

EARLY VIEW

RESEARCH PAPER

P O U L T R Y
S T U D I E S

Genome-Wide Patterns of Runs of Homozygosity in Barka Indigenous Chickens

Kiplangat NGENO^{1*}, 

¹Moi University, Department of Agriculture, Animal Science and Natural Resources, PO Box 3900, 30100, Eldoret, Kenya

Article History

Received: Jan 14, 2026

Accepted: Apr 19, 2026

First Online: May 05, 2026

*Corresponding Author

Tel: +254735218577

E-mail: kiplangat.ngeno@mu.ac.ke

Keywords

Genomic

Chicken

Breeding

Improvement

Abstract

Runs of homozygosity (ROH) provide insight into population history, inbreeding, and selection. In this study, genome-wide ROH patterns were evaluated in Barka indigenous chicken reared under scavenging systems in arid and semi-arid lands (ASALs), by stratifying segments by length and assessing their chromosomal distribution and gene content. Average ROH length varied across chromosomes, with the longest tracts observed on chromosomes 1, 2, 3 and 4, and shorter segments on chromosomes 11 and 28, indicating a heterogeneous genomic landscape. Gene-level analysis indicated that significant ($P < 0.05$) associations were confined to a small subset of loci within the intermediate ROH category (1-2 Mb), notably *LHX8* and *YAP1*. *LHX8* encodes a LIM homeobox transcription factor essential for oocyte differentiation and early folliculogenesis, while *YAP1* is a central effector of Hippo signalling pathway regulating cell proliferation, organ size and tissue homeostasis functions potentially linked to reproductive fitness and adaptive resilience under resource-limited ASAL conditions. These findings suggest that ROH patterns in ASAL-adapted Barka indigenous chickens reflect a combination of recent and historical demographic processes shaped by low-input scavenging systems. The limited but functionally relevant gene enrichment supports the role of localized selection on key biological pathways, while the broader genomic background remains largely neutral.

Introduction

Indigenous chickens are common in rural farms due to their ability to adapt to stressful environmental conditions, their resistance to endemic diseases, and low input requirements (Magothe et al., 2012; Ngeno et al., 2015). Egg production in indigenous chickens is, however, usually low and highly inconsistent among the different ecotypes. Certain indigenous chicken ecotypes have a superior egg-laying ability compared to others even under the same conditions. Variation in egg production among indigenous chicken ecotypes is caused by a combination of genetic, nutritional, environmental, and management factors (Muchadeyi and Dzomba, 2017). Genetically, some indigenous ecotypes may have inherent characteristics conducive to greater egg production, such as early sexual maturity

or improved feed conversion ratio. Farmers in rural areas practice traditional or informal selection for performance traits such as egg production, body weight, and brooding. Farmer or traditional informal selection is the selection of breeding individuals on the basis of visible characteristics e.g., frequency of egg laying, condition, or mothering ability, often following indigenous-experiential knowledge and local preference. Such selection criterion is transferred through generations. Inasmuch as they are not necessarily systematic or recorded, these selection activities place genetic pressure on the population and can affect the frequency of alleles for the desirable characteristics, e.g., egg production. These actions in the long run, put directional selection pressure on

certain characteristics and cause subtle genetic shifts in populations. In particular, selection on egg traits lead to fixation of alleles with positive effects, which manifest as long regions of homozygosity (ROH) in the genome. ROHs are continuous stretches of homozygous runs (Zhang et al., 2015) that can be the result of selection, inbreeding, or genetic drift and may indicate past and present selection pressure. ROH indicates that the individual has received identical copies of genetic information from parents (Ceballos et al., 2018; Curik et al., 2014; Purfield et al., 2012). ROHs naturally exist in individuals as a consequence of inbreeding or intense selection pressure and have speculated involvement with genes controlling disease susceptibility, intelligence, and production performance in individuals (Ceballos et al., 2018; Purfield et al., 2012). ROH analysis has been gaining traction and is now being used more and more to understand the evolutionary history of a population, measure the level of inbreeding and track genomic evolution in homozygosity in given environments (Ceballos et al., 2018; Curik et al., 2014; Panigrahi et al., 2023)

This study aimed to characterize genome-wide patterns of ROH in Barka indigenous chicken populations reared under scavenging systems in arid and semi-arid lands (ASALs), with the goal of inferring population history, inbreeding dynamics, and signatures of selection. Specifically, the study sought to (i) quantify and compare ROH length distributions across chromosomes, (ii) identify genomic regions and candidate genes enriched within distinct ROH categories, and (iii) evaluate the potential functional relevance of these regions in relation to adaptation, reproductive fitness, and resilience under low-input, resource-constrained environments. Understanding genome-wide ROH patterns and associated functional loci provides critical insights into the genetic architecture, adaptive potential, and sustainable improvement of indigenous chickens in ASAL scavenging systems.

Materials and Methods

Sample collection and DNA extraction

Blood samples were collected from 24 birds from Barentu sub-region in Eritrea. Blood was drawn from the wing vein using syringes and preserved on FTA Whatman Qiagen cards (Krambrich et al., 2022). DNA extraction was done following the Nucleomag kit and genotyping was done using genotyping-by-sequencing (GBS).

Quality analysis of marker data and data filtering

Sex chromosomes were excluded from the dataset with VC Ftools (Danecek et al., 2011). All other single nucleotide polymorphisms (SNPs) data were filtered using PLINK v1.9 with the following cut-offs: a sample call rate >90%, a SNP call rate >90%, and a minor allele frequency (MAF) of >0.01.

