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Review article

Genetic improvement of poultry: A review on conventional breeding to biotechnological approaches

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A B S T R A C T

Biotechnology in animal production is expanding at a quicker rate than plant production. The major goal of this review is to learn about the traditional breeding and many biotechnologies employed in poultry species for genetic improvement. The conventional breeding system primarily focuses on many selection processes, such as selective breeding, and progeny testing. To optimize genetic improvement, molecular genetic techniques must be used in conjunction with conventional poultry breeding procedures. Since a large number of DNA-level genetic polymorphisms have been identified over time, genetic markers have become crucial in the breeding of poultry as they may be used to assess the genetic basis of phenotypic diversity. Artificial insemination (AI) is the most frequently used animal biotechnology in terms of reproduction, genetics, and breeding, allowing for considerable genetic improvement. Molecular DNA markers can also be employed to characterize and protect animal genetic resources, as well as for genetic improvement via marker-assisted selection (MAS). Numerous windows of opportunity for the direct production of transgenic poultry are also indicated by the different stages of germ cell development. This review incorporates current advancements and new insights about poultry production genetic improvement methods, and the ability to alter avian development. Specific alternatives will help to make more informed decisions about the future deployment of acceptable biotechnologies in the poultry sector. At that point, we will be able to confidently state to any audiences that poultry genetic improvement has been accomplished through the use of traditional breeding and biotechnology tools.

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1. INTRODUCTION

Poultry species are becoming significantly more important as a source of eggs and meat for human consumption. Since the domestication of the species, different developed varieties of poultry has been selected mostly for productivity features. Additionally, certain native poultry breeds have been identified, indicating that disease resistance and other traits

can still be improved. Apart from providing meat and eggs, research in the fields of "Poultry Genetics, Breeding, and Biotechnology" has vastly advanced since the first draft of chicken genome is published in 2004 (Lee, 2021). Any technological application that alters goods or procedures for a particular use while utilizing biological systems, living organisms, or their derivatives is referred to as biotechnology. Among the several biotechnological approaches, artificial insemination (AI) is the geneticist's preferred approach for sustaining pedigree mating. Because broad-breasted turkeys are biologically incapable of natural mating, artificial insemination is the only way for them to reproduce. In guinea fowl, AI is used to reduce the size of the male flock because under traditional breeding one male is used for two to three females (Kharayat et al., 2016; Mohan et al., 2018).

In the 1990s, the primary focus of animal breeding efforts shifted from quantitative to molecular genetics. Previously, the main focus of the conventional breeding system was on progeny testing and numerous selection procedures, including selective breeding. It is critical to incorporate molecular genetic approaches alongside traditional poultry breeding methods in order to maximize the poultry breeding program. Over the years, several genetic polymorphisms at the DNA sequence level have been found and utilized as markers to assess the genetic basis for observed phenotypic diversity (Singh et al., 2014). These markers are crucial for chicken breeding. Concerns in poultry genomics fall into four categories: (a) the mapping and isolation of genetic markers (b) mapping quantitative trait loci (QTL) (c) identification of candidate genes, and (d) discovery of genes (Burt, 2002). Molecular markers are genetic markers that can detect genetic variability at the level of the DNA sequence (Emara and Kim, 2003).

The use of molecular breeding, for example, is a recent technological development in poultry genetic enhancement. The first advancement that affected the breeding of chickens was the identification of genetic markers. Later, less detailed maps were created by numerous research groups, and in 2000, a consensus linkage map containing over 2,000 such genetic markers were published (Thiruvenkadan and Prabakaran, 2017). The initial draft of the chicken genome assembly has been available since March 2004, and its quality is continually improved. The most recent version, which is available at www.ensembl.org, forecasts that the chicken genome will have 24,000 genes. The "black box" of knowledge regarding the genetic basis of poultry features relevant to commercial poultry breeding can only be opened with the help of the chicken genome assembly, which is an incredibly valuable resource. Studying candidate genes is one way to use the chicken genome information; another way is to employ an intermediary ("gray box") approach by utilizing information on a large number of genetic markers that were acquired indirectly from the sequencing process.

