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LETTER

Genomic conservation of Mongolian horses promoted by preservation of the intangible cultural heritage of Naadam in Mongolia

Togtokh Mongke[1] | **Undarmaa Budsuren**[2] | **Aertengqimike Tiemuqier**[1] | **Elif Bozlak**[3,4] | **Barbara Wallner**[3] | **Samdanjamts Dulamsuren**[5] | **Dorjsuren Daidiikhuu**[6] | **Saruuljargal Amgalan**[7] | **Tana An**[1] | **Baoyindeligeer Mongkejargal**[1] | **Wenbo Li**[1] | **Sarula Borjgin**[8] | **Manglai Dugarjaviin**[1] | **Haige Han**[1]

[1]Inner Mongolia Key Laboratory of Equine Science Research and Technology Innovation, College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China

[2]School of Animal Science and Biotechnology, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia

[3]Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna, Vienna, Austria

[4]Vienna Graduate School of Population Genetics, University of Veterinary Medicine Vienna, Vienna, Austria

[5]Mongolian Association of Zootechnicians and Livestock Breeders, Ulaanbaatar, Mongolia

[6]Ulaanbaatar Veterinary Office, Veterinary monitoring and risk assesment devision, Ulaanbaatar, Mongolia

[7]Arkhangay Veterinary Office, Arkhangai, Mongolia

[8]Department of Grassland Ecology and Animal Husbandry and Veterinary, Xilingol Vocational College, Xilinhot, China

Correspondence
Haige Han and Manglai Dugarjaviin, Inner Mongolia Key Laboratory of Equine Science Research and Technology Innovation, College of Animal Science, Inner Mongolia Agricultural University, Hohhot 010018, China.
Email: hanhaige@126.com and dmanglai@163.com

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Abstract

Uncontrolled crossbreeding is a major challenge to the conservation of landrace horses in East Asia. Understanding the factors driving this trend is crucial for effective conservation efforts. Here, we investigate the genomic makeup of 40 Mongolian Naadam racehorses and 21 Asian landrace horse breeds through analyzing whole-genome resequencing and Y chromosome data. Our results show that crossbreeding practices are linked to horse-racing traditions. Regions characterized by strong horse-racing traditions and a lack of crossbreeding regulations exhibit significant levels of exotic genetic introgression, as observed in populations from Inner Mongolia and Central Asia. However, in Mongolia, despite having strong horse-racing traditions, the implementation of policies aimed at preserving traditional horse-racing culture effectively reduces exotic introgression. These results suggest that horse-racing traditions are the main driver of crossbreeding practices. Our research highlights that the preservation of traditional values in landrace horses, achieved through carefully managed horse-racing activities, can lead to successful conservation outcomes.

KEYWORDS
genomic conservation, genomic resources, intangible cultural heritage, Mongolian Naadam racehorses, uncontrolled crossbreeding

1 | INTRODUCTION

Since the mid-20th century, globalization has significantly contributed to the dissemination of high-yielding livestock breeds, exerting a profound influence on the conservation of landrace breeds (Berthouly-Salazar et al., 2012; Roosen et al., 2005; Tisdell, 2003). According to the Food and Agriculture Organization (FAO), over 70% of landrace breeds are currently at risk of extinction (FAO, 2022). Among these, horses are particularly susceptible, with 33% facing imminent threats and 101 horse breeds have become extinct (FAO, 2022).

The Mongolian horse serves as a compelling illustration of the enduring bond between humans and equines, providing a distinctive case study for understanding human social transformation. The history of Mongolian horses, spanning from ancient domestication to the modern era, reflects the dynamic evolution of the human–equine relationship. Horse husbandry in Mongolia dates back to the Middle Bronze Age (Fages et al., 2019; Librado et al., 2021; Ventresca Miller et al., 2022). Traditional Mongolian horse husbandry has developed into a comprehensive system that includes horse breeding, training, and utilization. Rooted in centuries of knowledge and experience, this system is dedicated to ensure the well-being of these horses. Today, Mongolian horses continue to be bred in accordance with the traditional nomadic herding philosophy, which prioritizes genomic diversity as a means to prevent inbreeding depression (Hund, 2008). Therefore, Mongolian horses stand as one of the oldest domestic horse breeds globally, serving as crucial genomic resources, especially in light of the extinction of wild ancestor populations of domestic horses (Librado et al., 2021).

In recent decades, the impact of international trade has resulted in a substantial influx of exotic horse breeds, notably Thoroughbreds and Arabians, into Mongolia. Traditional horse-racing reveals that the F1 or F2 generations resulting from the crossing of Mongolian and Thoroughbred (or Arabian) horses exhibit superior speed compared to local Mongolian horses, primarily due to their increased wither height, with F1 and F2 generations usually being taller than purebred Mongolian horses. This has led to widespread crossbreeding, motivating herders and breeders to prioritize racing crossbreeds over Mongolian horses in traditional horse-racing. In particular, exotic stallions have assumed a central role in "improvement" programs due to their greater effectiveness in crossbreeding compared to exotic mares. This trend has resulted in the marginalization of landrace stallions in the breeding system, posing a significant risk to the dynamics of traditional horse-racing culture and the genomic resources of Mongolian horses.

