J. Dairy Sci. 105:6783–6794
https://doi.org/10.3168/jds.2022-22081

Tunisian camel casein gene characterization reveals similarities and differences with Sudanese and Nigerian populations

N. Letaief,[1,2] S. Bedhiaf-Romdhani,[2] W. Ben Salem,[3] A. A. S. Mohammed,[4] G. Gaspa,[4] and A. Pauciullo[4]*

[1]National Agronomic Institute of Tunisia 1082, Tunis, Tunisia
[2]Laboratory of Animal and Forage Production, National Agricultural Research Institute of Tunisia, University of Carthage, Ariana 1004, Tunisia
[3]Animal and Pasture Agency, Tunis 1002, Tunisia
[4]Department of Agriculture, Forest and Food Sciences, University of Torino, 10095 Grugliasco (TO), Italy

ABSTRACT

Milk is a primary protein source that has always played a role in mammalian health. Despite the intensification of research projects on dromedary and the knowledge of the genetic diversity at the casein loci, the genetic structure of the Tunisian camel population still needs exploration. This study sought to determine the genetic diversity of 3 casein gene variants in 5 Tunisian camel ecotypes: c.150G>T at *CSN1S1* (α_{S1}-casein), g.2126A>G at *CSN2* (β-casein), and g.1029T>C at *CSN3* (κ-casein). The obtained results were compared with data published on Sudanese and Nigerian camels to establish the level of differentiation within and between populations. A total of 159 blood samples were collected from 5 Tunisian camel ecotypes and the extracted DNA was genotyped by PCR-RFLP. A streamlined genotyping protocol was also developed for *CSN3*. Results indicated that allele T was quite rare (0.06) at *CSN1S1* for all ecotypes. Minor allele frequency was found for G (0.462) in *CSN2* except for Ardhaoui Medenine ecotype who deviated from the average *CSN2* allele frequency of the total population. Allele C showed minor allele frequency of 0.384 in *CSN3*. Among the Tunisian population, GAT (0.343) was the most represented haplotype in all ecotypes except for Ardhaoui Medenine, where GGC (0.322) was the most frequent one. Significant differences in heterozygosity and local inbreeding were observed across the Tunisian, Sudanese, and Nigerian populations, although the global fixation index indicated that only 2.2% of the genetic variance is related to ecotype differences. Instead, phylogenetic analysis revealed a closer link between the Tunisian and Sudanese populations through a clade subdivision with 3 main branches among the ecotypes. This study represents the first attempt to understand casein gene variability in Tunisian camels; with further study, milk traits and genetic differentiation among populations can be associated with the history of camel domestication.

Key words: casein genes, ecotypes, genetic diversity, Tunisian camel population

INTRODUCTION

Climate change effects have raised concerns for livestock production and productivity globally (FAO, 2017) as it affects all aspects of an animal's feed and life (Henry et al., 2018). Camels attract particular interest with regard to climate change because they are used for meat, milk, and fiber production in some of the hottest and most hostile environments on Earth (Abri and Faye, 2019). Camels produce more milk for longer periods during drought than any other domestic animal adapted to arid lands (Al-Jassim and Sejian, 2015). Intensive dairy farms are being developed in Tunisia (Kamoun and Jemmali, 2012), Saudi Arabia (Musaad et al., 2013), and the United Arab Emirates (Nagy and Juhasz, 2016) because camel milk is an important source of protein for people currently living in these areas (Konuspayeva et al., 2009).

As in other mammals, whey and caseins are the major protein groups in camel milk (Al Kanhal, 2010). Casein is the larger of the 2 and has 4 components (α_{S1}, β, α_{S2}, and κ), each of which is encoded, respectively, by single-copy gene *CSN1S1*, *CSN2*, *CSN1S2*, and *CSN3*. Casein gene variability has been established as the key determinant to qualitative and quantitative differences in the production of milk for many species. Already well-studied in farm animals, such as cattle (Caroli et al., 2009), sheep (Ordás et al., 1997; Giambra et al., 2010), goats (Caroli et al., 2006; Cosenza et al., 2008), and buffalo (Cosenza et al., 2008; Pauciullo et al., 2021), a rising interest in camel milk has prompted study of the different variants of these genes in camels.

Received March 14, 2022.
Accepted April 27, 2022.
*Corresponding author: alfredo.pauciullo@unito.it

The first investigation of *Camelus dromedarius* milk casein genes identified the A and B variants of the *CSN1S1* gene (Kappeler et al., 1998). Later, Sudanese and Nigerian camel population studies discovered new variants in *CSN1S1* (Shuiep et al., 2013; Erhardt et al., 2016), *CSN3* (Pauciullo et al., 2013), and *CSN2* (Pauciullo et al., 2014). A recent study that fully described the casein gene cluster in dromedary camel revealed the assembly of the 4 genes in a DNA fragment of about 190 kb (Pauciullo et al., 2019). Deciphering the structure and polymorphism in the casein gene in the camel species allowed researchers to investigate casein gene variability in different camel populations (Othman et al., 2016; Yelubayeva et al., 2018; Redwan et al., 2018; Nowier and Ramadan, 2020). Nevertheless, to our knowledge, no studies have been conducted in Tunisia where camel population includes roughly 80,000 productive females per 2017 statistics produced by the Office d'Elevage et de Pâturage (OEP, 2017).

Camels are of great socioeconomic importance in Tunisia, especially for people living in the country's southern region (Jemli et al., 2018). The only reared ecotype is Maghrebi that is differentiated by tribal affiliation and geographic origin: Abidi, Merzougui, Gueoudi, Ghiloufi, and Chambi in Kebili region, and Ardhaoui in Medenine, Gabes, and Tataouine regions (Ould Ahmed et al., 2010; Chniter et al., 2013). Despite the heterogeneity of these ecotypes, no explicit genetic differences have been demonstrated (Chniter et al., 2013). This study had 2 goals. The first was to genotype the casein gene cluster in Tunisian camel ecotypes. The second was to compare them with data published for Sudanese and Nigerian camels to establish similarities and differences based on their genetic diversity. In the process, a faster protocol for the *CSN3* genotyping was developed.

MATERIALS AND METHODS

Ethics Statement

Blood was sampled respecting ethical treatment and animal welfare national legislation. Regional public veterinarians at the Commissariat Régional au Développement Agricole (CRDA) in Kebili, Medenine, Sousse, and Tozeur governorates gave permission to handle the animals. Verbal consent was also obtained from the camel owners who took part in this study.

Animals

A total of 159 whole blood samples were collected from unrelated, southern and central region Maghrebi Tunisian camels from both sexes (14 males and 145 females) belonging to different ecotypes: Abidi (n = 12), Aradhaoui Medenine (n = 30), G'oudi (n = 12), Merzougui (n = 52), and Sahli (n = 53; Figure 1). Camels were between 1 and 22 yr old. The blood samples (5 mL) were taken from the jugular vein by veterinarians during national vaccination campaigns, collected in tubes containing K_2-EDTA as the coagulant, and stored at −20°C until DNA isolation was performed.

DNA Extraction and Amplification Conditions

Genomic DNA was extracted from leucocytes using the standard phenol-chloroform method described by Sambrook and Russell (2001), and it was resuspended in 100 μL of Tris-EDTA buffer. The DNA concentration and optical density ratio (260/280) were determined using NanoDrop ND-1000 Spectrophotometer (Thermo-Scientific).