Effective methods for avian transgenesis have been researched for many years due to the high level of interest in poultry industry and research. For the development of transgenic animals, foreign DNA must be transferred into the germ line, resulting in stable transgene transmission across multiple generations. According to research, foreign DNA can be efficiently injected into the germ line to make transgenic chickens (Bednarczyk et al., 2018; Chapman et al., 2005; McGrew et al., 2004). Although genetic linkage mapping (Burt et al., 1998) was the beginning of avian genomics, our understanding of the chicken genome has evolved substantially in subsequent years. The chicken genome assembly is a priceless resource that holds the key to opening the "black box" of knowledge on the genetic basis of commercial poultry breeding (Burt et al., 2005). Aspects of SNP-based genome-wide marker coverage are also being used for specific reasons. Taken together, we believe that within the next couple of years, genomic-based selection processes will be an integral part of any poultry breeding program. As a result, the goal of this study is to emphasize conventional breeding, molecular techniques and their value in chicken breeding progress. The primary goal of this review paper is to analyze the many biotechnological technologies utilized in poultry breeding for genetic improvement.

2. CONVENTIONAL BREEDING IN POULTRY

In poultry breeding, traditional breeding methods have been successfully implemented, resulting in a significant rate of genetic improvement (Thiruvenkadan and Prabakaran, 2017; Smith, 1985). In poultry, nucleus breeding systems are used, in which parents' breeding values, sibling performance, and own performances are used to make selection decisions, although the generation gap is nearly zero, selection precision is low, particularly for sex-limited and carcass traits. As a result, improving selection precision is projected to result in a faster rate of genetic improvement in poultry breeding. To enhance genetic progress, traditional poultry breeding procedures combine qualitative and quantitative genetics, physiology of reproduction, computer science, statistics and poultry husbandry in an extremely dynamic manner (Siegel et al., 2006). The measuring of the attribute accurately is the first step in this approach (phenotype). For instance, in layers, number of eggs, egg weight, feed intake, shell quality, and mortality; in meat birds, juvenile body weight, fat content, breast meat production, and so on. Based on quantitative genetics theory, the geneticist employs the "infinitesimal model" to scan the genome for genetic variation in these qualities (Hill, 2014). Each attribute is impacted by numerous genes, each with little effect on the trait, according to the infinitesimal model. Advanced statistical techniques, such as restricted maximum likelihood (REML) and best linear unbiased predictor (BLUP), are used to assess genetic variation and quantify the breeding value of each chicken or turkey. To maximize the overall economic reaction to the commercial product, the selection is based on intricate indices that best incorporate personal and family information for a variety of aspects. It's important to note that the foundation of quantitative genetic theory is the assumption of the linearity of genetic effects, the quantity and distribution of genetic effects, and the organization of the genome. Essentially, these are merely guesses, as we do not yet have a thorough understanding of the genes involved and all of their activities. If we have a better understanding of the biological and molecular components of reproduction, development, and growth, the conventional selection process will be more effective. As a result of more precise selection decisions, the introduction of genetic markers may speed up the rate of genetic progress. Furthermore, detecting markers connected to QTLs will improve our understanding of poultry genetic architecture, which will be very useful for making selection decisions. For example, if we find a QTL that contributes to an unfavorable association between two key variables, such as growth rate and viability, we may be able to manage or anticipate undesirable selection effects. For the genetic improvement of chickens, several breeding and selection techniques were used over time (Table 1).

Table 1. The progression of selection methods over time.

Technique/Methodology	Decade
Mass selection	1900
Trap nesting	1930
Hybridization	1940
Artificial insemination	1960
Osborne index in layers	1960
Family feed conversion testing	1970
Selection index	1980
Individual feed conversion testing	1980
BLUP breeding value estimation	1990
DNA markers	2000

Source: (Thiruvenkadan and Prabakaran, 2017; Saxena and Kolluri, 2018)

Geneticists must balance traits related to growth and reproduction while creating or maintaining a strain of fowl (Table 2).

3. GENETIC MARKERS IN POULTRY BREEDING

Quantitative genetic methods view the bird as a "black box," with numerous genes contributing to the development of traits under study. This mystery is now being explored by molecular genetics, which shows how individual genes affect the way attributes are expressed phenotypically (Thiruvenkadan and Prabakaran, 2017). There were a lot of types I markers like restricted fragment length polymorphisms (RFLPs), expressed sequence tags (ESTs), and

Table 2. The most common criteria used to choose pure-line breeders

Growth-related traits	**Reproduction**
Growth rate	Egg number
Weight-for-age	Egg size
Feed efficiency	Hatchability of fertile eggs
Meat (breast) and carcass yield and body conformation	Fertility
Livability	Libido
Skeletal integrity	Mature weight and age
Feathering-cover, rate, and color	Aggressiveness (±)
Adaptation to heat distress	Adaptation to heat distress

Source: (Leeson and Summers, 2009; Saxena and Kolluri, 2018)

Single nucleotide polymorphisms (SNPs), as well as type II markers like RAPDs, mini- and microsatellites, AFLP, and so on are used in poultry breeding. Type II markers are preferred because they are abundant in the genome and highly polymorphic, however, SNPs, a third generation marker, are being used in a range of genetic applications (Reshma and Das, 2021).

