Microsatellite markers reveal low genetic differentiation among southern African *Camelus dromedarius* populations

M. Nolte¹, A. Kotzé^{2,3}*, F.H. van der Bank^{1#} and J.P. Grobler⁴

 ¹Department of Zoology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa
² ARC Animal Improvement Institute, P. Bag X2, Irene 0062, South Africa
³ Department of Plant Sciences: Genetics, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa
⁴ Department of Biodiversity, School of Molecular and Life Sciences, University of Limpopo, P. Bag X1106, Sovenga 0727, South Africa

Abstract

We report new demographic and genetic data on southern African camel (*Camelus dromedarius*) populations. Results from questionnaires on demography indicated that approximately 476 camels were extant in South Africa, Namibia and Botswana in 2003. We have sampled 234 camels for genetic analysis using a microsatellite marker set consisting of 12 loci. Results indicated little differentiation between camels from southern Africa, the Sudan or an outgroup from the family Camelidae, the alpaca (*Lama pacos*). Analysis of molecular variance (AMOVA) showed that -0.09% of total variation reside between species, 0.26% between the two southern African camel populations and 99.83% within populations. A coefficient of population differentiation (R_{ST}) indicated low levels of differentiation between southern African camel populations, with no specific pattern observed in pair-wise comparisons of 16 populations. An assignment test conformed to known population. There was no evidence of loss of genetic diversity in any individual population. Parentage analysis confirmed the utility of the microsatellite marker set for elucidating uncertain paternity. The results are discussed with reference to the management history of camels in the southern African region and the importance of population and parentage verification in the light of the many historic translocations.

Keywords: Camel, Camelus dromedaries, genetic differentiation, genetic variation, microsatellites

[#] Corresponding author. E-mail: fhvdb@na.rau.ac.za

* Present address: National Zoological Gardens of South Africa, P.O. Box 754, Pretoria 0001, South Africa

Introduction

Species of the family Camelidae provide us with a range of products and services, including wool, meat, milk, blood and draught power. Camels were domesticated about 3 000 years ago and are most numerous in the arid areas of Africa (with approximately 11.5 million animals present in this region in 1992), particularly in the arid lowlands of Eastern Africa (Schwartz & Dioli, 1992).

In southern Africa, the first introduced camels (of unknown origin) arrived in Namibia via Walvis Bay in 1891. These animals were used for postal and military transport duties between Windhoek and Walvis Bay. More camels for military patrolling duties arrived in the years that followed. A large number of camels were used during the Second World War (1939-1945) in fighting units. It is believed that 3 000 camels were introduced into Botswana, Namibia and South Africa in that period. Kalkfontein-North was used as the breeding station to supply the South African Defence Force and Police Service with camels, which used the animals for patrolling and anti-poaching operations.

The introduction of modern all-terrain vehicles caused a sharp decline in the use of camels. Due to neglect of the remaining animals, the castration of bulls to lower their level of aggression during the rutting season, ongoing slaughtering, the limited number of breeding animals and a high infant mortality, numbers declined to an estimated 450 by the year 2000 (Ramsay *et al.*, 2001).

In recent years, the use of camels in southern African has again increased, mostly due to eco-tourism. It is envisaged that camels might also assume a role in agriculture in arid regions and with management of bush encroachment. It is therefore important to manage the remaining camel populations in southern Africa properly to ensure continued survival and optimal production.

Table 1 Site number, description of site, origin of population, number of individuals sampled (n) for camel and alpaca populations studied, average heterozygosity (H), average number of alleles (A) and the percentage of animals that classified to the correct population in an assignment test. Southern African populations marked with an asterisk were considered true breeders after analysis of returned questionnaires

