8 | Putatively selected genes (top 0.1 % level Fst values) (**file is provided as an electronic format**).

Table S3.9 | Functional annotation of the candidate genes based on Fst analysis between High *versus* Low precipitation groups using DAVID annotation web tool (**file is provided as an electronic format**).

Table S3.10 | List of selected genome regions and related candidate genes based on *ZHp* test (*ZHp* ≤ -4.0) in high precipitation group **(file is provided as an electronic format**).

Table S3.11 | Functional annotation for high precipitation group based on Hp analysis based on DAVID annotation web tool **(file is provided as an electronic format**).

Table S3.12 | List of selected genome regions and related candidate genes based on *ZHp* test (*ZHp* ≤ -4.0) in the low precipitation group **(file is provided as an electronic format**).

Table S3.13 | Functional annotation for low precipitation group based on Hp analysis based on DAVID annotation web tool **(file is provided as an electronic format**).

Table S.3.14 | Overlapped of the candidate selected region based on Fst analysis with the value based on Hp/*ZHp* analysis in each group **(file is provided as an electronic format).**

Chapter 4

Table S4.1 | List of sweep region and overlapped genes in Sudan savanna ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.2 | List of sweep region and overlapped genes in Northern guinea savanna ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.3 | List of sweep region and overlapped genes in Southern guinea savanna ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.4 | List of sweep region and overlapped genes in Derived guinea savanna ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.5 | List of sweep region and overlapped genes in Mid-altitude (plateau) ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.6 | List of sweep region and overlapped genes in the Rainforest ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.7 | List of sweep region and overlapped genes in the Freshwater swamp ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.8 | List of sweep region and overlapped genes in the Mangrove ecotype based on *ZHp* test (**file is provided as an electronic format**)

Table S4.9 | Shared genes identified in all warm-arid and warm semi-arid ecotypes (Sudan savanna, Northern Guinea savanna, Southern Guinea savanna, Derived savanna)

Gene ID	Gene name (Description)	SSN	NGS	SGN	DSN	Total
ENSGALG00000047413	*Novel (lncRNA)*	Yes	Yes	Yes	Yes	4
ENSGALG00000010572	*TSHR thyroid stimulating hormone receptor [Source:NCBI gene;Acc:428900]*	Yes	Yes	Yes	Yes	4
ENSGALG00000054958	*Novel / MEOX, AGMO (nearby gene)*	Yes	No	Yes	Yes	3
ENSGALG00000011930	*OVSTL ovostatin-like [Source:NCBI gene;Acc:425757]*	No	Yes	Yes	Yes	3
ENSGALG00000039224	*HHLA1 HERV-H LTR-associating 1 [Source:NCBI gene;Acc:101749300]*	Yes	Yes	No	No	2
ENSGALG00000027900	*5S_rRNA / MEOX, AGMO (nearby gene)*	Yes	No	No	Yes	2
ENSGALG00000039533	*EFR3A EFR3 homolog A [Source:NCBI gene;Acc:420327]*	No	Yes	Yes	No	2
ENSGALG00000030128	*Novel*	No	Yes	Yes	No	2
ENSGALG00000002080	*DOCK2 dedicator of cytokinesis 2 [Source:NCBI gene;Acc:427612]*	No	Yes	No	Yes	2
ENSGALG00000016602	*ARHGAP6 Rho GTPase activating protein 6 [Source:NCBI gene;Acc:418642]*	No	Yes	No	Yes	2
ENSGALG00000022875	*Novel*	No	Yes	No	Yes	2
Total shared genes		5	9	6	8	

*SSN: Sudan Savanna, NGS: Northern Guinea Savanna, SGN: Southern Guinea Savanna, DSN: Derived Savanna

Table S4.10 | Shared genes identified in all warm-humid ecotypes (Humid Rainforest, freshwater swamp, and Mangrove swamp)

Gene ID	Gene name (Description)	HRF	HFW	HSM	Total
ENSGALG00000047413	*Novel (lncRNA)*	Yes	Yes	Yes	3
ENSGALG00000010572	*TSHR thyroid stimulating hormone receptor [Source:NCBI gene;Acc:428900]*	Yes	Yes	Yes	3
ENSGALG00000014425	*NCAPG non-SMC condensin I complex subunit G [Source:NCBI gene;Acc:422822]*	Yes	Yes	No	2
ENSGALG00000052351	*Novel*	Yes	Yes	No	2
ENSGALG00000014421	*LCORL ligand dependent nuclear receptor corepressor like [Source:NCBI gene;Acc:422820]*	Yes	Yes	No	2
ENSGALG00000041635	*CAMK2D calcium/calmodulin dependent protein kinase II delta [Source:NCBI gene;Acc:422688]*	No	Yes	Yes	2
ENSGALG00000028001	*COMMD2 COMM domain containing 2 [Source:NCBI gene;Acc:425044]*	No	Yes	Yes	2
ENSGALG00000048323	*Novel*	No	Yes	Yes	2
ENSGALG00000010427	*TM4SF4 transmembrane 4 L six family member 4 [Source:NCBI gene;Acc:771806]*	No	Yes	Yes	2
ENSGALG00000010412	*WWTR1 WW domain containing transcription regulator 1 [Source:NCBI gene;Acc:100859902])*	No	Yes	Yes	2
ENSGALG00000012129	*CCDC93 coiled-coil domain containing 93 [Source:NCBI gene;Acc:424277]*	No	Yes	Yes	2
ENSGALG00000027591	*TSC22D2 TSC22 domain family member 2 [Source:NCBI gene;Acc:100859827]*	No	Yes	Yes	2
ENSGALG00000010411	*RNF13 ring finger protein 13 [Source:NCBI gene;Acc:396303]*	No	Yes	Yes	2
Total shared genes		5	13	10	

*HRF: Humid Rainforest, HFW: Humid Freshwater swamp, HSM: Humid Savanna Mangrove

Table S4.11 | Shared genes identified in all warm-arid and warm-humid ecotypes

Gene ID	Gene name (Description)	SSN	NGS	HRF	HFW	HSM	Total
ENSGALG00000010572	*TSHR thyroid stimulating hormone receptor [Source:NCBI gene;Acc:428900]*	Yes	Yes	Yes	Yes	Yes	5
ENSGALG00000047413	*Novel (lncRNA)*	Yes	Yes	Yes	Yes	Yes	5
ENSGALG00000014425	*NCAPG non-SMC condensin I complex subunit G [Source:NCBI gene;Acc:422822]*	No	Yes	Yes	Yes	No	3
ENSGALG00000014421	*LCORL ligand dependent nuclear receptor corepressor like [Source:NCBI gene;Acc:422820]*	No	Yes	Yes	Yes	No	3
ENSGALG00000012129	*CCDC93 coiled-coil domain containing 93 [Source:NCBI gene;Acc:424277]*	No	Yes	No	Yes	Yes	2
ENSGALG00000049908	*Novel*	No	Yes	Yes	No	No	2
ENSGALG00000002080	*DOCK2 dedicator of cytokinesis 2 [Source:NCBI gene;Acc:427612]*	No	Yes	Yes	No	No	2
ENSGALG00000011930	*OVSTL ovostatin-like [Source:NCBI gene;Acc:425757]*	No	Yes	No	Yes	No	2
ENSGALG00000026901	*Novel (lncRNAs)*	No	Yes	No	Yes	No	2
Total shared genes		2	9	6	7	3	

* SSN: Sudan Savanna, NGS: Northern Guinea Savanna, HRF: Humid Rainforest, HFW: Humid Freshwater swamp, HSM: Humid Savanna Mangrove

Table S4.12 | Shared genes identified in all warm semi-arid and warm-humid ecotypes

Gene ID	**Gene name (Description)**	**SGS**	**DSN**	**HRF**	**HFW**	**HSM**	**Total**
ENSGALG00000010572	*TSHR* thyroid stimulating hormone receptor [Source:NCBI gene;Acc:428900]	Yes	Yes	Yes	Yes	Yes	5
ENSGALG00000047413	Novel (*lncRNA*)	Yes	Yes	Yes	Yes	Yes	5
ENSGALG00000017002	*FNDC3A* fibronectin type III domain containing 3A [Source:NCBI gene;Acc:418863]	Yes	No	No	Yes	No	2
ENSGALG00000002080	*DOCK2* dedicator of cytokinesis 2 [Source:NCBI gene;Acc:427612]	No	Yes	Yes	No	No	2
ENSGALG00000011930	*OVSTL* ovostatin-like [Source:NCBI gene;Acc:425757]	Yes	Yes	No	Yes	No	2
ENSGALG00000026901	*Novel (lncRNAs)*	No	Yes	No	Yes	No	2
ENSGALG00000037014	*TSNARE1 t-SNARE domain containing 1 [Source:NCBI gene;Acc:420304]*	Yes	No	No	Yes	No	2
ENSGALG00000009107	*NRXN1 neurexin 1 [Source:NCBI gene;Acc:395398]*	No	Yes	No	No	Yes	2
Total shared genes		5	6	3	6	3	

*SGS: Southern Guinea savanna, DSN: Derived savanna, HRF: Humid Rainforest, HFW: Humid Freshwater swamp, HSM: Humid Savanna Mangrove

Table S4.13 | Shared genes identified in Mid-altitude and All Warm arid, Warm semi-arid ecotypes

