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Genetic Diversity and Population Assignment of Arabian Horses

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MAS, HAA, RME, HHB, SIR, EGC and ASF designed and planned the study. MAS, HAA, SIR and ASF performed the experiments. MAS, HAA, SIR and ASF collected the data while MAS, HAA, SIR and ASF analyzed it. MAS, HAA, SIR and ASF wrote the manuscript.

Key words
Al Khamsa, Arabian horse, Genetic diversity, Parentage

ABSTRACT

A total of 229 Arabian horse hair samples including 120 samples from El-Zahraa stud and 89 from two private farms in addition to 20 hair samples of Dutch Warmblood were genotyped by 16 microsatellite markers. The purposes of this study were; firstly, to investigate the current status of the genetic diversity and inbreeding of Arabian horse populations reared in Egypt. Secondly, to examine the traditional maternal based strain classification system "Al Khamsa" using samples of native Arabian horses reared in the El-Zahraa stud based on 16 microsatellite markers. El-Zahraa stud showed high inbreeding (F_{IS} = 0.110) and should be corrected by modifying mating system through avoiding excessive use of certain sires in breeding program. Across the five basic strains of the Arabian horse, nine loci showed 13 private alleles with the Seglawi recorded six and the Abeyan recorded no private alleles The highest Nei genetic distance and pairwise F_{ST} values were recorded between Abeyan and Hamdani while the lowest were recorded between Kehilan and Seglawi. The cluster pattern of the individual phylogenetic tree and STRUCTURE plots of the five basic horse strains indicate that there was no sharp demarcation between those five strains, and the influence of the dame line and the traditional maternal lines classification of the El-Zahraa Arabian horses was unclear. The results of this study confirm the applicability and efficiency of these 16 STR markers for assessing genetic diversity but not in examining the traditional maternal based strain classification system using native Arabian horses from Egypt.

INTRODUCTION

The Arabian horse is considered the world's oldest purebred breed (Głażewska, 2010). From historical records, the Bedouins (the original breeder of the horse in the Arabian Desert) used traditional methods to maintain the purity of the Arabian horse. These included avoiding any mating between Arabian horses and non-Arabian horses and by maintaining strictly separated strains (van Lent and Upton, 1999; Chmiel *et al*., 2006). According to the Arabian Horse Association, the Arabian horse breed consists of five strains "Al Khamsa" based upon dam line, each with unique characteristics. The five basic strains descended from the Al Khamsa were known as the Kehilan, Seglawi, Abeyan, Hamdani and Hadban. In other lists, Muniqi and Dahman replace Abeyan and Hamdani. Each strain from the Al Khamsa has specific body colors and morphological characteristics. The Kehilan strain was known for its masculine power and size and their colors were gray and chestnut. The Seglawi was noted for its refinement and feminine elegance, commonly bay color. Abeyan strain is similar to the Seglawi with more white markings. The Hamdani horse was one of the largest strains with an athletic and large boned build, gray and bay colored. Hadban strain was considered a smaller version of the Hamdani with few white markings (Forbis, 1976; Chmiel *et al*., 2006; Hendricks, 2007; Lynghaug, 2009). Individuals within each strain of Al Khamsa are expected to share and have similar STR alleles because they are descendant from common mother ancestors.

Egypt, although not an area of origin, has been a focal point for breeding Arabian horses for the past 200 years. The Egyptian Agriculture organization (EAO) is considered one of the most significant organizations in

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Egypt which plays a vital role in keeping the purity of Arabian horses all over the Egyptian country (Day, 1938). El-Zahraa Stud is the biggest governmental farm having about 450 horses, and about 650 private studs founded under supervision of EAO. Genetic diversity within breeds is needed for long-term genetic improvement of livestock breeds and to prevent low performance due to inbreeding (Engelsma *et al*., 2012). Decreased population genetic diversity and inbreeding can be associated with declines in population fitness and an increase in the expression of deleterious genes (Keller and Waller, 2002; Tarr *et al*., 2014). Actually, there are at least three recessive lethal genetic diseases segregate within the Arabian horse (Brosnahan *et al*., 2010; Aleman *et al*., 2018). This finding throw the light on the importance of evaluating and managing the inbreeding coefficient within the Arabian horse populations based on genomic tools as the pedigree-based investigation may not properly measure the loss of genetic diversity due to historical events (Al Abri *et al*., 2017). Breed registry authorities for Arabian horses have adopted parentage testing programs for breed registration processes, studbook creation, and to assure maintaining the purity of the blood of the horses of the Arabian breed throughout the world (van Lent and Upton, 1999).

