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## An integrated analysis of genomics and environment uncovers the determinants of local adaptation in chicken populations

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### Abstract

Examining the genetic signatures associated with historical temperature driven selection provides valuable insights into local adaptation and the potential impacts of climate change on long term population dynamics. Given its parallel domestication history with human activity and remarkable genetic diversity, the chicken emerges as a crucial species for investigating genetic adaptability. Despite the significance of understanding how chickens express genetic adaptability to both tropical and icy environments, limited research has explored this aspect. This study conducted whole-genome sequencing of domesticated chickens, encompassing various breeds arranged based on breeding environment temperatures, ranging from more tropical to more arctic, and identified SNPs indicated a significant connection with apparent fluctuations in allele frequencies. This research analyzed a total of 87 whole genome sequences sourced from various populations, including 3 domestic chickens, 6 Red jungle fowl, 4 kadaknath chickens, 8 Kashmir Faberella, 2 Aseel chickens. The dataset comprised publicly available sequence data with coverage ranging from 8× to 14, along with recently sequenced genomes having an average depth of 30. Across all 87 genomes, we identified a total of 91,053,192 autosomal single nucleotide polymorphisms (SNPs). The findings provide a valuable tool for the selective breeding of hens and provide a glimpse into the temperature-related stress might lead to selection impacts that produce adaptive genetic adaptations in poultry for tropical and neutral climates.

**Keywords:** Novelgenes, chicken adaptation, genomic sequence, populations

## 1. Introduction

The global livestock sector is indeed facing significant challenges due to climate change. The impacts of extreme weather events and global warming pose serious threats to the well-being of livestock and the ecosystems they rely on. The consequences of climate change on livestock are multifaceted and include effects on their physiology, access to food and water, and increased disease prevalence (Clark, 2020). As the demand for livestock products is projected to double by 2050, it becomes crucial to find sustainable solutions to improve livestock production while mitigating the impacts of climate change. One of the strategies is to focus on utilizing or developing climate-resilient breeds of livestock. Indigenous cattle populations have demonstrated superior adaptation to their particular agro-climatic circumstances. These local breeds have evolved over generations to cope with their specific environmental challenges, making them valuable resources in the face of changing climate conditions (Li, *et al.*, 2022, Srivastava, *et al.*, 2019). Additionally, certain livestock species, such as domestic chickens, have demonstrated remarkable environmental tolerance, as they can be found in diverse climates ranging from tropical to temperate regions worldwide. Researchers can find the genes responsible for stress adaptation by examining the genetic components of regional adaptations in such widely distributed and adaptable species. The knowledge can then be used to improve livestock breeds by combining desirable productivity traits with resilience to climate-related stressors (Brüniche-Olsen, *et al.*, 2021). Identifying and understanding stress adaptation genes can lead to more informed breeding programs that promote climate change resilience in livestock. By incorporating these resilient genetic traits, breeders can create livestock breeds that are better equipped to withstand the challenges posed by climate change while still meeting the increasing global demand for livestock products. This approach represents a vital step in ensuring the sustainability of the livestock sector and its ability to provide for the growing population and improved living standards (Bertorelle, *et al.*, 2022).

Adeboye, *et al.*, 2020 investigated the variance in microsatellites and genome-wide single nucleotide polymorphisms (SNPs). They also carried out whole genome sequencing utilizing a pooled sequencing technique (Pool-Seq) to describe 2 million SNPs throughout the genome as a complement to the microsatellite data. However, if significant marker specific biases are found, it may cause researchers to be cautious when interpreting the findings of studies that only use microsatellite data.