No.	Description of site	Origin of population	n	H (A)	Assignment
1*	Khai Apple Game Reserve, Kathu, Northern Cape	Askham, Northern Cape	7	0.387 (2.1)	100%
2*	Olifantshoek, Northern Cape	Mier region, Northern Cape	7	0.345 (1.9)	100%
3*	Jack and Fanie Maritz – Arabian Horse Breeding Farm, Northern Cape	Not known	10	0.372 (2.6)	70%
4*	Upington, Northern Cape	Witdraai, Northern Cape	12	0.479 (2.8)	75%
5*	Koppieskraal at Rietfontein, Northern Cape	Witdraai, Northern Cape	13	0.416 (2.6)	61.5%
6	Camel Farm, Swakopmundt, Namibia	Askham, Koppieskraal (locality 5) and Grootfontein (locality 9)	5	0.422 (2.3)	60%
7	Zelda Guest Farm – Buitepos, Gobabis, Namibia	Sishen and Kuruman, Northern Cape.	2	0.347 (1.7)	50%
8	Otavi Garage, Otavi, Namibia	Sishen and Kuruman, Northern Cape.	2	0.403 (1.8)	100%
9*	Grootfontein Amri, Grootfontein, Namibia	Descendants from Sishen and Kuruman, as well as Swakopmundt (locality 6).	13	0.409 (2.8)	92.3%
10	Grootfontein Blauw, Grootfontein, Namibia	Upington and Mier, Northern Cape	4	0.447 (2.3)	75%
11	Imhoff Farm – Kommetjie, Western Cape	Koppieskraal (locality 5)	4	0.432 (2.2)	75%
12*	Kaalfontein, Gauteng	Koppieskraal (locality 5) and auctions throughout South Africa	54	0.481 (3.8)	46.3%
13	Congo Caves, Oudtshoorn, Western cape	Koppieskraal (locality 5)	4	0.393 (2.2)	100%
14*	Reitzh Safaris, Windhoek, Namibia	Not known	12	0.465 (3.2)	33.3%
15*	Newcastle, KwaZulu Natal	Not known	11	0.423 (3.2)	72.7%
16	Botswana, Tshabong region	Witdraai, Northern Cape	75	0.483 (4.1)	80%
17	Sudanese camel population	Sudan	127	-	-
18	Alpaca; Groeneweide Farm, Western Cape	South America	33	-	-

Previous genetic studies on Camelidae in Dubai, Germany, Australia, Kenya and Ethiopia mainly reporting the development of new microsatellite loci in camels (Han *et al.*, 2000), alpacas and llamas (Lang *et al.*, 1996; Obreque *et al.*, 1998; Penedo *et al.*, 1998a; b). Sasse *et al.* (2000b) described the development of a microsatellite marker set for parentage and an identity verification test for dromedary racing camels. No studies regarding the population genetics of the southern African camel population have been reported.

The aims of this study are to determine (1) the numbers and geographic distribution of C. *dromedarius* in southern Africa, (2) the relationships between the Botswana, Namibian and South African camel populations and (3) if a specific microsatellite set and procedures can be used for parentage determination of C. *dromedarius* from southern Africa.

Material and Methods

Questionnaires were distributed to all known camel owners in southern Africa, to determine the number of extant camels in the region. The questionnaires also aimed to establish the status of populations, i.e. which farmers were truly breeding camels compared to persons who simply owned animals (possibly castrated) bought from elsewhere.

A total of 234 *C. dromedaries* from southern Africa (Botswana, Namibia and South Africa) was then physically sampled (Table 1). To gauge the relative significance of population differentiation values, we included two additional datasets: (1) we were kindly allowed to include data for 127 Sudanese camels typed by the ARC (Animal Improvement Institute - Agricultural Research Council, Irene) and (2) we sampled 33 alpacas (*Lama pacos*, Family Camelidae) as an outgroup.

Choice of sample for DNA isolation depended on the extent to which animals could be approached and handled. For tame animals, whole blood samples were obtained from the jugular vein using 7 mL EDTA tubes. Between 20-30 hairs from each animal were also plucked from the tail and stored in plastic ziplock bags. For free-roaming and untamed animals, skin samples were obtained using biopsy darts, with samples stored in 70% ethanol after collection. All the biological samples were deposited in the Bio-Bank at the ARC for future use in species identification, forensic cases, etc.

