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Comparative Genomics of Galliformes and the Evolutionary Dynamics of Domesticated Chickens

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Abstract Galliformes birds include pheasants, partridges, quails, turkeys, etc., which are relatively primitive and diverse groups on the avian evolutionary tree. In recent years, with the development of high-throughput sequencing and comparative genomics, a large number of Galliformes genomes have been analyzed, providing an unprecedented opportunity for in-depth study of their phylogenetic relationships, domestication history and functional gene evolution. This study reviews the basic characteristics of Galliformes genomes and cross-species comparative datasets, summarizes the latest phylogenetic analysis results and species divergence time estimates, and focuses on the dynamic changes in the genome of domestic chicken (*Gallus gallus domesticus*) during domestication, including the reshaping of genetic diversity, gene introgression from wild relatives, and genomic selection pressure caused by artificial breeding. The evolutionary trajectories of functional genes related to important economic traits of domestic chickens are further compared, and the key gene variations and their origins that affect the appearance, behavior, physiology and other traits of domestic chickens are listed. Finally, the progress of regional comparative genomic research is discussed using the East Asian domestic chicken lineage as an example, revealing the genetic structure of Chinese native breeds, evidence of multi-origin domestication and adaptive evolution mechanisms. This study uses systematic comparative genomic analysis to reveal the evolution of Galliformes genomes and the molecular mechanisms behind chicken domestication, providing a theoretical basis and reference framework for poultry breeding and resource protection.

Keywords Galliformes; Comparative genomics; Phylogeny; Chicken domestication; Functional gene evolution

1 Introduction

Galliformes is an ancient and diverse group in the class Aves, including Phasianidae, Turkey Family, Guinea Fowl Family, etc., with a total of more than 300 bird species. In the phylogenetic tree of birds, Galliformes and Anseriformes together form a basal new bird evolutionary lineage (i.e., land birds in the present-day jaws), which is considered to be a group that radiated rapidly near the Cretaceous-Paleogene mass extinction. The representative species of Galliformes, the domestic chicken (*Gallus gallus domesticus*), evolved from the red junglefowl and is one of the earliest domesticated animals by humans, and is widely raised around the world. The domestic chicken not only has important economic value, but is also a model organism, playing a key role in developmental biology, immunology and genetics research (Lawal et al., 2020). In recent years, with the development of sequencing technology, the genome sequences of domestic chickens and their closely related wild species have been continuously improved, and high-throughput comparative genomics has provided a new means for studying avian evolution.

The origin and domestication process of the domestic chicken have long been controversial. The traditional view is that domestic chickens originated from the red junglefowl in Southeast Asia based on morphology and archaeological records, but there are different opinions on the specific time and place of domestication. Molecular systematic studies have gradually clarified this issue: large-scale analysis using whole genome variation data shows that all domestic chickens are phylogenetically clustered into a monophyletic group, and the closest wild relative is a specific subspecies of red junglefowl-*Gallus gallus* spadiceus in Myanmar and South China. Molecular clock calculations show that domestic chickens diverged from this subspecies about 9 500 ± 3 300 years ago, which roughly coincides with the period of climate warming in the Holocene (Wang et al., 2021).

However, archaeological evidence shows that true domestication may have occurred in the middle and late Neolithic period thereafter. This suggests that the domestication of domestic chickens is a gradual process that may involve long-term genetic exchanges in wild populations and multi-stage domestication events. Similar phenomena are also reflected in other domestic animals: for example, the two main groups of domestic cattle have hybridized with closely related species such as yaks during their diffusion, thereby increasing genetic diversity and environmental adaptability (Li et al., 2017). Therefore, analyzing the signals of artificial selection and gene flow during domestication through large-sample whole-genome comparison has become an effective way to solve the mystery of the origin and evolution of domestic chickens.

The chicken genome was first sequenced and published in 2004, pioneering the study of bird genomes. Since then, the rapid progress of sequencing technology has enabled the publication of high-quality genomes of multiple Galliformes species, laying the foundation for comparative genomics research. Through cross-species genome comparison, the conservation and variability of genome structure can be revealed, gene deletions or new events occurring in specific branches can be identified, and molecular markers of lineage differentiation and trait domestication can be detected in combination with population genetics analysis (Ouyang et al., 2022). As a model species, the rich variety and clear history of artificial selection of domestic chickens provide ideal materials for studying domestication selection using comparative genomic methods. For example, through pan-genome analysis, the shared and unique gene sets of different chicken breeds can be identified, thereby discovering genes and variants missed in the reference genome. For another example, the combination of genome-wide association analysis (GWAS) and selection scanning can locate loci that are strongly selected during the domestication and variety differentiation of domestic chickens.

This study will systematically summarize the current status of comparative genomics research on Galliformes species, focusing on analyzing the dynamic changes in the genome and adaptive evolutionary mechanisms of chickens during domestication and breeding, comparing the genome characteristics of representative species of Galliformes, including assembly quality, chromosome composition and repeat sequences, reconstructing the phylogenetic relationship of Galliformes based on genomes, estimating the differentiation time of domestic chickens and closely related wild species, analyzing the selected genes related to speciation, and explaining the genome changes selected by humans during the domestication of chickens, such as the improvement of the genetic basis of phenotypic traits, changes in genetic diversity and genomic structural variation. By comparing the genomes of various East Asian chicken breeds in China, the genetic structure, domestication origin and adaptive genetic characteristics of local breeds are analyzed. Through multi-level comparative genomic research, this study hopes to provide a systematic review for understanding the evolutionary differentiation mechanism of Galliformes species and revealing the dynamics of chicken domestication, and provide a theoretical basis and data support for the formulation of future poultry breeding strategies.

2 Genome Features of Galliformes and Comparative Dataset

2.1 Sequenced representative species and assembly quality

With the development of high-throughput sequencing and assembly technology, high-quality genome sequences of many representative species of Galliformes have been completed in recent years, thus constructing a comprehensive comparative data set. The reference genome of domestic chicken has been continuously improved since its first release. At present, the genomes of multiple breeds or individuals have been assembled with high precision, even including previously missing microchromosome sequences and complex repetitive regions. For example, Xu Luohao et al. constructed a complete genome map based on the Huiyang Bearded Chicken in China, supplementing the 6 microchromosomes and a large number of repetitive sequences missing from the reference genome. This complete assembly reconstructed the chromosomes of ancestral vertebrates and analyzed the centromere sequence of domestic chickens, which greatly promoted our understanding of the avian genome architecture (Huang et al., 2018). In addition to domestic chickens, the genomes of other Phasianidae species such as ring-necked pheasants (*Phasianus colchicus*), turkeys (*Meleagris gallopavo*) and quails (*Coturnix japonica*) have also been sequenced. Meng He et al. assembled a high-quality reference genome of pheasant through multi-platform assembly, with a genome size of about 0.99 Gb and 23 058 predicted protein-coding genes. The turkey genome is slightly larger (~1.1 Gb), and a draft was published in 2010 by multi-platform sequencing and upgraded to the chromosome level in recent years (Barros et al., 2022). The Japanese quail genome is about 1.0 Gb, and a chromosome-level sequence has also been assembled. The genome sizes of Galliformes birds are relatively similar, ranging from 0.95 Gb to 1.3 Gb, which are significantly smaller than mammalian genomes. These high-quality genomic datasets provide a basis for cross-species comparisons, allowing us to examine the evolutionary differences of Galliformes species from a whole-genome scale.

2.2 Comparative analysis of genome size and chromosomal structure

Galliformes birds have highly conserved karyotype characteristics: relatively few large chromosomes and a large number of microchromosomes coexist, which is one of the important characteristics of bird genomes. For example, the domestic chicken has 2n=78 (i.e., 39 pairs of chromosomes), including 8 pairs of macrochromosomes, 30 pairs of microchromosomes, and sex chromosomes ZW/ZZ. The chromosome composition of other Phasianidae species is roughly similar to that of the domestic chicken, although the specific chromosome number may differ slightly (such as turkey 2n=80). Comparison of the chromosome colinearity of different species revealed that most autosomes remain highly colinear within the Galliformes, and only a few chromosomes have undergone fusion or splitting. For example, a comparison of the chromosomes of the ring-necked pheasant and the domestic chicken showed that most of the chromosome structure of the pheasant is consistent with that of the domestic chicken, with rearrangements only in a few segments (He et al., 2020). A significant difference is the microchromosome region where the major histocompatibility complex (MHC) genes are located: compared with the red junglefowl, the ancestor of the domestic chicken, the pheasant has undergone three large inversion rearrangements in this region, forming a unique gene arrangement pattern. This structural variation suggests that the pheasant immune genome has undergone special evolutionary events that may affect its immune function. In general, the genome of Galliformes is quite conservative in terms of chromosomal macrostructure, and the genome size of each species is not much different, mostly about 1.0 Gb. However, local structural variation is still widespread, especially in certain trait-related regions and sex chromosomes.

