Bioscience Evidence 2025, Vol.15, No.1, 20-32
http://bioscipublisher.com/index.php/be

Research Insight Open Access

Structural Variation of Chicken Genome: Genetic Basis in Domestication and Evolution

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Bioscience Evidence, 2025, Vol.15, No.1 doi: 10.5376/be.2025.15.0003
Received: 12 Dec., 2024
Accepted: 13 Jan., 2024
Published: 21 Jan., 2025

Preferred citation for this article:
Hong Z.M., and Lin X.F., 2025, Structural variation of chicken genome: genetic basis in domestication and evolution, Bioscience Evidence, 15(1): 20-32 (doi: 10.5376/be.2025.15.0003)

Abstract The domestic chicken (Gallus gallus domesticus) is one of the most common domestic animals around us. It is widely distributed around the world, closely related to human life, and is an animal with high economic value. In recent years, with the development of high-throughput sequencing technology, scientists have found that the impact of "structural changes" in the genome (such as large changes such as duplication and deletion of gene segments) on the domestication and trait evolution of chickens may be more important than we thought. Starting from the structure of the chicken genome, this article explains the types of variation, detection technology, and its important role in evolution and artificial selection. The article focuses on the origin mechanism of SV, the distribution differences among different chicken species, and its impact on gene expression, phenotype, and domestication traits (such as feather color, comb shape, growth rate, and egg-laying ability). Finally, a specific case, the duplication variation of the TBC1D1 gene region in broilers, is used to explore the mechanism of SV in the rapid growth trait of broilers. At the end of the article, we look forward to the application prospects of emerging technologies such as single-cell analysis, pan-genome construction, and genomic selection in SV research and poultry breeding. Structural variation is an important genetic basis in the process of domesticated chickens. In-depth research on the mechanisms and functions contained therein will help reveal the genetic nature of complex traits and promote the development of precision breeding.

Keywords Structural variation; Chicken genome; Domestication and evolution; Gene regulation; Genomic selection

1 Introduction

As the most numerous domesticated animal in the world, the domestic chicken (Gallus gallus domesticus) holds significant importance for human society (Wang et al., 2020; Tan et al., 2023). Research has shown that domestic chickens originated from the subspecies of red chickens in Southeast Asia, and after domestication, they have been spread and hybridized multiple times to form today's abundant breeds (Wang et al., 2020). During the long domestication process, the phenotype of domestic chickens underwent significant changes, including behavior, morphology, and physiology (Chen et al., 2022). Genetic variation is the fundamental reason driving these phenotypic diversities, among which structural variation (SV) of the genome is particularly noteworthy (Chen et al., 2022; Wang et al., 2024).

Structural variation generally refers to the addition, subtraction, or rearrangement of genome segments longer than 50 bp, including large segment deletions, duplications, insertions, inversions, and translocations, as well as copy number variations (CNVs) caused by these events (Wang et al., 2024). Compared to single nucleotide polymorphism (SNP), structural variations cover a wider range of genomic regions and may have a more significant impact on gene function and phenotype (Chen et al., 2022; Wang et al., 2024). Many studies have confirmed that structural variation plays a key role in the evolution of complex traits of animals and plants, such as human obesity, diabetes and other diseases, and the adaptive evolution of animals and plants are closely related to structural variation (Chen et al., 2022). In domesticated animals, structural variation is considered one of the important genetic foundations driving the differentiation of domestication traits (Chen et al., 2022).

However, due to technological limitations, the detection and analysis of structural variations have always been challenging, and there has been relatively little attention paid to them in the past

(https://news.bioon.com/article/f6cf861994cd.html). In recent years, with the development of high-throughput sequencing and bioinformatics technology, our understanding of the structural variations in the chicken genome has been continuously deepened (Chen et al., 2022; Zhang et al., 2022).

This article summarizes the main types and detection techniques of structural variations in the chicken genome, elucidates the origin and mechanism of structural variations in chicken domestication and evolution, focuses on the impact of structural variations on gene function and phenotypic traits, and lists typical cases of structural variations related to domestication traits (such as appearance, productivity, behavior, etc.), especially the role of TBC1D1 locus duplication in rapid growth domestication of broiler chickens. We also looked forward to the application prospects of emerging technologies such as single-cell sequencing and pan genomics in structural variation research and poultry breeding. Through this review, we hope to enhance our understanding of the genetic basis of genomic structural variations during the domestication and evolution of domestic chickens, and provide references for further analysis of the molecular mechanisms underlying complex traits.

2 Classification and detection of structural variations

2.1 Types of structural variation

According to genome rearrangement patterns, SVs can be divided into two categories: balanced and non-equilibrium (Wang et al., 2024). Balanced SV includes inversion and translocation, which refers to the rearrangement of chromosome segments in direction or position, but the total genome length remains unchanged (Wang et al., 2024). Unbalanced SV alters genome copy number, including insertions, deletions, and duplications (Wang et al., 2024). Among them, large fragment copy number changes caused by insertion or deletion are commonly referred to as copy number variation (CNV) (Wang et al., 2024) (research progress on copy number variation in poultry breeding). In addition, there are some special types, such as new sequence insertions caused by mobile element insertions, or complex structural variations such as chromosome circularization and attachment (Chen et al., 2022). These types of mutations can often combine to produce more complex structural variations. For example, tandem duplication, accompanied by insertion, forms a complex event of segmental duplication and insertion into new positions (Wang et al., 2024).