Following these filters, 36,037 SNPs from 32 autosomal chromosomes from 24 individual birds were available for downstream analysis.

Runs of homozygosity detection

Runs of homozygosity were identified using PLINK v1.9 (Chang et al., 2015; Purcell et al., 2007). Quality-controlled genotype data were analyzed using a sliding window approach to detect continuous homozygous segments across the genome, a method commonly used in population genomic and livestock studies (Peripolli et al., 2017; Zhi et al., 2023). ROH were defined using the following parameters: a minimum ROH length of 500 kb (--homozyg-kb 500), minimum of 50 SNPs per ROH (--homozyg-snp 50), minimum SNP density of 1 SNP per 50 kb (--homozyg-density 50), and maximum gap between consecutive SNPs of 1000 kb (--homozyg-gap 1000). A sliding window of 50 SNPs was applied, allowing up to five missing genotypes per window (--homozyg-window-missing 5) and no more than one heterozygous SNP per window (--homozyg-window-het 1) to account for potential genotyping errors and missing data. These thresholds are consistent with previously published studies and recommended practices for minimizing false-positive ROH detection while retaining biologically meaningful autozygous segments (Ceballos et al., 2018; Curik, I., Ferenčaković, M. and Sölkner et al., 2014; Ferenčaković et al., 2011; Ferenčaković et al., 2013; Peripolli et al., 2017). The resulting output were subsequently processed in R version 4.2.3 for downstream analyses, including ROH detection and classification.

Identification of runs of homozygosity islands

Runs of homozygosity islands were identified to detect genomic regions exhibiting a high frequency of homozygosity across individuals, which may indicate signatures of selection or reduced recombination. SNP-wise ROH prevalence was calculated as the proportion of individuals in which a given SNP occurred within a ROH segment (Equation 1).

$$\text{Prevalence} = \frac{\text{Number of ROH segments containing the SNP}}{\text{Total number of individuals}}$$

Equation 1

SNPs with missing genotype calls (NA) were excluded to reduce bias in both allele frequency and ROH prevalence estimates (Ferenčaković et al., 2011). The remaining markers were ranked in descending order of ROH prevalence, and the top 1% of SNPs exhibiting the highest values were selected as candidate loci for ROH island detection, consistent with thresholds widely applied in livestock genomic studies (Goli et al., 2024; Peripolli et al., 2017; Tan et al., 2024; Zhang et al., 2015). To characterize the genomic architecture of homozygosity, ROH islands were stratified by physical length into five categories (0.5-1 Mb, 1-2 Mb, 2-4 Mb, 4-8 Mb, and > 8 Mb).

This classification facilitates inference of both ancient and recent inbreeding patterns as well as putative selection signatures, following established frameworks in population and livestock genomics (Ceballos et al., 2018; Peripolli et al., 2017).

Data processing and statistical analyses were conducted in R (v4.2.3) using the packages *data.table*, *dplyr*, and *tidyr*. These packages were used to import, clean, and restructure large-scale SNP and ROH datasets, including filtering markers with missing genotype calls, summarizing SNP-wise ROH prevalence, and generating analysis-ready data tables through grouping and aggregation operations. Data reshaping between long and wide formats was performed to facilitate downstream analyses. Visualizations were generated using *ggplot2*, including plots of ROH prevalence across the genome, chromosomal distribution of ROH islands, and summaries of ROH segment length classes.

Functional annotation of runs of homozygosity islands

To identify candidate genes and biological processes potentially influenced by selection, ROH islands were mapped to the reference chicken genome assembly (*Gallus gallus* GRCg6a). Genomic coordinates of ROH islands were intersected with annotated gene regions to identify overlapping protein-coding genes and regulatory elements. This approach enables the identification of genomic regions enriched for homozygosity that reflect selection pressure, adaptation, or population-specific breeding history (Ceballos et al., 2018; Peripolli et al., 2017).

Functional enrichment analysis of genes located within ROH islands was performed using the *g:Profiler* (Kolberg et al., 2023). Gene lists were uploaded to the platform to identify significantly enriched biological functions, including Gene Ontology (GO) categories; Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) as well as Genomes (KEGG) pathways. To account for multiple comparisons and reduce false-positive findings, Benjamini-Hochberg false discovery rate (FDR) correction was applied, and only terms with adjusted FDR < 0.05 were considered significantly enriched (Benjamini and Hochberg, 1995; Kanehisa et al., 2021; Kolberg et al., 2023).

Results

Genome-wide average length of runs of homozygosity across chromosomes

The distribution of average ROH length varied across chromosomes, ranging from 0.80 Mb to 6.20 Mb (Figure 1). The average length of ROH varied across chromosomes in the Barka indigenous chicken population. Chromosome 4 exhibited the longest average ROH length (6.20 Mb), followed by chromosome 1 (4.79 Mb), chromosome 3 (4.48 Mb), and chromosome 2 (4.02 Mb). Moderate ROH lengths were

