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Invited Review Article

Genomic tools in poultry breeding: Harnessing molecular markers for progress

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Abstract

Molecular markers serve as essential tools for discerning and tracing genetic variances within diverse organisms. This comprehensive review sheds light on the multifaceted applications of molecular markers within the realm of poultry genetics and breeding. Noteworthy markers like microsatellites, single nucleotide polymorphisms (SNPs), and restriction fragment length polymorphisms (RFLPs) have been instrumental in tasks ranging from genetic diversity assessment to the pinpointing of quantitative trait loci (QTLs), parentage verification, sex determination, and the diagnosis and resistance of diseases. These markers have played a pivotal role in the formulation of breeding initiatives, aimed at preserving genetic diversity, selecting superior breeding stock with coveted attributes, and enhancing resistance against illnesses. In sum, molecular markers exert a profound influence on poultry genetics and breeding, with a foreseen surge in their utilization as novel markers and technologies emerge. Their application holds great potential for augmenting the efficiency and efficacy of breeding programs, as well as advancing the well-being of avian populations.

Keywords: Breeding, Molecular marker, QTL, RFLP, SNP

Highlights

- Molecular markers are essential tools for analyzing genetic variations.
- The review emphasizes the versatile applications of molecular markers in poultry genetics and breeding.
- These markers play a crucial role in breeding programs: preserving genetic diversity, selecting superior breeding stock, and enhancing disease resistance.
- They hold potential for improving the efficiency and effectiveness of breeding programs and the well-being of avian populations.

INTRODUCTION

Genetic markers, which are essential tools for linkage and association studies, are particular DNA sequences with a defined position on a chromosome. To track a person, a tissue, a cell, a nucleus, a chromosome, or a gene, genetic markers might be used as tags or experimental probes; because they are biological characteristics that are determined by allelic forms of genes or genetic loci and can be passed down from one generation to the next. DNA segments of the genome, known as molecular genetic markers, can give molecular information, allowing the differentiation of taxa (Patwardhan *et al.*, 2014; Grover and Sharma, 2016). In systematics investigations, the use of DNA sequences as genetic markers has been beneficial in identifying species and discovering new ones as well as revealing association between organisms (Sites and Marshall, 2003). The genes for dwarf and blue-eggshell features may assemble in one person through hybridization (Cui *et al*., 2019). DNA markers have been very valuable in revealing the extent and distribution of variation in a diversity of species (Hailu and Asfere, 2020).

Genetic markers can be made using distinct DNA sections from the nuclear or mitochondrial genomes. Each genetic marker's degree of sequence variation significantly affects its resolution and utility (Blasco-Costa *et al.*, 2016). Mitochondrial DNA (mtDNA) has a larger degree of sequence variation than nuclear DNA (nDNA) due to its faster rate of evolution and may serve as a reliable source of genetic markers for species taxonomy resolution at lower levels; however, it is maternally inherited, which can limit its use in certain studies (Hwang and Kim, 1999; Le *et al.*, 2000; Blouin, 2002; Allio *et al.*, 2017). The protein-coding genes for cytochrome c oxidase subunit I (COI) and NADH dehydrogenase subunit 1 (NAD1) as well as the 12S and 16S ribosomal RNA (rRNA) genes are examples of genetic markers found in mtDNA. Nuclear rRNA genes

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have highly conserved sequences, which makes them a potential source of genetic markers for resolving higher taxonomic levels for species (Hwang and Kim, 1999; Patwardhan *et al.*, 2014; Choudhary *et al.*, 2015). Because of a higher rate of nucleotide substitution, the internal transcribed spacer (ITS) regions of nDNA have more sequence variation than nuclear rRNA genes (Hwang and Kim, 1999; Blouin, 2002; Vilas *et al.*, 2005; Choudhary *et al.*, 2015). The choice of genetic markers for each application is made more difficult by the different characteristics of the genetic markers, despite the fact that many different types of genetic markers are acceptable for molecular systematics and identification reasons. Because of this, it is crucial to constantly keep a few key variables in mind when choosing the right DNA marker approaches to help achieve a specific set of research goals (Nadeem *et al.*, 2018). The introduction of molecular marker technologies brought about a level of precision in breeding that was before unattainable (Reddy *et al.*, 2021). If differences known as polymorphisms exist in the marker nucleotide sequences between or among individuals or species, DNA markers can be useful for determining the individual genotypic differences in the same or different species. Molecular marker polymorphisms result from a variety of DNA mutations that alter the nucleotide sequences of different organisms (Mandal *et al.*, 2018).

Classical markers and DNA markers are the two distinct types of genetic markers used in genetics (Xu, 2010). Classical indicators include morphological, cytological, and biochemical ones.

