



Genetic Diversity Analysis of Kachchhi-Sindhi Horses and Repercussions for Conservation in India

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ABSTRACT

Background: The Kachchhi-Sindhi horse is a native Indian breed, mainly found in the Gujarat and Rajasthan regions of India, with an estimated population of about 4,000 horses. A comprehensive genetic assessment to understand the genetic diversity and population structure of this breed is essential for conservation strategies and for maintaining the health and vitality of the breed.

Methods: To determine the genetic diversity and population structure of the Kachchhi-Sindhi breed, we genotyped 50 unrelated, healthy adult horses using 30 microsatellite DNA loci. The genetic data were analyzed to calculate the number of alleles, observed and expected heterozygosity and the inbreeding coefficient (F_{IS}). We also tested for Hardy-Weinberg equilibrium deviations and marker neutrality. We used Structure analysis to assess the admixture within the breed. Besides, population structure analysis was done with principal coordinates analysis (PCoA). Alongside mode shift tests, as well as Sign, Standardized differences and Wilcoxon sign rank tests, were made for assessing signs of a potential population bottleneck recently.

Result: Our study disclosed substantial genetic diversity in the Kachchhi-Sindhi breed. Key genetic parameters averaged an average number of alleles of 10.10±3.18, with an effective number of alleles of 5.15±1.88 and observed heterozygosity of 0.90±0.11, indicating significant genetic variation in the population. The inbreeding coefficient (F_{IS}) was very low at -0.17±0.19, indicating limited inbreeding. While 14 of the 30 microsatellites showed deviations from Hardy-Weinberg equilibrium, most markers were considered neutral, with only four showing non-neutral behaviour, according to the Ewens-Watterson neutrality test. Population analysis by PCoA revealed a single large but fragmented population of Kachchhi-Sindhi horses. Further tests, such as the mode-shift test and M ratio value, showed no evidence of a recent bottleneck in the breed. These are crucial for guiding future breeding and conservation efforts for the Kachchhi-Sindhi breed.

Key words: Conservation, Genetic diversity, Indian native horse, Marker, Sindhi.

INTRODUCTION

Before the beginning of mechanized vehicles, the horse was commonly used as a draft animal and riding on horseback was one of the imperative means of transportation. Presently, horses are an excellent means of transportation in the mountains and hilly terrains. India has seven distinct breeds of horses; Marwari, Kathiawari and Kachchhi-Sindhi are the horses of north northwestern region used for sports and transport, Spiti and Zanskari as pack animals for high high-altitude temperate Himalayan region and Bhutia and Manipuri as pack and transport ponies of the eastern region. Kachchhi-Sindhi breed of horse has been accessioned (INDIA_HORSE_0417_KACHCHHISINDHI_07007) recently by ICAR-National Bureau of Animal Genetic Resources, Karnal (<http://www.nbagr.res.in/reghorse.html> retrieved on 6/9/2019). Most of the information on phenotypic characterization is confined mainly to Marwari, Kathiawari, Spiti, Zanskari, Bhutia and Manipuri breeds (Gupta *et al.*, 2012). But, literature on phenotypic characterization of Kachchhi-Sindhi horse is very scanty. Research on phenotypic traits in indigenous livestock holds considerable importance, as numerous studies across various animal species in India have demonstrated associations between physical characteristics and traits related to production and

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performance (Pandey *et al.*, 2001; Dixit *et al.*, 2009; Chakraborty and Dhaka, 2012; Nehara *et al.*, 2013; Atay and Gokdal, 2016; Khan *et al.*, 2017; Islaam *et al.*, 2018). Gaining insights into these associations is crucial for enhancing selection strategies and improving breeding programs in native animal populations. Diverse aspects of research have been done for the equines, including management, conservation, genetics and reproduction

(Bhardwaj *et al.*, 2017; Bhardwaj *et al.*, 2019; Bhardwaj *et al.*, 2021).

The Kachchhi-Sindhi horses are famous for its 'Rewal chal' (a unique style of running). These horses dominantly exist in the western-northern border of India adjoining Pakistan. The breeding tract of these horses is Surat, Navsari, Kachchh district of Gujarat and Jaisalmer-Barmer districts of Rajasthan in India. Most familiar colors in the Kachchhi-Sindhi horses are bay and chestnut. Roman nose, ears curved at tips but not touching each other, short back, short pastern length, broader hoof for better grip and docile temperament are major characteristics of these horses. On an average, these horses stand 148 cm height; have a body length of ~140 cm, a heart girth of ~165 cm, an ear length of ~15 cm and a face length of ~61 cm (Pal *et al.*, 2021). Horse keepers sustain horses in intensive as well as extensive system of rearing. It has also been ascertained from various sources that horse number is declining rapidly, however breed population statistics are not available. There is, therefore, an urgent need to conserve this breed.