Gene ID	Gene name (Description)	MA	SSN	NGS	SGS	DSN	Total
ENSGALG00000010572	*TSHR* thyroid stimulating hormone receptor [Source:NCBI gene;Acc:428900]	Yes	Yes	Yes	Yes	Yes	5
ENSGALG00000047413	Novel (*lncRNA*)	Yes	Yes	Yes	Yes	Yes	5
ENSGALG00000011930	*OVSTL* ovostatin-like [Source:NCBI gene;Acc:425757]	Yes	No	Yes	Yes	Yes	4
ENSGALG00000039224	*HHLA1* HERV-H LTR-associating 1 [Source:NCBI gene;Acc:101749300]	Yes	Yes	Yes	No	No	3
ENSGALG00000022875	*MOG* *myelin oligodendrocyte glycoprotein [Source:NCBI gene;Acc:421050]*	Yes	No	Yes	No	Yes	3
ENSGALG00000014425	*NCAPG* non-SMC condensin I complex subunit G [Source:NCBI gene;Acc:422822]	Yes	No	Yes	No	No	2
ENSGALG00000026901	Novel	Yes	No	Yes	No	No	2
ENSGALG00000014421	*LCORL* ligand dependent nuclear receptor corepressor like [Source:NCBI gene;Acc:422820]	Yes	No	Yes	No	No	2
ENSGALG00000017002	*FNDC3A* fibronectin type III domain containing 3A [Source:NCBI gene;Acc:418863]	Yes	No	No	Yes	No	2
ENSGALG00000037014	*TSNARE1* t-SNARE domain containing 1 [Source:NCBI gene;Acc:420304]	Yes	No	No	Yes	No	2
Total shared genes with Mid-altitude ecotype		10	3	8	5	4	

*MA: Mid-altitude (Plateau), SSN: Sudan Savanna, NGS: Northern Guinea Savanna, SGN: Southern Guinea Savanna, DSN: Derived Savanna

Table S4.13 | Shared genes identified in Mid-altitude and warm humid ecotypes

Gene ID	Gene name	MA	HRF	HFS	HSM	Total
ENSGALG00000010572	*TSHR* thyroid stimulating hormone receptor [Source:NCBI gene;Acc:428900]	Yes	Yes	Yes	Yes	4
ENSGALG00000047413	Novel (*lncRNA*)	Yes	Yes	Yes	Yes	4
ENSGALG00000014425	*NCAPG* non-SMC condensin I complex subunit G [Source:NCBI gene;Acc:422822]	Yes	Yes	Yes	No	3
ENSGALG00000052351	Novel	Yes	Yes	Yes	No	3
ENSGALG00000014421	*LCORL* ligand dependent nuclear receptor corepressor like [Source:NCBI gene;Acc:422820]	Yes	Yes	Yes	No	3
ENSGALG00000053797	Novel	Yes	Yes	No	No	2
ENSGALG00000011930	*OVSTL* ovostatin-like [Source:NCBI gene;Acc:425757]	Yes	No	Yes	No	2
ENSGALG00000052961	Novel	Yes	No	Yes	No	2
ENSGALG00000017002	*FNDC3A* fibronectin type III domain containing 3A [Source:NCBI gene;Acc:418863]	Yes	No	Yes	No	2
ENSGALG00000026901	Novel	Yes	No	Yes	No	2
ENSGALG00000047983	Novel	Yes	No	Yes	No	2
ENSGALG00000037014	*TSNARE1* t-SNARE domain containing 1 [Source:NCBI gene;Acc:420304]	Yes	No	Yes	No	2
ENSGALG00000014485	*LDB2* LIM domain binding 2 [Source:NCBI gene;Acc:395631]	Yes	No	Yes	No	2
Total shared genes with Mid-altitude ecotype		13	6	12	2	

*MA: Mid-altitude (Plateau), HRF: Humid Rainforest, HFW: Humid Freshwater swamp, HSM: Humid Savanna Mangrove

Table S4.14 | Mean weighted *FST* between ecotypes **(file is provided as an electronic format)**

Table S4.15 | Range of weighted *FST* and *ZFST* value in the top 1% signature selection windows for all ecotypes comparison **(file is provided as an electronic format)**

Table S4.16 | Total number of signature selection windows in the top 1% overlapped with genes and non-overlapped with genes **(file is provided as an electronic format)**

Table S4.17 | Total number of signature selection windows with weighted $F_{ST} \geq 0.3$ and $ZF_{ST} \geq 7.0$ for all ecotypes comparison **(file is provided as an electronic format)**

Table S4.18 | Candidate sweep regions ($ZF_{ST} \geq 7.0$) between Sudan Savanna *versus* others **(file is provided as an electronic format)**

Table S4.19 | Sweep regions ($ZF_{ST} \geq 7.0$) between Northern Guinea Savanna *versus* others **(file is provided as an electronic format)**

Table S4.20 | Sweep regions ($ZF_{ST} \geq 7.0$) between Southern Guinea Savanna *versus* others **(file is provided as an electronic format)**

Table S4.21 | Sweep regions ($ZF_{ST} \geq 7.0$) between Derived Savanna *versus* others **(file is provided as an electronic format)**

Table S4.22 | Sweep regions ($ZF_{ST} \geq 7.0$) between Mid-Altitude a *versus* others **(file is provided as an electronic format)**

Table S4.23 | Sweep regions ($ZF_{ST} \geq 7.0$) between Rainforest *versus* others **(file is provided as an electronic format)**

Table S4.23 | Sweep regions ($ZF_{ST} \geq 7.0$) between Freshwater swamp *versus* Mangrove **(file is provided as an electronic format)**

S4.25 | Major selective genes concerning the morphological, physiological and behavioral signatures in eight ecotypes, identified from both *ZFST* and *ZHp* **(file is provided as an electronic format)**

S4.26 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Northern guinea savanna **(file is provided as an electronic format)**

S4.27 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Southern guinea savanna **(file is provided as an electronic format)**

S4.28 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Derived savanna **(file is provided as an electronic format)**

S4.29 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Mid-Altitude **(file is provided as an electronic format)**

S4.30 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Rainforest **(file is provided as an electronic format)**

S4.31 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Freshwater swamp **(file is provided as an electronic format)**

S4.32 | List of top 1% windows based on *ZFST* analysis from Sudan savanna *versus* Mangrove swamp **(file is provided as an electronic format)**

S4.33 | List of top 1% windows based on *ZFST* analysis from Northern guinea savanna *versus* Southern guinea savanna **(file is provided as an electronic format)**

S4.34 | List of top 1% windows based on *ZFST* analysis from Northern guinea savanna *versus* Derived savanna **(file is provided as an electronic format)**

S4.35 | List of top 1% windows based on *ZFST* analysis from Northern guinea savanna *versus* Mid-Altitude **(file is provided as an electronic format)**

S4.36 | List of top 1% windows based on *ZFST* analysis from Northern guinea savanna *versus* Rainforest **(file is provided as an electronic format)**

S4.37 | List of top 1% windows based on *ZFST* analysis from Northern guinea savanna *versus* Freshwater swamp **(file is provided as an electronic format)**

S4.38 | List of top 1% windows based on *ZFST* analysis from Northern guinea savanna *versus* Mangrove swamp **(file is provided as an electronic format)**

S4.39 | List of top 1% windows based on *ZFST* analysis from Southern guinea savanna *versus* Derived savanna **(file is provided as an electronic format)**

S4.40 | List of top 1% windows based on *ZFST* analysis from Southern guinea savanna *versus* Mid-Altitude **(file is provided as an electronic format)**

S4.41 | List of top 1% windows based on *ZFST* analysis from Southern guinea savanna *versus* Rainforest **(file is provided as an electronic format)**

S4.42 | List of top 1% windows based on *ZFST* analysis from Southern guinea savanna *versus* Freshwater swamp **(file is provided as an electronic format)**

S4.43 | List of top 1% windows based on *ZFST* analysis from Southern guinea savanna *versus* Mangrove swamp **(file is provided as an electronic format)**

S4.44 | List of top 1% windows based on *ZFST* analysis from Derived savanna *versus* Mid-Altitude **(file is provided as an electronic format)**

S4.45 | List of top 1% windows based on *ZFST* analysis from Derived savanna *versus* Rainforest **(file is provided as an electronic format)**

S4.46 | List of top 1% windows based on *ZFST* analysis from Derived savanna *versus* Freshwater swamp **(file is provided as an electronic format)**

S4.47 | List of top 1% windows based on *ZFST* analysis from Derived savanna *versus* Mangrove swamp **(file is provided as an electronic format)**

S4.48 | List of top 1% windows based on *ZFST* analysis from Mid-Altitude *versus* Rainforest **(file is provided as an electronic format)**

S4.49 | List of top 1% windows based on *ZFST* analysis from Mid-Altitude *versus* Freshwater swamp **(file is provided as an electronic format)**

S4.50 | List of top 1% windows based on *ZFST* analysis from Mid-Altitude *versus* Mangrove **(file is provided as an electronic format)**

S4.51 | List of top 1% windows based on *ZFST* analysis from Rainforest *versus* Freshwater swamp **(file is provided as an electronic format)**

S4.52 | List of top 1% windows based on *ZFST* analysis from Rainforest *versus* Mangrove swamp **(file is provided as an electronic format)**

S4.53 | List of top 1% windows based on *ZFST* analysis from Freshwater swamp *versus* Mangrove swamp **(file is provided as an electronic format)**

Supplementary Figures

Chapter 3

Figure S3.1 | The proportion (%) of variant categories based on 92,000 SNPs in eighty-seven Nigerian indigenous chicken (warm region population)

