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Full Length Research Paper

Genetic variability of camel (*Camelus dromedarius*) populations in Saudi Arabia based on microsatellites analysis

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The genetic diversity and population genetic structure of dromedary camels (*Camelus dromedarius*) are poorly documented in Saudi Arabia. The present study was conducted to address some of these genetics using four Saudi Arabian camel populations namely; Magaheem (MG), Maghateer (MJ), Sofr (SO) and Shual (SH). Genomic DNA was extracted from the hair roots of 160 camels, 40 individuals from each population. Sixteen microsatellite markers were used to genotype these 160 camels. Out of these 16 markers, only microsatellite *VOLP67* did not produce any polymerase chain reaction (PCR) amplicons. There were 139 alleles generated by the 15 microsatellites loci with a mean of 9.27 alleles per locus. Four of the microsatellites loci studied in MG, eight in MJ and six in both SO and SH were found to be deviated from Hardy-Weinberg equilibrium. The fixation genetic indices (F_{st}) among the four populations were very low, ranging from 0.006 (between SH and SO) to 0.017 (between MG and MJ), indicating low population differentiation among the four Saudi camel populations. No significant heterozygote excess or bottleneck in most nearest past was detected in the four camel populations as indicated by sign, standardized differences and Wilcoxon tests, along with the normal L shaped distribution of mode-shift test. The present study showed that the microsatellite markers are powerful tools in breeding programs, although there is a need for applying more microsatellites in order to be able to discriminate fairly between camel populations of Saudi Arabia.

Key words: Camels, *Camelus dromedarius*, microsatellite markers, Saudi Arabia, genetic variability.

INTRODUCTION

Camels are unique animals in many aspects and cannot be compared with other farm animals in their physiological responses and adaptation to arid environments (Schwartz, 1992). In arid zones, from north-western India and the lowlands of Afghanistan to the extremity of the Arabian Peninsula and Somalia to the south and westward across the African deserts, the Arabian camel is found to be a better provider of food than cattle and sheep, which are severely affected by the heat, scarcity of water and forage (Sweet, 1965). Camel populations in Saudi Arabia are named according to their area of origin or recognized based on some morphological characteristics (Al-Hazmi et al., 1994). The coat color is the most common morphological character used for the classification of camels in Saudi Arabia. For instance, Saudi camels are classified into three main phenotypic

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Abbreviations: MG, Magaheem; **MJ,** Maghateer; **SO,** Sofr; **SH,** Shual.

populations, namely Magaheem (black), Maghateer (white), and Al-Homr and Al-Sofr (brown), whereas Elamin and Wilcox (1992) indicated four major ecotypes, namely (i) the Magaheem or the Najdi, (ii) the Wadha, which has been restricted to Hejaz area, (iii) the Al-Hawara that inhabits the northern area, and (iv) the Omani, which is used mainly for riding and racing.

The past century was characterized by the development of quantitative theory and methodology towards the accurate selection of economically important genetic traits in livestock as well as the prediction of genetic responses to biotic and abiotic stresses (Hines, 1999; Walsh, 2001). It is, however, the development of molecular biological methods during the past decades that created exciting new means for studying livestock genetics and animal breeding. However, genetic studies on camels are scarce and the extent to which dromedary populations in Saudi Arabia are genetically differentiated is poorly documented. Local breeding management practices of the different ethnic groups may favor genetic differentiation (Kaufmann, 1998), but on the other hand, gene flow also likely exists between the populations. The present study was, therefore, undertaken to determine the genetic diversity in four populations of Saudi Arabian camels and to infer the relationship among them. Genetic variability assessment on camels is important to preserve genetic resources and to develop future breeding programs to improve camel productivity.

