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Research Insight **Open Access**

A Comparative Study of Genetic Diversity in Wild and Domestic Water Buffalo Populations

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Abstract Buffalo are important livestock animals, and their genetic diversity plays a key role in species evolution, farm production, and future breeding. Wild buffalo (*Bubalus arnee*) and domestic buffalo (*Bubalus bubalis*) show clear lineage differences and have a complex evolutionary history. Domestic buffalo are mainly divided into two groups: river type and swamp type. In this study, we used mitochondrial DNA, microsatellite markers, SNP data, and whole-genome sequencing to compare the genetic diversity of wild and domestic buffalo. Because of habitat loss and small population size, wild buffalo now show lower genetic diversity and stronger inbreeding. Domestic buffalo have been shaped by long-term human selection, so their population structure is different. River-type buffalo have been strongly selected for milk traits, while swamp-type buffalo still keep high geographic separation and more uniform physical features. Genomic analysis also shows clear signals of domestication and artificial selection, including several selection sweep regions. We also found gene flow at different levels between river and swamp types, and between domestic buffalo and wild buffalo. This study points out that protecting the wild buffalo gene pool is very important. It also suggests that breeding programs for domestic buffalo should maintain genetic diversity, make good use of genomic selection, and improve hybrid strategies. These results can support future buffalo breeding, resource management, and biodiversity conservation.

Keywords Water buffalo; Genetic diversity; Domestication and artificial selection; Genomic differentiation; Population conservation and breeding strategies

1 Introduction

The global buffalo population (*Bubalus bubalis*) has exceeded 200 million, distributed across five continents. However, its main concentration area and economic influence are still in Asia, which accounts for more than 95% of the global buffalo population (Zhong et al., 2020; Shen et al., 2024). Due to its good adaptability to high temperature, high humidity and low-quality forage, water buffaloes can often maintain good production performance in environments where other domestic animals have difficulty surviving. In India alone, buffalo milk production accounts for more than half of the national milk production. The high milk fat content and high nutritional value in its milk are particularly valued (Nagarajan et al., 2015; Mishra et al., 2023).

The genus Bubalus has both wild and domestic types. The wild water buffalo (*Bubalus arnee*) is seen as the ancestor of the domestic water buffalo (*Bubalus bubalis*) (Curaudeau et al., 2021; Villamor et al., 2022). The domestication of buffaloes is not straightforward. It happened in different places on its own, so two main domestic groups appeared: the river buffalo (*B. bubalis*) and the marsh buffalo (*B. bubalis* carabanensis or *B. kerabau*) (Si et al., 2024). he river buffalo has 50 chromosomes. It is found mostly in the Indian subcontinent, the Middle East, and the Mediterranean. People know it for its high milk yield and its wide range of traits. The marsh buffalo has 48 chromosomes. It mainly lives in Southeast Asia and China. It is used more for farm work and meat. It shows clear genetic differences between regions, but its appearance does not vary much (Hassan et al., 2022). Wild water buffaloes are bigger and have a more aggressive nature. Today, only small and scattered groups remain in parts of South and Southeast Asia. They are now considered a critically endangered species.

In water buffaloes, genetic diversity is very important. It not only shapes how they evolve, but also affects their use in farming. When the genetic diversity is higher, it becomes easier to improve and keep important traits, like milk yield, reproduction, disease resistance, and the ability to live in different environments (Macciotta et al., 2021; Sikdar et al., 2021; Khan et al., 2022). For wild water buffaloes, keeping their genetic diversity is even more important. It helps the species survive, and it also keeps the original genetic base for domestic buffaloes. In recent years, with the fast development of tools like microsatellites, single nucleotide polymorphisms (SNPs), and whole-genome sequencing, researchers can now check and manage genetic diversity more clearly and more accurately.

Although the importance of genetic diversity in water buffaloes has been widely recognized, there is still a significant lack of systematic understanding of the comparative genetic diversity between wild and domesticated water buffaloes, especially at the genomic level. Most previous studies have focused on a single group (wild or domestic) or a specific population, lacking a comprehensive genomic comparison covering wild, riverine and swamp buffalo. This study conducts a comparative analysis of wild and domestic buffalo populations through extensive sampling and advanced genomics methods and provides references for the management of livestock genetic resources and biodiversity conservation.

