J. Agric. Sci. Technol. (2024) Vol. 26 (5): 973-982

Genetic Variations and Bottleneck Demographic Studies in Kurdish Horse Breed Using 17 Microsatellite Markers

H. R. Seyedabadi[1], and H. Emrani[1]*

ABSTRACT

The Kurdish horse is one of the most valuable and original pure genetic reserves in Iran. According to historical evidence, this breed dates back to more than 2,500 years ago. In this population, genetic variations were analyzed using 17 microsatellite markers recommended by International Society for Animal Genetics (ISAG). Genomic DNAs were extracted from the hair roots of 761 Kurdish horses. DNA fragments were amplified by multiplex PCR reaction using fluorescently labeled primers, and determined by capillary electrophoresis. Average number of alleles per locus was 4.29 (from 6 alleles in HTG7 to 17 alleles in ASB17). The mean value of the observed heterozygosity was 0.721, ranging from 0.491 (HTG7) to 0.838 (VHL20), while expected heterozygosity ranged from 0.523 (HTG7) to 0.839 (VHL20) with a mean of 0.752. The PIC value was from 0.708 (HMS6) to 0.856 (ASB17) with a mean of 0.782. The inbreeding coefficient ranged from -0.012 (ASB2) to 0.183 (HTG6) with a mean of 0.040. Deviation from Hardy-Weinberg equilibrium (P< 0.05) was found in 11 loci. The total exclusion probability of the 17 microsatellite loci was 0.9999. Kurdish horse revealed bottleneck event under two models of microsatellite evolution for sign and standardized differences for Infinite Alleles Model (IAM) and Stepwise Mutation Model (SMM). Based on this study, the effectiveness of 17 microsatellite markers for parentage verification and assignment test of Kurdish horse is confirmed. These results may facilitate conservation programs for the studied breeds and raise preserve their genetic variation.

Keywords: Bottleneck effect, Genetic reserves, Infinite alleles model, Stepwise mutation model.

INTRODUCTION

The horse is a great mammal befit for its speed, endurance, and strength. Horses are members of the Equidae family, the horses' role in human history and culture make it one of the most momentous domestic animals (Budiansky, 2004). The horse has played an important role in the history of Iran for many centuries (Fotovati, 2000), but with the advancement of technology, its role has been reduced and, as a result, its number is gradually declining. The history of horse breeding in Iran is very long due to its agricultural, cultural, and historical importance. Horse breeds in Iran mainly include Caspian, Turkmen, Dareshuri, Persian Arabian, and Kurdish (Fotovati, 2000). Because some of these breeds are considered endangered, it is important to know about their genetic structure. The The latest official statistics on the number of Iranian horse population in the country, published in (FAO, 2021), shows about 131000, of which the Kurdish horse is about 1500 (https://data.un.org/Data.aspx?d=FAO&f=itemCode%3A1096). The Persian Kurdish Horse, with its unique characteristics such as great sagacity and high strength in mountainous areas, and its inherent talent in playing Dressage and Polo amongst all the other Iranian horse breeds, is one of the most precious and main pure genetic reserves in

[1] Animal Science Research Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Islamic Republic of Iran.
* Corresponding author, e-mail: h.emrani@areeo.ac.ir

[DOI: 10.22034/JAST.26.5.973]

Iran. The main habitat of the Persian Kurdish horse is commonly Kermanshah and Kurdistan province. However, the distribution of this horse population has developed to the Iranian provinces of Azerbaijan, Kerman, Hamadan, Isfahan, and Elam, as well as parts of Turkey and Iraq. The Food and Agriculture Organization (FAO, 2022) reported that many horse populations all over the world are at risk of extinction. Among mammalian species, sheep, cattle and horses have the largest numbers of breeds at risk. However, rabbits (61%), horses (33%) and sheep (30%) are the species with the largest proportions of breeds at risk. Cattle are the species with the largest number of breeds (159) reported as extinct. Large numbers of extinct breeds of sheep (107), horses (101), and pigs (70) are also reported (FAO, 2022).

Therefore, it is important to know the genetic diversity of these populations to design conservation programs. Genetic diversity studies in livestock focus on assessing genetic variation within and between breeds. To better manage livestock resources, it is necessary to know the genetic structure of livestock populations such as population size and genetic variation within and between populations. The genetic variation in populations can be calculated using genetic and molecular information. In the case of lacking or incomplete pedigree data, it would be better to use molecular markers to characterize a population structure. (Delgado *et al*., 2014). Microsatellites are mainly used to determine the genetic variation within and between different populations of the horse (Aberle and Distl, 2004; Sargious *et al*., 2021a, b, c; Štohlová Putnová and Štohl, 2021; Cozzi *et al*., 2022). Microsatellites were identified for the first time in Swedish horse breed by Ellegren *et al*. (1992) and Marklund *et al*. (1994). Microsatellite markers are one of the most efficient types of molecular markers because of their high polymorphism, wide genome coverage, co-dominant inheritance, and good reproducibility. Microsatellite markers have been widely used to identify the genetic structure of Iranian horses. (Seyedabadi *et al*., 2017; Amirinia *et al*., 2007; Rahimi-Mianji *et al*., 2015; Moridi *et al*., 2013). However, little information is available for the genetic structure of the Kurdish horse using microsatellites markers in Iran (Khamisabadi, *et al*., 2020). This study aimed to determine historical population bottlenecks, genetic diversity, and describe the setup of polymorphic microsatellites for the paternity testing of the Kurdish horse.

