



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com



Original article

Genetic diversity and population genetic structure of six dromedary camel (*Camelus dromedarius*) populations in Saudi ArabiaAhmed Hossam Mahmoud^{a,*}, Faisal Mohammed Abu-Tarbush^a, Mohammed Alshaik^b, Riyadh Aljumaah^b, Amgad Saleh^c^a Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia^b Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia^c Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:

Received 28 October 2019

Revised 17 November 2019

Accepted 27 November 2019

Available online 14 December 2019

Keywords:

Breeding programs

Gene flow

Microsatellite

Population structure

Saudi Arabia

ABSTRACT

Camels are an integral and essential component of the Saudi Arabian heritage. The genetic diversity and population genetic structure of dromedary camels are poorly documented in Saudi Arabia so this study was carried out to investigate the genetic diversity of both local and exotic camel breeds. The genetic diversity was evaluated within and among camel populations using 21 microsatellite loci. Hair and blood samples were collected from 296 unrelated animals representing 4 different local breeds, namely Majaheem (MG), Maghateer (MJ), Sofr (SO), and Shaul (SH), and two exotic breeds namely Sawahli (SL) and Somali (SU). Nineteen out of 21 microsatellite loci generated multi-locus fingerprints for the studied camel individuals, with an average of 13.3 alleles per locus. Based on the genetic analyses, the camels were divided into two groups: one contained the Saudi indigenous populations (MG, MJ, SH and SO) and the other contained the non-Saudi ones (SU and SL). There was very little gene flow occurring between the two groups. The African origin of SU and SL breeds may explain their close genetic relationship. It is anticipated that the genetic diversity assessment is important to preserve local camel genetic resources and develop future breeding programs to improve camel productivity.

© 2019 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Arabian camels belong to the genus *Camelus* that contains only two species, one-humped (*C. dromedarius*) and two-humped (*C. bactrianus*) camels. Arabian camels were domesticated in southern Arabia thousand years before they were known in the north part of Arabia (Wardeh, 1989). Camels are unique animals in many aspects and cannot be compared with other farm animals in their physiological responses or adaptation to arid environment (Schwartz and Dioli, 1992; Sweet, 1965). Due to the unique characteristics of camel, the work on evaluation of breeds/strains is an advantage. The camels of Saudi Arabia are mainly dromedaries, the one-humped camels. They are an integral and notable compo-

nent of the Kingdom heritage, a symbol of its nomadic traditions. Saudi Arabian rapid modernization has moved camel caravans to be a part of the past, although the animal still provides transportation for some bedouin in remote areas. The total population of dromedary is estimated to be around 1.6 million camels within the Arabian Peninsula, about 53% found in Saudi Arabia (FAO, 2011). Genetic studies on camels are scarce; therefore, the genetic diversity assessment of Saudi Arabian camels is important to preserve their genetic resources and to improve their breeding programs. The development of molecular biology during the past decades has offered new tools in many biotechnological disciplines including livestock genetics and animal breeding (Hines, 1999; Walsh, 2001). These biotechnological tools can be applied specifically to improve camels' productivity, preserve their genetic resources and develop successful breeding programs. Numerous studies on the genetic relationships among and within farm animals breeds have been reported using different genetic markers (Mannen et al., 1998; Ming et al., 2017; Hedayat-Evrigh et al., 2018). Among these genetic markers, microsatellites have been extensively used for assessing the genetic diversity and relationships within and among closely related farm animal populations (Geng et al.,

* Corresponding author.

E-mail address: ahmahmoud@ksu.edu.sa (A. Hossam Mahmoud).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

2003; Li et al., 2004; Yang et al., 2004). Microsatellites are valuable genetic markers due to their dense distribution in genomes, easy to develop and inexpensive. Microsatellite analysis simply measures the nucleotide length of different alleles at a particular locus across different individuals. Allelic diversity represents how many different alleles are present throughout a population for each locus. Both Allelic diversity and heterozygosity can provide a direct measurement of genetic diversity in animal populations. As in other domestic animal species, microsatellites in camels are highly informative polymorphic markers and becoming the technique of choice for investigating genetic diversity and population studies (Al-Swailem et al., 2009; Mburu et al., 2003; Schulz et al., 2005). In Saudi Arabia, genetic studies on camels are scarce and the extent to which dromedary populations are genetically differentiated is poorly documented. The present study was, therefore, carried out to (1) determine the genetic diversity of four local populations and two exotic ones, existing in Saudi Arabia and to (2) infer the genetic relationship within and among them. It is anticipated that the assessment of genetic diversity of Saudi camels is important to preserve their genetic resources and to develop future breeding programs to improve camel productivity.

