Journal of Applied Biosciences 39: 2626 - 2634

ISSN 1997–5902

Genetic variations between camel breeds using microsatellite markers and RAPD techniques

Karima F. Mahrous[1], Hassan A. I. Ramadan[1,2] Sekena H. Abdel-Aziem[1], Mohamed Abd-El Mordy[3], and Dalia M. Hemdan[1]

[1] Department of Cell Biology, National Research Centre, Egypt
[2] Department of Biological Sciences, Faculty of Science, King Abdul Aziz University, P. O. Box 80203-, Jeddah 21589, Kingdom of Saudi Arabia
[3] Department of Zoology, Faculty of Science, Ain Shams University, Egypt

***Correspondence to:** Prof. Karima F. Mahrous. Cell Biology Department, National Research Centre, El -Tahrir Street, 12622. Dokki, Giza, Egypt E-mail: l_fathy@yahoo.com. ; nadanahl@yahoo.com Fax: +202-33370931, +202-3766187
Original submitted in 22nd Decemeber 2011. Published online at www.biosciences.elewa.org on March 9, 2011.

ABSTRACT

Objective: The genetic polymorphism within and among camel breeds; Baladi, Somali, Sudani, Maghrabi and Mowallad was detected by three microsatellite loci (YWLL44, YWLL08 and YWLL59) and 12 random primers.

Methodology and results: A total of 70 blood samples were collected from camel breeds (without harming the animals). Fifteen blood samples were taken from each of the first four breeds and only 10 samples from the fifth one (Mowallad). Genomic DNA was extracted from whole blood of each sample. Random amplified polymorphic DNA (RAPD) and Microsatellites techniques were used for analysis of DNA. The results showed that, Sudani was the highest polymorphic breed for the microsatellite locus YWLL44, while Somali was the lowest one. In the 2nd locus YWLL08, Baladi was the highest polymorphic breed and Sudani was the lowest one. Regarding the locus YWLL59, Somali and Sudani showed maximum polymorphism, while Maghrabi and Mowallad showed minimal polymorphism. The RAPD- PCR primers used in this study were OPA02, OPA07, OPA08, OPA09, OPA10, OPA12, OPC03, OPC04, OPC05, OPC06, OPC08 and OPC20. They amplified a total of 288 bands, of which 190 (65.9 %) were polymorphic.

Conclusions and application of findings: The RAPD results showed genetic variation between and within camel breeds. The phylogenetic relationship between the five camel breeds showed two groups. The first group includes Baladi, Maghrabi and Mowallad, while the second group includes Somali and Sudani. The results showed also that Mowallad breed was very close to both Baladi and Maghrabi breeds and this confirm the origin of Mowallad breed as a hybrid between Baladi and Maghrabi breeds. In general, the low genetic distances between the five studied breeds can give an insight about their origin and evolution.

Key words: Microsatellite Markers, RAPD Markers, Camels, Genetic Diversity, Genotype

INTRODUCTION

Camels are in the taxonomic order Artiodactyla, sub-order Tylopod, and family Camiladae. This family of mammalian animals is comparatively small. There are two genera within this family. The

genus *Camelus* consists of *Camelus dromedarius*, dromedary camel (one hump) and *Camelus bactrianus*, Bactrian camel (two humps). Studies of genetic variation in camels using protein electrophoresis revealed little or no genetic polymorphism. (Scott *et al*., 1992.; Khanna and Tandon, 1997)

On the other hand, DNA analysis technique including RAPD (Random amplified polymorphic DNA) was found to be a powerful tool in detecting genetic variation in camels (Shereif and Alhadrami,1996.; Al-Swailem *et al*,2007.; Al-Swailem *et al*, 2008) Many researchers employed RAPD technique to characterize and estimate genetic distances between breeds (Williams *et al*., 1990; Welsh and McClelland, 1990**)** and genetic diversity within breeds (Apostolidis *et al*., 2001; El-Seoudy *et al*., 2005; Eroglu and Arica, 2009**)**.

Random amplified polymorphic DNA markers have been used successfully in estimating genetic relatedness among various populations of sheep, cattle, goat, buffalo, chicken, camel and horse (Okumus and Kaya, 2005; Rahman *et al*., 2006; Abdel-Rahman and Hafez ,2007; Al-Swailem *et al*., 2007; Mahrous *et al*., 2007; Mahfouz *et al*., 2008; Mahrous *et al*., 2010, respectively).

Other DNA analysis techniques, such as DNA fingerprinting, using minisatellite or microsatellite sequences, appear to be more powerful in detecting genetic variation in camels (Schulz *et al*., 2005) and many other animal species.

Microsatellites are ideal for determining paternity in population genetic studies and recombination mapping. It is also the only molecular marker that provides clues about which alleles are more closely related. Microsatellites owe their variability to an increased rate of mutation compared to other 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(Lettier *et al*., 2006).

Microsatellites were considered as the markers of choice for the molecular characterization of livestock genetic resources. To date, several microsatellite loci have been characterized in South American camelids (Lang *et al*., 1996. Obreque *et al*., 1998; Penedo *et al.*, 1998, 1999[a] and 1999[b]., Sarno *et al*., 2000) and dromedary camels (Sasse *et al*., 2000). Recent studies (Jianlin *et al*., 2000; Mburu *et al*., 2003; Al-Swailem *et al*., 2009) have demonstrated the usefulness of New World Camelidae microsatellite loci as genetic tools for the study of dromedary and bactrian camelids. However, there have been no in-depth studies of the genetic diversity and relationships among populations of these two species using microsatellite DNA markers.

This study was carried out to address the following objectives: (1) to identify polymorphism within and between five different breeds of one-humped camels found in Egypt (2) to assess the purity of these breeds, and (3) to estimate the genetic relationship between them.

