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ANIMAL GENETICS

Genetic Diversity, Parentage Verification, and Genetic Bottlenecks Evaluation in Iranian Turkmen Horse[1]

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Abstract—The present study was undertaken to genetically evaluate Turkmen horses for genetic diversity and to evaluate whether they have experienced any recent genetic bottlenecks. A total of 565 individuals from Turkmen horses were characterized for within breed diversity using 12 microsatellite markers. The estimated mean allelic diversity was (9.42 ± 1.78) per locus, with a total of 131 alleles in genotyped samples. A high level of genetic variability within this breed was observed in terms of high values of effective number of alleles (4.70 ± 1.36), observed heterozygosity (0.757 ± 0.19), expected Nei's heterozygosity (0.765 ± 0.13), and polymorphism information content (0.776 ± 0.17). The estimated cumulative probability of exclusion of wrongly named parents (PE) was high, with an average value of 99.96% that indicates the effectiveness of applied markers in resolving of parentage typing in Turkmen horse population. The paternity testing results did not show any misidentification and all selected animals were qualified based on genotypic information using a likelihood-based method. Low values of Wright's fixation index, F_{IS} (0.012) indicated low levels of inbreeding. A significant heterozygote excess on the basis of different models, as revealed from Sign and Wilcoxon sign rank test suggested that Turkmen horse population is not in mutation-drift equilibrium. But, the Mode-shift indicator test showed a normal 'L' shaped distribution for allelic class and proportion of alleles, thus indicating the absence of bottleneck events in the recent past history of this breed. Further research work should be carrying out to clarify the cause of discrepancy observed for bottleneck results in this breed. In conclusion, despite unplanned breeding in Turkmen horse population, this breed still has sufficient genetic variability and could provide a valuable source of genetic material that may use for meeting the demands of future breeding programs.

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INTRODUCTION

Archeological documents hold Iran as one of the oldest domestication and breeding center of the horse in the world. On the basis of geographical localization, at least seven separate populations of native horses, Caspian, Kurdish, Gharabagh, Dareshouri, Baluchi, Asil, and Turkmen (Fig. 1) are distributed in Iran. The Turkmen horse breed is considered as one of the most ancient horse breed in the world. It is breed in the north, east of Caspian Sea, Golestan and Northern Khorasan provinces, mainly in Raz and Jargalan regions at 100 km to Boojnord city, the center of Northern Khorasan province (Fig. 2). Steadily and unfortunately our attentions to these valuable animals decline, because it has not been used so widely as a means of transport in war and peace, communications as well as agricultural progress. This situation has been reduced horse keepers and breeders in the world and then in Iran as the other countries the horse populations are taken the risk of endangered species. The horse breeds that have suffered a substantial decline in population size may have elevated levels of inbreeding which can lead to an overall decline in fitness (inbreeding depression) and increased risk of extinction. These effects are believed to be the result of increased homozygosity leading to the increased expression of deleterious recessive alleles. In recent years, the issue of maintaining biodiversity as a major element of environment preservation has been discussed globally. To this end, preserving biodiversity of indigenous species, especially of those of economic interest must represent a relevant aspect in the scientific research activity. For four decades now, FAO (Food and Agriculture Organization) has included in its agenda the problem of preserving, evaluating and using animal genetic resources [1]. One of the difficulties in implementing a selective breeding program in horse stocks is maintaining pedigree information. A correct pedigree is important for any domestic horse breed whether rare or not. For breeds that are common, an incorrect pedigree can frustrate breeding plans for selective improvement of the breed. For rare breeds, correct pedigrees are important for developing

[1] The article is published in the original.

breeding strategies. Another difficulty in managing a selective breeding program is loss of genetic variability and increases in inbreeding as a result of the inadvertent mating of related individuals. The effects of inbreeding in horses will result in a decrease in genetic diversity, which will limit the potential for genetic gain from artificial selection. Once reliable pedigree information is available, mating can be arranged in order to minimize inbreeding [2]. In livestock, the use of paternity testing primarily aims to confirm the relationship between individuals and to help in criminal investigations [3–8]. Such assays have wide acceptance among the entities responsible for keeping pedigree registries. Genetic characterization is the first step in the conservation of breeds. This information could serve as a guideline for future breeding strategies [4, 9]. Genetic analysis using molecular markers can provide valuable information about current levels of genetic variation. This information can then be used to make predictions about how particular management strategies will influence genetic variation in the herd.