Microsatellites are considered a marker of choice for evaluation of genetic diversity and individuals assignment in different animal species including horses (Khanshour *et al*., 2013; Sargious *et al*., 2014). Genetic diversity studies of the Arabian horses reared in Egypt based on microsatellite markers are scant (Mahrous *et al*., 2011; Sargious *et al*., 2014), so that in the present study we aimed to investigate the current status of the genetic diversity and inbreeding of Arabian horses in the El-Zahraa and two private studs reared in Egypt as well as examine the traditional maternal based strain classification system "Al Khamsa" using samples from native Arabian horses reared in Egypt based on microsatellite markers.

MATERIALS AND METHODS

Horse samples

A total of 229 Arabian horse hair samples representing diverse set of Egyptian populations were examined, including 120 samples from El-Zahraa stud (Egyptian Agricultural Organization (EAO), Ain Shams, Cairo, Egypt). Moreover, 48 and 41 hair samples collected from two private farms located in Cairo governorate, Egypt were tested. In addition to the Arabian populations, 20 hair samples of Dutch Warmblood harness type horses were collected from El-Ferosiah Club, El Gezirah, Cairo, Egypt and were used as an out-group. To examine the traditional maternal based strain classification system "Al Khamsa" we included 82 samples with known mother ancestor of Arabian horse from El-Zahraa stud; Kehilan (n = 23), Seglawi (n = 21), Abeyan (n = 9), Hamdani (n = 12), Hadban (n = 17). The experiment was carried out in accordance with the guidelines laid down by the Institutional Animal Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt and in accordance with the local laws and regulations.

DNA extraction and microsatellite analysis

Total DNA was extracted from hair follicles using EZ-10 Spin Genomic DNA Minipreps purification kit following the manufacturer's protocol. A total of 16 microsatellite markers (*AHT4, AHT5, ASB17, ASB23, HMS6, HMS7, HTG4, VHL20, HMS3, ASB2, HTG10, HMS2, HMS1, HTG6, HTG7* and *CA425*) specific to *Equus caballus* were used in this study. All markers are included in the panel recommended by the International Society for Animal Genetics for diversity studies and parentage verification. The 16 microsatellites are amplified in one multiplex reaction using Stockmarks; horse genotyping kit (Cat. No.: PN4336407 – Applied Biosystem - USA) according to the method described by (Sargious *et al*., 2014). Fragment sizes of microsatellite alleles were determined using Genetic analyzer 3500 (Applied Biosystem-USA) with the aid of Liz standard. The data obtained is further analyzed using Gene Mapper V 4.1 software (Applied Biosystem, USA).

Marker polymorphisms and populations diversity

Number of alleles (N_A), effective number of alleles (N_e), observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated using GENALEX version 6 software (Peakall and Smouse, 2006). Polymorphic information content (*PIC*) was calculated using CERVUS version 3 software (Marshall *et al*., 1998). Hardy Weinberg Equilibrium (*HWE*), *F-statistics* [fixation coefficient of an individual within a subpopulation (F_{IS}), fixation coefficient of an individual within the total population (F_{IT}), and fixation coefficient of a subpopulation within the total population (F_{ST})] per locus were estimated by GENEPOP version 3.4 program (Raymond, 1995).

Relationships and population structure

A phylogenetic tree was constructed based on the Reynolds's genetic distance (D_A) by using the neighbor-joining (NJ) method (Saitou and Nei, 1987). These processes were conducted using POPULATIONS version 1.2.30 software (http://bioinformatics-org/~tryphon/populations/).