Table 2 Sequence (5'-3') and labels of 16 primer pairs used to amplify microsatellite regions in the
Camelidae. Also listed are the total numbers of alleles resolved for each primer pair, and the range of sizes
(bp) of fragments generated. Loci marked with asterisks were analysed in alpaca and camels from the Sudan,
and loci in bold gave poor resolution

Loons	Labal	Forward Primer	Reverse Primer	Size	Alleles
Locus	Laber	Sequence	Sequence	Range	(n)
VOLP03*	5' TAMRA	AGACGGTTGGGAAGGTGGTA	CGACAGCAAGGCACAGGA	144-176	8
VOLP08	5' JOE	CCATTCACCCCATCTCTC	TCGCCAGTGACCTTATTTAGA	144-148	3
VOLP10*	5' FAM	CTTTCTCCTTTCCTCCCTACT	CGTCCACTTCCTTCATTTC	249-267	6
VOLP32	5' JOE	GTGATCGGAATGGCTTGAAA	CAGCGAGCACCTGAAAGAA	256-262	3
VOLP67*	5' FAM	TTAGAGGGTCTATCCAGTTTC	TGGACCTAAAAGAGTGGAG	149-203	19
YWLL02	5' FAM	GTGCACTCAGATACCTTCACA	TACATCTGCAATGATCGACCC	290-304	6
YWLL08*	5' JOE	ATCAAGTTTGAGGTGCTTTCC	CCATGGCATTGTGTTGAAGAC	134-172	12
YWLL09	5' TAMRA	AAGTCTAGGAACCGGAATGC	AGTCAATCTACACTCCTTGC	158-162	3
YWLL38*	5' JOE	GGCCTAAATCCTACTAGAC	CCTCTCACTCTTGTTCTCCTC	182-188	3
YWLL44*	5' FAM	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA	090-114	6
LCA33	5' FAM	GAGCACAGGGAAGGATATTCA	ACAGCAAAGTGATTCCATAATACA	136-164	7
LCA37	5' FAM	AAACCTAATTACCTCCCCCA	CCATGTAGTTGCAGGACACG	132-133	2
LCA56	5' JOE	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG	133-169	16
LCA63	5' TAMRA	TTACCCAGTCCTTCGTGGG	GGAACCTCGTGGTTATGGAA	190-254	14
LCA66*	5' JOE	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA	240-244	3
LCA77	5' FAM	TGTTGACTAGAGCCTTTTCTTCTTT	GGGCAAGAGAGACTGACTGG	233-263	15

The Wizard® Genomic DNA Purification Kit (Miller *et al.*, 1998) and High Pure PCR Template Preparation Kit (Vogelstein & Gillespie, 1979) were used to obtain DNA from blood and skin samples. No extraction kit was used to obtain DNA from hair. Two hair follicles of each sample were cut into a reaction tube after which the PCR mixture was added.

A camelid microsatellite set was compiled using published data for South American Camelids, alpacas and llamas. The set contained 16 primers with highest polymorphism in published trials (Lang *et al.*, 1996; Obreque *et al.*, 1998; Penedo *et al.*, 1998a; b; Jianlin *et al.*, 2000; Sasse *et al.*, 2000a). Specific modifications and primer sequences are described in Table 2. Reaction mixtures for amplification consisted of 50 – 200 ng DNA or two hair follicles, 1.48 pmol of each primer, 0.44 mM of each dNTP, 4.09 mM MgCl₂, 1 x reaction buffer, 1.25U Taq (Supertherm Gold – Southern Cross Biotechnology ©), 1.28 µL Tween, with ddH₂O added to a total volume of 7.7 µL. Reaction conditions were 10 min at 95 °C, followed by 35 cycles each of 45 s at 94 °C, 80 s at 58 °C and 60 s at 72 °C, and with a final extension step of 10 min at 72 °C.

The samples were analysed on a denaturing polyacrylamide gel (PAGE) using an ABI377 DNA sequencer and the internal size standard GeneScanTM Rox-350. Data were collected and analysed using GeneScanTM (version 3.1) and GenotyperTM (version 2.0) software (Applied Biosystems). Standard coefficients of genetic variability (average heterozygosity – H and average number of alleles per locus – A) were calculated using POPGENE version 1.31 (Yeh & Yong, 1999). To determine the distribution of genetic diversity between species, populations and within populations, an Analyses of Molecular Variance (AMOVA – Michalakis & Excoffier, 1996) was used, as implemented in ARLEQUIN software (Schneider *et al.*, 2000). Differentiation among geographic locations sampled was estimated from stepwise mutation model based, R_{ST} values (Slatkin, 1995) and gene flow (effective number of individuals exchanged between populations in each generation, Nm) were calculated using the RST CALC software (Goodman, 1997).

