



The role of parentage studies in Arabian and Bactrian camel's pedigree verification

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Abstract

Molecular marker technologies have revolutionized the way animal genetics research is conducted. The development of DNA-based genetic markers has had a revolutionary impact on animal genetics. For commercial breeders, DNA typing offers a new and powerful test for collecting information on their animals and to enhance genetic improvement through the selection of high performance progeny. The identification of markers for traits of economic importance will also facilitate the selection of superior animals in the future. The present study was conducted to evaluate the use of RAPD markers in parentage relations and to investigate misidentification paternity frequency in self and cross-species of Arabian and Bactrian camels. Some mating groups and offspring's exhibited a homogeneous pattern indicative of a very low level of DNA polymorphism and the others showed a low heterogeneous polymorphism. Cluster analysis revealed two main clusters of the S (Sufer) group with 89-92% similarity, two main clusters of the M (Magaheem) group and the sire of this group is 76% genetically similar to the dams and their offspring, one main cluster of the G (Shogeh) group and one offspring out of group with 91% similarity. On the other hand, cluster analysis of the B (Bactrian) group revealed three main clusters. Cluster A consisted of dam 2 and 3 pregnant from Arabian sire. Cluster B includes 4 samples and subdivided into 2 subgroups; Subgroup A includes dam 10 Bactrian pregnant from Arabian sire and its offspring, Subgroup B includes mother 12 Bactrian pregnant from Arabian sire and its offspring with 0.84-0.89 similarity matrix. Cluster C consisted of 3 subgroups; Subgroup A includes Bactrian dam pregnant from Arabian sire and Bactrian dam pregnant from Bactrian sire, Subgroup B includes Bactrian dam pregnant from Arabian sire and the offspring of Bactrian dam and Bactrian sire, Subgroup C includes 3 samples: 2 Bactrian dams pregnant from Bactrian sire and one of their offspring with 0.89-0.92 similarity matrix. Amplification products of the sires, dams and offspring's revealed the presence of common and specific markers. Thus, RAPD-DNA-based markers are powerful tools for parentage studies in camel.

Key words: Saudi camels, parentage, molecular markers, RAPD.

Introduction

The camelidae family comprises the Old World Camelini (or dromedary with one hump and Bactrian with two humps) and the New World tribes (namely the llamas, alpacas, guanacos and vicunas)¹. From the seventeenth century onward, a series of European travelers, anthropologists and veterinary scientists have amassed an important record of observations of the intentional cross-breeding of Bactrian and dromedary camels². In 1985 Tapper reported that hybrids were not allowed to breed, as their offspring would be vicious and dangerous. Statements to the effect that the dromedary-Bactrian crosses were infertile³ are incorrect, and ample evidence demonstrates the contrary⁴. Archaeo-zoologists have identified faunal evidence of camel hybrids at Mleiha in the United Arab Emirates, Troy in western Turkey and Pella in Jordan. Chronologically, the earliest evidence dates to the Roman or Parthian period⁵⁻⁶. In 1998⁷, at the Camel Reproduction Centre in Dubai, United Arab Emirates, Rama was born - the world's first viable cross between an Old World camel and a South American camelid. Although the species within each group can hybridize among themselves to produce fertile offspring, it is only recently that a hybrid between New and Old World camelids has been reported⁸. Dromedary hybrids, called Bukhts, are larger than either

parent, have a single hump and are good draft camels. The females can be mated back to a Bactrian to produce ¾-bred riding camels. These hybrids are found in Kazakhstan. As with most hybridization, the aim in crossing camels has been to produce a better camel, in this case a more robust individual, stronger as a pack animal.

Parentage misidentification is harmful due to the reduction in annual genetic earnings of the population and because it endangers an efficient genetic improvement program. Parentage testing of animals has long been used by breed societies to maintain the reliability of their pedigree records. This in turn helps to enhance genetic progress in breeding programs. The accuracy of parentage tests is therefore very important to the animal industry. Accurate determination of paternity is also valuable to commercial animal producers who wish to multiple-sire mate their herds and allocate offsprings to their correct sires without the need to test the dams. This enables producers to trace the fecundity of their offsprings and to identify those sires and sire lines producing high performing progeny.