The Naadam Festival, an ancient national event deeply rooted in Mongolian history, dating back to the era of Genghis Khan (Walker, 2008), serves as a celebration of Mongolian culture and heritage, with horse-racing as a central tradition (UNESCO, 2023). The festival is not limited to Mongolia; it is also celebrated in Inner Mongolia and Xinjiang region of Central Asia. In Mongolia, participation in the Naadam Festival is restricted to male horses, and racing distances vary based on the ages of the horses. For example, 2-year-old colts race 10–12 km, 3-year-old colts race 12–14 km, 4-year-old colts race 16–18 km, 5-year-old colts race 22–24 km, stallions typically race 22–24 km, and geldings (6-year-old and above) race 24–26 km (ММСУХ, 2023). In 2010, the Naadam Festival was inscribed on the Representative List of the Intangible Cultural Heritage of Humanity (ICH) by the United Nations Educational, Scientific and Cultural Organization (UNESCO) (UNESCO, 2023).

The preservation of this nomadic intangible cultural heritage in Mongolia is critically dependent on the active participation and motivation of the broad local herder community, especially in the context of uncontrolled crossbreeding. Since 2011, prompted by UNESCO, the Mongolian government has been systematically identifying Mongolian Naadam racehorses on the basis of wither height. The wither height criteria are 127 cm for 2-year-old, 132 cm for 3-year-old colts, 137 cm for 4-year-old colts, 140 cm for 5-year-old colts and stallions, and 141 cm for geldings (ММСУХ, 2023). Simultaneously, crossbreeds have been banned from participating in Naadam horse-racing. In contrast, Naadam horse-racing in Inner Mongolia and the Xinjiang region of Central Asia does not impose restrictions on crossbreeds.

In this study, we conducted whole-genome resequencing (WGS) with a depth of 15x on 34 Mongolian Naadam racehorses (Table S1). The newly sequenced data were integrated with genomic datasets from 21 Asian populations, which included six Mongolian Naadam racehorses (Do et al., 2014; Han et al., 2022; Librado et al., 2015; Liu et al., 2019), as well as three exotic international breeds (Liu et al., 2019; Han et al., 2022), and five Przewalski's horses (Liu et al., 2019), totaling 248 individuals (Table S2). Additionally, we examined 77 Mongolian Naadam racehorses (Table S1) for Y chromosome (Y-chr) diversity. Our study aims to elucidate the outcomes of efforts to preserve Intangible Cultural Heritage (Naadam) by assessing the genomic makeup of Naadam racehorses.

2 | METHODS

A full description of overall methods and materials is shown in the Supporting Information Materials and Methods. In this study, we extracted and sequenced genomic DNA from 34 Naadam racehorses and downloaded the genomes of 214 other horse samples from the National Center for Biotechnology Information's (NCBI) Sequence Read Archive and the European Variation Archive (Tables S1 and S2). We mapped raw reads to equine reference genome EquCab3.0 (Kalbfleisch et al., 2018) and conducted genotype calling using a modified pipeline from the Genome Analysis Toolkit Best Practices pipeline (McKenna et al., 2010; Van der Auwera et al., 2013). We filtered SNP dataset using VCFtools (Version: 0.1.13) (Danecek et al., 2011). We used Plink (Version: 1.90b7) (Chang et al., 2015) and ADMIXTURE (Version: 1.3.0) (Alexander et al., 2009) software to investigate the population structure. To visualize the results from the principal component analysis (PCA) and ADMIXTURE analyses, we used the R programming language software (Version: 4.1.2) (https://www.R-project.org). We visualized the structure pie chart map using QGIS Geographic Information System (Version: 3.2.8) (http://qgis.org). To investigate the history of crossbreeding of Asian landrace breeds, we used neighbor-joining (NJ) tree to determine which of the landrace individuals were genetically closest to exotic horse breeds using FastME (Version: 2.1.5) (Lefort et al., 2015). To assess and test for the occurrence of exotic gene flow, we used TreeMix (Version:1.12) (Pickrell & Pritchard, 2012). We examined genome-wide heterozygosity and quantified the extent of ROH in Naadam horses using VCFtools (Danecek et al., 2011) and PLINK (Chang et al., 2015). To investigate Y-chr introgression, we mapped the whole genome sequencing data from 34 Naadam racehorses (Table S1) to the LipY764 assembly (Felkel et al., 2019) using BWA-aln (Li & Durbin, 2009) and used freebayes (Garrison & Marth, 2012) for variant calling, followed by filtering using methods described in a previous study (Bozlak et al., 2023). We reconstructed a maximum parsimony tree using 1946 filtered Single nucleotide polymorphisms (SNPs), which were combined with 24 horses from a previously reported dataset (Felkel et al., 2019) to create the phylogenetic tree. We used 27 kompetitive allele specific PCR (KASP) markers (Dataset S1) to detect the Y-chr introgression in the extensive sample of Naadam horses ($n = 77$) using the BIO-RAD CFX-96 qPCR instrument following standard KASP protocols. Naadam racehorse Y-chr haplotypes (Dataset S2) were visualized using a median-joining network created with PopART (Version: 1.7) (Leigh & Bryant, 2015). We used the previously reported (Felkel et al., 2018) horse Y-chr haplotype phylogeny as the framework for this visualization.