As an added measure of population uniqueness, an assignment test was used to measure how often individual camels were classified to their correct populations of origin. GENECLASS (Cornuet *et al.*, 1999) software was used to do an assignment test through a likelihood-based Bayesian method. The genotypes obtained from individuals of the three southern African populations were used for this test. To evaluate the suitability of the chosen microsatellite set for parentage studies, genotypes of five calves were tested against genotypes of their known parents and a set of data for a non-related bull. CERVUS Version 2.0 (Marshall *et al.*, 1998) was used for this analysis.

Results and Discussion

From the questionnaires it was estimated that 476 camels were extant in the southern African region in 2003, and these were found in the Gauteng, Northern Cape, Western Cape and KwaZulu-Natal provinces of South Africa; in the Windhoek, Swakopmundt, Grootfontein and Gobabis districts of Namibia and in Botswana. It was established that nine true camel breeders exist in southern Africa. Breeders are indicated in Table 1.

In the genetic study, four microsatellite primer pairs (YWLL02, YWLL09, LCA33 and LCA56) did not provide satisfactory results. The remaining 12 primer pairs (Table 2) provided interpretable results, and these loci were used in subsequent analysis.

Differentiation between *C. dromedarius* and *L. pacos*, and between the regional populations of *C. dromedarius* (southern Africa and the Sudan) was calculated using AMOVA. The data obtained from Sudan included results for seven corresponding loci, hence seven rather than 12 loci (Table 2) were used for this comparison of the overall pattern of differentiation. The AMOVA values indicate very little differentiation among groups, with -0.09% of variation between species, 0.26% between the two camel populations and 99.83% within populations (P < 0.01). Since negative AMOVA values denote completely pannictic taxa, the results suggest extremely close identity between the genera *Camelus* and *Lama*. Similarly, the between-population component of 0.26% suggests little differentiation between camels from southern Africa and the Sudan. The latter results conform to population history since a component of the southern African camel population probably originated from the Sudan.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Khai	-	0.144	1.621	3.186	2.317	0.945	2.965	1.076	4.960	19.207	0.947	1.507	1.428	0.506	4.874	23.059
2 Olifantshoek	*0.635	-	0.330	0.237	0.414	0.104	0.078	0.157	0.160	0.150	0.416	0.522	0.479	0.847	0.189	0.167
3 Arabian Horse Farm	0.134	*0.431	-	6.151	4.788	2.549	0.725	1.847	2.123	4.009	0.819	6.349	6.133	1.013	3.846	3.433
4 Upington	0.073	*0.513	0.039	-	5.074	1.612	0.669	1.514	1.680	22.238	0.901	3.356	6.767	1.115	4.695	12.636
5 Koppieskraal	0.097	*0.376	0.050	0.047	-	0.685	0.537	0.686	1.194	3.858	8.895	23.563	25.058	5.856	1.706	6.606
6 Camel Farm	*0.209	*0.707	0.089	0.134	0.267	-	0.728	2.023	1.458	2.440	0.266	0.701	0.723	0.259	1.930	1.188
7 Zelda Guest	0.078	*0.763	0.257	0.272	0.318	0.256	-	6.011	-6.636	0.878	0.309	0.488	0.485	0.189	5.321	0.906
8 Otavi	0.189	*0.614	0.119	0.142	0.267	0.110	0.040	-	43.064	0.771	0.333	0.706	0.994	0.317	-5.772	0.844
9 Grootfontein,	0.048	*0.609	0.105	*0.130	*0.173	*0.146	-0.039	-0.006	-	1.882	0.577	1.109	1.150	0.385	19.276	2.061
10 Grootfontein,	-0.013	*0.625	0.059	0.011	0.061	0.093	0.222	0.245	0.117	-	0.793	2.385	1.789	0.589	2.558	-3.956
11 Kommetjie	0.209	*0.375	0.233	0.217	0.027	*0.485	0.447	0.429	*0.302	0.240	-	3.530	1.858	5.282	0.648	1.082
12 Kaalfontein	*0.142	*0.324	0.038	*0.069	-0.011	*0.263	0.339	0.261	*0.184	0.095	0.066	-	12.456	3.860	1.516	2.954
13 Botswana	*0.149	*0.343	0.039	0.036	0.010	*0.257	0.340	0.201	*0.179	0.123	0.119	0.020	-	4.575	2.021	2.389
14 Oudtshoorn	*0.331	0.228	0.198	0.183	0.041	*0.491	0.570	0.441	*0.394	0.298	0.045	0.061	0.052	-	0.520	0.721
15 Windhoek	0.049	*0.570	0.061	0.051	0.128	0.115	0.045	-0.045	-0.013	0.089	*0.279	*0.142	*0.110	*0.325	-	3.160
16 Newcastle	-0.011	*0.600	0.068	0.019	0.036	*0.174	0.216	0.228	*0.108	-0.067	*0.188	*0.078	0.095	*0.257	0.073	-