We investigated the genetic structure and clustering of the sampled populations using a Bayesian clustering

procedure implemented in STRUCTURE with the admixture method (Pritchard *et al*., 2000). We did 50 runs for each different value of *K* with 50,000 iterations following a burn-in period of 20,000. Pair wise comparisons of the 50 solutions of each *K* value were run along with 50 permutations using CLUMPP software (Jakobsson and Rosenberg, 2007). The CLUMPP software calculated the highest pair wise similarity index (*H*) and outputs a mean of the permuted matrices across replicates after aligning the cluster membership coefficients of these replicate. Finally, the clustering pattern with the highest *H* value and best Δ *K* value was graphically displayed for the selected *K* value using DISTRUCT software (Rosenberg, 2004).

RESULTS

Marker polymorphisms and populations diversity

Across the three studied Arabian horse populations the total number of alleles was 116 with locus *ASB17* recorded the highest value (12) and locus *HMS6* recorded the lowest value (4). The estimated means of N_A, N_e, H_O and H_E were 7.250, 3.320, 0.611 and 0.670 respectively. The mean values of F_{IS} and F_{ST} for the 16 studied loci were 0.073 and 0.033 respectively. All loci except *HTG6*, *HTG7*, *HMS1* and *CA425* showed deviation from *HWE* (Table I). In respect to the within population genetic diversity, the three Arabian populations showed medium genetic diversity (N_A = 5.625; N_e = 3.057; H_O = 0.613 and H_E = 0.645) as compared to other domestic horse breeds. El-Zahraa population showed high and positive value of F_{IS} (0.110) while the other two Arabian populations recorded low and positive values (Private 1= 0.009 and Private 2= 0.011). In contrast, the Dutch Warmblood population showed high genetic diversity (N_A = 6.313; N_e = 4.323; H_E = 0.773; F_{IS} = -0.039) as shown in Table II.

Relationships and populations structure

The clustering pattern of the neighbor-joining phylogenetic tree indicates the close relationship of the three Arabian populations, and this was supported by the STRUCTURE plot. The most probable structure clustering of the four studied populations was at *K* = 2 (Figs. 1 and 2). The Dutch Warmblood population was assigned independently into its own cluster while the remaining three Arabian populations (El-Zahraa, Private 1 and Private 2) were grouped together forming one cluster.

Examining the traditional maternal based strain classification system "Al Khamsa"

The genetic diversity of the five basic strains of the Arabian horse "Al Khamsa" is shown in Tables III and IV. Across the five basic strains of the Arabian horse; the genetic diversity indices were medium (N_A = 4.025; N_e = 2.740; H_O = 0.625 and H_E = 0.624) with locus *AHT4* recorded the highest and locus *HMS1* recorded the lowest values. In respect to the within population genetic diversity, the five basic strains of the Arabian horse showed medium genetic diversity with Seglawi recorded the highest N_A = 4.313 and P_A = 6.000 and Abeyan recorded the highest H_O = 0.657 and H_E = 0.648. The frequency and size of private alleles across the five basic strains are shown in Table VI. Nine loci showed 13 private alleles with Abeyan recorded non-private allele. The pairwise Nei genetic distance and F_{ST} values between the five studied horse strains recorded the lowest values between Kehilan and Seglawi (0.029 and 0.012 respectively), while the highest values were recorded between Abeyan and Hamdani (0.077 and 0.032 respectively). *HTG4*, *HMS7*, *AHT5* and *CA425* loci showed the highest F_{ST} values (0.043, 0.038, 0.054 and 0.041 respectively) and the greatest variation in allele frequency distribution across the five basic strains of the Arabian horse as shown in Table III and Figure 3. Allele 136 bp of the *ATH5* locus showed the highest frequency (0.708) in Hamdani and the lowest in Seglawi (0.238). The individual phylogenetic tree and STRUCTURE diagram showed one cluster pattern for the five basic strains of the Arabian horse (Figs. 4 and 5).

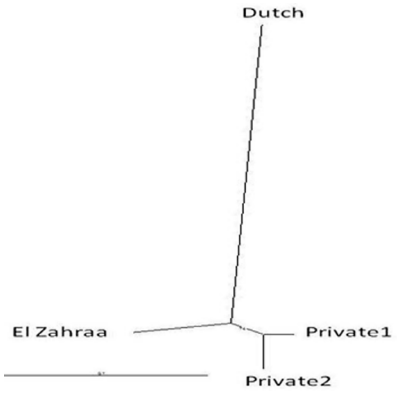

Fig. 1. Neighbor-joining phylogenetic tree of the three Arabian and the Dutch Warmblood horse populations based on16 microsatellite loci.