As an introduction of foreign horse breed in the country and also lack of sound breeding programs for conservation of native horse breed, presently only a few hundred true Turkmen horses are in existence. To avoid further loss of potential unique genes and to preserve the genetic diversity within breed, characterization of genetic structure based on molecular genetic markers is of priority. Application of microsatellite markers in assessment of the biodiversity levels in Iranian Turkmen horses breed has not been done yet and this is the first research work for characterization of genetic structure and parentage verifications based on tests with ISAG's (international society for animal genetics) standards microsatellite paternity markers in this breed.

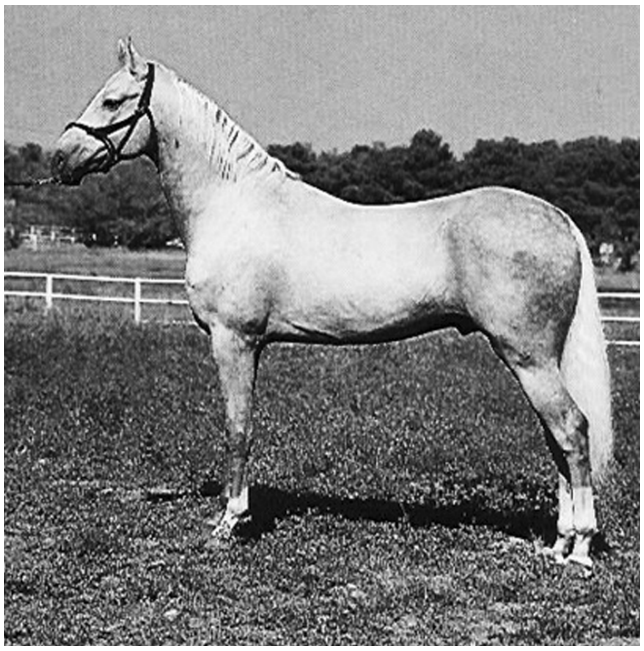

Fig. 1. A typical Turkmen horse [copyright Mobarak-Andish Institute].

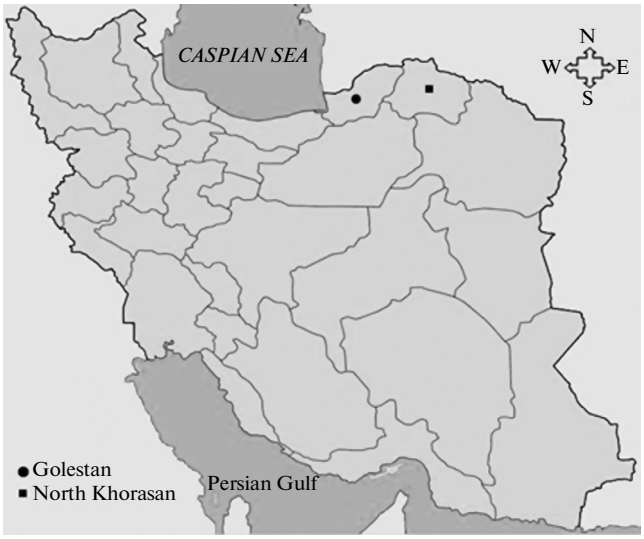

Fig. 2. Main geographical location of the Turkmen horse breed sampled at the present study.

MATERIALS AND METHODS

Genomic DNA Isolation

The animals were randomly chosen by their breeders who were able to document their pedigrees (parents, offspring). Blood samples were collected from 565 individuals of Turkmen horses in EDTA treated tubes as an anticoagulant. The samples were kept in a cooling chain, transferred to the laboratory and stored at −20°C until further analysis. Genomic DNA was extracted using NucleoSpin Blood Quick Pure Kit (Macherey & Nagel, Düren, Germany) according to the manufacture's recommendations.