2. Materials and methods

2.1. Animal resources and DNA isolation

Five raising-camels regions, covering East, West, North, South and middle localities in Saudi Arabia, were visited for sampling camels. Hair and blood samples were collected from 296 camels representing 4 indigenous breeds, 50 Magaheem (MG), 50 Magha-teer (MJ), 50 Sofr (SO) and 50 Shaul (SH), in addition to two exotic ones, 48 Sawahli (SL) and 48 Somali (SU). The collected samples were kept in -20°C until use. Information about camel breeds, e.g. history of sampled populations, were also recorded. DNA was extracted using the QIAgen DNeasy blood and tissue kit (Hildane, Germany) following the manufacturer's instructions.

2.2. Polymerase chain reaction (PCR) and fragment analysis

Twenty one microsatellite primer-pairs (FAO, 2000) were used to genotype the 296 camels (Table 1). To test the usability of the 21 microsatellites in genotyping sampled camels, pooled DNA preparations from apparently distant populations were firstly tested. PCR gradients were also used to obtain the optimum annealing temperatures for every primer-pair of each microsatellite marker. The amplification was performed using the Gene Amp PCR System 9700 thermocycler (Applied Biosystems, Warrington, UK) according to Mahmoud et al. (2012). The amplification protocol included an initial denaturation step for 2 min at 94°C , followed by 35 cycles at 94°C for 30 s, $50\text{--}60^{\circ}\text{C}$ annealing temperature (depending on the primer-pair used) for 30 s and 72°C for 30 s. The final step of the amplification protocol was an extension step at 72°C for 5 min. Agarose gels were used to check the amplification reproducibility from DNA preparations. The amplified PCR products were multiplexed based on their fluorescent dye and sizes. Each $0.5\ \mu\text{l}$ of multiplexed PCR products were mixed with $9.25\ \mu\text{l}$ of HiDi formamide and $0.25\ \mu\text{l}$ of GeneScan[®] LIZ standard Applied Biosystems. This mixture was immediately denatured at 95°C for five minutes and chilled on ice for five minutes. Two microliters of the previous mixture were loaded into DNA capillary ABI Prism[®] 3500 Genetic Analyzer. The used fluorescent dyes were FAM, NED, VIC and PET. The raw data were collected using genetic analyzer data collection software version 3.0.

2.2.1. Genetic analyses

The basic parameters for each locus and populations, including allele frequencies, observed number of alleles (N_a), effective number of alleles (N_e), observed (H_o) and expected (H_e) heterozygosity values were calculated using Cervus version 3.0.3 (Kalinowski et al., 2007). Wright's F-statistics (F_{IS} , F_{ST} , and F_{IT}) within and among the camel populations were calculated by using GenePop version 4.0.10 (Raymond, 1995). Deviations from Hardy-Weinburg equilibrium were also calculated by using the GenePop. Factorial correspondence analysis (AFC) 2D plots were performed on a table of allele frequencies using GENETIX 4.05.2 (Belkhir et al., 2004) to infer population differentiation (Jombart et al., 2009). Population structure was estimated using Structure v.2.3 (Pritchard et al., 2000) through the population admixture model. Simulations were run with Markov Chain Monte Carlo (MCMC) of 2.2×10^6 iterations after a burn-in of 2×10^5 . Six independent simulations for different K values (1–8) were performed to identify the most probable clustering solution through examining the modal distribution of DeltaK (ΔK) (Evanno et al., 2005). The results from multiple runs for each K were concatenated by clump (Jakobsson and Rosenberg, 2007).

3. Results and discussion

The present study included 296 individuals of dromedary camels belonging to six populations: MG, MJ, SO, SH, SL and SU. The camel individuals were genotyped using 21 microsatellite loci (Table 1). Out of the 21 microsatellite primer-pairs, 19 successfully generated scorable polymorphic fingerprints from the sampled animals. The VOLP67 and CMS25 primer-pairs did not produce any PCR products. The total number of alleles (N_a), mean effective number of alleles (N_e), observed (H_o) and expected (H_e) heterozygosities were estimated for the six populations. The number of alleles per locus ranged from 7 to 23, with an average of 13.3 alleles per locus (Table 2). This range was comparable with that observed by Spencer et al. (2010) in Australian dromedary camels with an average of 13.18. However, Mehta et al. (2007), Al-Swailem et al. (2009), Nolte (2003) and Schulz et al. (2010) reported an average of 10.7, 3.81, 10.3 and 10.3 alleles per locus in Indian, Saudi, South African, Canarian camel populations, respectively.

The total number of alleles generated from the sampled camels using the 19 microsatellite primer-pairs was 253 alleles. The observed alleles varied within SL, SU, MG, MJ, SH and SO populations, with a total numbers of 158, 139, 161, 155, 168 and 162, respectively (Table 2). It is proposed that the number of alleles in a population is highly dependent on the sample size and the presence of unique alleles with low frequencies (Kalinowski, 2004). Generally, as the sample size increases, the number of alleles increases. Al-Swailem et al. (2009) reported a total number of 61 alleles generated from 99 animals using 20 microsatellite primer-pairs. The other previous camel studies varied in the total number of alleles where different numbers of individuals and microsatellites were used (Spencer et al., 2010; Nolte, 2003; Schulz et al., 2010; Spencer and Woolnough, 2010).