It is worth noting that sex chromosomes are highly heteromorphic in Galliformes birds, especially the W chromosome of females, which is severely degraded, carries very few genes, but is rich in repetitive sequences and transposons. Studies have shown that the transposable element density of chicken W chromosomes exceeds 55%, which is much higher than the average level of less than 10% in the genome, and contains more than half of the complete endogenous retroviral copies of the whole genome. This "repetitive sequence shelter" phenomenon makes the W chromosome an accumulation of harmful mutations, which may cause female individuals to carry a higher mutation load (Peona et al., 2021). At the same time, the length and composition of W chromosomes in different species also vary. For example, the W chromosome of domestic chicken carries about 40 Mb of sequence, while the W chromosome of turkey is smaller. These differences indicate that during the evolution of Galliformes, sex chromosomes have undergone rapid transposon expansion and sequence loss. In terms of minichromosomes, all species generally have about 30 pairs of minichromosomes, which are rich in genes, high in GC content, and low in repetitive sequences, and are gene-dense regions. However, it was not until recently that many minichromosomes were completely spliced out through long-read sequencing and graphical assembly. These new data help to discover the core genes hidden on minichromosomes.

2.3 Variation in repetitive elements and regulatory sequences

Repetitive sequences, especially transposable elements (TEs), play an important role in genome evolution. A typical feature of bird genomes is that the transposon content is relatively low, generally accounting for only 7%~15%. The genomic repetitive sequences of Galliformes species generally follow this rule, but there are certain variations between different groups. For example, about 9%~10% of the domestic chicken genome is transposon sequences, among which LINE-1 (CR1) type transposons are the most abundant. In contrast, the proportion of transposons in passerine birds such as sparrows can be as high as 30%, reflecting that different evolutionary lineages have different degrees of inhibition of transposon expansion. Recent studies have found that the dynamic balance of transposons in bird genomes mainly depends on the balance of amplification and deletion rates, rather than just limited amplification. In Galliformes, there is also evidence that different species have experienced different transposon expansion histories. For example, analysis of the ring-necked pheasant genome found that the CR1 subfamily common in some chickens has fewer copies in pheasants, while some elements of DNA transposons are relatively abundant. This may be related to species-specific genomic defense mechanisms or population history. In addition, the abundant endogenous viral sequences on the W chromosome of domestic chickens may have an impact on female physiology and interspecific hybrid infertility.

In addition to repetitive sequences, regulatory elements in the genome (such as enhancers and promoters) are also changing in evolution. Comparative genomes can identify conserved non-coding sequences and predict the loss or creation of functional regulatory elements. Studies have shown that despite the early divergence between bird orders, there are cross-species conserved enhancer clusters near multiple development-related genes. However, some regulatory sequences have also undergone significant lineage-specific changes in Galliformes. For example, compared with turkeys, domestic chickens may lack a conserved sequence upstream of feather development genes, suggesting that the loss of these elements may be related to the feather traits of specific breeds (Ouyang et al., 2022). For another example, structural variations or insertions have been found near the promoters of hormone receptor genes that regulate growth and reproduction in different chicken breeds, which may be regulatory changes caused by artificial selection. With the progress of multi-species pan-genome and map comparisons, scientists have begun to draw a picture of the evolution of regulatory elements in the genomes of Galliformes birds, including which sequences are highly conserved and functionally important, and which sequences have mutated, deleted or newly formed in specific lineages. Overall, comparative analysis of genomic repeat sequences and regulatory elements reveals the mechanism by which the genomes of Galliformes species achieve adaptive evolution through detailed changes while maintaining overall stability, providing clues for further exploring the genetic basis of trait differences.

3 Phylogeny and Species Divergence

3.1 Whole-genome-based phylogenetic tree reconstruction

The use of whole genome data for phylogenetic reconstruction has become an effective means to determine the evolutionary relationship of birds. Traditionally, systematic classification studies have been conducted on Galliformes based on mitochondrial DNA or a few nuclear gene sequences, but the results are uncertain due to factors such as inconsistencies between gene trees and species trees and hybridization. With the help of high-throughput genome sequencing, researchers can obtain whole-genome SNPs or colinear sequences of many species of Galliformes to construct high-resolution phylogenetic trees. Kimball et al. (2021) collected ultraconserved element (UCE) sequence data of 130 species of Galliformes birds and constructed a large-scale phylogenetic supermatrix containing all genera. The resulting internal phylogenetic tree is basically consistent with the previous results based on mitochondria, but with higher resolution and stronger support. The overall topology shows that the branches within the Phasianidae (including chickens, pheasants, peacocks, etc.) are clearly differentiated, for example, the genus Phasianus and the genus Gallus are grouped separately; and the positions of subfamily groups such as partridges and bamboo partridges are also more reliably determined. These big data analyses based on the whole genome help to solve evolutionary nodes that were previously difficult to determine.

A significant finding is that several major lineages of modern chickens form two large branches on the whole genome phylogenetic tree: one is the domestic chicken breeds in northern Eurasia and their closely related wild types, and the other is the local native chickens in central and southern China. Before the emergence of these two major lineages, some earlier differentiated domestic chicken individuals mainly came from southwest China and Southeast Asia. This result supports the possibility that there may be multiple early domestication centers or diffusion paths for domestic chickens, rather than simple diffusion after a single origin. This is consistent with the comprehensive analysis of nuclear DNA and mitochondrial evidence, suggesting that domestic chickens have undergone a complex migration and hybridization process after domestication. In contrast, the subspecies of red jungle fowl all form monophyletic groups on the genome tree, and are clearly differentiated from each other. This means that the pedigree structure of wild jungle fowl is relatively stable, while domestic chickens have mixed multiple genetic components due to artificial propagation. The cross-species genome phylogenetic tree also confirms that Gallus is a relatively early differentiated branch in the Phasianidae, and is quite different from other Phasianidae groups (such as Phasianus and Pavo).

When constructing a genome phylogenetic tree, it is necessary to avoid interference from factors such as incomplete pedigree sorting (ILS). To this end, researchers used the co-alescent method and the method of sampling different gene segments to test controversial nodes. The family-level genome analysis of Stiller et al. (2024) showed that even with the whole genomes of 218 bird family representatives, some deep nodes still had a certain degree of gene tree inconsistency. Similar problems may also exist within the Galliformes due to ancient rapid radiation. With the improvement of data volume and analysis methods, the phylogenetic relationships of the main groups of Galliformes have been basically clarified. If ancient genome data can be introduced in the future, it is expected to further calibrate the branching time and detect traces of prehistoric hybridization, adding information of the time dimension to the phylogenetic tree.

3.2 Divergence times among chicken, junglefowl, and related taxa

Accurately estimating the divergence time of domestic chickens and their closely related wild species will help to understand the spatiotemporal background and speed of domestic chicken domestication. Using the whole genome molecular clock method, Wang et al. (2020) used multiple statistical methods to infer the separation time of the domestic chicken lineage and the subspecies of red junglefowl. Among them, the divergence time between the domestic chicken and the G.g. spadiceus subspecies was about 9.5 thousand years ago (present), while the divergence with other red junglefowl subspecies (such as Javan junglefowl and Indian junglefowl) was earlier. It is worth noting that this estimate is not equivalent to the time when domestication actually began, because the specialized morphology and archaeological remains of domestic chickens appeared later after genetic separation. In fact, ancient DNA studies have found that some key alleles for domestic chicken domestication (such as TSHR gene mutations) did not increase rapidly in frequency in European domestic chickens until about 1 000 years ago. This suggests that after the initial domestication, the domestic chicken may have experienced a long period of free-range and slow evolution, and then, driven by the development of human civilization and breed improvement, more intense selection and differentiation occurred in the last one or two thousand years.