Parental relations between individuals may be proved using several markers. Any attribute that can be readily detected and its

inheritance traced can serve as a marker. At first, polymorphism of morphological markers and biochemical polymorphisms were used for this purpose. These marker categories do not give conclusive results, and due to the great number of genetic systems need for an adequate final result, paternity testing once done, has its use limited due to costs. In the past, allozyme and protein markers have been popular in animal genetics research. Recently, DNA has been exploited for species identification due to its stability at high temperatures and its structure being conserved within all tissues of an individual. Recent advancements on molecular biology and the constant discovery of new molecular markers are significantly helping solve these limitations. This has resulted in the development of restriction fragment length polymorphism (RFLP) and species specific DNA probes⁹⁻¹⁰, random amplified polymorphic DNA (RAPD)¹¹⁻¹² and microsatellite and polymerase chain reaction (PCR) assays¹³.

RAPDs have all the advantages of a PCR-based marker, with the added benefit that primers are commercially available and do not require prior knowledge of the target DNA sequence or gene organization. Other advantages of RAPDs are the ease with which a large number of loci and individuals can be screened. RAPD markers have been used for analysis of population structure in marine algae¹⁴, analysis of genetic impact of environmental stressors¹⁵ and analysis of genetic diversity¹⁶.

In a previous work, Al-Swailem *et al.*¹⁷ developed and explored the potential application of RAPD markers in assessment of genetic diversity and/or similarity among the Saudi camel subtypes of different colors. Such system of classification may fit the requirements for the development of camel production and the improvement of the standard of their herders. The present study was conducted to evaluate the use of RAPD markers in parentage relations and to investigate misidentification paternity frequency in Arabian and Bactrian camels.

Materials and Methods

Animal material: Samples of four groups of self and cross-species of Arabian and Bactrian camels (Tables 1-5) were collected from the Camel Research Center, King Faisal University, Al-Hassa.

Table 1. Group S (Sufer) Arabian ♂ (Ashaal) x Arabian ♀ (Sufer) & offspring (Sufer).

Code	Sample	Description
S0	G33 (Sire)	Shogeh (Father of Sufer)
S1	S1	Dam 1 (8 months)
S2	S2	Dam 2 (8 months)
S3	S3	Offspring ♀ (7 months)
S4	S4	Offspring ♀ (7 months)
S5	S5	Offspring ♀ (10 months)
S6	S6	Offspring ♀ (10 months)
S7	S7	Offspring ♂ (7 months)
S8	S8	Offspring ♂ (7 months)
S9	S9	Offspring ♂ (7 months)
S10	S10	Offspring ♂ (7 months)

Table 2. Group M (Magaheem) Arabian ♂ (Magaheem) x Arabian (Magaheem) ♀ & offspring (Magaheem).

Code	Sample	Description
M0	M31(Sire)	Magaheem (Father)
M1	M21	Adult dam 1
M2	M22	Offspring ♀ of dam 1 (5 months)
M3	M23	Adult dam 2
M4	M24	Offspring ♂ of dam 2 (6 months)
M5	M25	Adult dam 3
M6	M26	Offspring ♀ of dam 3 (3 months)
M7	M27	Adult dam 4
M8	M28	Offspring ♀ of dam 4 (6 months)
M9	M29	Adult dam 5
M10	M30	Offspring ♀ of dam 5 (6 months)

Table 3. Group G (Shogeh) Arabian ♂ (Shogeh) x Arabian ♀ (Shogeh) & offspring (Shogeh).

Code	Sample	Description
G0	G32 (Sire)	Shogeh (Father of Shogeh)
G1	G11	Dam 1
G2	G12	Offspring ♂ of dam 1 (5-6 months)
G3	G13	Dam 2
G4	G14	Offspring ♀ of dam 2 (4-5 months)
G5	G15	Dam 3
G6	G16	Offspring ♀ of dam 3 (5-6 months)
G7	G17	Dam 4
G8	G18	Offspring ♂ of dam 4 (5 months)
G9	G19	Dam 5
G10	G20	Offspring ♂ of dam 5 (5 months)

Table 4. Part I of Group B: Arabian ♂ x Bactrian ♀ (Pregnant Bactrian ♀ and offspring).