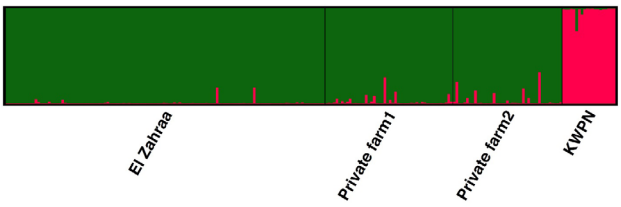

Fig. 2. Structure clustering of the three Arabian and Dutch Warmblood horse populations obtained for *K* = 2.

Table I. Observed (N_A) and effective (*Ne*) number of alleles, polymorphism information content (*PIC*), observed (H_O) and expected (H_E) heterozygosities and *F*-statistics (F_{IS}, F_{ST}, and F_{IT}) across the three studied Arabian horse populations.

Locus	N_A ±SE	*Ne* ±SE	*Ho* ±SE	H_E±SE	F_{IS} ±SE	F_{ST} ±SE	F_{IT} ±SE	*HWE*
VHL20	9.000	3.233	0.694	0.691	-0.003	0.002	-0.002	***
HTG4	5.000	2.581	0.603	0.613	0.000	0.031	0.031	***
AHT4	9.000	6.129	0.789	0.837	0.036	0.041	0.075	***
HMS7	7.000	3.965	0.694	0.748	0.063	0.021	0.083	***
HTG6	6.000	3.208	0.633	0.688	0.060	0.042	0.099	n.s
AHT5	5.000	3.474	0.727	0.712	-0.022	0.005	-0.017	***
HMS6	4.000	2.375	0.545	0.579	0.096	0.027	0.120	***
ASB23	7.000	2.208	0.428	0.547	0.171	0.099	0.254	***
ASB2	11.000	3.754	0.647	0.734	0.096	0.046	0.138	***
HTG10	7.000	4.549	0.718	0.780	0.075	0.014	0.088	***
HTG7	5.000	3.006	0.740	0.667	-0.115	0.013	-0.101	n.s
HMS3	8.000	2.343	0.350	0.573	0.392	0.002	0.393	***
HMS2	8.000	3.530	0.592	0.717	0.166	0.033	0.194	***
ASB17	12.000	4.634	0.732	0.784	0.045	0.043	0.086	***
HMS1	7.000	1.970	0.383	0.492	0.154	0.132	0.266	n.s
CA425	6.000	2.163	0.505	0.538	0.061	0.004	0.065	n.s
Mean	7.250±0.552	3.320±0.279	0.611±0.034	0.670±0.025	0.073±0.027	0.033±0.009	0.104±0.029	
Total mean [a]	8.875±0.569	3.579±0.292	0.626±0.031	0.695±0.023	0.064±0.023	0.061±0.008	0.121±0.027	

[a] Total mean= after including Dutch Warmblood population. ****P*<0.001 and n.s stands for not statistically significant.

Table II. Observed (N_A) and effective (*Ne*) number of alleles, observed (H_O) and expected (H_E) heterozygosities, and fixation coefficient of an individual within a subpopulation (F_{IS}) per population.

Population	N	N_A ±SE	ne	N_e ±SE	*Ho* ±SE	H_E ±SE	F_{IS} ±SE	*HWE*
El-Zahraa	120	6.688	68.250	3.595	0.604	0.686	0.110	***
Private1	48	5.188	80.400	2.831	0.626	0.636	0.009	***
Private2	41	5.313	67.830	2.818	0.605	0.620	0.011	***
Dutch	20	6.313	65.240	4.323	0.781	0.773	-0.039	n.s
Mean		5.625±0.241	72.160±4.122	3.057±0.143	0.613±0.023	0.645±0.017	0.040±0.026	
Total mean		5.875±0.199	70.430±3.390	3.392±0.144	0.655±0.021	0.679±0.015	0.023±0.022	

N, Number of genotyped animals; ne, effective population size = $4*N_m*N_f/(N_m+N_f)$; ****P*<0.001 and n.s stands for not statistically significant.