Bomblies and Peichel 2022 examined the difficulties in evolutionary biology associated with locating the genes underlying ecologically significant traits and comprehending the fitness implications of natural variation at these loci. Population genomics and quantitative genetics are the two main strategies, and they contend that combining these approaches can be a potent strategy to accomplish their goal. Zhao, *et al.*, 2022 sought to understand the genetic underpinnings of hens' ability to tolerate frigid temperatures using whole-genome and transcriptome sequencing techniques. To investigate the significance of immunological and metabolic processes in cold tolerance, Genes with differential expression were subjected to functional enrichment analysis carried out. It took additional research with larger sample sizes and consideration of other factors to fully comprehend the intricate nature of cold adaptation processes in chickens.

Lawal, *et al.*, 2020 compared native village chicken populations to the RJF's main ancestor of domestic chickens, to look for genome-wide evidence of positive selection. Using the pool heterozygosity method, six RJF samples and native village chicken populations were studied. Additional research with bigger and more varied sample sizes would be required to fully comprehend the genetic complexity and variety of domestic village chicken populations

worldwide.

James, *et al.*, 2022 investigated the evolutionary change that occurs in chickens, concentrating on how the contribution of various genotypes to the following generation impacts their propensity for survival and reproduction. The importance of using genomic methods, including single nucleotide polymorphism (SNP) genotyping, whole genome, transcriptome sequencing, and whole genome sequencing, was emphasized in determining functional genomics areas connected to adaptation. The objective was to increase the productivity and sustainability of chicken breeds that are globally confronted with similar production issues.

Silva, *et al.*, 2020 looked into the molecular mechanisms used by the hens. This top predator lives on the Indian subcontinent to adapt to the physiological demands of low oxygen levels and high temperatures. It entailed examining the falcon's transcriptomes and contrasting the gene expression patterns in various groups. The outcomes also showed that genes involved in oxygen transport were concentrated in differentiated modules, and variations were discovered, indicating that this gene may be interested in the adaptation process.

In order to find selection signs connected to phenotypes or biological processes related to hypoxic responses, meat qualities, disease resistance, and coat color. Zhang, *et al.*, 2021 used whole-genome sequencing to examine the genetic diversity. They compared the sequences to the assembly of the ovine Oar\_v4.0 genome after searching the sequences for SNPs and indels, or single nucleotide polymorphisms. The study results give important information for future breeding and conservation efforts in these livestock populations and a better knowledge of the genetic basis of adaptive and production qualities in Tibetan sheep.

Wang, *et al.*, 2020 comprehend how genome-wide patterns of genetic diversity among individuals, groups, and species are shaped by natural selection. They use experimental and analytical approaches to identify and investigate the impact of choice on particular genomic areas. By implementing this, scientists can learn more about how natural selection affects genetic variation in populations and species.

Arab, *et al.*, 2019 investigated Persian walnuts genetic variation and population structure, the main place where this species originated. They discovered the genetic variety prevalent throughout the genome by using next-generation sequencing (NGS) technology to assess the genetic data from the sampled walnut trees. Overall, the research advances understanding of walnut genetics and paves the way for additional research and breeding initiatives in this significant nut-producing species.

According to Maiorano, *et al.*, 2022, to locate and analyze genomic areas selected in Nelore cattle, a breed well-known for its economic value in the beef market and its capacity to thrive in tropical environments. They utilized two key strategies, haplotype differentiation and allelic differentiation while using whole-genome sequencing. However, additional research with larger sample sizes and complementary techniques was required to validate and build upon these findings.

The study's principal goal is to find the genetic and environmental factors that contribute to local adaptation in chicken populations. They see the genetic fingerprints connected to local adaptation by analyzing chicken populations' genomic variations and patterns across various geographic regions. They also look into any potential environmental influences on the selected pressures affecting the people of chickens in these areas, such as climate, geography, and ecological issues. The thorough examination of genomic and environmental data reveal the

precise genetic modifications that have developed in response to regional ecological conditions, revealing important details about the mechanisms behind the dynamics and adaptation of the chicken population.