Code	Sample	Description
B1	B1	Bactrian ♀ pregnant (7 months) from Arabian ♂
B2	B2	Bactrian ♀ pregnant (6 months) from Arabian ♂
B3	B3	Bactrian ♀ pregnant (6 months) from Arabian ♂
B4	B4	Bactrian ♀ pregnant (4 months) from Arabian ♂
B10	B10	Bactrian ♀ pregnant from Arabian ♂
B11	B11	Offspring ♀ of dam 10
B12	B12	Bactrian ♀ pregnant from Arabian ♂
B13	B13	Offspring ♀ of dam 12
B14	B14	Bactrian ♀ pregnant from Arabian ♂

Table 5. Part II of Group B: Bactrian ♂ x Bactrian ♀ (Pregnant Bactrian ♀ and offspring).

Code	Sample	Description
B5	B5	Bactrian ♀ pregnant (7 months)
B6	B6	Bactrian ♀ pregnant (6 months)
B7	B7	Bactrian ♀ pregnant (4 months)
B8	B8	Offspring ♀ (15 days old)
B9	B9	Offspring ♂ (15 days old)

DNA isolation: Total genomic DNA was extracted from blood samples using the DNA Isolation Kit for Mammalian Blood (Roche Diagnostics, GmbH, Mannheim, Germany) according to the instruction's manual.

DNA markers assay: DNA markers were determined by using fifty decamer primers. PCR amplifications were performed in 25 ml reactions containing 10 hg of DNA, 10-20 pmol each primer, 1.5 mM MgCl₂ and 2 U *Taq* polymerase (Roche Diagnostics GmbH), 100 mM of each dNTP (DNA polymerization mix, Pharmacia) and 1x PCR buffer. The PCR amplification program – performed on a Flexigene thermal cycler – consisted of an initial denaturation temperature of 94°C for 5 min, then 40 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 1 min and a final extension step at 72°C for 10 min. Repeatability and reproducibility were achieved using two different thermal cyclers in two labs at KACST and adopted for all amplifications. The same PCR conditions were used in the dromedary and Bactrian. PCR products were analysed on 1.4% (w/v) agarose gels containing ethidium bromide (0.5 mg/ml) in 0.5% TBE. DNA fragments were visualized by UV-transilluminator and documented using Gel Doc System 2000 (Bio Rad). Data were collected and analysed using Bio-Rad data base software. The similarity coefficient and unweighted pair group method with arithmetic averages (UPGMA) was used for size calling and cluster analysis.

Results and Discussion

The results of samples tested with RAPD markers are illustrated in Figs 1-8. Variants of these markers appear as distinct bands. Every animal displays one or more bands. Animals with one band have inherited the same variant from each parent (both bands being in the same position) whereas those with two bands have inherited a different variant from each parent.

Some mating groups and offspring's exhibit a homogeneous pattern indicative of a very low level of DNA polymorphism and the others showed a low heterogeneous polymorphism. Amplification products of the sire, dams and offspring's of Sufer revealed three common markers of molecular size 320 and 1100 bp (Fig. 1) and 700 bp (Fig. 2). On the other hand, Magaheem showed two common markers of molecular size 480 bp (Fig. 3) and 1400 bp (Fig. 4). Also, Shogeh revealed two common markers of molecular size 1200 bp (Fig. 5) and 500 bp (Fig. 6). Bactrian dams and their offspring's revealed the presence of one specific common marker of molecular size 800 bp (Fig. 7). However, all Arabian and Bactrian dams and offspring's showed a common marker of molecular size 1100 bp (Fig. 8). This may indicate the presence of common alleles.