In this study, CVRL06 was the lowest polymorphic microsatellite locus with seven alleles detected in the studied camel populations (Table 2). Vijn et al. (2007) reported that CVRL06, amongst 23 loci, was also the lowest polymorphic locus with 3 alleles in Indian Bikaneri breed. Although Mariasegaram et al. (2002) found that CVRL08 had the lowest number of alleles; they recorded 3 alleles of CVRL06 in dromedary camels. Moreover, 3 and 4 alleles of CVRL06 were detected in Tunisian Kebili and Medenine camel populations, respectively (Ahmed et al., 2010). On the other hand, YWLL08 was the most polymorphic locus with 23 alleles (Table 2). Many studies reported different numbers of YWLL08 alleles.

Table 1
The 21 Microsatellite primer-pairs used to genotype the sampled camels collected from Saudi Arabia.

No.	Locus	Primer (5'→3')	Annealing Temp.	Size range (bp)
1	YWLL08	F- ATCAAGTTTGAGGTGCTTTCC R- CCATGGCATTGTGTGAAGAC	55 °C	133–180
2	YWLL09	F-AAGTCTAGGAACCGGAATGC R-AGTCAATCTACACTCCTTGC	55 °C	138–180
3	YWLL38	F-GGCCTAAATCCTACTAGAC R- CCTCTCACTCTGTCTCTCCTC	60 °C	174–192
4	YWLL44	F-CTCAACAATGCTAGACCTTGG R- GAGAACACCGCTGGTGAATA	60 °C	86–120
5	YWLL59	F-TGTGCAGCAGTTAGGTGTA R- CCATGTCTCTGAAGCTCTGGAA	58 °C	96–136
6	VOLP03	F-AGACGGTTGGGAAGGTGGTA R-CGACAGCAAGGCACAGGA	60 °C	129–206
7	VOLP08	F-CCATTACCCCATCTCTC R-TGCCAGTGACCTTATTTAGA	55 °C	142–180
8	VOLP10	F-CTTTCTCCTTTCCTCCTACT R-CGTCCACTTCCTTCATTTTC	55 °C	231–268
9	VOLP32	F-GTGATCGGAATGGCTTGGAA R-CAGCGAGCACCTGAAAGAA	55 °C	192–262
10	VOLP67	F-TTAGAGGGTCTATCCAGTTTC R-TGGACCTAAAAGAGTGGAG	55 °C	142–203
11	LCA66	F-GTGCAGCGTCCAAATAGTCA R-CCAGCATCGTCCAGTATTCA	58 °C	212–262
12	CVRL01	F-GAAGAGGTGGGGCACTAC R-CAGGAGATATCCATTGAA	55C	188–253
13	CVRL05	F-CCTTGGACCTCTGTCTCTG R-GCCACTGGTCCCTGTCTATT	60C	148–174
14	CVRL06	F-TTTTAAAAATTCTGACCAGGAGTCTG R-CATAATAGCCAAAACATGGAAACAAC	60 °C	185–205
15	CVRL07	F-AATACCCTAGTTGAAGCTCTGTCTCT R-GAGTGCCTTTATAAATATGGGTCTG	55 °C	255–306
16	CMS13	F-TAGCCTGACTCTATCCATTTCCTC R-ATTATTGGAAITCAACTGTAAGG	55 °C	238–265
17	CMS17	F-TATAAAGGATCACTGCCTTC R-AAAATGAACCTCCATAAAGTTAG	55 °C	135–167
18	CMS18	F-GAACGACCCTTGAAGACGAA R-AGGAGCTGGTTTTAGGTCCA	60 °C	157–188
19	CMS25	F-GATCCTCTGCGTTCTTATT R-CTAGCCTTTGATTTGGAGCAT	58 °C	93–128
20	CMS50	F-TTTATAGTCAGAGAGAGTGCTG R-TGTAGGGTTCATTGTAACA	55 °C	129–190
21	CMS121	F-CAAGAGAACTGGTGGAGATTTC R-TTGATAAAAAATACAGCTGGAAG	60 °C	128–166

Table 2
Number of alleles of each microsatellite locus for the six different camel populations from Saudi Arabia.