3 | RESULTS

Population structure of Naadam racehorses: To characterize the genomic structure of the Naadam racehorses, we conducted a population genomic analysis using a dataset of 881,486 high-confidence biallelic SNPs from 248 individuals, encompassing Asian landrace and exotic populations. The PCA delineated Asian populations into three distinct geographic genetic clusters: Central and Northeast Asia, Qinghai-Tibet Plateau, and Southwestern China. Notably, Naadam racehorses clustered closely with populations from Central and Northeast Asia (Figure 1a). When exotic breeds (Arabian horse, Thoroughbred, and Akhal-Teke) were included, the PCA revealed that only six of the 40 Naadam racehorses separated from the Asian clusters. Additionally, Inner Mongolian, Elunchun, Yili, and Kazakh horses exhibited a notable trend of separation from the Asian clusters (Figure 1b).

Admixture analysis mirrors the geographical origins and breeding history. With $K = 6$ as the optimal model for ancestral populations (Figures S1 and S2), we identified six major ancestral lineages concentrated in Central and Northeast Asia, the Qinghai-Tibetan Plateau, Southwest China, Arabian and Akhal-Teke, as well as Thoroughbred and Przewalski populations (Figure 1c, d). Notably, Thoroughbred and Arabian horses made relatively high genetic contributions to Inner Mongolian (11.6%), Elunchun (14.2%), Yili (23.2%), and Kazakh (16.6%) horses. In contrast, Mongolian horses and Mongolian Naadam racehorses exhibited a comparatively smaller genetic influence from Thoroughbred and Arabian horses, approximately 6.7%–7.8% only (Figure 1c, d).

Footprints of crossbreeding between exotic breeds and Asian landrace breeds: To comprehensively explore genetic differentiation and gene flow, we employed two