Microsatellite Markers and Multiplex PCR

Turkmen horses were characterized for within breed diversity using 12 microsatellite markers. Multiplex PCR reactions were carried out in a total volume of 30 μL, containing 5 μL 10× PCR buffer, 0.17 mM dNTP-mix, 2.5 mM $MgCl_2$, 100 ng DNA, and 2 units *Taq* DNA polymerase (ABGene). In the 8-plex PCR the following microsatellite markers, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, AHT4, and VHL20 [10–14] were used (Table 1). The markers AHT5, ASB2, HMS2, and HTG10 [10, 12, 13, 15] were used in the 4-plex PCR with a total volume of 15 μL (Table 2). For each PCR reaction one primer was 5'-end labeled with commercially fluorescent labels TAMRA, FAM, and HEX. The thermo-cycling conditions included an

Table 1. Primer sequences were used in an 8-plex PCR for amplification of the microsatellite loci in Turkmen horse population

Locus	Chromosome number	Fluorescent label	Primer sequences [5'–3']	Reference
HMS3	9	TAMRA	F: CCA ACT CTT TGT CAC ATA ACA AGA R: CCA TCC TCA CTT TTT CAC TTT GTT	[10]
HMS6	4	HEX	F: GAA GCT GCC AGT ATT CAA CCA TTG R: CTC CAT CTT GTG AAG TGT AAC TCA	[10]
HMS7	1	FAM	F: AAC CGC CTG AGC AAG GAA GT R: GCT CCC AGA GAG TTT ACC CT	[10]
HTG4	9	FAM	F: CTA TCT CAG TCT TGA TTG CAG GAC R: CTC CCT CCC TCC CTC TGT TCT C	[11]
HTG6	15	HEX	F: CCT GCT TGG AGG CTG TGA TAA GAT R: GTT CAC TGA ATG TCA AAT TCT GCT	[11]
HTG7	4	TAMRA	F: CCT GAA GCA GAA CAT CCC TCC TTG R: ATA AAG TGT CTG GGC AGA GCT GCT	[12]
AHT4	24	FAM	F: AAC CGC CTG AGC AAG GAA GT R: GCT CCC AGA GAG TTT ACC CT	[13]
VHL20	30	FAM	F: CAA GTC CTC TTA CTT GAA GAC TA R: AAC TCA GGG AGA ATC TTC CTC AG	[14]

Table 2. Primer sequences were used in a 4-plex PCR for amplification of the microsatellite loci in Turkmen horse population

Locus	Chromosome number	Fluorescent label	Primer sequences [5'–3']	Reference
AHT5	8	HEX	F: ACG GAC ACA TCC CTG CCT GC R: GCA GGC TAA GGG GGC TCA GC	[13]
ASB2	15	HEX	F: CCA CTA AGT GTC GTT TCA GAA GG R: CAC AAC TGA GTT CTC TGA TAG G	[15]
HTG10	21	TAMRA	F: CAA TTC CCG CCC CAC CCC CGG CA R: TTT TTA TTC TGA TCT GTC ACA TTT	[12]
HMS2	10	TAMRA	F: CTT GCA GTC GAA TGT GTA TTA AAT G R: ACG GTG GCA ACT GCC AAG GAA G	[10]

initial denaturation at 95°C for 15 min, followed by 30 cycles of 1 min at 94°C, 1 min at 60°C for annealing temperature and 1 min at 72°C. A final elongation step was carried out at 72°C for 10 min. The cycling conditions were the same in both multiplex PCR. DNA sequencing was performed with the Dye Primer Cycle Sequencing Ready Reaction-21-M13 kit (Applied Biosystems) following the supplied protocol. The sequencing products were separated in an automated ABI 377 DNA sequencer. DNA fragment sizes were determined with GeneScan (version 2.1) and the genotypes of the horses were established using Genotyper (version 2.0) software.

Data Analysis

Expected heterozygosity is a useful measure of informativeness of a locus. The loci with expected heterozygosity of 0.5 or less are in general not very useful for large-scale parentage analysis. The overall expected heterozygosity across all loci which are simply the arithmetic average of the average heterozygosities at each locus. Cervus software (version 2.0) [16] was used to calculate allele frequencies, expected and observed heterozygosities and parentage assessment [17]. Polymorphic information content (*PIC*) is a measure of informativeness related to expected heterozygosity. The *PIC* value of the microsatellite loci

was calculated on the basis of observed allele frequencies [18].

$$PIC_i = 1 - \sum_{j=1}^{n} P_{ij}^2,$$

where P_{ij} is the frequency of the jth allele for the ith marker, and summed over n alleles. The effective allele number (estimates the reciprocal of homozygosity) was calculated according Kimura and Crow [17], using POPGENE software [19].