2 Evolutionary Background of Buffalo Populations

2.1 Evolutionary origin and phylogenetic relationship between wild and domestic buffaloes

The history of buffaloes (genus Bubalus) shows that they split early and have complex family links. Wild and domestic buffaloes also differ a lot. Studies based on DNA and body traits say that the two main domestic types, the river buffalo and the swamp buffalo, did not come from the same group. They came from different wild Asian buffalo (*Bubalus arnee*) groups. These groups separated about 900 000 years ago, and the two types later developed on their own in different regions (Zhong et al., 2020; Sun et al., 2020).

The currently critically endangered wild buffalo is generally regarded as the common ancestor of the two domestic types, but the divergence between river and swamp buffalo predates domestication and can be compared to the degree of divergence between major cattle species, such as the common cattle and the zebu. Recent genomic studies even support the classification of river and swamp buffalo as two subspecies or even different species, while the tamarau (*Bubalus mindorensis*) and the anoa (*Bubalus depressicornis*) also provide a richer background for the phylogenetic relationships within the genus Bubalus (Curaudeau et al., 2021; Cailipan et al., 2023).

2.2 Domestication history: timeline, centers, and evidence from archaeological and genomic studies

River buffaloes were first domesticated in the western part of the Indian subcontinent (present-day India and Pakistan) around 6 300 BP (Zhong et al., 2020; Nagarajan et al., 2015). Both archaeological records (such as buffalo remains from the Indus Valley Civilization) and mitochondrial DNA studies support this time and geographical center, and suggest that the domestication process may have involved multiple gene introgressions from wild buffaloes. River buffaloes spread westward from their domestication center, eventually reaching Egypt, the Balkan Peninsula, and Italy, and formed multiple breeds with high diversity in production performance and phenotype (Sun et al., 2020).

The swamp buffalo was independently domesticated in the border regions between southwestern China and northern Indochina, with an estimated domestication period between 3 000~7 000 BP. Studies on mitochondrial and Y-chromosome diversity all support this region as the domestication center of the swamp buffalo, with populations in southern China and northern Indochina exhibiting the highest genetic diversity (Sun et al., 2020). Ancient DNA research from Neolithic sites in China also suggests the possible existence of now-extinct local wild buffalo species in the past.

2.3 Historical geographical distribution and factors influencing population differentiation

During the Pleistocene, climate fluctuations profoundly affected the differentiation and distribution of wild buffaloes. Ice age conditions prompted the separation of major lineages and the formation of different genetic pools. Subsequently, river and marsh water buffaloes spread from their respective domestication centers. River water buffaloes migrated westward from the Indian subcontinent, while marsh water buffaloes spread eastward and southeastward from the border of China/Indochinese Peninsula. Both experienced the founder effect, population bottleneck and localization adaptation during their migration processes. Marsh buffaloes exhibit a strong systemic geographical structure and extremely low gene flow. River buffaloes are relatively weak in geographical structure, but more abundant in phenotype and genetic diversity, which is related to breed formation, hybrid breeding and the selection of traits such as milk production and draft capacity (Si et al., 2024; Dutta et al., 2020). After river buffaloes entered the Mediterranean region and South America, the local groups further mixed and formed unique regional types.

3 Genetic Diversity of Wild Water Buffaloes

3.1 Current protection status and population bottleneck

The wild water buffalo (*Bubalus arnee*) is an endangered animal. There are fewer than 4 000 left in the world. About 90% of them stay in two small and separate areas in India, in Assam and Chhattisgarh (Pacha et al., 2021). These buffaloes face many problems. Their homes are getting smaller or damaged. People still hunt them. Livestock bring diseases. They also mix and breed with domestic buffalo. The group in central India is in very bad shape. It is almost gone. It needs fast help through protection and reintroduction work (Bora et al., 2024). In Nepal, the only wild group lives in the Koshi Tappu Wildlife Reserve. But it also faces human pressure, poor living conditions, and gene mixing with domestic buffalo (Khulal et al., 2021).

3.2 Genetic variation patterns revealed by mitochondrial DNA, microsatellites and SNP markers

Sequencing results of the mitochondrial genome of wild buffaloes revealed genetic similarities between the two major populations in India, but significant differences from domesticated buffaloes, especially in the obvious structural changes of multiple tRNA secondary structures (Pacha et al., 2021). Bayesian phylogenetic tree analysis indicates that wild buffaloes and domestic buffaloes form sister groups, showing a close evolutionary relationship and revealing clear genetic differentiation.

Even though the wild populations are small, they still keep some genetic variation, but it is usually lower than that of domestic breeds. Studies based on SNPs are still limited, but the current findings already show a trend of genetic changes caused by past bottlenecks. Using different types of molecular markers together is very important for checking genetic health, finding hybrid events, and helping with conservation work (Si et al., 2024).