observed on chromosomes 12 (3.66 Mb), 5 (3.67 Mb), 13 (3.41 Mb), and 25 (3.90 Mb). Intermediate ROH lengths ranging between 2.00 and 3.00 Mb were detected on chromosomes 6 (2.45 Mb), 7 (2.55 Mb), 8 (2.63 Mb), 9 (2.70 Mb), and chromosome 22 (2.42 Mb). Shorter ROH segments (1–2 Mb) were distributed across the remaining chromosomes, including chromosome 10 (1.84 Mb), 14 (1.82 Mb), 15 (1.47 Mb), 17 (1.37 Mb), 18 (1.18 Mb), 19 (1.20 Mb), 20 (1.36 Mb), 21 (1.42 Mb), 23 (1.32 Mb), 24 (1.62 Mb), 26 (1.37 Mb), 27 (1.30 Mb), and 33 (1.02 Mb). The shortest (<1 Mb) average ROH length was observed on chromosome 11 (0.94 Mb), followed by chromosome 28 (0.80 Mb), indicating reduced homozygosity in these genomic regions. Overall, macrochromosomes (chromosomes 1–5) exhibited longer ROH segments compared with microchromosomes, which generally displayed shorter ROH lengths across the genome.

Runs of homozygosity islands

Genome-wide distribution of SNP prevalence and ROH islands are shown in Figure 2. SNP prevalence across chromosomes revealed a heterogeneous distribution of homozygosity signals. SNP prevalence values ranged from near zero to approximately 0.78 across autosomes (chromosomes 1–33), indicating variable levels of shared homozygosity among individuals. SNPs exceeding the top 1% prevalence threshold (≥ 0.375) were designated as ROH island SNPs and highlighted in red, whereas SNPs below this threshold were classified as non-ROH island SNPs and shown in grey. A total of 263 SNPs met the top 1% threshold and were distributed across multiple chromosomes, suggesting the presence of distinct genomic regions under potential selection pressure rather than a single dominant locus. Notably, clusters of high-prevalence SNPs were observed on several chromosomes, including chromosomes 1, 3, 5, 7, 8, 12, 13, 20, 26, and 27, where SNP prevalence frequently exceeded the defined threshold. Some chromosomes exhibited pronounced peaks, with prevalence values approaching 0.70–0.78, indicating strong and shared homozygosity among individuals within these genomic regions. In contrast, the majority of SNPs fell below the threshold and were broadly distributed across all chromosomes. Distribution of identified ROH islands by chromosome and length category are shown in Table 1. A total of seven ROH islands were identified across six chromosomes (1, 3, 4, 5, 8, 9, and 26), with segment lengths ranging from 529 kb to 1.35 Mb. These ROH islands were primarily concentrated within the short-to-intermediate length categories (0.5–1 Mb and 1–2 Mb). Two ROH islands were classified within the 1–2 Mb category, located on chromosomes 1 and 8. The ROH island on chromosome 1 spanned 1.04 Mb (18,328,149–18,432,096 bp) and contained 9 SNPs, while the region on chromosome 8 represented the largest ROH island, spanning 1.35 Mb (28,743,130–30,094,989 bp) and

containing 67 SNPs. The remaining five ROH islands were categorized within the 0.5–1 Mb class, distributed across chromosomes 3, 4, 5, 9, and 26. Specifically, chromosome 3 exhibited an ROH island spanning 732 kb (41,806,994–42,539,190 bp) with 6 SNPs, while chromosome 4 contained a 966 kb region (5,784,506–5,881,180 bp) also comprising 6 SNPs. On chromosome 5, an ROH island of 834 kb (21,927,999–22,762,436 bp) was identified with 12 SNPs, suggesting moderate SNP clustering within this region. Additional ROH islands were detected on chromosome 9 and chromosome 26, spanning 529 kb (7,778,755–8,308,223 bp) and 537 kb (17,644–554,553 bp), respectively. These regions contained 9 SNPs and 11 SNPs, respectively, indicating moderate levels of shared homozygosity.

Functional enrichment analysis of ROH-associated genes

The identified ROH islands were extracted and annotated using the Variant Effect Predictor (VEP) to assess potential functional consequences of the underlying variants. A total of 24 genes were located within ROH regions spanning 1–2 Mb, while a single gene was identified within the 0.5–1 Mb ROH category, indicating a size-dependent distribution of gene content across ROH classes (Table 2).

Functional enrichment analysis of genes located within ROH islands identified significant Gene Ontology (GO) categories and KEGG pathways (Table 2). The enriched biological process (BP) terms were primarily associated with metabolic processes, cellular processes, biological regulation, and developmental processes. In the molecular function (MF) category, the most represented terms included binding activity, catalytic activity, and molecular regulator activity. For cellular component (CC), enriched terms were predominantly related to membrane components, intracellular organelles, and protein-containing complexes. KEGG pathway analysis revealed enrichment in pathways associated with metabolic pathways, signal transduction, immune-related pathways, and cellular growth processes. The number of genes contributing to each enriched category varied, with metabolic and regulatory pathways containing the highest gene counts.

Runs of homozygosity were stratified by length and assessed for gene content and statistical significance. Generally, statistically significant associations were confined to a limited subset of genes within specific ROH length categories, while the majority of identified genes did not demonstrate significant enrichment (Table 2 and Appendix 1). In the short ROH category (0.5–1 Mb), a single gene, *AFDN*, was identified with a nominal association ($P > 0.05$). In the intermediate ROH category (1–2 Mb), two genes, *LHX8* and *YAP1*, showed significant association ($P = 0.01$). A broader set of genes within this ROH class did not reach statistical significance ($P > 0.05$), including *PDGFD*, *CEP126*, *TRPC6*, *PDE4B*, *SIGIP1*, *MIER1*, *SLC35D1*, *SERBP1*, *GADD45A*, *GNG12*, *WLS*, *MCCC2L*,

RPF65, *DEPDC1*, *LRR40*, *SRSF11*, *ANKRD13C*, *CTH*, *PTGER3*, *ZRANB2*, *NEGR1*, and *SLC44A5*.

