422. Whole genome sequencing reveals genetic diversity and heat-stress adaptation in Nigerian indigenous chickens

A.A. Gheyas[1*], M. Rachman[2], O. Bamidele[3], T. Dessie[4], J. Smith[1] and O. Hanotte[2,4]

[1]Centre for Tropical Livestock Genetics and Health (CTLGH), Roslin Institute, University of Edinburgh, EH25 9RG, Edinburgh, United Kingdom; [2]School of Life Sciences, University of Nottingham, NG7 2RD, Nottingham, United Kingdom; [3]African Chicken Genetic Gains (ACGG), Department of Animal Sciences, Obafemi Awolowo University, Ile Ife 220282, Nigeria; [4]LiveGene – CTLGH, International Livestock Research Institute (ILRI), P.O. Box 5689, Addis Ababa, Ethiopia; almas.gheyas@roslin.ed.ac.uk

Abstract

Poultry is a crucial sector for the livelihoods and food security of millions of people in Nigeria. Here we present the first large scale whole-genome sequencing analysis on Nigerian indigenous chickens from different agro-climatic conditions, investigating their genetic diversity and adaptation to tropical hot climates. We observe a large genetic diversity but low levels of population differentiation. Selection signature analyses were performed to identify candidate genes in relation to heat-stress adaptation including those specific to extreme hot-humid or hot-arid conditions. These results have important implications for the conservation of genetic diversity and breeding improvement of chickens for thermo-tolerance.

Introduction

Indigenous livestock populations from different geographic regions constitute important genetic resources for conservation as they represent adaptation to local agro-climatic conditions. Native tropical breeds are particularly crucial, as climate change and global warming is forcing many temperate regions to experience tropic-like conditions and such breeds may hold a genetic solution for climate-resilience.

Nigeria is a tropical lowland country, where poultry farming plays a crucial role in the economy and the livelihood of millions. Nigeria has one of the largest stocks of chickens in Africa, but over half of its birds are still raised in extensive backyard farming systems, which are predominantly represented by unimproved indigenous breeds. Even though these local unimproved breeds have poor productivity, they show superior adaptive ability to harsh tropical climates. The Nigerian agro-climate offers an excellent opportunity to dissect thermo-tolerance in chickens, both under hot-humid and hot-arid conditions. Whilst most Nigerian geographic regions experience very high temperatures (except in the high plateaus), the climate varies from very wet conditions in the coastal south (annual rainfall >3,500 mm, temperature up to 32 °C) to substantially dry conditions in the Sahel region of the north-west and north-east (annual rainfall <600 mm, temperature up to 41 °C). The genetic characterisation of these indigenous chickens (IC) is crucial for conservation of genetic and adaptive diversity and for elucidating the molecular mechanisms of environmental adaptation, particularly to heat-stress.

Most of the genetic studies on Nigerian IC were on mtDNA, with none examining whole-genome sequence (WGS) data. Here we examine the genetic diversity in Nigerian IC with WGS data, and explore genomic signatures for positive selection in response to heat-stress.

Materials & methods

Chicken sampling and genome sequencing. Blood samples were collected from 120 village chickens from 14 populations representing different agro-ecological zones (AEZs) (Figure 1). WGS was performed on the Illumina HiSeqX platform at 30x paired-end coverage.

R.F. Veerkamp and Y. de Haas (eds)

DOI: 10.3920/978-90-8686-940-4_422, © A.A. Gheyas *et al.* 2022

Population	# Samples	Agro-ecology
Sabiyal	6	Sudan Savanna (Arid/Semi-arid)
Jiga	4	Sudan Savanna (Arid/Semi-arid)
WatKaru	10	MidAltitude
DakanKaru	10	MidAltitude
Senchi	4	Northern Guinea Savanna
KWT	6	Northern Guinea Savanna
Ogbondoroko	10	Southern Guinea Savanna
Sanchitagi	10	Southern Guinea Savanna
Gitta Mbasha	10	Derived Savanna
Karu	10	Derived Savanna
Okpofe	10	Humid Savanna/Rain Forest
Odenkume	9	Humid Savanna/Rain Forest
Isiokpo	10	Humid Savanna/ Fresh water Swamp
Degema	10	Humid Savanna/Mangrove

Figure 1. (A) Nigerian agro-ecological map showing sampling locations; (B) details of the studied chicken populations.