MATERIALS AND METHODS

Hair samples, including follicles, were collected from 160 individuals of the Arabian camel (*Camelus dormedarius*) from different localities in Riyadh region, 40 samples represented each of the following indigenous populations: Magaheem (MG), Maghateer (MJ), Shual (SH) and Sofr (SO). DNA was extracted from hair samples using the QIAgen DNeasy blood and tissue kit (Hilden, Germany) following the manufacturer's instructions. Sixteen microsatellite primer-pairs were used to genotype the 160 camels (Table 1). Polymerase chain reaction (PCR) amplifications were carried out in a 25 μl reaction volume containing 100 ng of template DNA and 2 μl of each 10 μM primer. To reduce the possibility of cross contamination and variation in the amplification reactions, master mixes containing all PCR reagents including the Kapa Taq polymerase enzyme (KAPA Biosystems, Boston, MA, USA) except DNA templates and primers were used. The amplification program was performed using the Gene Amp PCR system 9700 thermocycle (Applied Biosystems, Warrington, UK). The amplification protocol was initial denaturation step for 2 min at 94°C, followed by 35 cycles at 94°C for 30 s, 50 to 60°C annealing temperature (depending on the microsatellite primer-pair) (Table 1) for 30 s and 72°C for 30 s. The final step of the amplification protocol was the extension step at 72°C for 5 min. All the reactions were carried out on 96 well PCR plates (Applied Biosystems, Warrington, UK). The microsatellite primers were labeled with dyes FAM, NED, VIC and PET (Applied Biosystems, Warrington, UK) and microsatellite data were analyzed in the ABI Prism® 3130 Genetic analyzer (Applied Biosystems, Warrington, UK). Each analyzed PCR reaction contained GeneScan® LIZ 500 molecular weight standards (Applied Biosystems, Warrington, UK).

Statistical analysis

The basic parameters for each locus and populations, that is, allele frequencies, observed number of alleles (N_o), effective number of alleles (N_e), observed (H_o) and expected (H_e) heterozygosities, and polymorphic information content (*PIC*) values for each locus, were calculated using Cervus version 3.0.3 (Kalinowski et al., 2007). Wright's *F*-statistics (F_{is}, F_{st}, and F_{it}) and number of migrants (N_m) within and among the camel populations were calculated by using GenePop version 4.0.10 (Raymond and Rousset, 1995; Takahata, 1983). Deviations from Hardy-Weinburg equilibrium (HWE) were also calculated by using GenePop. The bottleneck version 1.2.02 software was applied to determine if there had been past bottlenecks in population size and locality (Cornuet and Luikart, 1996). PAUP version 4.0b10 (Swofford, 2003) was used to identify unique microsatellite haplotypes and assess the phylogenetic relationships between the 160 camels. The unweighted pair-group method with arithmetic mean (UPGMA) and neighbor-joining (NJ) algorithms implemented in Phylogenetic analysis using parsimony (PAUP) were used to construct phylogenetic trees based on microsatellite molecular markers. To test the reliability of tree topography (Felsenstein's, 1985), bootstrap test was applied by using Efron's (1982) bootstrap resampling with1000 replications.

RESULTS AND DISCUSSION

The present study included 160 individuals of dromedary camels belonging to four populations: MG, MJ, SO and SH. The camel individuals were genotyped using 16 microsatellite loci (Table 1). All the microsatellite primer-pairs successfully generated PCR amplicons except *VOLP67* primer-pair which failed to produce DNA bands. The fifteen microsatellite loci were polymorphic. The total number of alleles (N_o), mean effective number of alleles (N_e) and observed (H_o) and expected (H_e) heterozygosities estimated for the four populations is given in Table 2. The number of alleles per locus ranged from 3 to 18, with an average of 9 alleles per locus (Table 2). The locus *CMS17* had four alleles and was the least polymorphic, while the locus *CVR01* was highly polymorphic with 18 alleles. A total of 139 alleles were detected among the 160 camels by applying the 15 microsatellite loci. The total number of alleles observed in MG, MJ, SO and SH populations were 102, 105, 95 and 100, respectively. Out of these 139 alleles, 33 were designated as private alleles that is, only observed in one population. Out of these 33 private alleles, ten were observed in MG and eight in both MJ and SO populations. SH population had only seven private alleles. The number of alleles observed in the present study is higher compared to other previous studies on different camel populations for example, Kachchhi and Saudi camel populations (Mehta et al., 2007; Al-Swalim et al., 2009). On the other hand, Schulz et al. (2010) reported that the observed number of alleles per locus ranged from 2 to 22 in the Canarian camels. The effective number of alleles was less than the observed number of alleles (Table 2), suggesting a large number of alleles at low frequency.

The mean observed heterozygosity values were 0.665,

Table 1. The 16 primer-pairs used to amplify microsatellite regions in the *Camelus dromedaries* from Saudi Arabia.