MATERIALS AND METHODS

Sample Collection and DNA Isolation

First, 761 hair samples of Kurdish horses were collected from different regions of Iran. Samples were collected from male and female and all horses registered in the Studbook. Total DNA was extracted from the hair follicles by the methods described by QuickGene DNA tissue kits (FUJIFILM, Japan) and the concentration and purity of the extracted genomic DNAs were determined using a NanoDrop spectrophotometer at optical density of 260 and 280 nm.

Microsatellite Amplification

Samples were genotyped for genetic diversity studies and paternity tests using a set of 17 microsatellites recommended by the International Society for Animal Genetics (ISAG) (Table 1). Microsatellites were amplified using fluorescently labeled primers and following the PCR conditions given by Dimsoski (2003). Thermal cycling included initial denaturation at 94°C for 5 minutes, 30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 1 minute and final extension at 72°C for 10 minutes. The PCR products were separated on the 3130×l Genetic Analyzer (Applied Biosystems). Allele sizes were determined with the software package GeneScan 3.7 using a LIZ 500 bp internal size standard (Applied Biosystems).

[DOI: 10.22034/JAST.26.5.973]

Table 1. Microsatellite names and primer information.

Locus	Localization on chromosome	Repeat motif	Size range (bp)	Fluorescent dye	Reference
AHT4	24q14	(AC)nAT(AC)n	144–164	6-FAM	Binns *et al*. (1995)
AHT5	8	(GT)n	126–144	VIC	Binns *et al*. (1995)
ASB2	15q21.3-q23	(GT)n	216–250	VIC	Binns *et al*. (1995)
ASB17	2p14-p15	(AC)n	87–129	PET	Binns *et al*. (1995)
ASB23	3q22.1-q22.3	(TG)n and (TG)n TT(TG)4	175–211	VIC	Irvin *et al*. (1998)
CA425	28q18	(GT)n	226–246	PET	Eggleston *et al*. (1997)
HMS1	15	(TG)n	170–186	PET	Guerin *et al*. (1994)
HMS2	10	(CA)n(TC)2	222–248	NED	Guerin *et al*. (1994)
HMS3	9	(TG)2(CA)2TC(CA)n and (TG)2(CA)2TC(CA)nGA(CA)5	148–170	NED	Guerin *et al*. (1994)
HMS6	4	(GT)n	151–169	VIC	Guerin *et al*. (1994)
HMS7	1q25	(AC)2(CA)n	165–185	6-FAM	Guerin *et al*. (1994)
HTG4	9	(TG)nAT(AG)5AAG(GA) 5, ACAG(AGGG)3	127–139	6-FAM	Ellegren *et al*. (1992)
HTG6	15q26-q27	(TG)n	84–102	VIC	Ellegren *et al*. (1992)
HTG7	4	(GT)n	118–128	NED	Marklund *et al*. (1994)
HTG10	21	(TG)n and TATC(TG)n	95–115	NED	Marklund *et al*. (1994)
LEX3	Xq	(TG)n	142–164	PET	Coogle *et al*. (1996)
VHL20	30	(TG)n	87–105	6-FAM	Van Haeringen *et al*. (1994)

Statistical Analysis

The percentage of observed heterozygosity was calculated for the population. Average expected theoretical heterozygosity from Hardy-Weinberg assumptions was calculated using Equation (1) (Hedrick, 1999):

$$H_e = 1 - \sum_{i=1}^{n} p_i^2 \quad (1)$$

Where, *pi* is the *i*th allele frequency. Polymorphic Information Content (PIC) was calculated using Equation (2) (Botstein *et al.,* 1980):

$$PIC = 1 - (\sum_{i=1}^{k} p_i^2) - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2 p_i^2 p_j^2 \quad (2)$$

Where, *pi* and *pj* are frequencies of corresponding alleles. Effective number of alleles (ne) was calculated using Equation (3) (Hedrick, 1999):

$$n_e = 1 / \sum_{i=1}^{n} p_i^2 \quad (3)$$

This parameter (n_e) gives an indication of the relative influence of the alleles. Standard diversity including the Number of alleles (Na), effective Number of alleles (Ne), observed (Ho), and expected (He) Heterozygosity, Polymorphic Information Content (PIC), Probability of Exclusion (PE), and Combined Probabilities of Exclusion (CPE) were calculated using Cervus 3.0 (Kalinowski *et al*., 2007). Testing of the Hardy–Weinberg equilibrium and Wright's Fixation Indices (FIS) were calculated using GenAlEx software version 6.5 (Peakall and Smouse, 2006).