3.3 Evidence of inbreeding, fragmentation and genetic drift

Studies show that many wild buffalo groups do not match Hardy–Weinberg balance, and this means their mating is not fully random. It also suggests that inbreeding may be taking place (Pacha et al., 2021). The presence of back-crossed animals and the ongoing mix with domestic buffalo make the genetic situation even harder to understand. Over time, this mixing can reduce the wild genetic types and may bring harmful traits into wild herds (Khulal et al., 2021). When the population goes through a bottleneck, genetic drift becomes stronger. As a result, some alleles may disappear just by chance, and the overall genetic diversity drops (Bora et al., 2024).

4 The Genetic Diversity of Four Domestic Water Buffaloes

4.1 Comparison between river water buffaloes (such as Indian and Pakistani breeds) and marsh water buffaloes (such as Southeast Asian breeds)

River-type buffalo mainly live in South Asia, the Middle East, and the Mediterranean. They are well known for producing a lot of milk. In countries like India, Pakistan, and Italy, they play an important role in the dairy industry. In contrast, swamp-type buffalo are common in Southeast Asia and southern China. They are strong and can live well in wetland areas, so people traditionally use them as working animals in rice fields (Zhong et al., 2020).

The two types of buffalo also have different chromosome numbers. River-type buffalo have 50 chromosomes, while swamp-type buffalo have 48. They also show clear differences in appearance and genetic structure. River-type buffalo include many breeds, and their body size, milk yield, and adaptability vary a lot. Swamp-type buffalo, although spread across a wide area, look very similar to each other and show more uniform traits.

Whole genome sequencing and SNP chip analysis further confirmed the segregation of the two types of buffaloes in systematic clustering (Si et al., 2024; Lu et al., 2020; Luo et al., 2020; Sun et al., 2020). Among river buffaloes, varieties such as Murrah, Nili-Ravi and Mediterranean exhibit high genetic diversity and are subject to intensive selection centered on milk-use traits. Marshland buffaloes also maintain high diversity in both maternal and paternal genetic lineages, but the phenotypic differences among populations are relatively small. This is likely related to their main use as draft animals and the relatively weak artificial selection pressure.

4.2 Genetic variations of different varieties and regional populations

River buffaloes, especially those from India and Pakistan, generally show high genetic diversity, which has been confirmed by microsatellite markers, SNP data and genome-wide analysis (Macciotta et al., 2021). Studies on Indian varieties such as Murrah, Banni and Jaffarabadi have found that the diversity within these groups is rich, while the differences among varieties are moderate (Dutta et al., 2020; Rehman et al., 2021). The Mediterranean and European buffalo populations originated from the ancient introduction of river buffaloes and formed unique genotypes in regions such as Hungary and Germany, which is closely related to hybridization and local adaptation (Noce et al., 2021). Although swamp buffalo are very different from river buffalo at the genetic level, they show clear geographic differences. In some isolated areas of Southeast Asia and China, their genetic diversity inside the population is quite low (Lu et al., 2020). The highest genetic diversity is found near the China–Indochina border, which is seen as an early domestication center. As the buffalo moved north or south from this area, their genetic diversity became lower.

The body shape and look of swamp buffalo are quite similar in different places. This may mean that their daily work and the environment around them limit how much their traits can change. But at the molecular level, they still have a lot of genetic variation. In some areas, certain groups also have special haplotypes and alleles. This shows that protecting local genetic resources is very important (Saputra et al., 2021).

4.3 The Influence of artificial selection, breeding practice and domestication on genetic structure

In river buffalo, people have focused on selecting animals with high milk yield, good reproduction, and strong disease resistance. After many years, this made some specialized dairy breeds that now show their own genetic patterns. Genomic work has found clear selection signals in several genes. For example, CSN2 is related to milk traits, *MC1R* affects coat color, and other genes are linked to reproduction. These signals are very strong in breeds like the Italian Mediterranean and the Indian Murrah (Sun et al., 2020; Si et al., 2024; Pauciullo et al., 2025). Such long-term selection increased the number of good alleles in these breeds, but it also reduced overall genetic diversity in some high-production groups, especially those living at the edge of their range, such as the Italian river buffalo.