2.2 Progress in detection technology

Traditionally, large chromosomal structural variations can be detected through microscopic techniques such as karyotype analysis and fluorescence in situ hybridization (FISH), but the resolution is limited （ https://news.bioon.com/article/f6cf861994cd.html ）With the development of genome sequencing technology, data analysis based on high-throughput sequencing has become the mainstream method for SV detection (Zhang et al., 2022). Short read long sequencing (such as Illumina) combined with multiple algorithms can detect different types of SVs: Paired End Mapping (PE) method uses sequencing reads to identify SVs by aligning them with the reference genome and inserting abnormal fragment length and direction (Wang et al., 2024); The Split Read Segment (SR) method divides the read segment from the mutation breakpoint and compares it to accurately locate the insertion or breakpoint position (Wang et al., 2024); The Read Depth (RD) method detects changes in copy number by statistically comparing changes in coverage depth (Wang et al., 2024); There are also detections based on whole genome alignment or graphical genomics, which can reveal structural differences between assemblies (Shi et al., 2023).

However, the short read rectangular method often lacks sensitivity to large and complex SVs (Zhang et al., 2022). In recent years, long read sequencing techniques such as PacBio and Nanopore have provided higher continuous read lengths, making it possible to detect long fragment insertions and complex rearrangements (Zhang et al., 2022). For example, using PacBio sequencing can detect more than double the number of SVs in chicken breeds compared to the short read rectangle method (Zhang et al., 2022). Ouyang Fengzheng et al. (2020) successfully identified candidate genes for pig body height traits by combining CNV detection with QTL mapping (using copy number variation whole genome association analysis and quantitative trait locus mapping to jointly identify candidate genes for pig body height traits) (using copy number variation whole genome association analysis and quantitative trait locus mapping to jointly identify candidate genes for pig body height traits). In addition, new technologies such as single-cell sequencing and optical profiling have also been applied to structural variation

research, making it possible to analyze SVs at the single-cell level (https://news.bioon.com/article/f6cf861994cd.html).

2.3 Challenges in Poultry Genome Testing

The avian genome has some peculiarities, such as the presence of numerous microchromosomes (short in length but enriched in genes) and a high proportion of repetitive sequences, which pose challenges for SV detection (Chen et al., 2022). Taking chickens as an example, there are about 30 pairs of microchromosomes in the 2n=78 chromosome, and their high GC content and repetitive sequences can easily lead to short read sequencing alignment errors or assembly gaps (Chen et al., 2022). Therefore, traditional short read sequencing has lower accuracy in detecting SV on microchromosomes (Zhang et al., 2022). The application of long read technology has significantly improved this situation: Lin et al. (2023) used PacBio long read sequencing and combinatorial algorithm to identify 7550 CNV regions in 8 duck breeds. Similarly, studies on chickens have shown that using multiple detection tools simultaneously and taking the intersection results can improve the credibility of SV identification. In the future, with the reduction of sequencing costs and algorithm optimization, we are expected to more comprehensively and accurately depict the structural variation map of the chicken genome (Zhang et al., 2022).

3 Chicken Genome: Overview and Structural Features

3.1 General Features of the Chicken Genome

The domestic chicken genome has a size of approximately 1.05 Gb, consisting of 39 pairs of chromosomes, including about 8 pairs of macrochromosomes and 30 pairs of microchromosomes. Microchromosomes, typically shorter than 20 Mb in length, are a distinctive feature of the chicken genome, characterized by high gene density and elevated GC content (Chen et al., 2022). Due to the large number and sequence complexity of microchromosomes, early studies faced significant gaps in these regions. Recent advancements, particularly the release of next-generation reference genomes (GRCg6a and GRCg7b), have greatly improved the assembly and annotation of microchromosomal regions (Rice et al., 2023). Repetitive sequences-such as transposable elements and low-copy repeats-constitute a substantial portion of the genome and serve as hotspots for non-allelic homologous recombination, driving the formation of structural variations (Chen et al., 2022).

3.2 Known Hotspots of Structural Variation

Existing studies have shown that the distribution of SVs in the chicken genome is not random, and its distribution has hotspot areas. Chen et al. (2022) compared the measured SV frequency with the Poisson random distribution and identified 113 SV high-density segments in the chicken genome (≥33 SVs in a 500 kb window), containing a total of 6,180 SVs. Among them, the number of SVs on chromosome 2 is the largest, which indicates that macrochromosomes, especially larger chromosomes, may accumulate more structural variations (Zhang et al., 2022). In these SV hotspot regions, there are approximately 768 genes, which are enriched in functional pathways such as stem cell maintenance, metabolic regulation, and cell proliferation (Zhang et al., 2022). These research results indicate that some functional gene cluster regions may be more prone to SVs due to repetitive sequences or structural characteristics. Conversely, some regions with important and conserved gene functions (such as basic metabolic pathway genes) have fewer structural variations.

3.3 Genome Structures Associated with SVs

The major histocompatibility complex (MHC) of chickens is located on microchromosome 16, contains several immune-related genes, and exhibits a high degree of copy number variability (Chen et al., 2022). In a 2019 bird study, Minias et al. found that the copy number of MHC genes in different bird species varies with life history and pathogen exposure pressure, indicating that immune-related structural variation is driven by natural selection. In chicken populations, copy number variation in the MHC region may affect differences in disease resistance and vaccine response, which is one of the current hot topics in poultry breeding. Relatively speaking, the macroscopic structure of the chicken genome is relatively stable, but there are a large number of structural variations at the microscopic level, and the distribution of variations is regionally heterogeneous. These structural features provide clues for us to understand the evolutionary history and domestication traits of chickens (Zhang et al., 2022).