Discussion

Genome-wide average length of runs of homozygosity across chromosomes

The distribution of average ROH across chromosomes (Figure 1) in the Barka indigenous chicken population revealed substantial variation, with longer ROH segments predominantly located on macrochromosomes and shorter segments observed on microchromosomes. Chromosome 4 exhibited the longest average ROH length (6.20 Mb), followed by chromosomes 1, 3 and 2. This pattern is consistent with previous genomic studies in indigenous chicken populations, where macrochromosomes typically harbour longer ROH due to lower recombination rates and larger genomic sizes, which increase the probability of extended homozygous segments forming over generations (Ceballos et al., 2018; Ferencakovic et al., 2011; Ferenčaković et al., 2013).

The presence of long ROH segments on chromosomes 1–4 in the Barka indigenous chicken suggests historical inbreeding, genetic drift, or selection pressures acting on these genomic regions. Indigenous chicken populations raised in ASAL environments often experience small effective population sizes, limited gene flow, and adaptation-driven selection, all of which contribute to the formation of long ROH segments (Mastrangelo et al., 2023; Peripolli et al., 2017). In ASAL production systems, chickens are typically managed under extensive scavenging conditions, which promote adaptation to harsh environmental stressors such as heat stress, disease pressure, and feed scarcity. These selective pressures may drive fixation of beneficial alleles, resulting in extended ROH regions on macrochromosomes.

Moderate ROH lengths observed on chromosomes 5, 12, 13 and 25 further support the presence of population-specific selection and demographic history. Such intermediate ROH segments are generally associated with older inbreeding events or long-term population structure, rather than recent inbreeding (Ceballos et al., 2018). This observation aligns with the evolutionary history of indigenous chickens in ASAL regions, where populations have undergone gradual adaptation over multiple generations rather than intensive artificial selection typical of commercial breeds (Muchadeyi and Dzomba, 2017; Rege et al., 2020).

Chromosomes with intermediate ROH lengths (2–3 Mb), including chromosomes 6–9 and 22, likely reflect ancient demographic events combined with moderate selection pressure. These regions may harbour genes associated with environmental adaptation, thermotolerance, immune response and

metabolic efficiency, traits that are critical for survival in ASAL environments (Fleming, 2016; Rostamzadeh Mahdabi et al., 2025).

Short ROH segments (1-2 Mb) distributed across most microchromosomes suggest higher recombination rates and increased genetic diversity in these genomic regions. Microchromosomes in chickens are known to exhibit higher gene density and recombination rates, which reduces the formation of long homozygous segments (Groenen et al., 2009; Burt, 2002). The shortest ROH observed on chromosomes 11 and 28 (<1 Mb) further supports reduced homozygosity and potentially greater genetic diversity in these regions, which may contribute to adaptive flexibility in Barka indigenous chickens raised in ASAL environments.

Generally, the observed ROH distribution pattern indicates that Barka indigenous chickens maintained in ASAL regions exhibit signatures of both historical inbreeding and adaptive selection. The predominance of longer ROH segments on macrochromosomes suggests genomic regions potentially involved in adaptation to harsh environments, while shorter ROH segments on microchromosomes reflect retained genetic diversity necessary for resilience under extensive production systems. These findings are consistent with genomic patterns reported in indigenous and locally adapted chicken populations worldwide (Mastrangelo et al., 2018; Muchadeyi and Dzomba, 2017; Rostamzadeh Mahdabi et al., 2025; Tan et al., 2024).

Runs of homozygosity islands

Results of SNP prevalence analysis provided details regarding the genetic composition of the Barka chicken population. The prevalence of specific SNPs indicates that such genetic variations are either selectively favoured or an adaptive feature important for survival or productivity. High-frequency SNPs such as those in the top 1% category are often associated with traits that are under intense natural or artificial selection, including disease resistance, reproductive success, or egg production (Hu et al., 2020). Such SNPs may be potential candidates for use in the development of breeding programs that would enhance desired traits in the population. However, the prevalence of SNPs also raises important questions about genetic diversity. The areal concentration of high-prevalence SNPs might simply indicate regions of the genome with low genetic variation, perhaps due to selective breeding or ongoing evolutionary pressures (Ceballos et al., 2018; Hu et al., 2020; Ngeno et al., 2015). Where this is so, the resultant genetic homogeneity could increase the threat of inbreeding depression, reducing the general adaptive capacity and environmental resistance of the population to change or disease outbreaks (Hoffmann and Sgrò, 2011; Li et al., 2022). On the other hand, the grey-marked lower prevalence SNPs are rarer genetic variants that contribute to the overall genetic diversity of the population. Even though such variants may not

