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Microsatellite analysis for parentage testing of the Arabian horse breed from Syria

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Abstract: Parentage testing is very important for genetically determining the accuracy of the pedigree of domestic animals. The Arabian horse population in Syria might be expected to have a high level of homozygosity because the traditional method of breeding results in such a small population. For this reason, using a set of highly polymorphic markers such as microsatellites is required for parentage testing. The purpose of this study was to analyze the usefulness of 16 STR markers for routine parentage testing in 94 samples from registered and nonregistered horses collected from Syria. The mean number of alleles was 5.69 per locus in the registered group and 7.69 in the nonregistered group. The nonregistered group had a mean polymorphic information content (PIC) of 0.715, which was significantly higher than the PIC in the registered group. The individual probability of exclusion per locus ranged from 32% for HTG7 to 80% for ASB17 in the registered group and 41% for HTG7 to 84% for ASB17 in the nonregistered group. The results of this study indicate no serious loss of heterozygosity and confirm that 13 STR markers can be used in parentage testing with high efficiency for the Arabian horses from Syria.

Key words: Arabian horse, parentage testing, microsatellite, Syrian horse population

1. Introduction

The horse of the desert, the drinker of the wind, the runner, the *Asil*, the pure blood; these are some of the descriptions of the Arabian horse. The Arabian is commonly believed to be one of the oldest horse breeds (1), and it has been very influential throughout the world (2) as it has been involved in the formation of many horse breeds, such as the Thoroughbred (3), Lipizzan (4), and many new Brazilian breeds (5). Furthermore, pure bred Arabians are found in Europe, Australia, and the United States (6).

In horses, parentage testing has been of particular importance for breed registration processes, studbook creation, and validation. In general, parentage testing in animals is important for checking the genetic accuracy in progeny testing and in the selection for traits (7), while accurate pedigree information is very important for a successful animal breeding program (8) and for conservation plans of animal populations (9).

As a well-established breed, the Arabian might be expected to have a high level of homozygosity, because of the manner in which the *Bedwi* (the traditional breeder of the horse in the Arabian Desert) has managed this breed by strict breeding rules, which include breeding only horses sharing maternal lineages and avoiding crossing with other breeds or horses of uncertain origins (6). This manner of breeding becomes problematic in small populations, especially when the effects of natural selection are negated by breeding far away from the desert conditions under which the Arabian horses developed. In such circumstances, the use of a set of highly polymorphic markers is required for reliable parentage testing. Recently, DNA-based methodologies for genetic marker-testing, using polymerase chain reaction (PCR) technology, provided a more powerful alternative to blood typing and protein polymorphism tests, particularly the analysis of short tandem repeat (STR) loci (or microsatellites) (10,11). The stability, ease, and accuracy of typing these codominant markers, together with their high levels of polymorphism and wide-spread distribution in the genome, make microsatellite loci an attractive source of information of genetic structure (12). Compared to earlier blood typing, the use of STRs allows for the use of hair samples, rather than blood samples. In addition, STRs can provide an indication of the levels of inter- and intrabreed variability (13). For these reasons, STR loci have been

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widely used to investigate the genetic structure and pedigree analyses of different horse breeds (9,10,14–16) and STRs have been used successfully in the analysis of small populations of closely bred animals (17).

The purpose of this study was to determine if a panel of 16 STR markers was sufficient to validate the parentage of Arabian horses collected directly from local breeders in Syria. This is the first in-depth study of the Arabian horse breed originating from the Arabian Desert, which may more closely reflect the original status of the genetic structure of the Arabian horse breed.

2. Materials and methods

2.1. Sampling and DNA extraction

The Syrian horse population consists of 8 registered groups or *RASANs*: Hadbaa, Hamadania, Dahmaa, Kahlila, Abian, Saklawia, Shwemat, and Muanakii. These groups are all considered to be "pure" Arabian. There is also a large population of nonregistered horses, which are known as local horses. Ninety-four hair samples were collected from different regions of Syria. Forty-nine samples were from nonregistered horses, while the remaining 45 consisted of samples from all of the registered groups of the Arabian horses in Syria. The animals from different RASANs were pooled for this analysis. Total DNA was extracted from the hair follicles using a Puregene® DNA purification kit according to the manufacturer's instructions.