In contrast, swamp buffalo have faced much weaker artificial selection. Their breeding has focused more on keeping working ability and environmental adaptability, not improving production traits. Because the two types of buffalo were domesticated in different places and at different times, and later moved into new areas with different environments, they slowly developed their own genetic differences. Over time, they formed clear and separate population structures (Lu et al., 2020; Luo et al., 2020). New genomic tools, such as SNP chips and whole-genome sequencing, make it easier to find regions that may be under selection. These tools also help scientists look back at the population history of domestic buffalo and see how human activities have influenced their development (Macciotta et al., 2021; Rehman et al., 2021). A key challenge today is how to improve important traits while still keeping enough genetic diversity in buffalo around the world.

4.4 The role of gene flow, genetic infiltration and variety improvement projects

In the Philippines and Brazil, both types of buffaloes live in the same areas. Because of old and recent movement of animals, and because people sometimes cross them on purpose, their genes have become mixed (Luo et al., 2020). Genomic studies also show signs of hybridization in these groups. Some genes related to useful traits, like higher milk yield, often move from one type to the other. It is also common to bring fluvial-type genes into marsh-type buffalo groups.

With the growth of molecular genetics and genomics, many breeding programs now try to increase production but also avoid losing too much genetic diversity. High-density SNP chips and reference genomes make it possible to track genetic changes, find key alleles, and set up more accurate breeding plans. By using genomic selection together with marker-assisted breeding and inbreeding control, researchers can speed up genetic improvement while still keeping enough diversity in the population.

5 Comparative Genomic Analysis: Wild and Domestic Buffalo Populations

5.1 Key genomic differentiation patterns: allele frequency, heterozygosity, and effective population size

Wild water buffaloes are seen as important ancestral gene pools, so they usually show high genetic diversity at the species level. But their population is now very small and highly scattered. Because of this, their heterozygosity keeps dropping, and the runs of homozygosity keep growing. These patterns suggest that they went through strong bottlenecks in the past and also experienced inbreeding.

Domestic water buffaloes (both river and marsh types) show a different and more mixed situation. Some of them, like the Italian Mediterranean river buffalo, still keep a fairly high heterozygosity (Ho ≈ 0.46). But others have much lower genetic diversity. This often happens in groups that live in isolated areas or have been under strong human selection pressure for a long time. Factors such as the founder effect, artificial selection, and geographic isolation all play a role in this decline (Pineda et al., 2024; Si et al., 2024; Pauciullo et al., 2025). FST values between wild buffalo, river buffalo, and marsh buffalo around the world show that their genetic differentiation ranges from moderate to high. The gap between river buffaloes and marsh buffaloes is especially large.

For wild water buffaloes, their very small population and fragmented habitats mean their effective population size (Ne) is low. With such a small Ne, they are easily affected by genetic drift and may lose some useful adaptive alleles. Domestic buffaloes generally have larger populations, but Ne can still fall in certain breeds, especially those that have been under strong selection or kept in closed breeding systems. For example, some buffalo breeds in Iran show an Ne as low as 32, which suggests that better germplasm management is needed to avoid further loss of genetic diversity.

5.2 Selection signals related to domestication (such as milk production, draft capacity, disease resistance)

Strong selective sweeps (selective sweeps) were detected in several gene regions related to milk production in river-type buffaloes, including CSN2 (β -casein) and DGAT1, which have key effects on milk quantity and milk composition (Si et al., 2024; Li et al., 2025; Pauciullo et al., 2025) (Figure 1). Genetic diversity often decreases around these areas, reflecting the long-term and intense artificial breeding of dairy breeds such as Murrah and Italian Mediterranean buffaloes. Similarly, genes related to coat color (such as *MC1R*), reproductive performance and metabolic pathways also have hard selective sweeping and soft selective sweeping, further promoting the differentiation between domestic buffaloes and wild ancestors (Luo et al., 2020).

Marshy buffaloes are mainly selected for draft capacity and environmental adaptability, and their genomes also show selection signals related to muscle development, energy metabolism and stress response (Zhang et al., 2022). Comparative genomic studies have also found that river-type and marshy water buffaloes exhibit convergent selection for traits such as disease resistance and environmental adaptability, and some loci even show parallel evolution with cattle species.

5.3 Evaluation of gene infiltration and Hybridization between wild and domestic water buffaloes

Genomic work with SNP chips and whole-genome sequencing shows that gene flow is common in places where the two buffalo ranges meet, or where domestic buffalo enter wild areas (Si et al., 2024; Pauciullo et al., 2025). In countries such as the Philippines and Brazil, researchers have also found gene introgression between river-type and swamp-type buffalo (Pineda et al., 2024). This introgression can add more genetic diversity and bring in useful alleles. But it may also break the genetic identity of wild buffalo.