$$N_e = \frac{1}{H_i} = \frac{1}{\sum_{i=1}^{n} P_i^2},$$

where N_e is the effective allele number and H_i is the homozygosity of each locus. The exclusion probability of exclusion (*PE*) and the accuracy of the paternity assessment were estimated as described by Marshall et al. [20]. In brief, for homozygous AA, exclusion occurs if the candidate parent is neither AA nor any of the $k - 1$ heterozygotes AX. For heterozygous offspring AB, exclusion occurs if the candidate parent is neither AA, BB, any of the $k - 1$ heterozygotes AX nor any of the $k - 1$ heterozygotes BX. The heterozygous candidate parent AB occurs both in the set of genotypes AX and the set of genotypes BX. Defining the probability of genotypes AA, AB, AX and BX as $p(ii)$, $p(ij)$, $p(ix)$ and $p(jx)$ respectively and summing across all pair wise genotypic combinations, the average probability of exclusion (*PE*) at locus E with k codominant alleles was calculated with the following model:

$$PE = 1 - \left\{ \sum_{i=1}^{k} \sum_{x=1}^{k} p(ii)p(ix) + \frac{1}{2} \sum_{i \neq j}^{k} \sum_{x=1}^{\bar{k}} p(ij)p(ix) + p(jx) - p(ij) \right\}.$$

The combined average probability of exclusion (*CPE*), across n independently inherited loci was calculated based on Marshall et al. [20]:

$$CPE = 1 - \prod_{E=1}^{n} [1 - PE].$$

In order to measure an individual's inbreeding coefficient, detailed pedigree information is required. In recent years, the developments of molecular techniques offer the opportunity to determine accurate pedigrees in populations. Such approaches have the advantages that pedigree information is not required. The within population inbreeding (heterozygote deficiency) was calculated as the difference between observed and expected heterozygosity [21].

$$F_{IS} = \left[\frac{H_o - H_e}{H_e} \right],$$

where F_{IS} is the coefficient of inbreeding, the deviation of the observed heterozygosity of an individual relative to the heterozygosity expected under random mating (Hardy–Weinberg equilibrium); H_o is the observed frequency of heterozygous individuals and H_e is the expected frequency of heterozygous in the population. When $F_{IS} > 0$ it signifies more inbreeding than is expected at random, and when $F_{IS} < 0$ it indicates that inbreeding occurred less often than would be expected at random. The probability of random mating in the population was estimated by chi-square (χ^2) and likelihood ratio tests (G^2) to examine Hardy-Weinberg equilibrium using Cervus software program. The POPGEN program was used to examine linkage disequilibrium (LD) among microsatellite loci. This program estimates correlation coefficients according to Weir [22]. Bottleneck events were tested using two methods. The first method consisted of three excess heterozygosity tests using three possible mutation models; infinite allele model [IAM, 17], stepwise mutation model [SMM, 23] and two phase model [TPM, 24] which tests the population for presence of any significant heterozygotic excess. TPM analysis was run with mutation model proportions of 95% SSM and 5% IAM as recommended by Piry et al. [25]. The default parameters of 30% variance and 1,000 iterations were used for TPM estimates. The proportion of stepwise mutations in TPM was set at 90%, as recommended by Cornuet and Luikart [26]. The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart et al. [27]. Loss of rare alleles in bottlenecked populations is detected when one or more of the common allele classes have a higher number of alleles than the rare allele class. These two methods were conducted using Bottleneck computer program [25].

RESULTS AND DISCUSSION

All loci were observed to be polymorphic and a total of 113 distinct alleles were detected across 12 microsatellites in Turkmen horse population. The numbers of alleles was ranged from 6 (HTG7) to 12 (HTG10), with a mean of 9.42 ± 1.78 per locus (Table 3). FAO has recommended a minimum of four distinct alleles per locus for proficient judgment of genetic differences between breeds [21]. Consequently, all 12 microsatellite markers used in this study showed sufficient polymorphism for evaluating genetic variation within the studied horse population. The mean effective number of alleles in the Turkmen horse population was 4.70 ± 1.36, ranging from 2.51 (HTG4) to 6.97 (VHL20). Genetic markers showing *PIC* values higher than 0.5 are normally considered as informative in population genetic analyses [18]. All marker loci employed in this study were informative since the average *PIC* value calculated at 0.776 ± 0.17 (Table 4). The mean number of alleles per microsatellite locus in Turkmen horses was higher to that reported in the Asian, European,