4 Origins and Evolution of Structural Variations in Chickens

4.1 Molecular Mechanisms

The structural variation of the chicken genome is driven by a variety of molecular mechanisms. Among them, non-allelic homologous recombination (NAHR) is one of the important sources. When there are large segments of highly similar repetitive sequences in the genome, these regions may be mispaired during cell division, resulting in the incorrect duplication or loss of some genes (Chen et al., 2022). For example, the widely distributed repetitive sequences in the chicken genome provide incorrect "templates" for such errors, causing changes in gene copy number and generating CNV events (Chen et al., 2022). Another mechanism is non-homologous end joining (NHEJ). When a double-stranded DNA break occurs, the cell directly connects the two ends of the break for repair. However, this "simple and crude" repair method is often not accurate enough and can easily lead to the insertion or deletion of small segments of genes (Wang et al., 2024). If multiple breakpoints are misconnected, complex structural variations such as chromosome segment translocation and inversion may occur. In addition, the activity of transposons also affects the structure of the genome. They insert new sequences into the genome by inserting into new sites, and may mediate NAHR or induce chromosome breakage by providing microhomologous sequences (Wang et al., 2024). The insertion of LINE-1 transposons in the buffalo genome activates the expression of the ASIP gene, causing these buffaloes to show an albino phenotype; similarly, in the chicken genome, the special eggshell color of Dongxiang green-shell laying hens is derived from the 4.2 kb retroviral sequence inserted upstream of its SLCO1B3 gene, and its insertion activates the specific expression of this gene in the eggshell gland. (Zhang et al., 2020). The combined action of these mechanisms may not only cause genetic defects, but also provide a source of diversity for species evolution. Through this dynamic genomic change, organisms produce new traits to adapt to environmental needs.

4.2 Evolutionary Time Scale

Some structural variations already existed in wild populations before the domestication of chickens, while others were generated and retained during the domestication of chickens (Chen et al., 2022; Zhang et al., 2022). Large-scale population genomic analysis shows that the genomic differences between domestic chickens and red junglefowl are not only reflected in SNPs, but also in a large number of CNV regions. In their study, Chen Xia et al. (2022) compared the genomes of wild red junglefowl with those of multiple domestic chicken breeds and found that compared with red junglefowl, domestic chickens have 235 unique CNVRs involving 255 protein-coding genes, which are mainly enriched in pathways such as nervous system development, immune response, and reproductive function. These variations are likely to have been selected during the domestication process, thus promoting the domestication adaptation of domestic chicken behavior, immunity, and reproductive characteristics. Similarly, different breeds of domestic chickens have their own unique structural variations. White Leghorn chickens have a unique high-copy repeat coverage of the NTRK3 gene, which may be associated with high egg production performance; while White Rock chickens have structural variations in genes such as DOCK3 and AKR1B1L, which enable them to grow rapidly (Chen et al., 2022) (Figure 1). These studies show that due to different domestication directions (meat-producing type, egg-laying type, ornamental type, etc.), domestic chicken breeds have obvious differentiation in genomic structural variation.

4.3 Comparison of SV and SNP Rates in Domestic Chickens

The domestication process of domestic chickens is not a simple one-time event. Wang Mingshan et al. (2020) analyzed the genomic data of 863 domestic chickens and found that domestic chickens were first domesticated from a specific subspecies of red junglefowl. But for a long time afterwards, domestic chickens had many hybridizations with other subspecies and some wild relatives in Southeast Asia. This process made it possible for some structural variants to enter the gene pool of domestic chickens from wild chickens through "gene introgression". For example, the yellow skin of domestic chickens comes from an allele of gray junglefowl (Wang et al., 2020). Although this example was formed through SNP variation, it shows that the variation of wild relatives also affects the traits of domestic chickens. Similarly, some structural variations found only in domestic chickens may also come from different wild populations. Guo et al. (2018) found that the beard trait of Huiyang bearded chickens is related to a specific structural variation. This variation may be the result of long-term separate

evolution of local chicken species and cannot be found in other chicken species (Lin et al., 2023). In addition, due to the high intensity of artificial selection, the genetic diversity of commercial chicken breeds has decreased. Many harmful or useless structural variations have been eliminated, while useful variations have been retained (Zhang et al., 2022). For example, in today's white-feathered broilers, a mutation type related to the TBC1D1 gene has almost become a "standard" (Rubin et al., 2010). This also shows that artificial selection has a great impact on genome structure.