The polymorphic regions of the *CSN1S1* and *CSN2* genes were amplified using primers as suggested by Shuiep et al. (2013) and Pauciullo et al. (2014), respectively. Conversely, a new set of primers was designed for the *CSN3* gene by DNASIS Max 3.0 software (Hitachi Software) using GenBank sequence HE863813.1 as the reference. The complete list of primers used and their details is reported in Table 1. The PCR reaction was optimized in the Bio-Rad T100 Cycler and conducted in a total volume of 15 μL containing 50 ng of genomic DNA, 1× PCR buffer (Promega), 2.5 m*M* of $MgCl_2$, 5 pmol of each primer, dNTP, each at 200 μ*M*, and 2.5 U of Taq DNA Polymerase (Promega). The PCR thermal profile for *CSN1S1* and *CSN2* was performed according to Shuiep et al. (2013) and Pauciullo et al. (2014), respectively. For *CSN3*, the targeted fragment at 230 bp was amplified by PCR under following conditions: 95°C for 3 min, 40 cycles at 95°C for 30 s, 61°C for 40 s, 72°C for 20 s, and final elongation at 72°C for 5 min.

Restriction Fragment Length Polymorphism

The PCR amplicons of *CSN1S1*, *CSN2*, and *CSN3* genes were digested with *SmlI*, *HphI*, and *AluI*, respectively. Briefly, 10 μL of each PCR product was digested with 0.5 μL of the specific restriction enzyme under different incubation temperatures (Table 1). Digestion products were immediately separated by electrophoresis on agarose gel and stained with ethidium bromide. The bands were then visualized under UV light and their size was determined using specific DNA ladders.

Statistical Analysis

Allele and genotype frequencies were calculated using Popgene software v. 1.32. To test the distribution of the genotypes at Hardy-Weinberg equilibrium, the

chi-squared (χ^2) test was performed. The same software was used to evaluate the effective number of alleles, Nei's genetic diversity, and Shannon information index. To analyze between- and within-ecotype diversity, the pairwise fixation index ($\mathbf{F_{ST}}$) and the global fixation index ($\mathbf{G_{ST}}$) were figured by FSTAT v. 2.9.3.2 software (Goudet, 2022). PAST software v. 3.18 (Hammer et al., 2001) was employed to compute the graphical F_{ST}

Figure 1. Geographic distribution of the identified ecotypes within the Tunisian camel population.

Table 1. Sequences and annealing temperatures of the primers, restriction enzymes, incubation temperature, and gel concentration of *CSN1S1*, *CSN2* and *CSN3* genes

SNP	Primer name	Primer sequence[1] (5′→3′)	Temperature (°C)	Size (bp)	Endonuclease	Incubation[2]	Gel concentration (%)	Reference
CSN1S1	Cd CSN1S1 Ex4 F	F: TGAACCAGACAGCATAGAG	58.5	930	*SmlI*	2 h at 55°C	1.5	Shuiep et al., 2013
c.150G>T	Cd CSN1S1 Ex6 R	R: CTAAACTGAATGGGTGAAAC						
CSN2	Cd CSN2 5′F	F: GTTTCTCCATTACAGCATC	60.0	659	*HphI*	2 h at 37°C	2.5	Pauciullo et al., 2014
g.2126A>G	Cd CSN2 Int1 R	R: TCAAATCTATACAGGCACTT						
CSN3	Cd CSN3 F3	F: CACAAAGATGACTCTGCTATCG	64	488	*AluI*	ON at 37°C	3.5	Pauciullo et al., 2013
g.1029T>C	Cd CSN3 Int1 R	R: GCCCTCCACATATGTCTG						
	Cd CSN3 5′ F4	F: TCCTCCTATGCACTTCAC	61	230	*AluI*	ON at 37°C	3.5	Current study
	Cd CSN3 Ex1 R	R: CTGACAGGCACAAGGGAA						

[1]F = forward; R = reverse.
[2]ON = overnight.

matrix and the dendrogram by unweighted pair group method with arithmetic mean cluster analysis (1,000 bootstraps). Linkage disequilibrium between loci was calculated with HaploView v. 4.2 (Barrett et al., 2005), whereas casein haplotype frequencies were estimated using PHASE v. 2.1 software (Stephens and Donnelly, 2003). Significant differences between genotypes, haplotypes and genetic diversity parameters [observed heterozygosity (**Ho**), expected heterozygosity (**He**), allele richness, F_{ST}, number of effective alleles (**Ne**), and Shannon information index] were determined by *t*-test for independent means, one-way ANOVA and Mann-Whitney pairwise test using SAS software v. 9.00. To control for type I error associated with multiple comparisons involving single sampling sites, sequential Bonferroni adjustment was performed.

RESULTS

The 159 Maghrebi camels were genotyped for SNPs c.150G>T, g.2126A>G, and g.1029T>C located on genes *CSN1S1*, *CSN2*, and *CSN3*, respectively. The first 2 loci showed genotyping patterns that agreed with previous studies (Shuiep et al., 2013; Pauciullo et al., 2014, 2019). In the *CSN3* gene, the new set of primers resulted in amplification of a 230 bp-length fragment. Three patterns were obtained after digestion with *AluI*. The homozygous samples were characterized by 3 fragments for genotype C/C (151, 41, and 38 bp) and 2 bands for genotype T/T (189 and 41 bp). The heterozygous samples exhibited 4 fragments (189, 151, 41, and 38 bp). Therefore, the genotype discrimination was accomplished by the polymorphic band of 189 bp (Figure 2).

Allele and genotype frequencies are reported in Table 2. In the total population, alleles G and T at *CSN1S1* had frequencies of 0.934 and 0.066, respectively. The major allele ranged between 0.913 and 0.958 among the different ecotypes. No homozygous T/T genotypes were found in the Tunisian population at the *CSN1S1*. Regarding *CSN2* and *CSN3*, 3 genotypes and 2 alleles were revealed for both loci. On average, the minor allele frequencies were G (0.462) for the *CSN2* and C (0.384) for the *CSN3*, the latter varying between 0.167 for the Abidi and 0.467 for the Ardhaoui Medenine ecotype. The allele frequencies did not deviate from the Hardy-Weinberg equilibrium for all loci. The distribution of casein haplotypes in the Tunisian camel population is given in Table 3. Eight haplotypes were observed. No significant differences were found among the Tunisian ecotypes, even though differences do exist among populations ($P < 0.05$; Table 3).