Any phenotype that can be viewed or evaluated is a genetic marker, as is the genetic basis for a genetic test used to assess phenotypic variability. Morphological and productive traits (physically evaluated features), biochemical markers (gene products), and molecular markers (DNA analysis) are the three basic categories of genetic markers (Teneva and Petrović, 2010). A DNA marker, also referred to as a molecular marker, is a section of DNA that indicates mutations or variations and can be used to identify polymorphism (base deletion, insertion, and substitution) between various genotypes or alleles of a gene in a given population or gene pool (Yadav et al., 2017; Singh et al., 2014). Depending on the application and species involved, the ideal DNA marker for use in marker-assisted breeding (MAB) should have the following qualities:

- A high amount of polymorphism
- Uniform distribution over the whole genome
- Co-dominance in expression
- Divergent allelic characteristics
- One copy and no pleiotropic influence.
- Cost-effectiveness to use
- Automation and simple assay/detection
- High availability and duplicated or multiplexing suitability
- Genome-specific
- There should be no negative impact on the phenotype

A genetic marker is a genomic locus that can be used to determine an individual's allele(s). In an outbred or crossbred population, MAS during introgression has been documented along with parental control, varietal identification, loci affecting quantitative traits (i.e., QTL mapping), and varietal identification (Soller and Beckmann, 1983; Smith and Simpson, 1986). The present chicken genetic map, covering around 4,000 cM, contains at least 1,965 loci in 50 linkage groups. About 235 of these loci share known genes with humans or other animals. The remaining loci are characterized by molecular DNA markers such as microsatellites, amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), CR1 elements, and others. A third-generation genetic map of humans uses single nucleotide polymorphisms (SNPs), which have made it possible to map complex traits using linkage disequilibrium (Emara and Kim, 2003). The chicken has been the subject of the majority of investigations, and there is currently a genetic linkage map of over 2000 loci spanning the majority of the 1200-Mb and 4000 cM genome (Schmid et al., 2000).

Categorization of molecular markers

DNA markers are now widely used in the fields of genetics and breeding, as well as the characterization and management of germplasm as a result of significant advancements made in the development of molecular techniques that facilitate the quick identification of markers of interest. More types of DNA markers have lately been discovered using new, sophisticated, and widely available techniques. Based on the methods used to discover DNA polymorphism in livestock, different types of molecular

markers are usually grouped into three main categories (Yadav et al., 2017).

a) Non-PCR-based or hybridization-based molecular markers: the most prevalent example of this class of marker is RFLPs.
b) PCR-based DNA markers: RAPDs, SSRs, and AFLPs.
c) Sequencing-based DNA markers and DNA chips: e.g. SNPs.

Restriction fragment length polymorphisms (RFLPs)

RFLPs were the first DNA markers used to construct the first generation genomic map (Williams, 2005). Synthetic oligonucleotides were used as probes in this hybridization-based marker technology, which were fluorescently tagged to hybridize DNA (Teneva et al., 2013; Salisu et al., 2018). This approach is commonly used in livestock species for nucleic acid definition, detection, and diagnostics, as well as the description of genes polymorphisms, genetic linkage map construction, and recombinant DNA technology (Beuzen et al., 2000). The production of co-dominant markers, which allow the separation of homozygote and heterozygote circumstances in a diploid organism, is one of the advantages of RFLP markers. Other notable characteristics of these markers include selective neutrality, stability, and reproducibility. However, the major disadvantages of RFLP markers require higher quality and larger quantities of starting DNA, which are not always available. Additionally, the procedure is time-consuming and labor-intensive. Furthermore, RFLPs are limited in their ability to identify whole genome variation in animals, and the low diversity found in chickens owing to inbreeding makes many RFLP sites useless (Yadav et al., 2017).

Randomly amplified polymorphic DNA (RAPD)

RAPD approach uses *in-vitro* amplification to randomly amplify the unknown nuclear DNA loci (Welsh et al., 1990; Williams et al., 1990). When detection is paired with polyacrylamide gel electrophoresis, several primers in the range of 5 to 21 nucleotides are commonly utilized and have proven to be successful. The genetic differences between or within some taxa of interest have been quantified using RAPD technology (Bardakci, 2001). In comparison to RFLP, the RAPD methodology provides a quick, easy, and inexpensive method of generating molecular data. Due to its high polymorphism, just small amount of DNA is required for PCR amplification in the absence of DNA sequence information. This is one of the main reasons why the RAPD method has been successful in a variety of phylogenetic and taxonomic studies in poultry (Salisu et al., 2018; Yadav et al., 2017). However, one significant downside of the RAPD methodology is that RAPD primers are extremely sensitive to PCR conditions, which may result in poor reproducibility when compared to other methods (RFLP, SSR or SNPs). Furthermore, the outcome of each primer's amplification profile typically includes numerous unique loci within the genome, making it difficult to discriminate between individuals who are heterozygous and homozygous (Bardakci, 2001).

