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Molecular Markers and Genetic Variation in Water Buffalo: Insights for Conservation and Breeding

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Abstract This study provides a comprehensive overview of water buffalo biodiversity, focusing on their global distribution, domestication history, and genetic variation across populations. Various molecular markers, including SSRs, SNPs, and AFLPs, are discussed for their applications in genetic studies, alongside the benefits of next-generation sequencing for marker discovery. A case study on the application of SNP markers demonstrates their utility in conservation, highlighting population bottlenecks and inbreeding trends. Furthermore, this study explores the use of molecular markers in breeding programs, emphasizing marker-assisted selection and genomic selection for genetic improvement. While acknowledging current limitations, the study underscores the potential of emerging technologies to bridge research and practical applications. The findings aim to support sustainable conservation and breeding initiatives, ensuring the genetic resilience and productivity of water buffalo populations.

Keywords Water buffalo; Genetic diversity; Molecular markers; Conservation; Breeding programs

1 Introduction

Water buffalo (*Bubalus bubalis*) are a crucial livestock species, significantly contributing to the global agricultural economy through the provision of milk, meat, skins, and draft power. They are primarily found in Asia and support more people per capita than any other livestock species (Mishra et al., 2023). The economic importance of water buffalo is underscored by their role as a major source of milk and meat in many countries, with their milk yield being a focal point for improvement in breeding programs (Liu et al., 2018; Du et al., 2019).

Genetic diversity is vital for the conservation and breeding of water buffalo, as it ensures the resilience and adaptability of populations to changing environmental conditions and disease pressures (Anas et al., 2023). The identification and understanding of genetic variation within and between buffalo breeds are essential for effective breeding strategies aimed at enhancing desirable traits such as milk production, feed conversion efficiency, and reproductive performance (Uffo et al., 2017; Mishra et al., 2020). Genetic diversity also plays a critical role in maintaining the distinct status of breeds, as seen in the Nili breed, which has maintained its separate breed status despite close evolutionary relationships with other breeds.

Molecular markers, such as single nucleotide polymorphisms (SNPs) and microsatellites, are indispensable tools in genetic studies, providing insights into the genetic architecture of economically important traits (Pérez-Pardal et al., 2017; Khan et al., 2022). These markers facilitate the identification of candidate genes associated with traits like milk yield and quality, enabling targeted breeding programs (Liu et al., 2018; Deng et al., 2024). The use of genome-wide association studies (GWAS) and other genomic tools has advanced the understanding of genetic variation and trait association in water buffalo, supporting the development of genomic resources for breeding and conservation (Macciotta et al., 2021).

This study attempts to explore the genetic variation in water buffalo using molecular markers, discuss their implications for conservation and breeding, and provide an overview of the genetic basis of economically important traits. By leveraging genomic data and molecular markers, this study aims to enhance breeding programs and contribute to the sustainable management and conservation of water buffalo populations.

2 Water Buffalo: Biodiversity and Population Structure

2.1 Global distribution and domestication history

Water buffaloes (*Bubalus bubalis*) are primarily distributed across Asia, where they play a significant role in agriculture due to their contributions to milk, meat, and draft power (Mishra et al., 2023). The domestication of buffaloes dates back approximately 3000 to 6000 years, with two main types recognized: the river buffalo, which is more common in India and Pakistan, and the swamp buffalo, found mainly in Southeast Asia (Figure 1) (Rehman et al., 2021). The genetic diversity within these populations is influenced by their domestication history and geographical distribution, which has led to distinct phenotypic and genetic characteristics (Anas et al., 2023).

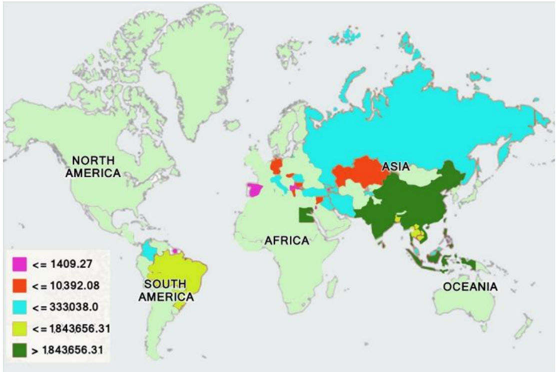

Figure 1 Geographical distribution of buffalo population (Adopted from Rehman et al., 2021)