2.2. Microsatellite analysis

Sixteen microsatellite markers (Table 1) specific to *Equus caballus* were used in this study. Fifteen are recommended by the International Society for Animal Genetics, and one X chromosome marker, LEX3, was also typed. The 16 microsatellites were amplified in 3 multiplex reactions as follows: (8plex: AHT4, AHT5, ASB17, ASB23, HMS6, HMS7, HTG4, and VHL20; 5plex: LEX3, HMS3, ASB2, HTG10, and LEX3; and 3plex: HMS2, HTG6, and HTG7) (15). Each reaction had a final volume of 12 µL, containing 50 ng of genomic DNA, from 0.07 to 0.8 pmol of primers, 1X PCR buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, and 1 U AmpliTaq for the 8plex, while for the 3plex and 5plex, 1 U ChoiceTaq was used. For microsatellite amplification, a hot start procedure was used, in which the genomic DNA and primers were combined and heated at 95 °C for 5 min. The temperature was then lowered and held at 85 °C for 10 min for the addition of the remaining reagents. Thirty-five cycles were as follows: 95 °C for 1 min, either 56 °C (5plex) or 60 °C (for 8plex) for 30 s, and 72 °C for 1 min of annealing. The cycling was completed with a final extension at 72 °C for 15 min. The PCR products were separated by electrophoresis on a 6% polyacrylamide gel using the ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The fragment sizes of the microsatellite alleles were determined using the STR and computer software (www.vgl.ucdaviess.edu/STRand). Alphanumerical nomenclature was used for allele size designation in accordance with the International Society for Animal Genetics. All of the tests were repeated at least 3 times, and both positive and negative controls were used in each reaction.

2.3. Statistical analyses

Standard diversity indices were calculated using Cervus 3.0 (26). These include: the number of alleles (Na); effective number of alleles (Ne = 1/(1 – He)); observed (Ho) and expected (He) heterozygosity (calculated from allele frequencies assuming Hardy–Weinberg equilibrium) (27); polymorphic information content (PIC), which is a measure of informativeness related to the expected heterozygosity (28); frequency of the most common allele (FNA); probability of exclusion (PE); and combined probabilities of exclusion (CPE) (7).

Table 1. The 16 markers used in the study.

Locus	Chromosome	Reference	Locus	Chromosome	Reference
ASB17	2	(18)	LEX33	4	(22)
HMS2	10	(19)	VHL20	30	(23)
LEX3	X	(20)	HTG6	15	(24)
ASB23	3	(21)	AHT4	24	(25)
ASB2	15	(18)	AHT5	8	(25)
HMS7	1	(19)	HTG4	9	(24)
HMS3	9	(19)	HMS6	4	(19)
HTG10	21	(14)	HTG7	4	(14)

3. Results

The PCR amplicons ranged between 93 bp and 211 bp in size. As shown in Table 2, the total Na was 91 in the registered group, with a mean of 5.69 per locus, and 123 alleles in the nonregistered group, with a mean of 7.69. The Na per locus ranged from 3 for HTG6 and HTG7 to 8 for ASB2 in the registered group, while in the nonregistered group, the Na ranged from 4 for HTG7 to 14 for ASB17. The Ne varied from 1.86 for HTG7 to 5.464 for ASB17 in the registered group and from 2.141 for HTG7 to 5.988 for ASB17 in the nonregistered group. The mean Ne was 3.747 in the registered group and 4.476 in the nonregistered group. The observed heterozygosity per locus in the registered group varied from 0.362 for HTG7 to 0.915 for LEX33 and from 0.469 for HMS2 to 0.878 for ASB17 for the nonregistered group, with means of 0.694 and 0.711, respectively. The lowest PIC value for both groups was for HTG7 (0.385 in the registered group and 0.454 in the nonregistered group), while the highest value was for ASB17 (0.781 in the registered group and 0.803 in the nonregistered group). The mean PIC was 0.657 in the registered group and 0.715 in the nonregistered group. The individual PE ranged from 32% at the HTG7 locus to 80% at ASB17 for the registered group and 41% at the HTG7 locus to 84% at ASB17 for the nonregistered group. The CPEs for all of the loci were more than 99.999% in each group. The Figure shows the CPE values for both groups as a function of the number of microsatellite loci.