MATERIALS AND METHODS

Animal Material: A total of 70 Venous blood simples chosen at random from some farms in Cairo were collected from camel breeds (Somali, Sudani, Maghrabi, Fallahi (Baladi), and Mowallad). Fifteen samples were taken from each of the first four breeds and only 10 samples from the fifth one (Mowallad).

DNA Isolation: Genomic DNA was extracted from whole blood by phenol-chloroform method described by John *et al*. (1991)**.**

RAPD and Microsatellite analysis: RAPD-PCR was carried out using 12 random oligonucleotide primers (Table 1). The RAPD -PCR reactions were set up in 25 µL reaction volume in 0.2 mL thin walled PCR tubes (Axygen). The reaction mixture was as follows: One microlitre of the template DNA (10 ng/µL), 2.5 µL of 10x PCR buffer (Bangalore Genei with 15 mM $MgCl_2$), 5 µL of 1 mM dNTP mix, 1 Unit of *Taq* DNA polymerase (Bangalore Genei) and 1 µL of primer (15 pmol or 50 ng). PCR was performed in a thermal cycler with a heated lid (Perkin- Elmer Gene Amp2400). The cycling conditions used were initial denaturation at 94 °C for 5 min followed by 45 cycles each of 1 min denaturation at 94 °C, 45 seconds annealing at 36 °C and 1 min elongation at 72 °C. This was followed by a final extension for 5 min at 72 °C. After amplification

reactions, five microlitres of loading dye were added to each tube and 15 µL of the product was run on 1.5% agarose gels. Standard molecular weight markers were also run with the sample. Electrophoresis was performed at 60 V for 1.5 h., After electrophoresis the gels were stained with ethidium bromide and were examined through a gel documentation system.

For Microsatellite analysis, three loci were used to study camel breeds (Table 2). These loci comprised highly polymorphic short tandem repeat (STR) sequences (Lang *et al*., 1996).

Microsatellite Amplification: The PCR reaction was carried out in 25 µl volume containing 50 ng of genomic DNA, 2 units of Taq DNA polymerase (Dyanzyme), dNTPs mix at 2 mM concentration of each nucleotide triphosphate (Q-Biogene), 10 pmol of each primer pair (the primers were synthesized and purified in Metabion GmbH,Germany), In addition to buffer and double distilled water. Amplification conditions for the three primers were as follows: one cycle of 5 min. at 95^{o}C, and 30 cycles of 30 s at 94^{o}C, 30 s at 57^{o}C and 1 min at 72^{o}C. Electrophoreses of the PCR products was done using 12% nondenaturing polyacrylamide gel and allele bands were detected by UV lamp after ethidium bromide staining.

Statistical analysis: Statistical analysis of the data was performed using the Popgene version 1.31 developed by Francis *et al*., (1999).

Homozygosity and heterozygosity were computed according to Levene (1949) and Nei (1978). The genetic variation and F-statistics (Fis and Fst) were computed using Nei (1987) and genetic distance was computed using Nei (1972)**.**

RESULTS

RAPD- PCR: To analyze genetic diversity of Baladi, Somali, Sudani, Maghrabi and Mowallad, primers of three microsatellites derived from llama genomic DNA and twelve random primers with varying GC contents (eight sequences 60% and four sequences 70%) were used. The same PCR program was used to standardize the evaluation of the primers. All primers gave clear reproducible amplified polymorphic DNA products.

The proportion of the polymorphic bands was the highest with the primer G05 (94.4%) followed by A09 (85.7%), OA12 (84.8 %), C20 (80%), C08 (70%), C03 (69.2%), A10 (68.4), A08 (65.5), C06 (60%), A02 (56.5), A07 (47%) and C04 (45%). The RAPD results showed enough genetic variation between and within camel breeds. Reproducible polymorphic bands with varying frequencies among the seventy camel samples were obtained with all primers used. Some DNA bands exhibited a kind of specificity for identification of breeds, whereas some other bands are useful in expecting other camel breeds. Some common bands were recorded as monomorphic (species specific). The highest percent (55%) was scored with primer OPC-04, while the lowest percent (5.6%) was recorded with primer OPC-05 (Table 1). The choice of primer to be used can greatly affect the amount of polymorphism generated.

Table 1: Sequence, operon codes and GC content of the random primers used to study variation in camel species.

Primer	Sequence 5 `⟶3`	GC%	Total amplified Fragments	Polymorphic fragment
OPA02	TGC CGA GCTG	70	23	13
OPA07	GAA ACG GGTG	60	51	24
OPA08	GTGACGTAGG	60	29	19
OPA09	GGGTAACGCC	70	7	6
OPA10	GTG ATC GCAG	60	38	26
OPA12	TCGGCGATAG	60	33	28
OPC03	GGG GGT CTTT	60	13	9
OPC04	CCGCATCTAC	60	31	14
OPC05	GAT GAC CGCC	70	18	17
OPC06	GAA CGG ACTC	60	10	8
OPC08	TGG ACC GGTG	70	20	14
OPC20	ACT TCG CCAC	60	15	12
Total			288	190

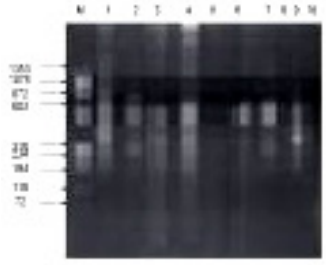

Figure 1

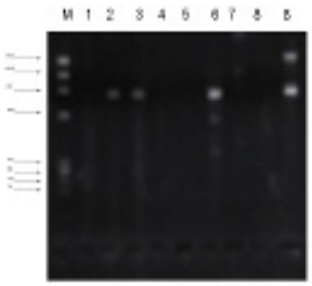

Figure 2

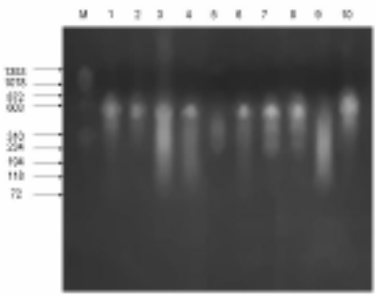

Figure 3

Figure 1: Random amplified polymorphic DNA (RAPD) profile generated by primer OPA02 in camels breeds. Lane M = molecular marker (Φx174 DNA HaeIII digest).Lane 1, 2, 3& 4 Baldi, Lane 5, 6, 7, 8 Sudani. Lane 9& 10 somali.