Locus	Primer-pair (5'→3')	Annealing temperature (°C)	Size range (bp)	Reference
YWLL08	ATCAAGTTTGAGGTGCTTTCC CCATGGCATTGTGTTGAAGAC	55	133-180	Lang et al. (1996)
YWLL38	GGCCTAAATCCTACTAGAC CCTCTCACTCTTGTTCTCCTC	60	174-192	Lang et al. (1996)
YWLL44	CTCAACAATGCTAGACCTTGG GAGAACACCGCTGGTGAATA	60	86-120	Lang et al. (1996)
VOLP03	AGACGGTTGGGAAGGTGGTA CGACAGCAAGGCACAGGA	60	129-206	Obreque et al. (1998)
VOLP08	CCATTCACCCCATCTCTC TCGCCAGTGACCTTATTTAGA	55	142-180	Obreque et al. (1998)
VOLP32	GTGATCGGAATGGCTTGAAA CAGCGAGCACCTGAAAGAA	55	192-262	Obreque et al. (1998)
VOLP67	TTAGAGGGTCTATCCAGTTTC TGGACCTAAAAGAGTGGAG	55	142-203	Obreque et al. (1998)
LCA66	GTGCAGCGTCCAAATAGTCA CCAGCATCGTCCAGTATTCA	58	212-262	Penedo et al. (1999)
CVRL01	GAAGAGGTTGGGGCACTAC CAGGCAGATATCCATTGAA	55	188-253	Mariasegaram et al. (2002)
CVRL05	CCTTGGACCTCCTTGCTCTG GCCACTGGTCCCTGTCATT	60	148-174	Mariasegaram et al. (2002)
CVRL06	TTTTAAAAATTCTGACCAGGAGTCTG CATAATAGCCAAAACATGGAAACAAC	60	185-205	Mariasegaram et al. (2002)
CMS9	TGCTTTAGACGACTTTTACTTTAC ATTTCACTTTCTTCATACTTGTGAT	55	227-256	Evdotchenko et al. (2003)
CMS13	TAGCCTGACTCTATCCATTTCTC ATTATTTGGAATTCAACTGTAAGG	55	238-265	Evdotchenko et al. (2003)
CMS17	TATAAAGGATCACTGCCTTC AAAATGAACCTCCATAAAGTTAG	55	135-167	Evdotchenko et al. (2003)
CMS50	TTTATAGTCAGAGAGAGTGCTG TGTAGGGTTCATTGTAACA	55	129-190	Evdotchenko et al. (2003)
CMS121	CAAGAGAACTGGTGAGGATTTTC AGTTGATAAAAATACAGCTGGAAAG	60	128-166	Evdotchenko et al. (2003)

0.605, 0.662, and 0.605 for MG, MJ, SO, and SH camel populations, respectively (Table 2). These are higher than

Table 2. Total number of alleles (*No*), mean effective number of alleles (*Ne*) and observed (*Ho*) and expected (*He*) heterozygosities for each locus of the four different Saudi camel populations (MG, MJ, SH, and SO).