Amplified fragment length polymorphism (AFLP)

The AFLP method is a simple and inexpensive finger-printing approach that produces multi-locus and consistent genetic fingerprints, providing more relevant information (Vos et al., 1995). The underlying concept of AFLP polymorphism was the insertion, deletion, or substitution of nucleotides between and at restriction sites. Following amplification of a subset of complete pieces, allows for easy separation of the produced DNA fragments. Though the basic purpose of AFLP is the same as that of RFLP, which is polymorphism, it allows for the simultaneous analysis of several loci as an alternative to study one locus at a time (Jun et al., 2004; Negrini et al., 2007).

A large number of polymorphic genetic markers that can be automatically genotyped can be found using the AFLP approach, which offers a dependable, rapid, and affordable method for doing so. The discovery of genetic polymorphisms, evaluation and characterization of breed resources, measurement of the association between breeds, construction of genetic maps, and identification of genes in the main species of farm animals have all been

accomplished with the help of AFLP technology (Ajmone-Marsan et al., 2002; Jun et al., 2004; Negrini et al., 2007; Negrini et al., 2006; De Marchi et al., 2006; Buntjer et al., 2002). Apart from microsatellites, the finest molecular approach for population genetics and genome typing is AFLP technology (Yadav et al., 2017). Despite its many advantages, AFLP has numerous drawbacks, including the need for more DNA (300-1000ng each reaction) and being technically more complex than RAPD. However, with the current availability of kits and automation, the procedure may become more widely applicable (Salisu et al., 2018; Karp et al., 1997).

Microsatellite marker/simple sequence repeats (SSRs)

Microsatellites are DNA segments scattered across the genome that have a variable number of copies (often 5-50) of sequence motifs with two to five base lengths (Scherf and Pilling, 2015). They're polymorphic and abundant, and they're frequently located in non-coding sections of genes (Duran et al., 2009; Moxon and Wills, 1999). Short tandem repeats (STRs), simple sequence repeats (SSRs), and simple sequence tandem repeats (SSTR) are all terms used to describe microsatellite loci. Microsatellite-derived markers can be used to map genes that affect more valuable attributes. This method is usually more effective, although it has the flaw of precisely estimating the size of DNA. They are the most valuable markers in estimating genetic diversity within and between poultry breeds because of their co-dominant nature and high mutation rate (Salisu et al., 2018; McCouch et al., 1997).

Microsatellite markers have recently emerged as the most important genetic markers in animal genetic characterization research (Civanova et al., 2006; Sunnucks, 2000). These markers have several advantages over other types of markers, including the ability to identify many SSR alleles at a single locus using a simple PCR-based screen, the need for relatively little DNA for screening, and the ability to automate allele identification and sizing (Schlötterer, 2000). Recent report suggests that microsatellites may play a key role in the evolution of the chicken genome (Bilska and Szczecińska, 2016; Moxon and Wills, 1999). Microsatellites are preferred over RFLP markers in livestock improvement because of the wide range of molecular applications, which include genetic characterization studies, population structure analysis (Arora and Bhatia, 2004), genetic variability and inbreeding estimation (Mateus et al., 2004), paternity determination (Luikart et al., 1999), phylogenetic relationships among populations (Saitou et al., 1987), disease diagnostics, forensic and marker-assisted breeding among others (Teneva et al., 2013; Ritz et al., 2000; Thomas and Anilkumar, 2008; Montoya et al., 2007). However, one of the most significant disadvantages of this method is that it is extremely costly and time-consuming. Furthermore, null alleles can develop as a result of mutations in the primer annealing sites, which can cause heterozygotes to be misclassified as homozygotes.

Single nucleotide polymorphisms (SNPs)

A SNP in chicken refers to a nucleotide substitution that results in a change in the DNA sequence at a particular location in the genome (Scherf and Pilling, 2015). To put it another way, an SNP marker is a single base replacement in a DNA sequence (Beuzen et al., 2000). SNPs are a valuable genetic variation resource for population studies and genome mapping since they account for more than 90% of all individual differences (Frohlich et al., 2004). Seidel (2009) describes SNP markers as a potent new technique for genetic selection that can be used in genomic selection. These types of markers are becoming more and more sought-after in the creation of molecular markers due to their frequency in the genome of any organism (both coding and non-coding regions) and their ability to identify hidden polymorphism that is typically not found by other genetic markers and approaches (Rasal et al., 2017).

