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Genetic Diversity of Arabian Horse from Stud "Borike" (Bosnia and Herzegovina) Using Microsatellite Markers

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ABSTRACT

The first evaluation of genetic diversity of Arabian horse from stud "Borike" (Bosnia and Herzegovina) was performed in this study. Genomic DNA was extracted from whole blood collected from 20 Arabian horses. A total of 17 microsatellite loci amplified by PCR. Average number of alleles per locus was 4.29, varying from 3 (HMS7, AHT5, HMS3, HMS1 and CA425) to 8 (HTG6). The observed heterozygosity ranged from 0.4 (HMS1) to 0.8 (ASB23, HTG7) with a mean of 0.6291 while expected heterozygosity ranged from 0.5088 (HMS1) to 0.7938 (HTG6) with a mean of 0.6529. The PIC values fluctuated from 0.4023 (HMS1) to 0.7643 (HTG6) with a mean of 0.5901. The inbreeding coefficient ranged from -0.0082 (CA425) to 0.4213 (HTG10) with a mean of 0.0621. Deviation from Hardy-Weinberg equilibrium ($p<0,05$) was found in five loci (HTG6, HTG10, HTG7, ASB17 and LEX3). HTG6 locus showed the highest, while the locus HMS1 showed the lowest genetic diversity. The results suggest that major loss of genetic diversity does not affect the population of Arabian horse sampled from stud “Borike” and indicate that Arabian horse from Bosnia and Herzegovina belongs to the modern Arabian horse populations.

INTRODUCTION

Arabian horse is one of the oldest and the most influential breeds in the World, with worldwide distribution. It has been involved in the genetic conformation of many other horse breeds, such as the Thoroughbred and the Lipizzan [1,2]. Arabian horse has also been involved in the formation of Bosnian and Herzegovinian mountain horse [3]. Historical records indicate introduction of Arabian horses into Bosnia and Herzegovina (B&H) during Ottoman Empire era. The stud “Borike” was established in 1895 and Arabian horses have been breed and rose in B&H ever since. Nowadays, Arabian horse population from B&H is well adapted to ecological and geographical conditions of this region [3].

The discovery of polymorphism in short tandem repeat (STR or microsatellites) loci and the introduction of polymerase chain reaction (PCR) methodology have led to the establishment of extremely powerful “universal” method for individual identification and for parentage control in humans and animals [4]. Microsatellites are highly polymorphic genetic markers with co-dominantly inherited alleles that are relatively easy to score. Microsatellites are repeat regions of two to seven nucleotide units that occur primarily in non-coding regions of DNA [2]. Microsatellites have been employed to construct linkage maps, examine population genetic structure, genetic variation, molecular evolution studies, and studies of gene flow, forensic sciences and as parentage testing markers [2,5].

Genetic diversity of Arabian horse are has been investigated by using microsatelites, including Algerian Arab horse [6], Spanish Arab horse [7], Arabian horses from Charmahal-va-bakhtiari province [4], Romanian Arabian horse [8], Polish Arabian horse [9], Syrian Arab horse [1], Middle Eastern Arabian and Western Arabian horse populations [10], Iranian Arab horse [2] and European Anglo-Arab horse [5].

However, genetic diversity of Arabian horse from B&H using microsatellites has not been investigated yet. In this paper we report the results of the first analysis of genetic diversity of Arabian horse population from B&H by using 17 microsatellite markers recommended by the International Society for Animal Genetics (ISAG).

