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Genome-wide analysis of inbreeding patterns and candidate genes for adaptation in indigenous chickens of Botswana

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ABSTRACT

Indigenous chickens are an important genetic resource in Botswana, providing protein, cultural value and economic benefits to rural communities. The Tswana chicken, comprising multiple strains including Dwarf, Nakedneck, and Normal, has been shaped by domestication, local adaptation and traditional management practices. However, the lack of structured breeding strategies raises concerns about genetic erosion. This study evaluated genomic inbreeding patterns and signatures of selection in Tswana chicken strains using genome-wide SNP data from the 60 K iSelect chicken array. After quality control in PLINK v1.9, 52,719 SNPs from 81 chickens were retained for analysis. Runs of homozygosity (ROH) analysis and complementary selection signature approaches (F_{ST} , iHS , Rsb , and $xp-EHH$) were applied and results revealed generally low levels of inbreeding F_{ROH} (Dwarf: 0.01396; Nakedneck: 0.00921; Normal: 0.01216), with most ROH segments being short (<8 Mb). This may indicate that homozygosity largely reflects historical demographic processes rather than recent widespread inbreeding. Long ROH segments were observed only in a subset of Normal individuals, suggesting localized recent relatedness rather than strain-wide effects. A prominent ROH hotspot consistently detected on GGA5 (1.92–3.75 Mb) was shared across all strains and harbored candidate genes including *NELL1*, *ANO5* and *SLC17A6* which are implicated in skeletal integrity, neuromuscular function and metabolic processes. In addition, four (4) candidate genes on GGA8 and GGA15 (*BRINP3*, *C1orf21*, *ISCU*, *RNF2*) were also identified by multiple analytical methods. Collectively, the results indicate low current inbreeding, measurable but moderate population structure and candidate genomic regions potentially associated with adaptation to local production environments. These findings provide a genomic foundation for the sustainable management of Tswana chicken genetic resources.

Introduction

Chickens are believed to have been domesticated in the geographical regions of South and Southeast Asia (Kanginakudru et al., 2008; Liu et al., 2006). This evolutionary event led to the emergence of various breeds including the indigenous chickens that are widely present across all agroecological zones of the African continent. Shaped by domestication, natural selection and local adaptation pressures indigenous chickens exhibit distinct phenotypic and genomic traits, including resilience to diseases and efficiency on limited feed (Elbeltagy et al., 2019; Qi et al., 2024). The common indigenous chicken in Botswana, is the Tswana and consists of five strains; dwarf, rumpless, frizzled, nakedneck and normal (Badubi et al., 2006). These chickens are

generally reared under free range backyard conditions with low husbandry requirements by small-scale poultry farmers serving as an excellent protein source and income (Mwacharo et al., 2013). However, due to relentless pursuit of economic gains and absence of strong breeding strategies, these chickens may face genetic dilution and replacement leading to extinction (Sithole et al., 2025).

The scanning of whole genome using single nucleotide polymorphisms and advanced statistical analysis allows for monitoring and examination of genomic variability within and across populations (Elbeltagy et al., 2019; Tan et al., 2024). By analyzing for runs of homozygosity (ROH) and identifying selection signatures, conservation strategies can be designed to protect these valuable genetic resources from erosion and extinction. ROHs are contiguous segments of the

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genome that are inherited from a common ancestor without any recombination (Ceballos et al., 2018). Such patterns can result from small effective population sizes, geographic isolation, or ongoing selection for local adaptation (Mastrangelo et al., 2023). Studies on ROH have been reported in other livestock species including cattle (Mulim et al., 2022), pigs (Jiang et al., 2022), goats (Li et al., 2022), and chickens (Wang et al., 2023). Furthermore, understanding selection signatures, that is, genomic regions that exhibit a deviation from neutral expectations due to selective pressures (Mastrangelo et al., 2023) provides a deeper comprehension of how these chickens have evolved and thrive in Botswana's environment. Studies on selection signatures using different methods have revealed different adaptive genes among indigenous chicken population globally (Elbeltagy et al., 2019; Mastrangelo et al., 2023). For instance, in west Africa, candidate genes associated with thermotolerance, and immune response have been documented on indigenous Nigerian chickens (Rachman et al., 2024).

Preliminary analyses on the genetic diversity and population structure of indigenous Tswana chickens have been reported using genome-wide SNP data (Machete et al., 2021). The patterns of genetic differentiation showed that most of the indigenous Tswana chickens strains clustered together except for the dwarf strain (Machete et al., 2021). In addition, the different strains of Tswana chickens have showed low effective population sizes, indicating risk of extinction (Machete et al., 2021). However, to date, no comparative genome-wide search for signatures of selection and patterns of genomic inbreeding has been conducted in these chicken strains. Therefore, the present study, uses genome-wide information from the 60 K iSelect chicken SNP array and *iHS*, *Rsb*, *xp-EHH*, and ROH approaches in comparative analyses of indigenous Tswana chicken strains populations to detect signatures of selection and unravel the effect of selection and environmental pressure on these important local genetic resources. The study further uses ROH to unravel genomic inbreeding patterns. This study will contribute to the sustainability of local animal genetic resources and support food security as well as the cultural heritage of Botswana's rural communities.

Materials and methods

Ethical statement

This study received ethical approval from Botswana University of Agriculture and Natural Resources (Approval No. 2020-13).

Sampling of animals and study area

The sampling of animals and the study area are well elaborated by Machete et al. (2021). Briefly, a total of 84 free range indigenous Tswana chickens; Normal strain ($n = 39$), Nakedneck strain ($n = 32$), Dwarf strain ($n = 13$) of Tswana chickens from locations in the Kweneng and Southern districts in Botswana were used. EDTA vacutainers bottles were used to collect blood from the chickens. Firstly, wings were plucked from the sites of blood collection which were either the medial metatarsal vein on the leg or brachial vein on the wing using a 23-gauge 1-in needle. The samples were then stored at -20°C prior to DNA extraction.

DNA extraction and genotyping

DNA was extracted using NucleoMag® B-Beads by mixing 24 μl of beads with 360 μl of MB2 buffer, shaking for 5 minutes, and separating the beads with the NucleoMag SEP magnetic separator. The supernatant was discarded, and the washing steps were repeated with MB3, MB4, and MB5 buffers. Finally, 50 μl of DNA elution buffer was added, and samples were shaken at 56°C for 10 min to obtain purified genomic DNA for SNP genotyping. Genotyping was performed at the Agricultural Research Council-Biotechnology Platform, Pretoria, South Africa, using the Illumina chicken iSelect SNP 60 BeadChip and Infinium assay on the

HiScan SQ platform. SNP calling was done with GenomeStudio v2.0, and data were converted to PLINK (v1.07) format for quality control based on population genetic parameters.

Data analysis

Genome-wide SNP data was analysed to assess population structure, estimate genomic inbreeding and detect signatures of selection among the three Tswana chicken strains (Nakedneck, Normal and Dwarf) (Fig. 1). The workflow consists of the following main steps;

- Quality control and filtering of SNPs to clean up the data making it more reliable for further analysis
- The use of Principal component analysis (PCA) and admixture analysis to evaluate genetic relationships and population structure.
- Estimating genetic differentiation and identifying candidate regions under selection using F_{ST} , *iHS*, *Rsb*, and *xp-EHH* statistics
- Assessing genomic inbreeding and homozygosity patterns through Runs of homozygosity (ROH) analysis
- Identification and annotation of candidate genes detected by more than 1 method

Quality control. PLINK v1.9 software (Purcell et al., 2007) was used to perform the quality control (QC) of the dataset. The analysis was conducted on the dataset allowing the inclusion of extra chromosomes through the *-allow-extra-chr* option. The *-autosome* flag was used to focus exclusively on autosomal chromosomes, excluding sex chromosomes from the analysis. To filter out poorly represented markers, the *-geno 0.05* threshold was applied, removing SNPs with a missing genotype rate greater than 5 %. Similarly, the *-mind 0.05* parameter was set to exclude individuals with more than 5 % missing genotype data. The filtering of minor allele frequency (MAF), Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) pruning were not applied in this analysis to preserve the full spectrum of genetic variation.

Principal Component Analysis. To conduct principal component analysis (PCA), PLINK v1.9 software (Purcell et al., 2007) was used. Pairwise genetic distances between individuals were computed using the *-distance-matrix* option. The resulting symmetric distance matrix was read into R v.4.3.2 software (RStudio Team, 2020) and classical multidimensional scaling (MDS) was performed using the *cmdscale* function with eigenvalue decomposition enabled. The first ten eigenvectors were extracted to examine the distribution of genetic variance across axes. The proportion of variance explained by each axis was calculated by dividing individual eigenvalues by the sum of all positive eigenvalues and expressing the result as a percentage. Individual coordinates were merged with strain identifiers (Dwarf, Nakedneck, and Normal) derived from the *mdist.id* file. Ordination plots were generated using the *ggplot2* package in R, with individuals represented by strain-specific colors and symbols to facilitate visual discrimination among groups. The first two principal coordinate axes were plotted, and the percentage of total variance explained by each axis was displayed on the respective axes.