For other species of Galliformes, phylogenetic and molecular clock analysis also gave a corresponding time frame. Ultra-conserved element analysis showed that the approximate radiation time of each genera of Phasianidae was around the middle Miocene 20 million years ago. The emergence of the *Gallus gallus* genus is speculated to be in the late Miocene, and then the various species of jungle fowl gradually differentiated in the Pliocene. By the Pleistocene (about 2 million years ago), several species of modern jungle fowl (red jungle fowl, gray jungle fowl, Ceylon jungle fowl, green jungle fowl) had each taken shape. This is basically consistent with the estimation of mitochondrial DNA. As a descendant of the red jungle fowl, the genome of the domestic chicken is highly similar to that of the wild type, and the differentiation time is short and occurred during the process of human domestication. Therefore, the genomic differences are mainly reflected in some artificial selection markers, rather than the overall large genetic distance. It is worth mentioning that some studies have tried to more accurately characterize the genomic composition of domestic chickens at different times through ancient genome methods. For example, a study measured the DNA of European domestic chicken bones about a few hundred years ago and found that the frequency of some domestication-related alleles (such as TSHR mutations) was significantly lower than that of modern domestic chickens. This directly confirms the role of modern selection in human breed improvement.

Combining various evidences, the timeline of domestic chicken evolution can be outlined: red junglefowl have survived and reproduced in Southeast Asia and southern China for a long time. During the holocene environmental changes, one of the geographical populations began to associate with humans and was gradually domesticated and utilized around 6 000~5 000 BC (Cai et al., 2022). In the next few thousand years, domestic chickens spread to various parts of Eurasia with the spread of agriculture, and the indigenous chicken populations in different regions were genetically mixed with local red junglefowl or other junglefowl species to a certain extent (Guo et al., 2022). In the Middle Ages and modern times, people carried out targeted breeding for cockfighting, viewing and high yield purposes, which accelerated the differentiation between different strains. Therefore, the formation of genetic diversity in modern domestic chickens is a complex process that includes slow domestication in prehistoric times, migration and hybridization in historical periods, and recent artificial breeding. The estimation of the differentiation time of the whole genome provides us with a starting point, and further detailed characterization requires a comprehensive perspective combining archaeology, historical documents, and functional genomics research.

3.3 Core gene sets under selection during speciation

Speciation involves the generation of isolation mechanisms and the accumulation of adaptive differences. Among Galliformes, different species have their own characteristics in ecological niches, behaviors, and morphology. Genomic studies can help find gene groups associated with these differences. Comparing recently differentiated closely related species (such as domestic chickens and red junglefowl subspecies) is an effective strategy for finding selected genes. Through whole genome scanning, Wang et al. (2020) identified a group of core genes that may have been selected during the differentiation of domestic chickens and red junglefowl. These include several genes related to reproductive development, such as changes in the expression regulation of SLC family genes in testicular development. These genes themselves have not undergone non-synonymous mutations, but show signals of selection loss in the domestic chicken population, indicating that selection may target their regulatory regions. The thyroid hormone receptor gene TSHR is a known marker of domestication in chickens. It encodes the thyroid stimulating hormone receptor and plays a role in seasonal reproductive regulation. A mutation in the TSHR gene that causes an amino acid substitution (Gly558Arg) is nearly fixed in domestic chickens, but is extremely rare in wild jungle fowl. This mutation is believed to reduce the reproductive response of domestic chickens to seasonal sunlight, allowing them to lay eggs and reproduce throughout the year. Ancient DNA studies have confirmed that the TSHR mutation became common in European domestic chickens in the Middle Ages, suggesting that this mutation was strongly artificially selected after it spread in domestic chickens. Interestingly, the natural frequency of this mutation in the ancestral subspecies of domestic chickens (G. g. spadiceus) is also not low (about 94%), suggesting that humans may have preferentially domesticated wild populations carrying this favorable mutation, thus laying the foundation for later domestication. This reflects the importance of "pre-adaptive" genetic variation in the process of speciation and domestication.

At a higher level of comparison, different Galliformes species show their own unique adaptive characteristics, and selection has shaped the corresponding genomic differences. For example, the ring-necked pheasant has significant sexual dimorphism and complex feathers. Comparative genomic analysis found that genes related to calcium ion signaling and feather coloring in its genome may have undergone accelerated evolution. The study also found a large rearrangement in the MHC gene region of pheasants, speculating that the evolution of its immune system was driven by disease selection pressure. Another example is that guinea fowl (Numididae) may have different selection patterns for water metabolism and heat regulation genes compared to pheasant species living in arid environments in Africa. Studies have reported that genes that control feather coverage and skin evaporation in the guinea fowl genome show signs of positive selection (Ouyang et al., 2022). In addition, strains with extreme traits, such as “naked neck chickens” (no feathers on the neck), are believed to originate from mutations in the FGF20 gene; “curly feather chickens” (curled feather shafts) are related to loss-of-function mutations in the keratin gene KRT75. These mutations may have been extremely rare in wild species at first, but they were fixed under artificial selection, demonstrating the strong influence of humans on specific traits during species formation.

4 Genomic Dynamics of Chicken Domestication

4.1 Human-driven selection and its molecular impact on phenotypes

Human selection played a decisive role in the evolution of domestic chickens from wild jungle fowl. In order to meet different production and aesthetic needs, humans exerted directional selection pressure on many traits of domestic chickens during domestication and subsequent breed improvement, including docile temperament, higher meat and egg production performance, and diverse appearance characteristics (Cai et al., 2022). These selections significantly changed the phenotype of domestic chickens in a short time scale dominated by humans, and also left traces of linkage disequilibrium and selection sweeps in the genome. Through genome scanning, researchers have identified a series of candidate genes related to important economic traits of domestic chickens, which show high-frequency variation or specific haplotypes in domestic chicken breeds, but rarely appear in wild red jungle fowl, reflecting the cumulative effect of artificial selection.

Domestication first changed the comprehensive traits of domestic chickens, such as behavior and physiological rhythm, making them easier to raise and breed. The most typical case is the aforementioned TSHR gene mutation, which weakens the restriction of photoperiod on reproduction and enables domestic chickens to lay eggs all year round. This mutation meets the human need for continuous egg sources, so it is almost fixed in domestic chickens and becomes one of the genetic markers of domestic chicken domestication. At the same time, the THSR mutation also affects metabolism and behavior, making domestic chickens more docile and easy to manage. Another important physiological trait is that domestic chickens grow faster and are larger than wild jungle fowl, which is related to human selection for broiler production. Whole-genome association studies have found that genes related to the growth hormone pathway in domestic chickens, such as IGF1 and GHR, have specific allele combinations that can increase growth rate (Ouyang et al., 2022). Pan-genome analysis further reveals hidden variations that control growth traits: for example, in small chicken breeds, there is a specific deletion in the promoter of the IGF2BP1 gene, which leads to decreased expression, thereby limiting body growth. This deletion does not exist in large breeds, showing that people use this gene variation when breeding chickens for different purposes. Other studies have shown that genes related to hunger and feeding regulation in the chicken genome, such as NPY, may also have undergone selective changes, making domestic chickens more willing to eat and easier to grow fat to adapt to the rich feed in the domestic environment.

Driven by both human aesthetics and practicality, the morphological diversity of domestic chickens far exceeds that of wild jungle fowl, and many traits are the product of artificial selection. For example, coat color and skin color are important appearance characteristics of domestic chickens: wild jungle fowl males have gorgeous feathers while hens have gray-brown feathers, but the feather colors of domestic chicken breeds can be white, black, red, mottled, etc. Genomic studies have found that a variety of pigmentation-related genes are subject to selection. Different alleles of the MC1R gene cause the coat color to change from red to black, which is the dominant genetic pattern. The allele of this gene is fixed in black-feathered breeds (Dharmayanthi et al., 2022). The color of the skin and tibia is determined by genes such as BCO2 and EDNRB2: yellow-skinned chickens carry an inactivating mutation in the BCO2 gene, which causes the carotenoids in corn feed to be deposited under the skin; some white-feathered black-bone chickens have excessive melanin deposition in the dermis of the skin due to large fragment duplications in the adjacent region of the EDN3 gene, presenting a black skin phenotype.