To accomplish the goal of finding evidence of fluctuations in Kurdish horse population sizes, Bottleneck V1.2.02 Software (http://www.ensam.inra.fr./URLB, was used to detect deviations from mutation drift equilibrium. This method developed by Cornuet and Luikart (1996) consisted of three excess heterozygosity tests: Sign test, standardized differences test, and Wilcoxon

[DOI: 10.22034/JAST.26.5.973]

sign-rank test. The probability distribution was established using 1000 simulations under the three models: Infinite Allele Model (IAM), Two-Phase Model of Mutation (TPM), and Stepwise Mutation Model (SMM).

RESULTS AND DISCUSSION

A total of 183 alleles were detected across the 17 loci analyzed. Statistical results of 17 microsatellites loci for Kurdish horse are reported in Table 2. The basic diversity indices (Bottleneck analysis) like observed number of alleles (Ho), expected Hardy-Weinberg Equilibrium (HWE) Heterozygosity (He), and the expected mutation drift equilibrium heterozygosity (H_{eq}) under different mutation models are presented in Table 3.

The number of alleles per marker varied from 7 for HTG7 and HMS6 to 17 for ASB17 (Figure 1) and the across loci Ho value ranged from 0.491 for HTG7 to 0.838 for VHL20 while the He value varied from 0.523 for HTG7 to 0.839 for VHL20 (Table 2). The mean of heterozygosity values for the whole dataset were 0.721 for Ho and 0.752 for He. The average number of alleles per locus was 10.76±0.673 for the Kurdish horse population. How informative a locus is depends upon the Na exhibited by the locus and the frequency distribution of these alleles in the population (Ozkan *et al*., 2009). The mean Na in the present study was higher than that seen in other Iranian horse breed (Seyedabadi *et al*., 2017; Amirinia *et al*., 2007; Rahimi-Mianji *et al*., 2015). All marker loci used in this study were informative, since the average PIC value was calculated at 0.782. The lowest PIC value was for HMS6 (0.708), while the highest value was for ASB17 (0.856).

Microsatellite markers showing PIC values higher than 0.5 are generally considered as informative in horse population. Based on the classification of Botstein *et al*. (1980), PIC> 0.5 is highly informative, 0.25< PIC< 0.5 is middle informative and PIC< 0.25 is slightly

Table 2. Statistical results of microsatellites loci for Kurdish horse.[a]

Loci	Na	Ne	I	H_O	H_e	PIC	F_{IS}	PE	HWE
VHL20	11.000	6.227	1.969	0.838	0.839	0.814	0.001	0.852	ns
HTG4	10.000	3.109	1.437	0.664	0.678	0.808	0.022	0.657	***
AHT4	10.000	4.588	1.717	0.787	0.782	0.802	-0.006	0.763	ns
HMS7	8.000	4.509	1.662	0.782	0.778	0.775	-0.005	0.764	ns
ASB2	12.000	4.900	1.820	0.806	0.796	0.824	-0.012	0.796	***
ASB17	17.000	6.145	2.102	0.837	0.837	0.856	0.000	0.859	***
AHT5	11.000	3.905	1.568	0.716	0.744	0.817	0.037	0.710	***
HMS6	7.000	4.286	1.577	0.768	0.767	0.708	-0.002	0.735	ns
ASB23	15.000	5.462	1.868	0.823	0.817	0.831	-0.007	0.816	***
HTG10	12.000	4.746	1.790	0.779	0.789	0.725	0.013	0.778	ns
HMS3	11.000	4.705	1.729	0.787	0.787	0.818	0.000	0.777	ns
HTG6	8.000	3.107	1.262	0.554	0.678	0.724	0.183	0.576	***
HTG7	7.000	2.095	0.986	0.491	0.523	0.818	0.061	0.445	***
HMS2	11.000	5.087	1.848	0.766	0.803	0.724	0.047	0.810	***
CA425	11.000	2.850	1.392	0.673	0.649	0.801	-0.037	0.635	**
HMS1	8.000	2.956	1.322	0.654	0.662	0.729	0.011	0.584	**
Mean (Sd)	10.562 (0.459)	4.292(0.257)	1.628(0.059)	0.732 (0.082)	0.745 (0.21)	0.785 (0.46)	0.019	0.9998	-

[a] Na: Observed Number of alleles, Ne: Effective Number of alleles, I: Information Index, Ho: Observed Heterozygosity, He: Expected Heterozygosity, PIC: Polymorphic Information Content, F_{IS}: Fixation Index, PE: Exclusion Probabilities, and HWE: Hardy Weinberg Equilibrium.

[DOI: 10.22034/JAST.26.5.973]

Table 3. Bottleneck analysis under three microsatellite evaluation models in Kurdish horse.