Locus	SL ^a	SU	MG	MJ	SH	SO	Total
CMS 121	9	9	11	9	9	9	15
CVRL 05	12	7	10	8	9	10	14
VOLP 08	11	7	5	6	4	5	15
YWLL 08	15	16	13	15	17	16	23
YWLL 38	5	4	7	6	7	5	10
CMS17	6	5	6	5	10	5	11
CMS13	8	7	7	7	6	9	10
CMS18	5	5	6	5	7	7	10
CVRL 06	5	3	5	4	4	5	7
LCA 66	7	8	10	9	10	10	13
VOLP 32	5	4	4	6	6	4	9
VOLP 03	11	11	9	9	12	10	17
CVRL07	12	7	15	11	11	9	16
CVRL 01	15	12	16	16	12	18	21
CMS50	14	14	10	11	13	11	22
YWLL44	6	7	7	8	10	9	13
YWLL59	4	3	6	5	6	5	8
YWLL09	4	4	7	6	8	8	9
VOLP10	4	6	7	9	7	7	10
Mean	8.316	7.316	8.474	8.158	8.842	8.526	13.316
SE	0.899	0.841	0.777	0.754	0.754	0.849	1.095

^a The breed abbreviations SL, SU, MG, MJ, SH and SO are as follows: Sawahli, Somali, Majaheem, Maghateer, Shaul and Sofr, respectively.

Spencer et al. (2010) found 24 alleles of YWLL08 in dromedary racing camels. Other studies reported variable number of YWLL08 alleles ranging from 9 to 20 in different camel populations, e.g. Indian Bikaneri and Australian camels (Mahmoud et al., 2012; Vijn et al., 2007; Spencer and Woolnough, 2010; Hashim et al., 2014; Eltanany et al., 2015; Nolte, 2003; Schulz et al., 2010). According to Bishop et al. (1994), there is a positive relationship between the number of dinucleotide repeats and the number of alleles at a given locus, explaining why YWLL08 (size range 133–180 bp) has more detected alleles than CVRL06 (size range 185–205 bp).

The heterozygosity level of a microsatellite marker varies from species to species and sometimes among different breeds of the same species (Guichoux et al., 2011). In the present study, the average heterozygosity observed in SL, SU, MG, MJ, SH and SO populations were 0.914, 0.929, 0.765, 0.717, 0.743 and 0.713, respectively (Table 3), whereas their expected values were 0.707, 0.702, 0.700, 0.667, 0.695 and 0.691, respectively (Table 3). Generally, the observed heterozygosity values were higher than expected, with the lowest expected heterozygosity value of 0.667 detected in MJ population. However, the highest value was 0.707 in SL population. The highest observed heterozygosity value was in SU camel population, whereas the lowest value was in SO population. The average observed heterozygosity in the present study (0.713–0.929) was higher than reported by previous studies in Tunisian (0.460), Arabian (0.552) and Australian camel populations (0.455) (Spencer et al., 2010; Nolte, 2003; Kalinowski, 2004). Vijn et al. (2007) observed heterozygosity values of 0.580, 0.570, 0.560 and 0.600 for Bikaneri, Jaisalmeri, Kutchi and Mewari populations, respectively. The study of Al-Swailem et al. (2009) on Saudi camels have dealt with three breeds of camels including; Magaheem, Sofr and Shogeh. They found very little variation between the three breeds and low heterozygosity concluding that the three types are closely related. The preliminary study carried out by Mahmoud et al. (2012) on MG, MJ, SO and SH populations from Riyadh area, Saudi Arabia, showed that the observed heterozygosities ranged from 0.605 to 0.665.

The maximum N_e values of YWLL08 locus were 7.200, 9.198, 7.764 and 6.803 for SL, SU, MJ and SO populations, respectively. However, N_e in MG Population was 7.123 for CVRL07 locus and

6.649 in SH population for locus CMS50 locus. The means of effective number of alleles were 3.938, 2.944, 3.770, 3.599, 3.774 and 3.590 for SL, SU, MG, MJ, SH and SO populations, respectively. Australian camels displayed an average effective number of alleles of 3.44 whereas Bikaneri camels displayed the highest average effective number of alleles of 4.40 (Mehta et al., 2007; Vijn et al., 2007).

Animal breeds with constant gene and genotype frequencies are expected to be in Hardy-Weinberg Equilibrium (Falconer and Mackay, 1996). In the present study, the number of loci that deviated from the Hardy-Weinberg Equilibrium were 14, 17, 16, 12, 14 and 13 in SL, SU, MG, MJ, SH and SO populations, respectively (Table 4). It has been shown that the number of microsatellite loci that deviated from Hardy-Weinberg Equilibrium (11, 5, 6 and 6, respectively) in Bikaneri, Jaisalmeri, Kutchi and Mewari camel breeds were less than those that followed it (12, 18, 17 and 17, respectively) (Vijn et al., 2007). A previous study on Saudi Arabian populations, half of used microsatellite loci were in Hardy-Weinberg Equilibrium (Al-Swailem et al., 2009). On another study in Saudi Maghaeem, Majahteer, Sufr and Shual dromedarian camels, most of the loci (11/4, 7/8, 9/6 and 9/6, respectively) followed the Hardy-Weinberg Equilibrium ($P < 0.01$) (Mahmoud et al., 2012).