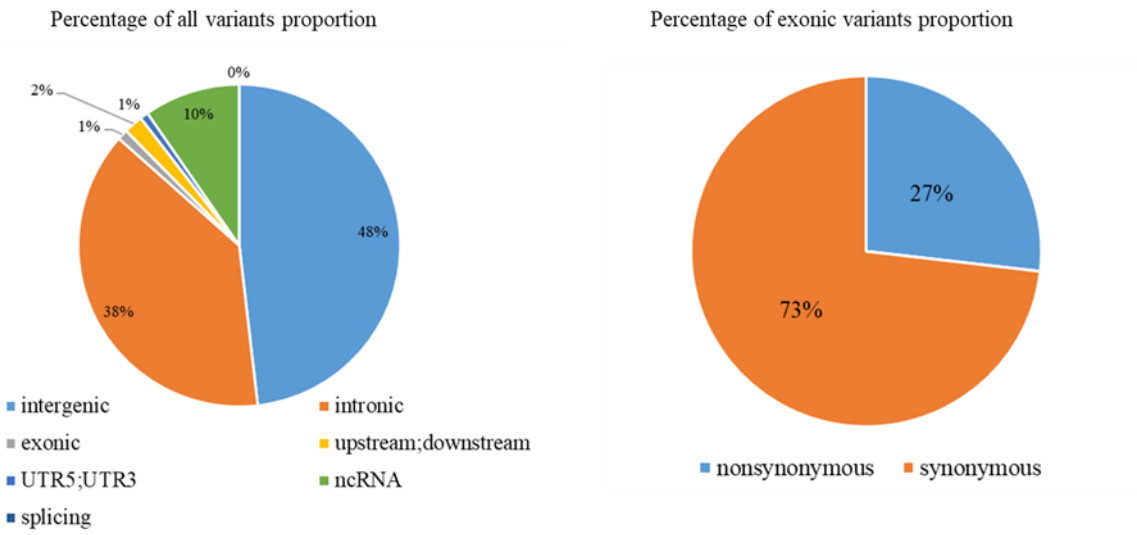


Figure S3.2 | Histogram of *Hp* and *ZHp* values in eighty-seven Nigerian indigenous chickens (warm region population)

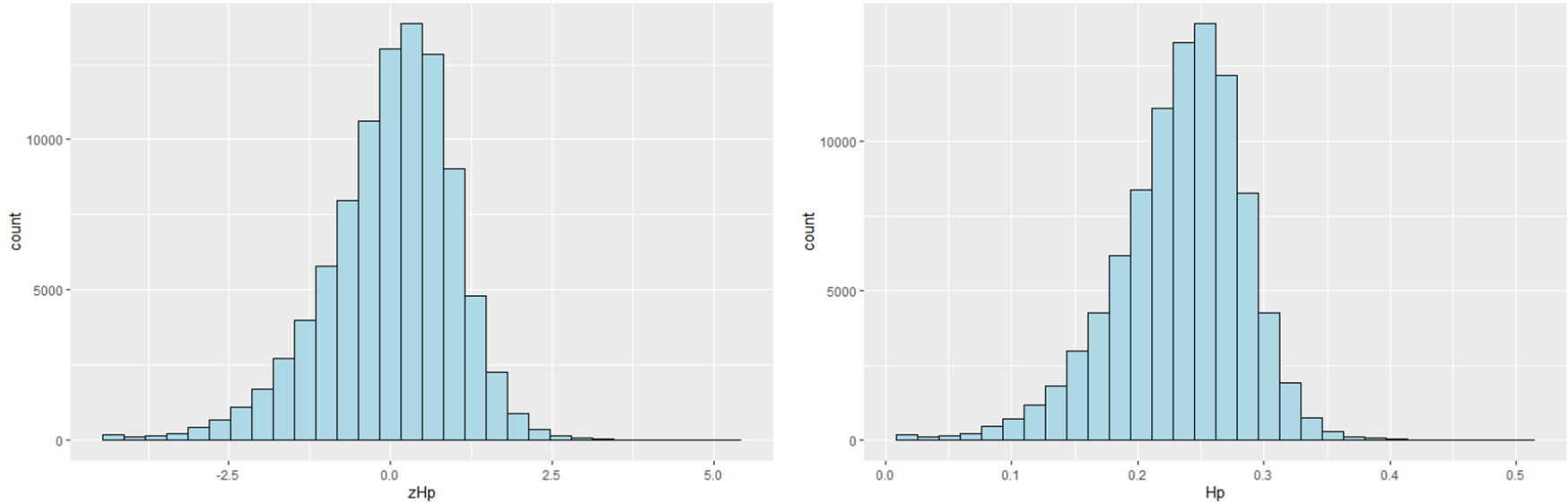


Figure S3.3 | Histogram of *FST* and *ZFST* high precipitation vs low precipitation

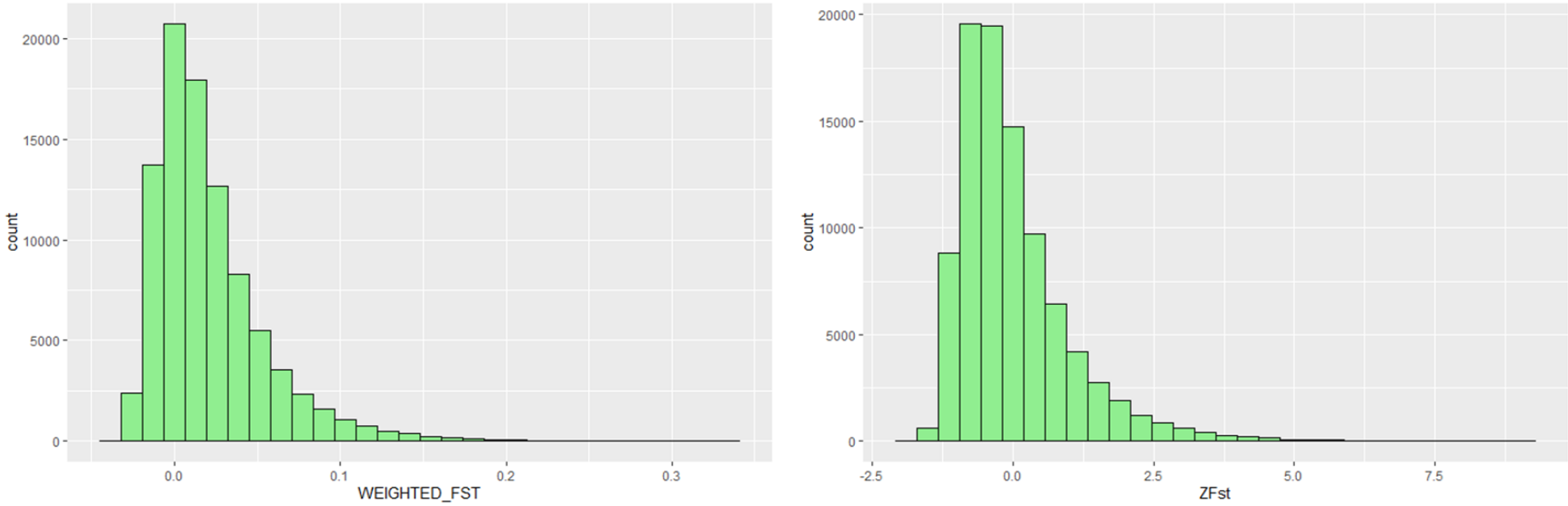


Figure S3.4 | Histogram of SNPs count, *Hp* and *ZHp* values in high precipitation region based on *Hp* analysis