The genetic diversity statistics for the 3 markers investigated are reported in Table 4, Supplemental Table

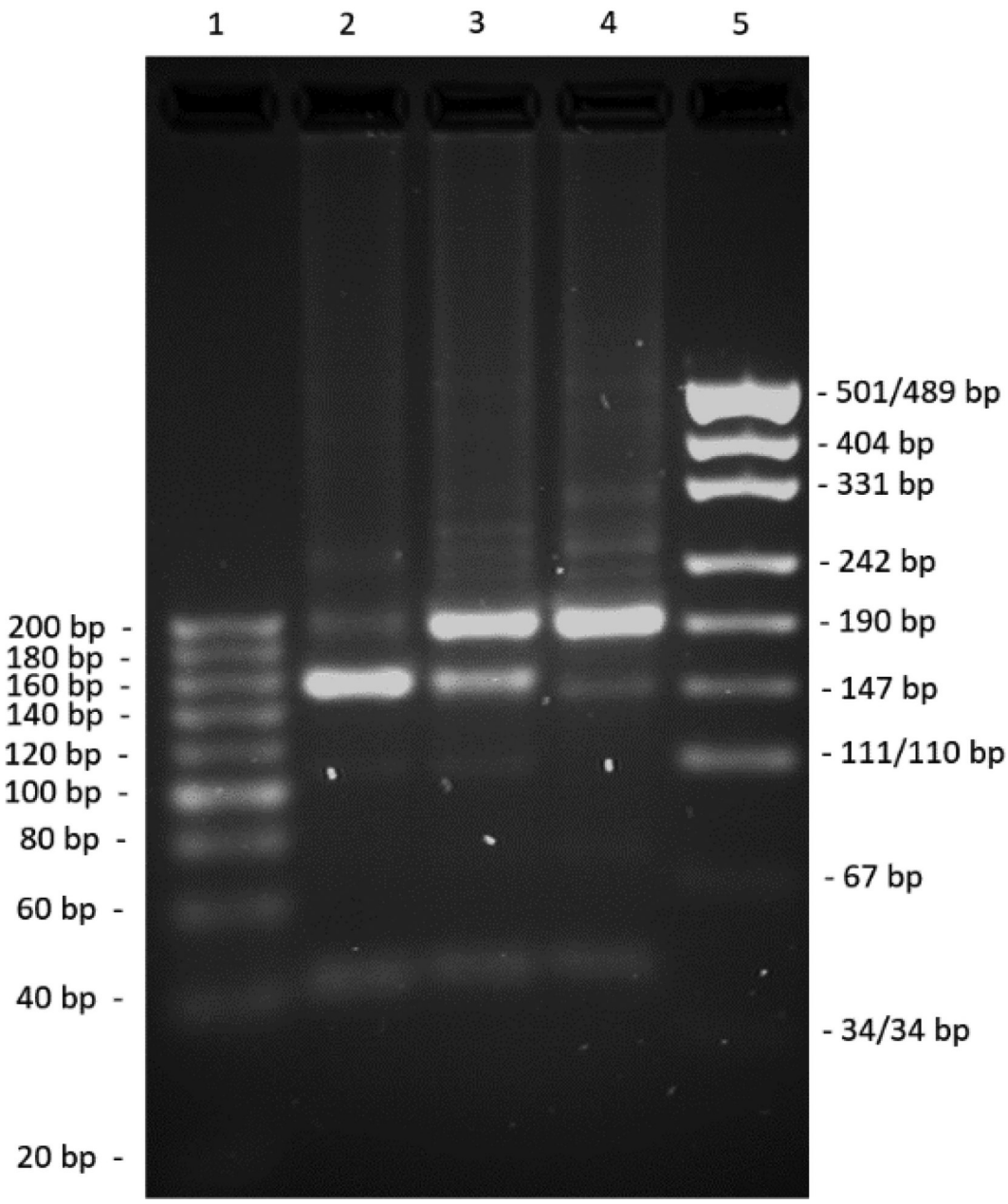

Figure 2. Genotyping of the *CSN3* by PCR-RFLP in the Tunisian camel population. Line 1: 20 bp DNA Ladder (Jena Bioscience); line 2: CC samples; line 3: CT samples; line 4: TT samples; line 5: pUC 19 DNA/MspI (*Hpa*II) Marker (ThermoFisher Scientific).

S1 (https://data.mendeley.com/datasets/vfyzwjk2p5/1; Pauciullo, 2022), and Figure 3. In all statistical analyses (He, Ho, allele richness, F_{ST}, Ne, and Shannon information index), SNP c.150G>T at *CSN1S1* exhibited lower values ($P < 0.05$) compared with other genetic variants, which made it a poor marker of variability as compared with others. This was very evident ($P \leq 0.002$) in the He value of 0.141 versus an average value of the other 2 loci of 0.487, but also for the effective number of alleles (Ne = 1.164 vs. 1.949 on average) and the F_{ST} value (0.009 vs. 0.036, on average). The linkage disequilibrium analysis revealed a low level of nonrandom association among the loci (average $D' = 0.253$ and $r^2 = 0.010$), which rendered it suitable for downstream analysis (genetic diversity within and between ecotypes).

The within-ecotype data (Table 4) showed that the lowest degree of heterozygosity, taken as a measure of genetic variability, was observed in Abidi (0.194). Conversely, the highest heterozygosity was detected in La-

Table 2. Genotypes and allele frequencies at the *CSN1S1*, *CSN2*, and *CSN3* genes in the Tunisian camel population

			CSN1S1					*CSN2*					*CSN3*				
			Genotype c.150G>T			Allelic frequency		Genotype g.2126A>G			Allelic frequency		Genotype g.1029T>C			Allelic frequency	
Ecotype	n	Type[1]	GG	GT	TT	G	T	AA	AG	GG	A	G	CC	CT	TT	C	T
Abidi	12	Obs.	11	1	0	0.958	0.042	4	4	4	0.500	0.500	1	2	9	0.167	0.833
		Exp.	11.02	0.96	0.02			3	6	3			0.33	3.33	8.33		
Ardhaoui	30	Obs.	27	3	0	0.950	0.050	6	15	9	0.450	0.550	11	6	13	0.467	0.533
Medenine		Exp.	27.07	2.85	0.07			6.07	14.85	9.07			6.53	14.93	8.53		
G'oudi	12	Obs.	11	1	0	0.958	0.042	5	5	2	0.625	0.375	3	3	6	0.375	0.625
		Exp.	11.02	0.96	0.02			4.69	5.62	1.69			1.69	5.62	4.69		
Merzougui	52	Obs.	43	9	0	0.913	0.087	14	27	11	0.529	0.471	16	15	21	0.452	0.548
		Exp.	43.38	8.22	0.39			14.54	25.91	11.54			10.62	25.76	15.62		
Sahli	53	Obs.	46	7	0	0.934	0.066	15	32	6	0.585	0.415	12	10	31	0.321	0.679
		Exp.	46.23	6.54	0.23			18.13	25.73	9.13			5.45	23.09	24.45		
Total	159		138	21	0	0.934	0.066	44	83	32	0.538	0.462	43	36	80	0.384	0.616

[1]Obs. = observed; Exp. = expected.

haoi (0.406) and Shanbali (0.403). These data were also confirmed by the local inbreeding ($\mathbf{F_S}$) that showed an excess of local inbreeding for Abidi (0.358) and excess of outbreeding in Lahaoi (−0.040) and Shanbali (−0.054). Significant differences between the entire populations were evidenced again for Ho ($P = 0.021$) and F_S ($P = 0.003$) statistics, whereas no differences were found for the other variability parameters (Table 4).

The G_{ST} among the ecotypes was 0.022 (Supplemental Table S1), whereas the pairwise F_{ST} matrix indicated that the Abidi and G'oudi populations were the most different (Figure 3a; Supplemental Table S1). Based on the same matrix, the phylogenetic relationships among the entire populations and ecotypes indicated a closer link between the Tunisian and Sudanese populations (Figure 3b). An interesting clade subdivision with 3 main branches among the ecotypes was revealed (Figure 3c).