In terms of ornamental traits such as crown type and feather type, humans have also carried out targeted breeding. The wild type of domestic chicken has a single comb, but a variety of variant comb types such as pea comb and rose comb have appeared in the breed. The pea comb trait is caused by an insertion in the first intron of the SOX5 gene, which causes the gene to be abnormally expressed and changes the comb development. The rose comb comes from an inversion on chromosome 7 containing the HMGA2 gene, which affects the structure of the comb and is related to egg production (Dorshorst et al., 2011). Feather traits such as silk feathers (downy feathers) originate from a missense mutation in the KRT75 keratin gene, which leads to abnormal rachis structure; tailless feathers and curly feathers are also caused by TBXT gene deletion and KRT75 mutation, respectively. These mutations usually reduce fitness in the wild, but they are preserved and fixed in specific strains under human selection, forming a rich variety resource of domestic chickens.

4.2 Domestication bottleneck and genetic diversity loss

The domestication process is often accompanied by population bottlenecks and inbreeding, which will leave traces of reduced genetic diversity in the chicken genome. Wild jungle fowl populations usually live in diverse environments and have large population sizes, with high levels of genetic diversity; however, domestic chickens, due to their origin from limited domesticated individuals and long-term artificial selection and directional breeding, have significantly reduced their effective population size and relatively narrowed their genomic diversity (Cai et al., 2022; Zhang and Lin, 2024). The degree of this change can be quantified by comparing indicators such as nucleotide diversity (π value), heterozygosity, and linkage disequilibrium. Studies have shown that the average nucleotide diversity of domestic chickens is about 60%~70% of that of wild red jungle fowl, with slight differences between different breeds. The genetic diversity of some highly intensively selected commercial breeds (such as white Leghorn chickens and white Rock chickens) is even lower, manifested as longer linkage disequilibrium regions and large runs of homozygosity. In contrast, some free-range local breed chickens have retained more diversity and are close to the level of wild jungle fowl due to less rigorous selection (Huang et al., 2020).

Another genetic consequence of the chicken domestication bottleneck is the accumulation of some recessive alleles associated with inbreeding depression and the loss of functional variation. Comparative genomic studies have found that some genes in domestic chickens have become "pseudogenized", that is, loss-of-function mutations have become fixed. This may be due to the bottleneck and random drift that make some deletion alleles that do not affect fitness popular in small populations. For example, some studies have observed that several olfactory receptor genes in domestic chickens have loss-of-function mutations, while they are functional in wild jungle fowl (probably because domestic chickens have reduced reliance on olfaction). Similarly, the W chromosome of domestic chickens has accumulated a large number of harmful mutations and repeated sequences due to long-term small populations and inbreeding. These facts show that the domestication bottleneck not only reduces allele diversity, but also changes the mutation load and selection balance of the genome, which may reduce the genetic adaptability of domestic chickens to environmental changes.

However, there are also multiple opportunities to introduce new genetic components during the domestication process, which partially alleviates the decline in diversity. During the process of dissemination, domestic chickens often hybridize with jungle fowl or native chickens in different regions, thereby acquiring foreign genes. This is genetically manifested as the embedding of fragments of some other jungle fowl species or exotic breeds in the chicken genome. For example, studies have found that the chromosomes of chickens in South Asia and Southeast Asia contain fragments from the gray jungle fowl (*G. sonneratii*), which may have been brought in through artificial introduction or trade. This gene flow increases the overall genetic diversity of chickens and counteracts the bottleneck effect (Guo et al., 2022). Population structure analysis also shows that there are signals of gene exchange between chicken strains from different geographical origins, especially between breeds in neighboring regions. These hybridization events increase the level of local genetic variation and sometimes introduce beneficial exogenous alleles into the chicken gene pool. For example, there is evidence that some disease resistance gene alleles of wild gray jungle fowl in Southeast Asia have entered local chickens through hybridization, increasing the latter's resistance to parasites.

The domestication of chickens accompanied by population bottlenecks has led to a decline in overall genetic diversity, but regional gene flow and multiple domestication centers have partially compensated for this loss, so that the global chicken population still retains a considerable amount of genetic variation. A study on the fine structure of the global domestic chicken population revealed that domestic chickens can now be divided into several genetic groups, each with a unique allele frequency pattern and related to its transmission and breeding history. This reminds us that when using domestic chicken genetic resources, we should fully protect the genetic diversity of different strains. Some traditional native chickens may carry rare beneficial mutations and have important conservation value (Huang et al., 2018). Molecular breeding techniques (such as whole genome selection) can help us monitor and maintain the genetic diversity of the population while improving production performance, and avoid excessive inbreeding leading to reduced adaptability.

4.3 Copy number variations and chromosomal rearrangements under selection

Genome structural variation (SV), including copy number variation (CNV), large deletions, insertions, inversions and translocations, plays an important role in the selection of domestic chicken traits. Some significant economic traits are not caused by single point mutations, but are related to larger structural variations. For example, the "rose crown" trait in domestic chickens comes from an inversion rearrangement of about 7 Mb on chromosome 7.

This inversion changes the regulatory region, resulting in changes in the coronoid morphology, and also affects the egg-laying performance of hens (because the HMGA2 gene carried by the inversion affects the secretion of reproductive hormones). For example, in bantam breeds, a duplication and inversion near the GH growth hormone receptor gene led to growth plate development disorders, shortening their limbs. By scanning the genomes of different chicken breeds for copy number variation, researchers have found multiple CNV regions associated with traits.

A study on chicken beak deformity traits showed that in chickens with long upper beaks, the LRIG2 gene had an increased copy number. GWAS analysis locked the CNV in this region as a candidate causal variant. Another study on egg production and reproductive traits found that the PRLR prolactin receptor gene had partial gene duplication in high-producing laying hens, which enhanced the prolactin signal and prolonged the peak egg-laying period (Bai et al., 2018). These examples show that changing gene dosage or structure through CNV can significantly affect the phenotype and become a target for artificial selection. Compared with point mutations, CNVs often affect large genomic regions and may change the expression of multiple genes at once, which is one of the mechanisms for rapid trait variation.

Another famous structural variation case in domesticated chickens is the FM (Fibromelanosis) locus of black-bone chickens. Black-bone chickens have black connective tissue and internal organs all over their bodies. This trait originates from a repeated insertion of about 100 kb on chromosome 20, which activates the abnormal expression of the EDN3 gene, resulting in the deposition of melanin all over the body. This structural mutation was originally extremely rare, but it was consciously selected and fixed in ancient China to form the black-bone chicken breed. Another example is the soft silky feathers of the "silk feather" chicken, which are caused by an allele of the KRT75 gene containing an 18 bp deletion. This deletion is located in the coding region and affects the keratin structure. The silky chicken also carries a dominant mutation that causes the skin on the tibia to be blue. This trait is similar to black skin, both of which originate from increased melanin deposition and are caused by structural variations of different genes. In terms of muscle development traits, the study found that in breeds with high leg muscle fat deposition, there was an increase in the number of copies of the AMPD1 gene, which changed the muscle metabolism pattern and made it easier to deposit fat (this was used to breed flavored broilers). It can be seen that people may have inadvertently selected various CNVs and structural rearrangements in different breeding directions, and these variations have left a significant mark in the genome.