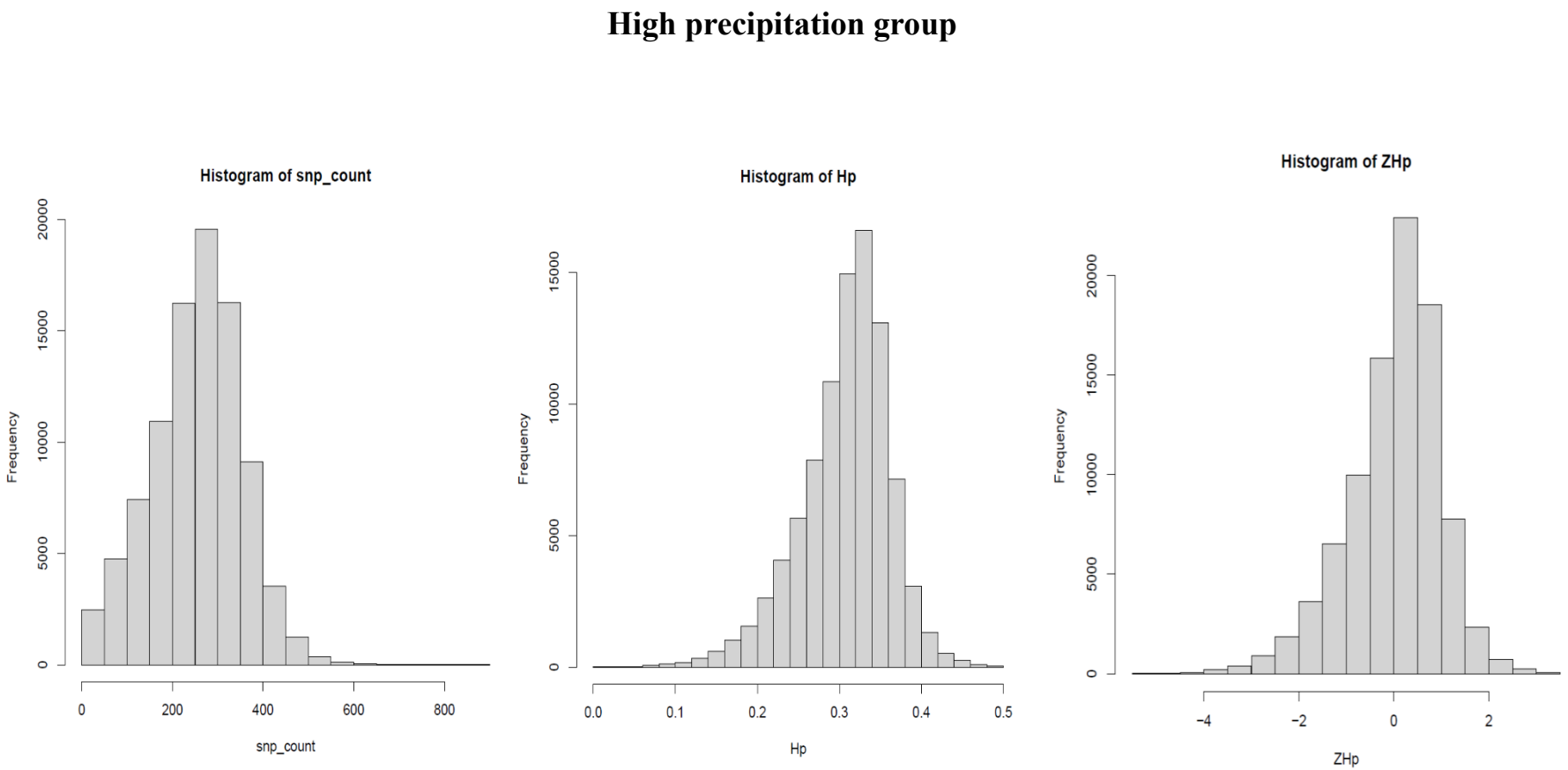


Figure S3.5 | Manhattan plot based on *ZHp* analysis in high precipitation group

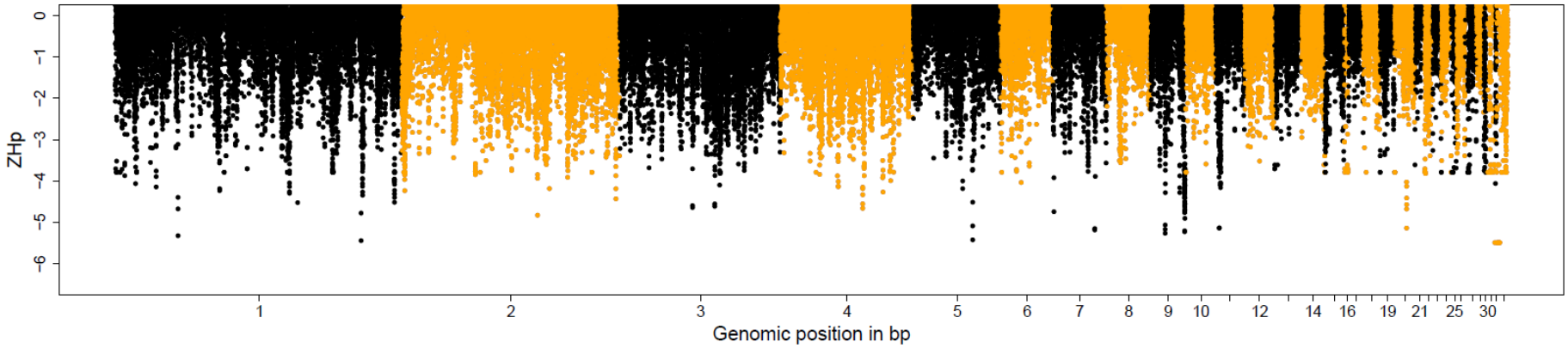


Figure S3.6 | Histogram of SNPs count, *Hp* and *ZHp* values in low precipitation region based on *Hp* analysis

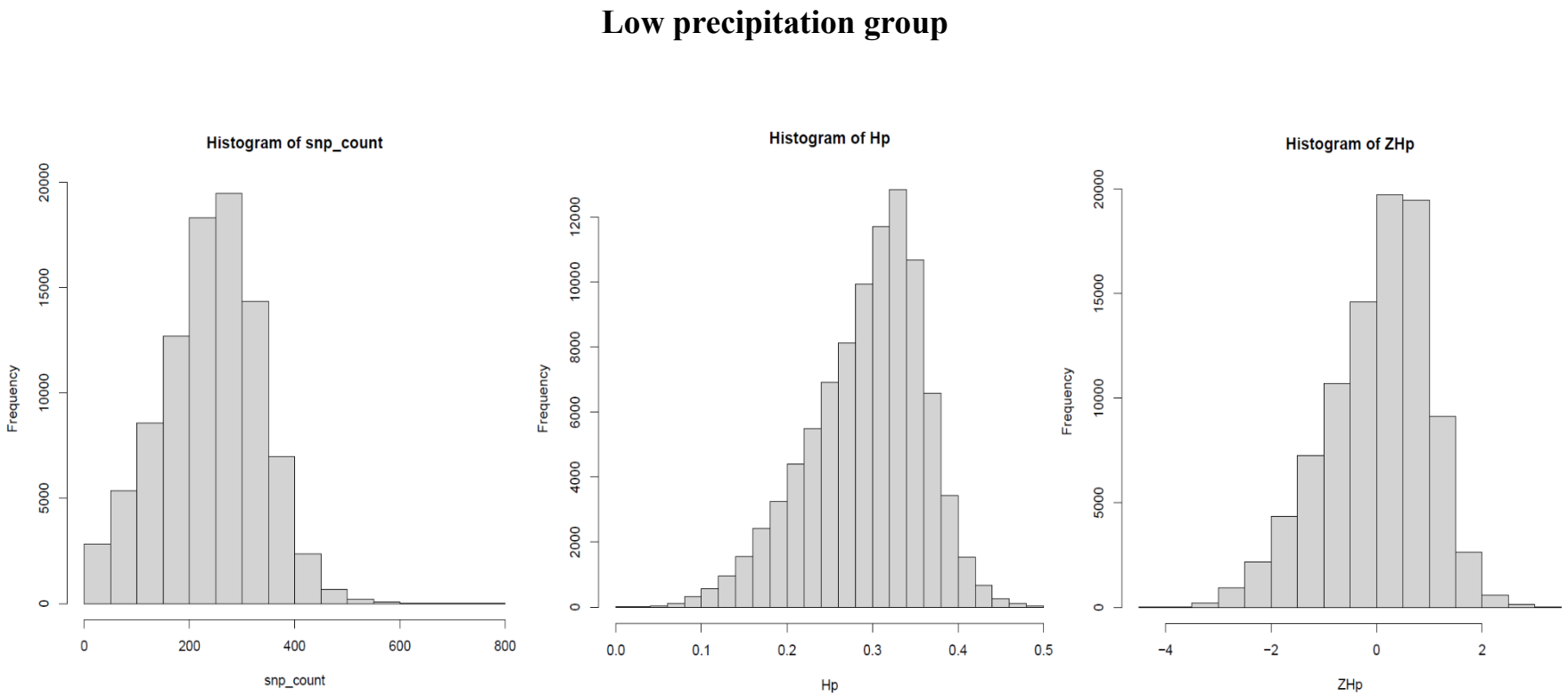
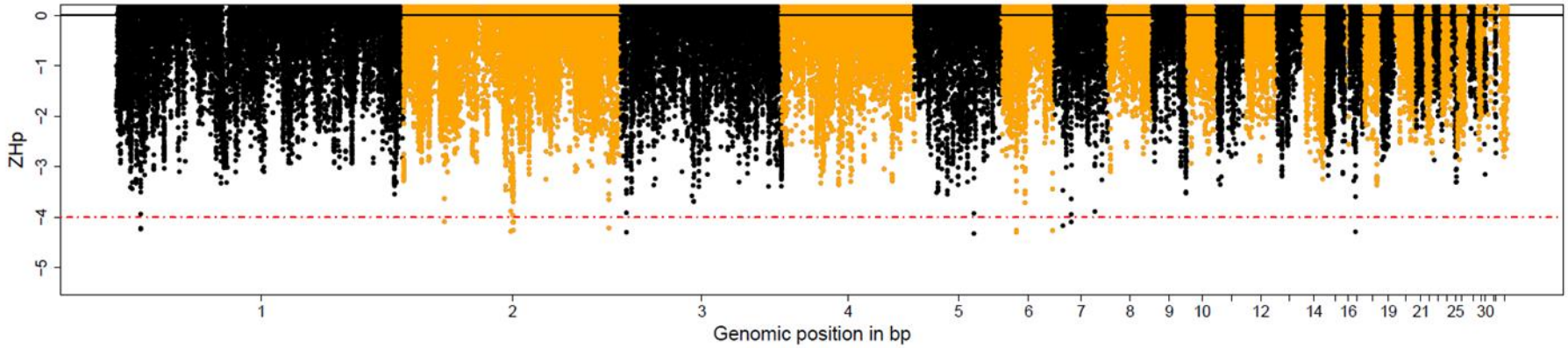


Figure S3.7 | Manhattan plot based on *ZHp* analysis in low precipitation group



Chapter 4

Figure S4.1 | Venn diagram showing shared genes identified in all warm-arid and warm semi-arid ecotypes (Sudan savanna, Northern Guinea savanna, Southern Guinea savanna, Derived savanna)