Table 3 Pair-wise genetic differentiation (R_{ST} values below the diagonal, and gene flow, Nm values above the diagonal) between 16 populations of *C. dromedarius*. The asterisks indicate R_{ST} values significantly (P < 0.05) different from zero

Genetic diversity (H) did not differ substantially between southern African (0.604 ± 0.060) and Sudan (0.680 ± 0.061) camels. By comparison, a higher H value of $0.757 (\pm 0.038)$ was calculated for alpacas. These biased values (since loci were specifically chosen on the basis of polymorphism) are comparable with values for other African species (where random microsatellite loci were applied), e.g. 0.73 for the African buffalo, 0.66 for the African lion, 0.69 for the black rhinoceros and 0.56 for the African wild dog (Frankham *et al.*, 2002). However, a random set of microsatellite markers would most likely produce lower H values for the camels. This could indicate slightly reduced genetic diversity in the camels studied.

Patterns of differentiation and diversity within the southern African regional group were determined by using the full set of 12 loci screened. Pair-wise R_{ST} values indicated significant (P < 0.05) differentiation between 39 out of 120 pair-wise combinations of populations (Table 3). Camels from Olifantshoek were most consistently different from other groups (Table 3). The remaining pair-wise comparisons did not suggest any specific pattern of differentiation. The trend of relative uniqueness in the Olifantshoek populations is also reflected by gene-flow values, with Nm values indicating less than one migrant per generation for all pair-wise comparisons between Olifantshoek and other population. By comparison, only 31 out of 105 other pair-wise comparisons yielded Nm values below one. Following Neigel (2002), we used Nm as a measure of relative differentiation only without implying any literal ecological meaning for values.

Results from the assignment test (Table 1) showed many incorrect classifications of animals, with 30.64% of camels not classified to their correct populations of origin. These results provide additional support for the hypothesis of low differentiation between populations. Within populations, the percentage of correct classifications showed some correlation to management history. For example, the Kaalfontein population, with the most diverse number of founders, also had the second lowest percentage of correct self-classifications at 46.3%. Four populations showed 100% correct assignments. In the case of Khai Apple and Olifantshoek, this is probably the result of single founder events followed by decades of breeding without further introductions. The results for Olifantshoek support the homogeneity in this population also evident from R_{ST} values. The Otavi and Outshoorn samples consisted of 2-4 animals and the 100% correct classifications and Namibian populations. The Botswana regional population, which has been excluded from South African and Namibian translocations in post war years, showed relative good identity with 80% of individuals classified correctly.

Coefficients of genetic diversity in the southern African populations were H = 0.345-0.483 and A = 1.7-4.1. Note that even the two numerically smallest populations, from Otavi Garage and Zelda guest farm, had H values of 0.347 and 0.403 respectively, comparable to other camel populations (although these values may be artificially high due to sampling error). As expected, the largest populations, with active breeding programmes and animals originating from diverse origins were also the most polymorphic, with H = 0.481 in the Kaalfontein population and H = 0.483 in the Botswana group. The results do not provide cause for concern on levels of diversity in individual camel populations. Broadly, similar levels of diversity across southern African camel populations probably reflect artificially induced gene flow, resultant from translocations among populations.