DISCUSSION

Camel genomics have remained relatively underdescribed, but a desire to improve breeding outcomes (Singh et al., 2015) for specific economic (Redwan et al., 2018; Sani et al., 2021) and adaptability traits has ignited research in this area over the past decade. Building on the described camel casein gene cluster (Pauciullo et al., 2019), genetic diversity was used to prove its association with milk yield and composition (Inostroza et al., 2020). Specifically, we examined *CSN1S1*, *CSN2*, and *CSN3* genes for SNPs polymorphisms using DNA extracted from Tunisian Maghrebi camels. The obtained results were then compared with other camel populations to offer useful information and gain better breeding advantages through the selection of more favorable genotypes.

During the study, a speedier protocol with easily interpreted results was created to genotype SNP 1029C>T on the *CSN3* gene by amplifying a smaller DNA fragment. Specifically, new primers amplified 230 bp, whereas those described previously amplified 488 bp (Pauciullo et al., 2013). This size reduction halved amplification time and allowed genotyping based on a single discriminant band of 189 bp, as opposed to the previous error-prone and difficult-to-discern protocol that relied on just 7 base pairs to discriminate between C/T and T/T genotypes. Using this new protocol, the *CSN3* T allele was observed in highest proportion (0.616) in the Maghrebi camels, which concurs with trends reported in Sudanese (0.62; Pauciullo et al., 2013), Egyptian (0.68; Othman et al., 2016), and Kazakh camels (0.89; Yelubayeva et al., 2018). The same alleles were also identified in Nigerian dromedaries, but in the Nigerian population, the C allele was most

Table 3. Haplotype frequencies detected for the SNPs c.150G>T, g.2126A>G, and g.1029T>C at the casein loci in Tunisian, Sudanese, and Nigerian dromedary camel populations

	Haplotype frequency							
Ecotype	GAC	GAT	GGC	GGT	TAC	TAT	TGC	TGT
Tunisian (n = 159)	0.153[b]	0.342	0.214[a]	0.225	0.009	0.034	0.008	0.015
Abidi	0.062	0.396	0.104	0.396	—	0.042	—	—
Ardhaoui Medenine	0.128	0.286	0.322	0.214	0.010	0.026	0.006	0.007
G'oudi	0.098	0.501	0.259	0.099	0.006	0.019	0.011	0.005
Merzougui	0.203	0.282	0.216	0.213	0.011	0.033	0.022	0.020
Sahli	0.151	0.392	0.161	0.231	0.009	0.033	—	0.024
Sudanese (n = 198)	0.348[a]	0.263	0.028[b]	0.269	0.007	0.026	0.004	0.052
Shanbali	0.415	0.126	0.003	0.354	0.019	0.067	0.000	0.013
Khali	0.277	0.401	0.039	0.203	0.016	0.037	0.001	0.023
Arabi	0.254	0.374	0.071	0.241	0.024	0.019	0.004	0.009
Lahaoi	0.347	0.239	0.039	0.269	0.001	0.001	0.015	0.087
Nigerian (n = 69)	0.187[ab]	0.226	0.290[a]	0.223	0.014	0.029	0.006	0.023
Overall frequency	0.230	0.277	0.176	0.239	0.010	0.029	0.006	0.031

[a,b]Different superscripts mean significant differences ($P < 0.05$).

frequent (0.549; Pauciullo et al., 2019). The 3 possible genotypes were found in all of the studies named above, except for the Kazakh population, in which no heterozygous genotypes were identified (Yelubayeva et al., 2018). The lack of the heterozygous genotypes in the Kazakh camel population could be related to the small number of individuals used in the study (18 camels) compared with Egyptian (n = 50), Nigerian (n = 69), Tunisian (n = 159), and Sudanese (n = 188) populations.

Instead, in the full Tunisian camel population, the highest frequency of the heterozygous genotype (0.522) was in *CSN2* and the minor allele was G (0.462). One ecotype, Ardhaoui Medenine, deviated from the average *CSN2* G allele frequency (0.550 vs. 0.462) of the total population, whereas in the Abidi camels, the 2 alleles (0.50) were perfectly balanced. Although the sample size was small and similar in both populations, this result was at odds with findings from studies of a similarly-sized population (Pauciullo et al., 2014; Nowier and Ramadan, 2020). The β-CN protein is both abundant and important to camel milk processing. Nowier and Ramadan (2020) proposed an association study of β-CN and milk chemical composition traits in Maghrebi camel breeds because high levels of the protein and acidity in A/A-genotyped individuals suggest an affinity for cheese processing. Our data confirmed that allele A is more frequent in the Tunisian popula-

Table 4. Marker and ecotype statistics for mean values of expected heterozygosity (He), observed heterozygosity (Ho), allele richness (AR), fixation index (F_{ST}) or local inbreeding coefficient (F_S), number of effective alleles (Ne), and Shannon's information index (I)

Variable	He	Ho	AR	F_{ST}	F_S	Ne	I
Locus							
CSN1S1 c.150G>T (n = 826)	0.141	0.152	1.857	0.009		1.164	0.269
CSN2 g.2126A>G (n = 792)	0.490	0.530	2.000	0.032		1.961	0.683
CSN3 g.1029T>C (n = 806)	0.484	0.326	2.000	0.041		1.937	0.676
Ecotype							
Tunisian (n = 159)	0.354	0.269[a]	1.971		0.245[A]	1.628	0.508
Abidi	0.303	0.194	2.000		0.358	1.491	0.439
Ardhaoui Médenine	0.370	0.267	1.930		0.280	1.692	0.526
G'oudi	0.358	0.250	2.000		0.303	1.617	0.499
Merzougui	0.388	0.327	1.972		0.158	1.721	0.558
Sahel	0.352	0.308	1.948		0.124	1.619	0.516
Sudanese (n = 198)	0.362	0.358[b]	1.970		0.017[B]	1.614	0.521
Shanbali	0.382	0.403	1.984		−0.054	1.619	0.516
Khali	0.333	0.293	1.964		0.121	1.543	0.499
Arabi	0.344	0.330	1.951		0.041	1.588	0.502
Lahaoi	0.390	0.406	1.984		−0.040	1.704	0.567
Nigerian (n = 69)	0.366	0.357[b]	1.919		0.024[B]	1.691	0.527

[a,b]Different superscripts mean significant differences ($P < 0.05$).

[A,B]Different superscripts mean significant differences ($P < 0.005$).