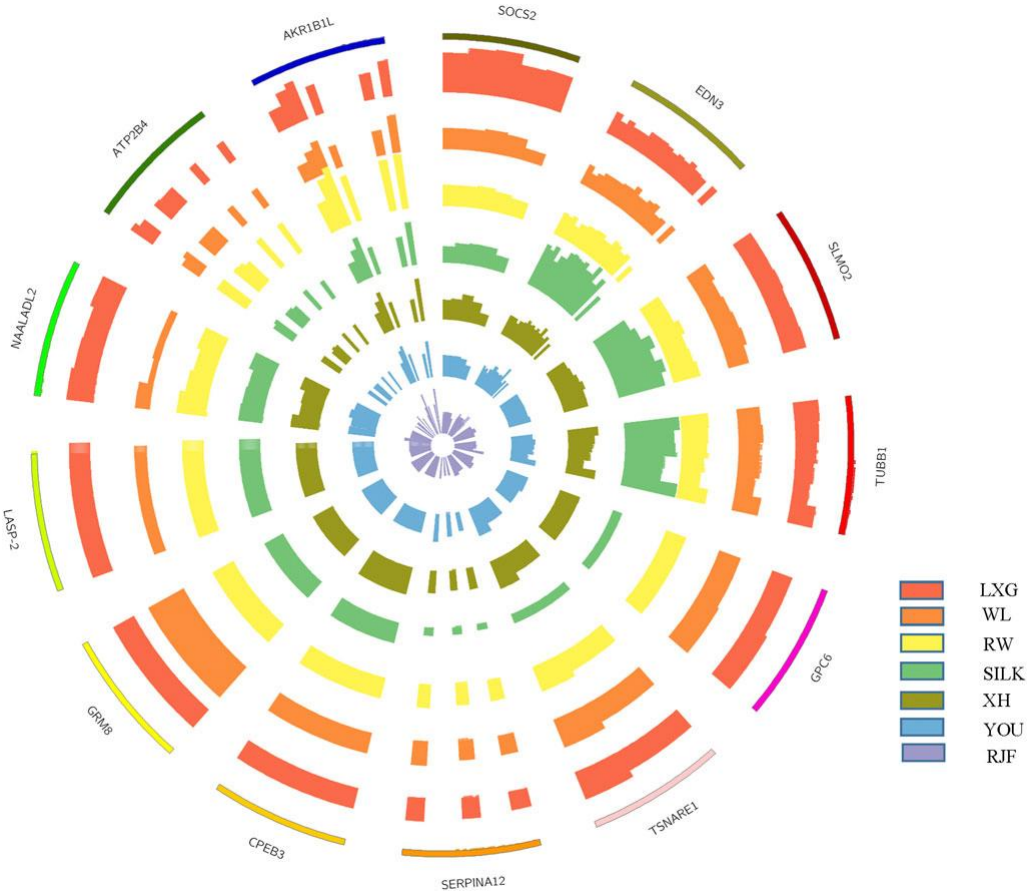

Figure 1 The schematization for a part of copy number variation genes distinguished in different chicken breeds. The outermost circle indicates the CNV-involved gene regions, with the thickness implying the normal diploid. The other circles from outside to inside indicate the breeds of Luxi Game fowl (LXG), White Leghorn (WL), Recessive White Rock (RW), Silkie (SILK), Xinghua (XH), Beijing You (YOU), and red jungle fowl (RJF) in turn, whose thickness proportionally means the estimated copy number

5 Functional Impacts of Structural Variations

Structural variation affects gene function and animal traits in many ways. These effects mainly include: changes in gene dosage, changes in position, and effects on the three-dimensional structure and epigenetic regulation of the genome (Chen et al., 2022; Wang et al., 2024).

5.1 Effect of gene dosage

When the number of copies of a gene increases or decreases, it directly affects its function. Sometimes, if a gene is deleted, it may lose its function completely or partially. This may cause some traits to manifest or weaken related pathways (Zhang et al., 2020). For example, in humans, if part of the SH2B1 gene is deleted, it may cause early-onset obesity. This is because when this gene is missing, the metabolic pathway has problems (Wang et al., 2024). In domestic chickens, studies have found that chickens with dark brown feathers lack an 8.3 kb fragment upstream of the SOX10 gene (Gunnarsson et al., 2011). Because of this deletion, SOX10 expression decreases and melanin synthesis decreases, so the feather color becomes dark brown. On the contrary, if the gene is repeated and the copy number increases, the function may be enhanced. For example, in pigs and cattle, the increase in the number of copies of the KIT gene causes problems in the development of pigment cells, resulting in white hair

(Chen et al., 2022). However, the effect of dosage is not absolute. Many times, there are "compensatory mechanisms" in cells to maintain balance. Sometimes, if a gene is deleted, another allele can make up for it; if the copy increases, sometimes there will be negative feedback to adjust the expression down (Zhang et al., 2020; Wang et al., 2024).

5.2 Effect of gene position

Structural variation may also change the location of genes or regulatory elements in the genome, which will also affect gene expression (Wang et al., 2024). For example, inversion can reverse the direction of the gene, which may interrupt the gene or move it away from the original enhancer; translocation can move a fragment to another position, which may make it controlled by a new promoter or enhancer. The rose-crowned chicken is an example. Studies have found that it has a 7.4 Mb inversion on chromosome 7. This changes the position and chromatin state of the *MNR2* gene, causing it to express abnormally, and the comb becomes rose-shaped (Zhang et al., 2020). For another example, the Huiyang bearded chicken has complex structural variations on chromosome 27, which moves the HOXB8 gene to a new position. At this position, it is expressed more in the chin skin, growing beards and ornamental hair (Lin et al., 2023; Zheng et al., 2023). These situations all show that when the position between regulatory elements such as enhancers and promoters and the genes they control is disrupted, gene expression may increase or decrease, thereby changing the appearance (Wang et al., 2024).

5.3 Three-dimensional structure and epigenetic influence

Now there are many studies showing that large structural variations can also affect the three-dimensional structure of chromosomes in the cell nucleus and change its epigenetic state (Zhang et al., 2024). Chromosomes have a structure called "TAD", which allows genes to be expressed more stably. If the boundaries of TAD are destroyed by structural variation, enhancers may control genes that they should not control. This may cause some problems, such as developmental abnormalities or cancer (Zhang et al., 2024). Although there are not many studies in this area in chickens, the results of studies in humans and other species indicate that chickens may have similar mechanisms. In addition, structural variation may also indirectly regulate genes by affecting the openness of chromatin or DNA methylation (Chen et al., 2022). For example, large duplications or insertions may make nearby areas "tighter", and the genes in these places will be silenced; while deletions may make that area more "loose", and gene expression will increase instead. Some studies have also found that CNV (copy number variation) can reorganize the three-dimensional genome structure and affect the interaction between regulatory elements and genes (Chen et al., 2022). The impact of structural variation on gene function is multifaceted. It may directly change the number and coding of genes, or it may work through complex regulatory networks and epigenetic mechanisms. Therefore, when studying the traits of domestic chickens, we cannot just look at the surface, but must also consider the impact of these potential structural variations (Wang et al., 2024; Zhang et al., 2024).

6 Structural Variations Related to Domestication Traits

Domesticated chickens have developed many special traits during the domestication process, such as changes in appearance, increased production, and even behavioral differences. Many of these changes are related to structural variation in the genome (Chen et al., 2022; Zhang et al., 2020). Let's take a look at how structural variation affects these traits with examples.