## **2. Materials and methods**

This research examined from the following sources, 87 complete genome sequences were obtained: Red jungle fowl ( $n = 6$ ), domestic chickens ( $n = 3$ ), kadaknath chicken ( $n = 3$ ) and Kashmir faberella ( $n = 8$ ), Aseel chicken, ( $n = 2$ ). The dataset included publicly accessible sequence data ranging from  $8\times$  to  $14\times$ , as well as recently genomes sequenced on average depth of  $30\times$ . 91,053,192 autosomal SNPs were identified in all 87 genomes. Additionally, 231 hens from four local breeds had blood samples taken for DNA extraction and PCR genotyping. Blood samples were taken from 1,399 native hens to examine the SNP chip 600 K for chickens. Every blood sample was drawn from the feather vein using an EDTA sodium anticoagulant tube, volume 5, and stored at 20 C. Principal component (PC) and hybridization analysis were performed using paternal SNPs that were filtered to take linkage imbalances into consideration. The objective was to gain insights into the genetic structure and diversity among the hens. Assembly of a whole genome sequencing library: 1.5lg of DNA was extracted from each sample. This research made sequencing libraries and used index codes with the DNA HT Sample Preparation Kit before sequencing each sample. All DNA samples were divided into 350 bp pieces, which were A-tailed, finalized, and assembled at the time. After purifying the PCR results, this study used the Agilent2100 Bioanalyzer to analyze and quantify these libraries.

### **Structure of populations and phylogenetic trees:**

Using TreeBest, this study created a neighbor joining (NJ) tree to illustrate the evolutionary connections based on genome-wide SNPs. The program FRAPPE was used to analyze using expectation maximization to study the population genetic structure approach. Using the GCTA tool, this research conducted a principal component analysis (PCA). The eigenvector's significance level was then calculated through the Tracey-Widom method experiment.

### **Genomic diversity**

Vcftools and Versican were used to calculate  $\theta_W$  and the horsepower, respectively. To detect Regions of Homozygosity, or ROH, are settings homozygous-homozygous density ten windowed 5000 homozygous windows, one homozygous-window-kb, 20 homozygous kb, and 100 homozygous SNPs. from PLINK's runs of homozygosity tool were utilized. Then, to calculate LD decay, this study used the Haploview program to calculate the squared correlation coefficient ( $r^2$ ). In addition to averaging throughout the entire genome, within a 500-kilobyte zone, the average  $r^2$  was counted.

### **Population Historical, differentiation time, and migration events:**

In paired sequential Markovian coalescence, a hidden Markov model (HMM) technique was used to reconstruct the demographic history of 119 samples. Initially, each sample's genotype was determined using the Samtools software. The consensus sequence was then converted using the tool "fq2psmcf" into a fasta-like format, which increased the precision of assumed past recombination events. The character shows if the bin  $[100i, 100i + 100]$  contains at least one heterozygote. The values chose for the parameters were  $\mu = 4 + 25 \times 2 + 4 + 60$  and  $r = 5$ ,  $t = 15$ ,  $N = 30$ . For hens, the rate of neutral mutation ( $\mu$ ) was 0.19. One hundred eight per generation, and the generational period ( $g$ ) was one year. Fastsimcoal2 was used to estimate divergent time, and easy was used to apply SNPs found in intergenic areas to modify site frequency spectrum (SFS). The mutation ratio and the number of generations per site were set to  $2E-9$ . The most likely scenario guided the development of the fitting model after 100 iterations of fastsimcoal2 were performed with different starting locations. Estimates of the population were produced using 40 cycles of

Expectation Conditional Maximization (–L40) and 100,000 runs per parameter file (–n100, 000). TreeMix used a maximum likelihood method to identify the patterns of gene flow and population relationships.