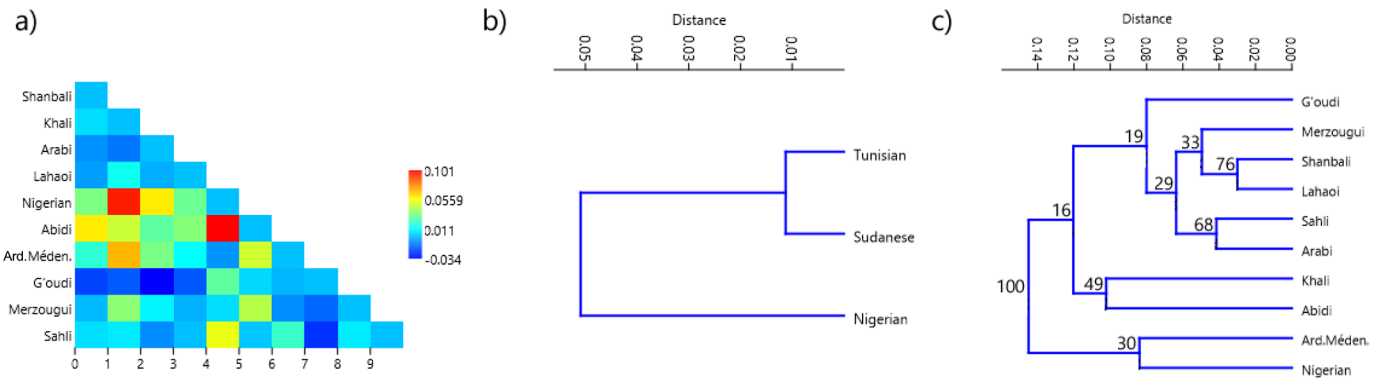

Figure 3. Output of F_{ST} statistics among Tunisian, Sudanese, and Nigerian camel populations represented by: (a) pairwise matrix; (b) phylogenetic relationships with camels grouped by geographical areas; (c) phylogenetic relationships with camels grouped by ecotype. Numbers indicate the percentage bootstrap support (1,000 replicates). The unit for evolutionary distances was computed as standard genetic distance.

tion, but at an average value of only 0.538. There is great opportunity for genetic improvement in all the ecotypes.

In the *CSN1S1* gene, T allele was quite rare (0.06). Its rarity was also reported in the Sudanese (0.094) and Nigerian (0.054) populations, with the presence of only one homozygous sample in both populations (Pauciullo et al., 2019). Our results confirmed this trend in Tunisian dromedaries. All *CSN1S1* marker statistics evidenced a limited variability of SNP c.150G>T across all the Tunisian ecotypes ($P < 0.05$) relative to the other 2 markers (Table 4). Despite the lack of T/T animals, all populations were in Hardy-Weinberg equilibrium. The most represented haplotype in the Tunisian dromedaries was GAT (0.342), whereas GAC (0.348) and GGC (0.290) were most frequent in Sudanese and Nigerian camels, respectively. Furthermore, GAC and GGC were significantly different ($P < 0.05$) among the populations, with GAC frequency higher in Sudanese (0.348) as compared with Tunisian (0.155) and Nigerian (0.187) populations. Conversely, GCC haplotype was higher in frequency in the Tunisian (0.211) and Nigerian (0.290) camels than in Sudanese dromedaries (0.028; $P < 0.001$). Given the casein cluster structure, the haplotype frequency differences among the populations should be further investigated in their association with milk traits, as suggested also by Pauciullo et al. (2019). Casein haplotypes have been used to select traits with economic benefits based on their correlations with milk yields, protein rates, and fat characteristics in cattle (Nilsen et al., 2009; Perna et al., 2016), sheep (Noce et al., 2016), and goats (Inostroza et al., 2020). Association studies between casein gene variants or haplotypes and camel milk traits could improve the camel dairy sector as well.

Although SNPs are less-suitable markers than microsatellite or mitochondrial DNA for classical molecular genetic diversity studies among populations, several factors make them useful: ubiquitous presence, even distribution across the entire genome, biallelism, and mostly co-dominant inheritance (Zimmerman et al., 2020). A review by Burger et al. (2019) makes clear that dromedary population structure is poorly documented at both the global and national scales. An absence of available genomic tools (SNP array) for camels led us to use investigated SNPs for differentiation analysis of Tunisian camels. Caseins have already served as powerful molecular modeling tools for evolutionary studies (Kawasaki et al., 2011). Their genetic characterization in less-investigated species is needed to understand the phylogenetic relationships among domesticated mammalian species and ecotypes.

If heterozygosity is used as the main measure of genetic diversity, data analysis reveals differences within ecotypes. The Abidi population had both the lowest degree of Ho (0.194) and the lowest values for Ne (1.491) and Shannon diversity index (0.439), which indicates low genetic diversity consistent with excessive local inbreeding ($F_S = 0.358$). Unfortunately, the absence of information from local breeders on the pedigree of their animals made it impossible to establish whether this resulted from unintentional selection, a case effect, the small sample size, or some combination of these events. The highest heterozygosity was observed among Sudanese ecotypes (Lahaoi and Shanbali), which in general, exhibited more genetic variability.

At the population level, genetic diversity statistics indicate significant differences in the values of Ho ($P = 0.021$) and F_S ($P = 0.003$), whereas other parameters showed no differences (Table 4). The G_{ST} of 0.022, according to interpretation by Balloux and Lugon-Moulin (2002), indicates little genetic differentiation between ecotypes (range: 0–0.05). In fact, only 2.2% of the genetic variance can be attributed to ecotype differences,

and the remainder must be ascribed to differences between individuals. Although this interpretation may be correct, it may not reflect all of the actual differentiation in the population. In fact, the effect of polymorphism (due to mutations) drastically reduces F_{ST} expectations, as Wright (1978) has already stressed. However, by using only a few SNPs on the casein cluster, our data were able to confirm the observations of other studies that evidenced little population differentiation using microsatellites. For instance, in 4 Kenyan indigenous camel breeds (Somali, Turkana, Rendille, and Gabbra), the global F_{ST} (0.009) denoted very little differentiation (Mburu et al., 2003). Similarly, the between-population component (0.26%) suggests little differentiation between camels from southern Africa and the Sudan (Nolte et al., 2005). Recent investigations of Australian camel populations have also reported low-structured populations with F_{ST} values < 0.027 and global $F_{ST} = 0.016$ (Spencer and Woolnough, 2010). Conversely, analyses of the Canarian camel population versus those originating from Arabia, Kenya, Pakistan, and Algeria (Schulz et al., 2010) found moderate levels of differentiation (F_{ST} statistics ranging from 0.095 to 0.116) in populations considered separately or grouped by geographic origin.

Pairwise average F_{ST} values showed that Nigerian (0.040), Abidi (0.036), and G'oudi (−0.010) populations differed significantly from other populations (Figure 3a; Supplemental Table 1). The pronounced differentiation observed in the Abidi and G'oudi, relative to other ecotypes may stem from a reduction of within-ecotype variability due to its small population size. Diversity has historically, and for this study, been determined based on ethno-geographic ecotype, as opposed to breed (Legesse et al., 2018). Therefore, further genetic and genomic characterization studies, especially in camels for whom intercountry univocal breed features are not standardized, could offer clarification.