Table 3. Observed (N_o) and effective (N_e) number of alleles, allele size range (bp), chi-square (χ^2) and likelihood ratio tests (G^2) for Hardy–Weinberg equilibrium (HWE) at each microsatellite locus in Turkmen horse population

Locus	Number of alleles		Allele size range range		Probability	
	N_o	N_e	min	max	χ^2	G^2
AHT4	10	5.27	148	166	0.005	0.069
AHT5	11	4.80	128	150	0.000	0.002
ASB2	11	6.17	230	262	0.059	0.032
HMS2	11	5.30	216	238	0.076	0.964
HMS3	9	4.82	146	168	0.009	0.162
HMS6	8	4.10	157	171	0.901	0.778
HMS7	9	5.19	169	185	0.000	0.015
HTG10	12	5.36	91	113	0.007	0.003
HTG4	7	2.51	129	141	0.013	0.016
HTG6	9	3.31	84	106	0.760	0.949
HTG7	6	2.57	116	128	0.000	0.000
VHL20	10	6.97	87	105	0.409	0.528
Mean ± Sd	9.42 ± 1.78	4.70 ± 1.36	–	–	–	–

Table 4. Observed (H_o) and expected heterozygosity (H_e), probability of paternity exclusion (*PE*: first and second parent), polymorphism information content (*PIC*), gene diversity estimates (F_{IS}) and Shannon's information index (*I*) across 12 microsatellite loci in Turkmen horse population

Locus	Heterozygosity		Paternity *PE*		Diversity index		
	H_o	H_e	PE^1	PE^2	*PIC*	F_{IS}	*I*
AHT4	0.783	0.810	0.433	0.610	0.769	0.034	1.835
AHT5	0.752	0.791	0.478	0.650	0.799	0.051	1.748
ASB2	0.853	0.835	0.586	0.741	0.858	–0.020	2.002
HMS2	0.812	0.811	0.490	0.662	0.809	0.001	1.814
HMS3	0.766	0.793	0.477	0.651	0.7996	0.035	1.744
HMS6	0.750	0.756	0.392	0.571	0.746	0.008	1.520
HMS7	0.786	0.804	0.525	0.692	0.825	0.023	1.786
HTG10	0.839	0.814	0.486	0.658	0.803	–0.031	1.909
HTG4	0.602	0.602	0.296	0.468	0.656	0.001	1.305
HTG6	0.714	0.698	0.424	0.602	0.767	–0.023	1.351
HTG7	0.582	0.611	0.282	0.427	0.642	0.048	1.148
VHL20	0.846	0.856	0.552	0.714	0.841	0.013	2.076
Mean ± Sd	0.757 ± 0.19	0.765 ± 0.13	0.9993	0.9999	0.776 ± 0.17	0.012 ± 0.02	1.69 ± 0.29
			Cumulative				

Sought and North American [28] and Arabian horse breeds [5].

The values of diversity measures at each locus are given in Table 4. The observed heterozygosity values across the 12 polymorphic marker loci ranged from 0.582 (HTG7) to 0.852 (ASB2), with a mean of 0.757 ± 0.19. The expected heterozygosity varied from 0.611 (HTG7) to 0.856 (VHL20), with a mean of 0.765 ± 0.13. The allele numbers and heterozygosity levels observed across the 12 studied loci indicate presence of reasonably high level of genetic variability in Turkmen horse population. The internal genetic structure of the studied Turkmen horse population revealed an increased allelic diversity for 12 microsatellites in relation to horse populations described in the literatures. With the same set of microsatellite markers,