MATERIAL AND METHODS

The sample included 20 Arabian horses raised in stud "Borike", B&H. DNA for genotyping analysis was extracted from whole blood. Using sterile needles and EDTA vacuum containers, the blood was drawn from *v. jugularis* of each animal. Salting out method of genomic DNA isolation, originally developed for human blood [11], was modified and adjusted for horse blood and to our laboratory conditions (3 ml of blood; 10 ml of Lysis buffer; 4 ml of PBS; 4 ml of Kern-lysis buffer; 150 µl of 20% SDS; 100 µl of Qiagen protease and 0,5 ml 6 M NaCl). The concentration of DNA extract was determined by spectrophotometry, using a spectrophotometer UV mini -1240 (*Shimadzu*). Nuclear DNA polymorphism was evaluated utilizing improved StockMarks® Equine Genotyping Kit (Applied Biosystems) designed for simultaneous amplification of 17 horse microsatellite loci. The size of amplified microsatellite fragments was analyzed using ABI Prism™ 310 Genetic Analyzer. Determination of the amplified fragments size was performed using GeneMapper ID v3.2 software. Allele size range, major allele frequency (f_M), number of different alleles (A_N), polymorphism information content (PIC) [12], observed heterozygosity (H_O), expected heterozygosity (H_E) [13], inbreeding coefficient (f) [14] and deviation from Hardy-Weinberg equilibrium (HWE) [15] was calculated using POWERMARKER 3.25 [16]. Number of effective alleles (A_E) was estimated as $1/\sum pi^2$, where p is allele frequency at given locus. Simple ratio between number of effective alleles and number of detected alleles (A_E/A_N) and major allele frequency index (If_M) were calculated as suggested by Pojskic [17]. Ratio indicates possible disproportion between the effective number of alleles and the number detected by direct counting. Major allele frequency index shows how greater the larger frequency is than the expected one, assuming equal frequencies of all detected alleles at a given locus. It is expressed as $f_M/(1/A_N)$ where; f_M represents the major allele frequency and A_N is the number of detected alleles at a given locus. Number of different genotypes (G_N) was estimated, since maximum number of possible genotypes (G_E) were calculated as $k(k+1)/2$, where k is number of detected alleles at given locus. Also, ratio between the number of detected genotypes and maximum number of possible genotypes (G_N/G_E) was estimated.

RESULTS

In this first evaluation of genetic diversity of Arabian horse from B&H genetic analysis was performed using 17 microsatellite loci. All the loci, reported in the study were amplified successfully. Results for allele size range, major allele frequency (f_M), major allele frequency index (If_M), number of different genotypes (G_N), maximum number of possible genotypes (G_E), G_N/G_E ratio, number of detected alleles (A_N), number of effective alleles (A_E), A_E/A_N ratio, expected heterozygosity (H_E), observed heterozygosity (H_O), polymorphism information content (PIC), inbreeding coefficient (f) and deviation from Hardy-Weinberg equilibrium (HWE) are given in Table 1. A total of 73 alleles were observed over all the loci. Alleles' size at individual loci varied between 79 and 251bp. PCR product size varied from 79–97 bp at locus HTG6 to 237–251 bp at locus ASB2. The number of alleles per locus ranged from 3 (HMS7, AHT5, HMS3, HMS1 and CA425) to 8 (HTG6) with a mean of 4.29. The highest ratio between the number of effective alleles and the number of detected alleles was established at ASB23 (0.9804), indicating that all detected alleles at a given loci were effective. The lowest value was observed at HMS2 locus (0.5128) showing that only half of the detected alleles were effective. Mean value for this ratio was 0.6709. The largest value of major allele frequency index was detected for HMS2 (2.7500) but with no statistical significance (P>0.05). The lowest value was detected for ASB23 locus (1.2000), also with no statistical significance (P>0.05) (Table 1.). Mean value of this parameter was 1.9162. The highest number of detected genotypes was observed for HTG6 locus (12) and the lowest for HMS1 locus (4). Average number of the detected genotypes across all loci was 7.12 (Table 1). Marked disproportion between the number of detected genotypes and maximum number of possible genotypes was detected for HTG6 locus (0.3333). On the other hand, the number of detected allele at ASB23 locus reached maximum number of possible genotypes (G_N/G_E=1.0000). The observed heterozygosity ranged from 0.4 (HMS1) to 0.8 (ASB23, HTG7) with a mean of 0.6291 and expected heterozygosity ranged from 0.5088 (HMS1) to 0.7938 (HTG6) with a mean of 0.6529.

Observed heterozygosity at loci AHT4, HMS7, AHT5, ASB23, ASB2, HTG7, HMS3, HMS2 was higher than the expected value. The PIC values fluctuated from 0.4023 (HMS1) to 0.7643 (HTG6) with a mean of 0.5901. The inbreeding coefficient ranged from-0.0082 (CA425) to 0.4213 (HTG10) with a mean of 0.0621. Statistically significant deviation from Hardy-Weinberg equilibrium (P<0.05) was found at five loci (HTG6, HTG10, HTG7, ASB17 and LEX3) **(Table 1).**

DISCUSSION

This paper elaborates the first data on genetic diversity of Arabian horse population from B&H. All the loci included in Stock Marks Kit, which was used for profiling, are dinucleotide repeats. Alleles' sizes at individual loci varied between 74 and 268bp [18].

Table 1. Allele size range, major allele frequency (f_M), major allele frequency index (If_M), number of different genotypes (G_N), maximum number of possible genotypes (G_E), G_N/G_E ratio, number of detected alleles (A_N), number of effective alleles (A_E), A_E/A_N ratio, expected heterozygosity (H_E), observed heterozygosity (H_O), polymorphism information content (PIC), inbreeding coefficient (f) and deviation from *Hardy-Weinberg* equilibrium (HWE) at 17 microsatellite loci in Arabian horses from stud "Borike" (B&H).