In the parentage analysis (Table 5), it was possible to verify the parentage of calves using the genotypes in Table 4. Five calves, their known parents and an unrelated male were used in the parentage determination. It was successful in all cases. For the first calf (K109) with the known female parent (K113) and the possible male candidate parents (K119 and K121), K119 is unambiguously the second parent (Table 5). A mismatch at the VOLP08 locus (where an alteration of a single allele occurred) was observed after the calf, known parent and candidate parent were compared (Table 4). This is possibly due to a mutation. Most published studies of parentage determination treat a mismatch between male and putative offspring as conclusive evidence for exclusion of that male from paternity. In practice, a mismatch could result either from a genuine non-relationship or from a laboratory typing error, a reality acknowledged for some time in human pedigree analysis (Thompson, 1976; Ashton, 1980; Lathrop *et al.*, 1993). When microsatellite markers are used, mutations (Queller *et al.*, 1993) and null alleles (Callen *et al.*, 1993; Phillips *et al.*, 1993; Pemberton *et al.*, 1995) may also generate mismatches between genuine relatives at measurable frequencies. Mutations are alterations of single alleles rather than pairs of alleles, and may not be independent of the previous allelic state; therefore, treating a mutation as an error is preferable to using it as a basis for exclusion (Marshall *et al.*, 1998). Thus, the mismatch found at the calf (K109) can be regarded as an error.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Khai	-	0.144	1.621	3.186	2.317	0.945	2.965	1.076	4.960	19.207	0.947	1.507	1.428	0.506	4.874	-23.059
2 Olifantshoek	*0.635	-	0.330	0.237	0.414	0.104	0.078	0.157	0.160	0.150	0.416	0.522	0.479	0.847	0.189	0.167
3 Arabian Horse Farm	0.134	*0.431	-	6.151	4.788	2.549	0.725	1.847	2.123	4.009	0.819	6.349	6.133	1.013	3.846	3.433
4 Upington	0.073	*0.513	0.039	-	5.074	1.612	0.669	1.514	1.680	22.238	0.901	3.356	6.767	1.115	4.695	12.636
5 Koppieskraal	0.097	*0.376	0.050	0.047	-	0.685	0.537	0.686	1.194	3.858	8.895	23.563	25.058	5.856	1.706	6.606
6 Camel Farm	*0.209	*0.707	0.089	0.134	0.267	-	0.728	2.023	1.458	2.440	0.266	0.701	0.723	0.259	1.930	1.188
7 Zelda Guest	0.078	*0.763	0.257	0.272	0.318	0.256	-	6.011	-6.636	0.878	0.309	0.488	0.485	0.189	5.321	0.906
8 Otavi	0.189	*0.614	0.119	0.142	0.267	0.110	0.040	-	43.064	0.771	0.333	0.706	0.994	0.317	-5.772	0.844
9 Grootfontein, Amri	0.048	*0.609	0.105	*0.130	*0.173	*0.146	-0.039	-0.006	-	1.882	0.577	1.109	1.150	0.385	-19.276	2.061
10 Grootfontein, Blau	-0.013	*0.625	0.059	0.011	0.061	0.093	0.222	0.245	0.117	-	0.793	2.385	1.789	0.589	2.558	-3.956
11 Kommetjie	0.209	*0.375	0.233	0.217	0.027	*0.485	0.447	0.429	*0.302	0.240	-	3.530	1.858	5.282	0.648	1.082
12 Kaalfontein	*0.142	*0.324	0.038	*0.069	-0.011	*0.263	0.339	0.261	*0.184	0.095	0.066	-	12.456	3.860	1.516	2.954
13 Botswana	*0.149	*0.343	0.039	0.036	0.010	*0.257	0.340	0.201	*0.179	0.123	0.119	0.020	-	4.575	2.021	2.389
14 Oudtshoorn	*0.331	0.228	0.198	0.183	0.041	*0.491	0.570	0.441	*0.394	0.298	0.045	0.061	0.052	-	0.520	0.721
15 Windhoek	0.049	*0.570	0.061	0.051	0.128	0.115	0.045	-0.045	-0.013	0.089	*0.279	*0.142	*0.110	*0.325	-	3.160
16 Newcastle	-0.011	*0.600	0.068	0.019	0.036	*0.174	0.216	0.228	*0.108	-0.067	*0.188	*0.078	0.095	*0.257	0.073	-