5 Evolution of Functional Genes Associated with Chicken Traits

5.1 Accelerated evolution in growth and development genes

In the process of domestic chickens from wild to domestic, the growth rate and body size have changed significantly. Compared with the small size and seasonal growth pattern of wild jungle fowl, domestic chickens show faster juvenile growth and larger adult weight under artificial breeding. Behind this phenotypic change is the accelerated evolution and functional variation of a series of growth and development regulatory genes. Comparative genomic analysis showed that several growth-related genes in the domestic chicken genome showed a higher non-synonymous mutation rate or selection pressure than wild jungle fowl. For example, the IGF1 (insulin-like growth factor 1) gene has specific haplotypes in different strains of domestic chickens, which is speculated to be the result of early domestication selection, which accelerates the bone and muscle growth of domestic chickens in the juvenile stage (Ouyang et al., 2022). In addition, multiple genes in the TH (thyroid hormone) signaling pathway have also mutated in domestic chickens, including a regulatory mutation in the THRA gene of the thyroid hormone receptor, which reduces the sensitivity of domestic chickens to thyroid hormones, thereby breaking the seasonal growth rhythm of wild-type chickens (Cai et al., 2022). Together, these changes have contributed to the high growth rate and reproductive capacity of domestic chickens throughout most of the year.

Pan-genome studies provide a new way to discover hidden mutations in growth and development genes. Wang et al. (2021) constructed a graphical pan-genome of chickens and found that in some breeds, there was a promoter deletion in the LEPR (leptin receptor) gene, which led to a weakened leptin signal, thereby increasing feed intake and growth rate. This mutation is more frequent in heavy meat breeds, indicating that humans may have unknowingly selected alleles that affect hunger and improved the growth efficiency of broilers. Similarly, a deletion mutation in IGF2BP1 (IGF2 messenger RNA binding protein 1) was detected in small ornamental chickens, causing these chickens to have limited growth and small body size. Introducing this mutation into ordinary chicken embryos can reproduce the reduced body size phenotype, verifying its functional effect. These examples show that accelerated evolution of growth genes often appears in the form of regulatory element mutations or gene dosage variations and is fixed by artificial selection.

In addition to body growth, the reproductive development cycle of domestic chickens is also different from that of wild jungle fowl. Domestic chickens reach sexual maturity at an earlier age, and the annual egg production of hens is much higher than that of jungle fowl. Related hormone and reproductive axis genes also show evolutionary changes after domestication. PRL (prolactin) and VIP (growth hormone release inhibitor) genes are important hormone genes that affect brooding and egg-laying intervals. Studies have found that domestic chicken breeds have variations in the upstream regulatory regions of these genes, which shorten the egg-laying cycle and make it difficult to brood. These variations are almost fixed in high-producing egg-laying chickens, but are rare in jungle fowl. For example, the FSHB (follicle-stimulating hormone beta subunit) gene related to the sexual maturity of roosters has a unique allele combination in some fighting cock breeds. It is speculated that this is a variation that humans have retained in order to breed fighting cocks with strong fighting spirit and late sexual maturity (Guo et al., 2022). Therefore, the functional genes in the growth and reproduction fields have undergone complex selection during the domestication of chickens. Some gene sequences have undergone accelerated evolution (more amino acid changes), while other genes have changed their expression patterns through regulatory evolution.

5.2 Regulatory mutations in feather color, comb shape, and egg-laying traits

The color of domestic chicken feathers is mainly determined by the ratio of eumelanin and phenomelanin. The MC1R gene (melanin receptor 1) controls the switch of melanin synthesis, and its active mutation is known to cause the whole body feathers to turn black. Studies have found that in black-feathered black-bone chickens and other breeds, the MC1R gene has amino acid substitutions such as L99P, which stabilizes the receptor configuration in an activated state, continuously stimulates melanin production, and thus darkens the feathers (Huang et al., 2020). In contrast, the MC1R allele carried by wild red junglefowl is less active, allowing the expression of brown-red feathers. Another important gene is ASIP (Agouti signaling protein), which regulates and antagonizes MC1R. The promoter variation of ASIP between different breeds leads to differences in the distribution of melanin in certain parts (such as the neck and wings), resulting in piebald or bicolor feathers (Ouyang et al., 2022). The genetic mechanism that controls white feathers is mainly the blocking of the pigmentation process: some white feather breeds lack the TYR (tyrosinase) gene, causing albinism; more common is recessive white, caused by the variation of the PMEL17 gene (silver gene) that prevents the pigment granules from aggregating into precipitation, and the feathers appear white. At present, through whole genome comparison, the differences between different white feather breeds have also been traced back to their specific gene combinations. For example, the recessive white allele of the White Rock chicken is located at a different locus than that of the White Leghorn chicken, which means that humans have selected the white feather mutation independently and multiple times in different regions.

The comb is an important trait of chickens, and its size and shape are determined by cartilage growth. The single comb is the wild type and is produced by the basic developmental program. The pea comb is caused by a mutation in the SOX5 gene: an inversion of about 1.2 Mb is inserted into the first intron of the SOX5 gene on chromosome 1, interfering with its normal expression, causing the comb to change from a single leaf to a three-leaf shape. The rose comb is caused by an inversion on chromosome 7, which includes the sequence of the imaging protein gene MNR2, changes the direction of cartilage differentiation, and makes the comb flat and vine-like. Genetic analysis shows that when the rose comb and pea comb genes interact, new crown types (such as walnut combs) will be produced. These complex phenotypes also have a molecular basis: the inversion of the rose comb and the SOX5 insertion of the pea comb work together to affect the distribution pattern of the blood vessels of the coronoid process, resulting in different crown phenotypes. It is worth noting that mutations in crown type genes often affect not only appearance but also reproductive performance. For example, rose crown mutations reduce sperm quality in roosters, while pea crown alleles are associated with ovarian function in hens. This may explain why these crown types are mainly fixed in ornamental or fighting cock breeds, while high-yielding laying hens are mostly single-combed.

The annual egg production of domestic chickens has increased significantly from more than 10 eggs in wild jungle fowl to more than 300 eggs in laying hens, and there are complex changes in the physiological regulatory network behind this. The secretion rhythm of prolactin (PRL) and gonadotropins (such as FSH and LH) has been artificially modified, allowing domestic chickens to lay eggs almost all year round without brooding. Population genetics studies have found that in high-producing egg-producing breeds, there is an insertion mutation in the promoter region of the PRLR (prolactin receptor) gene, which weakens the prolactin signal and inhibits brooding behavior. At the same time, the THSR gene of high-producing laying hens has been introduced, and its mutation also helps to break seasonal reproduction. At the same time, ovarian factor genes such as GDF9 and BMP15 related to follicle development carry specific haplotypes in high-producing chickens, increasing the probability of multiple ovulation. Recent epigenetic studies have also found that the DNA methylation patterns of high-producing laying hens on reproductive axis genes are systematically different from those of low-producing chickens, suggesting that egg-laying selection also works at the epigenetic level (Figure 1) (Pan, 2024; Shi et al., 2024). It can be seen that the improvement of egg-laying performance involves multi-gene and multi-level regulatory changes, including both DNA sequence variation and expression regulation and epigenetic modification changes. Comparative genomes can only capture changes at the sequence level. To fully analyze the genetic mechanism of egg-laying, it is necessary to combine transcriptome, methylome and other data.

5.3 Adaptive evolution of behavioral and neurological genes

Domestic chickens show significantly different behavioral characteristics compared to wild jungle fowl, including a more docile temperament, weaker flying ability and changes in social-level behavior. These behavioral differences are also the result of domestication selection. Humans prefer to retain docile and easy-to-manage individuals, thereby changing the neural regulation system of domestic chickens. Comparing the genomes and brain transcriptomes of domestic chickens and jungle fowl, it can be found that genes related to neural development and neurotransmitters have undergone adaptive evolution or expression regulation changes. Zhou et al. (2023) compared Luxi fighting chickens (highly aggressive) with ordinary laying hens and identified a group of candidate genes related to nervous system function at the whole genome level. Combined with RNA sequencing of brain tissue, it was found that the expression of these genes was significantly different between fighting chickens and laying hens. Among them, genes involved in neural development pathways (such as the CAMK2 pathway) are highly expressed in fighting chickens, which may give them stronger attack and response capabilities; while genes related to calmness and social behavior, such as AVPR1A (arginine vasopressin receptor), are more highly expressed in laying hens, making their temperament more docile. This change in gene expression pattern may be driven by genetic variation, such as mutations in regulatory regions or differences in epigenetic modifications. This study revealed that the domestication of behavioral traits has a complex genetic basis, but key regulatory factors can still be excavated through comparative genomics.