Admixture and cross entropy. The data was processed with the LEA package on R v.4.3.2 software (RStudio Team, 2020) to generate genotype data using the *ped2geno*, followed by running *snmf* for admixture analysis across multiple values of K. Furthermore, cross-entropy metrics were evaluated for all runs, and the best K value was selected based on the lowest cross-entropy. The optimal admixture matrix was extracted for further analysis and exported for visualization in plots with the *ggplot2* package.

F_{ST} . To compute F_{ST} values and identify genomic regions under selection, analyses were conducted using PLINK V.1.9 (Purcell et al., 2007) and R v.4.3.2 software's. Pairwise F_{ST} was calculated by executing the *-fst* command in PLINK V.1.9 (Purcell et al., 2007) with the input dataset and appropriate grouping files. The output F_{ST} data was imported into R, where negative values were removed, and the top 0.1 % F_{ST} values were identified using the 99.9th percentile threshold.

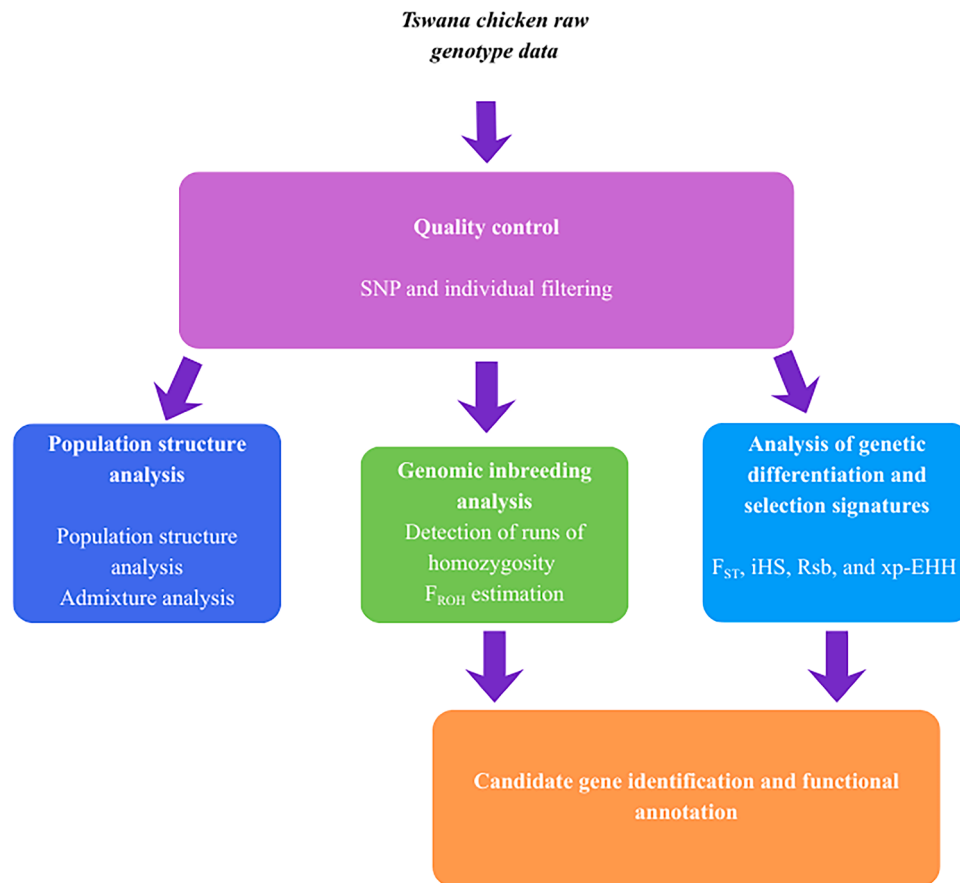


Fig. 1. Workflow of genome-wide SNP analyses for population structure, genomic inbreeding and selection signatures in Tswana chicken strains using various methods.

Manhattan plots were generated to visualize F_{ST} values, highlighting significant SNPs using a custom color scheme on *ggplot2*. To refine candidate regions, a 100 kb flanking window was defined around significant SNPs by calculating start and end positions. The significant SNPs and flanking regions were considered as regions of interest.

ROH analysis. The ROH analysis was conducted using the *detectRUNS* package in R v.4.3.2. Runs of homozygosity (ROH) were identified using a sliding window approach with a window size of 50 SNPs, a minimum of 50 consecutive SNPs, and a minimum ROH length of 1 Mb. A density threshold of 1 SNP per 50 kb was applied to account for the resolution of the 60 K SNP chip. The maximum gap allowed between consecutive SNPs was 1 Mb, with up to one opposing and two missing genotypes permitted within a window. Manhattan and bar plots were generated to visualize ROH distributions and patterns across groups. The top 0.1 % of SNPs by ROH count were extracted for further analysis, including defining 100 kb flanking regions around significant SNPs.

iHS, Rsb and xp-EHH analysis. To identify selection signatures in the study populations, we employed the integrated haplotype score (iHS), cross-population extended haplotype homozygosity (xp-EHH), and the standardized ratio of site-specific extended haplotype homozygosity (Rsb) statistics. PLINK v1.9 and Beagle 5.4 (Browning et al., 2021) were used to phase the data and the R package *rehh* (Gautier and Vitalis, 2012) was employed to compute the iHS for each SNP. The iHS statistic was computed to detect within-population selection signals by estimating extended haplotype homozygosity (EHH) with *scan_hh*, merging results per population, and calculating iHS values using *ihh2ihs* with frequency binning for bias correction. xp-EHH was used to detect signatures via *ies2xpeshh*, comparing haplotype decay between populations, and Rsb analysis was done using *ies2rsb* for standardized haplotype decay comparison. Manhattan plots were generated in *ggplot2* with

significant regions annotated using publicly available databases to identify genes under selection. Putative genes were identified using the *calc_candidate_regions* function, which applied a 100 kb window size overlap, considering regions as candidates if they contained at least 2 extreme SNP markers, representing at least 20 % of the total markers in the region. We considered genes detected by at least two methods as putative candidates and further prioritized those identified by three or more methods, focusing on the overlap among analyses to highlight common genomic regions consistently flagged across multiple approaches.

Functional annotation and candidate genes. Biomart was used for the functional annotation of genes which were identified as putative from the analysis (Harrison et al., 2024; Smedley et al., 2009). Additionally, the functions of these genes were obtained through a literature review on Google and the use of GeneCards (Stelzer et al., 2016).

Results

Quality control

A total of 57,636 SNPs were initially genotyped for each of the strains (Table 1). Post quality control filtering (5 % SNP call rate and removal of non-autosomal variants) and exclusion of poorly genotyped individuals, the Nakedneck, Normal and Dwarf strains retained 52,074, 51,905, and 51,783 SNPs, respectively. In total, three animals were removed, and 5,562, 5,731, and 5,853 SNPs were excluded from the Nakedneck, Normal, and Dwarf strains, respectively.

Table 1. Summary of quality control individual and SNP filtering in the Tswana chicken strains.

Table 1

Summary of SNP Filtering in the Tswana chicken strains.

Strain	n	Total SNPs before filtering	Removed animals	N	Excluded SNPs		Total SNPs removed	Total SNPs retained	Total SNPs retained (%)
					5 % SNP call rate	Non-autosomal variants			
Nakedneck	32	57636	0	32	1240	4322	5562	52074	90.3
Normal	40	57636	3	37	1409	4322	5731	51905	90.1
Dwarf	12	57636	0	12	1531	4322	5853	51783	89.8
Totals	84		3	81					

n = number of animals before filtering; N = number of animals retained after quality control.

Principal component analysis (PCA)

The PCA revealed a clustering pattern among Tswana chickens, with PC1 (4.96 %) and PC2 (6.03 %) jointly explaining 10.99 % of the total variance (Fig. 2). The Dwarf strain mainly grouped in the upper-left while some Dwarf individuals also overlapped with the Nakedneck and Normal strains near the origin. Some Normal individuals also formed loose clusters, notably scattering away from the other clusters.

Admixture analysis

The population structure was assessed by inferring the optimal number of clusters ($K = 2$ to $K = 6$) within the dataset, allowing for the detection of both inter-population differences and potential hidden substructure (Fig. 3). The most likely number of clusters was determined to be $K = 3$ (Fig. 4). The Nakedneck, Normal and Dwarf strains displayed similar genetic profiles, suggesting a shared ancestry among them. Within these populations, some Nakedneck individuals appeared less admixed, with several presenting as nearly pure lines. A similar, though less pronounced, pattern was also observed in certain Dwarf and Normal individuals, indicating varying degrees of admixture within strains.