Georgescu et al. [29] investigated structure of indigenous Romanian Hucul horse breed. The observed and expected heterozygosity per breed ranged from 0.662 and 0.676 (Hucul) to 0.759 (Thoroughbred) and 0.741 (Romanian Sport Horse), respectively. Vostry' et al. [30] investigated intra-line and inter-line genetic diversity in sire lines of the Old Kladruber horse based on 16 microsatellite loci. The estimated average for the observed heterozygosity across microsatellite loci was 0.637, while the estimated mean value of genetic diversity was 0.678. The heterozygosity observed for each of the microsatellites ranged from 0.374 to 0.82. Genetic diversity among Sanfratellano and three other Italian horse breeds were assessed using a set of 11 microsatellites. All the breeds showed a high level of gene diversity (H_e) ranging from 0.71 in Sicilian Oriental Purebred to 0.81 in Sicilian Indigenous [31]. Giacomoni et al. [32] studied Genetic diversity in the Pantaneiro horse breed using 10 microsatellite DNA markers. Values of H_o for each population for all the loci examined ranged from 0.49 (Nova Esperança) to 0.68 (Promissão). Advi and Banos [33] investigated Genetic diversity in the Greek Skyros horse using 16 microsatellite loci. Theoretical heterozygosity levels ranged from 0.41 to 0.79 with an average of 0.63 (±0.06), whereas mean observed heterozygosity was 0.66 (±0.06). The average expected heterozygosity value of 0.73 was measured in Korean native horse breed [34]. Tozaki et al. [35] investigated genetic variation among Asian horses using 22 microsatellite loci. It has been showed an average heterozygosity between 0.34 in the Tokara population of Japanese horses to 0.77 in the Bajandzargalan population of Mongolian horses. Khanshour et al. [5] reported the mean observed and expected heterozygosity of 0.694 and 0.712 for registered and 0.711 and 0.759 for nonregistered Arabian horse, respectively. The average heterozygosity observed in Turkmen horse population was higher than to the observed one in the Akhal-teke horse breed [36].

At the present study, the average Shannon's information index and *PIC* value for all loci assessed from the allele frequency data were 1.690 ± 0.29 and 0.776 ± 0.17, respectively. All the loci used in our study had *PIC* values higher than the required value of 0.60, pointing to the high degree of informativeness of these markers in evaluation of genetic diversity. Out of 12 microsatellite markers used at the present study, only ASB2, HTG10 and HTG6 loci showed higher observed heterozygosity than the expected values, whereas for the rest of the loci and excess of heterozygotes was calculated (Table 4). Heterozygote deficiency analysis revealed significant deviations from HWE at some of the loci ($P < 0.05$). It is, however, difficult to imagine the exact basis of this departure, although the presence of low frequency of null alleles segregating at these loci may be a possible reason. The neutrality of each marker tested by Ewens Watterson test [37] suggested that all loci except ATH4, ASB2, HMS7, and VHL20 were neutral indicating that homozygosity in Turkmen horse population might not be an outcome of selection (data not shown, available on request) . The within-population inbreeding estimate (F_{IS}) ranged between −0.002 and 0.048 with an average of 0.012. Thus, on an average, deficiency (1.2%) of heterozygote existed in the Turkmen horse population. All the 12 examined microsatellite markers, except ASB2, HMS2, HTG10, and HTG6 contributed to this observed heterozygote shortage. It is right time to initiate planned and organized breeding, as F_{IS} suggests moderate level of inbreeding in Turkmen horse population. The inbreeding detected in Turkmen horse population may be as a result of diminished population size, small breeding areas and/or with an insufficient number of breeding males in the breeding region. However, high levels of heterozygosity, *PIC* and low level of heterozygosity deficiency in Turkmen horse population reflect high genetic variability that can be exploited by horse breeders for planning breeding strategies and prioritizing the breed for its conservation.

The paternity testing results did not show any misidentification and all selected foals were qualified by compatibility of 12 microsatellite markers using a likelihood-based method. Table 4 shows the exclusion probabilities (*PE*) for each locus which are given the genetic information of both parents, was calculated based on allele frequency [38]. The obtained PE value for each polymorphic locus at the present study was ranged between 0.282 for HTG7 and 0.741 for ASB2 locus, with a combined average probability of exclusion of 0.999. Other studies reported similar values of cumulative probability of paternity exclusion (0.999) in Thoroughbred and Arabian horse [3, 5, 39]. The effectiveness of paternity testing is not only depends on the number of used microsatellites but on the level of informativeness that these markers provide. The level of informativeness of a microsatellite marker is determined by its values of heterozygosity, *PIC*, *PE* and gene diversity and these values are dependent on the number of alleles and on the frequency distribution of these alleles in the population [40]. The heterozygosity, *PIC* and *PE* values obtained for microsatellite markers used in our study indicated the high level of informativeness of these markers on the studied samples. So, these microsatellite markers (ISAG recommended markers for horse paternity), showed to be adequate to perform a paternity testing and for individual characterization on the sample population of Turkmen horse breed. Cornuet and Luikart [26] described heterozygosity excess as a method for detection of bottleneck. This method is based on the hypothesis that populations experiencing recent reduction in size develop an excess of heterozygosity at selectively neutral loci relative to the heterozygosity expected at mutation-drift equilibrium. Because bottlenecks influence the distribution of genetic variation within and among populations, the genetic effects of