Table 3 Pair-wise genetic differentiation, R_{ST} values below the diagonal, and gene flow, Nem values above the diagonal between 16 populations of *C. dromedarius*. The asterisks indicate R_{ST} values significantly (P < 0.05) different from zero

Table 4 The genotypes of the camel individuals used for parentage determination. The bold value marked with an asterisk denotes where a denovo mutation possibly occurred

ID	Vol	p10	Vo	lp67	Yw	v144	Lo	ca77	Lo	ca37	Vo	lp08	Vo	lp32	Yv	v1108	Yv	v1138	Lc	a66	Vo	lp03	Lc	a63
K109	261	261	155	185	107	107	231	231	135	135	147	*149	264	264	135	135	190	190	241	243	146	173	214	214
K110	261	261	155	155	107	107	231	231	133	133	147	147	264	264	135	167	190	190	239	243	146	173	216	218
K112	261	261	149	149	107	107	231	231	133	133	147	147	264	264	133	135	184	190	237	239	146	173	216	216
K113	261	263	155	185	105	107	231	231	131	135	147	147	264	264	135	167	190	190	241	243	171	173	214	214
K114	261	261	155	155	105	107	231	231	131	135	147	149	264	264	135	135	190	190	239	241	146	173	214	214
K115	261	263	155	155	107	107	231	231	131	131	147	147	264	264	133	133	190	190	239	239	171	173	214	216
K116	261	261	155	155	107	107	231	231	131	131	147	147	264	264	133	135	190	190	239	243	146	173	216	218
K118	261	263	155	185	107	107	231	231	133	135	147	147	264	264	135	135	184	190	237	239	146	173	214	216
K119	259	261	155	155	105	107	231	231	131	135	147	147	264	264	135	135	190	190	239	243	146	146	214	218
K121	261	261	155	155	107	107	231	231	133	133	147	147	264	264	135	167	184	190	237	243	146	146	216	218

Parentage analysis for a second (K110) and third (K112) calf did not result in any mismatches when comparisons were made with the known and candidate male parents. In both scenarios K121 is unambiguously the second parent, rather than K119. For the fourth (K114) and fifth (K116) calf, no mismatches occurred for the known male parent and candidate male parent (K119). The known historic information was therefore confirmed, validating the use of this microsatellite set for parentage determination.

Table 5 Parentage determination comparing the loci of the offspring (O), known parent (KP) and candidate
parent (CP). Comparison of the genuine male parent together with the known female parent and the offspring
is illustrated in bold. A mismatch occurred at one of the loci (VOLP08)

Calf	O ID	O loci typed	KP ID	KP loci typed	O + KP loci com- pare	O + KP loci mis- match	CP ID	CP loci typed	O + CP loci com- pare	O + CP loci mis- match	O + KP + CP loci com- pare	O + KP + CP loci mis- match
1	K109	12	K113	12	12	0	K119	12	12	0	12	1
1	K109	12	K113	12	12	0	K121	12	12	2	12	3
2 2	K110 <i>K110</i>	12 12	K118 K118	12 12	12 12	0 0	K121 K119	12 12	12 12	0 1	12 12	0 2
3	K112	12	K118	12	12	0	K121	12	12	0	12	0
3	K112	12	K118	12	12	0	K119	12	12	1	12	1
4 4	K114 <i>K114</i>	12 12	K109 <i>K109</i>	12 12	12 12	0 0	K119 K121	12 12	12 12	0 3	12 12	0 4
5	K116	12	K115	12	12	0	K119	12	12	0	12	0
5	K116	12	K115	12	12	0	K121	12	12	Ι	12	1

Conclusions

The results provide the first estimation of camel numbers in southern Africa. The genetic results from this study indicated that the southern African camel populations are closely related, confirming known historic information. The information will also provide a scientific base for future genetic management of camel populations. Calculated levels of genetic diversity can be used to decide which populations should be prioritised for introductions to maintain levels of genetic diversity and the coefficients of differentiation can be used to determine suitable donor populations. The latter is important to avoid mixing of closely related animals, a real possibility considering the history of translocations among southern African camel populations. It is also apparent from the results obtained that the particular microsatellite set will be effective in future paternity tests.

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