The average F_{IS} , F_{IT} and F_{ST} values were -0.165 , -0.090 and 0.063 , respectively (Table 5). The low F_{IS} and F_{IT} values may indicate low level of inbreeding within and among the studied populations, with low genetic differentiation among them. The F_{ST} values indicate that less than 1% of the total genetic variation was explained by a population difference, whereas the remaining 99% corresponding to differences among individuals.

To avoid the standard error of distance estimates, Barker (1994) suggested microsatellite loci with fewer number of alleles (>4) should be removed from the genetic distance studies. In our study, none of the alleles was removed from the genetic distance analysis as the lowest number of alleles per locus was 7. The pairwise genetic distance showed that the Saudi indigenous camel breeds (MG, ML, SO, and SH) are genetically close to each other. The pairwise genetic distances among these four breeds ranged between 0.023 and 0.041 (Table 6). The SL breed showed larger genetic distances with the previous four breeds with genetic distances ranged between 0.296 and 0.337 (Table 6). Interestingly, the exotic Somali

Table 3
Observed (H_o) and expected (H_e) heterozygosities of microsatellite loci for the six camel populations from Saudi Arabia.

Locus	SL ^a		SU		MG		MJ		SH		SO	
	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e	H_o	H_e
CMS121	1	0.747	1	0.727	0.78	0.774	0.72	0.755	0.8	0.741	0.6	0.722
CVRL05	1	0.781	1	0.747	0.76	0.735	0.68	0.689	0.48	0.646	0.62	0.653
VOLP08	0.958	0.724	0.938	0.706	0.42	0.485	0.48	0.424	0.5	0.459	0.44	0.455
YWLL08	0.979	0.861	1	0.891	0.62	0.827	0.6	0.871	0.66	0.846	0.7	0.853
YWLL38	1	0.714	1	0.634	0.66	0.646	0.58	0.597	0.5	0.519	0.48	0.572
CMS17	1	0.54	1	0.576	0.92	0.593	1	0.633	1	0.615	0.96	0.601
CMS13	1	0.736	1	0.73	0.56	0.617	0.48	0.568	0.64	0.654	0.58	0.673
CMS18	0.667	0.59	0.688	0.545	1	0.713	0.98	0.681	0.74	0.659	0.82	0.723
CVRL06	0.938	0.539	1	0.52	0.86	0.715	0.7	0.551	0.8	0.655	0.78	0.668
LCA66	0.896	0.787	1	0.735	0.8	0.727	0.62	0.695	0.62	0.762	0.56	0.69
VOLP32	0.792	0.607	0.875	0.599	0.76	0.517	0.28	0.282	0.54	0.451	0.52	0.471
VOLP03	0.917	0.645	0.792	0.75	0.82	0.708	0.84	0.679	0.88	0.729	0.9	0.76
CVRL07	1	0.843	0.979	0.801	1	0.86	0.92	0.856	1	0.843	1	0.789
CVRL01	0.958	0.838	0.958	0.84	0.8	0.819	0.78	0.729	0.82	0.789	0.68	0.781
CMS50	0.958	0.85	0.875	0.866	0.62	0.825	0.84	0.836	0.86	0.85	0.72	0.816
YWLL44	1	0.74	1	0.705	0.64	0.573	0.64	0.631	0.72	0.728	0.62	0.701
YWLL59	1	0.548	1	0.547	0.92	0.744	0.84	0.746	1	0.755	0.98	0.722
YWLL09	0.875	0.647	0.938	0.648	0.94	0.717	0.92	0.662	0.8	0.736	0.92	0.721
VOLP10	0.438	0.69	0.604	0.763	0.66	0.715	0.72	0.782	0.76	0.764	0.66	0.751
Mean	0.914	0.707	0.929	0.702	0.765	0.7	0.717	0.667	0.743	0.695	0.713	0.691
SE	0.033	0.025	0.027	0.025	0.036	0.024	0.043	0.033	0.039	0.027	0.04	0.024

^a The breed abbreviations SL, SU, MG, MJ, SH and SO are as follows: Sawahli, Somali, Majaheem, Maghateer, Shaul and Sofr, respectively.

Table 4
Microsatellite loci significantly deviated from Hardy-Weinberg equilibrium.