Most of this hybridization comes from human actions. Some crossings are planned to improve breeds, but many others happen by accident because people disturb habitats or change grazing patterns. With high-density SNP tools and newer population genetics methods, it has become much easier to find and measure these hybrid events. These tools let researchers look at gene flow in a clearer way and pick out hybrid animals in both wild and domestic groups.

5.4 Identification of conserved and differentiated genomic regions

Conserved genomic regions are usually associated with important biological functions such as reproduction, immune response and environmental adaptation, and these regions are maintained in both wild and domestic buffaloes. A large homogeneous island (ROH island) on chromosome 2 has been found in both river and marsh buffaloes. This region contains genes related to environmental adaptation and reproduction, suggesting that it is subject to strong purification selection and evolutionary conservation (Macciotta et al., 2021).

In comparison, the genome regions that show clear differentiation are usually linked with traits that people focus on during domestication and breeding. These traits include milk production, working ability, and disease resistance (Zhang et al., 2022; Li et al., 2025; Pauciullo et al., 2025). Selection sweeps in these regions, together with copy number variations (CNVs), make some helpful alleles stay and spread in domestic groups. Genes related to milk yield and coat color are strongly selected in dairy breeds. In working breeds, genes involved in muscle growth and stress resistance are more common and show stronger signals (Luo et al., 2020; Si et al., 2024).