have a large effect on observable traits, they are important for maintaining genetic resilience and plasticity (Berghof et al., 2024; Fleming, 2016). It is essential to keep these rare SNPs in breeding programs to prevent loss of genetic variation and maintaining population health in the long run. This grouping of SNPs into the top 1% by prevalence suggests the importance of recognizing the interaction of genetic markers and phenotypic characteristics. The top 1% of SNPs would suggest important genetic positions associated with traits of economic and ecologic interest such as egg production and climatic adaptability. These can be used as markers in the context of marker-assisted selection (MAS), a method of breeding that accelerates the selection of desirable traits (Sharma et al., 2024; Weiler and Fernando, 2020). A number of genomic regions with ROH in the Barka chicken genome were detected and their distribution was classified according to ROH segment lengths as earlier defined in literature. As the table indicates, most of the detected ROH islands were in the 0.5-1 Mb range, suggesting the occurrence of historical inbreeding or selection events that is indicative of historical population structure, long-standing or ongoing selective pressures. These segments were detected on chromosomes 3, 4, 5, 9, and 26, having ROH lengths from 529 kb to 966 kb and SNP numbers ranging from 6 to 12. Two regions on chromosomes 1 and 8, however, had longer ROH segments in the range of 1–2 Mb with lengths of 1039 kb and 1352 kb respectively. These larger ROH islands, particularly that on chromosome 8 with a considerably greater SNP density (67 SNPs), reflect relatively more recent inbreeding or targeted selection for certain traits linked to this locus.

The frequency of shorter ROH segments supports the argument that much of the autozygosity found is the result of past events and not the product of recent inbreeding. These results align with those of (Macharia et al., 2024), who reported that local chicken populations have shorter ROH, an aspect attributed to their historical conservation in large, fairly unstructured populations with minimal artificial selection practices. Furthermore, the occurrence of ROH islands in some genomic hotspots indicate the existence of regions under selective pressure, as suggested by (Macharia et al., 2024; Mastrangelo et al., 2023; Xu et al., 2022) in their investigation on Egyptian chickens. These findings justify the pursuit of additional downstream analysis into the functional role of the genes contained within these homozygous regions, which may be involved in adaptation, productivity, or hardiness traits in chickens.

Functional annotation of ROH-associated genes

The distribution of statistically significant genes across ROH length classes suggests a non-random enrichment of biologically functional loci within specific homozygosity tracts. Notably, the restriction of significant associations to shorter (0.5–2 Mb) ROH

categories support the hypothesis that relatively recent inbreeding or selection events preferentially capture functionally relevant variants, whereas longer ROH segments may largely reflect background autozygosity with limited functional enrichment. The identification of *LHX8* and *YAP1* as significant within intermediate ROH regions (1–2 Mb) is consistent with their established roles as key transcriptional regulators in developmental and cellular differentiation pathways. *LHX8* functions as an oocyte-specific transcription factor essential for early folliculogenesis and organ morphogenesis, highlighting its importance in reproductive and developmental biology (Liu et al., 2023). Similarly, *YAP1*, a central effector of the Hippo signalling pathway, regulates cell proliferation, lineage specification, and organ development, including cardiogenesis and tissue homeostasis (Abraham et al., 2025). The enrichment of such regulatory genes within ROH segments may reflect selective pressures acting on developmental and fitness-related traits. The presence of *AFDN* in shorter ROH (0.5–1 Mb) with marginal significance ($P > 0.05$) further supports the notion that shorter homozygous tracts may harbour loci involved in cell adhesion and signalling processes, although the weaker statistical signal suggests either reduced effect size or limited power for detection. This aligns with the broader observation that only a subset of ROH-associated genes exhibits measurable phenotypic or functional relevance.

In contrast, the majority of genes identified within the 1–2 Mb ROH class did not reach statistical significance, despite encompassing loci with known biological functions. For instance, *TRPC6* is implicated in calcium signalling, cell migration, and disease-related pathways including cancer progression and renal pathology (Formoso et al., 2020), while *CEP126* is associated with centrosomal function and regulatory genomic elements linked to transcriptional control (listed, 2022). The lack of statistical significance for these genes suggests that their presence within ROH regions may be incidental or that their effects are context-dependent and not captured under the current analytical framework.

Conclusion

Genome-wide ROH patterns in Barka indigenous chickens reared under ASAL scavenging systems reveal a heterogeneous distribution of homozygosity across chromosomes, reflecting both recent and historical demographic processes. The predominance of shorter ROH alongside a limited number of longer segments suggests generally low to moderate inbreeding with localized signatures of recent common ancestry. Importantly, significant gene enrichment was restricted to a small subset of loci within intermediate ROH (1–2 Mb), notably *LHX8* and *YAP1*, which are functionally linked to reproductive development and cellular regulation. This indicates that selection in these populations is targeted rather than widespread, likely

acting on key adaptive traits relevant to survival and productivity under harsh, low-input environments. Overall, the findings underscore the value of ROH analysis for understanding genetic diversity and identifying functionally important regions that may support resilience in indigenous poultry systems.

Acknowledgements

Author acknowledge personnel involved in the collection and processing of samples. Special thanks are extended to the chicken farmers for their cooperation and sample provision.