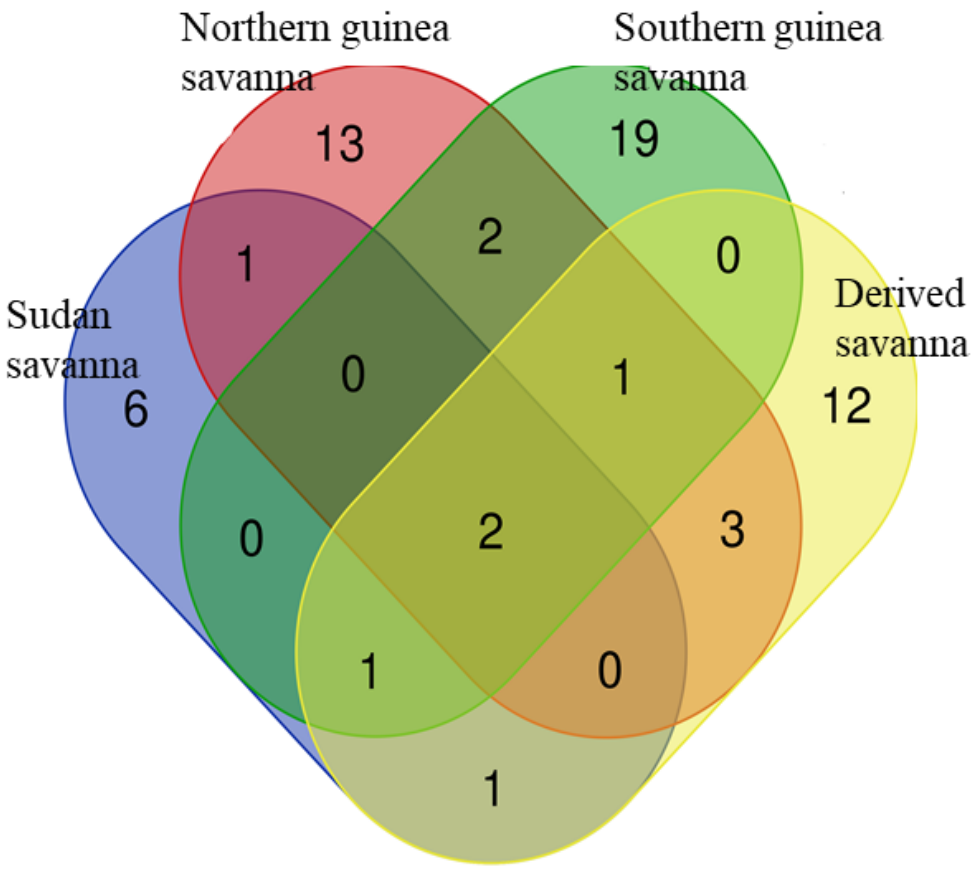


Figure S4.2 | Venn diagram showing shared genes identified in all warm-humid ecotypes (Humid rainforest, freshwater swamp, and Mangrove swamp)

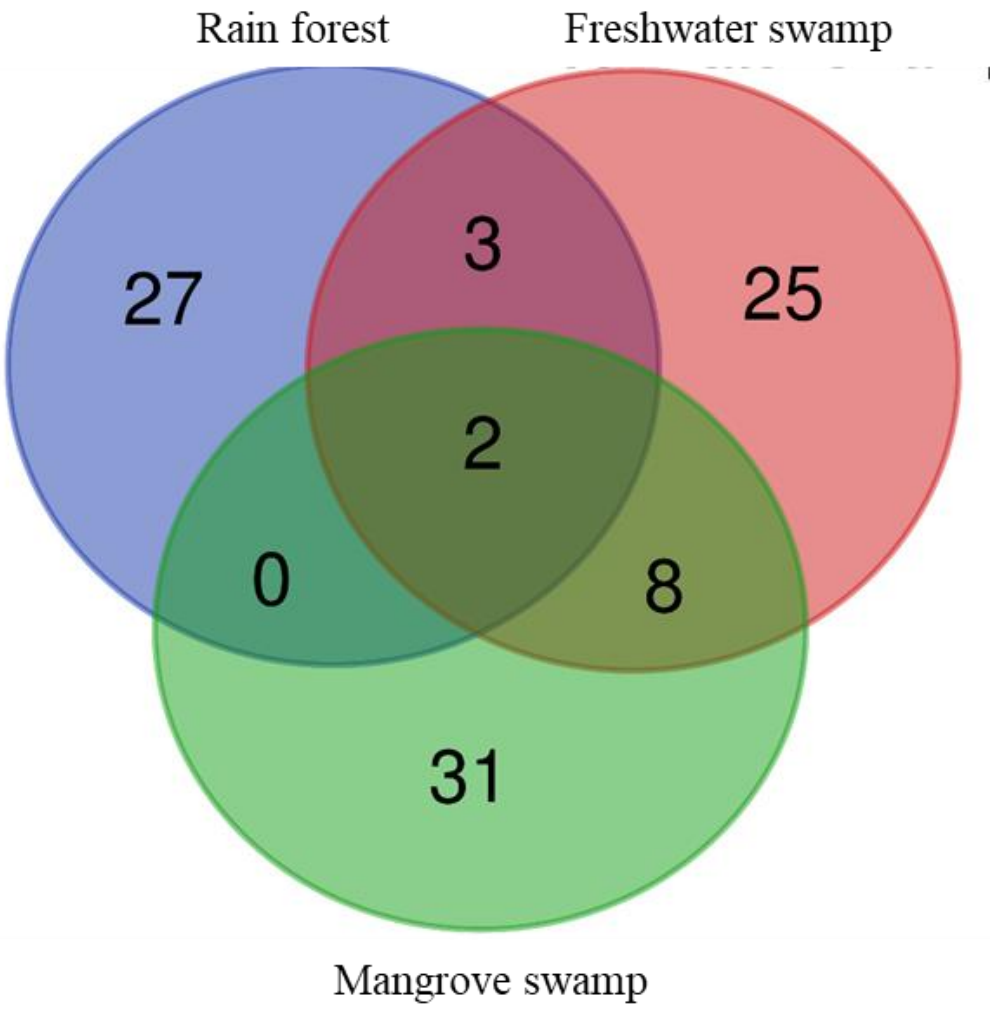


Figure S4.3 | Venn diagram showing shared genes identified in all warm-humid ecotypes (Humid rainforest, freshwater swamp, and Mangrove swamp) and warm-arid ecotypes (Sudan savanna and Northern guinea savanna)

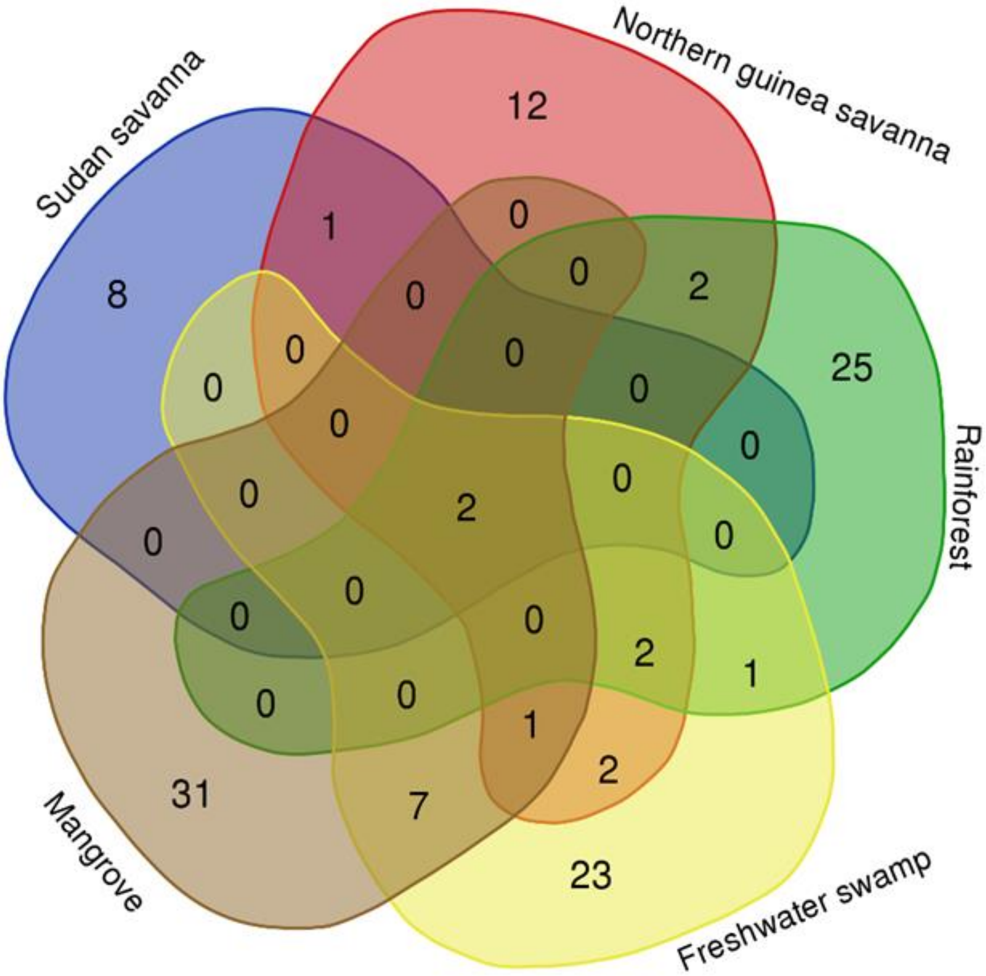


Figure S4.4 | Venn diagram showing shared genes identified in all warm-humid ecotypes (Humid rainforest, freshwater swamp, and Mangrove swamp) and warm semi-arid ecotypes (Southern guinea savanna and Derived savanna)