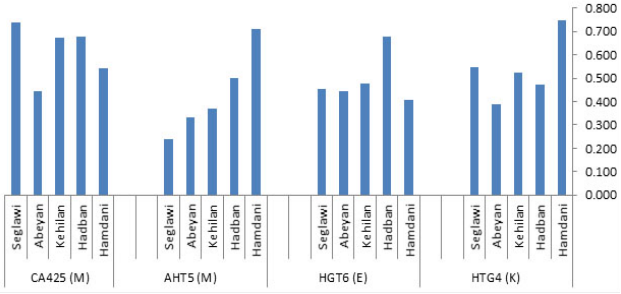

Fig. 3. Allele frequencies' distribution across the five basic horse strains "*Al Khamsa*".

DISCUSSION

Populations diversity

The number of alleles (N_A), the frequency distribution of these alleles (N_e) and heterozygosity are important indicators of genetic diversity. The mean N_A of this study was higher than that seen in Syrian registered Arabian horses (Khanshour *et al.*, 2013) and Arabian horses from stud Borike (Rukavina *et al.*, 2016), but was lower than that seen in Syrian non-registered Arabian horses (Khanshour *et al.*, 2013). This might be attributed to different microsatellite sets and different sample sizes,

Table III. Observed (N_A) and effective (*Ne*) number of alleles, observed (H_O) and expected (H_E) heterozygosities and *F*-statistics (F_{IS}, F_{ST}, and F_{IT}) across the five basic horse strains.

Locus	N_A ±SE	*Ne* ±SE	*Ho* ±SE	H_E±SE	F_{IS} ±SE	F_{ST} ±SE	F_{IT} ±SE	*HWE*
VHL20	7.000	2.364	0.634	0.593	-0.108	0.020	-0.086	ns
HTG4	4.000	2.390	0.562	0.583	0.000	0.043	0.044	ns
AHT4	9.000	4.392	0.840	0.799	-0.089	0.033	-0.053	ns
HMS7	4.000	3.375	0.718	0.726	-0.025	0.038	0.014	ns
HTG6	4.000	2.741	0.669	0.648	-0.071	0.027	-0.042	ns
AHT5	5.000	3.557	0.765	0.710	-0.115	0.054	-0.055	ns
HMS6	4.000	2.287	0.542	0.574	0.021	0.022	0.042	ns
ASB23	4.000	2.078	0.536	0.529	-0.051	0.019	-0.031	ns
ASB2	5.000	2.811	0.654	0.665	-0.019	0.010	-0.008	ns
HTG10	5.000	3.577	0.726	0.740	-0.015	0.026	0.011	ns
HTG7	4.000	2.593	0.789	0.633	-0.291	0.017	-0.270	ns
HMS3	6.000	2.397	0.417	0.599	0.278	0.032	0.302	***
HMS2	4.000	2.637	0.673	0.643	-0.089	0.031	-0.055	ns
ASB17	6.000	2.899	0.648	0.670	-0.001	0.023	0.022	ns
HMS1	3.000	1.425	0.279	0.301	0.041	0.023	0.063	ns
CA425	5.000	2.316	0.543	0.570	0.011	0.041	0.052	ns
Mean	4.025±0.122	2.740±0.090	0.625±0.020	0.624±0.014	-0.033±0.029	0.029±0.003	-0.003±0.028	

***P<0.001 and n.s stands for not statistically significant.

Table IV. Observed (N_A) and effective (*Ne*) number of alleles, private alleles (P_A) observed (H_O) and expected (H_E) heterozygosities, and fixation coefficient of an individual within a subpopulation (F_{IS}) for the five basic horse strains.

Population	N	N_A ±SE	N_e ±SE	P_A ±SE	*Ho* ±SE	H_E ±SE	F_{IS} ±SE
Kehilan	23	4.125	2.666	3.000	0.621	0.606	-0.052
Seglawi	21	4.313	2.797	6.000	0.571	0.623	0.059
Abeyan	9	3.688	2.801	0.000	0.657	0.648	-0.076
Hadban	17	4.125	2.710	3.000	0.651	0.621	-0.077
Hamdani	12	3.875	2.725	1.000	0.625	0.622	0.004
Mean	82	4.025±0.122	2.740±0.090	2.6±1.029	0.625±0.020	0.624±0.014	0.028±0.025