F_{ST} analysis and detection of selection signatures

The Weir and Cockerham's F_{ST} between the three chicken strains are summarized in Table 2. Genetic differentiation between the Nakedneck and Normal populations was low (0.012) consistent with a close genetic relationship. In contrast, the Dwarf population showed higher levels of

genetic differentiation, with values of 0.034 and 0.035 when compared with the Nakedneck and Normal, respectively.

Table 2. Pairwise Weir and Cockerham's F_{ST} estimates among the three Tswana chicken strains

The F_{ST} analysis identified a total of 88 annotated genes across the identified 95 divergent regions from the strain combinations (Supplementary file Table S1). The Dwarf vs. Nakedneck comparison yielded 41 genes, followed by Dwarf vs. Normal with 30 and Normal vs. Nakedneck with 17 (Fig. 5).

ROH analysis

The distribution of runs of homozygosity (ROH) varied among the Tswana chicken strains (Table 3). The Normal strain exhibited the highest total number of ROH (214), followed by Nakedneck (154) and Dwarf (91). Across all strains, ROH segments of 0–2 Mb were the most frequent, with Normal, Nakedneck, and Dwarf recording 152, 114, and 70 segments, respectively. ROH segments of 2–4 Mb were also common, observed in 47, 37, and 18 segments for Normal, Nakedneck, and Dwarf. Longer ROH segments (4–8 Mb) were less frequent, with 12 in Normal and 3 each in Nakedneck and Dwarf. Only the Normal strain contained segments in the 8–16 Mb category (3). Overall, the Normal strain consistently showed higher counts across most ROH length classes, whereas the Dwarf strain had the fewest segments in the shorter classes but maintained a presence in the intermediate lengths.

Table 3. Distribution of runs of homozygosity (ROH) across the three Tswana chicken strains

Mean ROH lengths across classes were comparable, ranging between

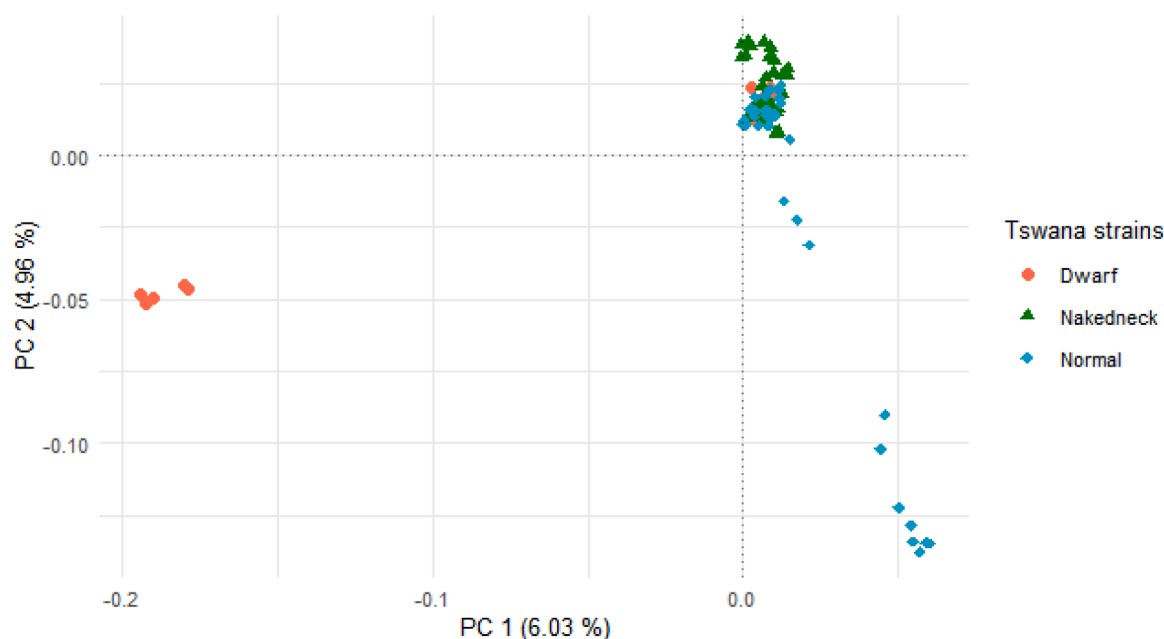


Fig. 2. Principal component analysis (PCA) of Dwarf, Nakedneck and Normal Tswana chicken strains based on genome-wide SNP data. Each point represents one individual, colored by strain. The percentage of total genetic variance explained by PC1 (6.03 %) and PC2 (4.96 %) is indicated on the axes.

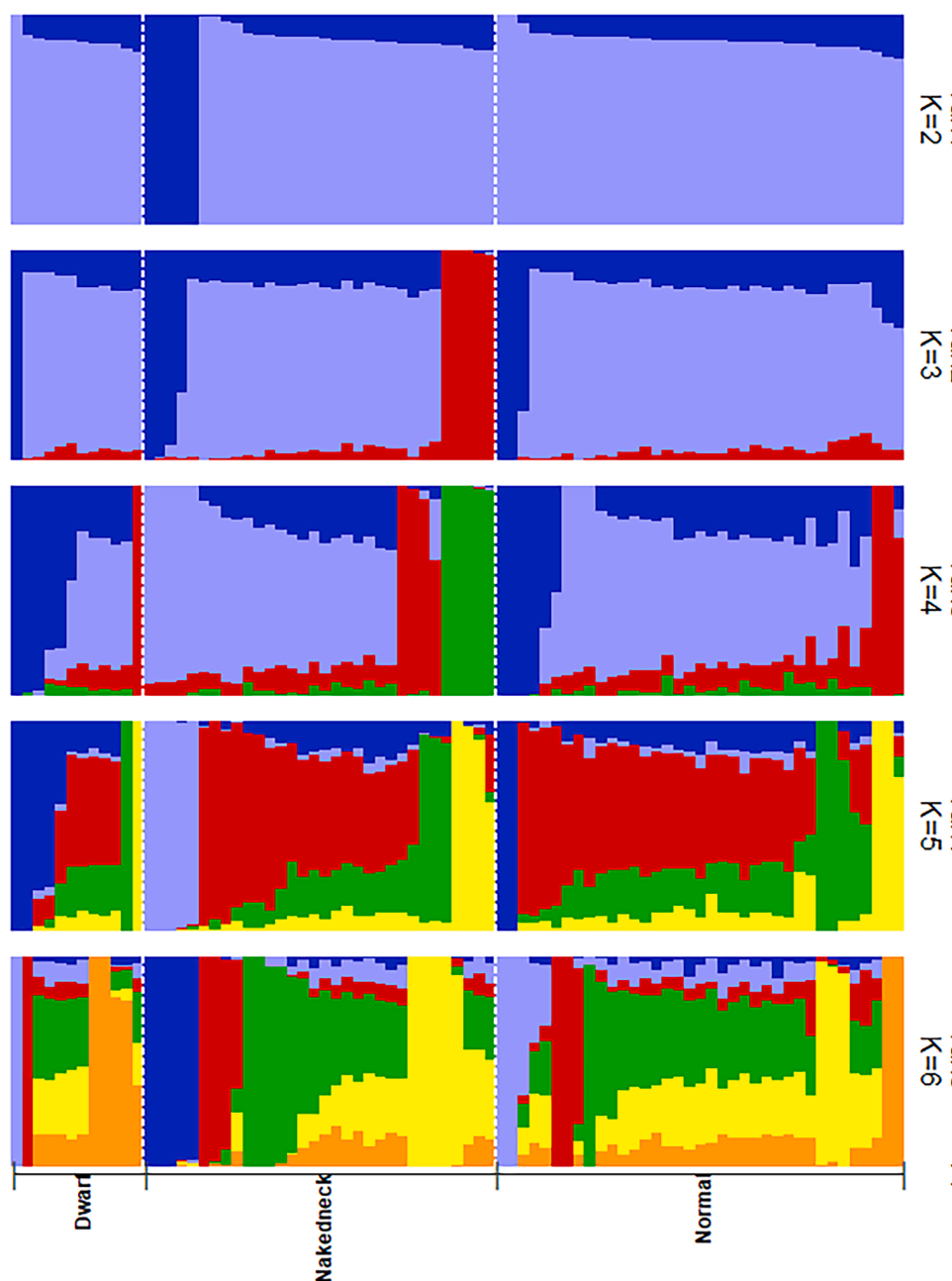


Fig. 3. Admixture analysis of Tswana chicken strains for $K = 2$ to $K = 6$ populations. Each vertical bar represents one individual and colors denote inferred ancestry proportions. Individuals are grouped by Tswana chicken strains; Dwarf, Nakedneck and Normal.