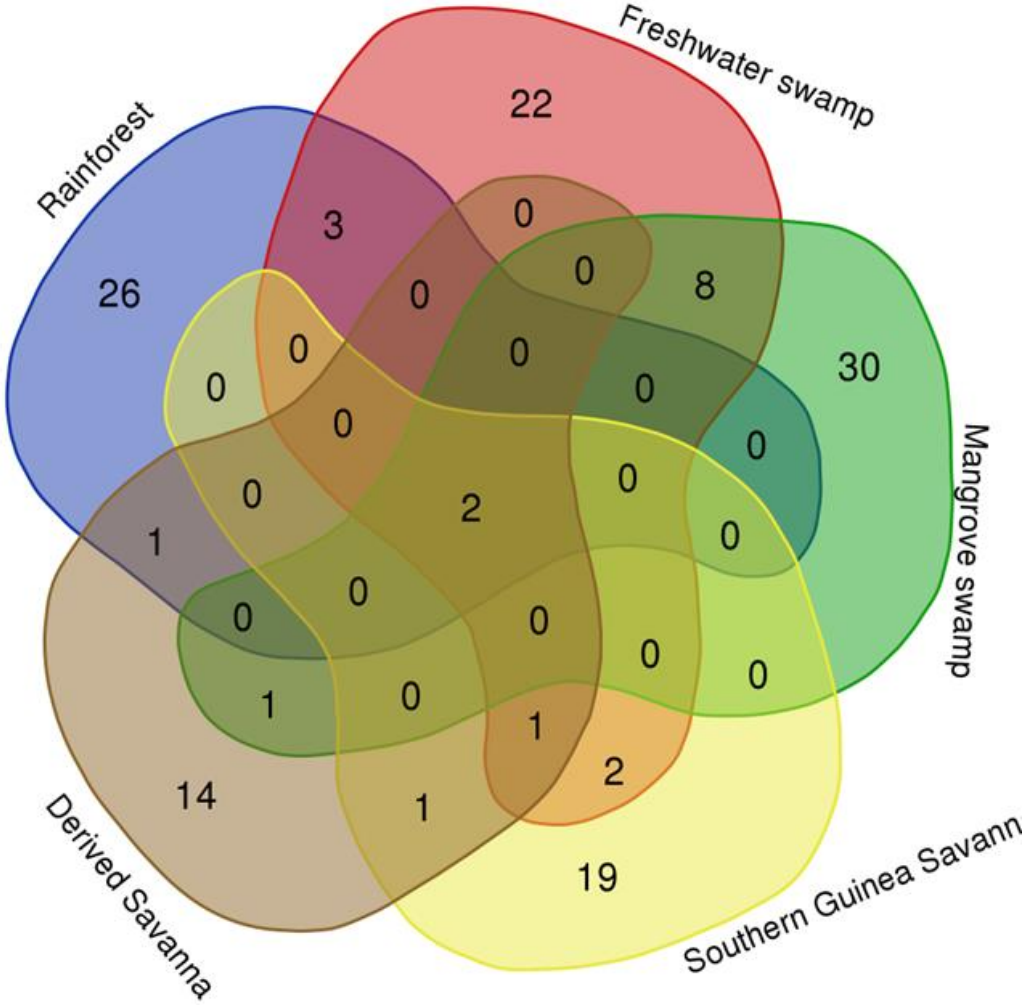


Figure S4.5 | Venn diagram showing shared genes identified in warm arid, warm semi-arid, and Mid-altitude ecotypes

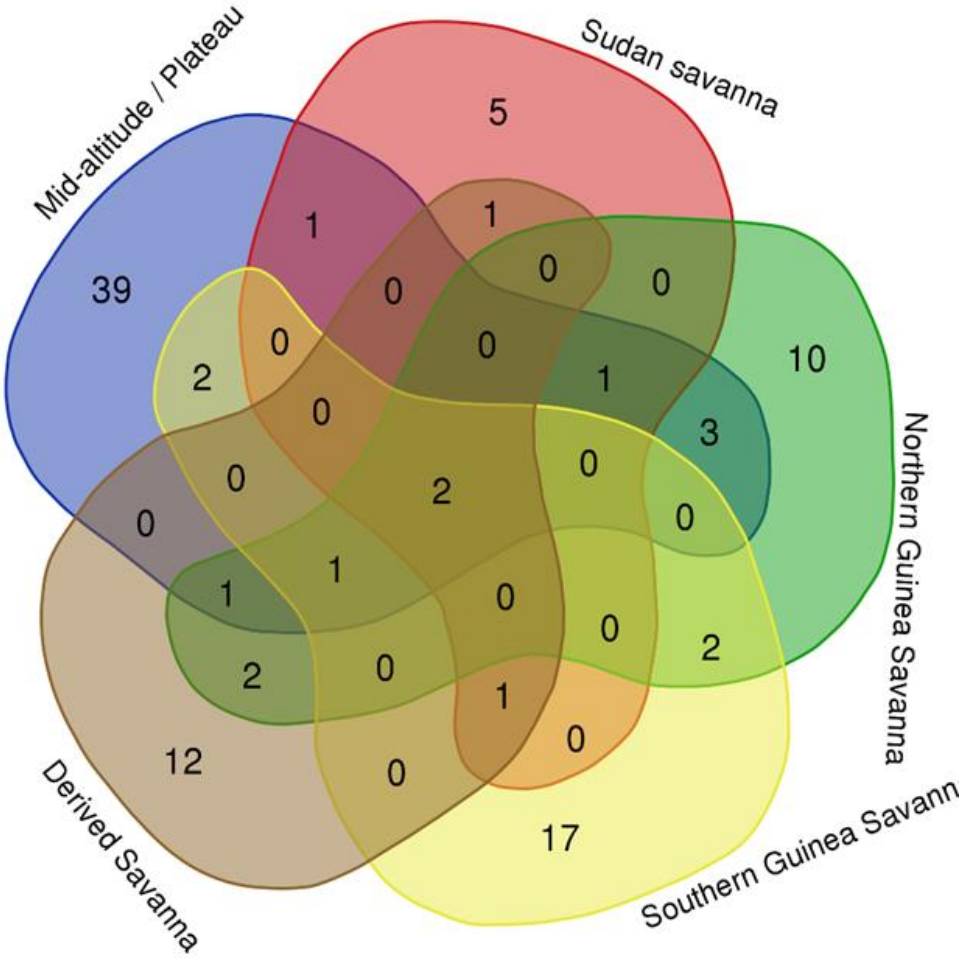


Figure S4.6 | Venn diagram showing shared genes identified in warm-humid (Rainforest, Freshwater swamp, Mangrove) and Mid-altitude ecotypes

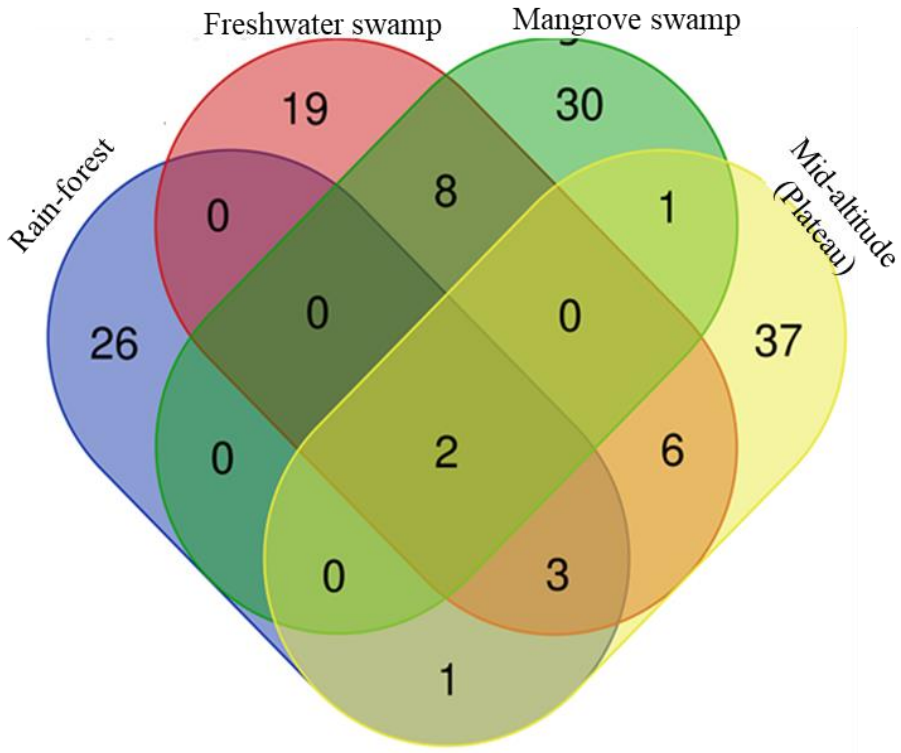
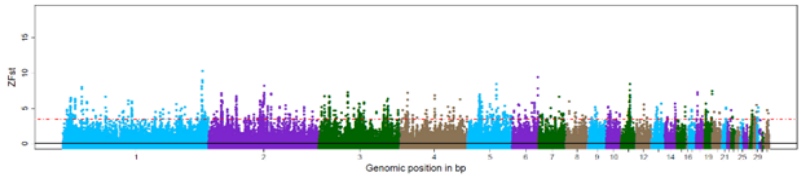
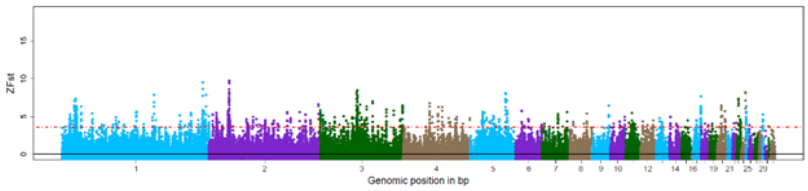