Figure 2: Random amplified polymorphic DNA (RAPD) profile generated by primer OPC06 in camels breeds. Lane M = molecular marker (Φx174 DNA HaeIII digest). Lane 1 Baldi . Lane 2 Magh. Lane 3, 8,9 Somali. Lane 4 & 5 Mowalad. Lane 6 Sudani

Figure 3: Random amplified polymorphic DNA (RAPD) profile generated by primer OPA10in camels breeds. Lane M = molecular marker (Φx174 DNA HaeIII digest). Lane 1& 2 somali. Lane 3, 4, 5 and 6 Maghrabi. Lane 7, 8 and 9 Mowald. Lane 10 Baldi.

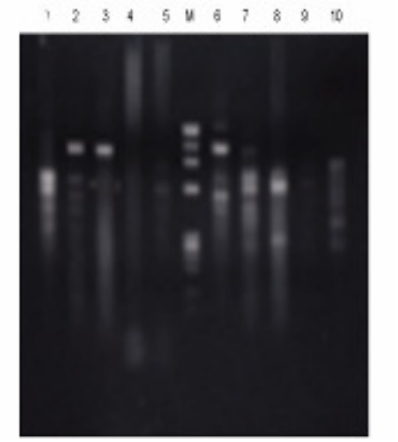

Figure 4

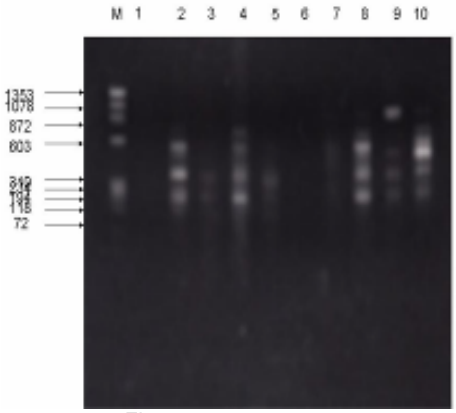

Figure 5

Figure 4: Random amplified polymorphic DNA (RAPD) profile generated by primer OPA09 & OPC20 in camels breeds. OPA09 represented by Lane 1 Baldi. Lane 2 Magh. Lane 3 Somali. Lane 4 Mowalad Lane 5 Sudani. Lane M = molecular marker (Φx174 DNA HaeIII digest). While OPC20 represented by Lne 6 Baldi. Lane 7 Magh. Lane 8 Somali. Lane 9 Mowalad Lane 10 Sudani

Figure 5: Random amplified polymorphic DNA (RAPD) profile generated by primer OPC04 in camel breeds. Lane M =molecular marker (Φx174 DNA HaeIII digest). Lane 1, 2, 3 and 4 Baladi. Lane 5, 6, 7, & 8 Sudani. Lane 9 & 10 Somali.

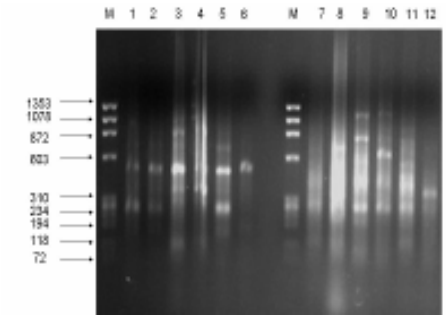

Figure 6

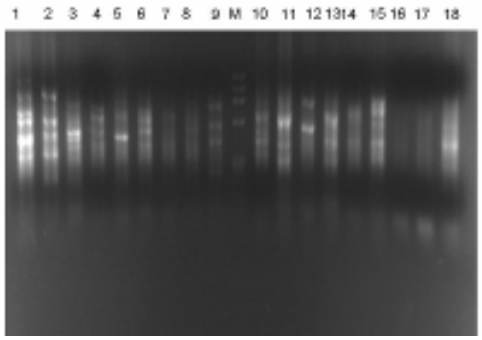

Figure 7

Figure 6: Random amplified polymorphic DNA (RAPD) profile generated by primer OPA07 & OPA12 in camels breeds. OPA07 represented by Lane M = molecular marker (Φx174 DNA HaeIII digest). Lane 1 Sudani. Lane 2 & 5 Baladi. Lane 3 & 4 Somali. Lane 6 Maghrabi. While OPA12 is shown in Lane 7 Sudani. Lane 8& 11Baladi. Lane 9 & 10 Somali. Lane 11 Maghrabi. Where is lane 12

Figure 7: Random amplified polymorphic DNA (RAPD) profile generated by primer OPA08 & OPC08 in camels breeds. OPA08: Lane 1& 2 Baladi. Lane 3 Somali. Lane 4 & 5 Sudani. Lane 6 &7 Maghrabi. Lane 8 &9 Mowald Lane M = molecular marker (PCR 100 bp Low Ladder). OPC08 represented by Lane 10 & 11 Baladi. Lane 12 Somali. Lane 13 & 14 Sudani. Lane 15 &16 Maghrabi. Lane 17 & 18 Mowalad.

