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Evaluation of the genetic variability of microsatellite markers in Saudi Arabian camels

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Abstract

A study was conducted to determine the genetic distances among Saudi Arabian camel types (Magaheem, Sufer and Shogeh) by microsatellite analysis. Ninety nine samples were analyzed by twenty microsatellite primers. The results of this study indicated that all types are very closely related; however, some alleles have a different distribution in the three types. The total number of alleles per locus ranged from 1 (LCA33) to 7 (YWLL38). The distribution of the allele frequencies is different. It is remarkable that numerous alleles are present in only one type, often appearing in relatively high frequencies. For instance the allele 211 of marker VOLP77 only exists within the Magaheem type with a relatively elevated frequency of 15% while the allele 148 of the marker LCA18 is only present in the Sufer type with a frequency of 12%. The panel of microsatellites evaluated in Saudi camels in the present study showed a very low heterozygosity and polymorphism and very high probability of genetic identity of thirty three individuals belonging to three types. Based on the results of this study, development of a test for camel type identification is possible. This study further showed that microsatellites are very useful and effective in the execution of future paternity tests as well as in the determination of genetic variation of camel types and differentiation within and between populations, even in closely related types, and for genetic diversity studies for selection and conservation of Saudi camels. This genetic study indicates that any further genetic loss should be prevented and the camel's fate is in our hands.

Key words: Genetic variation, microsatellite markers, heterozygosity, polymorphism, Saudi camels.

Introduction

In the light of continuously growing demand for better products, faster and more reliable methods of identification of individuals are essential. Identification methods such as typing of blood groups and biochemical polymorphism have proved their usefulness, but the discriminating power of these techniques is less than that of DNA markers. Moreover, the number of different tissues on which the typing can be done is very limited and represents a significant limitation of such methods. Studies of genetic variation in camels using protein electrophoresis revealed little or no genetic polymorphism [1] that also include Indian dromedary population [2]. On the other hand, DNA analysis technique viz. RAPD was found to be more powerful in detecting genetic variation in camels [3-5]. However, other DNA analysis techniques, such as DNA fingerprinting, using minisatellite or microsatellite sequences, appear to be more powerful in detecting genetic variation in camels [6] and many other animal species.

Microsatellites, or Simple Sequence Repeats (SSRs), are polymorphic loci present in nuclear DNA and organellar DNA that consist of tandem repeats of 1-4 base pairs in length [7]. They are typically neutral, co-dominant and are used as molecular markers which have wide-ranging applications in the field of genetics, including kinship and population studies. These markers often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number are ten or greater [8]. The repeated sequence is often simple, consisting of two, three or four nucleotides (di-, tri- and tetranucleotide repeats respectively), and can be repeated 10 to 100 times [9]. As there are often many alleles present at a microsatellite locus, genotypes within pedigrees are fully informative, in that the progenitor of a particular allele can often be identified. In this way, microsatellites are ideal for determining paternity, population genetic studies and recombination mapping. It is also the only molecular marker to provide clues about which alleles are more closely related [10]. Microsatellites owe their variability to an increased rate of mutation compared to other neutral regions of DNA. These high rates of mutation can be explained most frequently by slipped strand mispairing (slippage) during DNA replication on a single DNA double helix. Mutation may also occur during recombination during meiosis [11]. Microsatellites also are used for solving forensic cases such as stock theft and poaching. Thus microsatellites are used

to illustrate the effectiveness of parentage determination and to determine the genetic variation and differentiation within and between populations.

To date, little is available on DNA fingerprinting in camels either using RAPD or microsatellite markers [4]. The use of RAPD technique is not always reproducible and thus may lead to misinterpretation of results. However, the use of multilocus microsatellite oligos has proved to be valuable in genetic analysis of a variety of animals species including wild animal populations[6, 12-17]. The use of a common panel of markers in genetic diversity studies of a given species is desirable, as it is necessary to clarify the relationships existing among breeds and to make information compatible across studies [18]. Therefore, this study was undertaken to attempt the use of microsatellite-based DNA fingerprinting and to evaluate their effectiveness as a useful tool in the study of genetic structure and variation among Saudi Arabian camels.

Materials and Methods

Animal material: Blood samples of thirty three animals of three Saudi camel types (Magaheem, Sufer and Shogeh) were used in this study. Three replicates for each animal were used as a source of blood from the jugular vein of camels using Vacutainers (Becton Dickinson) containing EDTA as anticoagulant. All samples were provided by the Camel Research Center, King Faisal University, Al-Hassa.