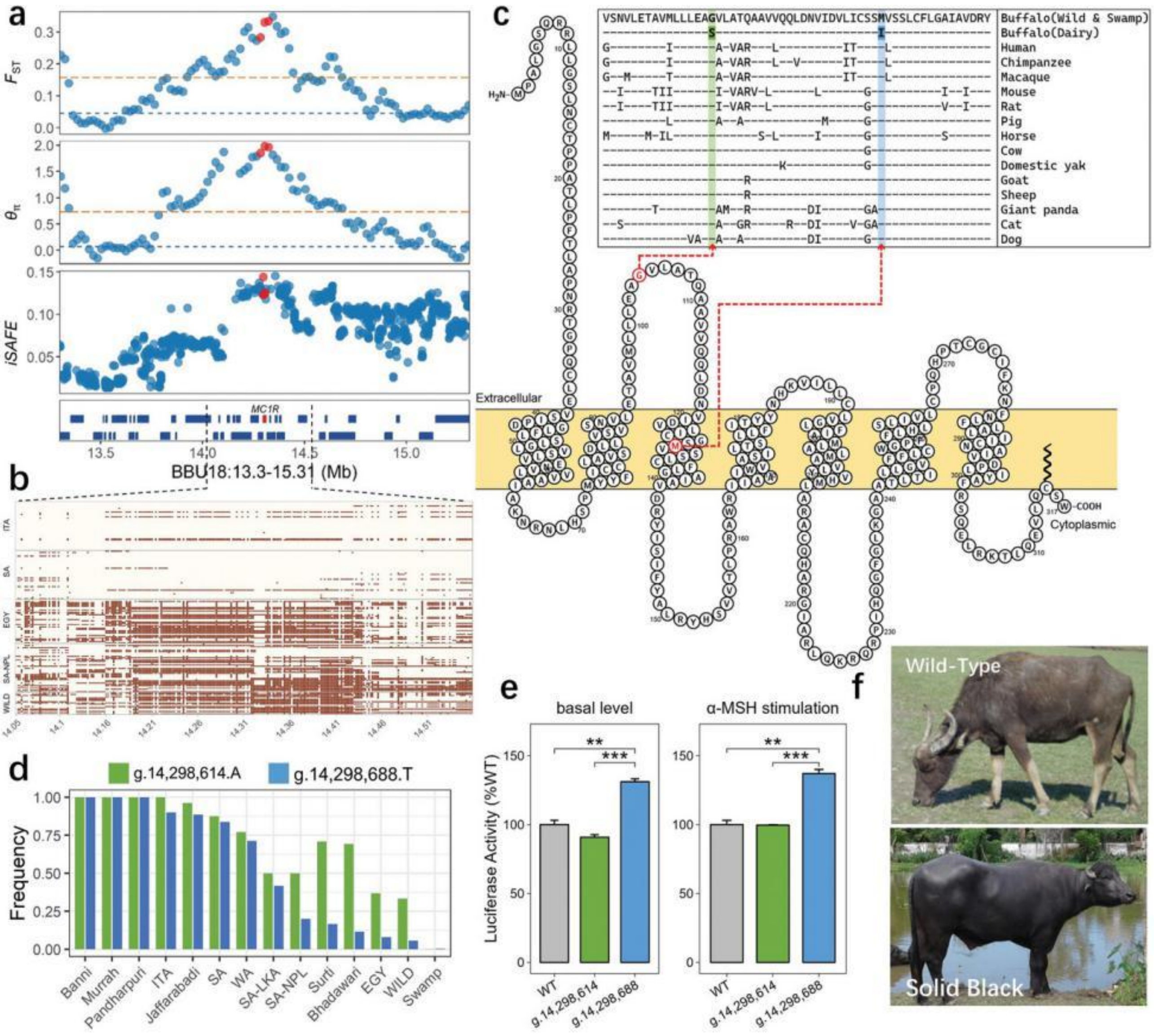

Figure 1 The selection signal, protein structure, and causal mutation of the *MC1R* gene (Adopted from Si et al., 2024)

6 Factors Driving Genetic Differentiation between Wild and Domestic Buffalo Populations

6.1 Environmental stress and ecological adaptation (wetlands, forests, agricultural landscapes)

Wild water buffaloes mainly inhabit wetlands and forest areas. These dynamic and challenging ecological environments prompt them to develop a series of genetic adaptations. They exhibit stronger resistance to local pathogens, physiological characteristics that adapt to water level changes, and behavioral strategies that effectively avoid predators. In contrast, domestic water buffaloes are raised and selected in agricultural landscapes. Human selection gradually replaces or supplements the pressure of natural selection, with a focus on improving production traits such as milk production, docility and draft capacity.

Wild water buffaloes' habitats include dense, waterlogged forests and wetlands, while domestic water buffaloes mainly live in irrigated farmlands and artificially managed pastures. This habitat difference is clearly reflected in its genetic composition, resulting in significantly different ecological adaptations between the two (Luo et al., 2020; Macciotta et al., 2021). The buffalo marsh type is more closely associated with wetland and paddy field ecosystems, retaining more morphological and genetic features from wild ancestors. On the contrary, river buffaloes, being selected for use in drier agricultural areas, exhibit higher milk production capacity and better heat tolerance.

6.2 Human-mediated factors: artificial selection, habitat fragmentation and cross-regional livestock mobility

Artificial breeding mainly aims to improve traits like milk yield, meat quality, and draft ability. River buffaloes have been under strong selection for milk traits for a long time. Because of this, they show large differences in appearance and performance, and some groups have become specialized breeds with high genetic diversity, such as the Italian Mediterranean buffalo (Luo et al., 2020; Zhong et al., 2020; Macciotta et al., 2021; Si et al., 2024; Pauciullo et al., 2025).

In contrast, marsh buffaloes were mostly used for farm work in the past. The selection pressure on them was much weaker. So, their traits look more similar overall, but they still show clear genetic differences between different regions. Habitat fragmentation often happens because of farmland expansion, city growth, and fences or other physical barriers. These barriers reduce gene flow between groups. When this happens, small groups may face more inbreeding and may lose genetic diversity (De Jager et al., 2020; Quinn et al., 2023; Colangelo et al., 2024; Karanja et al., 2025) (Figure 2). Cross-border trade, animal movement, or artificial hybridization can also move livestock from one region to another. This may bring new genetic resources to some groups, but it can also make different groups more similar and break the local gene pool that supports regional adaptation.