Locus	MG				MJ				SH				SO			
	N_o	N_e	H_o	H_e	N_o	N_e	H_o	H_e	N_o	N_e	H_o	H_e	N_o	N_e	H_o	H_e
CMS121	8	3.560	0.775	0.719	8	3.591	0.700	0.722	8	3.769	0.800	0.735	9	3.513	0.600	0.715
CVRL01	14	4.598	0.750	0.783	14	3.226	0.725	0.690	10	4.284	0.775	0.767	15	4.040	0.600	0.753
CVRL05	9	3.296	0.725	0.697	6	2.783	0.600	0.641	7	2.510	0.375	0.602	6	2.247	0.525	0.555
VOLP08	3	1.714	0.275	0.417	3	1.548	0.350	0.354	3	1.624	0.375	0.384	3	1.606	0.300	0.377
CVRL06	5	3.249	0.825	0.692	4	1.924	0.625	0.480	4	2.518	0.750	0.603	4	2.689	0.725	0.628
VOLP32	3	2.016	0.825	0.504	4	1.428	0.300	0.300	2	1.724	0.600	0.420	3	1.807	0.600	0.447
YWLL08	9	4.805	0.525	0.792	12	7.033	0.500	0.858	11	5.161	0.575	0.806	13	5.808	0.625	0.828
YWLL38	4	2.314	0.625	0.568	4	1.733	0.475	0.423	2	1.503	0.375	0.335	3	1.615	0.350	0.381
CMS9	9	3.936	0.650	0.746	7	3.330	0.725	0.700	9	5.047	0.825	0.802	5	2.656	0.775	0.623
CMS13	5	2.196	0.475	0.545	5	2.011	0.350	0.503	5	2.514	0.575	0.602	5	2.483	0.500	0.597
LCA66	8	3.337	0.750	0.700	7	2.719	0.575	0.632	6	3.616	0.525	0.723	6	2.502	0.450	0.600
VOLP03	6	2.716	0.775	0.632	10	2.548	0.800	0.608	10	2.885	0.900	0.653	10	3.865	0.875	0.741
CMS17	3	2.109	0.900	0.526	3	2.381	1.000	0.580	3	2.050	1.000	0.512	3	2.152	0.975	0.535
CMS50	9	4.923	0.550	0.797	9	5.229	0.800	0.809	9	5.594	0.825	0.821	6	4.644	0.650	0.785
YWLL44	7	2.207	0.550	0.547	6	2.572	0.550	0.611	7	3.219	0.650	0.689	9	2.960	0.525	0.662
Mean	6.80	3.132	0.665	0.644	6.33	2.937	0.605	0.594	6.33	3.201	0.662	0.630	6.66	2.972	0.605	0.615
SE	0.794	0.276	0.043	0.031	0.870	0.384	0.050	0.041	0.815	0.345	0.051	0.041	0.974	0.308	0.047	0.036

MG, Magaheem; MJ, Maghateer; SO, Sofr; SH, Shual.

those reported by other studies in Tunisian camels (0.460) (Ould Ahmed et al., 2010), and Australian camels (0.455) (Spencer and Woolnough, 2010). However, Schulz et al. (2010) showed that the observed heterozygosity in the Arabian camels was 0.552. Moreover, Vijh et al. (2007) observed heterozygosity values of 0.580, 0.570, 0.560 and 0.600 for Bikaneri, Jaisalmeri, Kutchi and Mewari camel populations, respect-tively. The expected heterozygosity values were 0.644, 0.594, 0.630 and 0.615 for MG, MJ, SO, and SH camel populations, respectively (Table 2). These expected heterozygosity values of Saudi camels were comparable to those observed in South African (Nolte et al., 2005), and Arabian camels (Schulz et al., 2010), but lower than that found in Sudanese camels (Nolte et al., 2005). Interestingly, the expected heterozygosity values of Saudi camels of the present study were higher than those reported from the United Arab Emirates camels (Mburu et al., 2003) and *Camelus dromedarius* populations from Australia (Spencer and Woolnough, 2010; Spencer et al., 2010).

The mean estimates of *F* statistics were F_{is} = -0.043, F_{it} = -0.025 and F_{st} = 0.018 (Table 3). All the three estimates of *F* statistic were significantly different from zero ($P < 0.01$). The within population inbreeding estimate, which represents the nonrandom union of gametes and deviation from HWE, revealed that there was some genotypes with several loci that followed HWE ($P < 0.05$). The number of loci that followed the HWE were 11 in MG, 7 loci in MJ, 9 loci in both SO, and SH populations. Although these markers indicated a deficiency and excess of heterozygotes, this does not explain the deviation from HWE. It is known that the migration natural processes of mutation, non random mating, genetic drift and both artificial and natural selection are factors that are known to cause deviations from HWE. The weak genetic differentiation (F_{st} = 0.018) (Table 3)

Table 3. *F*-statistics analysis for each of 15 microsatellite markers Saudi camel populations.

Locus	F_{is}	F_{it}	F_{st}
CMS 121	0.005	0.026	0.021
CVRL 01	0.047	0.059	0.012
CVRL 05	0.108	0.117	0.010
VOLP 08	0.151	0.157	0.007
CVRL 06	-0.217	-0.182	0.029
VOLP 32	-0.392	-0.341	0.036
YWLL 08	0.322	0.337	0.021
YWLL 38	-0.069	-0.043	0.025
CMS 9	-0.036	-0.005	0.030
CMS13	0.154	0.165	0.013
LCA 66	0.134	0.142	0.009
VOLP 03	-0.272	-0.256	0.013
CMS17	-0.799	-0.789	0.006
CMS50	0.120	0.137	0.019
YWLL44	0.094	0.109	0.017
Mean	-0.043	-0.025	0.018

Table 4. Gene flow measured in numbers of migrants (*Nm*; above diagonal) and F_{st} estimates (below diagonal) determined in pair-wise comparisons between four camel populations (MG, MJ, SH, and SO).