A classic behavioral domestication gene is SERPINE3, which was previously proposed as a "domestication gene" in fox domestication experiments. In the chicken genome, genes that affect neurotransmitter metabolism, such as SERPINE3 and MAOA (monoamine oxidase A), also show signs of selection: the variation composition near these genes in chicken breeds is significantly different from that in wild chickens (promoting changes in the levels of serotonin, dopamine, etc.), which may explain why chickens are less susceptible to fright and less aggressive. Other studies focused on circadian rhythms and stress response pathways, and found that several synchronously evolved amino acid substitutions accumulated in the ADCYAP1 (*PACAP* gene) sequence of chickens, which are speculated to be related to reduced vigilance and prolonged foraging time (Cai et al., 2022). Domestication-related variations have also been detected in the genes of the auditory and visual pathways of chickens. For example, regulatory mutations in the TH gene reduce the dependence of chicken chicks on the call of the hen, so that they can be artificially raised in batches.

In terms of social behavior, the hierarchical order (pecking order) of chickens is also a research focus. In the process of breeding docile chickens, some genes related to aggressive behavior may be indirectly selected. Comparison of the genomes of aggressive and docile breeds revealed that the EAAT3 glutamate transporter gene has unique mutations in docile chickens, which can enhance glutamate clearance and reduce neural excitability, and is speculated to be related to reduced aggression (Zhou et al., 2023). Similarly, the DRD2 (dopamine D2 receptor) gene showed an allele frequency bias in docile breeds, suggesting that the dopamine signaling pathway plays a prominent role in regulating the social behavior of chickens.

In the framework of adaptive evolution, changes in neural and behavioral genes can be regarded as the adaptation of domestic chickens to new ecological niches (artificial breeding environments). Compared with wild chickens, domestic chickens do not need to deal with predation pressure and long-distance foraging, but instead are high-density confinement and stable feed supply. The results of genome comparison showed that the BDNF (brain-derived neurotrophic factor) gene, which is related to stress and neural plasticity, showed reduced genetic diversity in domestic chickens, suggesting that they may have experienced a domestication bottleneck and selection, which reduced the behavioral differences between individuals in the flock. On the other hand, the OXTR (oxytocin receptor) gene, which is related to social communication, was found to have mutations in multiple breeds of domestic chickens, which may affect the hen’s protection of chicks and gregarious behavior. It is worth noting that local chickens bred in special environments such as high-altitude Tibetan chickens have different adaptive variations in neural regulatory genes. For example, a study found that the sympathetic nerve-related gene ADRA2A of Tibetan chickens has a unique mutation, which makes its heart rate regulation more effective in a hypoxic environment (Zhang et al., 2021). This shows that behavioral and physiological adaptation genes may be interrelated and jointly shape the adaptive traits of chickens.

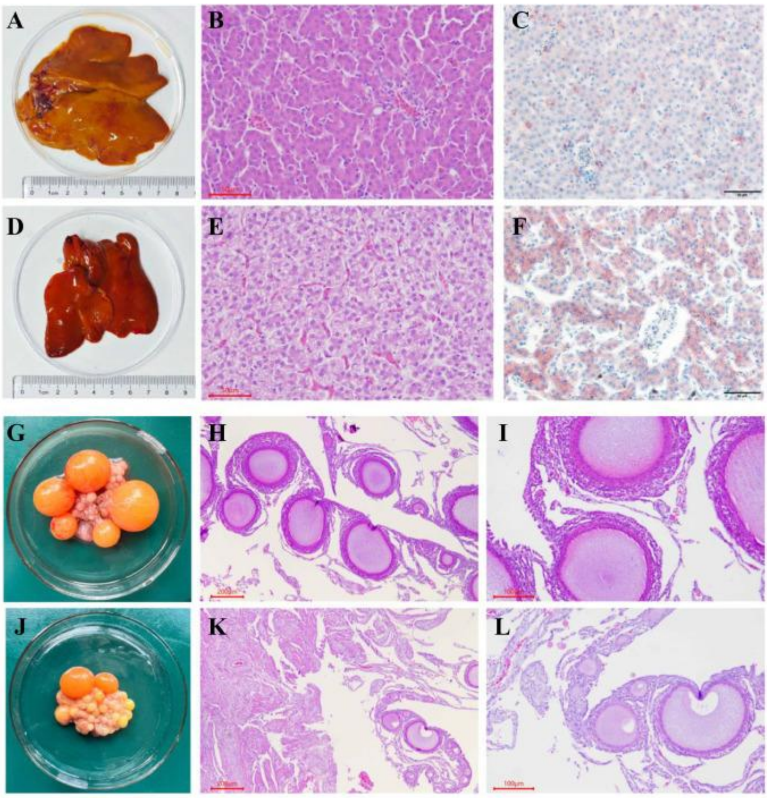

Figure 1 Ovarian morphological and histological characteristics of high-laying- and low-laying-chickens (Adopted from Shi et al., 2024)

Image Caption: (A): the liver from the high-laying group; (B, C): hematoxylin-eosin staining (H&E) and Oil Red O results of the liver of the high-laying group; (D): the liver of the low-laying group; (E, F): H&E and Oil Red O of the liver of the low-laying group; (G): the ovary of the high-laying group; (H, I): H&E of the ovary of the high-laying group (H 40 ×, I 100 ×); (J): the ovary of the low-laying group; (K, L): H&E of the ovary of the low-laying group (K 40 ×, L 100 ×) (Adopted from Shi et al., 2024)

6 Case Study: Comparative Genomics of East Asian Chicken Lineages

6.1 Genome comparisons between Chinese indigenous chickens and red junglefowl

China has rich local breed resources of domestic chickens. So far, there are more than 110 local chicken breeds listed in the National Catalogue of Livestock and Poultry Genetic Resources in my country, covering a variety of types such as yellow-feathered broilers, fast-growing white chickens, fighting chickens, and ornamental chickens. These native chicken breeds are similar to their wild ancestor, the Red Junglefowl, in the genome, and each has unique variations. The genomes of most Chinese native chickens are highly similar to those of the Red Junglefowl, and the shared allele frequencies account for the vast majority (>90%). This shows that the gene pool of domestic chickens is mainly derived from the genetic basis of the Red Junglefowl, and the domestication time is relatively short relative to the evolutionary scale, and a large number of random mutations have not accumulated. Especially in the conserved gene regions of the autosomes, such as basic metabolic and developmental genes, the sequences of native chickens and Red Junglefowl are almost the same. But at the same time, there are significant differences between native chickens and Red Junglefowl in some gene regions related to the adaptation of domestic chickens to the human environment and production performance. For example, a study of mitochondrial D-loop variation in 12 local chicken breeds in Guangdong Province found that they contained all the main maternal haplotypes of red junglefowl, but the frequency distribution was different. Most local chicken breeds are dominated by the B-lineage haplotype of red junglefowl, and a few, such as Huiyang bearded chicken, are also rich in the E-lineage haplotype of Southeast Asian junglefowl. This shows that Chinese native chickens share multiple points with red junglefowl in terms of maternal ancestry, but have also mixed in some genetic components of junglefowl from other regions, making their haplotype composition more diverse (Huang et al., 2018).

In terms of nuclear genome, the differences between Chinese native chickens and red junglefowl are mainly reflected in the trait gene regions that are subject to artificial selection. The researchers conducted whole-genome resequencing on multiple Chinese local breeds and found that each breed has some specific selection signal peaks. For example, Wuling Mountain Chicken has a high-frequency non-synonymous mutation in the EPAS1 gene, which may be related to the high-altitude environment in which it lives; the haplotype of Xianju Chicken near the TSHR gene is significantly different from that of Red Junglefowl, suggesting that special selection was made for its reproductive rhythm during domestication (Guo et al., 2022). Some local breeds have accumulated alleles that red junglefowl do not have due to their unique breeding history. For example, the colored shell egg-laying chicken breed carries a unique mutation in the SLCO1B3 transporter gene, resulting in a powder-blue eggshell, a trait that red junglefowl do not have (introgression of green shell egg-laying chicken genes from southern Tibet). Another example is that Sichuan fighting chickens retain a rare allele of the CACNA1C gene in red junglefowl, making it more excitable. These examples show that Chinese native chickens have inherited the common genome of red junglefowl and, through artificial or natural selection, have acquired some unique variations that distinguish them in phenotype and adaptability.