DNA markers

In order to identify variability between several genotypes or alleles of a gene for a certain sequence of DNA in a population or gene pool, scientists utilise DNA markers, which are classified as a fragment of DNA displaying mutations or variances. Such fragments can be discovered using specific molecular technologies and linked to specific genome locations. To simplify things, a DNA marker is a little section of DNA sequence that exhibits polymorphism between different individuals. Commonly used DNA markers are: RFLP (Restriction fragment length polymorphism), RAPD (Random amplification of polymorphic DNA), AFLP (Amplified fragment length polymorphism), VNTR (Variable number tandem repeat), SNP (Single nucleotide polymorphism) and SSR Microsatellite polymorphism (Simple sequence repeat).

Southern blotting, a nuclear acid hybridization technique (Southern, 1975), and PCR, a polymerase chain reaction technique, are the two fundamental ways to find the polymorphism (Mullis, 1990). The variation in DNA samples or polymorphism for a specific region of DNA sequence can be identified using PCR and/or molecular hybridization followed by electrophoresis (e.g., PAGE - Polyacrylamide gel electrophoresis, AGE - Agarose gel electrophoresis, CE - Capillary electrophoresis) based on the production features, such as band size and mobility. More detection methods, such as novel array chip techniques that use DNA hybridization in conjunction with labelled nucleotides and new sequencing techniques that identify polymorphism by sequencing, have been developed in addition to southern blotting and PCR. In general, point mutations resulting from single nucleotide substitutions, rearrangements including insertions or deletions, duplication of DNA sections, translocations, inversions, and errors in tandemly repeated DNA replication are the causes of marker polymorphisms in organisms (Selvakumari *et al.*, 2017). For marker-assisted breeding to be effective, depending on the species and application, the best DNA markers should have a high degree of polymorphism, uniform distribution across the entire genome, not concentrated in certain areas, co-dominance expression (to identify heterozygotes from homozygotes), well defined allelic characteristics (such that the various alleles can be quickly identified), single copy and no pleiotropic effect, affordable to use (development of inexpensive markers and genotyping), easy assay/detection and automation, unrestricted use, high availability, and appropriateness for multiplexing, allowing for the accumulation and sharing of data between laboratories, genome-specific in nature (especially with polyploids) as well as no detrimental effect on the phenotype.

DNA markers have evolved into several systems based on various polymorphism-detecting procedures or methods (southern blotting- nuclear acid hybridization, PCR- polymerase chain reaction, and DNA sequencing) (Amiteye, 2021). PCR- RFLP was shown to be a rapid and sensitive method for the detection of gene polymorphism. PCR-RFLP assay is a two-step reaction to identify multiple species after restriction enzyme digestion of PCR amplified DNA sequence (Alatafi and Kasturi, 2016). Recent modifications have, however, improved the RAPD technique into more efficient marker methods like sequence characterized amplified regions (SCAR), Sequence-related amplified polymorphism (SRAP) and cleaved amplified polymorphic sequence (CAPS) (Yang *et al.*, 2014; Babu *et al.*, 2021). A number of methods may be used to detect copy number variations, including single nucleotide polymorphism (SNP) arrays, sequencing and array comparative genome hybridization (aCGH). CNV detection becomes more reliable and accurate at the whole-genome level by using recent advances in next-generation sequencing (NGS) technology (Seol *et al.*, 2019).

Applications of molecular markers

Genetic diversity conservation: There has been an irreparable loss of genetic diversity among our local animal and bird breeds as a result of the widespread crossbreeding of exotic animals and birds with indigenous breeds in order to exploit heterosis. Insofar as it promotes an increased level of heterozygosity in the population, the conservation of genetic variety is crucial. Populations need genetic diversity to be able to adapt to upcoming environmental changes (Gholizadeh *et al.*, 2008). To ensure a long-term response to natural or artificial selection for features of economic or cultural importance, genetic diversity is required. Studies employing DNA markers should preserve potentially unique genes in populations since they would contribute more to biodiversity. The main goal of genetic diversity research is to comprehend the degree of population differentiation within a species. Several techniques for the detection of polymorphic loci can be employed to produce population-specific genetic markers, which can further help in the identification of various alleles in the population and thus can help in changing the population structure. Evaluation of genetic variability is made possible by the genetic characterization of populations, breeds, and species. Since molecular markers provide data on every part of the genome, they have been used to access this variability. In order to analyse genetic variants at the DNA level, the most used molecular approaches are RELP, RAPD, AFLP, microsatellites, and minisatellites (Gwakisa, 2002). Microsatellites exhibit a high degree of polymorphism among breeds and individuals (Deshmukh *et al.*, 2015). Microsatellites have been particularly helpful for generating integrated maps for plant species in which full-sib families are used for constructing linkage maps (Pereira *et al*., 2013; Souza *et al*., 2013), thus providing breeders and geneticists with a tool to link phenotypic and genotypic variation (Hayward *et al*., 2015). RAPD markers identify low genetic distance among native chicken (Ibrahim *et al.*, 2015).