The use of SNPs as markers for genetic study is becoming increasingly popular for a variety of reasons. They are initially more prevalent than other types of polymorphism, such as microsatellites, and provide a greater number of potential markers near or in any locus of interest. Secondly, some SNPs are situated in coding regions and affect protein function directly. These SNPs may be directly

responsible for some of the economic differences between people. SNPs have a higher potential as long-term selection markers since they are more stable in inheritance than microsatellites. Finally, when using DNA microarray technology for high throughput genetic analysis, SNPs are more trustworthy than microsatellites (Lipshutz et al., 1999). SNPs, on the other hand, are often biallelic systems, with only two alleles in a population. As a result, SNP markers have lower information content than multiallelic microsatellite markers. There are several serious technical issues that must be addressed (Hacia et al., 1999). These obstacles include the presence of secondary structures in the target and the challenge of changing hybridization conditions across the entire array because of changes in oligonucleotide annealing temperature. It can be challenging to prepare tagged genomic DNA segments with enough specific activity for hybridization to immobilize oligonucleotides, especially when a large number of loci need to be screened. However, identifying relevant SNPs that can be utilized to estimate the breeding value of chickens deems to be most difficult task.

The work on the chicken genome assembly contained an essay about SNPs in the chicken genome (International Chicken Genome Sequencing Consortium, 2004b). An SNP occurs when a single nucleotide in the genome- A, T, G, or C -varies between individuals of the same species. In this study, partial sequences of broiler, layer, and Chinese Silkie chickens were compared to the Red Jungle Fowl's whole sequence. Millions of SNPs were discovered as a result of this process, providing a valuable resource for genomics applications in poultry breeding.

4. QUANTITATIVE TRAIT LOCI (QTL) MAPPING AND MARKER-ASSISTED SELECTION (MAS)

QTL can be discovered in the genome through relationships between performance and the inheritance of genetic markers in an appropriate pedigree. A map of regularly distributed genetic markers throughout the genome is essential to this process. Muscling is an essential broiler trait, and a QTL for it is an example of QTL mapping (Burt, 2002). In the chicken, several QTLs have been identified for a variety of features, including muscling and body composition (Burt, 2002), body weight (Groenen et al., 2000), susceptibility to Marek's disease, and Salmonellosis resistance (Yonash et al., 1999). These QTL are now available for use in MAS (Spelman and Bovenhuis, 1998), and numerous poultry breeding businesses are investigating them.

Another strategy is MAS, which aids in the establishing of a link between certain chromosome segments and genetic variability of traits of interest. QTL mapping experiments are used to establish linkage. After the linkage marker has been established, it can be utilized as the target of a selection program instead of the actual trait. This method allows for the selection of males for genes that affect egg production and the selection of chicks at the day-old stage. It improves the precision of selection in poultry breeding programs by allowing for the selection of traits that are difficult to assess or have low heritability (disease resistance). Because breeding is concerned with discovering and utilizing the genetic basis of phenotypes, there is little question that applying molecular genetics knowledge to poultry breeding, i.e., molecular breeding, will completely transform our current selective breeding procedures. Furthermore, it is expected to have an overall impact the way poultry breeding industry involved in boosting production (Thiruvenkadan and Prabakaran, 2017).

Candidate gene identification

The final stage in establishing a causal link between a candidate gene and a genetic characteristic is to define a QTL. This is a challenging and time-consuming step. Understanding the fundamental biological principles that underpin quantitative features opens up new avenues for profit. Without family data, the definition of allelic variation at the causal gene allows for direct selection for the trait of interest. Candidate genes with functions related to the trait under research and that have been mapped to the region of interest may be found by looking at the genome map. This technique is unlikely until a dense gene map is developed, given that less than 500 genes have

been identified in the chicken so far (Schmid et al., 2000). Comparative gene mapping is a suggested method that uses anchor loci from genes that have been mapped in chickens to compare the maps of humans and chickens. These comparisons can be used to determine the gene content of a chicken QTL. Chicken chromosome 3 is compared to mouse and human homolog chromosomes (Burt and Cheng, 1998). The chromosomes of chickens and humans are more similar than the chromosomes of mammals. The number of conserved segments between chicken, mouse, and human gene maps can be estimated by comparing their gene maps (Waddington et al., 2000). The conclusion was unexpected at first: chicken and human genomes are more similar than mouse and human genomes (Burt et al., 1999). There were only 154 conserved segments between chickens and humans, of which 100 had already been identified (Schmid et al., 2000).