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

Microsatellite primers: A set of 16 microsatellite primers (Table 1) was used.

PCR amplification: PCR amplifications were performed in 25 µl reactions containing 5 ng of DNA, 10 pmol each primer, 1.5 mM $MgCl_2$ and 2.5 U *Taq* polymerase (Roche Diagnostics GmbH or Pharmacia), 200 µM of each dNTP (DNA polymerization mix, Pharmacia) and 1x PCR buffer. The PCR amplification program consisted of an initial denaturation temperature of 94ºC for 10 min, then 35-40 cycles at 94ºC for 1 min, 53-58ºC (depending on primer sequence) for 1 min and 72ºC for 1 min and a final extension step at 72ºC for 10 min. 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).

Repeatability and reproducibility: In the present investigation, PCR amplifications were processed in duplicate for all samples by two independent manipulators, in order to avoid any technical error. The same results (number and position of bands) were repeatedly displayed for each sample. Despite the changes in the PCR cycle numbers (35 or 40) and of the source of DNA polymerase, the profiles did not vary.

***Statistical analysis*:** Nei's genetic distance [19], Wahlund coefficients [20] and other standard genetic parameters were calculated based on microsatellite frequencies.

Table 1. Primer sequences and average number of alleles for 16 microsatellite primers.

Locus	Sequence Forward and Reverse (5'—> 3`)	No. of alleles	Size range (bp)
YWLL02	GTG CAC TCA GAT ACC TTC ACA TAC ATC TGC AAT GAT CGA CCC	5	298-318
YWLL44	CTC AAC AAT GCT AGA CCT TGG GAG AAC ACA GGC TGG TGA ATA	3	104-108
YWLL08	ATC AAG TTT GAG GTG CTT TCC CCA TGG CAT TGT GTT GAA GAC	4	164-204
YWLL09	AAG TCT AGG AAC CGG AAT GC AGT CAA TCT ACA CTC CTT GC	2	158-170
YWLL38	GGC CTA AAT CCT ACT AGA C CCT CTC ACT CTT GTT CTC CTC	7	236-246
VOLP03	AGA CGG TTG GGA AGG TGG TA CGA CAG CAA GGC ACA GGA	3	129-169
VOLP 10	CTT TCT CCT TTC CTC CCT ACT CGT CCA CTT CCT TCA TTT C	2	236-246
VOLP67	TTA GAG GGT CTA TCC AGT TTC TGG ACC TAA AAG AGT GGA G	4	152-182
VOLP77	TAT TTG GTG GTG ACA TT CAT CAC TGT ACA TAT GAA GG	2	220-262
VOLP32	GTG ATC GGA ATG GCT TGA AA CAG CGA GCA CCT GAA AGA A	2	278-290
LCA37	AAA CCT AAT TAC CTC CCC CA CCA TGT AGT TGC AGG ACA CG	5	162-166
LCA65	TTT TCC CCT GTG GTT GAA T AAC TCA GCT GTT GTC AGG GG	4	84-118
LCA33	GAG CAC AGG GAA GGA TAT TCA ACA GCA AAG TGA TTC CAT AAT ACA	6	147-167
LCA90	TAT AAC CCT GGT CTC GCC AA CCA AGT AGT ATT CCA TTA TGC G	1	237-248
LCA56	ATG GTG TTT ACA GGG CGT TG GCA TTA CTG AAA AGC CCA GG	6	165-191
LCA63	TTA CCC AGT CCT TCG TGG G GGA ACC TCG TGG TTA TGG AA	5	220-262

Results and Discussion

This study presents the first results on characterization of genetic variability through microsatellite molecular markers of the Saudi Arabian camel. The genetic measurements were calculated from allele frequencies of microsatellite loci genotyped on 3 replicates for each of the 33 animals. From the 20 microsatellite markers selected based on the results obtained from the Old and New World camelids, 16 were amplified under chosen technical specifications and 12 of those were polymorphic in ninety nine samples of three types included in the present study providing a total of 61 alleles. The total number of alleles per locus ranged from 1 (LCA33) to 7 (YWLL38) (Table 1). The distribution of the allele frequencies is different. It is remarkable that numerous alleles are present in only one type often appearing in relatively high frequencies. For instance the allele 211 of marker VOLP77 only exists within the Magaheem type with a relatively elevated frequency of 15% while the allele 148 of the marker LCA33 is only present in the Sufer type with a frequency of 12%.