It is worth mentioning that the protection of local chicken breed diversity is very important because they may contain precious alleles that red junglefowl do not have. Studies have found that the average heterozygosity of some local breeds is even higher than that of wild red junglefowl, which may be attributed to historical hybridization and large population size. For example, the microsatellite polymorphism and nucleotide diversity of well-preserved breeds such as Wenchang chicken and Xianju chicken are not lower than those of wild junglefowl. On the contrary, the genetic diversity of some over-cultivated commercial matching lines has significantly decreased, and it is necessary to broaden the genetic base by introducing local germplasm. Therefore, comparing the genomes of local chickens and red junglefowl is not only a scientific issue in studying domestication, but also can guide the utilization of genetic resources and breed improvement strategies. Through genome analysis, we can establish a "genomic profile" for each local breed, clarify its similar and different gene regions with red junglefowl, and use them in breeding in a targeted manner or maintain its characteristic traits.

6.2 Genetic structure and breeding history of local breeds

Due to geographical isolation and artificial breeding, Chinese local chicken breeds have formed their own relatively independent genetic structures. At the molecular level, different breeds have systematic differences in allele frequency and haplotype composition, which can be revealed through population structure analysis. Population genetic analysis of native chickens in various parts of China shows that they can be roughly divided into two main genetic clusters, northern type and southern type, as well as several transitional types. For example, Xinjiang Kazakh chickens, Shanxi Sanhuang chickens and other northern native chickens are closer to the red junglefowl in Tibet and Xinjiang, while Hainan Wenchang chickens, Yunnan Camellia chickens and other southern native chickens have more common ancestors with the Yunnan red junglefowl lineage (Guo et al., 2022). This pattern is consistent with the history of the spread of domestic chickens to the north and west: northern breeds may have originated from early domestic chickens spread through India and Xinjiang, and southern breeds are mostly derived from local domesticated chickens in South China.

Each local breed often has its own specific breeding history. For example, the Pudong chicken in the suburbs of Shanghai is known for its large body and early maturity. From the genome, it carries some alleles derived from Western broiler chickens, suggesting that it has been hybridized and improved with introduced foreign breeds in modern times. For another example, Anhui Xuanzhou chicken was famous for its high egg production in the 20th century. An obvious selection signal was found in the genome near NR5A2 (steroid hormone synthesis regulatory gene), corresponding to its domestication of high reproductive performance (Ouyang et al., 2022). For example, Shandong Longkou Sanhuang Chicken is famous for its yellow hair, yellow legs, and yellow beak. Its MC1R and BCO2 genotypes are significantly different from those of ordinary native chickens. They respectively fix the alleles that control less melanin and non-decomposition of carotenoids, thus showing a yellow body. These examples show that local breeds have formed a stable genetic structure under long-term artificial selection, that is, a specific combination of alleles, which makes them meet people's needs in terms of morphology and production performance.

Using whole genome data can also trace gene exchange events during the breeding process. Many local chicken breeds do not have a single origin, but are the product of multiple hybridizations in history. For example, a study of Chinese fighting cock breeds found that fighting cock breeds in different regions have considerable gene exchanges with each other (due to cross-regional competitions and trade), forming two major branches in the north and south in terms of genetic structure and mixed with local breed components. For example, although the Zhuang chicken in Guangxi and the Wuzhi fighting cock in Henan are located far away, they have common ancestral contributions genetically, indicating that there may have been fighting cock exchanges in the past (Figure 2) (Ren et al., 2023). For example, some yellow-feathered broiler breeds (such as Qingyuan Ma chicken and Hetian chicken) introduced the blood of foreign breeds such as White Rock to improve meat production performance after the reform and opening up. The genome structure analysis clearly detected that there were 15%-20% “exogenous blocks” on the chromosomes of these breeds, corresponding to the genetic fragments of foreign breed chickens. This introduction and hybridization history may lack detailed records in traditional pedigrees, but the genome can reveal it, thereby restoring the breeding process.

Figure 2 Geographical location of chickens (Adopted from Ren et al., 2023)

6.3 Identification of disease resistance and environmental adaptation genes

Local breeds are often formed under special environments or production conditions, and therefore have some stress resistance traits that commercial breeds do not have. For example, plateau Tibetan chickens are resistant to hypoxia, dryland fighting chickens are resistant to roughage, southern free-range chickens are resistant to humidity and heat, and some breeds are resistant to local diseases. These valuable adaptabilities are usually caused by genetic variations accumulated under long-term natural selection. By comparing the genomes of local chickens and ordinary breeds or red junglefowl, researchers can identify candidate genes related to these resistances and adaptabilities, providing references for genetic improvement and seed conservation.

Tibetan chickens are local chicken breeds living in the Qinghai-Tibet Plateau and can grow and reproduce normally in a low-oxygen environment. Zhong et al. (2022) conducted genomic analysis of chickens at different altitudes and found that there were positive selection signals in multiple genomic regions in Tibetan chickens. One of the significant candidate genes is HBAD, which encodes an abnormal hemoglobin delta subunit. HBAD has unique mutations in Tibetan chickens, which is speculated to improve the ability of hemoglobin to bind oxygen. Genes involved in blood coagulation and cardiovascular efficiency, such as HRG (histone release protein) and ANK2 (ankyrin 2), are also selected in Tibetan chickens. The mutations in these genes together give Tibetan chickens stronger oxygen transport and cardiopulmonary function to adapt to the high-altitude hypoxic environment. In contrast, lowland chickens rarely have these mutations. Therefore, introducing these alleles of Tibetan chickens into other chicken populations may improve the plateau adaptability of offspring.

Some local chickens have evolved strong disease resistance in the long-term coexistence with specific pathogens. For example, Pujiang chickens in the swamps of Sichuan have high resistance to chicken malaria parasites. Genome comparison found that the MHC-B complex genome type of Pujiang chicken is different from that of general chickens, with more alleles and higher heterozygosity (Fulton, 2020). This indicates that its immune system has a wider antigen presentation capacity, thus resisting diverse pathogens. For example, some free-range chickens in northern China are more tolerant to Marek's disease (a tumor caused by herpes virus). GWAS was conducted on sensitive and tolerant varieties, and CNV and haplotype differences in immune-related genes such as IRF2 and SMARCA4 were found to be associated with disease-resistant phenotypes. Further functional experiments confirmed that additional copies of the IRF2 gene help improve the antiviral ability of T cells. This discovery comes from the genetic comparison of local varieties and is instructive for breeding Marek's disease-resistant strains. Similarly, free-range chickens in the Jiangsu and Zhejiang areas are said to be more resistant to intestinal parasites, and multiple immune regulatory genes including candidate genes including CHIA (chitinase, anti-worm infection) have been detected to have undergone selection enrichment. In the future, molecular markers can be designed for these genes to carry out disease-resistant breeding.

Many local chickens can survive under nutrient deficiency or adverse climatic conditions. For example, mountain black-bone chickens can still grow on coarse grains and weeds, and their AMY2B (amylase gene) copy number is higher than that of ordinary chickens, and their starch digestion ability is stronger (similar to dogs being domesticated to produce high amylase). Hainan Wenchang chickens are not prone to heatstroke in hot and humid climates, which may be attributed to the high expression level of heat shock protein genes such as HSP70, and gene promoter mutations make them sensitive to heat stimulation. Control experiments show that the induced expression of HSP70 in Wenchang chickens is higher than that of ordinary white chickens under high temperature conditions (data has not yet been published), which may be related to SNPs in the promoter region of its gene. For example, in the cold winter in Northeast China, some stupid chicken breeds have thick down feathers and are cold-resistant. Their UGP2 gene (regulating brown fat heat generation) has a unique haplotype that helps them maintain body temperature. These adaptive traits are often the result of complex physiological mechanisms, which cannot be fully explained by one or a few genes, but genome comparisons can narrow the scope and find related gene networks. For example, through the combined analysis of multi-omics in chicken breeds in the cold regions of Northeast China, it was found that genes in the PPARA/PPARG (peroxisome proliferator-activated receptor) pathway all have synergistic selection signals, pointing to the role of fat metabolism in cold resistance.