Pairwise F_{ST} also suggested other ecotypes may be genetically indistinct, even though some level of differentiation clearly exists. To highlight the genetic relationship among global and populations and ecotypes, we plotted the pairwise F_{ST} matrix. When camels are geographically grouped, a separation is clearly visible between the Nigerian and 2 other populations (Figure 3b). The phylogenetic relationships among the ecotypes indicates a subdivision in clades with 3 main branches (Figure 3b). The Nigerian population and Ardhaoui Médenine ecotype seem to belong to an independent branch separated by a moderate bootstrap (30%). One of the other main branches is represented by Khali and Abidi ecotypes (49% bootstrap between them), whereas the other is represented by all other ecotypes. In general, a certain level of admixture is known to exist among Sudanese and Tunisian ecotypes, but it did not clearly differentiate. The Tunisian Merzougui and Sahli ecotypes are 2 such examples. The first is related to the closely-linked Sudanese Shanbali and Lahaoi ecotype (bootstrap value 76%), and the Sahli is related to Sudanese Arabi (bootstrap value 68%). Although these never-investigated ecotypes deserve further analyses, the observed minimal differentiation is similar to that generally found in other camels worldwide (Mburu et al., 2003; Nolte et al., 2005; Schulz et al., 2010; Spencer and Woolnough, 2010; Abdussamad et al., 2015; Almathen et al., 2016; Cherifi et al., 2017; Hossam Mahmoud et al., 2020). Although genetic drift events cannot be excluded in the analyzed ecotypes, our observations confirm what other authors reported (i.e., a drastic reduction in genetic diversity due to at least 2 bottlenecks during the history of camel domestication; Wu et al., 2014; Fitak et al., 2016, 2020).

In fact, overall, this study has revealed a low level of genetic diversity in casein genes among Tunisian, Sudanese, and Nigerian camels that may be explained by the close genetic relationship between African camel ecotypes and their weak genetic structure, as confirmed in other studies. For example, AlAskar et al. (2020) also found little genetic diversity across 27 camel types from 11 countries, with those from Asia exhibiting greater genetic variability than their African counterparts. Furthermore, the close genetic relationship between African breeds was confirmed by a recent study showing their clustering in a separated group of local Saudi camel population with very little gene flow occurring between the 2 groups (Hossam Mahmoud et al., 2020). Our findings also agree with those of Cherifi et al. (2017), whose characterization of 331 Algerian and Egyptian camels revealed a weak genetic differentiation between these 2 populations. The little genetic differentiation among modern camel population was also reported by Almathen et al. (2016). In fact, a little phylogenetic signal, was observed, thus indicating an extensive gene flow and consequently a limited genetic differentiation among dromedary populations of 4 geographically defined regions out of 5 including Tunisia, Nigeria, and Sudan.

CONCLUSIONS

This study may be considered as the first attempt to understand the genetic variability of casein genes in the Tunisian camel population. The allele frequency observed at casein loci offers opportunity for genetic improvement in all the ecotypes, for instance increasing the *CSN2* A allele frequency already proven by independent studies to be favorably related to milk composition. The significant differences found at haplotype

level should be further investigated as casein haplotypes have been associated with higher protein yield and economic benefits in other species. Furthermore, the results should inspire researchers to conduct future association studies that will enhance SNP genotyping technologies and provide powerful resources for animal breeding programs. In addition, the results will help Tunisian breeders improve their farm efficiencies as they work to meet the increasing demand for camel milk and the growing number of intensive and semi-intensive camel dairy farms in regions throughout the country.

ACKNOWLEDGMENTS

This work was supported by the GENEROUS Project with funds provided by the King Baudouin Foundation United States (KFBUS; grant no. PAUA_RIC_N_COMP_20_01). The authors thank Carmine Versace for his technical support and the camel owners in Tunisia for helping to provide and collect the samples. The author contributions included the following: A. Pauciullo and N. Letaief conceived and designed the experiments; N. Letaief, A. A. S. Mohammed and W. Ben Salem performed the experiments; A. Pauciullo and G. Gaspa analyzed the data; A. Pauciullo and S. Bedhiaf-Romdhani contributed reagents, materials, and analysis tools; N. Letaief and A. Pauciullo wrote the paper; A. Pauciullo, S. Bedhiaf-Romdhani revised the article critically for important intellectual content; and N. Letaief, S. Bedhiaf-Romdhani, W. Ben Salem, A. A. S. Mohammed, G. Gaspa and A. Pauciullo gave final approval of the version to be published. The authors have not stated any conflicts of interest.

REFERENCES

Abdussamad, A., P. Charruau, D. Kalla, and P. Burger. 2015. Validating local knowledge on camels: Colour phenotypes and genetic variation of dromedaries in the Nigeria-Niger corridor. Livest. Sci. 181:131–136. https://doi.org/10.1016/j.livsci.2015.07.008.

Abri, M. A. A., and B. Faye. 2019. Genetic improvement in dromedary camels: Challenges and opportunities. Front. Genet. 10:167. https://doi.org/10.3389/fgene.2019.00167.

Al-Jassim, R., and V. Sejian. 2015. Climate change and camel production: Impact and contribution. J. Camelid Sci. 8:1–17.

Al haj, O. A., and H. A. Al Kanhal., 2010. Compositional, technological and nutritional aspects of dromedary camel milk. Int. Dairy J. 20:811–821. https://doi.org/10.1016/j.idairyj.2010.04.003.

AlAskar, H., B. H. Alhajeri, F. Almathen, and H. Alhaddad. 2020. Genetic diversity and population structure of dromedary camel-types. J. Hered. 111:405–413. https://doi.org/10.1093/jhered/esaa016.

Almathen, F., P. Charruau, E. Mohandesan, J. M. Mwacharo, P. Orozco-terWengel, D. Pitt, A. M. Abdussamad, M. Uerpmann, H.-P. Uerpmann, B. De Cupere, P. Magee, M. A. Alnaqeeb, B. Salim, A. Raziq, T. Dessie, O. M. Abdelhadi, M. H. Banabazi, M. Al-Eknah, C. Walzer, B. Faye, M. Hofreiter, J. Peters, O. Hanotte, and P. A. Burger. 2016. Ancient and modern DNA reveal dynamics of domestication and cross-continental dispersal of the dromedary. Proc. Natl. Acad. Sci. USA 113:6707–6712. https://doi.org/10.1073/pnas.1519508113.

Balloux, F., and N. Lugon-Moulin. 2002. The estimation of population differentiation with microsatellite markers. Mol. Ecol. 11:155–165. https://doi.org/10.1046/j.0962-1083.2001.01436.x.

Barrett, J. C., B. Fry, J. Maller, and M. J. Daly. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Bitaraf Sani, M., J. Z. Harofte, M. H. Banabazi, S. Esmaeilkhanian, A. S. Naderi, N. Salim, A. Teimoori, A. Bitaraf, M. Zadehrahmani, and P. A. Burger. 2021. Genomic prediction for growth using a low-density SNP panel in dromedary camels. Sci. Rep. 11:7675.

Burger, P. A., E. Ciani, and B. Faye. 2019. Old World camels in a modern world–A balancing act between conservation and genetic improvement. Anim. Genet. 50:598–612. https://doi.org/10.1111/age.12858.

Caroli, A. M., S. Chessa, and G. J. Erhardt. 2009. Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. J. Dairy Sci. 92:5335–5352. https://doi.org/10.3168/jds.2009-2461.

Caroli, A., F. Chiatti, S. Chessa, D. Rignanese, P. Bolla, and G. Pagnacco. 2006. Focusing on the goat casein complex. J. Dairy Sci. 89:3178–3187. https://doi.org/10.3168/jds.S0022-0302(06)72592-9.