2.2 Genetic variation across populations

Genetic variation in water buffalo populations is crucial for their conservation and breeding. Studies using microsatellite markers have revealed significant genetic diversity within and between populations. For instance, research on Turkish water buffalo populations identified a high level of polymorphism and genetic diversity, indicating the presence of at least two major genetic clusters (Ünal et al., 2021). Similarly, Iranian buffalo populations also exhibit high genetic diversity, with a mean heterozygosity index indicating substantial variation within and between populations (Mahdiyeh et al., 2019). The use of molecular markers such as ISSR and microsatellites has been effective in assessing genetic diversity, which is essential for understanding population structure and planning conservation strategies (Gargani et al., 2010; Aytekin et al., 2011).

2.3 Significance of biodiversity in sustainable breeding

Biodiversity in water buffalo populations is vital for sustainable breeding programs. High genetic diversity allows for the selection of traits that improve productivity, disease resistance, and adaptability to environmental changes (Kenchaiwong et al., 2023). For example, the identification of SNP markers in Pakistani buffalo breeds has highlighted the potential for marker-based breeding programs aimed at enhancing milk production and disease resistance (Anas et al., 2023). Moreover, understanding the genetic architecture through whole-genome sequencing can aid in the development of breeding strategies that enhance the resilience and performance of buffalo populations globally (Rehman et al., 2021). Conservation efforts must focus on maintaining genetic diversity to ensure the long-term sustainability and productivity of water buffaloes (Gahlyan et al., 2024).

In summary, the global distribution and domestication history of water buffaloes have led to significant genetic variation across populations. This genetic diversity is crucial for sustainable breeding and conservation efforts, ensuring the continued economic and agricultural importance of water buffaloes worldwide.

3 Molecular Markers: Tools for Genetic Analysis

3.1 Overview of molecular marker types

Molecular markers are essential tools in genetic analysis, providing insights into genetic diversity, population structure, and evolutionary relationships. Among the most commonly used markers are Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs), and Amplified Fragment Length Polymorphisms (AFLPs). SSRs, also known as microsatellites, are highly polymorphic and widely used for assessing genetic diversity due to their abundance and variability in the genome (Uffo et al., 2017; Singh et al., 2018; Ünal et al., 2021). SNPs are single base-pair variations in the DNA sequence and are valuable for high-resolution mapping and genomic selection, offering insights into breed-specific traits and genetic characterization (Mishra et al., 2020; Anas et al., 2023). AFLPs, although less frequently used in buffalo studies, provide a broad genome coverage and are useful for detecting genetic variation without prior sequence information (Aytekin et al., 2011).

3.2 Advantages and limitations of molecular markers in buffalo studies

Molecular markers offer several advantages in buffalo genetic studies. SSRs are favored for their high polymorphism and ease of use with PCR, making them ideal for genetic diversity and population structure analysis (Singh et al., 2018; Ünal et al., 2021). SNPs provide high-throughput and precise genetic information, which is crucial for genomic selection and understanding breed-specific traits (Mishra et al., 2020; Anas et al., 2023). However, each marker type has limitations. SSRs require prior knowledge of the genome and can be labor-intensive to develop. SNPs, while informative, require extensive sequencing efforts and bioinformatics resources for analysis. AFLPs, although useful for initial diversity assessments, may lack the resolution needed for detailed genetic studies (Aytekin et al., 2011).

3.3 Role of next-generation sequencing in marker discovery

Next-generation sequencing (NGS) technologies have revolutionized the discovery and application of molecular markers in buffalo genetics. NGS allows for the rapid and comprehensive identification of SNPs across the genome, facilitating detailed genetic mapping and trait association studies. Techniques such as ddRAD sequencing enable the discovery of trait-specific SNPs, enhancing the understanding of genetic traits like milk production and disease resistance (Mishra et al., 2020). NGS also supports the development of high-density SNP arrays, which are instrumental in genomic selection and conservation strategies (Anas et al., 2023). The integration of NGS in buffalo studies accelerates marker discovery and enhances the precision of genetic analyses, supporting both conservation and breeding programs.