Sequence data processing and genomic analysis. Sequence reads were mapped against the chicken reference genome (GRcg6a) using the BWA-mem algorithm. SNP calling was performed following GATK best practice protocol for 'Germline short variant discovery' followed by Variant Calling Score Recalibration (VQSR) for initial filtration. Autosomal SNPs with minor allele frequency >0.05 and genotype quality >15.0 were used for genetic diversity and selection signature analyses (SSA). SSA was performed using Pooled Heterozygosity (*Hp*) (Rubin *et al.*, 2010) and *Fst* (Weir and Cockerham, 1984) approaches in overlapping sliding windows (20 kb size with 10 kb step) with at least 10 SNPs/window.

Results

Nigerian IC show large genetic diversity but low levels of population differentiation. The combined analysis of all samples identified over 17 M SNPs of which 24% (~4.1 M) are novel. This represents an average density of 1 SNP every 62 base pairs. The number of SNPs from different populations varied between 9 M (Jiga) and 12 M (Sanchitagi); 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 (0.0031-0.0035). Moreover, a low level of inbreeding (<0.05) was observed for all populations. PCA plots – using 4M LD-pruned SNPs – show overall weak population structure, with most populations clustering together except Degema, DakanKaru and Odenkume (Figures 2A, 2B). Admixture analysis predicted contributions from four ancestral gene pools (Figure 2C) but pairwise *Fst* analyses showed a generally low level of population differentiation (*Fst*<0.05), except in a few cases where moderate differentiation (0.05-0.08) was observed (Figure 2D).

SSA reveals candidate genes for heat-stress adaptations. The climatic conditions of the studied populations showed little variation in annual mean temperature (except populations from the MidAltitude region) (Figure 3A), but large variations in annual rainfall (Figure 3B). SSA was therefore performed in two ways. First, 12 populations (omitting WatKaru and DakanKaru) were combined for *Hp* analysis. The combined analysis allowed reduction of spurious signals from population structure and detection of genomic regions with extreme low heterozygosity (*zHp*<-4) from all the hot-climate populations. These genomic loci and overlapping genes are therefore candidates for heat-stress adaptation, irrespective of humid or arid conditions (Figure 4A). Second, *Fst* analysis was performed among population groups from hot-humid (Degema and Isiokpo) and hot-arid (Jiga and Sabiyal) climates (Figure 3B) to identify candidate regions/genes that show large differentiation (*Fst*>3.5) in relation to heat-stress adaptation under dry and humid conditions (Figures 4B-4D). Selection direction of the *Fst*-sweep regions was decided based on their *Hp* values within groups.