but it also is likely that the non-registered Syrian horses are not completely pure Arabian. In contrast, the mean N_e of our study was lower than that recorded by Syrian registered and non-registered Arabian horses (Khanshour *et al*., 2013). Value for our H_E mean was comparable to Arabian horses of Monies *et al*. (2011) and Rukavina *et al*. (2016). The high mean of N_A and lower mean of N_e and the positive mean for $F_{IS,}$ in addition to 12 loci showed significant deviation from *HWE*, indicate that there was non-random mating likely due to a selection program favoring some morphological characters. *HMS3* locus recorded the highest value for F_{IS} (0.392) and deviated from *HWE* in the three Arabian but not in the Dutch Warmblood populations. Moreover, only two alleles (160 bp and 164 bp) out of the eight alleles of the *HMS3* locus showed high frequency. This could be attributed to that these two alleles might be under some morphological or beauty related traits of selective interest in Arabian populations. However, Monies *et al*. (2011); Solis *et al*. (2005) and Achmann *et al*. (2001) previously reported some problems in *HMS3* locus genotyping, attributing these difficulties to be a result of non-amplification due to a base substitution in the flanking region of this locus. Such a problem was not noticed in our results as well as in other Arabian horses (Khanshour *et al*., 2013) and different horse breeds (Luis *et al*., 2007; Sereno *et al*., 2008). Regarding the within

population genetic diversity, the three Arabian populations showed moderate genetic diversity values. Although El-Zahraa and private 2 populations showed similar value for effective population size, El-Zahraa recorded higher F_{IS} value (0.110). This might be attributed to certain mating program in El-Zahraa stud favoring some beauty related traits.

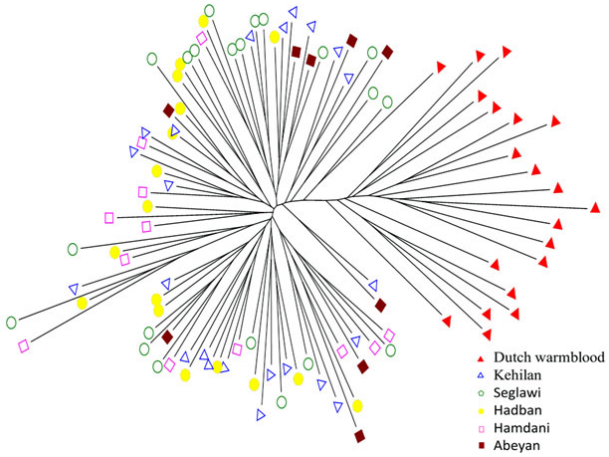

Fig. 4. Individuals neighbor-joining phylogenetic tree across the five basic horse strains "*Al Khamsa*" in addition to Dutch Warmblood as an out group.

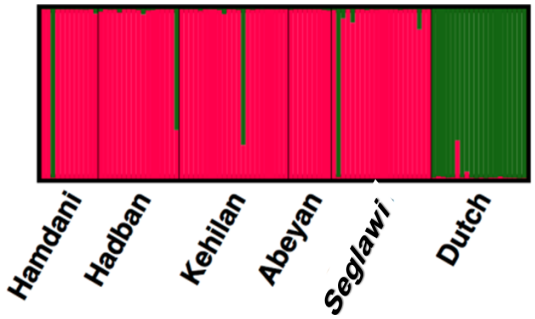

Fig. 5. Structure clustering of the five basic horse strains "*Al Khamsa*" obtained for $K = 2$.

Relationships and populations structure

The close relationship of the three Arabian horse populations as shown from clustering pattern in the neighbor-joining phylogenetic tree and STRUCTURE plots might be attributed to a single origin of the three populations (El-Zahraa stud). El-Zahraa stud is a governmental farm and considered the main source of pure Arabian horses to all Egyptian farms. This means that genetic diversity and inbreeding status of El-Zahraa stud is a very important issue as it might influence other Egyptian private farms.

Table V. Nei genetic distance (D_A: above diagonal) and pairwise F_{ST} (below diagonal) estimates for the 16 microsatellite loci between the five basic Arabian horse strains.