Previously, we have described the phenotypic and genetic diversity analysis of various indigenous horse and donkey breeds (Gupta *et al.*, 2005; Gupta *et al.*, 2012; Gupta *et al.*, 2013; Gupta *et al.*, 2014; Gupta *et al.*, 2018) and their conservation along with scientific management and breeding strategies (Pal *et al.*, 2013). To understand the genomic variability and phenotypic variance in horses, CNVs from six Indian horse breeds, namely, Manipuri, Zanskari, Bhutia, Spiti, Kathiawari and Marwari were also discovered using the equine genotyping array (Sharma *et al.*, 2023; Sharma *et al.*, 2025). The ROH islands in seven Indian horse (*Equus caballus*) breeds were also identified (Bhardwaj *et al.*, 2023). SNPs in myostatin and DMRT3 genes in Indian horses and donkeys were also elucidated (Sonali *et al.*, 2022; Sonali *et al.*, 2023).

The present study is an effort to genotypically characterize the Kachchhi-Sindhi horse breed based on a panel of ISAG-approved microsatellite markers. To the best of our knowledge, this is the first report on the genetic

characterization of Kachchhi-Sindhi horses. Proper managerial practices and conservation efforts will pave the way for the multiplication of this valuable equine genetic resource of India.

MATERIALS AND METHODS

Selection of animals and collection of blood samples

A total of 50 samples of KS horses (Fig 1) were selected from their breeding in Bhuj (Gujarat, India) and Jaisalmer (Rajasthan, India) (Fig 2). Horses of age 3-32 years were selected for the sampling. Five millilitres of whole blood were collected from the Jugular vein of the horses in a vacutainer containing EDTA. After blood sampling, all the samples are stored in ice and then temporarily transferred to 4°C before DNA isolation.

Genomic DNA isolation

Genomic DNA was extracted from the whole blood samples using the DNA isolation kit, following the recommended protocols by the ReliaPrep™ Blood gDNA Miniprep System (Promega, Madison/Wisconsin). The quality of the DNA was checked using 1% Agarose gel electrophoresis. The experiment was conducted at ICAR-National Research Centre on Equines (NRCE) in the year of 2024.

Microsatellite loci and genotyping

DNA samples were amplified by PCR using a set of 30 highly polymorphic microsatellite loci, which were internationally standardized by the International Society for Animal Genetics (ISAG) for evaluation of genetic diversity within and among horse breeds.

The PCR reaction was set up with 12.5 µL Promega GoTaq Green master mix (1X), 1 µL (10 µM) of each primer, 9.5 µL Nuclease Free Water and 1 µL of genomic DNA (100 ng/µL). PCR conditions involved the initial step at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, 57°C for 30 seconds and extension at 72°C for 30 seconds. The final extension was carried out at 72°C for 10 minutes. PCR products were analyzed in a 1.5% Agarose gel. The amplified samples



Fig 1: Kachchhi-sindhi horse.

were sequenced from Genosys Informatics Pvt Ltd, Delhi and AgriGenome Pvt Ltd, Kochi, Kerala.

Microsatellite statistical analysis

POPGENE (Yeh *et al.*, 1997) software was used to estimate basic population genetic descriptive statistics for each marker and population: observed number of alleles (N_a), effective number of alleles (N_e), Shannon Information Index (I), observed (H_o) and expected heterozygosity (H_e), Nei's (1973) expected heterozygosity (N_{ei}^{**}) and the Wright's F -statistics (F_{is}). The Hardy-Weinberg exact test and neutrality test. The pairwise mean genetic distance between individuals based on allele frequencies and Principal Coordinates Analysis (PCoA) were calculated using GenAlEx version 6.5 software (PEAKALL and SMOUSE, 2012). The Neighbour-joining tree based on genetic distance was constructed by MEGA ver. 11 (Tamura *et al.*, 2021). Population differentiation was further investigated by using a Bayesian clustering approach implemented in Structure version 2.3.4 software (Pritchard *et al.*, 2000). We used an admixture model with a burn-in of 50,000 iterations and 150,000 Markov chain Monte Carlo repetitions to estimate the probable number of genetic clusters (K) without giving any prior information. Population structure was used to estimate the number of groups. The Bayesian analysis set K values from 2 to 10 and examined the formation of colonies by group. Burn-in and MCMC repetitions (50,000 times and 1,50,000 times each, respectively) were conducted to estimate the optimal number of groups (ΔK values) and the ΔK value was estimated using Structure Harvester (Earl and VonHoldt, 2012), based on the rate of change in the log probability of data between successive K -values (Evanno *et al.*, 2005).