Locus	ko	He	IAM				TPM				SMM			
			H_{eq}	SD	DH/sd	Prob	H_{eq}	SD	DH/SD	Prob	H_{eq}	SD	DH/SD	Prob
VHL20	11	0.839	0.644	0.141	1.384	0.008	0.76	0.069	1.147	0.073	0.84	0.035	-0.041	0.415
HTG4	10	0.678	0.609	0.152	0.455	0.402	0.739	0.079	-0.777	0.178	0.825	0.037	-4.025	0.006
AHT4	10	0.782	0.624	0.145	1.095	0.093	0.735	0.078	0.605	0.315	0.824	0.037	-1.135	0.123
HMS7	8	0.778	0.539	0.164	1.451	0.019	0.667	0.11	1.017	0.114	0.779	0.049	-0.015	0.405
ASB2	12	0.796	0.668	0.131	0.98	0.114	0.778	0.062	0.295	0.463	0.854	0.029	-1.975	0.046
ASB17	17	0.837	0.751	0.097	0.89	0.162	0.849	0.04	-0.294	0.315	0.899	0.027	-2.252	0.01
AHT5	11	0.744	0.652	0.131	0.704	0.284	0.759	0.07	-0.211	0.345	0.841	0.035	-2.799	0.02
HMS6	7	0.767	0.509	0.174	1.484	0.02	0.632	0.12	1.119	0.07	0.743	0.062	0.386	0.43
ASB23	15	0.817	0.722	0.116	0.816	0.185	0.826	0.048	-0.19	0.351	0.885	0.021	-3.28	0.008
HTG10	12	0.789	0.67	0.12	0.992	0.125	0.782	0.06	0.123	0.488	0.855	0.029	-2.287	0.033
HMS3	11	0.787	0.647	0.131	1.069	0.102	0.759	0.07	0.403	0.407	0.84	0.034	-1.569	0.078
HTG6	8	0.678	0.547	0.168	0.781	0.231	0.668	0.108	0.094	0.445	0.775	0.052	-1.853	0.059
HTG7	7	0.523	0.516	0.174	0.042	0.428	0.64	0.109	-1.077	0.119	0.744	0.062	-3.58	0.01
HMS2	11	0.803	0.642	0.141	1.142	0.065	0.759	0.072	0.601	0.321	0.838	0.035	-0.99	0.144
CA425	11	0.649	0.652	0.134	-0.021	0.398	0.759	0.077	-1.43	0.089	0.84	0.034	-5.582	0.003
HMS1	8	0.662	0.549	0.165	0.689	0.285	0.678	0.096	-0.168	0.351	0.777	0.051	-2.258	0.039

Ko: Observed alleles, He: Expected HWE Heterozygosity, H_{eq}: Expected mutation drift equilibrium Heterozygosity, IAM: Infinite Allele Model, TPM: Two-Phase Model of Mutation, SMM: Stepwise Mutation Model, SD: Standard Deviation, DH: (Hobs-Hexp).

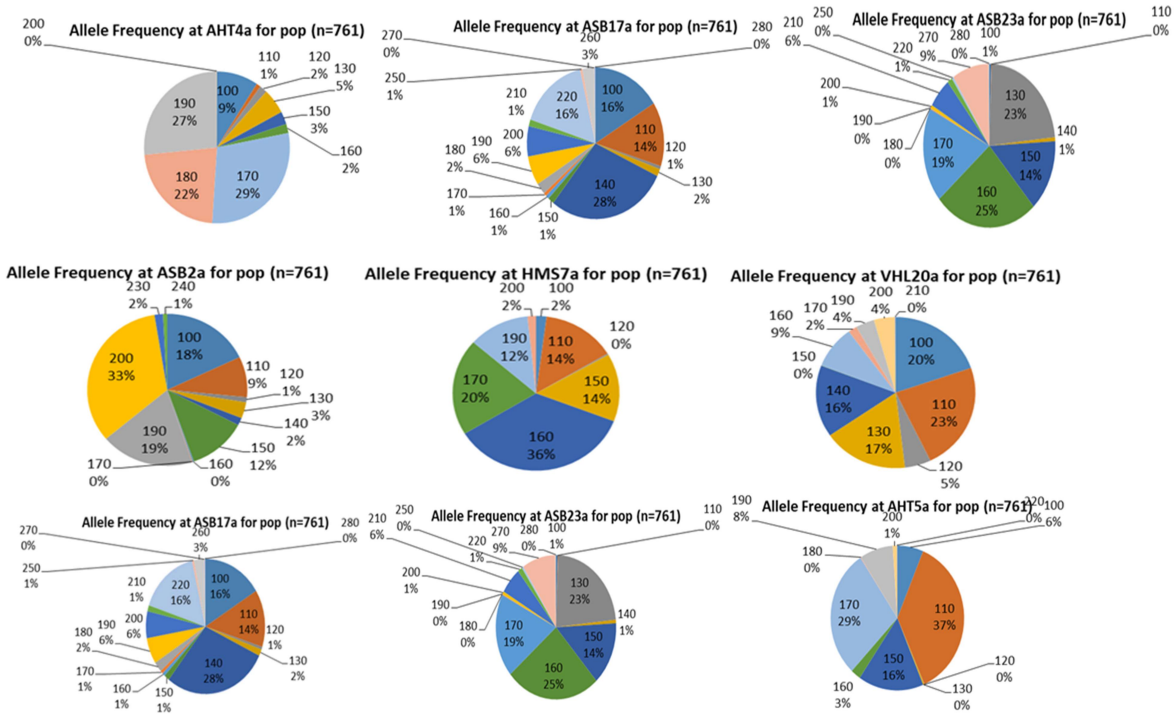

Figure 1. Alleles and allele's frequencies for the some microsatellite markers in Kurdish horse population.