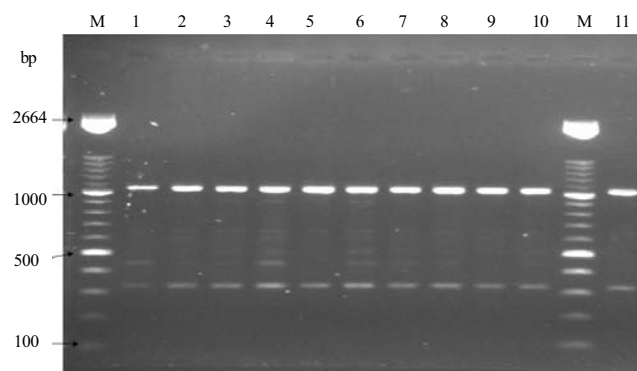


Figure 1. RAPD fingerprints detected in different individuals (Lanes 1-11) of Sufer camels (S0-10) using OPB-11 decamer primer. Lane M: Standard DNA molecular size marker.

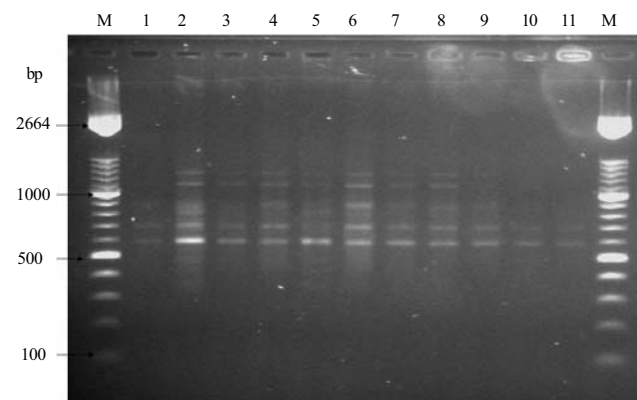


Figure 2. RAPD fingerprints detected in different individuals (Lanes 1-11) of Sufer camels (S0-10) using OPA-02 decamer primer. Lane M: Standard DNA molecular size marker.

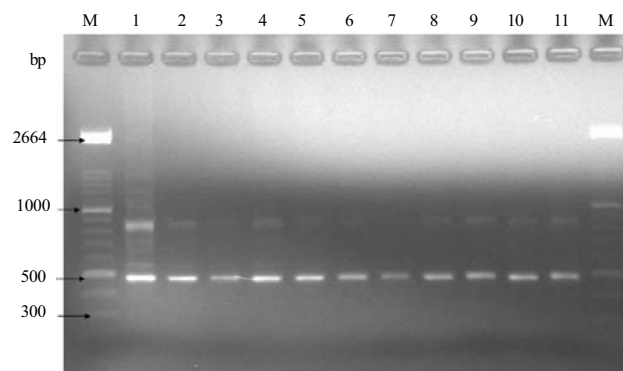


Figure 3. RAPD fingerprints detected in different individuals (Lanes 1-11) of Magaheem camels (M0-10) using OPB-01 decamer primer. Lane M: Standard DNA molecular size marker.

Parentage studies relies on the detection of genetically inherited markers that remain the same throughout the animal's life, and on the knowledge that all animals possess two copies of every gene (or marker), one of which was inherited from the sire and the other from the dam. If the marker shows variation, then the copy (or variant) inherited from the dam may be different to that inherited from the sire. Accurate parentage studies require breeders to identify possible parents since if considering a randomly selected

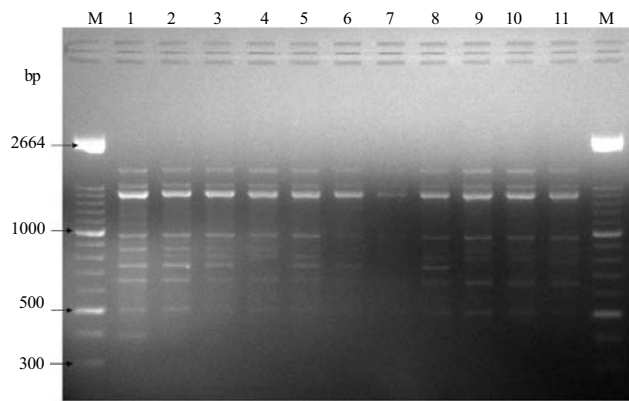


Figure 4. RAPD fingerprints detected in different individuals (Lanes 1-11) of Magaheem camels (M0-10) using OPB-08 decamer primer. Lane M: Standard DNA molecular size marker.