6.1 Changes in crown shape and head feathers

Chicken combs have many different shapes, such as bean combs, rose combs, and compound combs, all of which are related to structural variation.Bean combs are the most classic example. Studies have found that this is because a repetitive sequence is inserted into the intron of the SOX5 gene, affecting its expression, turning the original single-comb chicken into a bean comb (Dong et al., 2019; Zhang et al., 2022) (Figure 2). The rose comb is caused by a large inversion on chromosome 7, which disrupts the position of the regulatory element that controls the MNR2 gene, causing the comb to grow different protrusions (Zhang et al., 2020).The compound crown is a combination of the bean crown and the rose crown, and its formation is related to a repeated sequence near the EOMES gene on chromosome 2 (Zhang et al., 2020).The decorative feathers on the chicken's head, such as the

crest, are due to an inversion on chromosome 1, which affects the regulation of the HOXC gene, causing the top of the head to grow a crown like an "Afro" (Zhang et al., 2022).China's bearded chicken also has unique facial feathers, the so-called "beard" and "beard". Studies have found that this is a structural variation on chromosome 27, which changes the location and expression of HOXB8, allowing the chicken's face and throat to grow long feathers, and the wattles have become smaller (Zhang et al., 2020; Zheng et al., 2023).

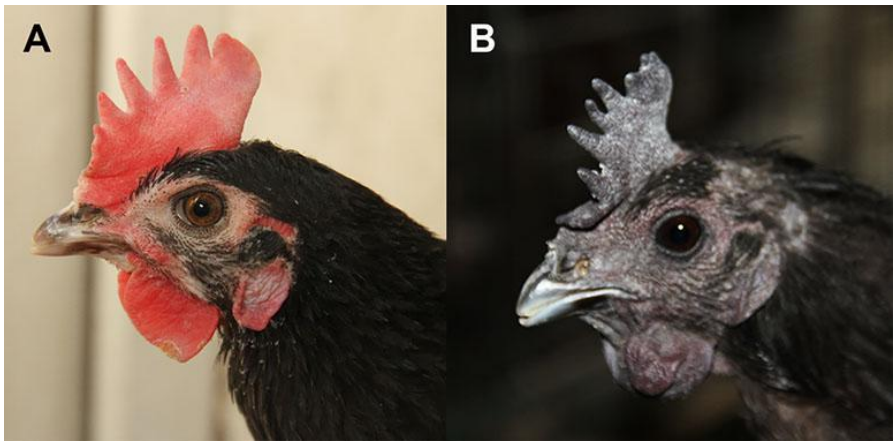

Figure 2 Comb color phenotype in Dongxiang blue-shelled chicken; A: Chicken comb with typical red color; B: Dark chicken comb, the wattle, face, and ear lobe also present fibromelanosis phenotype

6.2 Feather and skin color

The feather and skin colors of domestic chickens are also richer than those of pheasants, and these color changes are often caused by structural variations.For example, dark brown feathers are caused by the lack of an 8.3 kb fragment upstream of the SOX10 gene. The expression of SOX10 is reduced, and the feather color changes from black to dark brown (Zhang et al., 2020).The black skin and bones of the Silky-feathered Black-bone Chicken are very unique traits in domestic chickens. This is because there is a complex structural variation on chromosome 20, which contains multiple copies of the EDN3 gene. This gene promotes melanin production. When the copies increase, it is overexpressed during the embryonic period, causing melanin to be deposited in the skin and bones (Chen et al., 2022).The formation of blue-shelled eggs is also a good example. Dongxiang green-shelled laying hens have a 4.2 kb viral sequence inserted on chromosome 1. This insertion brings a new promoter, allowing the SLCO1B3 gene to be expressed in large quantities in the shell gland, resulting in an increase in biliverdin in the eggshell, turning it blue (Zhang et al., 2020).These examples show that structural variation can bring about a diverse appearance of domestic chickens by affecting the regulatory network of pigments.

6.3 Growth rate and body shape

The body shapes of broilers and ornamental chickens are very different, and structural variation also plays a role behind this.Broilers grow fast because some favorable variations are retained during the breeding process. For example, a repetitive structure of the TBC1D1 gene affects its function, causing the chicken's muscles to use glucose differently, thereby allowing the muscles to grow faster (Rubin et al., 2010).In ornamental chickens, such as the Chinese bantam chicken, there is an 11.9 kb deletion on chromosome 7, which contains the IHH gene. This deletion affects cartilage development and makes the chicken grow into a short-legged appearance (Zhang et al., 2020).Other studies have found that large and small chicken breeds differ on chromosomes 3 and 24. One of the genes, ADGRG6, has structural variations in large chickens, which may also be related to bone development (Tan et al., 2023).All of this shows that structural variations affect the growth path of chickens, thereby forming different body shapes.

6.4 Reproductive capacity and egg-laying performance

After being domesticated, the egg production of domestic chickens increased significantly. Some studies have shown that structural variation is also involved in this change.For example, a study found that HOXB8 in bearded chickens not only affects facial feathers, but also may regulate follicle-stimulating hormone, which may improve

their fertility (Guo et al., 2016; Zheng et al., 2023) (Figure 3).Other studies have found that comb color is also related to egg-laying performance. In red-crested chickens, structural variation in the two genes EDN3 and BMP7 may regulate pigments on the one hand and affect follicle development on the other hand (Cai et al., 2023). This shows that a variation may affect multiple traits at the same time.There are also two specific CNVs in the beak deformity strain of Beijing oily chickens. Studies have shown that this may affect the LRIG2 gene, thereby affecting feeding behavior and indirectly affecting egg production (Zhang et al., 2020).In addition, the weakening of nesting behavior in domestic chickens is also a typical domestication phenomenon. Although no clear structural variation has been found, there is speculation that hormone-regulating genes such as PRL may have mutated, affecting prolactin secretion, thereby reducing the tendency to nest and increasing egg production (Zhang et al., 2020).