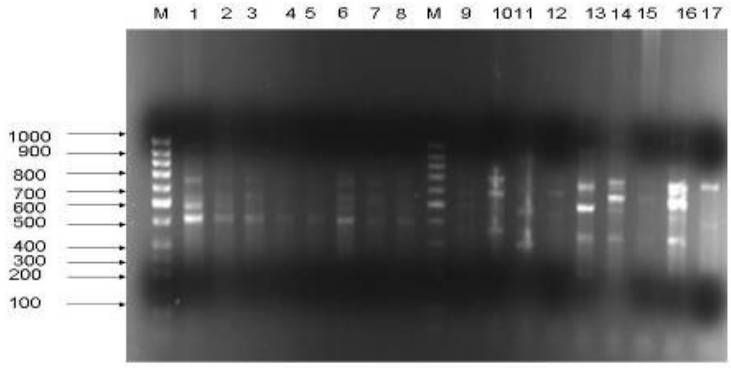

Figure 8: Random amplified polymorphic DNA (RAPD) profile generated by primer OPC03& OPC05 in camels breeds. OPC03: Lane M = molecular marker (PCR 100 bp Low Ladder). Lane 1,2 Baldi. Lane 3 Somali. Lane 4,5 Sudani. Lane 6, 7 Maghraby. Lane 8 Mowald while OPCO5: Lane 9, 10 Baldi. Lane 11 Somali. Lane12, 13 Sudani. Lane 14, 15 Maghraby. Lane 16, 17 Mowald

Microsatellites: The number of alleles for a certain locus in all breeds was studied. The average number of alleles ranged from 4.0 to 6.0 (Table 3). The highest number was found in locus YWLL44, while the lowest was shown by YWLL59. The overall mean for the number of alleles was 4.67 for all breeds. The mean value of the number of alleles for the three loci was 5. The mean number of alleles in the five breeds ranged between 4 and 5. The highest value (five) was detected in both Baladi and Maghrabi breeds, while the lowest one (four) was exhibited by Somali camels. In both Sudani and Mowallad, the mean number of alleles was (4.67). Considering the mean value for total allele frequency in the different breeds, it was found that this value ranged from 0.21 to 0.25. The highest value was expressed by Somali camels, while the lowest value was shown by Baladi and Maghrabi breeds. Considering the overall allele frequency for the three microsatellite, the value ranged from 0.25 to 0.18. The overall frequency mean value for the five breeds was 0.22. The highest value (0.25) was observed in the locus YWLL59, while the lowest one was found in the locus YWLL44.

Table 2: STR loci forward (F) and reverse (R) primers for PCR amplification, expected product size and references

STR name	PCR primer	Product length (pb range)	References
YWLL44	F: CTCAACAATGCTAGACCTTGC R: GAGAACACAGGCTGGTGAATA	108 -126	Sasse *et al*., 2000
YWLL08	F:ATCAAGTTTGAGGTGTTTCC R:CCATGGCATTGTGTTGAAGAC	130-174	Penedo *et al*., 1999a
YWLL59	F:TGTGCAGGAGTTAGGTGTA R:CCATGTCTCTGAAGCTCTGGA	113-131	Evdotchenko *et al*., 2003

Expected (He) and observed (Ho) heterozygosity for each breed within each locus are shown in Table (3). Values of Ho were higher than those of He for all loci in all breed. A clear differences were found between He and Ho in the three loci for each breed. The wide ranges of He and Ho were observed in Somali camels for locus YWLL44, in Sudani for Locus, YWLL08 and in Maghrabi for locus YWLL59. Incidentally, no observed homozygosity was recorded any of the loci in this study. The values of He in the five breeds ranged from 0.62 to 0.83 as shown in Maghrabi and Sudani camels. The lowest value was for the locus YWLL59, while the highest value was for the locus YWLL44. When values of He and Ho for each of the STR loci were compared in both Mowallad and all breeds (Table 3), it was found that values of He were nearly similar for all breeds and Mowallad for all loci. For all three loci, all breeds and Mowallad breed showed the highest He for locus YWLL44 and the lowest value for locus YWLL59. Values of Ho were the same for all breeds and Mowallad for all loci. The significant values of He and Ho were observed for all breeds and Mowallad on the three STR loci ($P<0.05$).

Table 3: Number of alleles and values of observed (HO), expected (He) heterozygosity for each locus in camel breeds, Inbreeding rate (Fis) values (Wright, 1978) for each locus in all camel breeds

Locus	Breed	No. of alleles	(Ho)	(He)	Fis value
YWLL44	Baladi	6	1.0	0.82	-0.2102
	Somali	4	1.0	0.78	-0.3342
	Sudani	6	1.0	0.83	-0.2095
	Maghrabi	6	1.0	0.80	-0.2179
	Mowallad	6	1.0	0.79	0.2279
	All breeds	6	1	0.80	-0.2399
YWLL08	Baladi	5	1.0	0.79	-0.2658
	Somali	4	1.0	0.78	-0.3333
	Sudani	4	1.0	0.73	-0.3600
	Maghrabi	5	1.0	0.79	-0.2711
	Mowallad	4	1.0	0.76	-0.3611
	All breeds	5	1	0.78	-0.3182
YWLL59	Baladi	4	1.0	0.72	-0.3663
	Somali	4	1.0	0.76	-0.3426
	Sudani	4	1.0	0.76	-0.3426
	Maghrabi	4	1.0	0.62	-0.4260
	Mowallad	4	1.0	0.63	-0.4294
	All breeds	4	1	0.70	-0.3813

Table (4) shows the values of genetic diversity for each locus in each breed. The highest value was in Sudani (1.70) for the locus YWLL44, in Baladi (1.50) for the locus YWLL08 and in Somali and Sudani breeds (1.35) for the locus YWLL59.