In summary, molecular markers such as SSRs and SNPs are invaluable in buffalo genetic studies, each offering unique advantages and facing specific limitations. The advent of NGS has significantly enhanced marker discovery, providing detailed insights into genetic diversity and aiding in the development of effective conservation and breeding strategies (Ramlachan, 2023; Gahlyan et al., 2024).

4 Insights into Genetic Variation in Water Buffalo

4.1 Genomic studies and key findings on genetic diversity

Genomic studies on water buffalo have utilized various molecular markers to assess genetic diversity. For instance, a study on Turkish water buffalo populations using 20 microsatellite markers revealed significant genetic diversity, identifying at least two major genetic clusters among the populations (Ünal et al., 2021). Similarly, research on Cuban water buffalo using 30 microsatellite markers highlighted the genetic variability within the population, which is crucial for conservation and breeding strategies (Uffo et al., 2017). In Pakistan, genomic characterization of buffalo breeds identified numerous breed-specific single-nucleotide polymorphisms (SNPs), emphasizing the need for conservation programs for distinct breeds like the Nili (Anas et al., 2023). Additionally, a 90K SNP genotyping assay developed for water buffalo has been instrumental in exploring genetic diversity and aiding in genetic selection (Mishra et al., 2020).

4.2 Population-specific markers and their applications

Population-specific markers have been identified in various studies, providing insights into the genetic makeup of different buffalo populations. In Pakistan, SNP markers specific to breeds such as Nili, Nili-Ravi, Azakheli, and Kundi were identified, which can be used for breed characterization and conservation efforts (Anas et al., 2023). In Trinidad and Tobago, microsatellite markers were used to genetically characterize the Buffalypso population, aiding in the establishment of conservation genetics programs (Figure 2) (Ramlachan, 2023). The use of heterologous microsatellite markers has also been effective in assessing genetic variability in buffalo species, supporting conservation and genetic improvement programs (Singh et al., 2018).

Figure 2 Buffalypso animals from a local herd in Trinidad and Tobago (Adopted from Ramlachan, 2023)

4.3 Phylogenetic studies using molecular markers

Phylogenetic studies using molecular markers have provided valuable insights into the evolutionary relationships among buffalo populations. A study on a lesser-known buffalo population in Central India used microsatellite markers to reveal genetic diversity and phylogenetic relationships with other well-established breeds, suggesting the need for conservation and improvement programs (Gahlyan et al., 2024). The use of SNP markers in phylogenetic studies has also been highlighted, with a 90K SNP chip distinguishing buffalo from different farms and identifying genomic regions involved in milk production (Iamartino et al., 2017). These studies underscore the importance of molecular markers in understanding the phylogenetic structure and guiding conservation strategies for water buffalo.

In summary, genomic studies have revealed significant genetic diversity in water buffalo populations, with population-specific markers playing a crucial role in conservation and breeding programs. Phylogenetic studies using molecular markers have further enhanced our understanding of the evolutionary relationships among buffalo populations, supporting targeted conservation efforts.

5 Case Study: Application of SNP Markers in Water Buffalo Conservation

5.1 Identification and selection of SNP markers for genetic monitoring

Single Nucleotide Polymorphisms (SNPs) are crucial molecular markers used in the genetic monitoring of water buffalo populations. The identification and selection of SNP markers involve analyzing genome sequences to detect allele differences among breeds. For instance, a study in Pakistan identified breed-specific SNP markers for the Nili, Nili-Ravi, Azakheli, and Kundi breeds, highlighting the importance of SNPs in maintaining breed diversity and aiding conservation efforts (Anas et al., 2023). Additionally, a 90K SNP genotyping assay was developed for water buffalo, which facilitates the analysis of genetic diversity and supports genomic selection approaches (Iamartino et al., 2017). These SNP markers are essential for monitoring genetic variation and ensuring the sustainable management of buffalo populations.

5.2 Analysis of population bottlenecks and inbreeding trends

The analysis of population bottlenecks and inbreeding trends is vital for understanding the genetic health of water buffalo populations. Studies have shown that genetic diversity can be assessed using SNP markers, which help identify inbreeding levels and potential bottlenecks. For example, the use of microsatellite markers in Cuban water buffalo populations revealed positive inbreeding estimates in several loci, indicating a need for careful management to prevent genetic erosion (Uffo et al., 2017). Similarly, the genetic characterization of Pakistani buffalo breeds using microsatellite markers showed low genetic diversity, suggesting historical bottlenecks and the necessity for conservation strategies to mitigate inbreeding (Hussain et al., 2017).