References

- Abraham, E., Kostina, A., Volmert, B., Roule, T., Huang, L., Yu, J., Williams, A. E., Megill, E., Douglas, A., & Pericak, O. M. (2025). A retinoic acid: YAP1 signaling axis controls atrial lineage commitment. *Cell Reports*, 44(5).
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), 289–300.
- Berghof, T. V. L., Bedere, N., Peeters, K., Poppe, M., Visscher, J., & Mulder, H. A. (2024). The genetics of resilience and its relationships with egg production traits and antibody traits in chickens. *Genetics Selection Evolution*, 56(1), 20.
- Ceballos, F. C., Joshi, P. K., Clark, D. W., Ramsay, M., & Wilson, J. F. (2018). Runs of homozygosity: windows into population history and trait architecture. *Nature Reviews Genetics*, 19(4), 220–234.
- Chang, C. C., Chow, C. C., Tellier, L. C. A. M., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*, 4(1), s13742–015.
- Curik, I., Ferenčaković, M. and Sölkner, J., Curik, I., Ferenčaković, M., & Sölkner, J. (2014). Inbreeding and runs of homozygosity: A possible solution to an old problem. *Livestock Science*, 166, 26–34.
- Curik, I., Ferenčaković, M., & Sölkner, J. (2014). Inbreeding and runs of homozygosity: A possible solution to an old problem. *Livestock Science*, 166, 26–34.
- 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. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156–2158.
- Ferencakovic, M., Hamzic, E., Gredler, B., Curik, I., & Sölkner, J. (2011). Runs of homozygosity reveal genome-wide autozygosity in the Austrian Fleckvieh cattle. *Agriculturae Conspectus Scientificus*, 76 (4), 325–329.
- Ferenčaković, M., Hamzić, E., Gredler, B., Solberg, T. R., Klemetsdal, G., Curik, I., & Sölkner, J. (2013). Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. *Journal of Animal Breeding and Genetics*, 130 (4), 286–293.
- Fleming, D. S. (2016). Examination of the genomic architecture of divergent poultry populations that underlies adaptation, tolerance, and resilience to environmental stressors. Iowa State University.
- Formoso, K., Susperreguy, S., Freichel, M., & Birnbaumer, L. (2020). RNA-seq analysis reveals TRPC genes to impact an unexpected number of metabolic and regulatory pathways. *Scientific Reports*, 10(1), 7227. <https://doi.org/10.1038/s41598-020-61177-x>

- Goli, R. C., Mahar, K., Chishi, K. G., Choudhary, S., Rathi, P., Sree, C. C., Haritha, P., Sukhija, N., & Kanaka, K. K. (2024). Runs of homozygosity assessment using reduced representation sequencing highlight the evidence of random mating in emu (*Dromaius novaehollandiae*). *Genome*, *68*, 1–8.
- Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479–485.
- Hu, G., Do, D. N., Gray, J., & Miar, Y. (2020). Selection for favorable health traits: a potential approach to cope with diseases in farm animals. *Animals*, *10*(9), 1717.
- Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). KEGG: integrating viruses and cellular organisms. *Nucleic Acids Research*, *49*(D1), D545–D551.
- Kolberg, L., Raudvere, U., Kuzmin, I., Adler, P., Vilo, J., & Peterson, H. (2023). g: Profiler—interoperable web service for functional enrichment analysis and gene identifier mapping (2023 update). *Nucleic Acids Research*, *51*(W1), W207–W212.
- Krambrich, J., Bringeland, E., Hesson, J. C., Hoffman, T., Lundkvist, Å., Lindahl, J. F., & Ling, J. (2022). Usage of FTA® Classic Cards for Safe Storage, Shipment, and Detection of Arboviruses. *Microorganisms*, *10*(7), 1–13. <https://doi.org/10.3390/microorganisms10071445>
- Li, G., Tang, J., Huang, J., Jiang, Y., Fan, Y., Wang, X., & Ren, J. (2022). Genome-wide estimates of runs of homozygosity, heterozygosity, and genetic load in two Chinese indigenous goat breeds. *Frontiers in Genetics*, *13*, 774196.
- Listed, N. authors. (2022). GeneCards®: The Human Gene Database. *Weizmann Institute of Science*.
- Liu, L., Liu, B., Wang, L., Li, C., Zhou, Y., Zhu, J., Ding, J., Liu, S., & Cheng, Z. (2023). Sohlh1 and Lhx8 are prominent biomarkers to estimate the primordial follicle pool in mice. *Reproductive Biology and Endocrinology*, *21*(1), 46.