Table 5. Results of the bottleneck detection tests on the Turkmen horse breed from the Sign and the Wilcoxon rank test

Test	IAM		TPM		SSM	
	HE		HE		HE	
	H_e/H_o	*Pr*	H_e/H_o	*Pr*	H_e/H_o	*Pr*
Sign test	7.09/12	0.001*	7.06/10	0.071	6.98/2	0.004*
Wilcoxon test		0.001*		0.002*		0.99

HE, Heterozygosity excess; H_e, expected number of loci with heterozygosity excess; H_o, observed number of loci with heterozygosity excess; *Pr*, probability. * Rejection of null hypothesis.

reductions in population size require evaluation. In the Sign test, under three models (IAM, TPM, and SSM) of microsatellite evolution, the expected number of loci with heterozygosity excess were 7.09, 7.06, and 6.98 for IAM, TPM, and SSM models, respectively (Table 5). The probability value obtained under this test was 0.001 (IAM), 0.071 (TPM), and 0.004 (SSM), and thus rejects the null hypothesis in both IAM and SSM models, indicating the population has undergone a recent genetic bottleneck. Using the Wilcoxon rank test the probability values were 0.001 (IAM), 0.002 (TPM), and 0.99 (SMM), indicating that the null hypothesis also is rejected in both IAM and TPM models, demonstrating that the Turkmen horse breed has experienced a recent genetic bottleneck. The results of the standardized differences test are not reported because 20 polymorphic loci are required for this test [26]. When a population goes through a bottleneck rare alleles tend to be lost and the average number of alleles per locus, or allelic diversity, is reduced. Heterozygosity, however, is not reduced proportionally, because rare alleles contribute little to heterozygosity [21]. The analysis of allele frequency distribution is shown in Fig. 3. The microsatellite alleles were organized into 10 frequency classes, which permit checking whether the distribution followed the normal L-shaped form, where alleles with low frequencies are the most numerous. The observed allele frequency distribution at the present study indicates the absence of bottleneck events in the recent past history of Turkmen horse breed. As assumed by Luikart et al. [27], populations that have experienced a long period of bottleneck should have high rates of inbreeding and low levels genetic variation. However, these were not observed in Turkmen horse population. The low levels of inbreeding (0.012 ± 0.027) and high level of observed allele number (9.42 ± 1.78) and heterozygosity (0.757 ± 0.19) might be due to the short time frame of the population bottleneck in Turkmen horse breed. However, further research work should be carrying out to clarify the cause of discrepancy observed for bottleneck results in this breed. The same discrepancy in bottleneck results has been reported in Portuguese Sorraia horse breed [36]. The present work contributes to the knowledge of population structure and assessment of existing genetic diversity in the Turkmen horse population. Further genetic analysis of other Iranian horse breeds and their comparisons need to be carried out to determine the phylogenic evolutionary relationships and genetic distances among the indigenous equine breeds. High priority action is necessary considering the breeding practices exercised by local horse owners, which may further weaken the diversity levels through the breeding of relatives. To make a start, providing the genetic management program through the Turkmen Horse Breeding and Consulting Corporation (THBC) is a great opportunity.

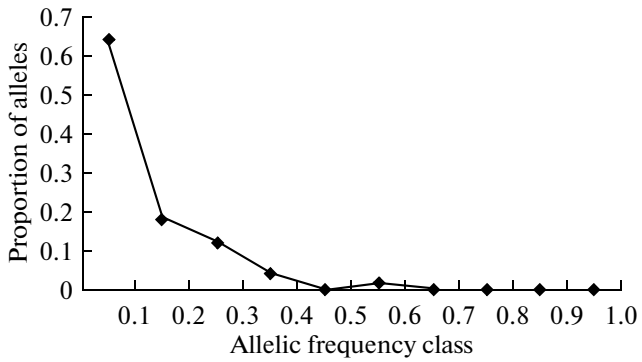

Fig. 3. Normal L-shaped curve of distribution of proportion of alleles in different allelic frequency classes.

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