#### **Genome-wide test for selective sweeps**

The fixation index  $h_p$  and  $(F_{ST})$  values ratios for these particular group pairs were obtained to determine genome-wide selection sweeps connected to temperature adaptation. Plots of the average SNP  $F_{ST}$  values were made in 20 kb steps inside 40 kb genomic bins. For the same containers, nucleotide diversity ( $p$ ) was estimated. The  $F_{ST}$  values underwent the following Z-transformation:  $Z(F_{ST})$  is calculated as  $(F_{ST} - \mu_{F_{ST}}) / \sigma_{F_{ST}}$ , where  $\sigma_{F_{ST}}$  represents the standard deviation and  $\mu_{F_{ST}}$  represents the mean  $F_{ST}$ . Log<sub>2</sub>-transformed  $h_p$  ratios were used. The empirical  $h_p$  ratio and log<sub>2</sub>

$(F_{ST})$  percentiles were evaluated and categorized. Under tight selective sweeps, the windows with log<sub>2</sub>( $h_p$  ratio) and  $Z(F_{ST})$  of the top 5% values simultaneously were labeled outliers. Each SNP's XP-EHH value was computed using the XPEHH software. 40 kb for the window size and 20 kb for the step size were used to determine the average XP-EHH values. Windows that scored in the leading 5% were XP-EHH chosen.

#### **Genomic analysis with arrays**

DNA extraction and Array analysis were conducted on the blood samples of chickens. Genomic DNA was taken from samples at GeneSeek, and genotyping was assessed the 600 k Array. The RJF genome was referenced in the annotation files of the genotyping array. Following that, this study used Axiom™ Analysis to perform genotype data quality screening. These filters have a 0.05 minor allele frequency and a 99% call rate for single nucleotide polymorphisms (SNPs).

#### **Transcriptome sequencing and analysis**

The RNA Collection Prep Kit for NEBNextUltra™ was used to create sequencing libraries. Then, using a cBot Cluster Generation System and the v3-cBot-HS TruSeq PE Cluster Kit, we clustered the index-coded samples. Bowtie was used to create a reference genome index, and the HISAT2 tool was used to align. Excellent RNA-seq readings reach the reference genome. The read counts for each gene were calculated using HTSeq. Following that, we estimated FPKM using read count and gene length data. To demonstrate the temporal expression pattern and validate the correctness of the transcriptome data, these tissues the hypothalamus were used. Four chickens of each breed were randomly chosen for their tissues. cDNA for tissues was created using the FastKing Super tissues Mix Kit from the whole RNA.

#### **Analysis of transcripts:**

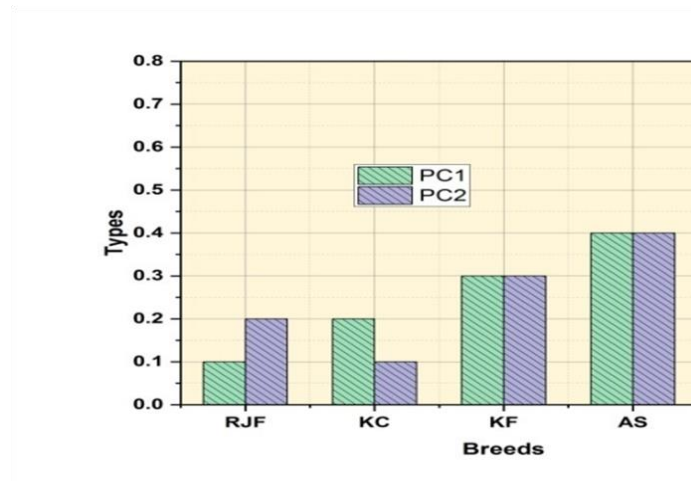
Alignments corresponding to the barcode adaptor, More than 3% of readings have unknown nucleotides (N), and more than 50% of reads have low quality (Q) To eliminated as part of the quality filtering techniques to achieve high quality, clean reads calculate the levels of gene expression, Rstudio set was used to perform tissue differential expression examination, which included comparing the tissue samples of RJF chickenis examining tissue samples from two different scenarios affecting the same breed.