Bottleneck events in the population were tested by three methods. The first method consisted of three excess heterozygosity tests developed by Cornuet and Luikart (1996): (i) Sign test, (ii) Standardized differences test and (iii) a Wilcoxon sign-rank test. The probability distribution was established using 1000 simulations under three models—Infinite allele model (IAM), stepwise mutation model (SMM) and two-phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart, (1998) using Bottleneck software (Piry *et al.*, 1999) (<http://www.ensam.inra.fr/URLB>).

RESULTS AND DISCUSSION

In the present study, all marker loci were observed to be polymorphic and a total of 323 alleles were detected across 30 microsatellites in KS horse population with observed number of alleles (N_a) per locus ranging from 5 (HMS5; ASB43) to 18 (UM11) with a mean value of 10.1 ± 3.18 and effective number of alleles (N_e) from 2.2252 (LEX78) to 10.8696 (UM11) with a mean value of 5.15 ± 1.88 . Shannon's information index (I), a measure of polymorphism, was observed to have minimum values in COR069 (1.1106) and maximum in UM11 (2.5588) and a mean value of 1.79 ± 0.36 exhibiting a moderate level of diversity or randomness.

The mean of observed heterozygosity (H_o) was found to be 0.90 ± 0.1 . The mean value of Levene's and Nei's expected heterozygosity were 0.78 ± 0.08 and 0.77 ± 0.08 respectively. This shows more heterozygosity than expected. The mean inbreeding coefficient (F_{IS}) was -0.17 ± 0.19 showing an excess of heterozygotes and avoidance of inbreeding (Table 1). The importance of monitoring genetic relationships and managing inbreeding through controlled mating strategies is well-documented in closed or small equine populations (Maftai *et al.*, 2022). The F_{IS} of -0.17 ± 0.19 likely reflects a combination of factors such as admixture (recent mixing between Kachi Sindhi and other breeds) and the inherent polymorphism of microsatellite markers inflating the heterozygosity. However, the large SE suggests caution in overinterpreting the result. Further analysis is critical to distinguish between true population processes and methodological artifacts. Fig 3 shows the allele frequency for thirty microsatellite loci tested in 50 individuals of Kachchhi Sindhi Horse.

The Chi-square (χ^2) and probability (G^2) tests were carried out to assess Hardy-Weinberg equilibrium. The HW equilibrium test determined that out of the thirty genotyped microsatellites, fourteen (HTG6, AHT5, HTG3, AHT4, TKY321, HMS3, TKY337, ASB23, LEX33, TKY312, VHL20, LEX78, HMS2 and AHT31) deviated equilibrium. from HW Out of these fourteen microsatellites, TKY321, ASB23, LEX33, LEX78, AHT31 have less observed heterozygosity than expected heterozygosity. The neutrality of each marker tested by Ewens–Watterson neutrality test indicated that out of 30 microsatellites, only four (EB2E8, TKY337, ASB23 and AHT31) were not neutral as their observed F values were outside the lower and upper limits of 95% confidence interval (Table 2).



Fig 2: Geographical location of the KS horse population sampled in the present study.

The genetic divergences among the populations based on allele frequencies were calculated according to the pairwise mean genetic distance (GD) between individuals with the help of GenAlEx. The phylogenetic tree was constructed using Neighbor-joining (NJ) method based on the pairwise mean genetic distance between individuals in MEGA software (Fig 4). Among the individuals, KS17 and KS25, KS26 and KS29 and KS42 and KS 43 were the closest (GD=44), indicating that they are more closely related compared to other pairs in the matrix. The largest difference was calculated for KS8 and KS49 and KS24 and KS49 (GD=67), indicating that they are more distantly related compared to other pairs in the matrix.

PCoA plot was constructed using GenAlEx. The first three principal coordinates from the genetic distance matrix explained 4.02%, 3.59% and 3.31% variation across large, noncontiguous (fragmented), single population, respectively (Fig 5). The highest ΔK value was obtained

with a K value of 5 (Fig 6) with mean $\ln P(K)$ -6574.40 value. Each colored segment corresponds to the proportion of individuals assigned to a hypothetical population or cluster. Successive preset K values were calculated under the admixture model and the option of correlated allele frequencies. Different colors represent genotypes belonging to different subpopulations. The proportions of the color bars represent the admixtures in the varieties as shown in (Fig 7). The Y-axis shows the estimated ancestry of each genotype from a particular subpopulation.