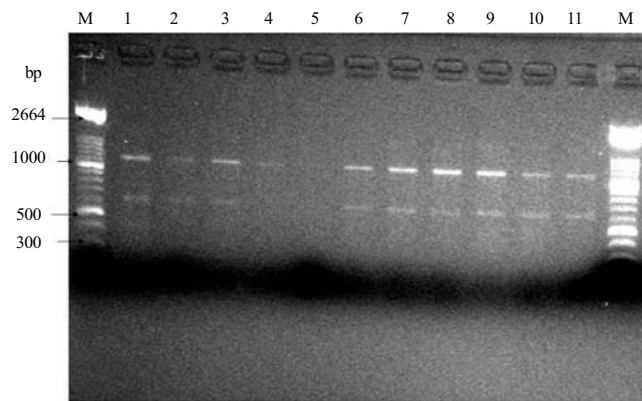


Figure 5. RAPD fingerprints detected in different individuals (Lanes 1-11) of Shogeh camels (G0-10) using OPA-11 decamer primer. Lane M: Standard DNA molecular size marker.

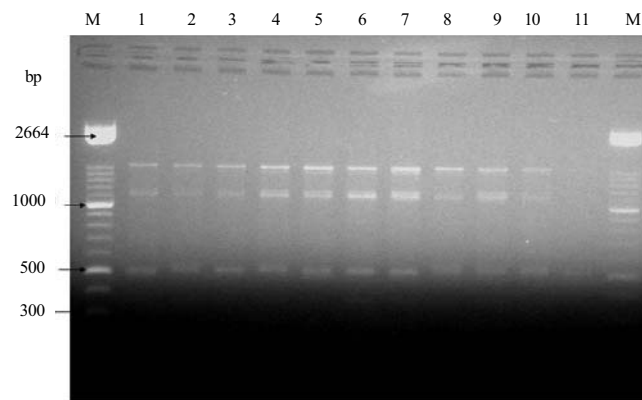


Figure 6. RAPD fingerprints detected in different individuals (Lanes 1-11) of Shogeh camels (G0-10) using OPB-20 decamer primer. Lane M: Standard DNA molecular size marker.

large group of individuals there could be more than one that qualifies as a parent. A good application for animal parentage testing is verification that the dam is correct and which of the sires on a particular farm are the actual sire¹⁸. Marker variation is reduced when animals are closely related and differs markedly between breeds of camel. Variation, and therefore accuracy, is reduced in those breeds of camel with a smaller gene pool.

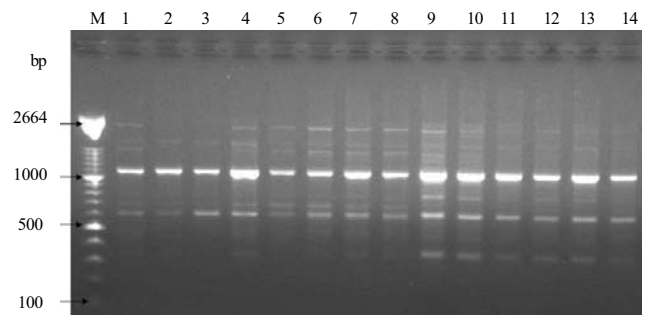


Figure 7. RAPD fingerprints detected in different individuals (Lanes 1-14) of Arabian and Bactrian camels (B1-14) using OPB-12 decamer primer. Lane M: Standard DNA molecular size marker.