The results of the pooled test for the deviation from Hardy-Weinberg equilibrium indicated that half of the alleles for both breeds are according to Hardy-Weinberg proportions with a

pooled test. A possible reason for this could be the high selection pressure commonly applied in camel breeding. Nei's genetic distance between types was 0.21, with genetic identity being 0.79. A maximum value of 1 for genetic identity means that all alleles over all loci have the same frequencies. This indicates that the three type camel populations are closely related. Considering the fact that the alleles in this study were nonfunctional nucleotide repeats that had been reported to have high mutation rate, it can be concluded that the identity between the three types is high. Wahlund coefficients were above 0.18 in 22 alleles and 9 loci. Thus, despite the fact that both lines have a common origin, different selection schemes that were applied caused differences in allelic distributions between the two types. This result is in agreement with that obtained by Schulz *et al*. [6] who found that the use of microsatellite markers can serve to identify individual's traceability and paternity relationship in dromedaries. Similar results were obtained by other authors using microsatellites to classify and identify Old and New world camelids [12, 14-17]. Also, Kaul *et al*. [13] propose that microsatellite markers may be used with reliability for studying the genetic diversity and for identification of individuals in Indian pig types.

Microsatellites have proved to be versatile molecular markers, particularly for population analysis, but they are not without limitations. Microsatellites developed for particular species can often be applied to closely related species, but the percentage of loci that successfully amplify may decrease with increasing genetic distance [21]. Point mutation in the primer annealing sites in such species may lead to the occurrence of 'null alleles', where microsatellites fail to amplify in PCR assays [21-22]. Null alleles can be attributed to several phenomena. Sequence divergence in flanking regions can lead to poor primer annealing, especially at the 3' section, where extension commences; preferential amplification of particular size alleles due to the competitive nature of PCR can lead to heterozygous individuals being scored for homozygosity (partial null). PCR failure may result when particular loci fail to amplify, whereas others amplify more efficiently and may appear homozygous on a gel assay, when they are in reality heterozygous in the genome. Null alleles complicate the interpretation of microsatellite allele frequencies and thus make estimates of relatedness faulty. Furthermore, stochastic effects of sampling that occurs during mating may change allele frequencies in a way that is very similar to the effect of null alleles; an excessive frequency of homozygotes causing deviations from Hardy-Weinberg equilibrium expectations. Since null alleles are a technical problem and sampling effects that occur during mating are a real biological property of a population, it is often very important to distinguish between them if excess homozygotes are observed. When using microsatellites to compare species, homologous loci may be easily amplified in related species, but the number of loci that amplify successfully during PCR may decrease with increased genetic distance between the species in question. Mutation in microsatellite alleles is biased in the sense that larger alleles contain more bases, and are therefore likely to be mistranslated in DNA replication. Smaller alleles also tend to increase in size, whereas larger alleles tend to decrease in size, as they may be subject to an upper size limit; this constraint has been determined but possible values have not yet been specified. If there is a large size difference between individual alleles, then there may be increased instability during recombination at meiosis [20]. Despite its complexity, this tandem repeated multi-loci microsatellites possess the three important features for a molecular marker, i.e. sensitivity, repetitiveness and discriminatory power. It will permit assessing the genetic polymorphism of Saudi camels. The microsatellite loci selected in our study indicate a lower genetic variation. Inbreeding may be the possible cause with the different populations being subjected to isolation.