[DOI: 10.22034/JAST.26.5.973]

informative (Botstein *et al*., 1980). The value of PIC similar to heterozygosity depends on the frequency of the allele. For genetic variation studies, researchers recommend using microsatellite markers with PIC values> 0.5, and values below this level are insufficient for parentage verification (Dierks *et al.*, 2007).

The individual PE ranged from 58% at the HMS1 locus to 86% at ASB17 for the registered group of Kurdish breed. Statistically significant deviations from the Hardy–Weinberg equilibrium were found in more than two-thirds of the microsatellite loci. There were many causes of disequilibrium such as small population, selection at or near the genomic locus, non-random mating, genetic drift and inbreeding. (Seyedabadi *et al*., 2017; Mahrous *et al*., 2011; Khanshour *et al*., 2013.

Other studies reported a high level of gene diversity (He) in Turkmen horse (0.73) and Caspian horse (0.74) in Iranian horse breeds using a set of 17 microsatellites (Seyedabadi *et al*., 2017a.b).

The inbreeding coefficient (F_{IS}) is a measure of relative heterozygote deficit and non-random mating in the population. Its value ranges between -1 (all individuals heterozygote), 0 (random association of alleles) and 1 (all individuals homozygote). If inbreeding is avoided, F= 0; negative F indices are usually from selection in favor of the heterozygotes, whereas positive values indicate that the considered population has an inbreeding system of mating (Liu *et al*., 2008).

In this study, the rate of F_{IS} (0.04) indicates a low level of inbreeding in Kurdish horse population, nevertheless, inbreeding index for locus HTG6 (0.183) was high in this population (Table 2).

The heterozygote deficiency (F_{IS}> 0) might be attributed to several factors, namely, sample relatedness, linkage with loci under selection, population heterogeneity, null alleles and inbreeding (Shahsavarani and Rahimi-Mianji, 2010).

When a breeder conducts a selective breeding program, his primary objective is to improve the population, not conserve gene and genotypic frequencies. Inbreeding and genetic drift are inevitable during a selective breeding program, because each act of selection creates a bottleneck event that accelerate the accumulation of inbreeding and magnify genetic drift (Emrani *et al*., 2011). However if the program is well defined, the variability will be preserved.

Bottlenecks influence the distribution of genetic variation within and among populations, thus the genetic effects of reductions in population size requires evaluation. To characterize it, sign, standardized differences and Wilcoxon sign rank tests were utilized. The values of average Heterozygosity (He) and their probabilities (H> He) in the sign test were calculated under three models of microsatellite evolution (IAM, SMM and TPM) and used to measure the expected number of loci with heterozygosity excess (Table 4). If the probability values for each model were less than 0.05, then, null hypothesis was rejected, indicating that the bottleneck event occurred in this model. Under null hypothesis in the sign test, the expected number of loci with heterozygosity excess in Kurdish horse was 10.17 and 9.96 for IAM and SMM, respectively (Table 4). The probability values were 0.00016 and 0.00009, respectively, which thus rejects the null hypothesis. These results indicate that, due to mutation-drift equilibrium, Kurdish horse has undergone a recent genetic bottleneck.

However, the expected number of loci with heterozygosity excess was 9.96 in TPM with probabilities 0.59448, meaning that the null hypothesis was accepted when using the Sign test.

The standardized difference test provided the *T*2 (probability) statistics equal to 3.736 (0.00009), 0.554(0.28964) and – 8.290 (0.00000) for the IAM, TPM and SMM models, respectively. The probability values were less than 0.05 for IAM and SMM, thus rejecting the null hypothesis of mutation-drift equilibrium under IAM and SMM.

[DOI: 10.22034/JAST.26.5.973]

Table 4. Test for null hypothesis under three microsatellite evolution models.

Test/Model	IAM	TPM	SMM
Sign test: number of loci with heterozygosity excess: Expected value (Probability)	10.17 (0.00016)* 1 loci with heterozygosity deficiency and 16 loci with heterozygosity excess.	9.96 (0.59448) 7 loci with heterozygosity deficiency and 10 loci with heterozygosity excess.	9.96 (0.00009)* 16 loci with heterozygosity deficiency and 1 loci with heterozygosity excess
Standard differences test: Ti values (Probability)	3.736 (0.00009)*	0.554(0.28964)	– 8.290 (0.00000)*
Wilcoxon rank test (Probability of heterozygosity excess)	0.00000*	0.22929	0.99997

* Rejection of null hypothesis/bottleneck.

These results indicate that Kurdish horse has undergone a recent genetic bottleneck.

Using the Wilcoxon rank test (a non-parametric test), the probability values were 0.00000 (IAM), 0.22929 (TPM) and 0.99997 (SMM) under these three models, indicating that the null hypothesis is rejected under IAM and the population under study has undergone a recent bottleneck.

In Miyako horses, the probability values for bottleneck models (Infinite allele model: 0.00000; Stepwise mutation model: 0.00026; and Two-phase model: 0.00000) suggested that Miyako horses have experienced a recent genetic bottleneck (Natsuko *et al*., 2017)

Populations exhibiting a significant heterozygosity excess would be considered to have experienced a recent genetic bottleneck. The sign test, standardized differences test, and Wilcoxon test all showed a significant heterozygosity excess under the IAM ($P < 0.05$). Also, the sign test and standardized differences test showed a significant heterozygosity excess under SMM. These results indicate that, due to mutation-drift equilibrium, Kurdish horse has undergone a recent genetic bottleneck. These recent bottleneck in the Kurdish horse population may be due to diminished population size and/or use of a limited number of stallion in the horse breeding farms.