5. GENOME SEQUENCING

Genome sequencing has increasingly become a standard technique in life science research. The chicken provided the clear "out-group" species (Siegel et al., 2006), and the National Human Genome Research Institute (NHGRI, USA) later assigned it "high priority" status due to its long history as a model organism that has been used in numerous significant discoveries and the existence of an international collaboration to map its genome. Beijing Genomics Institute (BGI) decided to add to the framework sequence of broiler, layer (White Leghorn), and Silkie chicken DNAs to create a dense SNP map when the NHGRI and the Washington University Genome Sequencing Center (WUGSC) decided to sequence the chicken genome (International Chicken Polymorphism Map Consortium, 2004). The red jungle fowl is the main 'wild-type' variety of the domestic chicken, and the genome sequenced came from a single female of the inbred red jungle fowl line (Abplanalp et al., 1992). This bird's DNA has been used in the past to make BAC (bacterial artificial chromosomes) libraries (Lee et al., 2003). BAC fingerprinting and hybridization techniques were used to create a physical "BAC contig" map from these BACs. On March 1, 2004, the first sequence assembly was made public, and the first analysis was published in December of that year (International Chicken Genome Sequencing Consortium, 2004).

In terms of evolutionary parallels to mammalian genomes, the chicken genome delivered on its promise. The sequences directly offer listings of genes, non-coding RNAs, and repetitive regions, as well as their particular sequences and configurations. This gene map is critical for identifying positional candidate genes that could encode interesting mutations or causal polymorphisms that lead to quantitative trait loci (QTL). The chicken genome sequence, for example, has already aided in the understanding of the molecular basis for a number of single gene mutant syndromes (Kerje et al., 2004; Dorshorst and Ashwell, 2009; Wright et al., 2009; Dorshorst et al., 2010). Genome sequences, on the other hand, are much more important in the construction of high-density linkage and association maps, as well as in transcriptome and proteomic investigations. About 3 million SNPs were generated by comparing partial sequences of various chicks to the reference red jungle fowl sequence (International Chicken Polymorphism Map Consortium, 2004). Initially, a collection of around 3,000 highly polymorphic, uniformly spaced SNPs were chosen and genotyped on 2,580 birds, representing the majority of global chicken variation (Muir et al., 2008). High-density SNP maps' most important effect is that it may enable breeders to completely bypass the QTL-encoding gene ascertainment stage and instead assess breeding values using genome-wide SNP profiles (GWMAS) (Meuwissen et al., 2001). The assembly of the chicken genome represents a significant step in elucidating all of the chicken's gene structures. This would necessitate a level of effort that is unimaginable now. Nonetheless, when new technologies become available, understanding of this entire subject will grow tremendously as a result of current and future research efforts in proteomics, metabolomics, and all other "-omics" fields.

Next generation sequencing (NGS)

Next-generation sequencing (NGS) techniques also make it possible to resequencing several commercial and experimental chicken lines at a low cost (Rubin et al., 2009; Eriksson et al.,

2008). Depending on their complexity and sophistication, sequencing techniques are categorized as first-generation sequencing (e.g. Sanger), second-generation sequencing (e.g. NGS), and third-generation sequencing (e.g. nanopore sequencing) (Nafea et al., 2023). The Sanger method is regarded as first generation sequencing and has stimulated the development of the next generation. It is based on the detection of tagged, partially digested fragments of two-dimensional fractionation (Heather and Chain, 2016). Despite advancements in first-generation sequencing techniques, they are no longer adequate (Pareek et al., 2011).

Sequencing of amplified DNA is used in second generation sequencing, which includes Solexa's sequencing by synthesis process (Heather and Chain, 2016). Third-generation sequencing, which includes methods like tSMS (True Single Molecule Sequencing) and SMRT (Single Molecule Real-Time), allows for the sequencing of a single DNA molecule without the need for pre-amplification (Schadt et al., 2010).

Application of next generation sequencing

1. **SNP marker discovery**: The chicken genome was re-sequenced using NGS, resulting in the discovery of 57,636 SNP markers. All of the newly identified SNPs were used to create a DNA microarray (chip) for polymorphism detection (Groenen et al., 2011).

2. **Copy number variation (CNV):** Copy number variation is a variation in the number of times a portion of the genome is repeated among people in a population. Pea-comb, cutaneous hyper pigmentation, dark brown plumage color, and late-feathering on chromosome Z are known chicken traits linked to CNV. Phenotypic diversity, such as genetic resistance to infectious illnesses, can be caused by CNV. For example, experimental laying hen lines showed minimal sensitivity to Marek's disease and a high inbreeding coefficient (Wang and Byers, 2014).