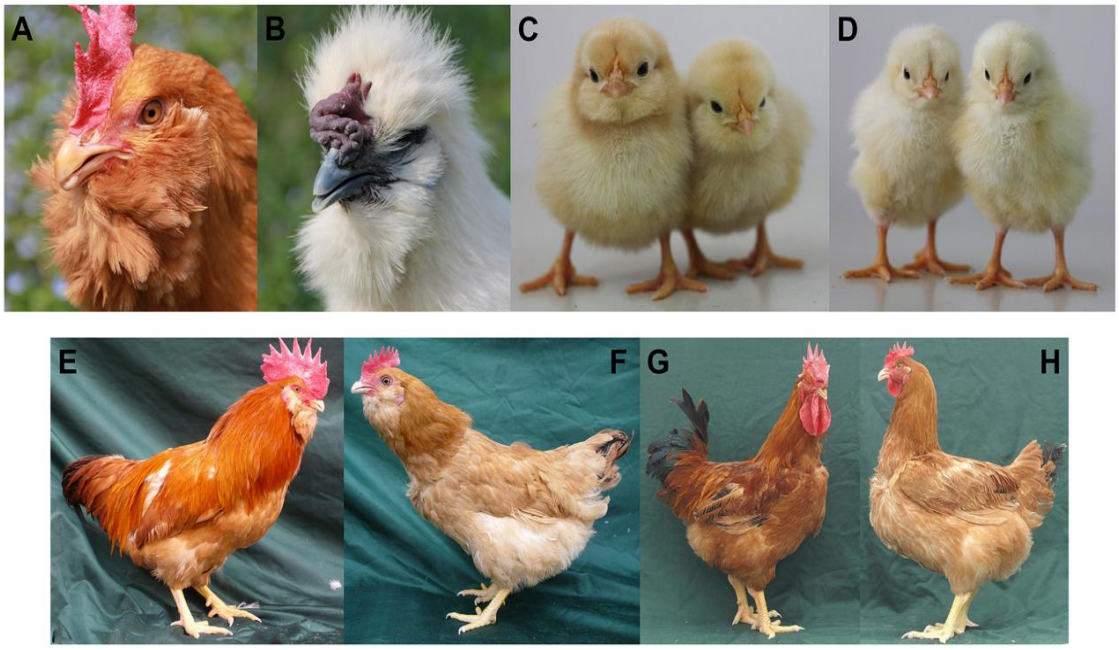

Figure 3 Muffs and beard phenotype in Chinese domestic chickens.
Image caption: A: Huiyang Bearded (HB) chicken; B: Silky-feather chicken with Muffs and beard; C: Newly hatched Mb chicks from the HB broiler breed; D: Newly hatched wild-type chicks from White Leghorn breed; Male (E) and female (F) Huiyang Bearded chickens that were founders of the HB & HQLA family. Male (G) and female (H) birds from the High quality chicken Line A were also founders of HB & HQLA family

6.5 Behavioral changes and environmental adaptability

The personality and adaptability of chickens to the environment are also affected by structural variation.Studies have found that fighting chickens have some repeated fragments containing genes related to neurodevelopment, such as SORCS2, which may make them more aggressive (Chen et al., 2022).Some unique CNVs have also been found in laying hens. These variations involve genes that regulate the reproductive axis and may be related to their tendency to be high-yielding.In terms of disease resistance, chickens resistant to Marek's disease have many deletion variations compared to susceptible chickens. These variations may delete some "susceptibility genes" and make them more immune (Zhang et al., 2020; Xu et al., 2021).These variations are "adaptive changes" that chickens make when facing diseases or environmental pressures, and were later preserved through artificial breeding.In general, whether it is fighting, defense, reproduction, or adapting to the living environment, these changes in behavior and ability may be related to structural variation.

7 Comparative Genomics of Wild and Domestic Chickens

By comparing the genomes of domestic and wild jungle fowl, we can see what changes domestic chickens have undergone during domestication, especially in terms of structural variation (Wang et al., 2020; Chen et al., 2022).In general, the genomic diversity of domestic chickens is lower than that of wild red jungle fowl. This can be seen at both the SNP and SV levels (Zhang et al., 2022). As early as 2008, Muir et al. found that many

commercial chicken populations had lost some rare alleles in wild chickens. Recent studies have also found that modern commercial chickens have fewer unique structural variations than some chickens that have not been artificially selected. This shows that during the artificial selection process, some variations have been retained, while others have been eliminated (Zhang et al., 2022).Simply put, structural variations that are useful in the wild but useless or harmful in captivity may have disappeared long ago. Variations that are conducive to domestication traits have gradually become more common in domestic chickens.

7.1 Some specific examples of structural differences

Domestic chickens do differ from wild jungle fowl in some gene regions. For example, in white chickens, the promoter region of the IGF2BP1 gene is deleted. This is related to the larger size of white chickens (Zhang et al., 2022). Wang Kejun et al. (2021) found through the establishment of a pan-genome of chickens that this deletion is common in large chickens and reduces the expression of IGF2BP1, which may in turn weaken the effect of the growth inhibition pathway, allowing chickens to grow faster.In contrast, wild jungle fowl and some small local chickens do not have this deletion, and they retain the complete promoter sequence. This may be related to their need to control growth rate in the natural environment.There are also some mutations that wild chickens have but domestic chickens do not. For example, there is a gene variant related to yellow skin in the gray jungle fowl, involving a deletion in the BCO2 gene. This mutation later entered the domestic chicken through ancient hybridization and became the source of yellow skin (Chen et al., 2022). Although this is a SNP, not a standard structural variation, it affects the phenotype and also shows that genes from wild species can flow into domestic chickens.In addition, there are structural differences between different subspecies of wild red jungle fowl. Wang Mingshan et al. (2020) found that these differences can help us determine which subspecies domestic chickens were domesticated from, and also help to infer the time and place of domestication.