Locus	SL ^a	SU	MG	MJ	SH	SO
CMS 121	0.000*	0.001*	0.906	0.698	0.054	0.145
CVRL 05	0.526	0.000*	0.310	0.000*	0.000*	0.310
VOLP 08	0.004*	0.033*	0.000*	1.000	0.854	0.983
YWLL 08	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
YWLL 38	0.000*	0.000*	0.034*	0.000*	0.000*	0.000*
CMS17	0.000*	0.000*	0.002*	0.000*	0.000*	0.000*
CMS13	0.000*	0.000*	0.931	0.980	0.988	0.373
CMS18	0.208	0.106	0.000*	0.000*	0.008*	0.000*
CVRL 06	0.000*	0.000*	0.000*	0.112	0.000*	0.000*
LCA 66	0.594	0.051	0.000*	0.000*	0.000*	0.000*
VOLP 32	0.032*	0.000*	0.000*	0.000*	0.000*	0.000*
VOLP 03	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
CVRL07	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
CVRL 01	0.712	0.000*	0.000*	0.999	0.288	0.269
CMS50	0.435	0.000*	0.000*	0.666	0.871	0.000*
YWLL44	0.000*	0.000*	0.000*	0.997	0.001*	0.000*
YWLL59	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
YWLL09	0.000*	0.000*	0.003*	0.006*	0.000*	0.000*
VOLP10	0.000*	0.000*	0.009*	0.000*	0.000*	0.300

^a The breed abbreviations SL, SU, MG, MJ, SH and SO are as follows: Sawahli, Somali, Majaheem, Maghateer, Shaul and Sofr, respectively.

* Microsatellite loci deviated from HWE at $P < 0.05$.

Table 5
F-statistics analysis for each of 19 microsatellite loci used for genotyping six Saudi camel populations.

Locus	F _{IS}	F _{IT}	F _{ST}
CMS121	-0.097	0.007	0.095
CVRL05	-0.068	0.074	0.133
VOLP08	-0.149	0.006	0.135
YWLL08	0.115	0.151	0.041
YWLL38	-0.146	-0.052	0.082
CMS17	-0.653	-0.480	0.105
CMS13	-0.071	-0.034	0.035
CMS18	-0.251	-0.221	0.024
CVRL06	-0.392	-0.277	0.082
LCA66	-0.023	0.023	0.045
VOLP32	-0.287	-0.151	0.106
VOLP03	-0.206	-0.118	0.073
CVRL07	-0.182	-0.158	0.020
CVRL01	-0.042	0.007	0.047
CMS50	0.034	0.069	0.037
YWLL44	-0.133	-0.104	0.026
YWLL59	-0.413	-0.346	0.048
YWLL09	-0.305	-0.272	0.026
VOLP10	0.139	0.168	0.033
Mean	-0.165	-0.090	0.063
SE	±0.044	±0.040	±0.009

SU breed was genetically distant from the Saudi breeds with pair-wise genetic distance ranged from 0.373 to 0.411 (Table 6). However, the pairwise genetic distance between SU and SL was 0.082 indicating close genetic relationship and most probably both are recently introduced to the Kingdom.

The 2D AFC based on allele frequencies grouped the accessions into two well differentiated clusters. The first principal component

Table 6
Pair-wise genetic distances among six camel populations from Saudi Arabia.

	SL ^a	SU	MG	MJ	SH	SO
SL	0.000					
SU	0.082	0.000				
MG	0.313	0.410	0.000			
MJ	0.298	0.405	0.041	0.000		
SH	0.337	0.411	0.041	0.038	0.000	
SO	0.296	0.373	0.033	0.033	0.023	0.000

^a The breed abbreviations SL, SU, MG, MJ, SH and SO are as follows: Sawahli, Somali, Majaheem, Maghateer, Shaul and Sofr, respectively.

(PC) explained 6.31% of the total variance and distinguished between these clusters. The first cluster included all SL and SU individuals and the second one included the remaining individuals from populations MG, MJ, SH and SO. Another way to visualize the eventual groups was to analyze the Arabian *C. dromedarius* microsatellite dataset using Structure with no prior distribution specified. Structure revealed that $K = 3$ had the highest ΔK , and has a strong peak at the “true” number of clusters, suggesting that the optimal number of K was 3 (Fig. 1A). In this analysis, the colors represent the three different clusters (Fig. 1B). Each bar in the graph represents an animal with its inferred proportion of genome admixture. The proportion of membership of each pre-defined population in each of the 3 clusters was 99.8% to the first cluster (red) for SL and SU breeds. This proportion reached 50% for MG, MJ, SH and SO to the second (green) and the third (blue) clusters, respectively. The bar plot graph shows a unique ancestral genome for both of SL and SU. However, camels from the MG, MJ, SH and SO appear sharing allele frequencies of the green and blue groups. The lowest genetic distance was observed between the second and third populations ($d_{2-3} = 1\%$). However, it reached 11% between the first and third populations.

4. Conclusion

Microsatellite markers were very useful to elucidate the genetic diversity within and among camel populations in Saudi Arabia. Based on the genetic analyses, the camels were divided into two groups: one contained the Saudi indigenous populations (Majaheem, Maghateer, Shaul and Sofr) and the other contained the non-Saudi ones (Somali and Sawahli). There was very little gene flow occurring between the two groups.