1 and 5 Mb in the short categories (<8 Mb), while the Normal strain was distinct in exhibiting an average length of 9 Mb in the longer classes (Fig. 5). At the chromosomal level, the Normal strain consistently displayed longer tracts on macrochromosomes, particularly GGA1 (2.43 Mb), GGA2 (2.41 Mb), and GGA9 (2.21 Mb), compared to the other strains. The Dwarf strain showed longer ROH on GGA5 (2.26 Mb), GGA12 (2.19 Mb), and GGA14 (2.57 Mb), whereas the Nakedneck strain exhibited elevated values on GGA6 (2.22 Mb) and GGA20 (2.58 Mb). Notably there were no values recorded in microchromosomes such as GGA 17,23 and 25 for the Dwarf, Nakedneck and Normal strains respectively. In terms of ROH proportions, the Normal strain contributed the highest percentages on GGA1 (21.0 %) and GGA2 (21.5 %), while the Dwarf strain showed enrichment on GGA1 (13.2 %), GGA2 (11.0 %), GGA3 (10.9 %) and GGA4 (9.9 %). The Nakedneck strain demonstrated intermediate distributions, with relatively high proportions on GGA1

(20.1 %), GGA2 (16.2 %), and GGA5 (13.6 %) (Fig. 6).

Inbreeding F_{ROH}

Dwarf chickens generally displayed low to moderate F_{ROH} values across most chromosomes, with occasionally elevated coefficients such as GGA22 (0.37) and GGA25 (0.50) (Fig. 7). Nakedneck chickens showed a broader distribution of values, with sporadic peaks including GGA20 (0.41) and GGA23 (0.33). Normal chickens also exhibited a low range of inbreeding coefficients, recording F_{ROH} on GGA17 (0.16), GGA21 (0.20) and GGA22 (0.34) (Fig. 7). Genome-wide F_{ROH} ranged from 0.0013 to 0.051, with the highest in Normal individuals N54 (0.051), N56 (0.040), N35 (0.034) and N9 (0.031). Some Dwarf and Nakedneck individuals, such as D7 (0.038) and K40 (0.037), also showed elevated inbreeding (Supplementary file Table S2). The

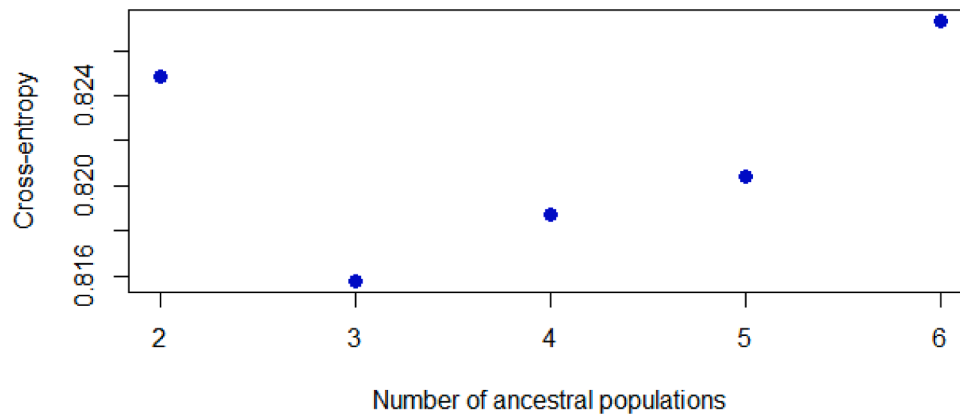


Fig. 4. Cross-entropy values from admixture analysis of Tswana chicken strains across $K = 2$ to $K = 6$ populations. The lowest cross-entropy at $K = 3$ indicates the most likely number of ancestral populations.

Table 2

Weir and Cockerham's F_{ST} between the four chicken populations.

Population	Nakedneck	Normal	Dwarf
Naked neck	0.00	-	-
Normal	0.012	0.00	-
Dwarf	0.034	0.035	0.00

F_{ST} : Fixation index.

genome-wide F_{ROH} among the strains was generally low with the Dwarf chickens recording the highest value (0.01396), followed by the Normal strain (0.01216), while the Nakedneck strain exhibited the lowest (0.00921) (Supplementary file Table S2).

ROH hotspots

The analysis of ROH hotspots identified overlapping regions on GGA5 across the three chicken strains. In the Dwarf strain, a hotspot spanning 1.92–3.58 Mb was detected, containing *NAV2*, *PRMT3*, *SLC6A5*, *NELL1*, *ANO5*, *SLC17A6* and *ANO3* (Table 4). The Nakedneck strain showed a similar hotspot between 1.92 and 3.03 Mb, which included *NAV2*, *NELL1*, *ANO5* and *SLC17A6*. In the Normal strain, the ROH regions extended from 2.42 to 3.75 Mb and contained *NELL1*, *ANO5*, *SLC17A6*, *ANO3*, *FIBIN* and *BBOX1*. An additional larger segment was observed in the Normal strain, encompassing *ALG14*, *CNN3*, *F3*, *ARHGAP29*, *ABCA4*, *GCLM*, *DNTTIP2*, *BCAR3*, *TMED5*, *FAM69A*, *EVI5* and *GFI1*.

Table 4 ROH Hotspots and putative genes identified in the Dwarf, Nakedneck and Normal Tswana chicken strains

iHS

The integrated haplotype score (iHS) analysis identified several candidate regions under potential recent positive selection in only the

Nakedneck and Normal strains (Fig. 8). In the Normal strain, eight (8) regions were detected across GGA1, GGA2, GGA3, GGA4, GGA10 and GGA20. The strongest signal was observed on GGA20 between 2.40 and 5.50 Mb. Additional notable regions included GGA1 (103.8–106.6 Mb), GGA2 (81.7–83.3 Mb), and GGA3 (54.5–56.4 Mb). In the Nakedneck strain, seven (7) candidate regions were distributed across GGA3, GGA4, GGA6, GGA8, GGA10, GGA11 and GGA15. The highest signal was detected on GGA3 (79.8–81.7 Mb), followed by regions on GGA4 (41.8–43.7 Mb) and GGA8 (13.9–15.7 Mb)

Table 5 shows candidate regions identified through iHS and the associated putative genes. In the Normal strain, the strongest cluster of selection signals was observed on GGA1 (104–106 Mb), harboring genes such as *IFNAR2*, *IL10RB*, *IFNAR1*, *IFNGR2*, *GART-B* and *SON*. Additional intervals included a region on GGA2 (81.89–83.19 Mb) containing *VSTM2A*, *FHOD3*, and *RPRD1A* and multiple adjacent loci on GGA3 (54.49–55.52 Mb and 55.87–55.93 Mb) spanning genes like *IFNGR1*, *IL22RA2*, *MAP3K5*, *MAP7*, *BCLAF1*, *HBS1L*, *ALDH8A1*, *SGK1*, and *SLC2A12*. Another locus of interest on GGA3 (55.26–55.35 Mb) was defined by *AHI1*. On GGA4 (36.80–38.56 Mb), several developmental and signaling genes were detected, including *LEF1*, *DKK2*, *PAPSS1*, and *CXXC4*. Additional signals were noted on GGA4 (60.89–62.31 Mb), encompassing *KLKB1*, *FRG1BP*, and *ASAHI1*. In the Naked neck strain, candidate regions were more dispersed across the genome. Notable

Table 3

Distribution of runs of homozygosity (ROH) across the three Tswana chicken strains.

Class	Dwarf	Naked neck	Normal
0–2	70	114	152
2–4	18	37	47
4–8	3	3	12
8–16	0	0	3
Total	91	154	214

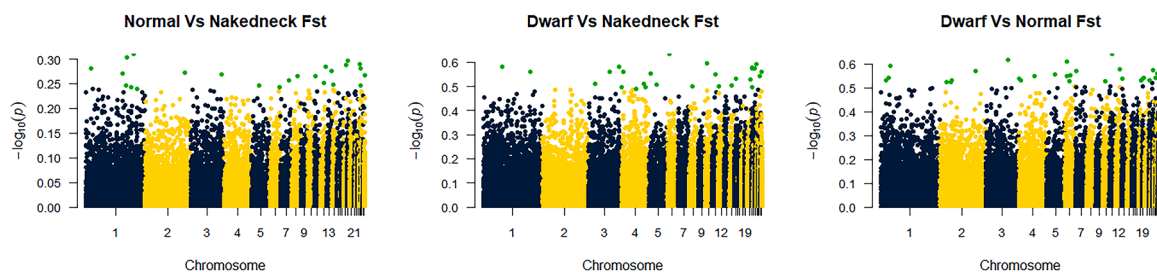


Fig. 5. Genome-wide Manhattan plots of Weir and Cockerham's F_{ST} for pairwise comparisons among Tswana chicken strains. The y-axis represents F_{ST} values for individual SNPs, plotted by chromosomal position. The horizontal dashed line indicates the 99.9th percentile threshold used to define highly differentiated loci.