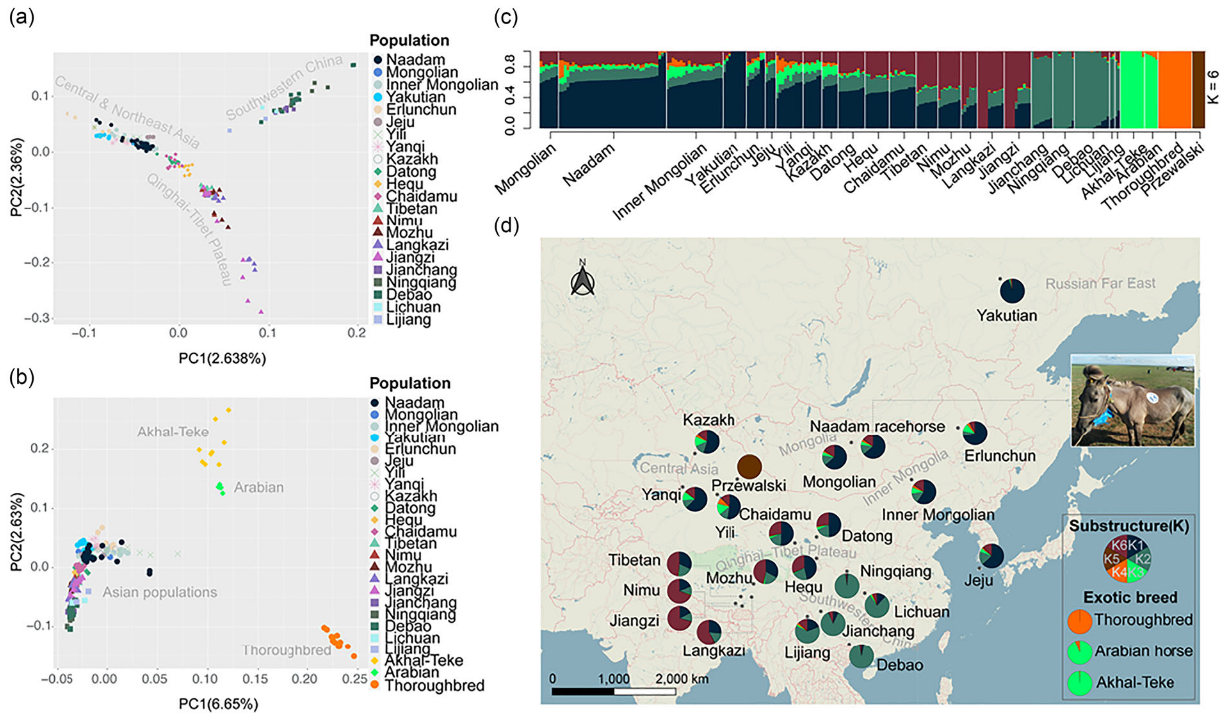

FIGURE 1 Genetic structure and geographic distribution of Naadam racehorses and Asian landrace breeds. (a) Principal component analysis (PCA) of Naadam racehorses and 21 Asian landrace populations, totaling 216 individuals. Different point shapes indicate the geographic origin of the landrace horses. (b) PCA of 22 Asian landrace populations and three exotic breeds, totaling 248 individuals. Different point shapes and colors represent the geographic origin of the horse populations. (c) Population structure and admixture patterns revealed by ADMIXTURE analysis. Each color represents a distinct genetic substructure, and individuals (vertical lines) are segmented based on the length of segments, indicating the admixture proportions from K genetic substructures. Ancestry proportions are displayed for $K = 6$. Six predominant genetic substructures were identified in horses from different geographic origins: Central and Northeast Asia (dark blue), Qinghai-Tibet Plateau (Tibetan red), Southwestern China (dark green), Akhal-Take & Arabian horses (light green), Thoroughbred (orange), and Przewalski's horses (brown). (d) Geographic distribution of genetic components percentages in Asian landrace populations. The circular legend in the bottom right corner provides a representation of the ancestral components corresponding to the colors on the pie charts. The dots on the map indicate the regions from which the samples were collected.

methods: NJ using FastME (Lefort et al., 2015) and maximum likelihood (ML) through TreeMix (Pickrell & Pritchard, 2012). The inferred topologies from NJ and ML analyses support the geographic distributions of the breeds revealed by PCA and admixture analysis (Figure 2a, b). Furthermore, integrating migration events into the cross-breeding history analysis, we found that the ML tree with eight inferred migration events displayed the optimal fit (Figure 2c, d; Figures S3 and S4), as determined by OptM (Fitak, 2021) and residual covariance matrix (Pickrell & Pritchard, 2012). Our analysis revealed significant genetic contributions from Thoroughbred and Arabian horses to several populations, including Inner Mongolian, Yanqi, Yili, Kazakh, and Naadam racehorses (Figure 2d). This influence was also evident in the NJ tree, where Central Asian breeds (Yili, Kazakh, Yanqi horses), Elunchun horses, and 10 out of 21 Inner Mongolian horses exhibited genetic similarity to Thoroughbreds and Arabian horses. Additionally, only six out of the 40 Naadam racehorses showed such genetic relatedness (Figure 2a).

Inbreeding and genetic diversity in Naadam racehorses: To explore the historical patterns of inbreeding, we assessed the inbreeding levels in the horse genomes through the analysis of runs of homozygosity (ROH) distributions. The Naadam racehorses, similar to many Asian landrace breeds, primarily displayed genomes marked by short ROH segments with average ROH segment length of 84.14 Mb (Figure 3a). In contrast, the Przewalski's horses (610.88 Mb) and exotic breeds such as the Thoroughbred (544.14 Mb), Arabian (396.7 Mb), and Akhal-Teke (321.16 Mb) exhibited extensive ROH segments (Figure 3a). Notably, Przewalski's horses, landrace Datong horses, and the exotic breeds displayed a significantly higher quantity of long ROH (>10 Mb), ranging from 28.32 to 69.24 Mb, indicating recent intentional mating practices involving very close relatives. The majority of landrace breeds displayed restricted quantities of long ROH segments (>10 Mb), ranging from 0 to 25.15 Mb, whereas the Naadam racehorses had 16.67 Mb (Figure 3a).