7 Concluding Remarks

Comparative genomics has become a powerful tool to reveal the mysteries of Galliformes evolution. First, at the macro-evolutionary level, comparative genomics has helped reconstruct more reliable phylogenetic relationships among Galliformes species and clarified some evolutionary nodes that have long been in doubt. The phylogenetic tree constructed using whole genome data supports the monophyly and evolutionary order of the main groups of Galliformes, and provides a quantitative estimate of the time of differentiation in combination with the molecular clock. These achievements have elevated traditional taxonomy to the genomic scale and established a new paradigm for avian systematics. Secondly, in terms of species and lineage formation, comparative genomics captures the molecular traces of natural selection during domestication and differentiation. A series of gene variants related to domestication and yield traits have been identified, such as TSHR, IGF2BP1, SOX5, etc. Their discovery directly benefited from cross-group genome comparisons. Thirdly, at the level of functional gene evolution, genome comparisons of different Galliformes species revealed the conservation and variation of many gene families. For example, it was found that birds have a number of protein-coding genes comparable to mammals, but they are distributed on microchromosomes, which changed our previous understanding that the number of bird genes was underestimated. This information has broadened the knowledge boundaries of vertebrate genomics. It is particularly worth emphasizing that comparative genomics provides an evolutionary perspective for studying model species such as domestic chickens, putting them back into the wild lineage for examination, and thus obtaining answers to some long-standing unsolved questions. For example, the origin and spread of domestic chickens were revealed through large-scale genome sampling and comparison, and the picture of origin subspecies and multi-center diffusion was determined. Another example is the huge phenotypic difference between domestic chickens and wild types. By comparing the selection sweep of the whole genome, we know that the genetic factors behind them are so numerous and each has its own mechanism. It can be said that comparative genomics has built a bridge between macroevolution and microgenes. The discoveries made in the ideal system of Galliformes have not only enriched avian evolutionary biology, but also provided a model for the evolutionary research of other domestic animals and wild animals.

As a typical representative of domesticated animals, the evolutionary dynamics of domestic chickens condense a microcosm of species adaptation to the human environment. By analyzing the changes in the genome before and after the domestication of domestic chickens, we can understand the species adaptation mechanism more broadly. First, the case of domestic chickens demonstrates the importance of pre-adaptive mutations: some mutations that are low-frequency in wild species (such as TSHR and yellow skin gene mutations) become advantageous in the new environment (domestic environment), and are therefore rapidly selected and fixed. This suggests that maintaining genetic diversity in wild populations is important for adaptation to potential environmental changes. Second, the domestication of domestic chickens reflects adaptation driven by rapid artificial selection. In just a few thousand years, domestic chickens have undergone significant changes in behavior, morphology, and physiology, and their speed far exceeds the rate of evolution under natural selection. This proves that the genome of a species has considerable plasticity and can reshape the phenotype through mutations in a few key genes under strong selection pressure. For example, only a SOX5 insertion can change the morphology of the comb, and an EDN3 duplication can make the skin darker. This reflects that there are certain "switch" nodes in the gene regulatory network, which have a large effect once mutated, opening up a shortcut for species adaptation. The evolutionary dynamics of domestic chickens show that gene flow and hybridization may play an active role in adaptation. During the process of spreading, domestic chickens have acquired additional beneficial mutations (such as disease resistance alleles of gray jungle fowl) by hybridizing with different wild jungle fowl, thus better adapting to different regional environments. This is consistent with the view that “hybridization promotes adaptation” found in more and more studies on wild species. When facing environmental stress, populations can expand their adaptability potential by introducing new mutations through hybridization. The multi-source background experienced by domestic chickens has given them a wide range of genetic diversity, enabling them to survive and reproduce in various climatic conditions around the world. This is also inspiring for current biodiversity conservation: maintaining moderate gene exchange or gene pool diversity between populations may improve their ability to cope with future environmental changes. Domestic chicken research also emphasizes the importance of coordinated trait changes. Domestication involves the simultaneous evolution of a series of traits, such as docile behavior often accompanied by changes in coat color (the famous “domestication syndrome”). Genomic analysis provides a possible explanation: some genes (or tightly linked gene clusters) have pleiotropic effects and affect multiple traits at the same time when selected. For example, the KIT gene affects both color spots and calmness, and similar reports have been made in the domestication of silver foxes. More evidence is needed in domestic chickens, but it has been observed that mutations in genes that control hormones often affect multiple aspects of reproduction and behavior (such as TSHR mutations that change seasonal rhythms and affect temperament). This suggests that when species adapt, the trade-offs and linkages at the genome level cannot be ignored, and many traits evolve together.

Looking to the future, comparative genomic studies of Galliformes and domestic chickens will be further expanded in multiple directions. First, more extensive genome sampling is an inevitable trend. Although there are some high-quality genomes of representative species, they are still insufficient for the entire lineage of Galliformes. The international “Bird 1K Project” and others will sequence more Phasianids species, including some rare or limited distribution species. As the data increases, we will improve the Galliformes phylogenetic tree, especially clarify the positions of some controversial small lineages, and calibrate the divergence time more accurately. For domestic chickens, large-scale population resequencing will cover more local breeds and ecotypes, allowing us to construct a full genome variation map and pedigree network for domestic chickens. Chinese scholars have sequenced thousands of chicken genomes, and in the future, the genome information of all major chicken species in the world may be covered. These data will push the domestication model of domestic chickens from a rough single-center model to a refined multi-region network model. Secondly, functional genomic verification will become a research focus. After a large number of candidate selection genes are identified through comparative analysis, the next step is to verify their functions through molecular biology and genetic experiments. For example, gene editing is used to knock out/repair corresponding mutations in chicken embryos or cell lines, and the effects on phenotypes or cell functions are observed to confirm causal relationships. At present, the in vitro culture and gene editing technologies of chicken embryos have become increasingly mature (such as CRISPR/Cas9-mediated primordial germ cell editing), and it is expected that functional verification can be carried out on key genes for domestication of domestic chickens (TSHR, SOX5, etc.). In addition, single-cell sequencing, ATAC-seq and other technologies can also be applied to chicken tissues to explore how mutations in regulatory elements change gene expression, thereby leading to trait changes. This will advance our understanding of domestication selection to the level of regulatory networks. Thirdly, paleogenomics has the potential to answer some unresolved questions in the evolution of domestic chickens. Although modern genomes reveal the overall changes after domestication, only ancient DNA can record the intermediate processes intuitively. In recent years, mitochondrial and partial nuclear DNA of chicken bones from hundreds or even thousands of years ago (such as medieval chickens in Europe) have been successfully sequenced. In the future, DNA from remains of early Neolithic chickens in China may be obtained, and compared with modern chickens, it will be possible to directly observe how allele frequencies evolve over time. In particular, it can be verified when and where mutations such as TSHR appeared and spread, and whether domestic chickens experienced severe bottlenecks when they were first domesticated. This information will greatly enrich the details of the domestication model. Ancient DNA can also identify the genetic characteristics of some extinct ancient breeds, giving us a more complete picture of the historical evolution of genetic diversity in domestic chickens. Finally, in terms of breeding applications, comparative genomics results will guide precision molecular breeding. Once we have identified the key mutations that affect traits such as meat and egg production, disease resistance and environmental tolerance, we can use marker-assisted selection (MAS) and gene editing to aggregate favorable mutations into target varieties. For example, molecular markers can be used to quickly screen whether breeders carry high-egg alleles to improve the efficiency of traditional breeding. For targets where there are no ideal alleles yet, such as further improving plateau tolerance, gene editing can be used to directly introduce the Tibetan chicken's EPAS1 mutation into commercial breeds, thereby creating a “super chicken”. Of course, this involves regulatory issues for transgenic/gene-edited animals, but it is technically feasible. Comparative genomics can also help broaden the genetic basis of breeding. By comparing the genomes with local breeds, breeders can target which breeds to introduce for hybridization to obtain the desired genetic variation. This avoids blind trial and error and improves the scientific nature of hybridization programs. It can be foreseen that future domestic chicken breeding will increasingly rely on whole genome selection (GS) and genome editing, and the knowledge provided by comparative genomics is the cornerstone.

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Conflict of Interest Disclosure

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