Cherifi, Y. A., S. B. S. Gaouar, R. Guastamacchia, K. A. El-Bahrawy, A. M. A. Abushady, A. A. Sharaf, D. Harek, G. M. Lacalandra, N. Saïdi-Mehtar, and E. Ciani. 2017. Weak genetic structure in northern African dromedary camels reflects their unique evolutionary history. PLoS One 12:e0168672. https://doi.org/10.1371/journal.pone.0168672.

Chniter, M., M. Hammadi, T. Khorchani, R. Krit, A. Benwahada, and M. B. Hamouda. 2013. Classification of Maghrebi camels (Camelus dromedarius) according to theirtribal affiliation and body traits in southern Tunisia. Emir. J. Food Agric. 25:625–634. https://doi.org/10.9755/ejfa.v25i8.16096.

Cosenza, G., A. Pauciullo, D. Gallo, L. Colimoro, A. D'avino, A. Mancusi, and L. Ramunno. 2008. Genotyping at the CSN1S1 locus by PCR-RFLP and AS-PCR in a Neapolitan goat population. Small Rumin. Res. 74:84–90. https://doi.org/10.1016/j.smallrumres.2007.03.010.

Erhardt, G., E. T. S. Shuiep, M. Lisson, C. Weimann, Z. Wang, I. E. Y. M. El Zubeir, and A. Pauciullo. 2016. Alpha S1-casein polymorphisms in camel (*Camelus dromedaries*) and descriptions of biological active peptides and allergenic epitopes. Trop. Anim. Health Prod. 48:879–887. https://doi.org/10.1007/s11250-016-0997-6.

FAO. 2017. The Future of Food and Agriculture: Trends and Challenges. FAO.

Fitak, R. R., E. Mohandesan, J. Corander, and P. A. Burger. 2016. The de novo genome assembly and annotation of a female domestic dromedary of North African origin. Mol. Ecol. Resour. 16:314–324. https://doi.org/10.1111/1755-0998.12443.

Fitak, R. R., E. Mohandesan, J. Corander, A. Yadamsuren, B. Chuluunbat, O. Abdelhadi, A. Raziq, P. Nagy, C. Walzer, B. Faye, and P. A. Burger. 2020. Genomic signatures of domestication in Old World camels. Commun. Biol. 3:316. https://doi.org/10.1038/s42003-020-1039-5.

Giambra, I. J., L. Chianese, P. Ferranti, and G. Erhardt. 2010. Short communication: Molecular genetic characterization of ovine alpha(S1)-casein allele H caused by alternative splicing. J. Dairy Sci. 93:792–795. https://doi.org/10.3168/jds.2009-2615.

Goudet, J. 2002. FSTAT 2.9.3.2, a program to estimate and test gene diversities and fixation indices. https://www2.unil.ch/popgen/softwares/fstat.htm.

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. Past: Paleontological statistics software package for education and data analysis. Palaeontol. Electronica 4.

Henry, B., R. Eckard, and K. Beauchemin. 2018. Adaptation of ruminant livestock production systems to climate changes. Animal 12(S2):S445–S456.

Hossam Mahmoud, A., F. M. Abu-Tarbush, M. Alshaik, R. Aljumaah, and A. Saleh. 2020. Genetic diversity and population genetic struc-

ture of six dromedary camel (*camelus dromedarius*) populations in Saudi Arabia. Saudi J. Biol. Sci. 27:1384–1389. https://doi.org/10.1016/j.sjbs.2019.11.041.

Inostroza, M. G. P., F. J. N. González, V. Landi, J. M. L. Jurado, J. V. D. Bermejo, J. Fernández Álvarez, and M. A. Martínez Martínez. 2020. Bayesian analysis of the association between casein complex haplotype variants and milk yield, composition, and curve shape parameters in Murciano-Granadina goats. Animals (Basel) 10:1845. https://doi.org/10.3390/ani10101845.

Jemli, M., H. Boulajfene, Z. Azouzi, W. Ben Salem, and S. Khaldi. 2018. Camel breeding development project in Tunisia. Rev. Mar. Sci. Agron. Vét. 6:256–259.

Kamoun, M., and B. Jemmali. 2012. Milk yield and characteristics of Tunisian camel. J. Anim. Sci. 1:12–13.

Kappeler, S., Z. Farah, and Z. Puhan. 1998. Sequence analysis of *Camelus dromedarius* milk caseins. J. Dairy Res. 65:209–222. https://doi.org/10.1017/S0022029997002847.

Kawasaki, K., A.-G. Lafont, and J.-Y. Sire. 2011. The evolution of milk casein genes from tooth genes before the origin of mammals. Mol. Biol. Evol. 28:2053–2061. https://doi.org/10.1093/molbev/msr020.

Konuspayeva, G., B. Faye, and G. Loiseau. 2009. The composition of camel milk: A meta-analysis of the literature data. J. Food Compos. Anal. 22:95–101. https://doi.org/10.1016/j.jfca.2008.09.008.

Legesse, Y. W., C. D. Dunn, M. R. Mauldin, N. Ordonez-Garza, G. R. Rowden, Y. M. Gebre, M. Y. Kurtu, S. Mohammed Ali, W. D. Whibesilassie, M. Ballou, M. Tefera, G. Perry, and R. D. Bradley. 2018. Morphometric and genetic variation in 8 breeds of Ethiopian camels (Camelus dromedarius). J. Anim. Sci. 96:4925–4934. https://doi.org/10.1093/jas/sky351.

Mburu, D. N., J. W. Ochieng, S. G. Kuria, H. Jianlin, B. Kaufmann, J. E. O. Rege, and O. Hanotte. 2003. Genetic diversity and relationships of indigenous Kenyan camel (*Camelus dromedarius*) populations: Implications for their classification. Anim. Genet. 34:26–32. https://doi.org/10.1046/j.1365-2052.2003.00937.x.

Musaad, A., B. Faye, and A. A. Nikhela. 2013. Lactation curves of dairy camels in an intensive system. Trop. Anim. Health Prod. 45:1039–1046. https://doi.org/10.1007/s11250-012-0331-x.

Nagy, P., and J. Juhasz. 2016. Review of present knowledge on machine milking and intensive milk production in dromedary camels and future challenges. Trop. Anim. Health Prod. 48:915–926. https://doi.org/10.1007/s11250-016-1036-3.

Nilsen, H., H. G. Olsen, B. Hayes, E. Sehested, M. Svendsen, T. Nome, T. Meuwissen, and S. Lien. 2009. Casein haplotypes and their association with milk production traits in Norwegian Red cattle. Genet. Sel. Evol. 41:24. https://doi.org/10.1186/1297-9686-41-24.

Noce, A., M. Pazzola, M. L. Dettori, M. Amills, A. Castelló, A. Cecchinato, G. Bittante, and G. M. Vacca. 2016. Variations at regulatory regions of the milk protein genes are associated with milk traits and coagulation properties in the Sarda sheep. Anim. Genet. 47:717–726. https://doi.org/10.1111/age.12474.

Nolte, M., A. Kotzé, F. H. van der Bank, and J. P. Grobler. 2005. Microsatellite markers reveal low genetic differentiation among southern African *Camelus dromedarius* populations. S. Afr. J. Anim. Sci. 35:152–161.