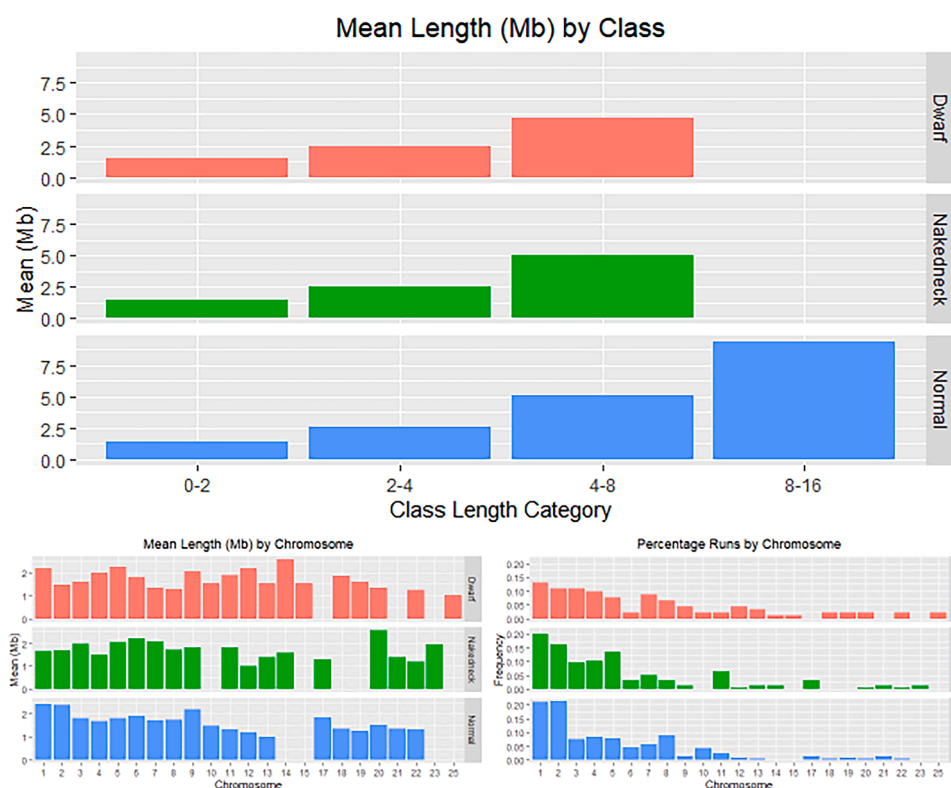


Fig. 6. Mean length and percentage by class and chromosomes of runs of homozygosity (ROH) across Tswana chicken strains.

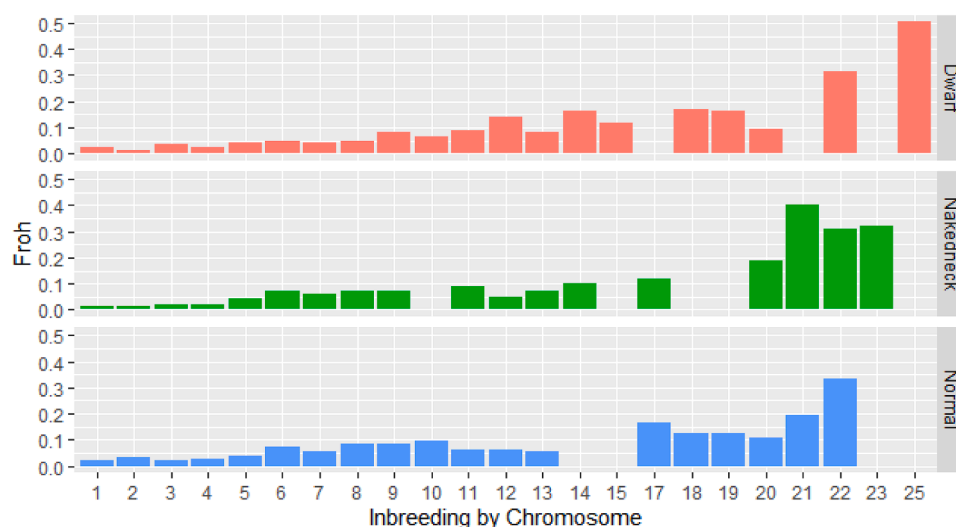


Fig. 7. Chromosome-wise inbreeding coefficients (F_{ROH}) for Dwarf, Nakedneck and Normal Tswana chicken strains. F_{ROH} (y-axis) represents the proportion of each chromosome (x-axis) covered by runs of homozygosity.

intervals included GGA3 (79.78–81.22 Mb), with genes such as *HTR1B*, *COL12A1* and *EEF1A1* and GGA4 (41.79–43.28 Mb) containing *AGA*, *GALNT6*, *GALNT7* and *CEP44*. A large segment on GGA6 (19.89–20.91 Mb) harbored a dense cluster of functional genes, including *FAS*, *IFIT5*, *KIF20B* and *TNKS2*. On GGA8 (13.93–15.65 Mb), selection encompassed genes such as *TGFBR3*, *HFM1*, *BARHL2* and *SELENOF*. A broad region on GGA10 (9.90–11.79 Mb) contained multiple genes, including pigmentation and metabolic candidates such as *SEMA6D*, *SLC24A5*, *FGF7*, *SQOR*, *TRPM7*, and *GATM*. Additional signals were detected on GGA11 (3.10–4.99 Mb), defined by *IRX3*, *TOX3* and *SLC12A4* and on GGA15 (4.95–6.53 Mb), which comprised a wide array of genes, including *EIF2B1*, *PITPNM2*, *P2RX7*, *ORAI1*, *SETD1A*, *SH2B3* and *PTPN11*.

Table 5 Putative genes identified by iHS in the Nakedneck and Normal Tswana chicken strains

Detection of Selection signatures by Rsb and xp-EHH analysis

Selection signature analyses using xp-EHH and Rsb identified multiple genomic regions in the Nakedneck vs Normal comparison only (Figs. 9 and 10). In the xp-EHH analysis, regions were detected on GGA4 (68.2–70.0 Mb) and GGA8 (7.8–9.9 Mb), containing genes such as *APBB2*, *PDS5A*, *TLR1A*, *ARAP2*, *C1orf21*, *RNF2*, and *BRINP3*. The Rsb analysis revealed additional regions on GGA6 (14.1–16.3 Mb) and GGA7 (5.5–7.3 Mb), with genes including *KCNMA1*, *DUSP13*, *TRPM8*, and

Table 4

ROH Hotspots and putative genes identified in the Dwarf, Nakedneck and Normal Tswana chicken strains.

GGA	Breed	Start (Mb)	End (Mb)	Genes
5	Dwarf	1.92	2.71	<i>NAV2, PRMT3, SLC6A5, NELL1</i>
		2.93	2.98	<i>ANO5</i>
		3.00	3.03	<i>SLC17A6</i>
		3.41	3.58	<i>ANO3</i>
5	Nakedneck	1.92	2.71	<i>NAV2, PRMT3, SLC6A5, NELL1</i>
		2.93	2.98	<i>ANO5</i>
		3.00	3.03	<i>SLC17A6</i>
		2.42	2.71	<i>NELL1</i>
5	Normal	2.93	2.98	<i>ANO5</i>
		3.00	3.03	<i>SLC17A6</i>
		3.41	3.58	<i>ANO3</i>
		3.60	3.70	<i>FIBIN</i>
		3.71	3.75	<i>BBOX1</i>

ADARBI. Notably, the region on GGA8 was detected by both xp-EHH and Rsb analyses. A summary of all the candidate genes is provided in [Supplementary Table S3](#).

Common genes across multiple tests

A total of four genes were identified by at least two analyses (xp-EHH, Rsb, Fst and iHS) ([Table 6](#)). These include *BRINP3*, *C1orf21*, and *RNF2* on GGA8, as well as *ISCU* on GGA15 detected in comparisons involving the Nakedneck and Dwarf strains ([Table 6](#)). These genes represent potential candidate regions under convergent selection identified by complementary haplotype-based and differentiation-based methods ([Table 6](#)).

Table 6: Candidate genes detected by three selection methods across Tswana chicken strains

Discussion

Understanding the genomic architecture of indigenous livestock is important for both conserving genetic diversity and supporting adaptation to changing environments. This study examined the inbreeding patterns and the selection signatures of indigenous Tswana chicken strains using genome-wide SNP data and multiple methods, including iHS, Rsb, and xp-EHH, alongside ROH analysis. These approaches allowed us to identify putative regions under selection and assess the extent of inbreeding across different Tswana chicken strains; Dwarf, Nakedneck and Normal. The findings highlight genomic regions of interest which may contribute to adaptation and give a foundation to better understanding and maintaining Tswana chicken population diversity.