### **3. Results**

This study analyze the result section in variant breeds in India such as Red jungle fowl(RJF),kadaknathchicken (KC), Kashmirfaberella(KF),Aseel chicken (AS),

### Population structure analysis

The PCA plot indicates that PC1 and PC2, the first and second primary elements, are able to differentiate among temperature stress in native chickens and that of RJF was split into PC1 and PC2, two distinct clusters. Relative gene expression in the liver refers to the quantification of gene activity compared to a reference sample. This analysis helps to understand the changes in gene expression levels, providing insights into liver functions, diseases, and responses to various conditions, such as drug treatments or environmental factors. Figure 1 shows that comparative gene expression and table 1 illustrate the types of relative gene expression.



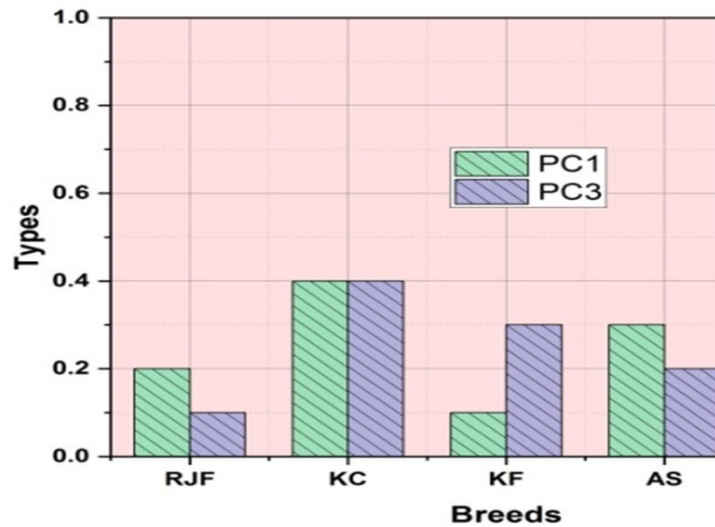
*Figure 1: Comparative gene expression*

**Table 1** Types of gene expression

Breeds	Types	
	PC1	PC2
RJF	0.1	0.2
KC	0.2	0.1
KF	0.3	0.3
AS	0.4	0.4

### Analyzing selection signatures

Genomic areas where natural or artificial selection modified genetic variation are referred to as selection signatures in chickens. Improve the breeding of chickens for desired attributes, including increased production or disease resistance; by identifying the genes linked to specific traits. The possibly selecting genetic areas were thought to be outliers. Several chicken breeds are shown in this table with identical values for the two kinds (PC1 and PC3). In the context of PC1 and PC3, these numbers, which range from 0.1 to 0.9, probably represent certain attributes or qualities connected to each breed. Figure 2 show that selection signatures and table 2 represents Principal Component (PC) scores for two components (PC1 and PC3) for different chicken breeds (RJF, KC, KF, AS). PC scores indicate the contribution of genetic variation to each component, reflecting breed relationships.



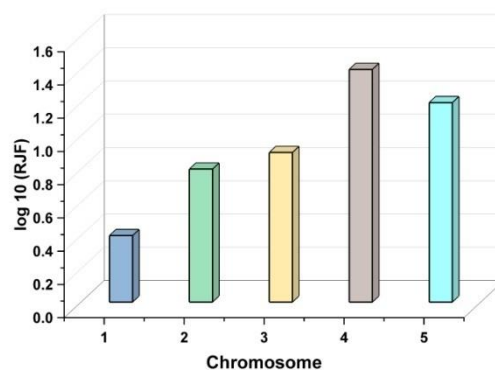
**Figure 2: Analyzing selection signatures**

**Table 2 Analyzing selection signatures**

Breeds	Types	
	PC1	PC3
RJF	0.2	0.1
KC	0.4	0.4
KF	0.1	0.3
AS	0.3	0.2

### Integrating omics findings with transcriptomic analysis

Integrating omics findings with transcriptomic analysis involves synthesizing data from various high-throughput techniques, such as genomics, proteomics, and metabolomics, to gain a comprehensive understanding of biological processes at the gene expression level, facilitating a more holistic approach to biological research. Figure 3 illustrates the omics findings in genomics and table 3 shows that omics findings in genomics logarithm base 10 of the recombination frequency (RF) for different chromosomes reveals varying degrees of genetic linkage. Chromosomes 1, 2, and 3 exhibit relatively lower recombination frequencies (0.4, 0.8, and 0.9, respectively), while chromosomes 4 and 5 display higher values (1.4 and 1.2, respectively).