Combined Probability of Exclusion (CPE) value for parentage testing in a horse should be higher than 0.9995 (Tozaki *et al.*, 2001; Seyedabadi *et al*., 2017a), that, in this study, the CPE using 17 microsatellite markers was higher than this amount. Other studies reported similar values of CPE in the Iranian horse (Seyedabadi *et al*., 2017a; Seyedabadi *et al*., 2017b; Amirinia *et al*., 2007).

Seyedabadi *et al* (2017) also reported a total Probability of Exclusion (PE) of 0.999 for 17 microsatellite loci used in Caspian horse parentage test. These results shows that our selected microsatellites have greater power of exclusion. The efficiency of paternity testing depends on the number of loci and the level of the informativeness that microsatellite markers provide. The level of informativeness of a microsatellite marker is specified by its values of heterozygosity, PIC, PE and genetic variation, and these values depend on the number and frequency of alleles in the population (Seyedabadi *et al*., 2017).

However, high levels of Polymorphism Information Content (PIC), heterozygosis, and a low level of inbreeding in Kurdish horse population indicate high genetic variability in this breed that can be used to design breeding programs by horse breeders.

CONCLUSIONS

This paper highlights the genetic structure of the Kurdish horse breed. As these data show, microsatellites are a useful tool for studying the population genetic structure among Iranian horse breeds. Our survey showed imperfection in the individual identification system and the ability of

[DOI: 10.22034/JAST.26.5.973]

genetic markers to resolve this defect. Also, these genetic markers would be of great importance for designing conservation program for Kurdish horse breed.

ACKNOWLEDGEMENTS

The authors acknowledged the reviewers for constructive comments on the manuscript. We gratefully acknowledge all farmers who took part in the present study, giving access to their animals. This work was financially supported by the Animal Science Research Institute of Iran.

REFERENCES

1. Aberle, K. S. and Distl, O. 2004. Domestication of the Horse: Results Based on Microsatellite and Mitochondrial DNA Markers. *Arch. Anim. Breed*., **47**: 517-535.
2. Amirinia, C., Seyedabadi, H. R., Banabazi, M. H. and Kamali, M. A. 2007. Bottleneck Study and Genetic Structure of Iranian Caspian Horse Population Using Microsatellites. *Pak. J. Biol. Sci.,* **10**: 1540-1543.
3. Binns, M. M., Holmes, N.G., Holliman, A. and Scott, A. M.1995. The Identification of Polymorphic Microsatellites Loci in the Horse and Their Use in Thoroughbred Parentage Testing. *Br. Vet. J.,* **151(1)**: 9–15.
4. Botstein, D., White, R. L., Skolnick, M. and Davis, R.W. 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphism. *Am. J. Hum. Genet*., **32**: 314-331.
5. Budiansky, S. 2004. *The Horse.* Microsoft Òencartao Encyclopedia.
6. Coogle, L,. Reid, R., Bailey, E. and Russ, M. 1996. Equine Dinucleotide Repeat Polymorphisms at Loci LEX002, -003, -004, -005, -006, -007, -008, -009, -010, -011, -012, -013 and -014. *Anim. Genet*., **27**: 126–127.
7. Cornuet, J. M. and Luikart, G.1996. Description and Power Analysis of Two Tests for Detecting Recent Population Bottlenecks from Allele Frequency Data. *Genetics,* **144**: 2001-2014.
8. Cozzi M.C., Valiati P., Longeri M., Ferreira C. and Ferreira S.A. 2022. Genetic Variability Trend of Lusitano Horse Breed Reared in Italy. *Animals,* **12(1):** 1-11.
9. Curi, R. A. and Lopes, C. R. 2002. Evaluation of Nine Microsatellite Loci and Misidentification Paternity Frequency in a Population of Gyr Breed Bovines. *Braz. J. Vet. Res Anim Sci.,* **39**: 129-135.
10. Delgado, J.F., De, Andres, N., Valera, M., Gutierrez, J. P. and Cervantes, I. 2014. Assessment of Population Structure Depending on Breeding Objectives in Spanish Arabian Horse by Genealogical and Molecular Information. *Livest. Sci.,* **168**: 9–16.
11. Dierks, C., Lohring, K., Lampe, V., Wittwer, C., Drogemuller, C. and Distl, O. 2007. Genome-Wide Searchfor Markers Associated with Osteochondrosi in Hanoverian Warm Blood Horses. *Mamm. Genome.*, **18**: 739-747.
12. Dimsoski, P. 2003. Development of a 17-Plex Microsatellite Polymerase Chain Reaction Kit for Genotyping Horses. *Croat. Med. J*., **44**: 332-335.
13. Ellegren, H., Johansson, M., Sandberg, K. and Andersson, L. 1992. Cloning of highly Polymorphic Microsatellites in the Horse. *Anim. Genet*., **23**: 133-142.
14. Eggleston-Stott, M., Del Valle, L.A., Bautista, M., Dileanis, D., Wictum, E. and Bowling, A. T. 1997. Nine Equine Dinucleotide Repeats at Microsatellite Loci UCDEQ136, UCDEQ405, UCDEQ412, UCDEQ425, UCDEQ437, UCDEQ467, UCDEQ487, UCDEQ502 and UCDEQ505. *Anim. Genet.*, **28**: 370–371.
15. Emrani, H., Amirinia, C. and Radjaee Arbabe, M. A. 2011. Genetic Variation and Bottleneck in Japanese Quail (*Coturnix japonica*) Strains Using Twelve Microsatellite Markers. *Afr. J. Biotechnol.*, **10(20)**: 4289-4295.
16. FAO. 2022. Commission on Genetic Resources for Food and Agriculture. Status and Trends of Animal Genetic Resource. Twelfth Session, FAO, Rome, Italy.
17. Fotovati, A. 2000. Persian Horse Breeds from Ancient Time to Present and Their Rules in Development of World Horse Breeds. *Asian-Australas. J. Anim. Sci*., **13**: 401–410.
18. Fujifilm Corp., Tokyo, Japan. http:// lifescience.fujifilm.com