In the present study, evidence for a bottleneck was not detected with any of the three methods. To characterize this, Sign Standardized differences and Wilcoxon sign rank tests were utilized. The values of average heterozygosity (H_e) and their probabilities ($H > H_e$) in the Sign test, under three models of microsatellite evolution-IAM, SMM and TPM-were calculated and used to measure the expected number

Table 1: Observed (N_a) and effective (N_e) number of alleles, Shanon Information Index(I), observed heterozygosity (H_o), expected heterozygosity (H_e), Nei's expected heterozygosity and FIS index in KS horses.

Locus	N_a	N_e	I	H_o	H_e	Nei**	F_{IS}
ASB2	14	6.2112	2.13	0.88	0.848	0.839	-0.049
EB2E8	9	6.1275	1.9	1	0.845	0.837	-0.195
HTG6	10	2.9994	1.494	0.92	0.673	0.667	-0.38
TKY333	8	3.858	1.53	1	0.748	0.741	-0.35
UM11	18	10.87	2.559	0.92	0.917	0.908	-0.013
ASB17	10	4.4763	1.818	1	0.784	0.777	-0.288
AHT5	12	7.3099	2.16	0.94	0.872	0.863	-0.089
HMS5	5	2.9087	1.213	0.96	0.663	0.656	-0.463
HTG3	10	4.4843	1.753	0.98	0.785	0.777	-0.261
HTG7	9	4.9751	1.794	1	0.807	0.799	-0.252
AHT4	13	5.4289	2.029	0.96	0.824	0.816	-0.177
TKY321	8	4.4326	1.684	0.74	0.782	0.774	0.0444
TKY394	7	2.7427	1.213	1	0.642	0.635	-0.574
UM32	10	5.9453	1.934	0.8	0.84	0.832	0.0382
HMS3	12	4.995	1.877	0.86	0.808	0.8	-0.075
TKY337	13	8.5911	2.326	0.98	0.893	0.884	-0.109
ASB23	10	7.0126	2.083	0.84	0.866	0.857	0.0203
LEX33	16	7.0721	2.277	0.84	0.867	0.859	0.0217
TKY297	11	6.6225	2.092	0.94	0.858	0.849	-0.107
TKY312	9	5.1073	1.794	0.84	0.812	0.804	-0.045
VHL20	15	5.5127	2.077	1	0.827	0.819	-0.222
ASB43	5	3.0139	1.17	1	0.675	0.668	-0.497
LEX78	7	2.2252	1.212	0.52	0.556	0.551	0.0556
HMS2	8	5.2301	1.833	1	0.817	0.809	-0.236
COR69	6	2.5176	1.111	0.94	0.609	0.603	-0.559
AHT31	7	5.1802	1.73	0.735	0.815	0.807	0.0895
COR22	7	4.0455	1.567	0.98	0.761	0.753	-0.301
HTG10	13	4.5413	1.859	0.74	0.788	0.78	0.051
SGCV28	11	5.8343	1.953	1	0.837	0.829	-0.207
COR7	10	4.2918	1.71	0.86	0.775	0.767	-0.121
Mean $\pm$ Sd	10.1 $\pm$ 3.18	5.15 $\pm$ 1.88	1.79 $\pm$ 0.36	0.90 $\pm$ 0.11	0.78 $\pm$ 0.08	0.77 $\pm$ 0.08	-0.17 $\pm$ 0.19

of loci with heterozygosity excess which was 17.95 and 17.64 for IAM and SMM with probabilities 0.00141 and 0.00008, respectively, under null hypothesis and thus rejects the null hypothesis indicating bottleneck under this model. However, the expected number of loci with heterozygosity excess was 17.90 in TPM with probability 0.08818, meaning that the null hypothesis was accepted when using the Sign test. These results indicate that, due

to mutation-drift equilibrium, the KS population has not undergone a recent genetic bottleneck. The standardized difference test provided the T2 (probability) statistics equal to 4.074 (0.00002), 0.670 (0.25128) and -6.249 (0.0000) for the IAM, TPM and SMM models, respectively. The probability values were less than 0.05 for IAM and SMM, thus hypothesis of mutation-drift equilibrium was accepted under TPM only. Using the Wilcoxon rank test (a non-parametric