Table 4: Shannon ingormation index (I) for each locus in each breed

Locus	Breed	I	Nei
	Baladi	1.67	0.77
	Somali	1.38	0.75
YWLL44	Sudani	1.70	0.82
	Maghrabi	1.62	0.82
	Mowallad	1.55	0.75
	Baladi	1.50	0.85
	Somali	1.39	0.78
YWLL08	Sudani	1.29	0.75
	Maghrabi	1.48	0.85
	Mowallad	1.29	0.81
	Baladi	1.27	0.78
	Somali	1.35	0.82
YWLL59	Sudani	1.35	0.78
	Maghrabi	1.06	0.75
	Mowallad	1.04	0.79

Table 5: Genetic diversity (I), F- value and Gene flow (Nm) for all breeds in each locus

The locus	I	Mean	S.D	FST	Gene flow (Nm)
YWLL44	1.6665	1.4822	0.2018	0.0279	8.6995
YWLL08	1.5137	1.4822	0.2018	0.0451	5.2913
YWLL59	1.2665	1.4822	0.2018	0.0199	12.3183

The gene flow is defined as the movement of genes from one population to another by way of interbreeding of individuals in the two populations. Nm values were calculated for the three STR loci as in previous (Table.5). Results showed that, the lowest Nm value was 5.2913 for the locus YWLL08, while the highest value was 12.3183 for the locus YWLL59. There is inverse proportion between Nm and Fst values, where high Nm means low Fst and vice versa. It was concluded that, high Nm values indicates little genetic differentiation. These values confirm a shared origin of the five camel breeds studied.

In microsatellities markers: Values of genetic distance between camel breeds were calculated by the application of Nei's formula (1972). The values are shown in Table (6) below the diagonal. The highest genetic distance was found between Sudani and Mowallad breeds (0.3188). The lowest value for genetic distance was found between Baladi and Somali (0.0431). This means that the range of genetic distance between camel breeds was small, which indicates a close genetic relationship between camel populations. This relationship is closer between Somali and Baladi camels, and higher between Mowallad and Sudani populations. With regard to the genetic identity, the values are found above diagonal in Table (6). High values for genetic identity means low values for genetic distance. These show that increasing the genetic distance decreases genetic identity and vice versa.

In RAPD-PCR: Genetic distance between the five breeds of camel was estimated using twelve primers pooled over primers indicated the lowest genetic distance between Baladi-Somali (Df=0.8686, Ds=0.1409) followed by Sudani-Somali (Df=0.8751, Ds=0.1334), Somali-Mawalad (Df=0.8833, Ds=0.1240), Somali-Maghrbi (Df=0.8870, Ds=0.1199), Sudani-Mawalad (Df=0.8983, Ds=0.1072), Baladi-Maghrabi (Df=0.8987, Ds=0.1068), Baladi-Sudani (Df=0.9061,

Ds=0.0986) . The Maghrabi-Mowalad breeds had the highest genetic distance (Df=0.9102, Ds =0.0941) among the breed pairs studied (Table 7). The similarity matrix between camel breeds shows an average genetic distance range from 0.8686 to 0.9102 with a mean of 0.89. Thus, the camel breeds tested in this study are not highly divergent (mean>0.6) at the DNA level.

Table 6: Nei's genetic distance for all loci and all breeds.

The breed	Baladi	Somali	Sudani	Maghrabi	Mowallad
Baladi	-------				
Somali	0.0431	-------			
Sudani	0.1146	0.0570	-------		
Maghrabi	0.0450	0.1068	0.2177	--------	
Mowallad	0.0706	0.1613	0.3188	0.0096	---------

Table 7: Nei's Unbiased Measures of Genetic Identity and Genetic distance

The breed	Baladi	Sudani	Somali	Maghrabi	Mowallad
Baladi	****	0.9061	0.8686	0.8987	0.9087
Sudani	0.0986	****	0.8751	0.9037	0.8983
Somali	0.1409	0.1334	****	0.8870	0.8833
Maghrabi	0.1068	0.1012	0.1199	****	0.9102
Mowallad	0.0957	0.1072	0.1240	0.0941	****

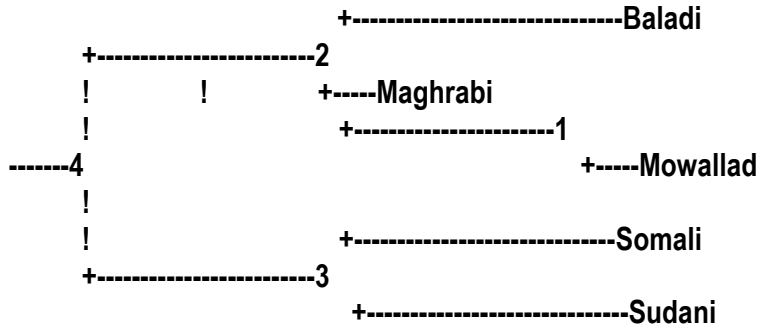

Figure 9: Phylogenetic relationships for the five camel breeds

The phylogenetic relationship between the five camel breeds showed two groups (Fig.9). The first group includes Baladi, Maghrabi and Mowallad, while the second group includes Somali and Sudani. The results showed also that Mowallad breed was very near to both Baladi and Maghrabi breeds. This confirms the origin of Mowallad breed as a hybrid between Baladi and Maghrabi breeds.

DISCUSSION

In this study, microsatellites and RAPD - PCR results have been used and compared for studing the genetic diversity between five breeds of camels. The RAPD technique is known to be sensitive to the amplification parameters (Williams *et al.*, 1993). The number of known microsatellite loci is limited in studying camels as compared to human and mice, however they are considered to be effective for molecular characterization (Han *et al*., 2000 and Al-Swailem *et al*., 2007). These results are in agreement with that of Sherief and Alhadrami 1996, who reported that different RAPD fragment patterns were obtained and found effective for detection of genetic variation in racing camels using seven Operon primers.