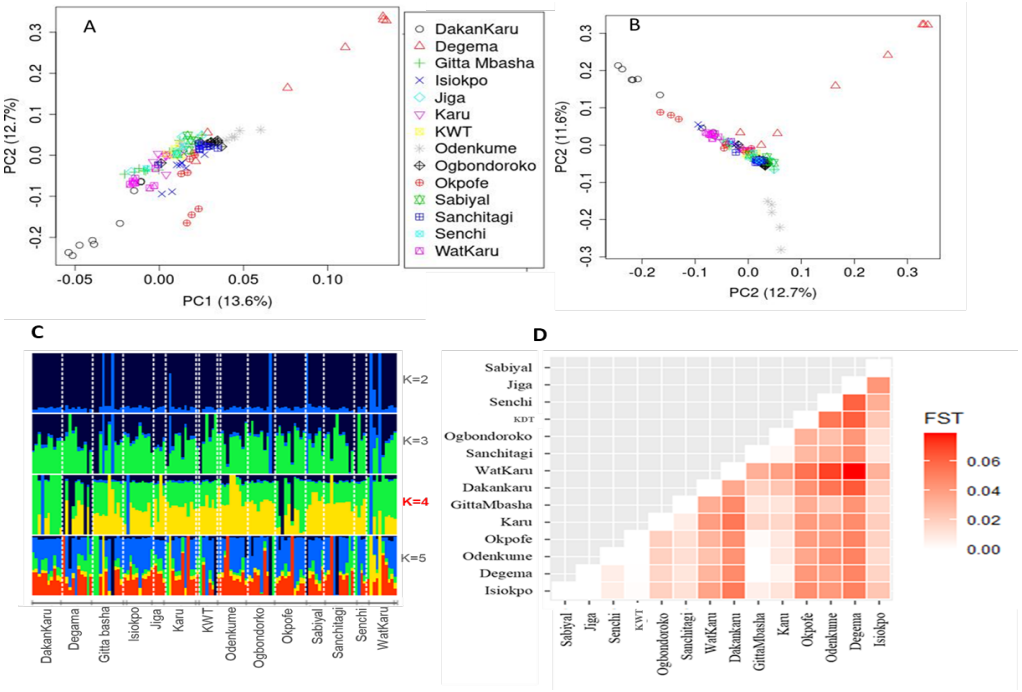

Figure 2. Population genetic diversity. (A & B) PCA plots showing population structure, (C) admixture analyses (K=4 is best prediction); (D) heat map of pairwise *Fst* values.

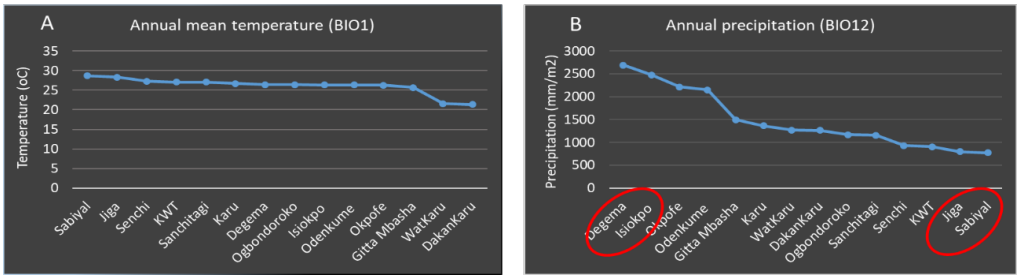

Figure 3. Population gradients based on (A) annual mean temperature and (B) annual precipitation (using the WorldClim database https://www.worldclim.org/data/bioclim.html).

Discussion

This is the first study performing a large scale WGS analysis on Nigerian IC to assess genetic diversity and identify genomic signatures of adaptive selection in relation to hot climates. Our study shows a large genetic diversity in Nigerian chickens that can be harnessed in breeding programmes for improvement of production and performance traits. The study also dissected candidate genes for general heat-stress adaptation (*Hp* analysis) as well as those important in either humid or arid conditions (*Fst* analysis). The *Hp*-based candidates have highly relevant biological functions associated with thermo-tolerance, e.g. *TSHR* – role in thermogenesis (possibly regulated by epigenetic mechanisms, as fixed in most chickens), *RYR1*-like genes (*LOC11233047* and *LOC101748756*) – involved in hyperthermia, *CYP450*-like genes