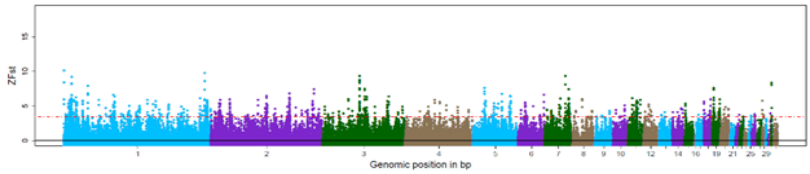
Figure S4.5 | Manhattan plot for *ZFST* analysis between Sudan Savanna vs other ecotypes



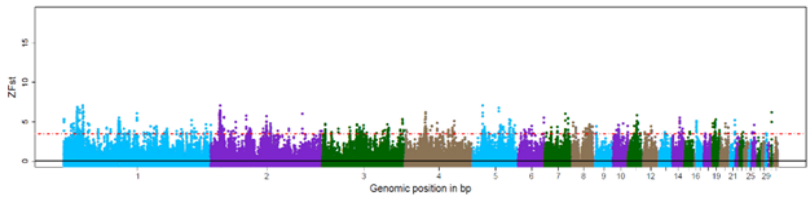
Sudan savanna vs Northern guinea savanna



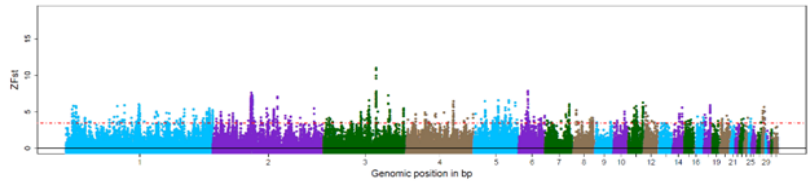
Sudan savanna vs Southern guinea savanna



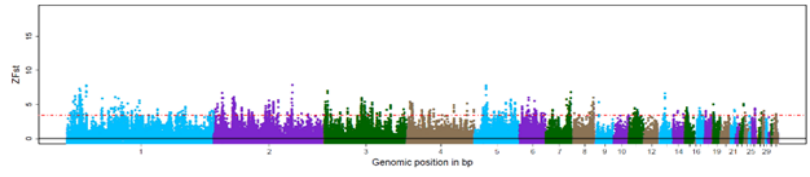
Sudan savanna vs Derived savanna



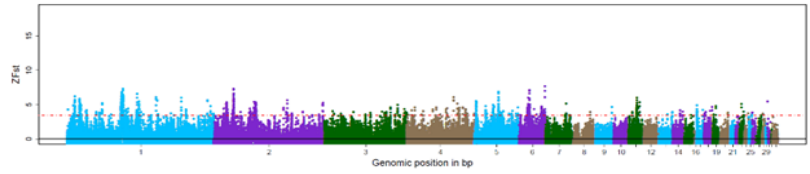
Sudan savanna vs Mid-Altitude



Sudan savanna vs Rain-forest

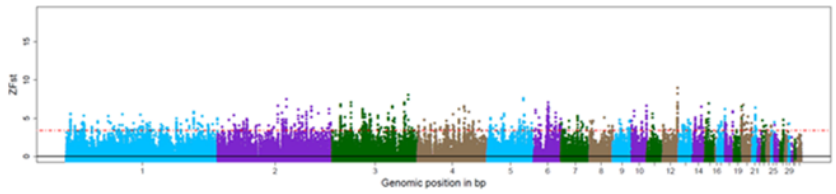


Sudan savanna vs Freshwater swamp

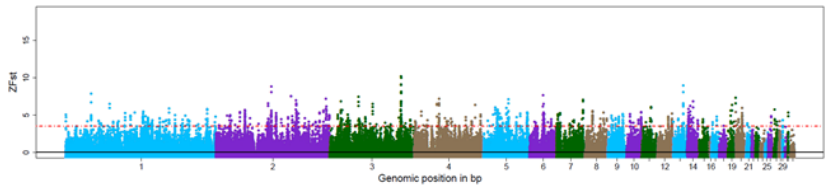


Sudan savanna vs Mangrove

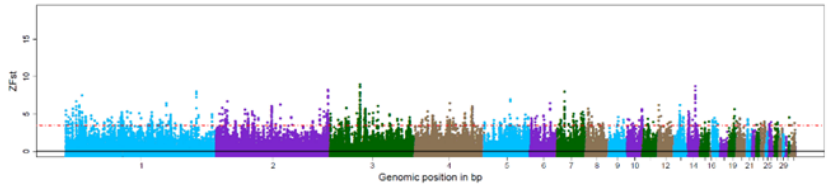
Figure S4.7 | Manhattan plot for *ZFST* analysis between Northern Guinea Savanna vs other ecotypes



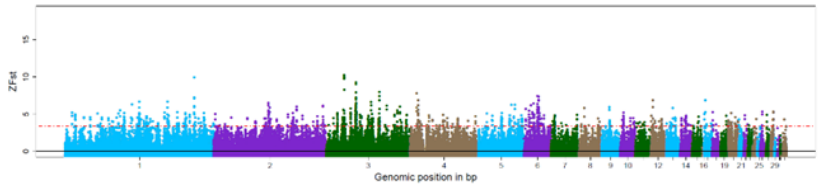
Northern guinea savanna vs Southern guinea savanna



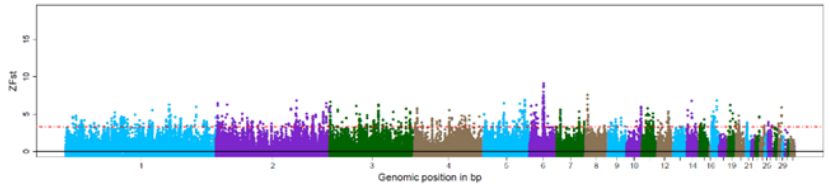
Northern guinea savanna vs Derived savanna



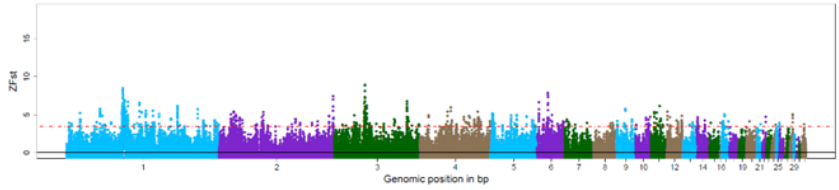
Northern guinea savanna vs Mid altitude



Northern guinea savanna vs Rain-forest

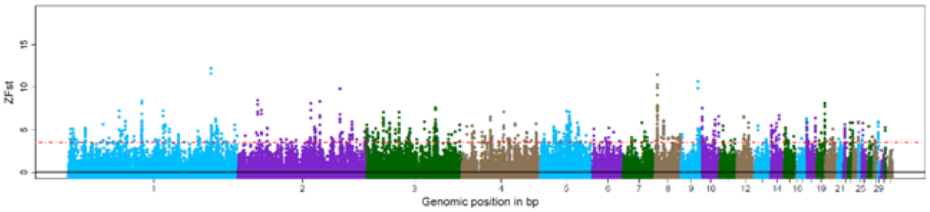


Northern guinea savanna vs Freshwater swamp

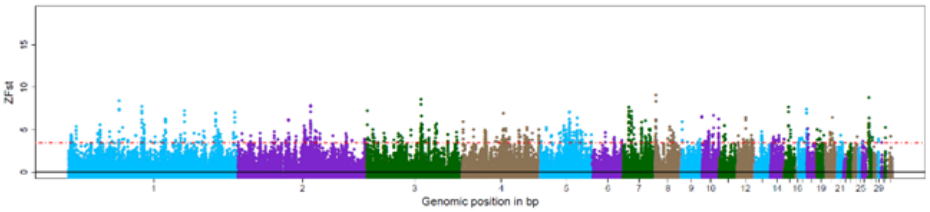


Northern guinea savanna vs Mangrove

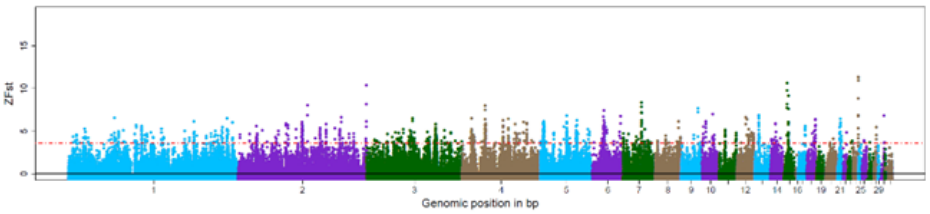
Figure S4.8 | Manhattan plot for *ZFST* analysis between Southern Guinea Savanna vs other ecotypes



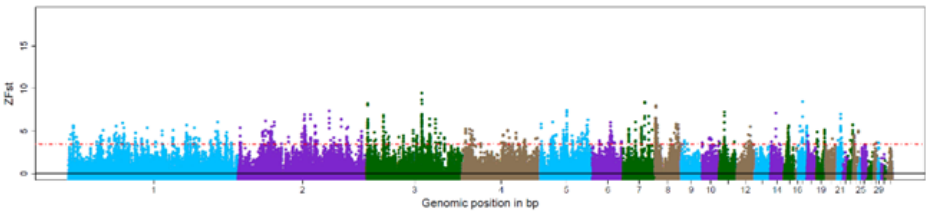
Southern guinea savanna vs Derived savanna