5.3 Conservation implications and recommendations

The application of SNP markers in water buffalo conservation has significant implications for developing effective conservation strategies. The identification of breed-specific SNPs allows for targeted conservation efforts, ensuring the preservation of genetic diversity within and among buffalo populations. It is recommended that conservation programs incorporate SNP-based genetic monitoring to track genetic variation and manage breeding programs effectively. For instance, the conservation of the Nili breed in Pakistan, which has maintained its distinct status despite close evolutionary ties with the Nili-Ravi breed, underscores the importance of SNP markers in conservation planning (Anas et al., 2023). Furthermore, the development of SNP chips, like the 90K SNP genotyping assay, provides a robust tool for genetic diversity studies and can guide genome-assisted selection programs to enhance productivity and disease resistance in water buffalo (Iamartino et al., 2017). These efforts are crucial for maintaining the genetic health and sustainability of water buffalo populations globally.

In summary, SNP markers play a pivotal role in the conservation and breeding of water buffalo by enabling precise genetic monitoring, identifying inbreeding trends, and informing conservation strategies to preserve genetic diversity (Rusdin et al., 2020; Kenchaiwong et al., 2023).

6 Applications of Molecular Markers in Breeding Programs

6.1 Marker-assisted selection (MAS) for desirable traits

Marker-assisted selection (MAS) is a powerful tool in breeding programs that utilizes molecular markers to select for desirable traits in water buffalo. Single Nucleotide Polymorphisms (SNPs) are among the most widely used molecular markers in animal breeding, allowing for the identification and selection of traits such as milk volume, age at first calving, and feed conversion efficiency (Mishra et al., 2020; Mishra et al., 2021). The use of SNPs enables breeders to enhance productivity by selecting animals with favorable genetic profiles, thereby accelerating genetic improvement (Du et al., 2019; Ramlachan, 2023). Additionally, microsatellite markers have been employed to assess genetic diversity and support MAS by identifying genetic variations linked to economically important traits (Uffo et al., 2017; Singh et al., 2018).

6.2 Genetic improvement strategies using molecular tools

Genetic improvement strategies in water buffalo have been significantly advanced through the use of molecular tools. Whole-genome sequencing and genome-wide association studies (GWAS) have been instrumental in uncovering candidate genes associated with productivity traits such as milk production and disease resistance (Du et al., 2019). These tools provide comprehensive genomic data that facilitate the understanding of complex traits and enable the development of targeted breeding strategies (Iamartino et al., 2017). The integration of these molecular tools into breeding programs allows for the precise selection of animals with superior genetic potential, ultimately leading to enhanced performance and adaptation (Marrero et al., 2016; Rehman et al., 2021).

6.3 Integration of genomic selection into breeding programs

The integration of genomic selection into breeding programs represents a transformative approach to improving water buffalo populations. Genomic selection leverages high-density SNP panels, such as the 90K SNP chip developed for river buffalo, to predict the genetic merit of individuals with high accuracy. This approach enables the selection of animals based on their genomic estimated breeding values (GEBVs), which are derived from genome-wide data rather than traditional phenotypic assessments (Rehman et al., 2021). By incorporating genomic selection, breeders can achieve faster genetic gains and improve traits such as milk yield, disease resistance, and overall productivity (Mishra et al., 2021). The use of genomic selection is particularly beneficial in managing genetic diversity and ensuring the sustainability of breeding programs (Marrero et al., 2016; Ünal et al., 2021).

In summary, the application of molecular markers in water buffalo breeding programs offers significant potential for enhancing genetic improvement. Marker-assisted selection, genetic improvement strategies using molecular tools, and the integration of genomic selection are key components that contribute to the advancement of breeding programs, ultimately leading to improved productivity and sustainability in water buffalo populations.