Al-Swailem *et al*., 2007 reported that the similarity matrix between Saudi camel subtypes showed an average genetic distance range from 0.73 to 0.92 with a mean of 0.82 and were not highly divergent at the DNA level. The genetic distance matrix using the (nweighted Pair Group Method of Arithmetic Means) dendrogram

among the populations (UPGMA algorithm) was computed to cluster the data and to draw the precise relationships among the tested camels. The obtained dendrogram shown in Fig. 9 illustrates the divergence between the used breeds and suggests their tree branching. Estimation of genetic relationships between the seventy samples of camel breeds. The intra-specific analysis of the RAPD patterns showed a rich polymorphism in the camel breeds. The main advantage of RAPD analysis is to obtain bands which gould be used as a DNA fingerprints. In this study RAPD results showed enough genetic variation between and within Egyptian camel breeds. These results are in agreement with Mehta *et al*., 2007 who studied the genetic differentiation of Indian camel breeds using the same method.

In addition, the branching of the phylogenetic trees was reasonably well correlated with the conventional criteria used for classifying Egyptian camel breeds (Al-Busadah, 1998). Although other methods can also be used, DNA fingerprinting of Arabian camel can be considered as an excellent resolving power for identifying these breeds and recognizing phylogenetic relationships among them. The branching indicated by the trees are reasonably well correlated with and supported the conventional morphological and physiological classification criteria.

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.

The present study represents the first step in identifying Egyptian camels breeds and their relationships. Especially at the molecular genetic level it deals with the investigation of certain microsatellite markers in the different camel breeds. The microsatellite or the molecular genetic markers or loci were chosen according to Lang *et al* (1996) who studied them in llama and alpaca (American camelids). The usefulness of microsatellites for the estimation of genetic variations among closely related populations has been documented by numerous studies (Barker *et al*., 1997; Machugh *et al*., 1997). Our results are in agree to those reported by Sasse *et al* (2000) but they are contradicting with Lang *et al* (1996) in American camelids, llama and alpaca, Jianlin *et al* (2000) and Gautam *et al*. (2004). In llama and alpaca, the number of alleles for the locus YWLL44 was 11, and the range of allele sizes ranged from 86-120 bp. In *Camelus dromedarius* and *Camelus bactrianus*, the number of alleles was three with a range of allele sizes from 105-109 bp in dromedary, and 101-112 bp in bacterian camels. In Jaisalmeri camel breeds, the number of alleles was three with a range of allele sizes from 105-109 bp. While Mehta and Sahan (2007) and Mehta *et al* (2007) found two alleles for the previous locus in the camels of Bikaneri and Kachchhi breed respectively with size ranging from 104-106 bp. Al-Swailem *et al* (2009) reported that the number of alleles was three with a range of allele sizes from 104-108 bp. in Saudi Arabian camel.

These discrepancies in the number of alleles and the limits of the range of allele sizes can be attributed to the difference in the species and the number of individuals analyzed. In addition, ecological conditions should not be neglected.

The second locus, YWLL08, has a total number of five alleles. This number was found in Baladi and Maghrabi camels. In the remaining breeds (Somali, Sudani and Mowallad) four alleles were detected. These data indicate that this locus, (YWLL08) like the previous one, (YWLL44) are considered to be polymorphic loci.

Lang *et al* (1996) detected 13 alleles for the locus YWLL08 in llama and alpaca. They reported that the range of allele sizes was from 135-177 bp. The results of Bustamante *et al* (2002) on llama indicated that only 11 alleles were found for the target locus, with size ranging from 131-153 bp. On guanaco, the same author and his colleagues published 14 alleles with range limits between 133-163 bp. Jianlin *et al* (2000) found nine alleles for the previous locus in *Camelus dromedarius*, and five in *Camelus bactrianus.* The range of allele sizes was 133-170 bp in the first species and 154-180 bp in the second. The number of alleles reported by Sasse *et al* (2000) in dromedary racing camels for the locus under discussion was 12, with size ranging from 134-172 bp. Comparing the present results with those published in other camelids**,** it was concluded that the polymorphic phenomenon is common, and the investigated populations have high genetic variability. Mehta and Sahan (2007) found seven alleles for the previous locus in the camels of Bikaneri breed with size ranging from 132-162 bp.

While in Kachchhi breed of camel, the number of alleles was six with a range of allele sizes from 132-158 bp Mehta *et al* (2007). Al-Swailem *et al* (2009) reported that the number of alleles was four with a range of allele sizes from 164-204 bp. in Saudi Arabian camels.

The third locus, YWLL59 showed a total of four alleles in studied camel breeds, with allele sizes ranging from 113 to131 bp. This differed from the data of Lang *et al*

(1996), Jianlin *et al* (2000), Sasse *et al* (2000) and Gautam *et al*. (2004). The first author reported ten alleles in American camelids, with a range of allele sizes from 96-136 bp.while the second author reported that two alleles of size 107 and109 bp for UAE. The last two authors reported 109-111 bp Kenyan camels and Jaisalmeri camel, respectively. One the other hand Mehta and Sahan (2007) and Mehta *et al* (2007) two alleles of size 115 and117 bp for Hikaneri and Kachchhi camel respectively.

Our findings indicated that the locus YWLL59 is polymorphic in camels found in Egypt, while the UAE and Kenyan camels were found to be monomorphic (Mburu *et al*., 2003). The presence of several alleles for a certain locus (Crawford *et al*., 1995) or its polymorphism is mainly due to certain mutations. Consequently, the appearance of new alleles in a population has the potential to change the genetic make-up of successive generations. Harmful mutations will likely not persist because the affected individual will either not survive or will have a limited fecundity. However, some mutations may be passed on to successive generations because an animal with that allele is better adapted to survive in its environment. So, it has a selective advantage. The selective advantage drives evolution, even if momentarily, in one direction or another. Evdotchenko *et al* (2003) studied the amplification of 23 loci in bacterian and dromedary camels, 19 loci in llama and 20 in alpaca. The different species had similar fragment lengths per locus, with more similarities between bacterian and dromedary and between llama and alpaca, respectively. No significant (P<0.05) deviation from HWE was observed for the groups of bacterian and dromedary, since microsatellite alleles of the same length can be different from species to another. So the new microsatellite loci can be efficiently used in several species for parentage control, gene mapping or phylogenetic analysis.