7.2 Hybridization and gene introgression

After domestication, domestic chickens have hybridized with wild relatives many times. Some gene fragments of wild species have been "mixed" into the genome of domestic chickens (Wang et al., 2020). For example, in white Leghorn chickens, some chromosome fragments from other subspecies of red jungle fowl can be found.Some fragments contain unique structural variations. These variations may allow white Leghorn chickens to retain some characteristics close to wild chickens, such as stronger flying ability or more acute reactions. This shows that some wild genes that help survival have been preserved through hybridization.Current genomic technology can well identify these introgressed fragments. By comparing with the gray jungle fowl, green jungle fowl, etc., we can find out which mutations are "foreign". These gene introgressions enrich the genetic diversity of domestic chickens and give us the opportunity to do "natural experiments": we can compare chickens with a certain structural variation with chickens without this variation to see how they differ in traits, so as to understand the role of this variation.

7.3 Comparison with other jungle fowl

In addition to the red jungle fowl, domestic chickens have other wild relatives, such as the gray jungle fowl, green jungle fowl and Ceylon jungle fowl. It is also meaningful to compare the genomes of domestic chickens with those of these species.If a structural variation is present in all jungle fowl species but not in domestic chickens, it may have been lost during domestication. If a variation is only present in domestic chickens and not in other jungle fowl species, it is likely that it is new to domestic chickens and has been selected and retained (Zhang et al., 2022).This comparison method is already common in other domestic animals and is now gradually emerging in chicken research. For example, in a recent pan-genome study, researchers spliced together the genomes of red junglefowl and a variety of domestic chickens and found about 9.9 Mb of "new" sequences that were not found in the original reference genome (Rice et al., 2023). Some of these new sequences only appear in red junglefowl.

8 Case Study: Structural Duplication at the TBC1D1 Locus and Growth Selection in Broiler Chickens

The TBC1D1 gene is an important gene that regulates glucose metabolism in mammals and poultry. When modern broiler breed selection emerged in the mid-20th century, a large-effect mutation located at the TBC1D1 locus spread rapidly and was fixed in almost all white-feathered broilers (Rubin et al., 2010). Rubin et al. (2010)

first discovered through whole genome resequencing that the region where the mutation was located showed a selection sweep signal in broilers, but this feature was not seen in red junglefowl and laying hens. This result suggests that mutations at the TBC1D1 locus are very important for the high growth traits of broiler targeted breeding (Rubin et al., 2010). Subsequently, multiple studies confirmed that the TBC1D1 mutation carried by broilers belongs to a structural variation haplotype, and its core variation may be a small fragment duplication insertion within the TBC1D1 gene, resulting in a frameshift in the coding sequence, thereby producing a loss-of-function allele (Rubin et al., 2010).

8.1 Expression and functional differences

The wild-type TBC1D1 protein is involved in insulin- and exercise-induced glucose transport in muscle and other tissues. In simple terms, the GAP protein encoded by this gene affects the localization of the glucose transporter GLUT4 on the cell membrane by regulating Rab GTPase activity (Rubin et al., 2010). When TBC1D1 function is lost (such as knockout mutation in mice), muscle cells are less sensitive to insulin, glucose uptake is reduced, the body exhibits a "lean body type" and is resistant to high-fat diet obesity (Rubin et al., 2010). In broilers, the TBC1D1 mutant allele is also expected to reduce the uptake and utilization efficiency of glucose by skeletal muscle. However, compared with the wild type, broiler individuals carrying the mutation may compensate for the reduced glucose utilization by consuming more feed, thereby achieving faster growth. In fact, in the high-growth and low-growth control pedigrees established in the 1950s, the TBC1D1 mutant type tended to be fixed in both lines, indicating that the mutation had appeared and was widely selected before the differentiation of broiler strains (Rubin et al., 2010). Therefore, it can be inferred that the partial loss of function caused by the structural variation of TBC1D1 is beneficial to broilers in an artificial high-nutrition environment: it may change the energy distribution, so that more energy is used for lean meat growth rather than fat deposition, thereby improving feed conversion efficiency and meat production.

In response to this inference, some studies have compared the phenotypes of chickens with different genotypes. The results showed that broilers carrying the TBC1D1 mutant haplotype tend to have a higher muscle ratio, relatively less fat deposition, and a carcass composition that is more in line with human requirements for meat quality (Tan et al., 2023). This is consistent with observations in mice: TBC1D1 functional loss will cause the body to be more inclined to lean meat. It is also worth noting that the TBC1D1 mutation may increase fasting blood glucose and insulin levels in broiler chickens, but it does not have a negative impact on survival in a breeding environment. Instead, it may promote growth due to continuous overnutrition. This suggests that some unfavorable mutations in the wild (such as those that may cause mild insulin resistance) become favorable traits in a domesticated environment and are artificially fixed (Rubin et al., 2010). This phenomenon is not an isolated case in the domestication of domestic animals, but TBC1D1 provides a particularly clear and important example.

8.2 Implications of artificial selection

The discovery of structural variation in the TBC1D1 locus reveals how artificial selection quickly shaped the genome during the domestication of domestic chickens. In just a few decades, billions of broiler chickens around the world almost all carry the TBC1D1 mutation haplotype (Rubin et al., 2010). This is an extremely dramatic change on an evolutionary scale, indicating that under high-intensity artificial selection pressure, even low-frequency mutations can sweep the population in a very short time (Rubin et al., 2010). For breeding, this case emphasizes the value of discovering large-effect structural variation. If breeders could directly detect the TBC1D1 genotype at that time, broiler strain improvement might be more targeted. In addition, this case also raises a topic for functional genomic research: if we can deeply analyze the mechanism of the effect of TBC1D1 mutation on muscle cell metabolism and growth signaling pathways, it will help us to adjust feeding and breeding strategies more reasonably. For example, can we use nutritional means to compensate for the metabolic side effects caused by TBC1D1 deficiency, or copy this favorable variation to other breeds through genome editing to improve their growth performance? These are all questions worth exploring in the future.