7 Challenges and Future Directions

7.1 Limitations of current molecular approaches

Current molecular approaches in studying water buffalo genetics face several limitations. One significant challenge is the underutilization of high-throughput technologies like whole-genome sequencing and genome-wide association studies (GWAS) due to the lack of a well-annotated and assembled reference genome for buffaloes (Rehman et al., 2021). Additionally, while microsatellite markers have been used to assess genetic diversity, they often show limited polymorphism and may not provide comprehensive insights into genetic variation (Uffo et al., 2017; Ünal et al., 2021). The existing SNP panels, primarily developed for cattle, are not fully applicable to buffalo, necessitating the development of buffalo-specific SNP chips (Iamartino et al., 2017). These limitations hinder the full exploration of genetic diversity and the potential for genomic selection in buffalo breeding programs.

7.2 Bridging the gap between research and practical applications

To bridge the gap between research and practical applications, it is crucial to translate molecular findings into actionable breeding strategies. This involves integrating molecular data with traditional breeding practices to enhance traits such as milk production, disease resistance, and climate resilience (Debaky et al., 2019). The development of buffalo-specific genomic tools, such as the 90K SNP chip, provides a foundation for genome-assisted selection programs, which can be used to improve economically important traits (Iamartino et al., 2017). Furthermore, fostering collaborations between researchers and breeders can facilitate the implementation of molecular breeding techniques in practical settings, ensuring that scientific advancements lead to tangible improvements in buffalo productivity and conservation (Du et al., 2019).

7.3 Emerging technologies and their potential for advancing genetic studies

Emerging technologies hold significant promise for advancing genetic studies in water buffalo. Techniques such as ddRAD sequencing and the development of trait-specific SNPs offer new avenues for identifying genetic markers associated with key traits like milk volume and feed conversion efficiency (Mishra et al., 2020). These technologies enable more precise genetic mapping and the identification of candidate genes for marker-assisted selection (Du et al., 2019). Additionally, the use of ISSR-PCR markers has been shown to effectively assess genetic diversity and structure, providing valuable insights for conservation genetics (Mokhnachova, 2022). As these technologies continue to evolve, they will enhance our understanding of buffalo genetics and support the development of more efficient and sustainable breeding programs.

In summary, addressing the limitations of current molecular approaches, bridging the gap between research and practical applications, and leveraging emerging technologies are critical steps toward advancing the genetic study and conservation of water buffalo. These efforts will contribute to the sustainable management and improvement of buffalo populations worldwide.

8 Concluding Remarks

The studies on molecular markers and genetic variation in water buffalo have revealed significant insights into the genetic diversity and structure of various buffalo populations. For instance, the evaluation of Turkish water buffalo populations using microsatellite markers demonstrated a clear genetic diversity, indicating the presence of at least two major clusters. Similarly, the analysis of Cuban water buffalo breeds using microsatellite markers highlighted the genetic variability within the population, which is crucial for conservation and breeding strategies. In Pakistan, the identification of breed-specific SNP markers has provided a deeper understanding of the genetic makeup of buffalo breeds, emphasizing the need for conservation programs. Additionally, the development of a 90K SNP genotyping assay has facilitated genomic analyses, aiding in genetic selection and diversity studies.

The findings from these studies have significant implications for conservation and breeding programs. The genetic diversity observed in Turkish and Cuban buffalo populations suggests the potential for developing targeted conservation strategies to preserve genetic resources. The identification of SNP markers in Pakistani buffalo breeds underscores the importance of genomic characterization in designing effective breeding programs to enhance productivity and maintain breed integrity. The use of advanced genotyping tools, such as the 90K SNP chip, can support the development of genome-assisted selection programs, thereby improving breeding efficiency and genetic gain. These insights are crucial for formulating strategies that ensure the sustainable management and utilization of buffalo genetic resources.

Future research should focus on expanding the genomic characterization of buffalo populations across different regions to build a comprehensive genetic database. This can be achieved by integrating various molecular markers, such as microsatellites and SNPs, to provide a holistic view of genetic diversity and population structure. Additionally, there is a need to explore the functional genomics of buffalo to understand the genetic basis of economically important traits, which can inform breeding decisions. Collaborative efforts between countries with significant buffalo populations can facilitate the sharing of genetic resources and knowledge, ultimately leading to improved conservation and breeding outcomes. Furthermore, the development of more precise and cost-effective genotyping technologies will enhance the ability to monitor genetic diversity and implement effective conservation strategies.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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