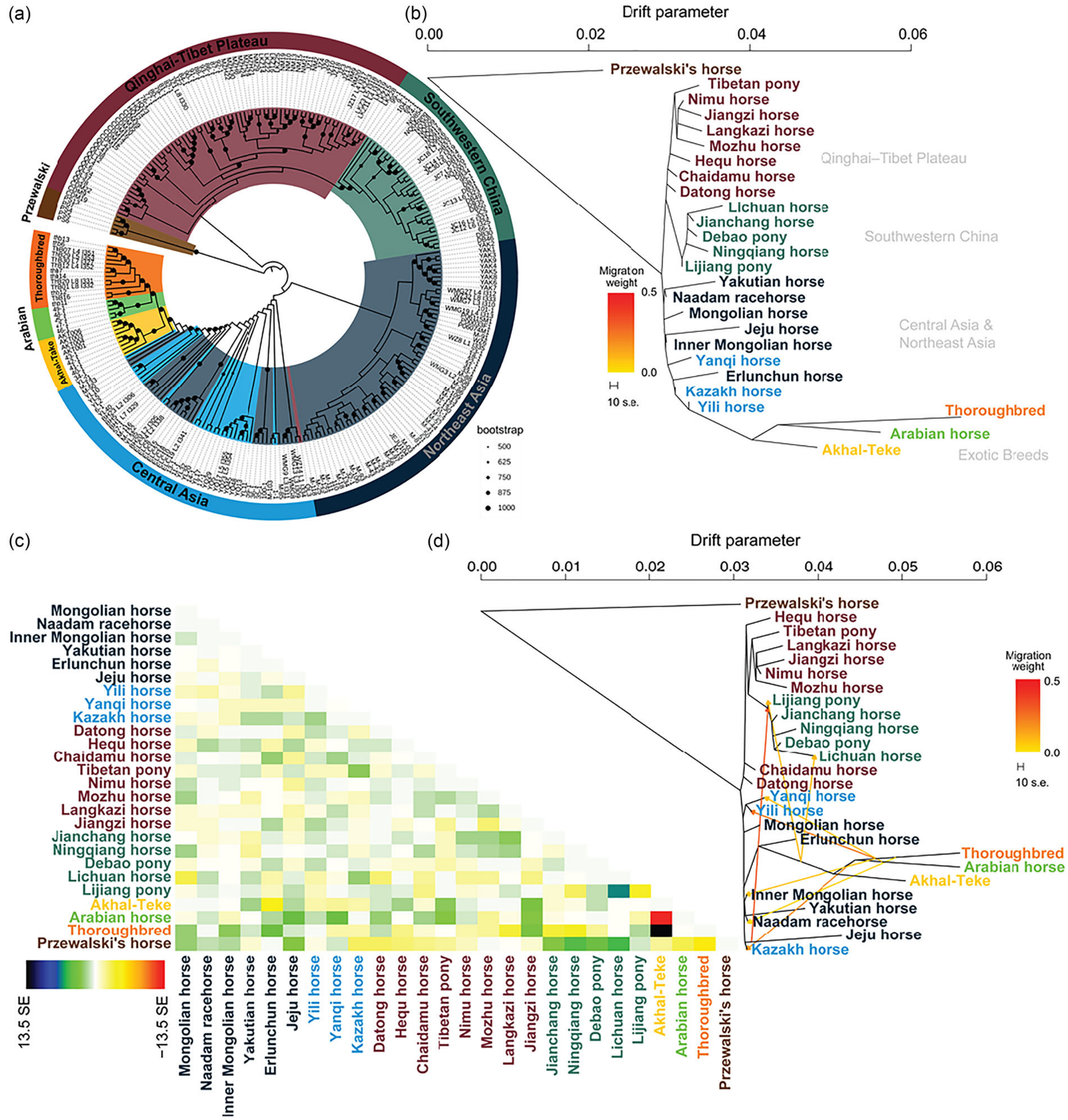

FIGURE 2 Phylogenetic relationships and migration events. (a) Neighbor-joining (NJ) tree of 22 Asian landrace populations, three exotic breeds, and Przewalski's horses. The NJ tree was constructed with 1000 bootstraps using a total of 248 individuals. The labels and numbers in the outer circle indicate sample identifiers and geographic origins. The dots on the branches represent percent bootstrap values based on 1000 replications. Only branches with bootstrap values exceeding 50% are indicated. Branch colors correspond to different geographic origins and exotic breeds. (b) Maximum likelihood tree of 22 Asian landrace populations and three exotic breeds. Przewalski's horses were used to root the tree. The breeds are colored based on their geographic origins: Central Asia (blue), Northeast Asia (dark blue), Qinghai-Tibet Plateau (Tibetan red), Southwestern China (dark green), Akhal-Take (yellow), Arabian horses (light green), Thoroughbred (orange), and Przewalski's horses (brown), the residual fit from this tree is shown in Figure S4. (c and d) Residual fit and maximum likelihood tree with migration events for Asian landrace populations and exotic breeds, exhibiting migration arrows from exotic breeds to Asian landrace populations. The scale bar indicates 10 times the average standard error of the values in the covariance matrix.