Nowier, A. M., and S. I. Ramadan. 2020. Association of β-casein gene polymorphism with milk composition traits of Egyptian *Maghrebi* camels (*Camelus dromedarius*). Arch. Anim. Breed. 63:493–500.

Office d'Elevage et de Paturage (OEP). 2017. Données sectorielles-2017-Effectifs du cheptel, 22June 2022. Accessed Jun. 22, 2022. http://www.oep.nat.tn/index.php/en/donnees-sectorielles/40-effectif-s-du-cheptel.

Ordás, J., A. Rando, C. Senese, and P. Masina. 1997. DNA polymorphisms of casein genes in Spanish dairy sheep. Small Rumin. Res. 26:9–13. https://doi.org/10.1016/S0921-4488(97)00009-6.

Othman, O. E., A. M. Nowier, and M. E. El-Denary. 2016. Genetic variations in two casein genes among Maghrabi camels reared in Egypt. Biosci. Biotechnol. Res. Asia 13:473–480. https://doi.org/10.13005/bbra/2057.

Ould Ahmed, M., F. Ben Salem, S. Bedhiaf, B. Rekik, and M. Djemali. 2010. Genetic diversity in Tunisian dromedary (*Camelus dromedarius*) populations using microsatellite markers. Livest. Sci. 132:182–185. https://doi.org/10.1016/j.livsci.2010.05.016.

Pauciullo, A. 2022. Letaief et al. - JDS - Supplementary Table 1. Mendeley Data, V1. https://doi.org/10.17632/vfyzwjk2p5.1.

Pauciullo, A., I. Giambra, L. Iannuzzi, and G. Erhardt. 2014. The β-casein in camels: Molecular characterization of the CSN2 gene, promoter analysis and genetic variability. Gene 547:159–168. https://doi.org/10.1016/j.gene.2014.06.055.

Pauciullo, A., S. Martorello, K. Carku, C. Versace, A. Coletta, and G. Cosenza. 2021. A novel duplex ACRS-PCR for composite CSN1S1–CSN3 genotype discrimination in domestic buffalo. Ital. J. Anim. Sci. 20:1264–1269. https://doi.org/10.1080/1828051X.2021.1952912.

Pauciullo, A., E. Shuiep, G. Cosenza, L. Ramunno, and G. Erhardt. 2013. Molecular characterization and genetic variability at κ-casein gene (CSN3) in camels. Gene 513:22–30. https://doi.org/10.1016/j.gene.2012.10.083.

Pauciullo, A., E. T. Shuiep, M. D. Ogah, G. Cosenza, L. Di Stasio, and G. Erhardt. 2019. Casein gene cluster in camelids: comparative genome analysis and new findings on haplotype variability and physical mapping. Front. Genet. 10:748. https://doi.org/10.3389/fgene.2019.00748.

Perna, A., I. Intaglietta, A. Simonetti, and E. Gambacorta. 2016. The influence of casein haplotype on morphometric characteristics of fat globules and fatty acid composition of milk in Italian Holstein cows. J. Dairy Sci. 99:2512–2519. https://doi.org/10.3168/jds.2015-10397.

Redwan, E. M., S. Korim, A. Samra, Y. Saad, H. A. Amhedar, and V. N. Uversky. 2018. Variability of some milk-associated genes and proteins in several breeds of Saudi Arabian camels. Protein J. 37:333–352. https://doi.org/10.1007/s10930-018-9782-x.

Sambrook, J., and D. W. Russell. 2001. Molecular Cloning: A Laboratory Manual. No. v. 1. Cold Spring Harbor Laboratory Press.

Schulz, U., I. Tupac-Yupanqui, A. Martínez, S. Méndez, J. V. Delgado, M. Gómez, S. Dunner, and J. Cañón. 2010. The Canarian camel: A traditional dromedary population. Diversity (Basel) 2:561–571. https://doi.org/10.3390/d2040561.

Shuiep, E. T. S., I. J. Giambra, I. E. Y. M. El Zubeir, and G. Erhardt. 2013. Biochemical and molecular characterization of polymorphisms of α_{S1}-casein in Sudanese camel (*Camelus dromedarius*) milk. Int. Dairy J. 28:88–93. https://doi.org/10.1016/j.idairyj.2012.09.002.

Singh, L. V., S. Jayakumar, A. Sharma, S. K. Gupta, S. Dixit, N. Gupta, and S. Gupta. 2015. Comparative screening of single nucleotide polymorphisms in β-casein and κ-casein gene in different livestock breeds of India. Meta Gene 4:85–91. https://doi.org/10.1016/j.mgene.2015.03.005.

Spencer, P., and A. Woolnough. 2010. Assessment and genetic characterisation of Australian camels using microsatellite polymorphisms. Livest. Sci. 129:241–245. https://doi.org/10.1016/j.livsci.2010.01.006.

Stephens, M., and P. Donnelly. 2003. A comparison of bayesian methods for haplotype reconstruction. Am. J. Hum. Genet. 73:1162–1169.

Wang, Z., G. Ding, G. Chen, Y. Sun, Z. Sun, H. Zhang, L. Wang, S. Hasi, Y. Zhang, and J. Li. 2012. Genome sequences of wild and domestic bactrian camels. Nat. Commun. 1:1202. https://doi.org/10.1038/ncomms2192.

Wright, S. 1978. Evolution and the genetics of populations: A treatise in four volumes: Vol. 4: Variability within and among natural populations. University of Chicago Press.

Wu, H., X. Guang, M. B. Al-Fageeh, J. Cao, S. Pan, H. Zhou, L. Zhang, M. H. Abutarboush, Y. Xing, Z. Xie, A. S. Alshanqeeti, Y. Zhang, Q. Yao, B. M. Al-Shomrani, D. Zhang, J. Li, M. M. Manee, Z. Yang, L. Yang, Y. Liu, J. Zhang, M. A. Altammami, S. Wang, L. Yu, W. Zhang, S. Liu, L. Ba, C. Liu, X. Yang, F. Meng, S. Wang, L. Li, E. Li, X. Li, K. Wu, S. Zhang, J. Wang, Y. Yin, H. Yang, A. M. Al-Swailem, and J. Wang. 2014. Camelid genomes reveal evolution and adaptation to desert environments. Nat. Commun. 5:5188. https://doi.org/10.1038/ncomms6188.

Yelubayeva, M., B. Buralkhiyev, V. Tyshchenko, V. Terletskiy, and Y. Ussenbekov. 2018. Results of *Camelus dromedarius* and *Camelus bactrianus* genotyping by alpha-S1-casein, kappa-casein loci, and DNA fingerprinting. Cytol. Genet. 52:179–185. https://doi.org/10.3103/S0095452718030040.

Zimmerman, S. J., C. L. Aldridge, and S. J. Oyler-McCance. 2020. An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. BMC Genomics 21:382. https://doi.org/10.1186/s12864-020-06783-9.

ORCIDS

N. Letaief https://orcid.org/0000-0002-3438-5119
S. Bedhiaf-Romdhani https://orcid.org/0000-0002-8602-9474
W. Ben Salem https://orcid.org/0000-0003-2380-9067
G. Gaspa https://orcid.org/0000-0003-1604-8917
A. Pauciullo https://orcid.org/0000-0002-3140-9373