A total of 84 chickens with 57 636 SNPs were initially considered, however three (3) Normal strain individuals were excluded due to low genotyping rates, likely because of the poor quality of the samples. No thresholds were imposed for Hardy-Weinberg Equilibrium (HWE) or

Minor Allele Frequency (MAF) to preserve signals of selection and rare variants that may be critical for understanding local adaptation. Rare alleles and deviations from HWE can themselves arise as consequences of selection, population structure, or recent demographic history and their removal may disproportionately eliminate loci of adaptive relevance ([Pearman et al., 2022](#)). Furthermore, ROH and haplotype-based analyses rely on extended patterns of linkage disequilibrium rather than independent SNP frequencies, therefore aggressive LD pruning can disrupt true homozygous segments and remove biologically meaningful information. [Meyermans et al. \(2020\)](#) demonstrated that LD pruning reduces genome coverage and decreases the number and length of detected ROHs in livestock datasets, potentially biasing inbreeding and selection comparisons and leading to missed regions of extended homozygosity. Previous studies of indigenous livestock populations have similarly retained genome-wide SNP variation without imposing strict HWE or MAF thresholds ([Mastrangelo et al., 2023](#); [Meyermans et al., 2020](#); [Sarviaho et al., 2024](#)). While the inclusion of low-frequency variants may increase background noise in allele-frequency-based statistics such as F_{ST} , this effect is mitigated by the use of stringent percentile thresholds and by focusing on clusters of extreme markers rather than isolated SNPs. Furthermore, candidate regions and genes were prioritized using more than one method. This overlap-based approach reduces method-specific bias and increases confidence in the identified selection signals compared to relying on a single statistic.

Post quality control, all three strains retained a comparable number of SNPs (89.8 %, 90.1 % and 90.3 %), which suggests that the retained data are of reliable quality. While the 60 K iSelect chicken SNP array was developed primarily from commercial breeds, the high retention of SNPs observed across the Tswana chicken strains indicates that the array provided reliable genomic coverage ([Groenen et al., 2011](#)). Although the first two principal components explained a modest proportion of the total genetic variance (~11 %), this pattern is consistent with expectations for indigenous livestock populations where differentiation is often distributed across multiple axes rather than concentrated in the leading components ([Ma and Shi, 2020](#)). Accordingly, PCA results are conservatively interpreted as illustrative of broad genetic relationships rather than definitive population separation.

The ordination plot revealed visible but modest distinctions among strains, with many Dwarf chickens clustering together while some overlapped with the Nakedneck and Normal strains, indicating partial differentiation alongside a possible shared ancestry. In this study, the birds were sampled from the Kweneng and Southern districts, which are geographically close. This proximity facilitates the exchange of breeding stock, whether through purchase or bartering, which may account for the observed clustering patterns. A similar trend was also reported in Tanzanian chickens, where the absence of physical barriers, close geographical proximity of sampling locations and free-range management practices allowed populations to cluster together genetically ([Luo et al., 2020](#); [Mushi et al., 2020](#)). Interestingly, a portion of the Normal strain was scattered away from the Dwarf and Nakedneck clusters, suggesting possible within strain variability. The Normal strain, which

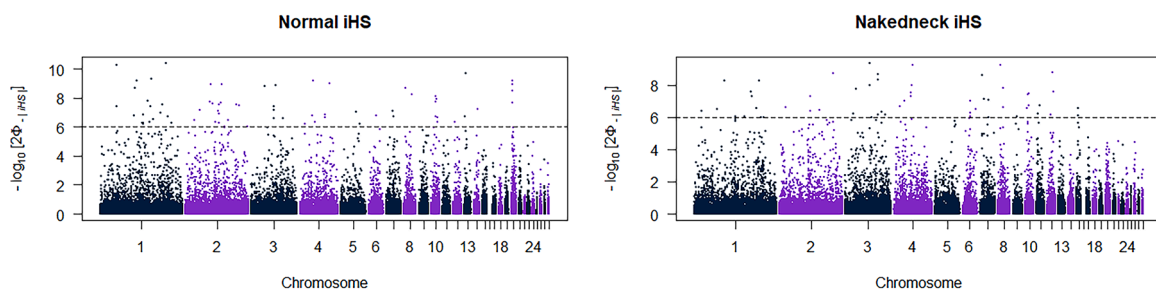


Fig. 8. Genome-wide selection signatures detected using integrated haplotype score (iHS) in Nakedneck and Normal strains. The y-axis shows standardized iHS values plotted by chromosomal position. The horizontal dashed line represents the top 0.1 % genome-wide threshold used to identify candidate regions under selection.

Table 5

Putative genes identified by iHS in the Nakedneck and Normal Tswana chicken strains.

Breed	GGA	Start (Mb)	End (Mb)	Genes
Normal	1	104.00	106.000	<i>N6AMT1, USP16, MAP3K7CL, BACH1, SOD1, HUNK, EVA1C, OLIG2, IFNAR2, IL10RB, IFNAR1, IFNGR2, GART-B, SON, ITSN1, MRPS6, SLC5A3, KCNE2, CLIC6</i>
	2	81.887	83.187	<i>VSTM2A, FHOD3, RPRD1A</i>
	3	54.493	55.523	<i>IFNGR1, IL22RA2, IL20RA, MAP3K5, MAP7, MTFR2, BCLAF1, HBS1L, ALDH8A1</i>
	3	55.867	55.930	<i>SGK1, SLC2A12</i>
	3	55.262	55.353	<i>AHI1</i>
	4	36.804	38.561	<i>OPN4-1, COL25A1, ETNPPL, LEF1, PAPSS1, DKK2, GIMD1, TBCK, INTS12, PPA2, CXXC4</i>
	4	60.894	60.915	<i>KLKB1</i>
	4	62.280	62.309	<i>FRG1BP, ASAH1</i>
	3	79.776	81.224	<i>HTR1B, IMPG1, FILIP1, TMEM30A, COX7A2, COL12A1, SLC17A5, EEFA1, CGAS</i>
	4	41.792	43.289	<i>AGA, GALNTL6, GALNT7, SAP30, CEP44</i>
	6	19.896	20.910	<i>TMEM72, LIPML5, LIPML4, STAMBPL1, FAS, IFT5, KIF20B, RPP30, PCGF5, HECTD2, TNKS2, NOC3L</i>
	8	13.936	15.651	<i>GLMN, TGFBR3, HFM1, ZNF644, BARHL2, KYAT3, GTF2B, SELENOF</i>
	10	9.900	11.789	<i>SEMA6D, SLC24A5, CTXN2, SLC12A1, DUT, GALK2, FGF7, DTWD1, SPATA5L1, C10H15ORF48, SQOR, BLOC1S6, SPPL2A, TRPM7, GABPB1, HDC, GATM, PDE8A, WHAMM, FAM103A1, TM6SF1, SH3GL3, ADAMTSL3, EFL1, MEX3B</i>
	11	3.103	4.990	<i>DPEP2, SLC12A4, IRX3, RPRGIP1L, AKTIP, TOX3</i>
	15	4.959	6.539	<i>EIF2B1, RILPL1, RILPL2, SBNO1, CDK2API, MPHOSPH9, PITPNM2, ABCB9, VPS37B, GPN3, ARPC3, ANAPC7, P2RX7, P2RX4, RNF34, ORAI1, TMEM120B, SETD1A, PSMD9, BCL7A, MLXIP, LRRCA43, B3GNT4, KNTC1, RAD9B, TCTN1, CCDC63, CUX2, SH2B3, ALDH2, MAPKAPK5, ADAM20L, TRAFD1, PTPN11, RPH3A, WSCD2, FICD, ISCU</i>
Nakedneck	3	79.776	81.224	<i>HTR1B, IMPG1, FILIP1, TMEM30A, COX7A2, COL12A1, SLC17A5, EEFA1, CGAS</i>
	4	41.792	43.289	<i>AGA, GALNTL6, GALNT7, SAP30, CEP44</i>
	6	19.896	20.910	<i>TMEM72, LIPML5, LIPML4, STAMBPL1, FAS, IFT5, KIF20B, RPP30, PCGF5, HECTD2, TNKS2, NOC3L</i>
	8	13.936	15.651	<i>GLMN, TGFBR3, HFM1, ZNF644, BARHL2, KYAT3, GTF2B, SELENOF</i>
	10	9.900	11.789	<i>SEMA6D, SLC24A5, CTXN2, SLC12A1, DUT, GALK2, FGF7, DTWD1, SPATA5L1, C10H15ORF48, SQOR, BLOC1S6, SPPL2A, TRPM7, GABPB1, HDC, GATM, PDE8A, WHAMM, FAM103A1, TM6SF1, SH3GL3, ADAMTSL3, EFL1, MEX3B</i>
	11	3.103	4.990	<i>DPEP2, SLC12A4, IRX3, RPRGIP1L, AKTIP, TOX3</i>
	15	4.959	6.539	<i>EIF2B1, RILPL1, RILPL2, SBNO1, CDK2API, MPHOSPH9, PITPNM2, ABCB9, VPS37B, GPN3, ARPC3, ANAPC7, P2RX7, P2RX4, RNF34, ORAI1, TMEM120B, SETD1A, PSMD9, BCL7A, MLXIP, LRRCA43, B3GNT4, KNTC1, RAD9B, TCTN1, CCDC63, CUX2, SH2B3, ALDH2, MAPKAPK5, ADAM20L, TRAFD1, PTPN11, RPH3A, WSCD2, FICD, ISCU</i>