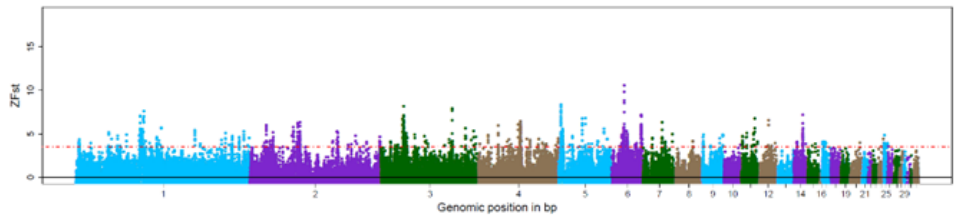
Southern guinea savanna vs Mid-Altitude



Southern guinea savanna vs Rain-forest

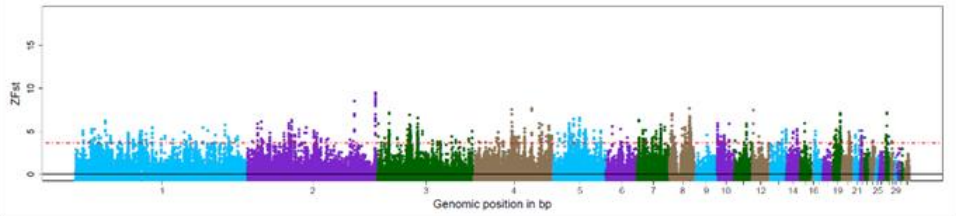


Southern guinea savanna vs Freshwater swamp

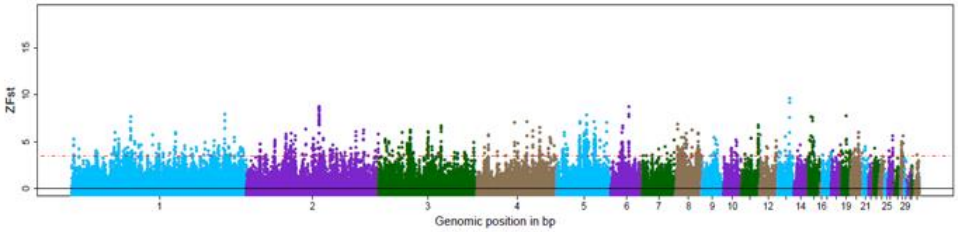


Southern guinea savanna vs Mangrove

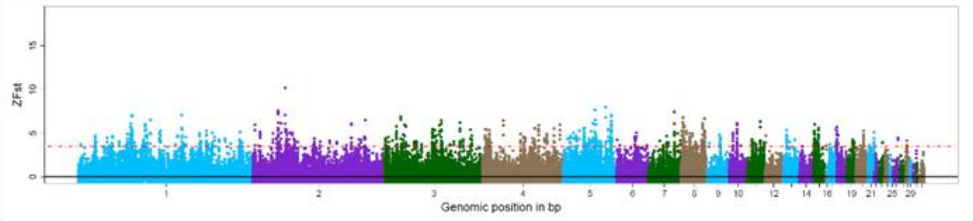
Figure S4.9 | Manhattan plot for *ZFST* analysis between Derived Savanna vs other ecotypes



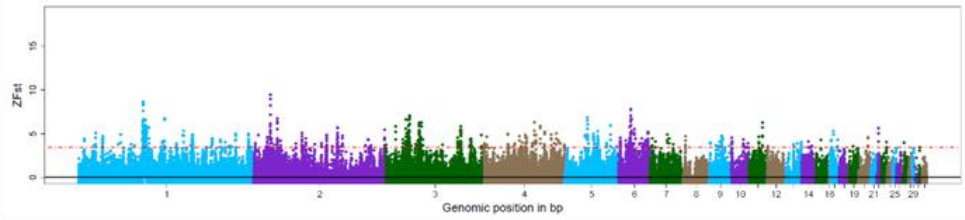
Derived savanna vs Mid-Altitude



Derived savanna vs Rain-forest

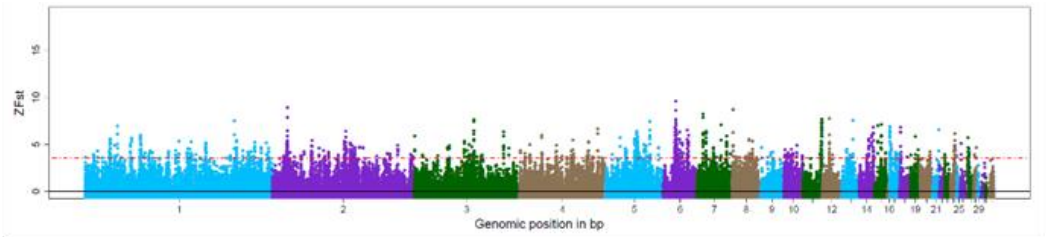


Derived savanna vs Freshwater swamp

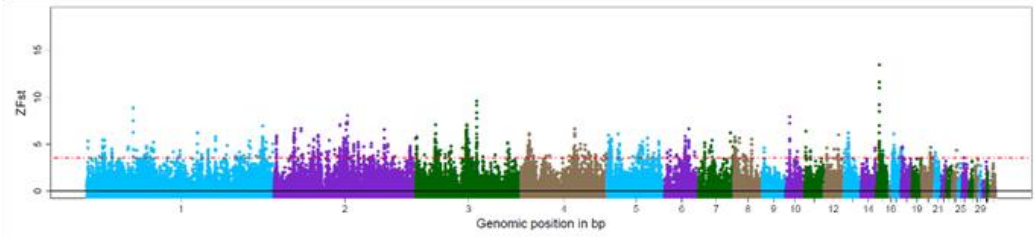


Derived savanna vs Mangrove

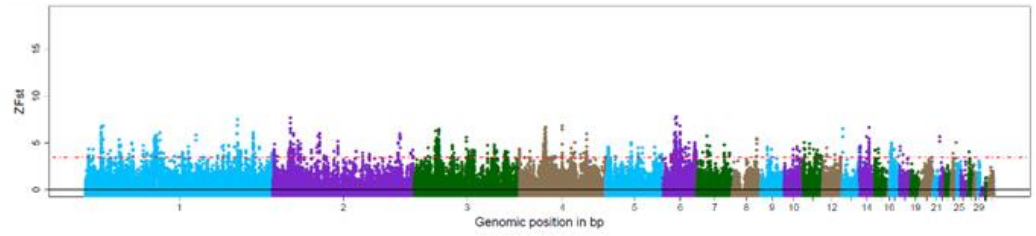
Figure S4.10 | Manhattan plot for *ZFST* analysis between Mid-Altitude vs other ecotypes



Mid-Altitude vs Rain-forest

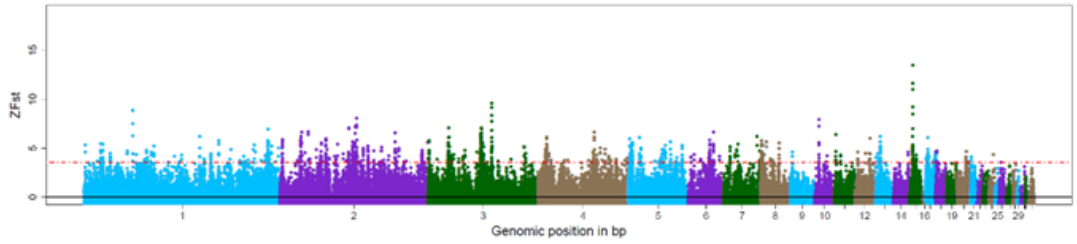


Mid-Altitude vs Freshwater swamp

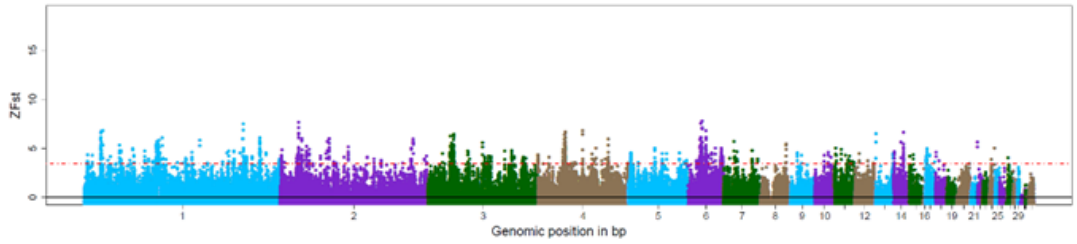


Mid-Altitude vs Mangrove

Figure S4.11 | Manhattan plot for *ZFST* analysis between Rainforest vs other ecotypes

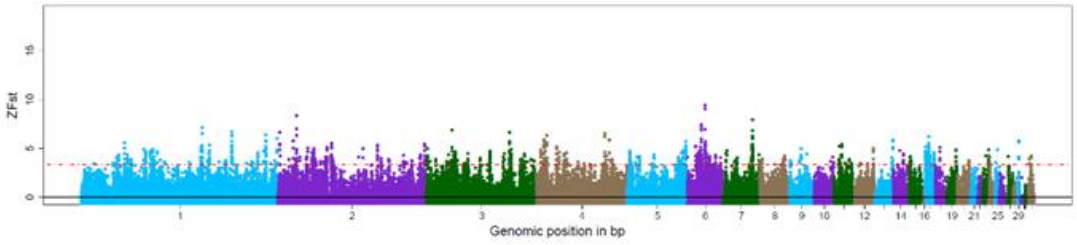


Rain-forest vs Freshwater swamp



Rain-forest vs Mangrove

Figure S4.12 | Manhattan plot for *ZFST* analysis between Freshwater swamp vs Mangrove ecotypes



Freshwater swamp vs Mangrove