9 Future Directions and Emerging Technologies

As genomic tools become more advanced, the study of structural variation in chickens has entered a new stage.

Now it is not only necessary to find these variations, but also to figure out what specific role they play. Future research can be roughly advanced from the following aspects.

9.1 Look at variations in single cells

In the past, we usually used DNA from a group of cells to analyze structural variation. The disadvantage of this is that we cannot see whether there are differences between different individual cells. But in fact, some variations, such as chromosome translocation or uneven copy number, may only appear in some cells.Now with single-cell sequencing technology, we can look at structural variation cell by cell. A study published in Science developed the "Genome-Shuffle-seq" technology, which can simultaneously create a large number of structural variations in vitro, and then label each cell with a "barcode" for tracking. This method can help us see the impact of variation on cells at a high throughput.In the study of chickens, in the future, early embryonic cells can be used to introduce specific variations, and then observe the changes in cell differentiation and proliferation, and then infer the impact on traits. If we can also combine the transcriptome data of single cells, we can see more clearly how the variation affects gene expression. This will help us to better understand how structural variation works.

9.2 Better genome assembly and annotation

Although the current chicken reference genome has been updated several times, some complex structural regions are still unclear. In the future, we can use new technologies such as third-generation sequencing, optical mapping, and Hi-C to piece together a more complete genome, and even build a "pan-genome" containing multiple chicken breeds.Warren et al. (2023) have already made attempts in this regard. They integrated the high-quality genomes of 30 breeds and made a "graphic pan-genome", which also included variations that were not in the previous reference. The results showed that compared with traditional references, this new method can reduce reference bias and more accurately detect complex variations such as K sites.In the future, we should also add more local chickens and wild jungle fowl to the pan-genome to describe the variation landscape of domestic chickens more completely. At the same time, in terms of genome annotation, we should also strengthen the functional analysis near structural variations. For example, we can use methods such as ATAC-seq and ChIP-seq to mark which are enhancers and promoters, so that we can determine whether a certain variation interrupts important regulation.

9.3 Use multiple omics together+functional verification

Structural variation generally does not directly change protein structure, but affects gene expression through regulatory networks. Therefore, we need to integrate multiple omics data, such as genome, transcriptome, epigenetic, metabolome, etc., and analyze them together.We can do eQTL analysis to first find out which genes a certain structural variation will affect expression, then use proteomics to see if there are changes in downstream pathways, and finally see if these changes are reflected in the phenotype. This "from gene to trait" approach has been successful in plants and humans, and it can also be done in chickens.On the other hand, with CRISPR/Cas9 technology, we can now ‘create’ specific structural variations to verify their functions. For example, a known variation, such as the mutation of TBC1D1, can be replicated in human cells or chicken embryonic cells, and then observe whether it has an effect on glucose metabolism or cell growth. Although it is still difficult to perform this operation in chickens, with the advancement of technology, it is expected that batch functional verification will be achieved in the future.

9.4 Application to breeding and variety protection

The study of structural variation must eventually be implemented in breeding and practical applications.At present, the genomic selection of domestic chickens mainly relies on SNP chips, but these chips cannot capture SVs. In the future, new molecular markers can be designed to specifically detect some useful SVs, such as TBC1D1 mutations or HOXB8 mutations in bearded chickens, to accelerate variety improvement.At the same time, structural variation can also help protect local breeds. Some local chickens may have unique SVs that can help them adapt to specific environments or improve the quality of meat and eggs. For example, Tibetan chickens may have CNVs that improve oxygen transport capacity. If it is really useful, we can introduce this variation into plain chickens through hybridization to make them more resistant to hypoxia.

10 Concluding Remarks

Genomic structural variations (SVs) represent a significant and often underappreciated form of genetic variation in the domestication and breed evolution of chickens. A growing body of research indicates that, compared to single-nucleotide polymorphisms (SNPs), SVs tend to exert larger phenotypic effects and can drive substantial trait differentiation in chickens over relatively short evolutionary timescales. Throughout the history of chicken domestication, it has been the emergence and accumulation of key SVs-such as the SOX5 repeat, EDN3 rearrangement, and TBC1D1 deletion-that endowed domestic chickens with morphological and productive traits distinct from their wild ancestors, enabling them to better adapt to human-managed environments and demands.

By altering gene dosage and regulatory networks, SVs have contributed to the development of major domestication traits such as increased egg production, accelerated growth, and docile temperament. At the same time, structural variants that were advantageous in the wild were often lost during domestication. Comparative genomics allows us to reconstruct these evolutionary footprints and understand the genetic trade-offs chickens underwent in adapting to human-controlled settings.

Looking ahead, the advancement of technologies such as long-read sequencing, single-cell genomics, and pan-genomics promises a more comprehensive identification of SVs across the chicken genome and more accurate assessment of their functional impact. In-depth functional genomics will illuminate the molecular mechanisms through which SVs influence gene expression and physiological pathways, offering new perspectives for dissecting complex trait genetics. Ultimately, this knowledge will serve poultry breeding: by incorporating beneficial SVs into breeding targets, we can improve economically important traits in a more directed manner while avoiding the accumulation of deleterious variants.

Moreover, cross-species comparisons should be strengthened to identify advantageous variants and stress-resistance genes lost in domestic chickens but retained in their wild relatives. Such alleles can be reintroduced through modern breeding techniques to meet future challenges posed by variable farming environments and shifting production demands.In conclusion, structural variation has provided a rich genetic foundation for the domestication of chickens. It not only records the evolutionary past but also points the way toward the future of breeding. As research continues to deepen, we will gain a clearer understanding of the genetic code underlying chicken domestication and evolution-and harness this knowledge to develop chicken breeds that are more productive, disease-resistant, and environmentally adaptable, writing a new chapter in the shared history of chickens and humankind.

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