4. Discussion

How informative a locus is depends upon the Na exhibited by the locus and the frequency distribution of these alleles in the population (8). The mean Na in the present study was higher than that seen in Sorraia, a breed that has a high level of inbreeding (29). Values of diversity statistics similar to those observed here for the Arabian breed have been

Table 2. Number of alleles (Na), number of effective alleles (Ne), observed (Ho) and expected (He) heterozygosity, polymorphic information content (PIC), probability of exclusion (PE), and combined probabilities of exclusion (CPE) for registered and nonregistered Arabian horses.

Group	Registered						Nonregistered					
Locus	Na	Ne	Ho	He	PIC	PE	Na	Ne	Ho	He	PIC	PE
ASB17	7	5.4	0.872	0.817	0.781	0.800	14	5.9	0.878	0.833	0.803	0.839
HMS2	7	5.2	0.872	0.809	0.771	0.789	9	5.8	0.469	0.830	0.799	0.831
LEX3	7	4.7	0.426	0.790	0.748	0.755	9	5.7	0.837	0.825	0.791	0.814
ASB23	6	4.2	0.702	0.767	0.723	0.731	8	5.0	0.735	0.803	0.766	0.785
ASB2	8	4.0	0.766	0.754	0.707	0.713	9	5.0	0.837	0.800	0.763	0.784
HMS7	6	4.2	0.787	0.766	0.717	0.709	8	5.1	0.673	0.805	0.766	0.781
HMS3	6	4.0	0.809	0.751	0.701	0.697	7	5.0	0.714	0.803	0.762	0.769
HTG10	5	3.9	0.766	0.749	0.698	0.689	8	4.5	0.694	0.782	0.743	0.762
LEX33	5	3.8	0.915	0.742	0.690	0.675	9	4.5	0.796	0.780	0.737	0.747
VHL20	5	3.4	0.745	0.709	0.657	0.650	8	4.4	0.714	0.774	0.732	0.742
HMS6	5	3.2	0.702	0.696	0.639	0.626	6	4.2	0.776	0.765	0.720	0.726
AHT4	6	3.3	0.596	0.698	0.634	0.604	6	4.1	0.735	0.760	0.712	0.711
AHT5	6	3.0	0.660	0.675	0.610	0.594	7	3.5	0.673	0.720	0.666	0.657
HTG4	6	2.5	0.553	0.607	0.560	0.559	5	2.8	0.633	0.648	0.595	0.581
HTG6	3	2.4	0.574	0.594	0.499	0.434	6	3.2	0.653	0.690	0.621	0.587
HTG7	3	1.8	0.362	0.463	0.385	0.329	4	2.1	0.551	0.533	0.454	0.408
Mean	5.69	3.7	0.694	0.712	0.657	CPE >0.9999	7.69	4.4	0.711	0.759	0.715	CPE >0.9999