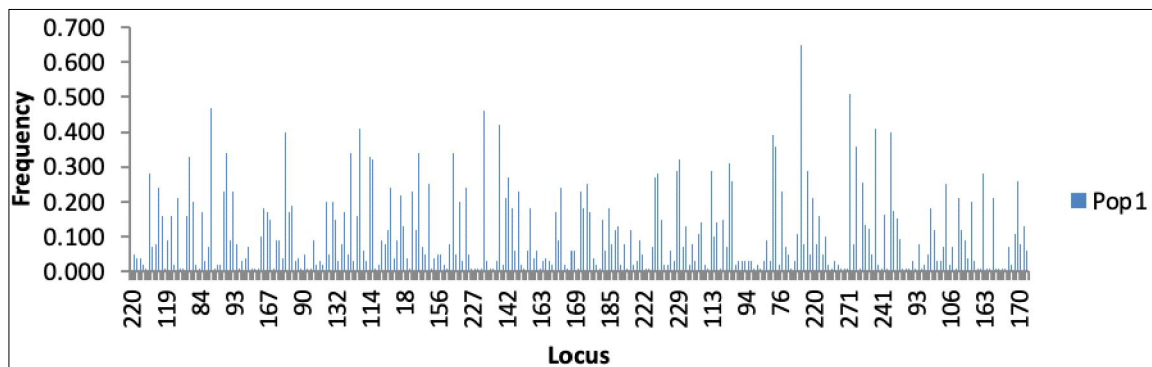


Fig 3: Allelic diversity for thirty microsatellite loci tested in 50 individuals of kachchhi sindhi horse.

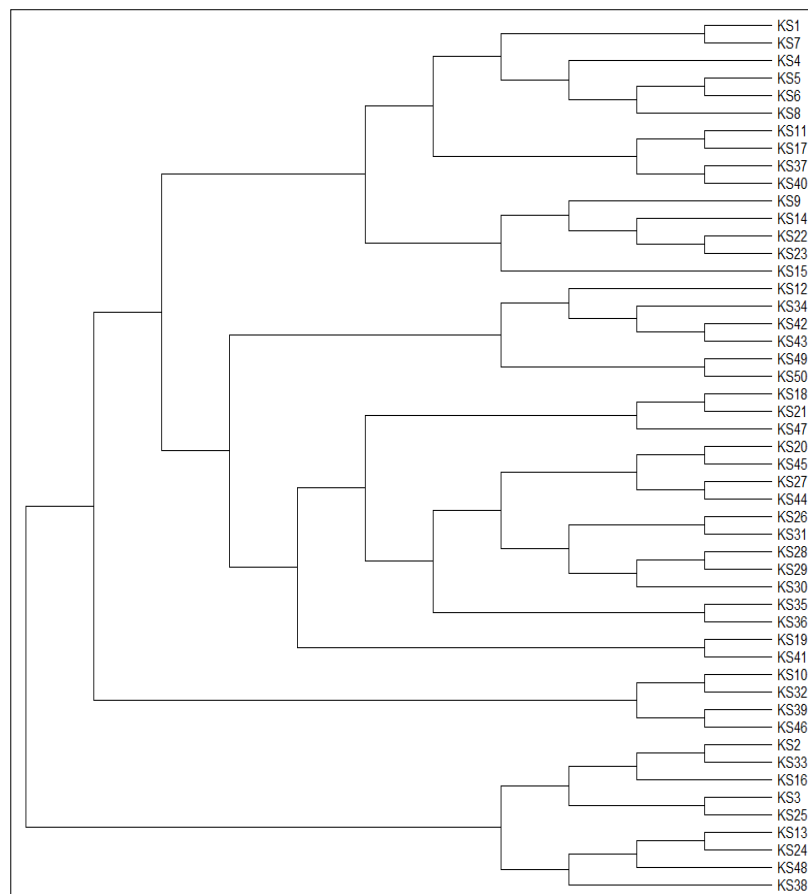


Fig 4: A neighbour-joining phylogenetic tree of the genetic distances (GD) among individual KS horses. The numbers in the tree are the identification numbers of individual horses.

test) the probability values were 0.0000 (IAM), 0.08860 (TPM) and 0.99986 (SMM) under these three models, indicating that the null hypothesis is accepted under TPM and SMM and the population under study has not undergone a recent bottleneck. Taking results from all the three tests together, it is clear that serious demographic bottlenecks have most probably not occurred in this breed. The Mode-shift indicator test was also utilized as a second method to detect potential bottlenecks. This test discriminates many bottlenecked populations from stable populations (Luikart 1997; Luikart and Cornuet 1997). A graphical representation utilizing allelic class and proportion of allele showed a normal 'L' shaped distribution (Fig 8). This distribution clearly reinforces the result that

the studied population has not experienced a recent bottleneck.