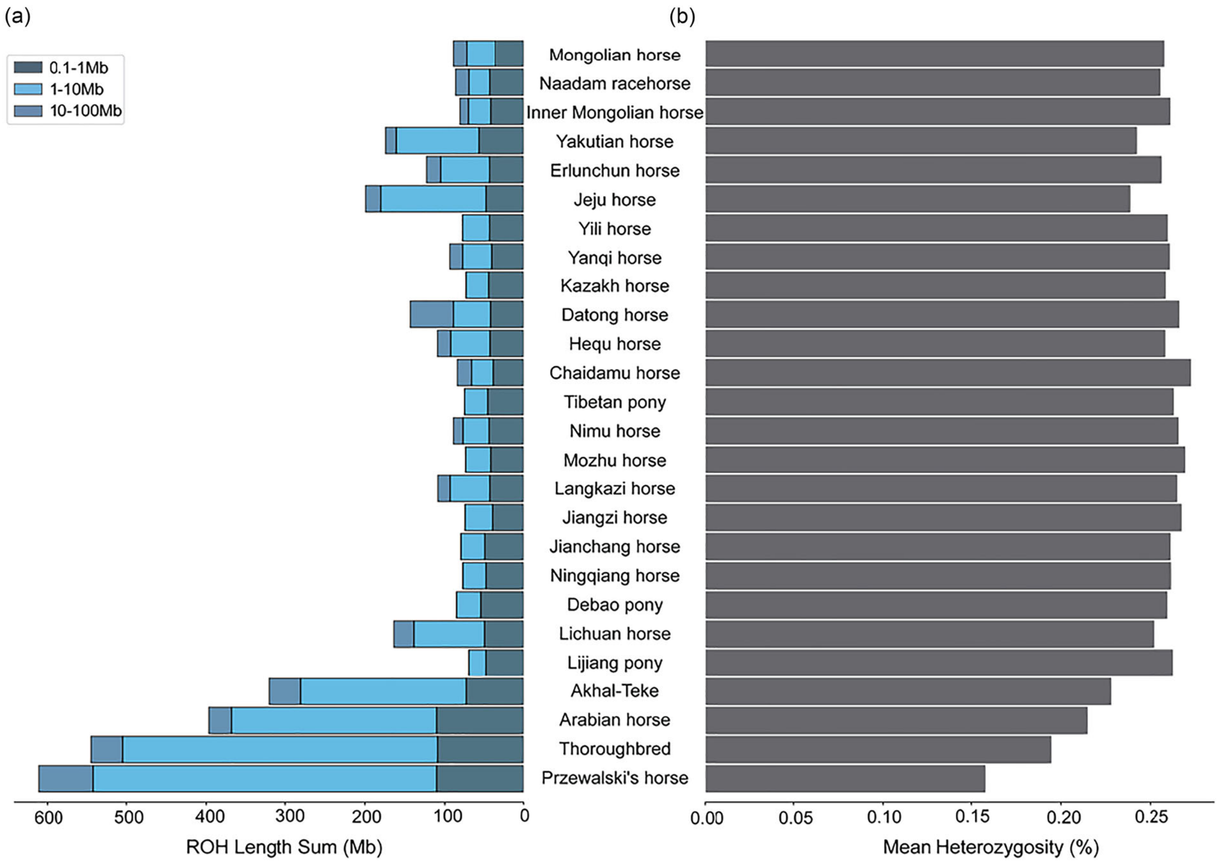

FIGURE 3 Inbreeding and heterozygosity of 26 horse populations. (a) Comparison of the summed lengths of runs of homozygosity in three specific length categories: short (0.1–1 Mb), medium (1–10 Mb), and long (10–100 Mb). (b) Genome-wide heterozygosity compared across Naadam horses and other horse populations.

Genetic diversity for the Naadam racehorses and for other horse breeds was estimated by genome-wide heterozygosity. As expected, our analysis revealed that Naadam racehorses and most Landrace horse breeds exhibited high mean heterozygosity levels compared to exotic breeds and Przewalski's horse (Figure 3b).

Limited exotic Stallion impact on Naadam racehorse patrilineages: To investigate the impact of exotic stallions on the patrilineages of Naadam racehorses, we analyzed Y-chr haplotypes. Y-chr data were extracted from the whole genome resequencing data using the Y-chr reference sequence LipY764 (Bozlak et al., 2023). In total, we identified 1946 SNPs, of which 280 were novel SNPs unique to Naadam racehorses. These data were integrated with 24 publicly available domestic horse data (Felkel et al., 2019), and then the Y-chr haplotype tree was reconstructed. Only four of 34 Naadam racehorses shared haplotype Ta and Tb-d which are specific to Thoroughbred and Arabian horses (Figure 4a). The majority of Naadam racehorses were distributed across multiple lineages on the Y-chr haplotype tree.

To validate this finding, we genotyped additional 77 Naadam racehorses for 27 Y-chr markers (Table S1 and Dataset S1). Only nine Naadam racehorses can be traced back to Arabian and Thoroughbred sire lines (haplotypes Ao and Ta) (Figure 4b). The majority of the Naadam racehorses located at the roots of the tree (Figure 4b and Dataset S2). Our results indicate that extensive local sire lines are involved in Naadam racehorse breeding.

4 | DISCUSSION

The conservation of landrace horses presents complex challenges where traditional cultural values intersect with uncontrolled breeding practices. Our research underscores the critical role of cultural preservation policies in maintaining the genomic integrity of landrace horses, especially in regions where strong horse-racing traditions incentivize the incorporation of exotic bloodlines.