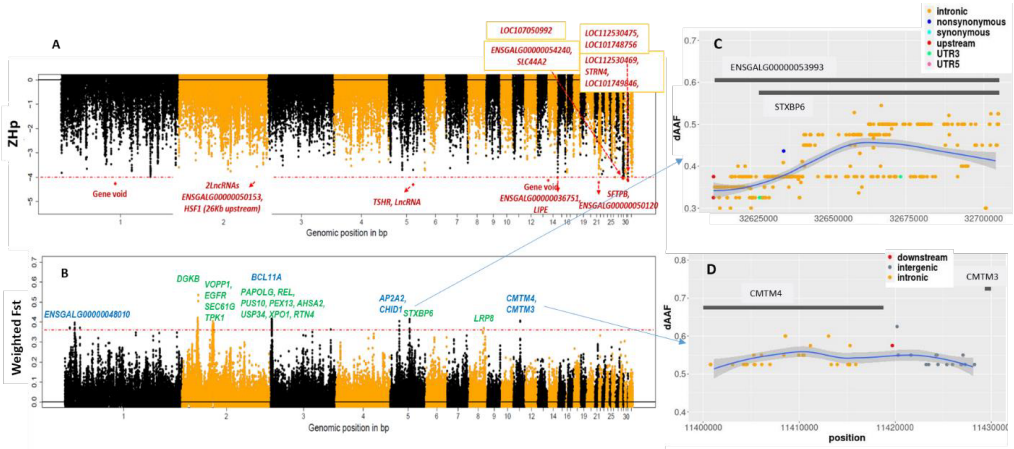

Figure 4. (A) Manhattan plot from *Hp* analysis based on 12 hot climate populations; (B) Manhattan plot for *Fst* analysis between hot-humid and hot-arid groups (candidate genes for humid climate are shown in green and for dry climate in blue); (C) and (D) showing close-up of two selection signature regions from humid and arid regions, respectively (Y axis represents difference in alternative allele frequency between the two groups)

(*LOC11253049* and *LOC101749846*) – roles in oxidative stress response, *SFTPB* – role in respiratory gaseous exchange (affecting heat loss from body), *LIPE* – lipid metabolism, *STRN4* and *SLC44A2* – roles in nervous system processes and immunity, and two lncRNAs with possible cis-regulatory effect on Heat Shock Factor 1 (*HSF1*) gene (Lin *et al.* 2017; Seo *et al.* 2020; Uniprot). The candidate genes associated with hot-arid conditions include: *CMTM3, CMTM4, BCL11A* and *CHID1* – all with roles in immune system development and/or functioning and *AP2A2* and *LRP8* with roles in lipid metabolism (Uniport). Prominent candidates from hot-humid climate include: *DGKB* – involved in many cellular processes including immunity (Zhong *et al.* 2008), *EGFR* – role in converting extracellular cues to appropriate response (Uniprot), *SEC61G* – involved in translocating proteins to Endoplasmic Reticulum, which has an important role in environmental and cellular stress response (Singh *et al.* 2021), *TPK1* – involved in heat and osmo-stress response (Reca *et al.* 2020); *AHSA2* – activator of Heat Shock Protein 90 (*HSP90*) gene (Uniprot), *RTN4* – with many roles including apoptosis, angiogenesis, and immunity (Uniprot). These results will have important implications for breeding improvement to develop heat-tolerant productive breeds for tropical small-holder farming systems.

References

Lin L., Lo L.H.Y., Lyu Q., and Lai K.O. (2017). J. Biol. Chem. 292(23):9451-9464. https://doi.org/10.1074/jbc.M116.772442

Reca S., Galello F., Ojeda, L, Pautasso, C., Cañonero L., *et al.* (2020) Biochim. Biophys. Acta. Gene Regul. Mech. 1863(9):194599. https://doi.org/10.1016/j.bbagrm.2020.194599

Rubin C.J., Zody M.C., Eriksson J., Meadows J.R., Sherwood E., *et al.* (2010). Nature 464:587-591. https://doi.org/10.1038/nature08832

Seo G., Han H., Vargas R.E., Yang B., Li X., *et al.* (2020). *Cell Reports* 32(1):107860. https://doi.org/10.1016/j.celrep.2020.107860

Singh M.B., Lohani N., and Bhalla P.L. (2021). *Front. Plant Sci.* 12: 661062. https://doi.org/10.3389/fpls.2021.661062

Uniport. Available at: https://www.uniprot.org/

Weir B.S. and Cockerham C.C. (1984). *Evolution* 38, 1358-1370. https://www.jstor.org/stable/2408641

Zhong X.P., Guo R., Zhou H., Liu C., and Wan C.K. (2008). *Immunol. Rev.* 224: 249–264. https://doi.org/10.1111/j.1600-065X.2008.00647.x