- Macharia, J. K., Kim, J., Kim, M., Cho, E., Munyaneza, J. P., & Lee, J. H. (2024). Characterisation of runs of homozygosity and inbreeding coefficients in the red-brown Korean native chickens. *Animal Bioscience*, *37*(8), 1355.
- Magothe, T. M., Okeno, T. O., Muhuyi, W. B., & Kahi, A. K. (2012). Indigenous chicken production in Kenya: I. Current status. *World's Poultry Science Journal*, *68*(1), 119–132.
- Mastrangelo, S., Ben-Jemaa, S., Perini, F., Cendron, F., Biscarini, F., Lasagna, E., Penasa, M., & Cassandro, M. (2023). Genome-wide mapping of signatures of selection using a high-density array identified candidate genes for growth traits and local adaptation in chickens. *Genetics Selection Evolution*, *55*(1), 20.
- Mastrangelo, S., Ciani, E., Sardina, M. T., Sottile, G., Pilla, F., Portolano, B., & Consortium, Bi. Ov. I. (2018). Runs of homozygosity reveal genome-wide autozygosity in Italian sheep breeds. *Animal Genetics*, *49*(1), 71–81.
- Muchadeyi, F. C., & Dzomba, E. F. (2017). Adaptation of Low Input Extensively Raised Chickens. *Poultry Science*, 211.
- Ngeno, K., Van Der Waaij, E. H., Megens, H. J., Kahi, A. K., Van Arendonk, J. A. M., & Crooijmans, R. (2015). Genetic diversity of different indigenous chicken ecotypes using highly polymorphic MHC-linked and non-MHC microsatellite markers. *Animal Genetic Resources/Resources Génétiques Animales/Recursos Genéticos Animales*, *56*, 1–7.
- Panigrahi, M., Rajawat, D., Nayak, S. S., Ghildiyal, K., Sharma, A., Jain, K., Lei, C., Bhushan, B., Mishra, B. P., & Dutt, T. (2023). Landmarks in the history of selective sweeps. *Animal Genetics*, *54*(6), 667–688.
- Peripolli, E., Munari, D. P., Silva, M., Lima, A. L. F., Irgang, R., & Baldi, F. (2017). Runs of homozygosity: current knowledge and applications in livestock. *Animal Genetics*, *48*(3), 255–271.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A. R., Bender, D., Maller, J., Sklar, P., De Bakker, P. I. W., & Daly, M. J. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics*, *81*(3), 559–575.
- Purfield, D. C., Berry, D. P., McParland, S., & Bradley, D. G. (2012). Runs of homozygosity and population history in cattle. *BMC Genetics*, *13*, 1–11.
- Rege, J. E. O., Ochieng, J., & Hanotte, O. (2020). Livestock genetics and breeding. In *The Impact of the International Livestock Research Institute* (pp. 59–102). CABI Wallingford UK.
- Rostamzadeh Mahdabi, E., Esmailzadeh, A., Han, J., & Wang, M. (2025). Comparative analysis of runs of homozygosity islands in indigenous and commercial chickens revealed candidate loci for disease resistance and production traits. *Veterinary Medicine and Science*, *11*(1), e70074.
- Sharma, P., Doultani, S., Hadiya, K. K., George, L. B., & Highland, H. N. (2024). Overview of marker-assisted selection in animal breeding. *HISTORY*, *9*, 11.
- Tan, X., Liu, L., Dong, J., Huang, M., Zhang, J., Li, Q., Wang, H., Bai, L., Cui, M., & Zhou, Z. (2024). Genome-wide detections for runs of homozygosity and selective signatures reveal novel candidate genes under domestication in chickens. *BMC Genomics*, *25*(1), 485.
- Weiler, J. I., & Fernando, R. L. (2020). Strategies for the improvement of animal production using marker-assisted selection. In *Gene-Mapping Techniques and Applications* (pp. 305–328). CRC Press.
- Xu, N.-Y., Liu, Z.-Y., Yang, Q.-M., Bian, P.-P., Li, M., & Zhao, X. (2022). Genomic analyses for selective signatures and genes involved in hot adaptation among indigenous chickens from different tropical climate regions. *Frontiers in Genetics*, *13*, 906447.
- Zhang, Q., Gulbrandtsen, B., Bosse, M., Lund, M. S., & Sahana, G. (2015). Runs of homozygosity and distribution of functional variants in the cattle genome. *BMC Genomics*, *16*, 1–16.
- Zhi, Y., Wang, D., Zhang, K., Wang, Y., Geng, W., Chen, B., Li, H., Li, Z., Tian, Y., & Kang, X. (2023). Genome-wide genetic structure of Henan indigenous chicken breeds. *Animals*, *13*(4), 753.