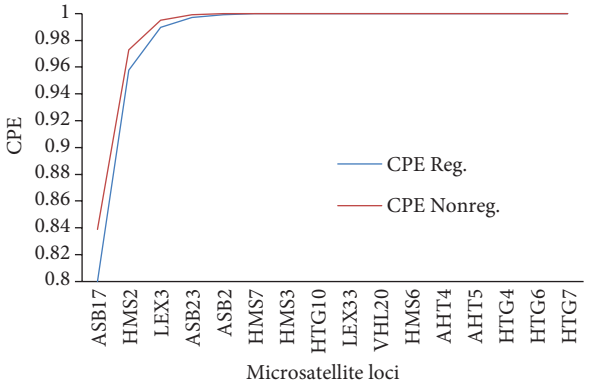

Figure. Combined probabilities of exclusion (CPE) as a function of the number of 16 microsatellite loci in registered (Reg.) and nonregistered (Nonreg.) Arabian horses.

recorded for a number of equine populations, including the Thoroughbred (30), Lipizzaner (16), Lithuanian native horse breeds (15), and Pantaneiro horses (9). However, the individual PE for 13 of the 16 loci used in this study was higher than that reported in the Thoroughbred (30). Furthermore, all 15 loci tested by Monies et al. (31) had lower PE values than we found using the same loci; this may be due to the different Arabian population that was used. Furthermore, some problems in genotyping the HMS3 locus were indicated by Monies et al. (31). Similar problems in HMS3 were noticed in the Potoka horse breed (32), which may be a result of nonamplification due to a base substitution in the sequence flanking the M allele priming site of the HMS3 (33). Such a problem was not noticed in our results as well in different horse breeds (9,10,13).

In this study, for the registered group, 7 microsatellite markers (ASB17, HMS2, LEX3, ASB23, ASB2, HMS7, and HMS3) had high PIC values (>7). A very high level of CPE (>0.99999) can be reached using only 6 of the 16 loci (Figure), which makes these markers highly valuable for use in parentage testing for these Arabian horses. Ellegren et al. (24) suggested that at least 10 microsatellite loci should be used to achieve maximum exclusion in horses, but our results show that fewer loci can give a relatively high exclusion power, similar to the results found by Sereno et al. (9). Two markers, HTG6 and HTG7, were found to have a PIC value of less than 0.5 for the registered group. As these markers are considered uninformative (28) and they are in the less efficient 3plex, these 2 loci plus HMS2 can easily be excluded from routine parentage testing for the Arabian horses with no significant loss of exclusion power.

The nonregistered group had a mean PIC of 0.715, which was significantly higher than the mean PIC in the registered group ($P < 0.0001$). This value reflects a higher level of variation in the nonregistered group compared to the registered horses. The difference of variation between the registered and nonregistered horses may be due to the restricted mating in the registered group, where registered horses must be mated within the same RASAN, while the nonregistered horses can be crossed with any horse. The higher Na indicates that horses other than those within the RASAN may have been introduced into this group or that the nonregistered group has retained alleles lost in the registered horses.

The heterozygosity in both groups was within the range of heterozygosity in different horse breeds (9,34). The heterozygosity levels were consistent with the high Na per locus seen in our study and indicate no serious loss of variability due to the breeding method employed by the local breeders in Syria compared to other horse breeds (34). Comparing the genetic variability of Syrian populations tested here with some other modern Arabian populations, we can recognize substantive disagreements in variability. In the Polish Arabian population, an increase in the inbreeding coefficient and reduction in the genetic diversity were found (35), and the same situation was reported in Spanish Arabian horses (36). The reduction in variability in these modern Arabian populations was due to the founder effect caused by a breeding policy that included mating between relatives. However, the mean heterozygosity for Syrian horses was a little bit higher than what was reported in a similar recent study about a different population of Arabian horses with some reservations on HMS3 locus (31).

The International Stud Book Committee (ISBC) has required that the CPE value for parentage verification and an individual identification in a horse be higher than 0.9995 (37). Here, we found that the CPE using 12 autosomal loci was greater than the value required by the ISBC. Based on these results, we confirm that the loci of the 8plex and 5plex PCR can be used in parentage testing with high efficiency for the Arabian horses from Syria. The data presented here will help solve the problems related to registration issues and will provide the breeders with an effective tool for confirming lineages. Genetic testing will also generate valuable data necessary for the conservation of this breed.

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