Decreased number of alleles for the second locus, YWLL08 in Sudani, Somali and Mowallad and the third locus, YWLL59 in Baladi, Somali, Sudani Maghrabi and Mowallad may be due to the presence of genetic drift within these breeds.

The highest Heterozygosity value for YWLL44, YWLL08 and YWLL59 was in Sudani, Baladi breeds and in Sudani and Somali breeds respectively. It can be inferred that these breeds have the maximum intra and inter breeds polymorphism, when comparing three loci, YWLL44 showed the highest genetic variation, while YWLL59 showed the lowest value. The observed and expected heterozygosity reported by Mehta and Sahan (2007) in Hikaneri camel, Jianlin *et al*. (2000) in dromedary and in Jaisalmeri camel by Gautam *et al*. (2004), Lang *et al*. (1996), Obreque *et al*. (1998) and Penedo *et al*. (1999a) in New World Camelids they detected difference between He due to more number of alleles at most of the loci. Shannon information index (I), Table (4) was highest in Sudani camels for YWLL44, while Somali ones showed the lowest value. It can be inferred that Sudani is the highest polymorphic breed for the YWLL44 locus, while Somali is the lowest one. In the 2nd locus, YWLL08, Baladi was the highest breed and Sudani was the lowest one. Somali and Sudani showed the highest polymorphism, while Maghrabi and Mowallad camels represent the minimal polymorphic breeds for YWLL59 locus. This is in concordance with heterozygosity test and Fis values. Where the highest polymorphic locus was YW0LL44 (1.67) and the lowest one was YWLL59 (1.27).

Genetic distance matrix for all loci indicated that Sudani camels were the most divergent from Mowallad, Maghrabi and Baladi at 0.32, 0.22 and 0.11 genetic distances. However Somali camels were divergent from Mowallad and Maghrabi in some extent with 0.16 and 0.11 genetic distances. The most related breeds were Mowallad and Maghrabi (0.01), Baladi and Somali (0.04), Baladi and Maghrabi (0.05), Sudani and Somali (0.06) and Baladi and Mowallad (0.07). The close relationship between Baladi, Maghrabi and Mowallad was expected, since, Mowallad is a hybrid between Baladi and Maghrabi. The low genetic distances between Baladi, Somali, Sudani, Maghrabi and Mowallad camels conform that they may have the same ancestor.

CONCLUSION

The genetic variations between Baladi, Somali, Sudani, Maghrabi and Mowallad camel breeds can be used as a basis for genetic improvement of their productivity. The results also provide an indication of the feasibility of the methodology employed, as well as the posible differentiation between and within these camel breeds reared in Egypt.

REFERENCES

Abdel-Rahman SM. and Hafez EE, 2007. Genetic similarity among the three Egyptian water buffalo flocks using RAPD-PCR and PCR-RFLP techniques Research Journal of Agriculture and Biological Sci. 3, 351-355.

Al-Busadah KA, 1998. Endocrine and Haematological Responses of the Camel (*Camelus dromedarius*) to Rehydration. Ph.D. thesis, University of Sheffield, U.K.

Al-Swailem AM, Al-Busadah KA, Shehata MM, Al-Anazi IO, Askari E, 2007. Classification of Saudi Arabian camel (*Camelus dromedarius*) subtypes based on RAPD technique. J. Food, Agric.Environ. 5,143-148.

Al-Swailem AM, Al-Busadah KA, Shehata MM, Askari E, 2008. The role of parentage studies in Arabian and Bactrian camel's pedigree verification. J. Food, Agric. Environ. 6, 280-285.

Al-Swailem AM, Shehata MM, Al-Busadah KA, Fallatah MH, Askari E, 2009. Evaluation of the genetic variability of microsatellite markers in Saudi Arabian camels. J. Food, Agric. Environ. **7**, 636 -639.

Apostolidis AP, Mamuris Z, Karkavelia E, Alifakiotis T, 2001. Comparison of Greek breeds of horses using RAPD markers. J. Animal Breed. Genet. 118, 47-56.

Barker SF, Moore SS, Hetzel DJS, Evans D, Byrne TnSGK, 1997**.** Genetic diversity of Asian water buffalo Microsatellite variation and comparison with protein –Coding loci.Animal Genet. 28, 103-115.

Bustamante AV, Zambelli A, De Lamo DA, Von Thungen J, Vidal-Rioja L, 2002. Genetic variability of guanaco and llama populations in Argentina. Small Ruminant Res. 44, 97-101.

Crawford AM, Dodds KG, Ede AJ, Pierson CA, Montgomery GW, Garmonsway HG, Beattie AE, Davies K, Maddox JF, Kappes SW, 1995**.** An autosomal genetic l. J. of Genetics and Cyto. inkage map of the sheep genome. Genet. 140, 703-724.

El-Seoudy AA, Abdel Gawad NM, Abu-Shady AM, Abdelsalam AZE , 2005. Biochemical and molecular characterization of some Egyptian goat breeds. Egypt34, 63-79.

Eroglu, D. and Arica ŞÇ, 2009. Molecular genetic analysisof three Turkish local silkworm breeds (Bursa Beyazı, Alaca and Hatay Sarısı) by RAPD-PCR method. Journal of Applied Biological Sci. 3, 15-18.

Evdotchenko D, Han Y, Bartenschlager H, Preuss **S**, Geldermann H**,** 2003**.** New polymorphic microsatellite loci for different camel species. Mol. Ecol. Not. 3, 431-434.

Francis C, Yeh Yang R, Boyle T, 1999. Popgene version 1.31: Microsoft window-based freeware for population genetic analysis. http://www.ualberta.ca/~fyeh/fyeh

Gautam L, Mehta SC, Gahlot RS, Gautam K**,** 2004**.** Genetic chracterisation of Jaisalmeri camel using microsatellite markers. Indian. Journal of Biotech. 3, 457-459.