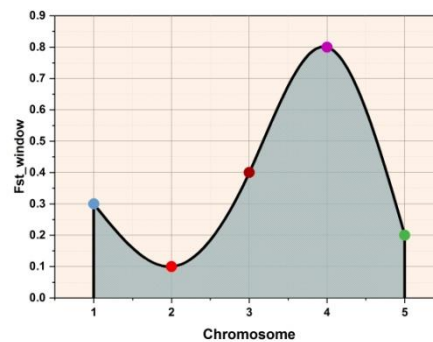
**Figure 3: omics findings in genomics**

**Table 3** omics findings in genomics

Chromosome	log <sub>10</sub> (RJF)
1	0.4
2	0.8
3	0.9
4	1.4
5	1.2

### Test gene chromosome

The test gene's location on a specific chromosome is not provided. Identifying a gene's chromosome is crucial for understanding its genomic context and potential functional relationships. We used two distinct methods, namely  $F_{ST}$  and  $\log_{10}$  (RJF), to investigate potential signature areas and genes linked to cold adaptation the chromosome is greater than 1 and  $P$ -values  $< 0.06$ . In the context of a cold environment, distinct numbers of differentially expressed genes were pinpointed in thyroid (0.8), pituitary (0.3), and tissues when comparing KC and KF chickens. Subsequent functional enrichment analysis revealed the presence of 3, 8, 4 significant  $F_{st\_window}$  term in the thyroid, hypothalamus, and pituitary, respectively. The table 4 displays  $F_{st}$  values for different chromosomes. Figure 4 shows that test gene chromosomes,  $F_{st}$  measures genetic differentiation among populations; higher values indicate greater differentiation. Chromosomes 4 and 3 show substantial differentiation (0.8 and 0.4, respectively), while chromosomes 2 and 5 exhibit lower differentiation (0.1 and 0.2). Chromosome 1 has moderate differentiation (0.3).

**Figure 4:** Test gene chromosome**Table 4** Test gene chromosome

Chromosome	Fst_window
1	0.3
2	0.1
3	0.4
4	0.8
5	0.2



#### 4. Conclusion

In this study, a comprehensive analysis of genomics and environmental factors elucidated the key determinants of local adaptation in chicken populations. The integrated approach revealed the intricate interplay between genetic variations and environmental conditions, providing valuable insights into the mechanisms underlying the adaptive evolution of chickens in diverse locales. This holistic understanding enhances the knowledge of avian adaptation and informs strategies for sustainable poultry breeding in different environments. In this research chicken breed was studied using whole genome sequencing. This study examined from the following sources, 87 complete genome sequences were obtained: Red jungle fowl (n = 6), domestic chickens (n = 3), kadaknath chicken (n = 3) and Kashmir faberella (n = 8), Aseel chicken, (n = 2). Following domestication, these breeds differentiated from one another and formed tight relationships according to patterns of genetic variation. Areas of the genome showed a signal of positive selection showing a distinctive difference due to environmental pressure for choice. These hens from tropical settings showed a decreased mutation frequency. In addition, Greater rates of SNPs in the gene are found in varieties from the RJF, KC, and KF, AS. These findings suggest that these extremely diverse species' genetic distinctions play a role in their ability to survive and adapt to severe conditions. The genetic improvements of chickens that will with stand catastrophic climate change and benefit from the genomic information we gained using this research. Future research may explore dynamic interactions between evolving genomics and changing environments to deepen to understanding of local adaptation in chicken populations, informing sustainable breeding practices and conservation efforts.

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