One of the primary challenges in the conservation of landrace horses is the tension between preserving traditional cultural values and meeting the demands of modern horse-racing. Over the past decades, policymakers in developing countries have often turned to crossbreeding and ignored traditional cultural values to improve the

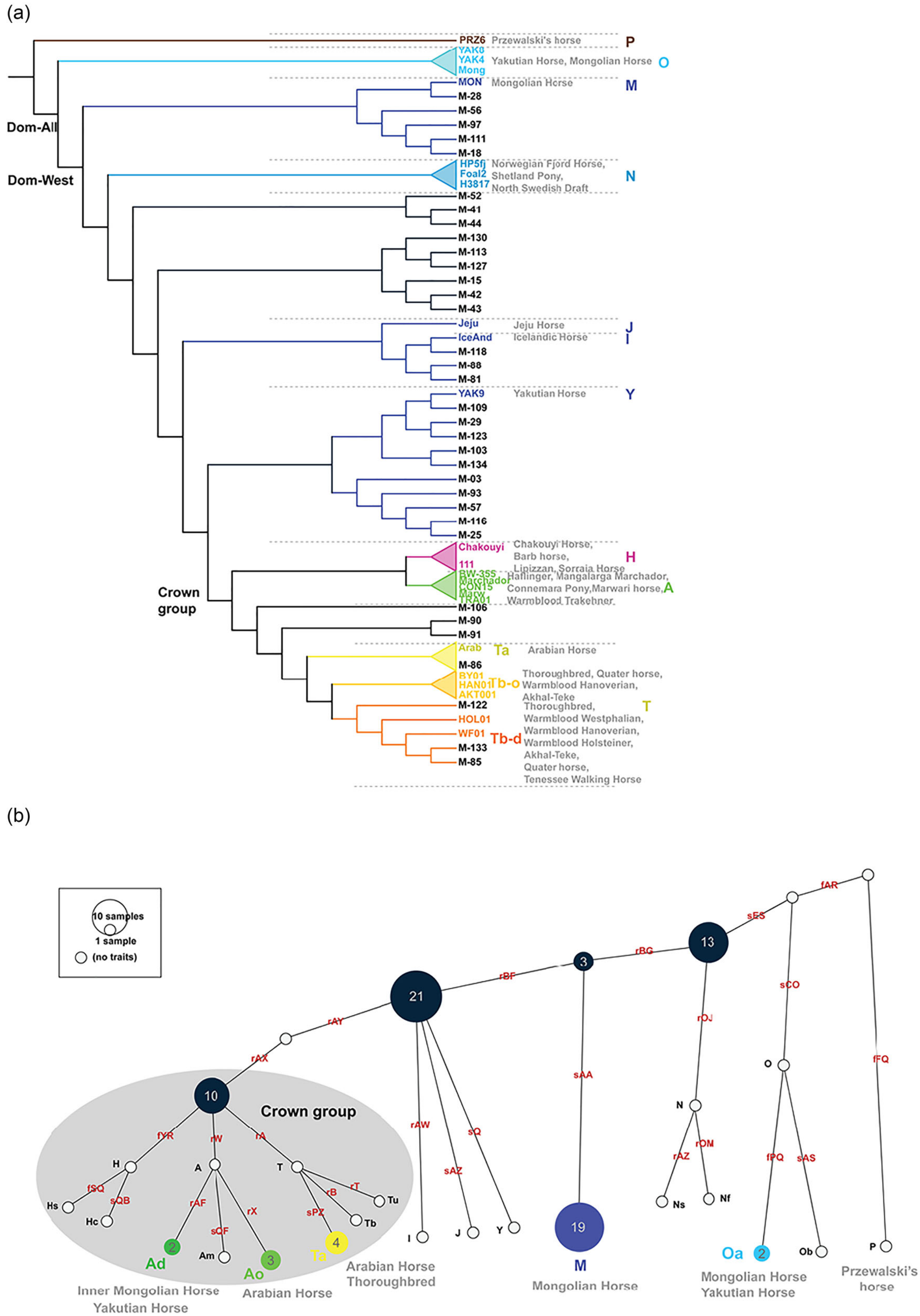

FIGURE 4 Phylogeny of Y-chr haplotypes. (a) Maximum parsimony (MP) tree for the 34 Naadam racehorse sequencing dataset, rooted with Przewalski's horse. Lineages previously reported (Felkel et al., 2019) are colored to define breed/geography-specific haplotypes. Assigned haplotypes are indicated on the right, and sample identifiers are labeled with numbers on the MP tree. (b) Y-chr haplotype tree of 77 Naadam racehorses. The tree was constructed using 27 breed/geography-specific haplotype markers and rooted with Przewalski's horse. Each haplotype is represented by a circle, with its size proportional to the number of individuals exhibiting that haplotype. The numbers within the circles denote the number of detected individuals. All detected haplotypes in Naadam racehorses are color coded and labeled according to their original breeds.

competitiveness of landrace breeds in modern horse-racing. However, these practices can undermine conservation efforts by substituting the unique genomic makeup of landrace populations. Our findings highlight the consequences of such initiatives, revealing a clear trend of exotic introgression in regions where crossbreeding is prevalent, such as Inner Mongolia and Central Asia. Our results suggest that uncontrolled crossbreeding has occurred in these regions. While these landrace horses are still grouped into Central & Northeast Asia, the issue of crossbreeding was not considered in previous study (Liu et al., 2019). Influenced by Thoroughbred and Arabian horses, the Central Asian and Inner Mongolian landrace populations exhibit a clear differentiation trend from the Asian landrace clusters, along with a high level of exotic introgression and close phylogenetic relationships with Thoroughbred and Arabian horses.