Han Y, Jewdoschenko D, Hue G, Reiver G, Geldermann H**,** 2000. Screening and analysis of new microsatellite loci in camels. Plant and Animal Genome VIII Conference, San Diego, CA, 9-12 .

Jianlin H, Mburu D, Ochieng J, Kaufmann B, Rege JEO, Hanotte O, 2000. Application of New World Camelidae microsatellite primers for amplication of polymorphic loci in Old World camelids. Animal Genet. 31, 404-419.

John SWM, Weitzner G, Rozen R, Scriver CR, 1991. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acid Res. 19, 408-412.

Khanna ND. and Tandon SN, 1997. Biochemical polymorphism in Indian camels. Annual Report, 1996/97, NRCC, Bikaner.

Lang KDM, Wang Y, Plante Y, 1996**.** Fifteen polymorphic dinucleotide microsatellites in llama and alpacas. Animal Genet. 27, 285-294.

Lettier G, Feng Q, de Mayolo AA, Erdeniz N, Reid RJ, Lisby M, Mortensen UH, Rothstein R. (2006). The role of DNA double-strand breaks in spontaneous homologous recombination in S. cerevisiae. PLoS Genet. 10;2(11):e194

Levene H, 1949**.** On a matching problem in genetics. Ann. Math. Stat. 20,91-94.

MacHugh DE, Shriver MD, Loftus RT, Cunningham P, Bradley DG, 1997. Microsatellites DNA variation and evolution, domestication and ghylogeography of Taurine and Zebu cattle (*Bos tourus* and *Bos indicus*). *Genet.* 146, 1071-1086.

Mahfouz ER, Othman OE, El Nahas SM, El Barody MAA**,** 2008. Genetic variation between some

Egyptian sheep breeds using RAPD-PCR. Research J of Cell and Molecular Biol. 2, 46-52.

Mahrous KF, Alam SS, Hassan AM, 2010. Genetic Variation Between Horse Breeds Using RAPD Markers. Nature and Sci. 8, 90-99.

Mahrous KF, Salem AMH, Hessen AF, Mohamed T M, Ebrahim MAM, Salem LM, 2007. Genetic diversity for milk yield genes in Egyptian native cattle using microsatellite markers. Ain shams science. Bullet. 45, 27-42.

Mburu DN, Ochieng JW, Kuria SG, Jianlin H, Kaufmann B, Rege JEO, Hanotte O, 2003. Genetic diversity and relationships of indogenous Kenyan camel (Camelus dromedarius) populations: implications for their classification. Animal Genet. 34, 26-32.

Mehta SC, Goyal A, Sahani MS, 2007. Microsatellite markers for genetic characterisation of Kachchhi camel. Indian Journal of Biotech. 6,336-339.

Mehta SC, and Sahan MS, 2007. Microsatillite markers for genetic. Characterisation of Hikaneri camel. Indian Journal of Animal Sci.77, 509-512.

Nei M., 1972. Genetic distance between populations. Am. Nat. 106, 283-292.

Nei M, 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89, 583-590.

Nei M, 1987. Molecular Evolutionary Genetics. Columbia University Press, New Yourk.

Obreque V, Coogle L, Henney PJ, Bailey E, Mancilla R, Garcia-Huidobro J, Hinrichsen P, Cothran EG, 1998. Characterization of 10 polymorphic alpaca dinucleotide microsatellites. Animal Genet. 29, 460-477.

Okumus A. and Kaya M, 2005. Genetic similarity by RAPD between pure lines of chickens. J. of Biol. Sci. 5, 424-426.

Penedo MCT, Caetano AR, Cordova K, 1998. Microsatellite markers for South American camelids. Animal Genet. 29, 398-413.

Penedo MCT, Caetano AR, Cordova K, 1999a. Six microsatellite markers for South American camelids. Animal Genet. 30, 161-168.

Penedo MCT, Caetano AR, Cordova K, 1999b. Eight microsatellite markers for South American camelids. Animal Genet. 30, 382-405.

Rahman MA, Rahman SMM, Jalil MA, Uddin SN, Rahman MM, 2006. Molecular characterization of Black Bengal and Jamuna Pari goat breeds by RAPD markers. American J. Of Anim. And Vet. Sci. 1, 17-22.

Sarno RJ, David VA, Franklin WL, Obrien SJ, Johnson WE, 2000. Development of microsatellite markers in the guanaco, Lama guanicoe: utility for South American camelids. Mol. Ecol. 9, 1919-1952.

Sasse J, Mariasegaram M, Babu R, Kinne J, Wernery U, 2000. South American camelid microsatellite amplification in Camelus dromedarius. Animal Genet. 31,33

Schulz U, Mínguez Y, Checa ML, García-Atance P, Dunner S, García D, Cañón J, 2005. The Majorero camel (*Camelus dromedarius*) breed. Animal Genetic Reso. 36, 61-72.

Scott AM, Binns MM, Holmes N, 1992. Genetic polymorphism in the camel and its use in parentage and identity testing. Proceedings of First International Camel Conference, Dubai, UAE. 1, 289-291.

Shereif NA. and Alhadrami GA, 1996. Detection of genetic variation in racing camels using random amplified polymorphic DNA (RAPD) technique. J. Camel Pract. Res. 3,91-94.

Welsh J. and McClelland M, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nuc. Acids Res. 18, 7213-7218.

Williams JGK, Hanodey MK, Rafalski JA, Tingey SV, 1993. Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzym. 218, 704-740.

Williams JGK, Kubelic AR, Livak KJ, Rafalski JA, Tingey SV, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18,6531-6535.

Wright S, 1978. Variability Withn and Among Natural Populations. Vol. 4. The Univ. Of Chicago Press, Chicago.