was previously reported as the most common in Botswana, may have gained diversity through historical cockerel exchange programs in the southern region of the country dating back to the 1970s (Badubi et al., 2006; Machete et al., 2021). Such practices likely contributed to the observed dispersion within strain in the Normal Tswana chicken. However, given the modest variance explained by the leading principal components, as well as limitations in sample size and SNP density, this pattern is interpreted cautiously. Rather than indicating established ecotypes, the results may show subtle population heterogeneity that warrants further investigation with larger and more geographically diverse samples. The admixture analysis further supports the genetic relationships identified in the PCA, with $K = 3$ emerging as the optimal number of clusters.

The current study adopted a conservative approach by using single-SNP F_{ST} values with fixed 100 kb flanking windows, rather than fully window-based or smoothed F_{ST} methods. Although windowed approaches can reduce stochastic noise, they may also obscure or dilute narrow signals of differentiation when applied to medium-density SNP arrays (Da Silva Ribeiro et al., 2022). The Nakedneck and Normal ($F_{ST} = 0.012$) were the least differentiated, suggesting a close genetic

relationship and shared ancestry. In comparison, the Dwarf strain exhibited moderately higher with F_{ST} values of 0.034 and 0.035 when compared with the Nakedneck and Normal strains, respectively. These findings are much lower than those detected in lean and fat broiler lines which had an average F_{ST} of 0.12 (Na et al., 2019). However, in Nigerian indigenous chickens, the F_{ST} value (0.03) were comparable to the Dwarf comparison but lower than the Nakedneck and Normal in the current study (Ajibike et al., 2022). As Machete et al. (2021) previously stated, the Nakedneck and Normal strains are postulated to originate from a common area in Asia or may be similar because they mate across strains. Size-assortative mating has been observed in birds, with body size influencing mating success through dominance hierarchies, mate choice and competitive advantage (Wang et al., 2019). Therefore, the pronounced small body size of the Dwarf strain could plausibly reduce mating opportunities with the larger Normal and Nakedneck birds, limiting gene flow and contributing to its observed differentiation. However, breed specific studies are needed to confirm this mechanism in the Tswana chicken.

The ROH parameters used in this study are consistent with those applied in recent 60 K chicken SNP analyses. For example, Kim et al. (2024) evaluated genome coverage of the 60 K chip and retained a density threshold of 1 SNP per 50 kb and a minimum ROH length of 1 Mb, while Macharia et al. (2024) applied similar sliding-window criteria using the Illumina 60 K array. Our parameter choices therefore fall within established ranges for medium-density chicken SNP panels and represent a conservative approach to minimize false positive ROH detection while ensuring adequate genome coverage. These strict parameters reduce the likelihood of spurious short ROH arising from uneven SNP spacing, which is particularly relevant in chickens due to the presence of macro and microchromosomes with heterogeneous recombination rates and SNP coverage (Groenen et al., 2011; Meyermans et al., 2020).

Our findings reveal that the Normal and Nakedneck strains exhibited the highest total ROH counts (214 and 154, respectively), while the Dwarf strain showed markedly fewer ROH (91), suggesting lower inbreeding levels and greater genetic diversity. Nearly all the detected ROH in the strains were short (<8 Mb) with only a small proportion (1.2 %) classified as long segments, which were observed exclusively in the Normal strain. These results were consistent with findings in native Korean and Italian chickens where short ROH (1-4 Mb) were dominant which indicates historical inbreeding or population bottleneck (Cendron et al., 2021; Macharia et al., 2024). The occurrence of long ROH segments in the Normal strain may point to recent demographic or management-related processes rather than widespread population-level inbreeding (Macharia et al., 2024). Long ROH are generally interpreted as signatures of recent common ancestry or localized inbreeding events affecting a limited number of individuals, whereas short ROH predominantly reflect older demographic history (Ceballos et al., 2018). In the present study, long ROH were detected in only a small subset of Normal individuals, suggesting that recent inbreeding may be confined to certain families or small household flocks rather than being common across the entire strain. Given that the Normal strain is the most common Tswana chicken population, it is possible that some sampled birds were more closely related.

Notably several chromosomes did not register ROH or contribute to the inbreeding coefficient. This is consistent with known biases of SNP panels such as the 60 K array which underrepresent microchromosomes (GGA11 up to GGA38) and therefore limit their detection (Groenen et al., 2011). Structural features of microchromosomes such as the high recombination rates reduce the likelihood of long continuous homozygous segments, making them less detectable in ROH analyses (Bosse et al., 2012). Consequently, most ROH in this study were consistently observed on the macrochromosomes (GGA1-5) and the intermediate chromosomes (GGA6-GG10). Additionally, certain chromosomes such as GGA16 have been documented to be short and lack sufficient informative alleles due to the presence of the major histocompatibility

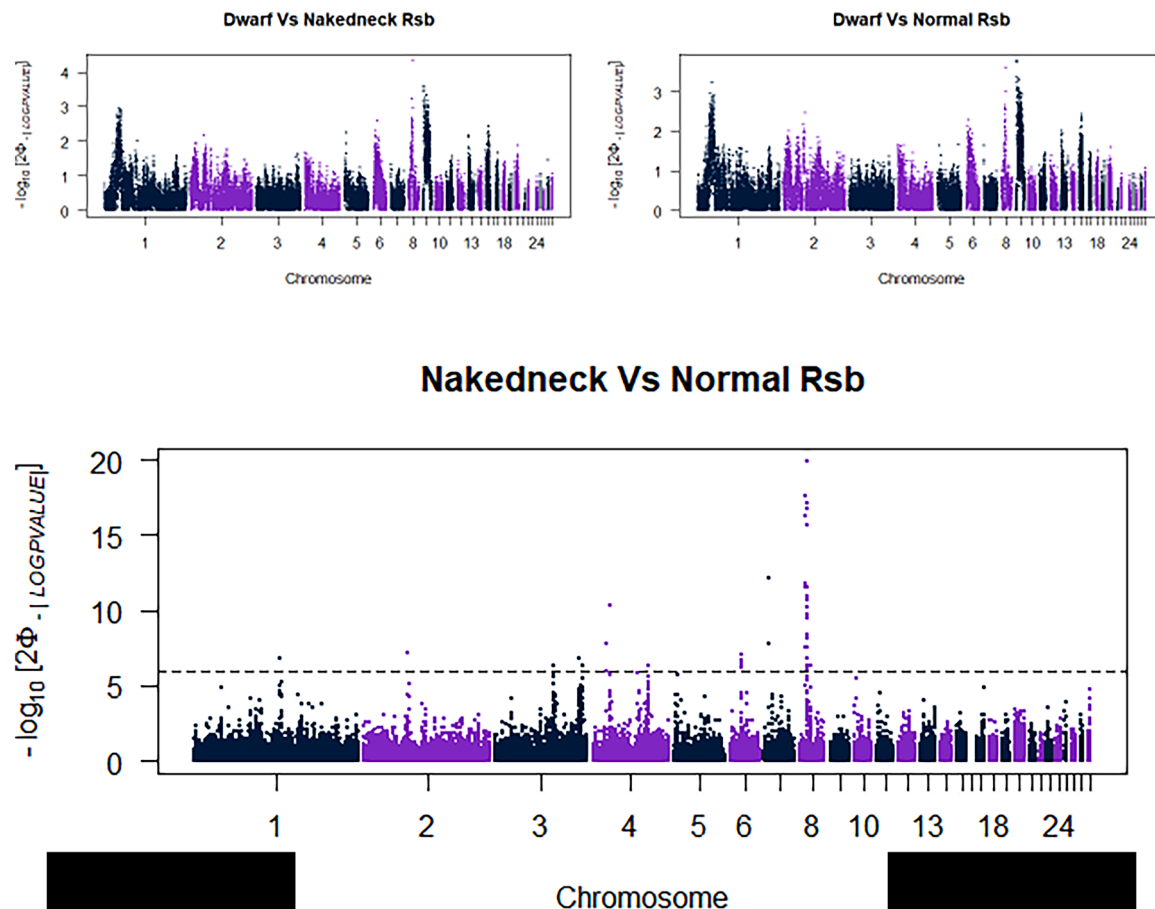


Fig. 9. Genome-wide selection signatures identified using Rsb for pairwise comparisons among Tswana chicken strains. The y-axis represents Rsb values, with the dashed line indicating the 99.9th percentile threshold.

complex (MHC) gene cluster, resulting in insufficient informative SNPs (Kim et al., 2025).