However, in the Qinghai-Tibet Plateau, Southwestern China, and Yakutia in the Russian Far East, landrace horses have traditionally adapted to harsh climatic and environmental conditions, such as high-altitude hypoxia (Liu et al., 2019), rugged terrain (Kader et al., 2015; Liu et al., 2022), and extreme cold (Librado et al., 2015). These landrace horses continue to serve traditional purposes, primarily in agriculture and pastoralism, rather than in horse-racing. As a result, traditional breeding practices in these regions have remained largely unchanged, focusing primarily on adaptation to local natural conditions, with minimal influence from exotic breeds. While we detect gene flow from exotic breeds to Lijiang and Lichuan horses, given the small sample size of only two individuals per breed, we do not address the influence of crossbreeding on these two horse populations in this study.

The central focus of our study is to evaluate the effectiveness of the Mongolian horse wither height-based identification policy in mitigating the impact of crossbreeding on the genomic integrity of Mongolian Naadam racehorses. Our findings suggest that implementing wither height criteria to limit the participation of crossbreeds in Naadam horse-racing has yielded favorable outcomes for conserving the genomic resources of Mongolian horses. Among Naadam racehorses, only six individuals stand out due to their elevated levels of exotic genome introgression. The percentage of exotic ancestry in these six individuals ranges from 10% to 30.9% (Figure 1c). This exotic introgression may be attributed to the bias of the wither height measurement, which effectively identifies F1 generations with 50% or more of exotic bloodline but may lead to misjudgments when the percentage is 30% or less. This discrepancy arises from the lower presence of exotic bloodlines in crossbred horses, leading to their wither height being closer to the average wither height of Mongolian horses.

The beneficial effects of the wither height-based identification policy are also evident in our Y-chr results. Thoroughbred and Arabian stallions are often utilized in crossbreeding programs within landrace populations, which ultimately restricts the breeding opportunities for landrace stallions. The preference for exotic stallion bloodlines has resulted in the predominance of their Y-chr haplotypes in landrace populations. A previous study reported that 56.7% of Inner Mongolian landraces have exotic sire lineages, implying the substantial involvement of exotic sires in the breeding process of Inner Mongolian horses (Han, Wallner et al., 2019). In contrast, our Y-chr results from whole-genome resequencing and KASP genotyping data revealed that only about 11% and 9% the Naadam racehorses in Mongolia have exotic paternal origins. Furthermore, we did not find a dominant sire preference phenomenon in Naadam racehorses; instead, they displayed a diverse range of sire lineages, which is consistent with a previous study that showed Mongolian horses have the highest diversity of paternal lineages among global modern horse breeds (Bozlak et al., 2023), suggesting that present-day Mongolian horses retain ancient paternal lineages.

Additionally, modern horse breeds have historically been managed through studbooks, where pedigrees are carefully maintained. However, intense selections for athletic performance often result in inbreeding depression and a collapse of genetic diversity, as observed in Thoroughbreds (Hill et al., 2022; McGivney et al., 2020; Wallner et al., 2017). In Mongolian Naadam racehorses, we observe a low level of ROH and a high level of heterozygosity. This pattern is consistent with the traditional Mongolian breeding practices that prioritize genetic diversity to prevent inbreeding depression.

Overall, the autosomal and Y-chr results provide compelling evidence that efforts to restrict crossbreeding in Naadam horse racing, driven by the policy of preserving intangible cultural heritage to maintain traditional equine values, and have proven effective in conserving the genomic resources of Mongolian horses. However, the limitations of the wither height-based identification policy also highlight the urgent need for genetic applications and pedigree registration to improve the reliability of purebred Mongolian horse identification.

AUTHOR CONTRIBUTIONS

Haige Han, Manglai Dugarjaviin, Togtokh Mongke, and Undarmaa Budsuren conceived the study. Tuyatsetseg Jambal, Undarmaa Budsuren, Samdanjamts Dulamsuren, Dorjsuren Daidiikhuu, and Saruuljargal Amgalan conducted biological sampling. Togtokh Mongke and Haige Han performed laboratory work. Togtokh Mongke conducted population genomic analyses. Aertengqimike

Tiemuqier, Tana An, Baoyindeligeer Mongkejargal, Wenbo Li, and Sarula Borjgin assisted with laboratory work. Elif Bozlak and Barbara Wallner performed Y chromosome analyses on WGS data. Togtokh Mongke wrote the manuscript with assistance from Haige Han, Barbara Wallner, Elif Bozlak, and Undarmaa Budsuren. All authors reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The whole genome sequencing data supporting the results of this study has been deposited in the National Center for Biotechnology Information (NCBI) (accession number:PRJNA1099933).

ORCID

Togtokh Mongke https://orcid.org/0000-0001-5092-1658
Haige Han https://orcid.org/0000-0002-0812-3000

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