Table 1. Distribution of ROH length categories across chromosomes

ROH length category	Chromosome	Start	End	Length kb	SNP count
0.5-1 Mb	3	41806994	42539190	732	6
	4	57845062	58811180	966	6
	5	21927999	22762436	834	12
	9	7778755	8308223	529	9
	26	17644	554553	537	11
1-2 Mb	1	183281490	184320096	1039	9
	8	28743130	30094989	1352	67

Table 2. Functional enrichment analysis of ROH-associated genes

ROH	P Value	Genes
0.5-1 Mb	>0.05	<i>AFDN</i>
1-2 Mb	0.01	<i>LHX8, YAP1</i>
	>0.05	<i>PDGFD, CEP126, TRPC6, PDE4B, SGIP1, MIER1, SLC35D1, SERBP1, GADD45A, GNG12, WLS, MCCC2L, RPE65, DEPDC1, LRRC40, SRSF11, ANKRD13C, CTH, PTGER3, ZRANB2, NEGR1, SLC44A5</i>

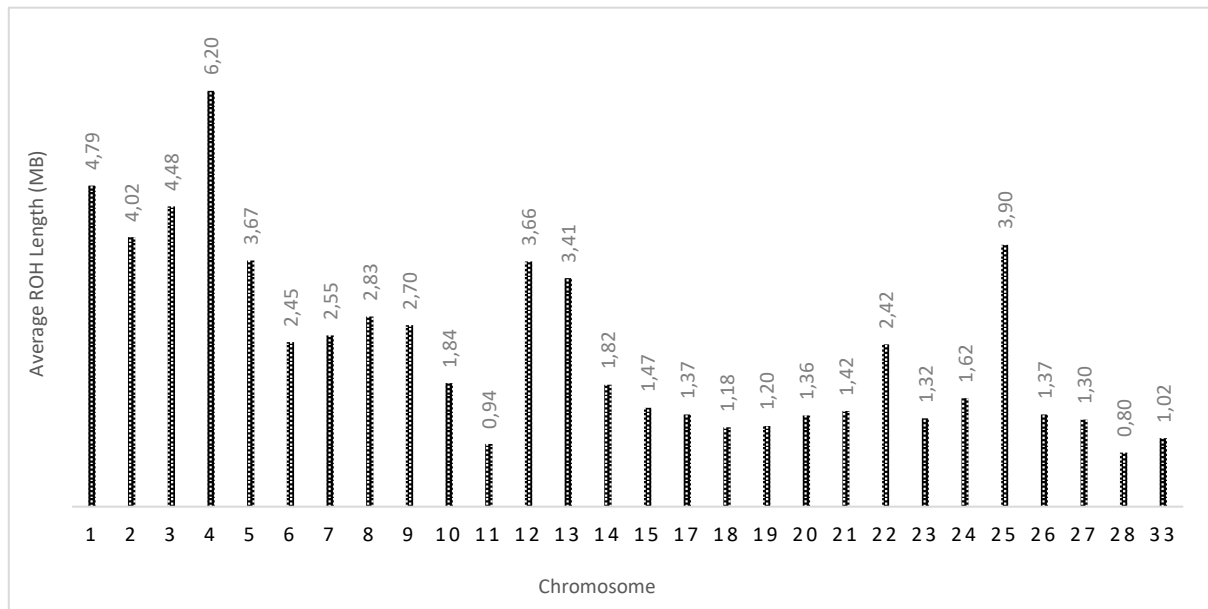


Figure 1. Chromosome-wise distribution of average ROH length

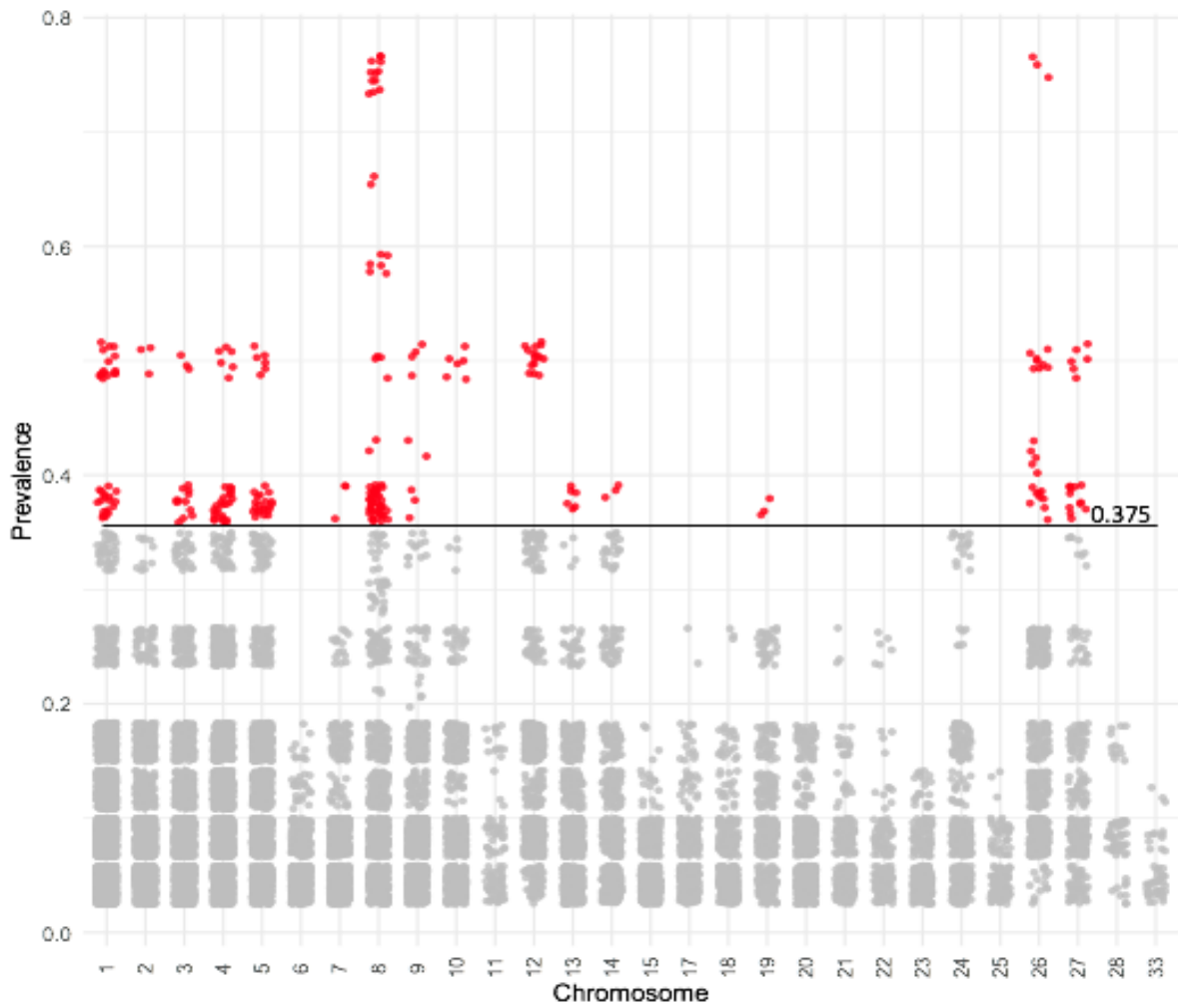


Figure 2. Genome-wide SNP prevalence across chromosomes with top 1% threshold highlighted (≥ 0.375). Red points represent candidate ROH island SNPs