Marker	Al. size	f_M	If_M	G_N	G_E	G_N/G_E	A_N	A_E	A_E/A_N	H_E	H_O	PIC	f	HWE
VHL20	92-104	0.4000	1.6000	7.00	10.00	0.7000	4.00	3.04	0.7605	0.6713	0.5500	0.6091	0.2053	0.1720
HTG4	126-136	0.5500	2.2000	7.00	10.00	0.7000	4.00	2.59	0.6472	0.6138	0.6000	0.5596	0.0480	0.4730
AHT4	144-158	0.5750	2.3000	6.00	10.00	0.6000	4.00	2.48	0.6211	0.5975	0.7000	0.5476	-0.1466	0.5810
HMS7	171-175	0.5000	1.5000	5.00	6.00	0.8333	3.00	2.38	0.7937	0.5800	0.7000	0.4918	-0.1822	0.7760
HTG6	79-97	0.2750	2.2000	12.00	36.00	0.3333	8.00	4.85	0.6061	0.7938	0.6000	0.7643	0.2681	0.0190
AHT5	130-138	0.4500	1.3500	5.00	6.00	0.8333	3.00	2.79	0.9292	0.6413	0.6500	0.5673	0.0120	0.4980
HMS6	160-168	0.3500	1.4000	9.00	10.00	0.9000	4.00	3.59	0.8969	0.7213	0.5500	0.6695	0.2615	0.1250
ASB23	186-192	0.3000	1.2000	10.00	10.00	1.0000	4.00	3.92	0.9804	0.7450	0.8000	0.6975	-0.0483	0.6540
ASB2	237-251	0.5000	2.0000	7.00	10.00	0.7000	4.00	2.93	0.7326	0.6588	0.7000	0.6084	-0.0370	0.5940
HTG10	89-103	0.3611	2.1667	8.00	21.00	0.3810	6.00	3.81	0.6353	0.7377	0.4444	0.6954	0.4213	0.0030
HTG7	118-130	0.4750	1.9000	5.00	10.00	0.5000	4.00	2.21	0.5525	0.5475	0.8000	0.4446	-0.4408	0.0470
HMS3	148-164	0.5000	1.5000	5.00	6.00	0.8333	3.00	2.38	0.7937	0.5800	0.6500	0.4918	-0.0953	0.5180
HMS2	218-236	0.5500	2.7500	8.00	15.00	0.5333	5.00	2.56	0.5128	0.6100	0.6500	0.5552	-0.0400	0.9340
ASB17	96-114	0.3750	2.6250	10.00	28.00	0.3571	7.00	4.08	0.5831	0.7550	0.7000	0.7190	0.0983	0.0190
LEX3	139-157	0.3750	1.5000	8.00	10.00	0.8000	4.00	3.43	0.8584	0.7088	0.5500	0.6545	0.2482	0.0490
HMS1	176-182	0.5750	1.7250	4.00	6.00	0.6667	3.00	2.04	0.6785	0.5088	0.4000	0.4023	0.2381	0.2230
CA425	230-242	0.4750	1.4250	5.00	6.00	0.8333	3.00	2.69	0.8979	0.6288	0.6500	0.5546	-0.0082	0.8020
Mean	-	0.4462	1.9162	7.12	11.37	0.6262	4.29	2.88	0.6709	0.6529	0.6291	0.5901	0.0621	-

Our results of allele's size range were in a previous described range. We observed an allele that overlaps with the expected size range (VHL20, HTG7 and HMS1) and falls outside of its expected size range (ASB17).

Average number of effective alleles per locus detected in our study was similar to Khanshour et al. [10] research on Western Arabian horse populations (2.12-3.41), but lower than that observed for Middle Eastern Arabian horse populations (3.30-4.23) [10]. The number of alleles observed at individual loci was highly variable in our study. The same extent of variation of the number of alleles (3 to 8) was described for Syrian Arab horses [1] and European Anglo-Arab horses [15]. Also, similar variation range (3 to 9) was described for Arabian horses in Charmahal-va-bakhtiari province [4] and Iranian Arab horses [2]. Average number of detected alleles per locus established in our study was similar to the results of Glovatzki-Mulls et al. [19] where mean number of alleles in Arabian horse population was 4.8, as well as comparable to Khanshour et al. [20-24] research on Western Arabian horse populations (3.0-5.67) and Tozaki et al. [5] research on Arabian horse population (4.5). On the other hand, in Juras et al. [20] research on genetic diversity of Arabian horses mean number of alleles was much lower (2.132) than the one we detected. In general, average number of alleles in our study was lower than reported in previous studies on Arabian horses (5.13-8.47) [1,4,6,8,10,21-26]. Generally, the differences among breeds and mean number of alleles may depend on number of analyzed of alleles, number of samples, set of chosen microsatellite markers as well as population structure.