Identification of disease carrier: The livestock/poultry farmer suffers significant losses in economic returns as a result of infectious illnesses. The majority of serious, incurable diseases are associated with faulty mammal or avian genomes rather than infectious disease-causing microbes, due to which the host genome's allelic differences determine a disease's susceptibility or resistance. One example to prove the above point is the variance in the host's reaction to the causal agent and the incubation period of bovine spongiform encephalopathy, which was caused by a specific RFLP in the Prion protein gene.

DNA polymorphism within a gene allows for the detection of heterozygous carrier animals or birds, which are otherwise phenotypically indistinguishable from healthy individuals. This helps to understand the molecular mechanism and genetic control of several genetic and metabolic disorders. The faulty recessive allele in carrier mammals and birds has been identified using the PCR-RFLP technique. The PCR-RFLP technique is utilised for species identification and differentiation because it makes use of the fact that SNPs are linked to the establishment or removal of a restriction enzyme recognition site. Disease resistance genes and fat deposition genes in chickens are identified using SNP (Arjunan *et al.*, 2019).

Determination of parentage: The exclusion principle is used to determine paternity in populations that are segregated generally. In other words, the existence of a unique parental pair at a specific genetic locus in the offspring of an allele not present in either of the putative parents essentially disqualifies the couple as biological parents. It has been observed that highly polymorphic DNA fingerprinting markers are particularly helpful for determining parentage (Mitra *et al.*, 1999). In programmes involving artificial insemination, molecular markers can be used to identify the sire. Molecular markers in candidate genes (cation channel of sperm 1, sperm-specific NHE, A kinase, anchor protein 4, pyruvate kinase, cytochrome oxidase, reproductive homeobox 5, cysteine rich secretory protein 2, phosphatidylethanolamine binding protein 1, Doppel, tissue inhibitor of metalloproteinase, etc.) are important for assessing semen quality and fertility in bulls (Singh *et al*., 2014). Some of the Y specific microsatellite biomarkers are also associated with some of the semen quality traits among crossbred bulls (Deb *et al*., 2013).

Marker-assisted selection: To improve the effectiveness of the conventional methods of breeding based on phenotypic data, this genetic engineering methodology involves the identification of genetic markers for selection. Utilizing superior genotypes by environment interaction, molecular marker analysis enables the identification of genomic regions and QTL that contribute to the genetic variance of a trait (Gholizadeh *et al.*, 2008). For the development of such economic features, selection for favourable QTL effects based on molecular marker research has much to offer. A total of 30 QTLs were detected by half-sib analysis, and seven QTLs were detected by full-sib analysis that was associated with fatty acid composition in Korean native chicken. Out of 30 QTLs, 12 were present in the thigh region, 18 were present in the breast region, and out of seven QTLs, three were present in the thigh region, and four were present in the breast region (Jin *et al*., 2018).

There was identification of five genomic regions in different chromosomes in which QTL for morphometric and mineral composition traits of the tibia bone in broiler and layer cross were reported (Faveri *et al*., 2019).

Transgenesis: This process involves integrating a gene or a portion of a gene from one person into the genome of another. The identification of the target genes serves as the foundation for this technique. The relevant genes can be mapped using molecular markers in this situation, which would be the first step in manipulating those genes. In order to multiply the transgenes, it is also possible to identify the animals or birds harbouring those using molecular markers. Many strategies have been developed as a result of the introduction of exogenous genes into recipient embryo tissues in order to enable their transfer into germ line. These techniques include the use of vector, DNA microinjection, chimeric chicken and Laser methods (Okon *et al*., 2015). Using any of the aforementioned methods will depend on how the foreign genetic material is introduced into the cells that will develop into germ cells. The majority of the developed methods will result in birds whose germ line has mosaic transgene insertions (i.e., only several cells in the gonad may carry the transgene). Consequently, effective targeting of the germ cells is necessary for the successful production of transgenic chickens because only these particular cells have the capacity to pass on the transgene to upcoming generations. (Scott *et al*., 2010).