Population	MG	MJ	SH	SO
MG	-	14.5	19.2	19.7
MJ	0.017	-	20.8	22.2
SH	0.013	0.012	-	39.7
SO	0.013	0.011	0.006	-

MG, Magaheem; MJ, Maghateer; SO, Sofr; SH, Shual.

observed among Saudi dromedary populations, as well as the low F_{is} values, may indicate that the cross-breeding is a likely explanation for the high heterozygosities observed within the Saudi dromedaries.

In the present study, the PIC ranged from 0.325 (*VOLP32*) to 0.821 (*YWLL08*), with a mean value of 0.581. In Kachchhi breed of Indian camel, Mehta et al. (2007), found that the polymorphic information content ranged from 0.277 to 0.765. Spencer et al. (2010), reported PIC values of 0.611, 0.905, 0.755, 0.863, 0.885, 0.602, 0.509 and 0.815 in *VOLP03*, *VOLP32*, *LCA66*, *CMS50*, *YWLL08*, *YWLL38*, *YWLL44* and *CVRL01* markers, respectively, with a mean value of 0.510. These PIC values were lower than our values, due to their lower number of alleles at these loci (Lang et al., 1996; Obreque et al., 1998). These high values of PIC indicated higher polymorphism in the Saudi dromedaries, suggesting the usefulness of the selected microsatellite markers for characterizing the genetic diversity of Saudi camels.

The values of number of migrants (N_m) were calculated among the four Saudi camel populations (Table 4). The highest gene flow (N_m = 39.7) (Table 4) was observed between SO and SH populations, whereas the lowest (N_m = 14.5) (Table 4) was observed between MG and MJ populations. The genetic distance between SO and SH populations was very small (F_{st} = 0.006) (Table 4). Vijh et al. (2007) showed that gene flow was limited between Jaisalmeri and Mewari camel breeds (N_m = 1.29) and high between Jaisalmeri and Kutchi breeds (N_m = 15.58). The N_m values among Tunisian camel breeds were 1.65 between Kebili and Medenine, 2.06 between Kebili and Tataouine and 6.65 between Medenine and Totaouine populations (Ould Ahmed et al., 2010). Nolte et al. (2003) observed N_m = 9.061 value between South African and Sudanese, 1.157 value between South African and alpaca and 1.388 value between Sudanese camel population and alpaca. Under the Sign, standardized different and wilcoxon rank tests, the four camel populations of present study showed no bottleneck.

Phylogenetic analysis using PAUP software was used to identify the unique haplotypes and assess phylogenetic relationship between the 160 camel individuals. All camel individuals represented unique