3. **Integration of viral DNA in chicken genome:** The retrovirus known as Avian Endogenous Retrovirus-HP (EAV-HP) integrated with the chicken genome before domestication and is still a part of it now. Whole genome sequencing can disclose the sequences of the viral and host genomes (Bushman et al., 2005; Hindmarsh and Leis, 1999).

4. **Identification of genes encoding production traits:** Using next-generation sequencing, it has also been possible to successfully pinpoint genes that regulate aspects of production like the quality of eggshells (Zhang et al., 2015).

6. ARTIFICIAL INSEMINATION (AI)

In modern poultry breeding, artificial insemination (AI) technology has made it possible to quickly transfer genetic material from a selective few exceptional males to a large number of females. AI has been gaining and well adopted technique for the poultry business (Benoff et al., 1981) as it allows rather more efficient use of males, compared to natural mating. This reduces the number of cockerels required for male gamete generation, which in turn lowers the immediate cost of poultry production (Benoff et al., 1981). AI was the first biotechnological method used to boost chicken production since it allowed for the widespread deployment of genetically superior cockerels with high productivity (Benoff et al., 1981). Ishikawa (1930) devised techniques that made artificial semen collecting easier in hens. Burrows and Quinn (1935) described an abdominal massage technique for hens, which was later enhanced and dubbed 'milking the male' (Burrows and Quinn, 1937). Quinn and Burrows (1936) invented the semen collection technology that was later used by AI. As a result, these scientists are known as the "Fathers of Avian AI," and their core technology is still used in various chicken species. With the invention of laying cages in Israel (Thumin, 1951) and Australia, AI became widely utilized (Skaller, 1951). AI was utilized to boost broiler fertility in the United States (Moultrie, 1956).

AI produces more viable offspring than traditional mating in bird species (Saeki and Nagomi, 1964; Brillard, 2003; Mohan et al., 2016). Even though natural mating can produce strong fertility rates, including AI into the reproductive process can increase such rates

even more (Gee et al., 2004). The price per unit of day-old chicks hatched is decreased due to the advantages of overall fertilization rate and hatchability (Brillard, 2003). In other poultry species as well artificial insemination is gaining momentum. When fertility in broiler breeds continues to decline as males are selected for growth, AI may become a more affordable method of managing broiler breeders (Reddy, 1995). Due to cloacal gland froth and poor semen volume, AI is challenging in quail (Mohan et al., 2002; Shit et al., 2010) and guinea fowls (Mohan et al., 2013). The oviduct cannot be everted in ducks and geese, unlike in chickens and turkey hens, which limits the necessity for AI in these species on a commercial level (Cooper, 1977). Cranes and other wild birds have been effectively adapted to AI techniques (Gee, 1995; Blanco et al., 2009), which is significant in the preservation of endangered species by assisting in the creation of sustainable and self-sustaining populations. Thirteen percent of the nearly 10,000 identified bird species are deemed endangered. This indicates that roughly 1,375 species are in jeopardy of becoming extinct (International Union for Conservation of Nature and Resources, 2018). However, a successful program of this approach's development in non-domesticated birds is necessary to help create sustainable, self-sustaining populations of critically endangered species. At the moment, AI is well established in most poultry species. The following are some of the benefits of AI in poultry:

1. Increased mating ratio: A cockerel may normally mate with six to ten hens. This ratio might be quadrupled with artificial insemination.

2. Older males that perform exceptionally well can be used for numerous generations. Natural mating, on the other hand, has a finite lifespan.

3. Even if a valuable male bird with leg damage, deemed not fit for natural mating but his semen can still be used for AI.

4. Elimination of preferential mating: Preferential mating can be eliminated if it is causing poor fertility.

5. Effective cross breeding: Despite the fact that cross breeding is very effective in natural condition, some hens won't mate with a male of a different color unless they have raised their young together. AI can help with successful cross-breeding in such circumstances.

7. TRANSGENESIS

A transgene is a foreign gene that has been integrated into the genome of a transgenic individual, and a transgenic product is the protein coded by the transgene. The steady incorporation of the transgene inside the host's genome, as well as its transmission to progeny through normal breeding programs, is referred to as transgenesis (Stella Cyriac' et al., 2012). Any of these genetic changes can occur in transgenic individuals, which may be advantageous for studying how genes work, altering animal or individual traits to produce high-value proteins, developing disease models in humans, or enhancing animal production or disease resistance (Houdebine, 2002; Felmer, 2004). In contrast to natural mutation, a transgenic chicken carries recombinant molecules that were purposely introduced by human intervention. Techniques for inserting unique genetic material into cells that will give rise to germ cells are used in all methods of creating transgenic fowl. The transgene can be introduced into germ cells such as mature oocytes and spermatozoa, newly fertilized egg zygote, early embryos, or primordial germ cells (PGC). The various stages of germ cell development also indicate many windows of opportunity for direct transgenic poultry production (Shuman, 1984).