[DOI: 10.22034/JAST.26.5.973]

19. Guérin, G., Bertaud, M. and Amigues, Y. 1994. Characterization of Seven New Horse Microsatellites: HMS1, HMS2, HMS3, HMS5, HMS6, HMS7 and HMS8. *Anim. Genet*., **25**: 62.
20. Hedrick, P. W. 1999. Genetics of Population. Second edition, Jones and Bartlett publishers, Sudbury, MA, USA. P375.
21. Kalinowski, S. T., Taper, M. L. and Marshall, T. C. 2007. Revising How the Computer Program CERVUS Accommodates Genotyping Error Increases Success in Paternity Assignment. *Mol. Ecol*., **16**: 1099-1106.
22. Khamisabadi, H. Badbarin, S. and Sharifi, R. S., 2020. Genetic Structure and Assignment Tests of Kurdish Horse Based on Microsatellite Markers. *Modern Genetics Journal (MGJ)*, **14 (4)**: 337–344.
23. Khanshour, A., Conant, A., Juras, R. and Cothran, EG. 2013. Microsatellite Analysis of Genetic Diversity and Population Structure of the Arabian Horse Breeds, *J. Heredity*, **104**(3): 386–398.
24. Liu, G. Q., Jiang, X. P., Wang, J. Y., Wang, Z. Y., Liu, G. Y. and Mao, Y. J. 2008. Analysis of Genetic Diversity of Yangzhou Chicken by Microsatellite Markers. *Int. J. Poult. Sci*., **7(12)**: 1237-1241.
25. Irvin, Z., Giffard, J., Brandon, R., Breen, M. B. K. and Bell, K. 1998.Equine dinucleotide repeat polymorphisms at loci ASB 21, 23, 25 and 37-43. *Anim. Genet*., **29**: 67.
26. Mahrous, K. F., Hassanane, M., Abdel, Mordy, M., Shafey, H. I. and Hassan, N. 2011. Genetic Variations in Horse Using Microsatellite Markers. *J. Genet. Eng. Biotechnol*., **9**: 103-109.
27. Marklund, S., Ellegren, H., Eriksson, S., Sandberg, K. and Andersson, L. 1994. Parentage Testing and Linkage Analysis in the Horse Using a Set of Highly Polymorphic Microsatellites. *Anim. Genet.* **25**: 19–23.
28. Moridi, M., Masoudi, A., Vaez Torshizi, R. and Hill, E. 2013. Mitochondrial DNA D-Loop Sequence Variation in Maternal Lineages of Iranian Native Horses. *Anim. Genet*., **44**: 209–213.
29. Natsuko, S., Teruaki, T., Hironaga, K., Julio, A., Masami M., Ryota M. and Masaki T.2017. Genetic Characterization of the Miyako Horse Based on Polymorphisms of Microsatellites and Mitochondrial DNA. *J. Vet. Med. Sci*., **79(1):** 218–223.
30. Ozkan, E., Soysal, M.I,, Ozder, M., Koban, E., Sahin, O. and Togan, İ. 2009. Evaluation of Parentage Testing in the Turkish Holstein Population Based on 12 Microsatellite Loci. *Livest. Sci*., **124**: 101–106.
31. Peakall, R. and Smouse, P. E. 2006. GenALEx 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Mol. Ecol. Notes,* **6**: 288–295.
32. Rahimi-Mianji, G., Nejati-Javaremi, A. and Farhadi, A. 2015. Genetic Diversity, Parentage Verification, and Genetic Bottlenecks Evaluation in Iranian Turkmen Horse. *Russ. J. Genet*., **51**: 916–924.
33. Sargious, M. A., El-Shawarby, R. M., Abo-Salem, M. E., EL-Shewy, E. A., Ahmed, H. A., Hagag, N. M. and Ramadan, S. I. 2021a. Genetic Diversity of Egyptian Arabian Horses from El-Zahraa Stud Based on 14 Microsatellite Markers. *Slov. Vet. Res.,* **58(2):** 55–62.
34. Sargious, M. A., Ahmed, H. A., El-Shawarby, R. M., Bakery, H. H., Ramadan, S. I., Cothran, E. G. and Farid, A. S. 2021b. Genetic Diversity and Population Assignment of Arabian Horses. *Pak. J. Zool*., **53**: 9-20.
35. Sargious, M. A., El-Shawarby, R. M., Abo-Salem, M. E., EL-Shewy, E. A., Ahmed, H. A., Hagag, N. M. and Ramadan, S. I. 2021c. Genetic Characterization and Parentage Assignment of Egyptian Arabian Horses Based on Two Microsatellite Panels. *Gene Rep.,* **23:** 101117.
36. Seyedabadia, H. R. and Savar Sofla, S. 2017a. Microsatellite Analysis for Parentage Verification and Genetic Characterization of the Turkmen Horse Population. *Kafkas Univ. Vet. Fak. Derg*., **23**: 467-471.
37. Seyedabadib, H. R., Savar Sofla, S. and Kazemi, E. 2017b. Genetic Characterization and Assessment of Demographic Bottleneck in Caspian Horse Population. *Cell Mol. Biol*., **63**: 92-96.
38. Shahsavarani, H. and Rahimi-Mianji, G. 2010. Analysis of Genetic Diversity and Estimation of Inbreeding Coefficient within Caspian Horse Population Using Microsatellite Markers. *Afr. J. Biotechnol*., **9(3)**: 293-299.