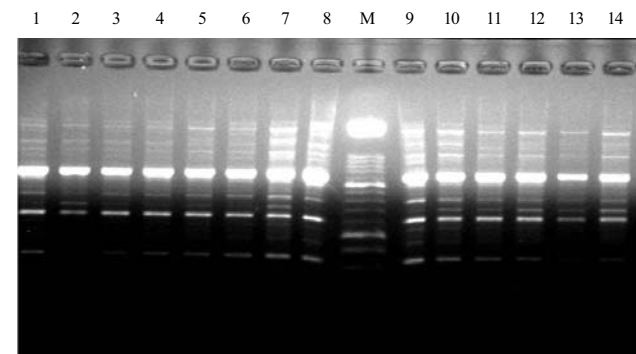


Figure 8. RAPD fingerprints detected in different individuals (Lanes 1-14) of Arabian and Bactrian camels (B1-14) using OPA-10 decamer primer. Lane M: Standard DNA molecular size marker.

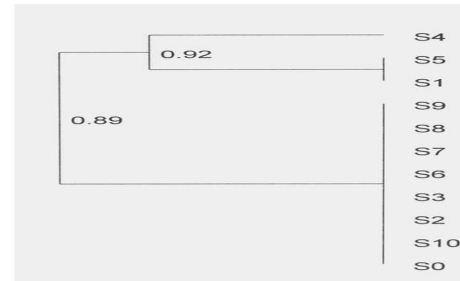


Figure 9. A dendrogram of phylogenetic relationships among eleven different individuals of Sufer camels (S0-10) based on Nei and Li Similarity Coefficient obtained from thirty RAPD reproducible primers.

Cluster analysis of the S (Sufer) group revealed two main clusters. Cluster A consisted of 2 samples (dam 3 and its offspring) with 89% similarity. Cluster B consisted of 3 samples (dam 1 and 2 offspring) with 0.89-0.92 similarity index (Fig. 9). Also, cluster analysis of the M (Magaheem) group revealed two main clusters. Cluster A consisted of 2 samples (dam 3 and its offspring). Cluster B consisted of 8 samples and is subdivided into three subgroups; Subgroup A includes the offspring dam 2, Subgroup B includes mothers 1 and 4, Subgroup C includes mother 2, offspring of dam 4 and dam 5 and its offspring. The father of this group is 76% genetically similar to the dams and their offspring (Fig. 10). Analysis of Group G (Shogeh) revealed one main cluster (includes 10 samples: sire, dam 5, dams 1; 2; 3 and 4 and their offspring) and one sample out of group which is the offspring of dam 5 with 91%

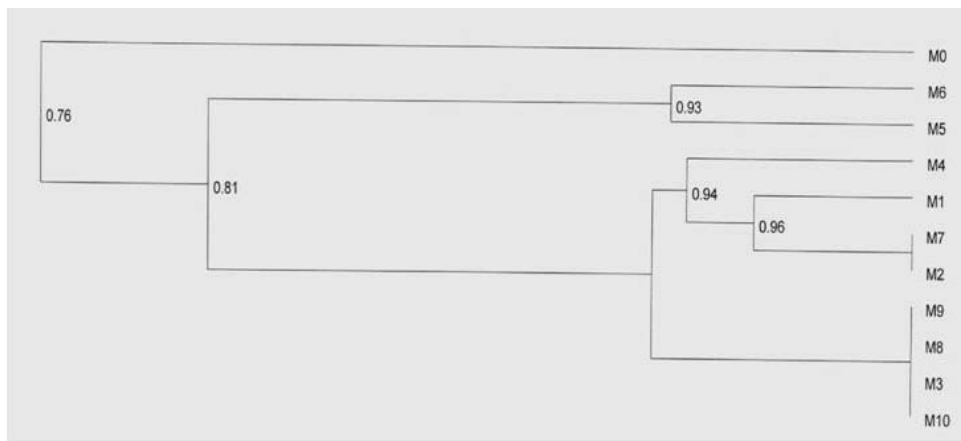


Figure 10. A dendrogram of phylogenetic relationships among eleven different individuals of Magaheem camels (M0-10) based on Nei and Li Similarity Coefficient obtained from thirty RAPD reproducible primers.

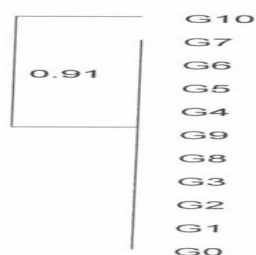


Figure 11. A dendrogram of phylogenetic relationships among eleven different individuals of Shogeh camels (G0-10) based on Nei and Li Similarity Coefficient obtained from thirty RAPD reproducible primers.