Compared to other Indian horse breeds, the KS breed exhibits more genetic variety. According to Gupta *et al.* (2005), the Marwari horse breed has 133 total alleles, with H_o of 0.5306 and H_e of 0.6535 and an average allelic diversity of 5.9. Koringa *et al.* (2008) found that the Kathiawari horse breed had 24 loci with 124 alleles, with H_o ranging from 0.1400 to 0.8600 and H_e from 0.1315 to 0.8480. The Kathiawari breed had a moderate amount of inbreeding, as indicated by its FIS of 0.1155, while the KS breed had a negative FIS value, which indicated a lower level of inbreeding in that breed. In the Zanskari horse breed, Behl *et al.* (2006) reported a mean of 5.80 alleles per locus and observed heterozygosity values ranging from

Table 2: Chi-square and G square probabilities (95% confidence level), observed F and upper (U95) and lower (L95) 95% confidence limits of expected F values across 30 polymorphic loci in KS horses.

Locus	Hardy-weinberg equilibrium test			Ewens-watterson neutrality test		
	Degrees of freedom	Probability of Chi-square	Probability of G-square	Obs. F	L 95*	U 95*
ASB2	91	0	0.638	0.161	0.115	0.333
EB2E8	36	0	0	0.163**	0.169	0.565
HTG6	45*	0.914	0.595	0.333	0.152	0.52
TKY333	28	0	0	0.259	0.178	0.633
UM11	153	0	0.997	0.092	0.088	0.245
ASB17	45	0	0	0.223	0.15	0.518
AHT5	66*	0.36	0.499	0.136	0.133	0.433
HMS5	10	0	0	0.343	0.266	0.832
HTG3	45*	0.278	0.315	0.223	0.154	0.521
HTG7	36	0	0	0.201	0.169	0.571
AHT4	78*	0.876	0.931	0.184	0.122	0.414
TKY321	29*	0.353	0.501	0.225	0.188	0.631
TKY394	21	0	0	0.364	0.206	0.694
UM32	45	0	0.007	0.168	0.154	0.529
HMS3	66*	0.326	0.997	0.2	0.128	0.401
TKY337	78*	0.094	0.447	0.116**	0.122	0.389
ASB23	45*	0.009	0.205	0.142**	0.154	0.525
LEX33	120*	0.001	0.988	0.141	0.099	0.304
TKY297	55	0	0.175	0.151	0.143	0.477
TKY312	36*	0.02	0.511	0.195	0.167	0.581
VHL20	105*	0.003	0.48	0.181	0.106	0.333
ASB43	10	0.001	0	0.331	0.263	0.832
LEX78	21*	0.076	0.184	0.449	0.211	0.71
HMS2	28*	0.124	0.006	0.191	0.188	0.647
COR69	15	0	0	0.397	0.228	0.762
AHT31	21*	0.137	0.12	0.193**	0.205	0.706
COR22	21	0	0	0.247	0.205	0.691
HTG10	78	0	0.998	0.22	0.119	0.405
SGCV28	55	0	0.471	0.171	0.139	0.488
COR7	45	0	0.705	0.233	0.153	0.488

*Deviate from HW.

**Deviate from neutrality.

0.48 to 0.74, with an FIS value of 0.219, indicating moderate inbreeding. Similarly, Chauhan *et al.* (2004) observed high genetic variability in the Spiti horse breed, with an average

of 4.8 alleles per locus and heterozygosity values ranging from 0.4091 to 0.7727. Compared to these breeds, the KS population exhibits higher allelic diversity and heterozygosity,