Population	Kehilan	Seglawi	Abeyan	Hadban	Hamdani
Kehilan	0	0.029	0.035	0.033	0.047
Seglawi	0.012	0	0.045	0.039	0.053
Abeyan	0.015	0.017	0	0.047	0.077
Hadban	0.009	0.013	0.017	0	0.043
Hamdani	0.024	0.023	0.032	0.021	0

Table VI. Private alleles among the five basic Arabian horse strains.

Pop	Locus	Allele (bp)	Frequency
Kehilan	*ASB2*	266	0.043
	HTG10	101	0.022
	ASB17	99	0.043
Seglawi	*VHL20*	97	0.024
	VHL20	99	0.048
	AHT4	160	0.024
	HMS6	165	0.024
	HMS2	222	0.025
	HMS1	182	0.048
Hadban	*VHL20*	103	0.029
	HMS3	152	0.029
	HMS1	188	0.029
Hamdani	*HMS3*	150	0.042

Traditional maternal based strain classification system "Al Khamsa"

Because the five Arabian horse strains "Al Khamsa" were based upon dam line, we expected that there could be private alleles and common alleles with high frequencies in each strain, because these alleles were descendant from their common mother ancestors. Although there were nine loci showing 13 private alleles, the frequency of these alleles were very low, so that it is very difficult to depend on these private alleles for differentiation between the five Arabian horse strains. The four loci (*HTG4, HMS7, AHT5* and *CA425*) showed the highest F_{ST} values and the highest variation in the allele frequency distribution among the five basic strains of the Arabian horses. For example, allele (*136 bp*) of the *ATH5* locus showed the highest frequency (0.708) in Hamdani while it was the lowest in Seglawi (0.238) strains. We might assume that allele (*136 bp*) of the *ATH5* locus came from and was a common allele in

the great grandmother of Hamdani individuals, but at the same time its high frequency in Hamdani strain might be paternal origin. For confirmation of this finding we need more samples from each strain and those samples should be collected from different countries to minimize the paternal effect on allele frequency. Consistent with this study across the three studied Arabian horse populations; *HMS3* locus recorded the highest value for F_{IS} (0.278) and deviated from *HWE* across the five studied Arabian horse strains Moreover, the 160 bp and 164 bp alleles out of the six alleles of the *HMS3* locus showed high frequency. This could be attributed to that these two alleles might be under some morphological or beauty related traits of selective interest in the five Arabian horse strains. The genetic distance (D_A) and the pair-wise population differentiation (F_{ST}) estimates showed the low genetic differentiation between the five studied horse strains. The close genetic relationship between Kehilan and Seglawi strains might be attributed to the introgression and to the gene flow between them. This result was consistent with those of Khanshour and Cothran (2013) who reported a closer relationship between individuals of Kehilan and Seglawi strains based on mtDNA D-loop and those two stains shared their maternal haplotypes more frequently than expected from pedigree registries.

The admixture cluster pattern of the individual phylogenetic tree and STRUCTURE plots for the five basic strains of the Arabian horse confirm the previous finding and indicate that there is no sharp demarcation between those five strains and the influence of the dame line and the traditional maternal family lines based on native Arabian horses from El-Zahraa farm in Egypt is unclear. Previously, Khanshour and Cothran (2013) concluded that there was no evidence that Arabian horse strains have clear subdivision depending on the traditional maternal based strain classification system by sequencing the whole mtDNA D-loop of 251 Arabian horses. We conclude from the current study that Locus *HMS3* should be interpreted with caution and should be analyzed in further studies based on different populations to test if it is linked to any morphological traits or if there were genotyping errors. The high F_{IS} (0.110) of El-Zahraa should be corrected by modifying mating system through avoiding excessive use of certain sires in breeding program. The four loci (*HTG4, HMS7, AHT5* and *CA425*) showing high variation in the allele frequency distribution among the five basic strains of the Arabian horses need more confirmation by genotyping more samples from each strain and those samples should be collected from different countries to minimize the paternal effects on allele frequency. Moreover, there was no clear evidence that Arabian horse five basic strains from El-Zahraa stud have clear subdivision depending on the traditional maternal based strain classification system based on 16 microsatellite loci. Future investigations aimed at determining the Arabian horse genetic diversity and examining the traditional maternal based strain classification system "Al Khamsa" based on Equine whole genome SNP array and mtDNA sequence are eagerly anticipated.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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