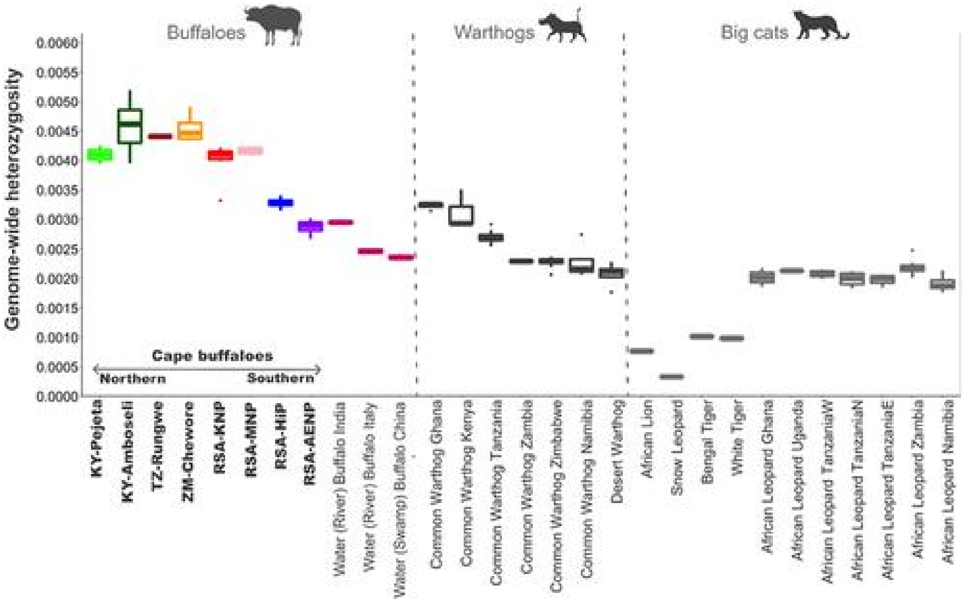

Figure 2 Genome-wide heterozygosity levels estimated for 58 cape buffalo samples (Adopted from Quinn et al., 2023)

6.3 Demographic events: Domestication bottleneck, Founder Effect and recent population decline of wild buffaloes

River type and marsh type water buffaloes were independently domesticated in different regions - river type originated in the Indian subcontinent, while marsh type originated in the border area between China and the Indochinese Peninsula. Each domestication event was accompanied by a bottleneck effect, resulting in a decrease in genetic diversity compared to wild ancestors. Subsequently, when the water buffaloes spread to new geographical areas, since only a small number of founding individuals formed the herds. Significant genetic drift and population differentiation occurred during the spread of river buffaloes to Europe and the Mediterranean region, as well as the transmission of marsh buffaloes in Southeast Asia.

Habitat loss, hunting, and competition with domestic water buffaloes have greatly reduced wild water buffalo numbers. Because of this drop, the chance of inbreeding becomes higher, and harmful alleles may become fixed in the population (De Jager et al., 2020; Quinn et al., 2023; Colangelo et al., 2024). Genomic studies also show a clear pattern: when a population goes through a strong bottleneck or stays isolated for a long time, it usually has more runs of homozygosity and less heterozygosity (Macciotta et al., 2021).

7 Implications for the Conservation and Breeding Procedures of Water Buffalo Populations

7.1 The Importance of preserving genetic diversity to combat diseases, climate change and environmental stress

A high level of genetic variation can help slow down the spread of infectious diseases. When a population has many different genes, it is more likely that some animals carry alleles that can resist certain pathogens. This point is very important today, because livestock production is becoming more intensive and animals move across regions and countries more often. Frequent transport makes it easier for diseases to spread quickly within and between populations.

Genetic diversity helps buffalo populations cope with new or changing environments. These changes may include higher temperatures, different rainfall patterns, and other climate pressures (Jaiswal et al., 2021; Rehman et al., 2021; Mishra et al., 2023). But when breeders focus too much on a few traits, such as milk yield or draft ability, they may reduce genetic variation without noticing it. This can lead to inbreeding depression and the buildup of harmful alleles. If the genetic base becomes too small, domestic buffalo may lose some ability to handle future problems, including new diseases or environmental stress.

7.2 Wild buffalo conservation strategies: protected areas, anti hybridization measures, and captive breeding

Establishing and effectively managing nature reserves is the foundation for preventing wild water buffalo from being affected by habitat loss, poaching, and human encroachment. These protected areas not only provide critical habitats for wild water buffaloes to maintain their natural behavior and ecological functions, but also serve as long-term monitoring and research bases. Merely designating protected areas is not enough, active management is also needed to reduce threats such as overgrazing, competition with livestock, and invasive alien species (Kherwar and Bhattarai, 2021; Khulal et al., 2021).

Hybridization, especially with domesticated or wild buffaloes, is a big threat to the genetic integrity of wild buffaloes. It can cause gene flow and make wild-type genotypes disappear. A good captive breeding program can work like a genetic resource bank. It can offer animals for release into the wild or help rebuild small populations. When making such plans, it is important to keep genetic diversity, prevent inbreeding, and use molecular tools to check genetic integrity. Setting up DNA sample banks and reference databases is also useful. Working with international teams and carrying out molecular research will further support both in situ and ex situ conservation efforts.

7.3 Breeding suggestions for domestic water buffaloes: maintain variability, optimize hybridization strategies and genomic selection pathways

Keeping a wide genetic base is very important. It helps avoid inbreeding depression. It also keeps alleles that may be useful in the future. We can do this by keeping a good effective population size. We should not use only a few males again and again. We can also bring in local breeds that are not used much. Using molecular markers, such as SNPs and microsatellites, can help us check genetic diversity. These tools also help us find rare alleles that need protection (Smith et al., 2020).