Our results of average HO were similar to results on Arabian horses previously reported by Conant et al. [21] (0.64), Glovatzki-Mulls et al. [19] (0.61), Iwanczyk et al. [23] (0.645), Khanshour et al. [10] (0.40-0.69), Luis et al. [24] (0.624), van de Goor et al. [25] (0.645) and Vega-Pla et al. [26] (0.6353). Juras et al. [20] reported lower HO values for Arabian horses (0.307). The average values of HO in our study were lower than those reported in other studies on Arabian horse populations (0.68–0.72) [1,4,6,8,10,22]. Deficiency of heterozygotes in Arabian horse was previously described by Di Stasio et al. [22]. According to Khanshour and Cothran [27] the number of alleles per locus in modern Arabian horse populations varied between 2.8 to 5.6 versus 5.1 to 8.5 in the old populations. In the modern Arabian horse populations HO values varied between 0.39 to 0.67 while the values ranged between 0.68 to 0.072 in the old populations. Modern populations generally exhibit lower values of diversity. Number of alleles and heterozygosity data indicate that Arabian horse population from B&H belongs to the modern Arabian horse population. The values of HE found in this study were similar to the values reported for Arabian horse populations by Georgescu [8] (0.665), Glovatzki-Mulls et al. [19] (0.63), Iwanczyk et al. [23] (0.646) and Khanshour et al. [10] (0.46–0.69). Lower HE values (0.327) were reported by Juras et al. [20]. Average values of HE reported in the literature for other Arabian horse populations, mostly ranged from 0.6725 to 0.772 [1,4,6,10,21,22,24-26]. Comparing our results of average number of alleles, average number of effective alleles, average HO and HE values with Khanshour et al. [10] results, we can assume that population of Arabian horse from B&H belongs to Western Arabian horse populations. These results supported our indication that Arabian horse population from B&H belongs to modern Arabian horses. Loci AHT4, AHT5, HMS7, ASB23, HTG7, HMS3 and CA425 showed higher observed heterozygosity than the expected values, whereas for the rest of the loci. observed heterozygosity was lower than expected. In the studied population, average values of HO and HE were similar. These results may indicate sufficient heterogeneity between animals. Low variability of Arabian horse populations has been shown in most studies [22,28,29]. The greatest differences between HO and HE, in our study was observed for HTG10 locus. The same locus showed the highest inbreeding coefficient, the highest deviation from HWE and substantial

heterozygote deficit. According to Galov et al. [30] highly significant deviation from HWE combined with substantial heterozygote deficit is likely to indicate locus-specific genotyping problem due to null alleles.

In population genetic analysis, genetic markers with PIC values higher than 0.5 are normally considered to be informative [31]. Mean PIC established in our study was 0.5901 although individual PIC values were lower than 0.5 at four loci: HMS7, HTG7, HMS3 and HMS1. At least 10 microsatellite loci should be used to achieve maximum exclusion in horses [1]. PIC values, observed in our work, suggested that 76.47% markers (13 microsatellite loci) were quite informative in terms of their suitability for genetic diversity studies while remaining loci were reasonably informative. HMS1 locus shows the lowest genetic diversity in our study. However, this locus, along with LEX3, is not among the markers recommended by the FAO for diversity studies [25]. An increased inbreeding coefficient was detected in our work. The same indicators were reported for Spanish Arabian horse population [7] and Polish Arabian horse population [9]. Possible reason may be consanguineous mating. The value for HTG10 locus is probably encumbered with locus-specific genotyping problem due to null alleles.

The results of the present study suggest that the population of Arabian horse sampled from stud "Borike" is not affected by major loss of genetic diversity and indicate that Arabian horse population from B&H belongs to the modern Arabian horse populations. When all observed parameters are taken into account, it can be concluded that HTG6 locus shows the highest, while HMS1 locus shows the lowest genetic diversity in Arabian horse population from B&H. However, assuming values of HO, HE and PIC around 0.6, we consider that loci HTG6, ASB23, ASB2 and ASB17 are the most polymorphic. An increase in the inbreeding coefficient and sufficient heterogeneity between animals indicate occurrence of consanguineous mating. The present research contributes to the understanding of population structure and current status of genetic diversity of the investigated population. Also, the results may assist horse breeders for advising breed management in designing breeding strategies.

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