[DOI: 10.22034/JAST.26.5.973]

39. Štohlová Putnová, L. and Štohl, R. 2021: The Assignment Success for 22 Horse Breeds Registered in the Czech Republic: The Machine Learning Perspective. *Czech J. Anim. Sci*., **66:** 1–12.
40. Tozaki, T., Kakoi, H., Mashima, S., Hirota, K. I., Hasegawa, T., Ishida, N., Miura, N., Choi-Miura N. H. and Tomita, M. 2011. Population Study and Validation of Paternity Testing for Thoroughbred Horses by 15 Microsatellite Loci. *J. Vet. Med. Sci*., **63**: 1191-1197.
41. Van Haeringen, H., Bowling, A. T., Stott, M. L., Lenstra, J. A. and Zwaagstra, K. A. 1994. A Highly Polymorphic Horse Microsatellite Locus: VHL20. *Anim. Genet*., **25**: 207.

مطالعه تنوع و تنگنای ژنتیکی اسب کرد به کمک ۱۷ جایگاه ریزماهواره

ح. ر. سیدآبادی، و ح ، عمرانی

چکیده

اسب کرد یکی از ارزشمندترین و اصیل ترین ذخایر ژنتیکی ایران است. بر اساس شواهد تاریخی، قدمت این نژاد به بیش از ۲۵۰۰ سال پیش باز می گردد در این جمعیت، تنوع ژنتیکی با استفاده از ۱۷ نشانگر ریزماهواره توصیه شده توسط انجمن بین المللی ژنتیک حیوانات (ISAG) در مورد تجزیه و تحلیل قرار گرفته است DNA.ژنومی از ریشه موی ۷۶۱ راس اسب کرد استخراج شد.قطعات DNA با واکنش Multiplex PCRبا استفاده از پرایمرهای نشاندار شده با فلورسنت تکثیر و با الکتروفورز مویرگی تعیین ژنوتیپ شدند. میانگین تعداد آلل در هر جایگاه ٤/۲۹ بود (از شش آلل در HTG7 تا ۱۷ آلل در.(ASB17 میانگین هتروزیگوسیتی مشاهده شده ۰/۷۲۱ با محدوده ۰/٤۹۱ (HTG7) تا ۰/۸۳۸ (VHL20) بود در حالی که هتروزیگوسیتی مورد انتظار از ۰/۵۲۳ (HTG7) تا ۰/۸۳۹ (VHL20) با میانگین ۰/۷۵۲ بود. مقدار PIC از ۰/۷۰۸ (HMS6) تا ۰/۸۵٦ (ASB17) وبا میانگین ۰/۷۸۲ بود.ضریب همخونی از ۰/۰۱۲ (ASB2) -تا ۰/۱۸۳ (HTG6) با میانگین ۰/۰٤۰ متغیر بود. انحراف از تعادل هاردی واینبرگ (۰/۰۵ (>pدر ۱۱ جایگاه مشاهده شد.قدرت تشخیص 17 (PE) جایگاه ریزماهواره ۰/۹۹۹ بود .اسب کرد برای دو مدل ارزیابی ریزماهواره درآزمون معنی داری و تفاوت استاندارد در مدل آلل نامحدود (IAM)و جهش مرحله ای) (SMM، تنگنای ژنتیکی نشان داد. بر اساس این مطالعه، کارایی هفده نشانگر ریزماهواره ای برای تایید والدین و اصالت اسب کرد مورد تایید می باشد. این نتایج ممکن است برنامه های حفاظتی را برای نژادهای مورد مطالعه تسهیل و تنوع ژنتیکی آنها را حفظ کند.

[DOI: 10.22034/JAST.26.5.973]