Improving crossbreeding plans can increase heterosis. It can also bring good traits from different breeds or groups into the target population. But crossbreeding needs careful control. If not, we may lose important adaptive genes in local breeds. Genomic selection uses high-throughput genotyping and whole-genome data. It can speed up genetic progress. At the same time, it can help keep and monitor genetic diversity (Jones et al., 2021).

7.4 Potential application of genetic resources of wild water buffaloes in domestic improvement

The unique alleles and adaptive characteristics contained in wild water buffaloes (such as disease resistance, environmental tolerance, and strong reproductive capacity, etc.) have greater application value in the context of climate change and the intensification of disease challenges. Through carefully designed genetic infiltration schemes, wild genetic variations can be reasonably introduced into domesticated populations to counteract genetic erosion or inbreeding effects caused by high-intensity selection (Zhong et al., 2020; Kherwar and Bhattarai, 2021; Rehman et al., 2021; Si et al., 2024).

To effectively utilize wild genetic resources, it is necessary to carry out systematic molecular research to identify and characterize the genetic differences between wild and domestic water buffaloes. Establishing DNA sample banks, genomic reference libraries and genotype databases will help screen candidate genes and alleles for the genetic infiltration of target traits.

8 The Future Direction of Genetic Diversity Research on Water Buffaloes

8.1 The need to conduct large-scale whole-genome resequencing for all buffalo lineages

Large-scale whole-genome resequencing of domestic water buffaloes of river and marsh types and their wild relatives is necessary for fully capturing their genetic diversity, identifying rare alleles and finely analyzing population structure (Rafiepour et al., 2020; Rehman et al., 2021; Arshad et al., 2025). Some resequencing studies involving multiple varieties and tens to hundreds of individuals have identified millions of single nucleotide variations and revealed the differentiation patterns among varieties, but these results still represent only a small part of global genetic diversity (Luo et al., 2020; Bian et al., 2024; Si et al., 2024). High-resolution genomic data can precisely locate genomic regions related to local environmental adaptation, disease resistance and production traits.

8.2 Develop high-density SNP chips for buffalo populations

Although some SNP chips for cattle have been used in water buffaloes at present, their applicability is limited due to the low polymorphism level and the existence of many information-free loci in the water buffalo genome. The development of buffaloe-specific SNP chips (such as the 90K SNP chip) has demonstrated higher resolution in population structure analysis, trait mapping and genomic selection, but further optimization is still needed to cover the genetic diversity of more species and subspecies (Khan et al., 2022).

Chip customization based on the genetic structure of river type, marsh type and their hybrid water buffaloes will achieve more accurate genotyping, enhance the ability to conduct GWAS on complex traits, and support the implementation of genomic selection breeding. High-density SNP arrays can also detect homozygous blocks, linkage disequilibrium patterns and selection imprints more effectively.

8.3 The integration of phenotypic, ecological and genomic data is used for the discovery of adaptive traits

By integrating GWAS with environmental association analysis, transcriptome analysis and other methods, researchers can identify candidate genes and regulatory networks that affect milk production capacity, disease resistance, growth rate and environmental adaptability (Mishra et al., 2023; Dai et al., 2025; Shen et al., 2025).

Establishing a large-scale and standardized phenotypic database, combined with high-precision environmental data, will help to analyze genotype-environmental interactions and identify the key gene loci that enable water buffaloes to maintain resilience under climate change and various environmental pressures.

8.4 Analyze the detailed domestication path of water buffaloes based on ancient DNA

Ancient DNA (aDNA) lets us look straight at how buffalo were first domesticated, how their groups moved around in the past, and whether they mixed with other groups in ways we can't see in living buffalo today. When researchers sequence DNA from old buffalo bones found at archaeological sites, they can see clearly when certain genetic changes happened. They can also find buffalo lineages that no longer exist and get a better idea of how today's domestic buffalo started (Si et al., 2024; Arshad et al., 2025).

When ancient DNA research is combined with modern genomic data and archaeological evidence, key controversies regarding the independent domestication of water buffaloes, their migration paths, and the impact of human selection pressure can be resolved. Such research can also reveal the domestication bottleneck, the founder effect and the genetic consequences of historical hybridization, deepening the understanding of the current genetic diversity formation mechanism of water buffaloes.

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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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