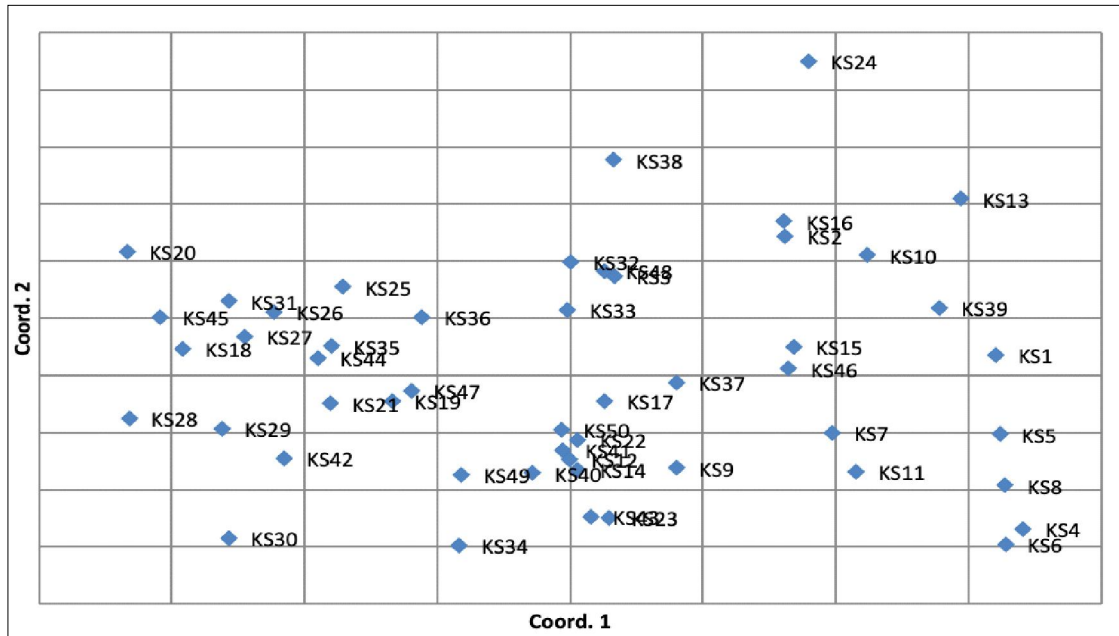


Fig 5: Principal co-ordinate (PCoA) analysis of KS horses analysis revealed a single, large, noncontiguous (fragmented) population.

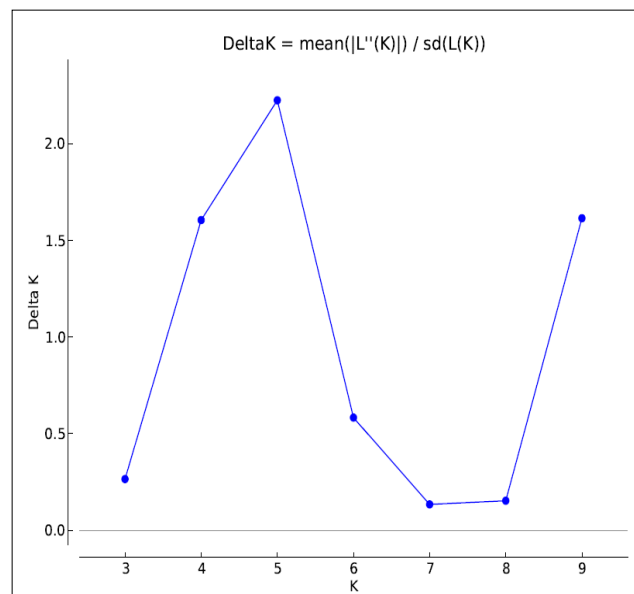


Fig 6: The maximum value of ΔK at $K=5$ was considered to be the appropriate number of populations for KS horse population.

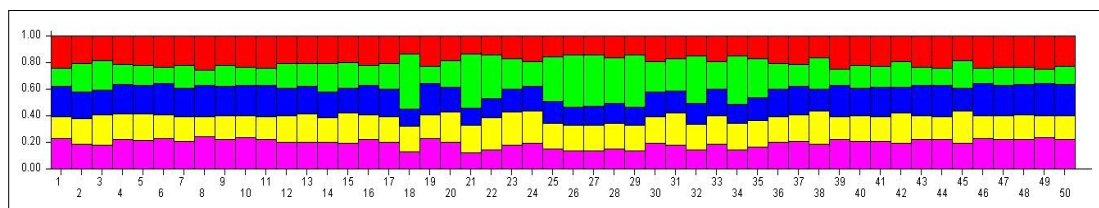


Fig 7: STRUCTURE analysis bar plot.