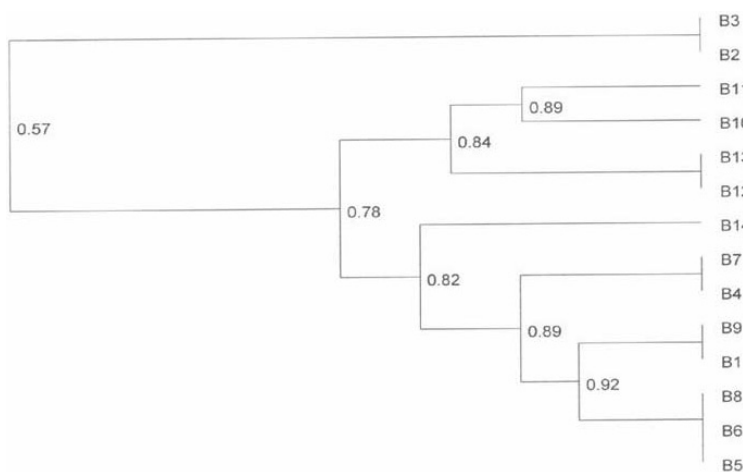


Figure 12. A dendrogram of phylogenetic relationships among eleven different individuals of Arabian and Bactrian camels (B1-14) based on Nei and Li Similarity Coefficient obtained from thirty RAPD reproducible primers.

similarity (Fig. 11). On the other hand, cluster analysis of Group B (Bactrian) revealed three main clusters. Cluster A consisted of dam 2 and 3 pregnant from Arabian sire. Cluster B includes 4 samples and subdivided into 2 subgroups; Subgroup A includes dam 10 Bactrian pregnant from Arabian sire and its offspring. Subgroup B includes mother 12 Bactrian pregnant from Arabian

sire and its offspring with 0.84-0.89 similarity matrix. Cluster C consisted of 3 subgroups; Subgroup A includes Bactrian dam pregnant from Arabian sire and Bactrian dam pregnant from Bactrian sire, Subgroup B includes Bactrian dam pregnant from Arabian sire and the offspring of Bactrian dam and Bactrian sire, Subgroup C includes 3 samples: 2 Bactrian dams pregnant from Bactrian sire and one of their offspring with 0.89-0.92 similarity matrix (Fig. 12).

All deviations were explained by heterozygote deficiency. The value of the Bactrian is lower than the Arabian although the mean allele is approximately the same. This is probably because the Bactrian population samples derived from one closed herd that may be less heterozygous as indicated by the lower heterozygosity values. These results are in harmony with that obtained by Mariasegaram *et al.*¹⁹ who isolated and characterized eight markers from amplifications of cross-species of Bactrian and *Lama pacos*. In 1998, at the Camel Reproduction Centre in Dubai, United Arab Emirates, Rama was born - the world's first viable cross between an Old World camel and a South American camelid⁷. Attempts to produce additional hybrids continue. The success of this project fuels discussion about the close genetic ties among all members of the family Camelidae. Several studies have reported that DNA fingerprinting markers tend to be shorter and less polymorphic in related species as compared with the species in which they were developed²⁰⁻²¹. The mean number of alleles, a useful measure of genetic diversity²², is strikingly different between the Arabian and Bactrian.

Finally, RAPD-DNA-based markers are powerful tools for parentage studies in camels. It is important to remember that while parentage exclusions are 100% accurate parentage qualifications are not. The accuracy of most animal parentage tests is greater

than 99% when both parents are included in the analysis and drops to around 95% when only one parent is included in the analysis. However, this accuracy will decrease when the potential parents are part of a large group of closely related animals. Again this is due to the fact that an animal closely related to an actual parent could possess marker alleles that would make it appear that animal is the correct parent. To prevent erroneous parentage qualifications breeders need to submit samples from all possible parents when first requesting parentage verification. If more than one sire and one dam qualify as parents of an offspring the laboratory can then test with additional DNA markers to sort out the actual parents.

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