Genome-wide F_{ROH} results also revealed low to moderate inbreeding across all strains, consistent with a population that maintains considerable genetic diversity. Further inspection showed that some individuals showed elevated coefficients with F_{ROH} values, with the highest observed in Normal individuals such as N54 (0.051) and N56 (0.040). A few Dwarf and Nakedneck chickens, such as D7 (0.038) and K40 (0.037), also exhibited relatively comparable inbreeding values. These findings are lower than the findings with previous studies in Russian (0.141) and Korean (0.143) chicken populations (Dementieva et al., 2020; Macharia et al., 2024). The small sample size, particularly of the Dwarf strain, coupled with its previously reported smaller effective population size by Machete et al. (2021), may contribute to the slightly higher mean observed for this strain compared to the Nakedneck and Normal strains. Although genome-wide inbreeding is currently low, proactive management of genetic diversity would help mitigate potential future risks.

The analysis of ROH also highlighted the same hotspot located on GGA5 (1.92–3.75 Mb) harboring genes such as *NELL1*, *ANO5* and *SLC17A6* in all three strains. Kimet et al. (2025) and Macharia et al. (2024) also identified the same region highlighting the same genes in the Yeonsan Ogye (YO) breed and Korean chickens, respectively. *NELL1* plays a role in bone development and has been highlighted as important in skeletal integrity of broilers (Elferink et al., 2012). Mutations in *ANO5* are implicated in muscle repair and membrane stability in mammals, with mutations leading to muscular dystrophies and skeletal abnormalities (Jarmula et al., 2019). *SLC17A6*, which encodes a vesicular glutamate transporter, may influence neurological functions and stress

responses, indirectly affecting behavior or feed efficiency (Serrano-Saiz et al., 2020). Collectively, the identification of these genes within the same ROH hotspot across all 3 strains point to their possible contribution to robust skeletal structure and muscle maintenance. These traits are relevant under free-range production systems, where locomotion, foraging efficiency and predator avoidance directly influence fitness and overall productivity. The recurrence of this locus across strains may represent a conserved genomic segment enriched for genes influencing musculoskeletal development. This region could serve as a candidate target for marker-assisted or genomic selection strategies aimed at improving structural resilience and survivability in low-input production systems. However, functional validation and association with relevant phenotypes would be necessary before direct implementation in breeding programs.

The detection of candidate genomic regions under potential selection revealed four genes located on GGA8 and GGA15, each identified by at least two selection methods, which are involved in key biological processes. These genes include *BRINP3*, *C1orf21*, *ISCU* and *RNF2*. Interestingly *BRINP3* has been associated with feeding behavior in sheep which provides functional context for its potential role in Tswana chickens (Zhao et al., 2024). *ISCU* was found to be significantly associated with egg laying interval in layers (Volkova et al., 2025). The functional roles of the identified candidate genes suggest plausible associations with observable traits such as body size, skeletal robustness, survivability and reproductive efficiency. These genomic regions therefore represent promising targets for future integrative studies in Tswana chickens.

One of the limitations of this study is sample size and its potential impact on the outcome of the chosen statistical analyses. Although

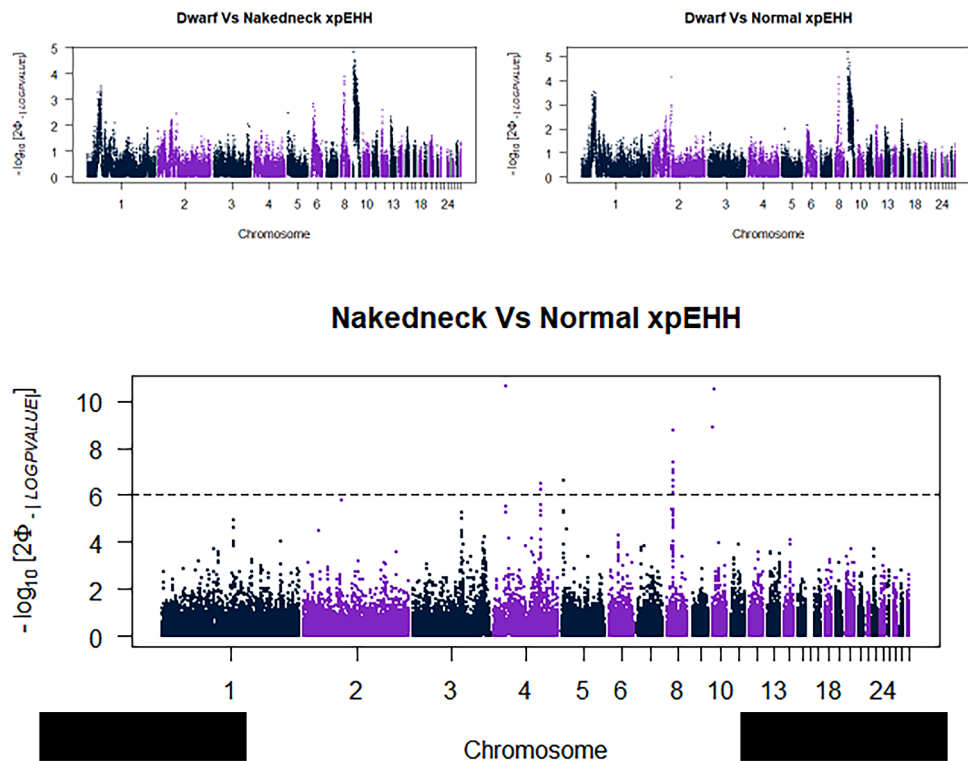


Fig. 10. Genome-wide selection signatures detected using xp-EHH for pairwise comparisons among Tswana chicken strains. The y-axis represents xp-EHH values and horizontal dashed line marks the 99.9th percentile threshold.

Table 6
Candidate genes detected by three selection methods across Tswana chicken strains.

Gene	Gene start (Mb)	Gene end (Mb)	Chr	Breeds/ Comparisons	Detected in	Test count
<i>BRINP3</i>	8.307	8.514	8	Nakedneck Vs Normal	xp-EHH, rsb	2
<i>C1orf21</i>	7.833	7.919	8	Nakedneck Vs Normal	xp-EHH, rsb	2
<i>ISCU</i>	6.536	6.539	15	Nakedneck Vs Dwarf, Nakedneck	F_{ST} , iHS	2
<i>RNF2</i>	8.034	8.080	8	Nakedneck Vs Normal	xp-EHH, rsb	2

Chr: Chromosome.

haplotype-based statistics such as iHS, Rsb and xp-EHH are sensitive to sample size and deviations from idealized assumptions, evidence from the present study indicate that Tswana chicken strains possess sufficient haplotype structure to support their exploratory application. In addition, the detection of runs of homozygosity across all strains, including shared ROH hotspots, demonstrates the presence of detectable shared haplotypes. Together, these features indicate that Tswana chicken populations deviate from random mating expectations and retain measurable haplotype structure, justifying the use of haplotype-based scans as first-pass, hypothesis-generating tools. Our findings contributes a foundational baseline for Tswana chicken strains, upon which future, larger-scale investigations can build.

Conclusions

This study provides a genome-wide assessment of inbreeding patterns and selection signatures in three Tswana chicken strains using medium-density SNP data. Overall genomic inbreeding levels were low

across strains, and the predominance of short ROH segments suggests that most homozygosity reflects older demographic processes rather than recent widespread inbreeding. The detection of long ROH in only a small subset of Normal individuals likely reflects localized or recent mating among related birds rather than strain-wide demographic effects. Moderate genetic differentiation was observed, particularly involving the Dwarf strain, while PCA and admixture analyses indicated partial clustering alongside shared ancestry among strains. These findings suggest the presence of measurable population structure without strong genetic separation. Selection scans and ROH hotspot analyses identified candidate genes associated with skeletal development, muscle maintenance and reproductive traits, which highlights genomic regions that may contribute to adaptation under low-input village production systems. Although constrained by sample size and SNP density, this study provides baseline genomic information for Tswana chickens and identifies candidate regions for further investigation. Maintaining genetic diversity within these strains will support their long-term resilience, productivity and cultural and economic importance in rural Botswana. Future studies incorporating larger sample sizes, broader geographic coverage and higher-density genomic data will help refine these findings and inform conservation and breeding strategies.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2026.106781.

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