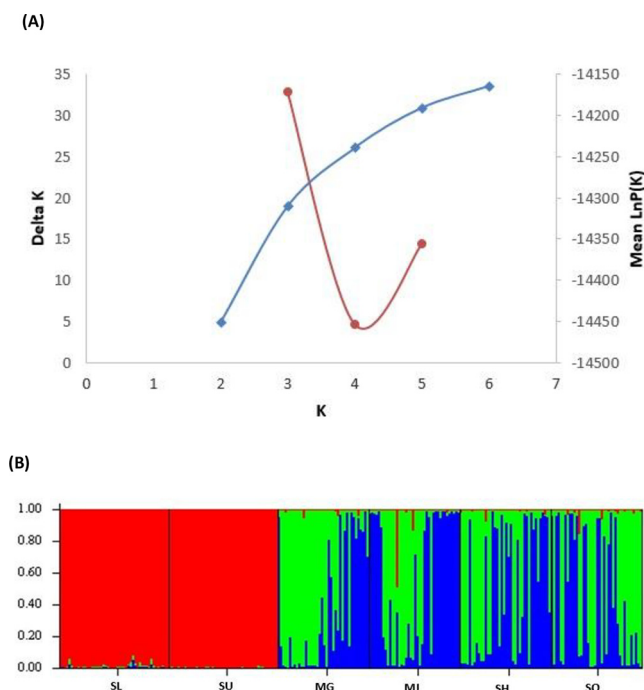


Fig. 1. Genetic structure of 296 unrelated camels representing six breeds from Saudi Arabia using Structure. (A) A plot of two graphical methods allowing detection of the true value for K. The blue (square) represents the mean of $\text{LnP}(k)$ over 8 runs for each K value analyzed the increase of the variance associated to $\text{LnP}(D)$ across different K values tested. The red (circle) represents the values of ΔK calculated, based on the methodology proposed by Evanno et al (2005). (B) A bar plot of individual ancestry proportions for the genetic clusters inferred using STRUCTURE for the optimal value of K estimated ($K = 3$).

Declaration of Competing Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgments

This Project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number (12-AGR2924-02).

References

- Ahmed, M.O., Salem, F.B., Bedhief, S., Rekih, B., Djemali, M., 2010. Genetic diversity in Tunisian dromedary (*Camelus dromedarius*) populations using microsatellite markers. *Livest. Sci.* 132, 182–185.
- Al-Swailem, S., Shehata, M.M., Al-Busadah, K.A., Fallatah, M.H., Ejaz, A., 2009. Evaluation of the genetic variability of microsatellite markers in Saudi Arabian camels. *J. Food. Agric. Environ.* 7, 636–639.
- Barker, J., 1994. A global protocol for determining genetic distances among domestic livestock breeds. Paper presented at the Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, Guelph and Ontario, Canada.
- Belkhir, B., Borsa, K.P., Chikhi, L., Raufaste, N., Bonhomme, F., 2004. GENETIX 405, logiciel sous Windows TM pour la génétique des populations. *Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France)*.
- Bishop, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D., Yoo, J., 1994. A genetic linkage map for cattle. *Genetics* 136, 619–639.
- Eltanany, M., Elfaroug Sidahmed, O., Distl, O., 2015. Assessment of genetic diversity and differentiation of two major camel ecotypes (*Camelus dromedarius*) in Sudan using microsatellite markers. *Arch. Anim. Breed.* 58, 269–275.

- Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14, 2611–2620.
- Falconer, D.S., Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*. Harlow UK Longman Science and Technology, England.
- FAO-statistics (2011). Available from: <<http://faostat.fao.org/>>.
- FAO, 2000. Domestic Animal Diversity Information System (DAD-IS 20) Available from: <http://dad.fao.org/>.
- Geng, R.Q., Chang, H., Yang, Z.-P., Sun, W., Wang, L.-P., Lu, S.-X., Tsunoda, K., Ren, Z., 2003. Study on origin and phylogeny status of Hu sheep. *Asian. Austral. J. Anim.* 16, 743–747.
- Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., Lepoittevin, C., Malausa, T., Revardel, E., Salin, F., 2011. Current trends in microsatellite genotyping. *Mol. Ecol. Resour.* 11, 591–611.
- Hashim, W., Ahmed, S.E.S., Makkaw, A.A., Yousef, G.M., Mehta, S., 2014. Twenty five polymorphic microsatellite loci used to genotype some camel types & subtypes from Sudan, Qatar, Chad and Somalia. *J. Camel. Pract. Res.* 21, 127–135.
- Hedayat-Evrigh, N., Khalkhali-Evrigh, R., Vahedi, V., Pourasad, K., Sharifi, R.S., 2018. Genetic Diversity and Population Structure of Old World Camelids (*Camelus dromedarius* and *Camelus Bactrianus*) in Iran Using Mitochondrial DNA. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi.*, 24.
- Hines, H.C., 1999. Blood groups and biochemical polymorphisms. In: Fries, R., Ruvinsky, A. (Eds.), *Genetics of Cattle*. CAB International, England.
- Jakobsson, M., Rosenberg, N.A., 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23, 1801–1806.
- Jombart, T., Pontier, D., Dufour, A., 2009. Genetic markers in the playground of multivariate analysis. *Heredity* 102, 330.
- Kalinowski, S.T., 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conserv. Genet.* 5, 539–543.
- Kalinowski, S.T., Taper, M.L., Marshall, T.C., 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* 16, 1099–1106.
- Li, C., Basarab, J., Snelling, W., Benkel, B., Kneeland, J., Murdoch, B., Hansen, C., Moore, S., 2004. Identification and fine mapping of quantitative trait loci for backfat on bovine chromosomes 2, 5, 6, 19, 21, and 23 in a commercial line of *Bos taurus*. *J. Anim. Sci.* 82, 967–972.
- Mahmoud, A., Alshaikh, M., Aljumaah, R., Mohammed, O., 2012. Genetic variability of camel (*Camelus dromedarius*) populations in Saudi Arabia based on microsatellites analysis. *Afr. J. Biotechnol.* 11, 11173–11180.
- Mannen, H., Tsuji, S., Loftus, R., Bradley, D., 1998. Mitochondrial DNA variation and evolution of Japanese black cattle (*Bos taurus*). *Genetics* 150, 1169–1175.
- Mariasegaram, M., Pullenayegum, S., Jahabar Ali, M., Shah, R., Penedo, M., Wernery, U., Sasse, J., 2002. Isolation and characterization of eight microsatellite markers in *Camelus dromedarius* and cross-species amplification in *C. bactrianus* and *Lama pacos*. *Anim. Genet.* 33, 385–387.
- Mburu, D., Ochieng, J., Kuria, S., Jianlin, H., Kaufmann, B., Rege, J., Hanotte, O., 2003. Genetic diversity and relationships of indigenous Kenyan camel (*Camelus dromedarius*) populations: implications for their classification. *Anim. Genet.* 34, 26–32.
- Mehta, S., Goyal, A., Sahani, M., 2007. Microsatellite markers for genetic characterisation of Kachchhi camel.
- Ming, L., Yi, L., Sa, R., Wang, Z., Wang, Z., Ji, R., 2017. Genetic diversity and phylogeographic structure of Bactrian camels shown by mitochondrial sequence variations. *Anim. Genet.* 48, 217–220.
- Nolte, M., 2003. *The genetic characterisation of Camelus dromedarius in Southern Africa*. University of Johannesburg.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- Raymond, M., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered.* 86, 248–249.
- Schulz, U., Minguez, Y., Checa, M., Garcia-Atance, P., Dunner, S., García, D., Cañón, J., 2005. The Majorero camel (*Camelus dromedarius*) breed. *Animal Genetic Resources/Recursos genéticos animales/Recursos genéticos animales*. 36, 61–71.
- Schulz, U., Tupac-Yupanqui, I., Martínez, A., Méndez, S., Delgado, J.V., Gómez, M., Dunner, S., Cañón, J., 2010. The Canarian camel: a traditional dromedary population. *Diversity* 2, 561–571.
- Schwartz, H. Jr., Dioli, M., 1992. *The one-humped camel (Camelus dromedarius) in eastern Africa*.
- Spencer, P., Wilson, K., Tinson, A., 2010. Parentage testing of racing camels (*Camelus dromedarius*) using microsatellite DNA typing. *Anim. Genet.* 41, 662–665.
- Spencer, P., Woolnough, A., 2010. Assessment and genetic characterisation of Australian camels using microsatellite polymorphisms. *LIVEST. SCI.* 129, 241–245.
- Sweet, L.E., 1965. Camel pastoralism in North Arabia. In: Leeds, A., Vayda, A.P. (Eds.), *Man, Culture and Animals*. AAAS, Washington, DC, pp. 129–152.
- Vijh, R., Tantiya, M.S., Mishra, B., Bharani Kumar, S., 2007. Genetic diversity and differentiation of dromedarian camel of India. *Anim. Biotechnol.* 18, 81–90.
- Walsh, B., 2001. Quantitative genetics in the age of genomics. *Theor. Popul. Biol.* 59, 175–184.
- Wardeh, M.F., 1989. *Arabian camels: Origin, Breeds, and Husbandry*. Al-Mallah Publishers, Damascus, Syria.
- Yang, Z., Chang, H., Sun, W., Gen, R., Mao, Y., Tsunoda, K., 2004. A comparison of two kinds of markers applied in analysis of genetic diversity in sheep and goat populations. *Asian. Austral. J. Anim.* 17, 892–896.