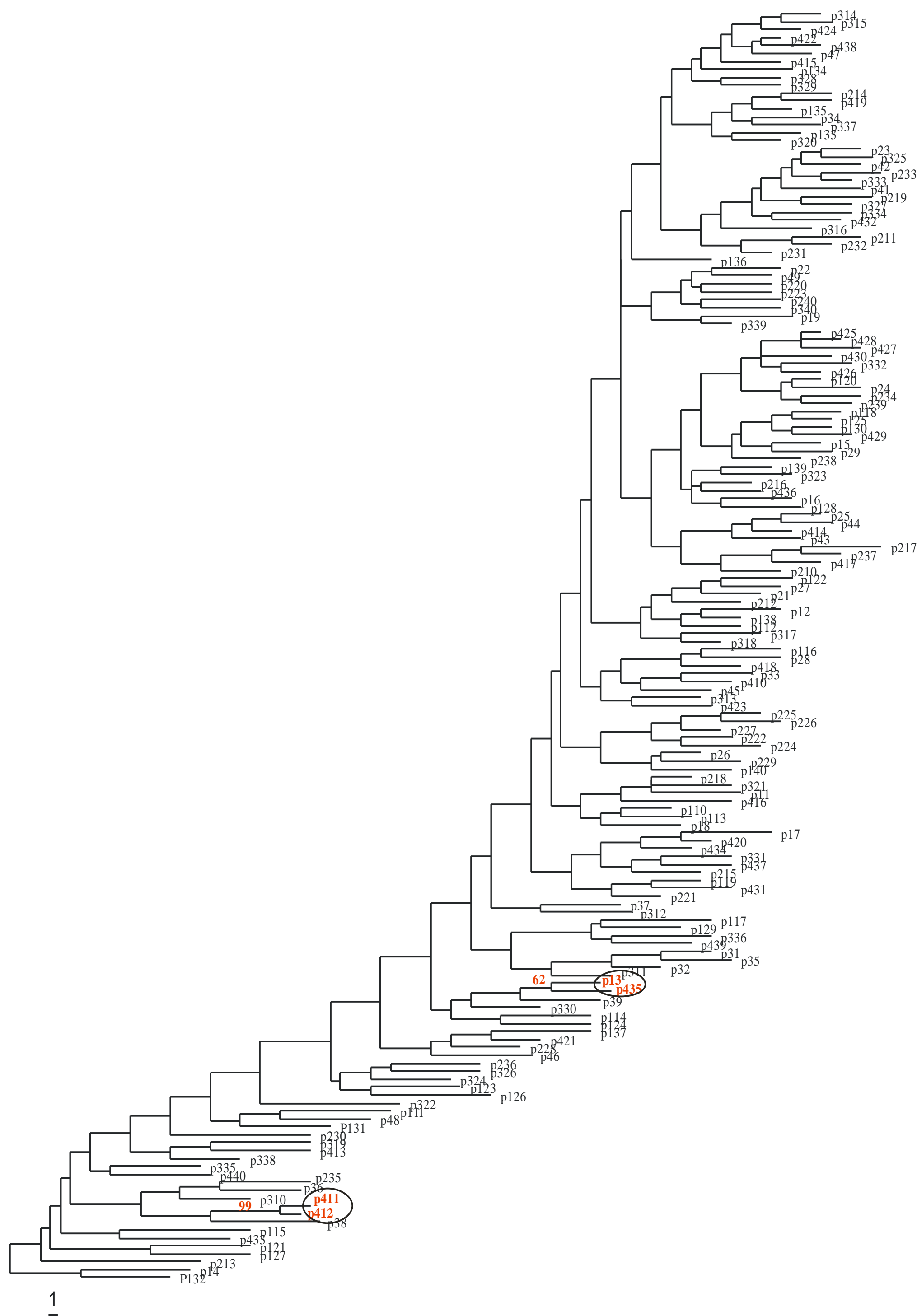

Figure 1. UPGMA dendrogram based on 139 microsatellite alleles generated from 160 camel individuals representing four populations. The scale bar under the tree represents one band difference. P in the sample identity refers to the population, followed by number 1 to 4 representing the population as follows: P1 = MG population, P2 = MJ, P3 = SH population, P4 = SO population. Each population has forty animals (1 to 40).

haplotypes (Figures 1 and 2). Generally, there were no significant bootstrap values supporting any meaningful cluster in neither UPGMA nor NJ trees (Figures 1 and 2). However, there were only two branches, each contained