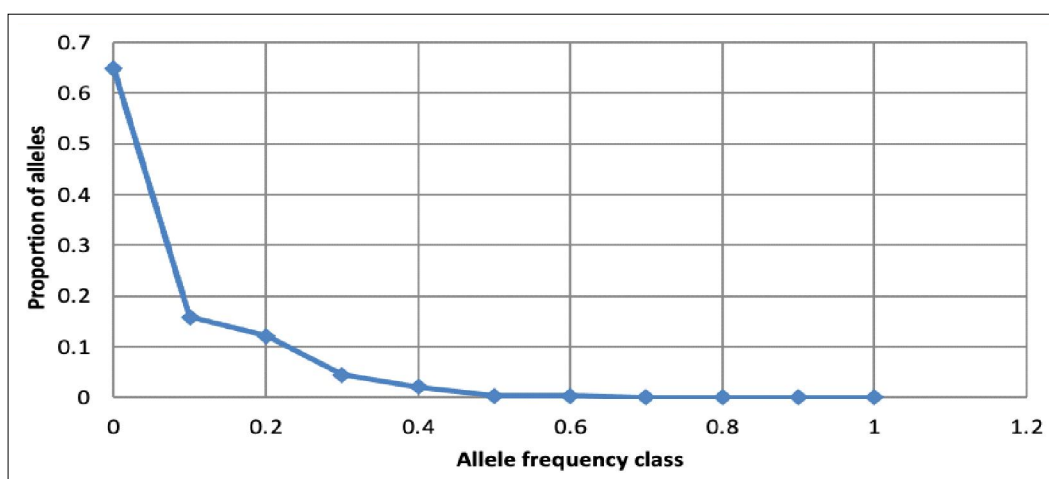


Fig 8: Graphic representation of the proportion of alleles and their distribution in KS horses.

which may reflect differences in breeding practices, population size, or historical genetic bottlenecks.

The excess heterozygosity observed in the KS breed ($H_o > H_e$) contrasts with findings in some other Indian breeds, such as the Kathiawari, where deviations from Hardy-Weinberg equilibrium (HWE) were observed at several loci, potentially due to the presence of null alleles or population substructure. In the KS breed, the negative F_{IS} value (-0.17 ± 0.19) suggests an avoidance of inbreeding, which may be due to deliberate breeding practices aimed at maintaining genetic diversity. However, the large standard error of the F_{IS} estimate (0.19) relative to its value indicates uncertainty in this interpretation, possibly due to the influence of the specific microsatellite markers used or the limited sample size ($n=50$) (Balloux *et al.*, 2004). Admixture analysis was crucial for studying the Kachchhi-Sindhi horse population with complex genetic ancestry. There has been a shared breeding tract of Kachchhi-Sindhi horse population with other breeds of horses in those regions. Historically, these breeds have coexisted in overlapping regions and can lead to potential genetic exchange due to interbreeding. Given the absence of strict pedigree records and the probable interbreeding, we hypothesized that Kachchhi-Sindhi horses might also exhibit admixture. This analysis helped confirm whether the breed has maintained genetic purity or if it has undergone significant gene flow from neighboring populations. STRUCTURE analysis provided actionable insights into the genetic distinctiveness, admixture history and diversity of populations like Kachi Sindhi horses. These findings could directly inform conservation priorities (e.g., protecting purebred vs. managing admixed groups), breeding programs (e.g., avoiding outcrossing that erases unique traits) and probable historical narratives by digging deeper (e.g., reconstructing past human-horse interactions in those regions). Quantifying ancestry and structure could bridge genetic data with practical decisions in breed management and cultural preservation. A Structure analysis could reveal the number

of genetic clusters (K) that best represent the population structure and the proportion of admixture in individual horses, indicating the genetic contributions from Kachchhi-Sindhi and other prevalent breed lineages. It was also able to provide an idea of whether the breed is genetically distinct or heavily admixed with other breeds. Population structure analysis in the KS breed revealed the presence of five distinct genetic clusters ($K=5$), suggesting substructure within the population. This substructure could contribute to the observed heterozygosity through a Wahlund effect, where mixing of genetically distinct subgroups inflates heterozygosity estimates. Similar substructure has been observed in other breeds, such as the Lusitano and Andalusian, where subpopulations within the breed have been linked to differences in allele frequencies and breeding practices (Petersen *et al.*, 2013). The lack of a significant relationship between individual inbreeding coefficients and homozygosity in the KS population aligns with findings in other equine populations, such as the Lipizzan horse, where no significant association was observed between inbreeding, microsatellite heterozygosity and morphological traits (Curik *et al.*, 2003).

CONCLUSION

The present work contributes to the knowledge of population structure and assessment of existing genetic diversity in the KS horse population. Further comparisons of other Indian horse breeds need to be carried out to determine the phylogeny evolutionary relationships and genetic distances among the indigenous equine breeds. The strong inference that the KS breed has not undergone major bottlenecks is also important for equine breeders and conservationists, as it suggests that any unique alleles present in this breed may not have been lost. The present work contributes to the knowledge of population structure, genetic characterization and assessment of existing genetic diversity in the KS horse population.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

The study was proposed in compliance with the already approved ethical clearance as granted by the IAEC, ICAR-NRCE, Hisar, Haryana, India. The procedure of DNA sampling by blood and hair was considered minimally invasive and a technique requiring minimal animal handling.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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