Sex determination of chicks: The sex of pre-implantation embryos can be determined using molecular markers. This can be done by employing DNA sequences particular to males or those found in the Y chromosome as probes. The advantage of using the PCR-based approach of sex determination is that it may be completed in less than five hours with almost 100% accuracy. It can be carried out at an early stage of the embryo and is less invasive than other cytogenetic techniques. Pre-implantation embryo sexing can be a useful strategy for enhancing a herd for a certain objective.

Use of microsatellite markers in poultry research

Chicken: About 100 RFLP markers made up the linkage map of chicken for the first time. Schmid *et al*. (2000) reported the chicken genome's first consensus linkage map. There were also reported chromosomal sites for 1……..965 markers, creating 50 linkage groups. There are 2,483 loci for the chicken in the Ark Database, 435 of which are unassigned genetic markers (Jacobsson *et al.*, 2004). Creating chromosome-specific libraries for macrochromosomes involves the isolation and sequencing of DNA fragments specific to a particular chromosome. Development of chromosome-specific libraries for the macrochromosomes will aid in the development of marker-saturated linkage maps for all those chromosomes. Chicken chromosome-specific libraries in a phage vector, macrochromosomes 1, 2, 3, and 4 were produced. These 'n' chromosome-specific libraries were used to create fifty two extra special (AC) type microsatellite markers. According to study findings, high density linkage maps for chicken macro chromosomes can be created using markers created from chromosome-specific libraries (Ambady *et al.*, 2002).

Japanese quail: Japanese quail, an economically significant avian species, offers a substitute for chicken, which is more frequently consumed. They are less expensive to start with, take up less room, and have strong export potential. The Japanese quail, or *Coturnix japonica*, is a little, rapidly developing bird that produces large quantities of eggs and meat. It is becoming more and more used as an experimental bird in both research and education. Japanese quail loci were amplified individually by 26% (31/120) of chicken primers, and 65% (20/31) of the amplified loci were discovered to be polymorphic. This was done to determine whether chicken microsatellite markers would work as genetic linkage markers in quail. The findings indicated that the majority of chicken markers are ineffective for research on Japanese quail. They came to the conclusion that more work should go into creating quail-specific markers as opposed to trying to modify chicken markers for use in quail.

Ensemble genome database

Ensembl (http://www.ensembl.org/) is a bioinformatics project that aims to arrange biological data according to huge genomic sequences. This all-inclusive resource provides consistent automated annotation of individual genomes together with the synteny and orthology links among them. The chicken's haploid genome is around 1.2×109 base pairs in size, which is about 40% smaller than that of mammals. The genome sequence is believed to have between 20,000 and 23,000 genes, which is a somewhat less amount than that of mammals. Chickens have five pairs of macrochromosomes (>40 Mb), five pairs of intermediate-sized chromosomes (20–40 Mb), and 28 pairs of microchromosomes (<20 Mb), according to the International Chicken Genome Sequencing Consortium (Wallis *et al*., 2004). National Library of Medicine (NCBI) has a detailed view of the *Gallus gallus* genomic map with updated chromosome-wise gene information, including different poultry breeds (https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_016699485.2).

Challenges and future directions

The integration of molecular markers has ushered in a new era in poultry genetics and breeding. However, there are still hurdles to overcome. Challenges include the high cost associated with genotyping and the ongoing need for validating marker-trait associations. Additionally, there are limitations in research infrastructure, such as inadequate access to scientific equipment and a shortage of technical expertise (Gamaniel and Gwaza, 2017). Essential utilities like reliable power and water supply are often lacking. Moreover, critical support services such as gene banks, *in vitro* storage facilities, animal holding areas, radiation shielding, disposal facilities, and computing and ICT services are often unavailable (Agarwal *et al*., 2020).

Looking ahead, research efforts should focus on refining techniques for marker-assisted selection and deepening our understanding of the avian genome. The integration of molecular markers into poultry genetics and breeding programs has laid the foundation for more efficient, sustainable, and ethically conscious practices in the poultry industry. With advancing technology and expanding knowledge of avian genomics, we can anticipate even more substantial progress, ultimately enhancing the productivity and welfare of poultry on a global scale.

Conclusion

The application of molecular markers in poultry genetics and breeding has significantly advanced our understanding of avian genetics and enhanced the efficiency of breeding programs. This brief review has highlighted several key points related to genetic diversity and conservation, which is vital for conservation efforts, ensuring that valuable genetic resources are preserved, trait selection and improvement, which allows breeders to make more informed decisions when selecting breeding stock, ultimately accelerating the breeding process, use of molecular markers for parentage testing and genomic selection has led to a significant reduction in the generation interval, molecular markers are instrumental in identifying genes related to disease resistance, reduces unintended genetic changes.

Conflict of interest: Authors have no conflict of interest in this study.

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