The first transgenic hens were successfully created, according to Salter et al. (1986), who used the replication-competent reticulo-endotheliosis virus (REV). There has been a lot of advancement in the field of transgenic poultry farming since then. The domestic chicken is about to enter the field of protein bioprocessing by becoming a significant animal bioreactor for the industrial production of therapeutic proteins in eggs. Many therapeutically important macromolecules have been produced utilizing transgenic chickens as bioreactors, including human parathormone (Lee et al., 2007), interferon (Rapp et al., 2003), and human

antibodies (Lillico et al., 2007). Scientists have recently succeeded in developing genetically modified chickens that do not spread the avian influenza virus to other birds via internal transmission (Lyall et al., 2011). This would not only protect the health of poultry, but it would also reduce the risk of human-to-human transmission of bird flu.

Transgenic chicken could be used as a bio-factory to produce a variety of medicinal and pharmaceutical proteins, as well as for functional genomics research and breeding. It is also possible to characterize the genetic variability of native poultry, which can be utilized to generate genetic resource data. This information can be used to impact adaption to hard environments, production, and illness and parasitism susceptibility (Kumari et al., 2014).

Future prospect of biotechnological approaches in poultry

In the coming years, increasingly important genes will be directly selected in poultry breeding programs. Aspects of genome-wide marker coverage by SNPs are also being used for specific reasons. Taken together, we believe that within a few years, genomic information-based selection processes will be an integral element of any poultry breeding program. Such selection processes, we feel, have the potential to become the backbone of the breeding program. After all, the genome is at the heart of genetic variation, and breeding enterprises rely on it for survival. As a result, molecular breeding technology encompasses all facets of a breakthrough development.

The completion of the elucidation of all of the chicken's gene structures is made possible by the assembly of the chicken genome. But before we can fully understand how the chicken's phenotypic performance is regulated, we need to walk the whole path from gene structure to gene function, gene expression, protein interactions, biochemical and signaling pathways, cellular function, and cell-cell communication. To achieve those, it would probably necessitate a level of consorted efforts to put together which might be beyond imagination at present time. However, as new technologies become available, study in the disciplines of proteomics, metabolomics, and all other "omics" will significantly advance our grasp of this subject. Once such knowledge is obtained, it is only a matter of time until it is put to use through targeted alteration of gene structure and function.

Directed gene manipulation is only possible with competent and effective technologies for bird genetic modification. The transfer of a transgene or gene construct to an avian embryo has shown to be significantly more challenging in avian systems than in mammalian systems (Mozdziak and Petitte, 2004). Even though current transgenics are mostly used in the pharmaceutical industry, these results pave the path for their use in chicken breeding for agricultural uses.

This, however, will take a long time. To begin, considerably more information about gene action in chickens is required before a viable plan for genetically modifying chickens for agricultural purposes can be developed. Henceforth, chicken genetic modification technologies still need to be greatly improved. Following the completion of these two procedures, it takes at least a few more years for a genetically modified breed to be established from conception to introduction. In light of this, we predict that the first genetically altered chicken with the potential to be used commercially in agricultural production will be ready in next twenty years.

8. CONCLUSION

Poultry contributed to research and development in addition to being a source of high-quality and nutritious proteins. Genomic research for poultry breeding has made great strides, with high-density SNP panels for broilers and layers being readily available. Additionally, poultry breeders are increasingly having access to a variety of statistical techniques for combining genomics into normal genetic analyses. In the future, genomics could play a significant role in assisting breeders with selection programs. Recent biotechnological technologies have proven to be effective in disease control, genetic variability estimation for boosting productivity and disease resistance, and poultry diversification. Application of biotechnology has played a key role in improving poultry health and production, and it will continue to do

so in the near future. We predict that within the next ten years, the basis of selective breeding will be considered in evaluating genetic variety at the genome level (DNA) as opposed to at the phenotypic level. This will have an impact on the organization of breeding programs as well as breeding's integration into the poultry production system. In the near future, genetically modified chicken breeds will be available owing to new information amassed along the route and the employment of cutting-edge technologies to develop and redesign the poultry genome. A number of biotechnology developments will be made gradually, and they will eventually be coupled with conventional techniques to become common tools for enhancing the overall genetic improvement of poultry.

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