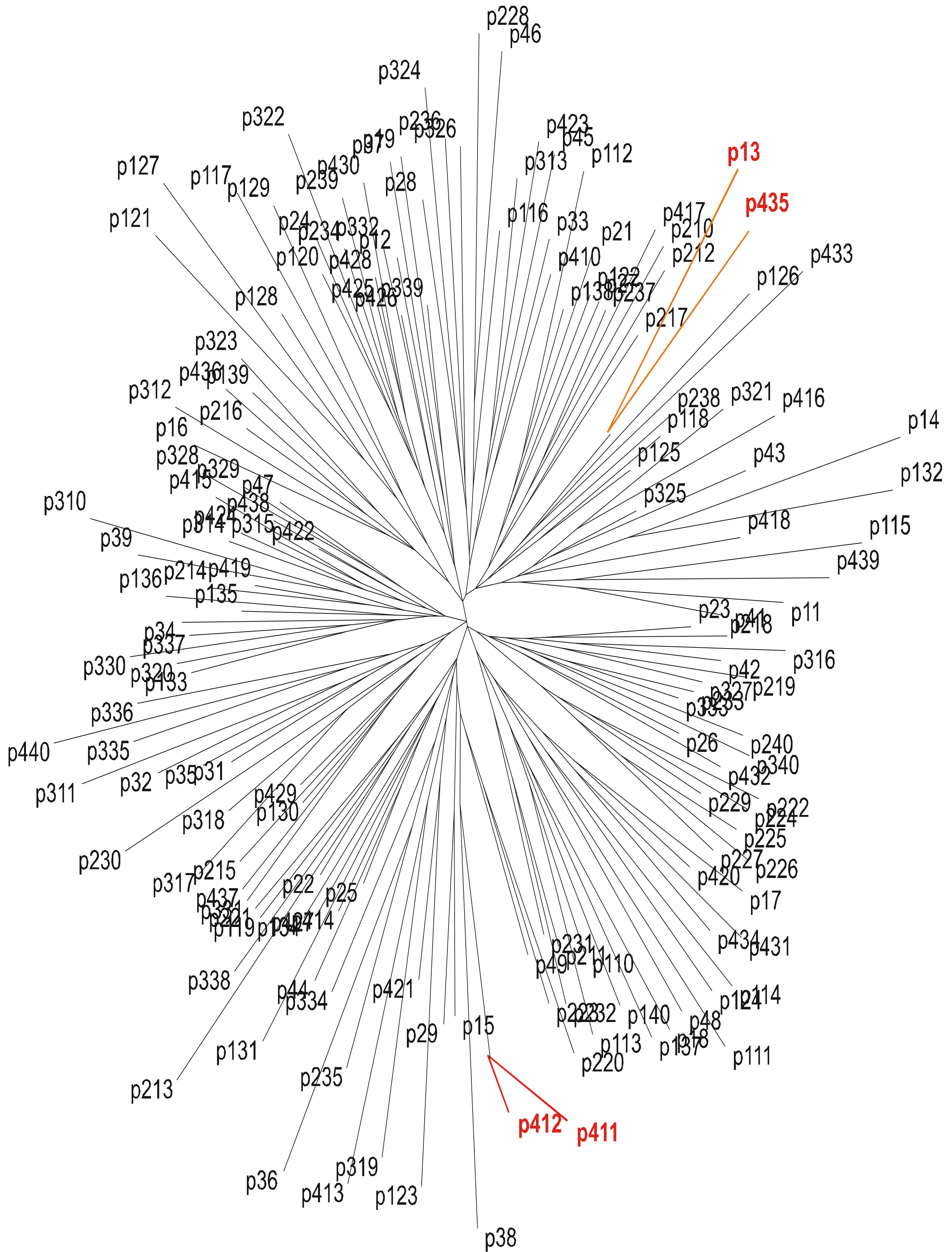

Figure 2. Unrooted neighbor joining (NJ) tree based on 139 bands generated from 15 SSR primers showing the genetic relationship between 160 camels representing four different populations. P in the sample identity refers to the population, followed by number 1 to 4 representing the population as follows: P1 = MG population, P2 = MJ, P3 = SH population, P4 = SO population. Each population has forty animals (1 to 40).

two animals, which received significant bootstrap values. The first branch was supported with 99% bootstrap value and contained P411 and P412 animals (SO population). The P411 and P412 individuals were a mother and its calf, so it is expected to have a high genetic relatedness. The second branch, supported with 62% bootstrap value, contained P13 (MG population) and P435 (SO population). These two individuals are from different populations and it is probably they may share a common genetic pool. Interestingly, most of UPGMA and NJ clusters contained individuals from different populations.

Conclusion

The genetic variation among the Saudi dromedaries may be due to higher levels of cross-breeding or a gain of genetic variation following genetic drift subsequent to migration from one area to another. Results of the present microsatellite analysis suggest a close genetic relationship and a common origin of some but not all of Saudi dromedary populations. There is extensive gene flow between the SO and SH populations. Microsatellite markers can be powerful tools in breeding programs. This study shows that more microsatellites are needed in order to identify more markers for assessing camel population differentiation. Furthermore, larger numbers of animals are required to establish a robust genetic analysis for genotyping and characterizing the camel population in Saudi Arabia.

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