The genetic distance matrix using the UPGMA algorithm was computed to cluster the data and to draw the precise relationships between the types. Cluster analysis of the Magaheem type revealed two main clusters. Cluster A consisted of 1 individual (offspring of Dam 2). Cluster B consisted of 10 individuals and is subdivided into four subgroups; subgroup A includes the offspring Dam 3, subgroup B includes Dam 1 and the sire, subgroup C includes Dam 5, and subgroup D includes Dams 2, 3 and 4 and offspring of Dams 1, 4 and 5. The father of this group is 89% genetically similar to the dams and their offspring (Fig. 1). Cluster analysis of the Sufer type revealed two main clusters. Cluster A consisted of 1 individual (offspring) with 69% similarity. Cluster B consisted of 2 subgroups; subgroup A includes the sire, Dam 1 and 3 offspring, and subgroup B includes Dam 2 and 4 offspring (Fig. 2). Also, analysis of Shogeh type revealed one main cluster (includes 10 individuals in two subgroups: sire, Dams 1, 3, 4 and 5 and their offspring in subtype A and 2 offspring in subtype B) (Fig. 3). These results correspond to the genetic distance, as populations with little to moderate genetic differentiation are genetically more similar, with high levels of gene flow. These values confirm and are in harmony with that obtained by Al-Swailem *et al*. [4-5] using RAPD markers to evaluate inter- and intra-genetic similarities and reported the significance of genetic markers in evaluating paternity relationships in Arabian camel types.

A phylogenetic analysis of the three camel types' populations was constructed. It is further clear from the results obtained, that mostly moderate genetic differentiation occurs in the Saudi camels. This indicates that outbreeding is limited, since little negative values are present, making isolation undesirable. Gene diversity may be limited by the use of isolated groups for breeding purposes. A variety of factors such as gene flow, mutation, selection, inbreeding, sample size and involuntarily breeding can disrupt the stability of a population and may lead to the change of genetic structures or profiles [23].

In conclusion, the panel of microsatellites evaluated in Saudi camels in the present study showed a very low heterozygosity and polymorphism and very high probability of genetic identity of thirty three individuals belonging to three types. This indicated that the Saudi camels are closely related with low genetic variation and differentiation that were observed, confirming known historic information that the populations derived from a single origin. Based on the results of this study, development of a test for camel type identification is possible. This study further showed that microsatellites are very useful and effective in the execution of future paternity tests as well as in the determination of genetic variation of camel types and differentiation within and between populations, even in closely related types and for genetic diversity studies for selection and conservation of Saudi camels. The information will also provide a scientific base for the execution of future studies. This genetic study indicates that any further genetic loss should be prevented. The camel's fate is in our

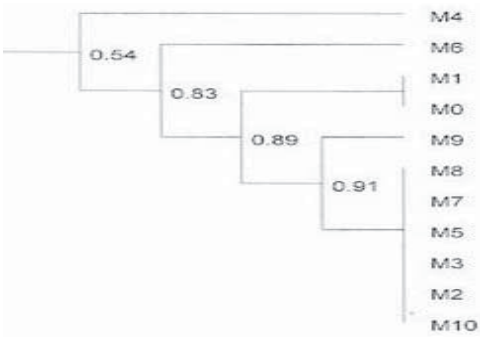

Figure 1. A dendrogram of phylogenetic relationships among eleven different individuals of Magaheem camels (M0-10) based on similarity coefficient obtained from microsatellite markers.

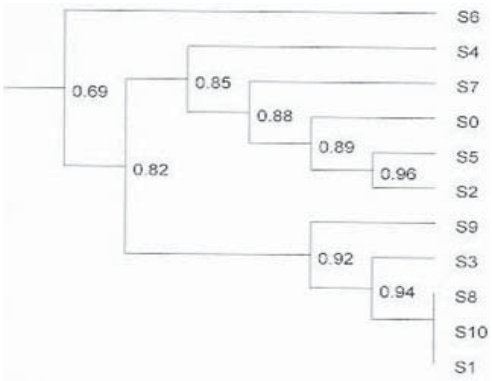

Figure 2. A dendrogram of phylogenetic relationships among eleven different individuals of Sufer camels (S0-10) based on similarity coefficient obtained from microsatellite markers.

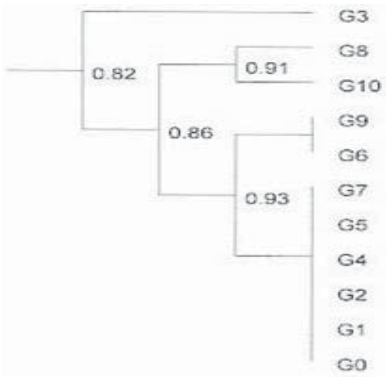

Figure 3. A dendrogram of phylogenetic relationships among eleven different individuals of Shogeh camels (G0-10) based on similarity coefficient obtained from microsatellite markers.

hands. Further studies are needed using a large number of samples to establish the most suitable microsatellite for particular genetic analysis such as identification and breed or type characterization.

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