Research Article

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Animal Science Journal, 3(1): 06-10, 2012
Available online at **www.isisn.org**
Print ISSN: 2220-9549
Online ISSN: 2220-9557

Preliminary genetic study of Libyan camels using DNA based RAPD and SSR markers.

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Present study reports the preliminary results from molecular analysis of the inter- and intra-population structure of important four Libyan camel breeds (Alarabia, Almahare, Altebesty and Alsertawiya). To date the majority of studies on Libyan camel groups involve the analysis of phenotypic and reproduction traits and to our knowledge this is the first study that adopts a molecular genetics approach to investigate the relationships within and between Libyan camel population. Molecular analysis was performed using PCR based RAPD and microsatellite markers systems. These DNA based marker systems were used to investigate the genetic polymorphism in four Libyan camel breeds. In conclusion, present study indicated existence of genetic variation among these four different camel breeds. However, detailed genetic investigations are still required.

Key words: Libyan camel genetic, camel breeds, Markers, RAPD, microsatellite loci

Arabic countries represent 70% of the camel world (FAO 1989). Camel genetic resource of Libya consist only of single humped (*Camolus dromedaries*). The Libyan camel like most local breeds has not undergone any systematic attempt to genetically improve current stocks (Hermas. 1998). Camels can live in relatively extreme environmental, living in areas not suitable for other animals including goats (Shraha. 1990). Therefore the economic status of camels and their potential as cattle is recently becoming evident. Due to their self-sustainability and general lack of dependence on farmers for continual assistance or sustenance the camel offers an attractive source of income for many Libyan farmers. Camels are a multipurpose animal, producing milk, meat, hair and hides. It can be reared in arid and semi-arid areas where the food is scarce (Hermas. 1997. Ismail and Mutari. 1991).

Very few camel molecular genetic studies exist in the literature (Hermas, 1998: Mburu et al. 2003: Shah. 2007) investigated genetic structure of Camel populations. These groups found that there was very little or no genetic variation between the individuals they included in the study. There are a number of PCR methods can identification of species such cattle, sheep, goat, chicken and pig but none of these methods can detection of camel materials, Shereif and Alhadrami (1996), recommended to use RAPD to study DNA for the animals

In an attempt to investigate intra- and inter-group structure we report here a preliminary analysis of 4 Libyan camel populations using Random Amplified Polymorphic DNA (RAPDs) and microsatellite loci. The purpose of this study was evaluation of intra- and inter - group genetic variability in the dromedary camels. This study was carrying out on thirty Libyan Camels from four different sub species: Alarabian (9), Altebesty (7), Almahare (7), and Alsertawe (7).

MATERIALS AND METHODS

Morphological study:

There are several breeds of camels in Libya including Alarabia, Almhare, Altebesty, Almaghrebia, Alsertawiya and furthermore other breeds coming from surrounding countries like Alsudania, Altchadia, Alnajaria. These are different in the phenotype and certain traits especially in shape, size and the color (Hermas 1998, Shraha 1990). All these breeds belong to single humped dromedary camels (*Camelus drom dromedaries*) and has 2n =74 chromosome (36 autosome pairs and 1 - sex chromosome. In this study we use four

different Libyan camel breeds, as fallowing Almahare breeds from Obaree (South Libya) and Altebestee breeds from Traghen (South Libya) Alarabia from Zweela (Morzouk region) and Alsertaweya. The camel's breeds were described based on external morphology and information collected from locals and our observations. The purpose of using PCR-RAPD, mitochondrial and microsatellite markers in this study was to determine the variation between four Libyan camel breeds and to estimate the genetic distance between these four breeds.

Genetic study:

Sample preparation:

Hair samples were collected from four different breeds as mentioned above, by cutting 1 cm from the root and placed in distilled water in eppendorf tub for 2 minutes, placed in distilled water twice. About 6-9 hair were picked and placed into 200 µl, PCR tube with hair follicle down to the bottom (the top part extending beyond the tub was cut off with clear scissors).

DNA isolation from camel hair:

Isolation of genomic DNA from camel hairs was carried out using Qiagen DNeasy Tissue Kit. About 6-9 hairs were used for each extraction following the recommendations of Roon et.al (2003) and Valderrama et al. (1999). Hair samples were washed with distilled water. By adding 30 µl in 1.5 ml eppendorf tub and left for 10 minutes. The follicle from each hair was then removed and added to a fresh eppendorf tube comprising 180 µL, Lysis Buffer provided with the Kit. 25 µl Proteinase K was added; the sample was vortexed and incubated at 55 °C in a water bath overnight.

Polymerase chain reaction

All PCR were set up in reaction volumes of 25 µl comprising 2µl template DNA, 5 µl buffer, 3 µl $MgCl_2$. 0.5 µl dNTPs, 1 µl of each primer (600 nM), 0.125 µl Taq polymerase, 12.3 µl H_2O. PCR reactions were performed for three types of marker systems i.e. RAPD and microsatellites sequences using different primers as mentioned in Table 1.

PCRs were conducted using a Peltier Thermal Cycler, DNA engine, DYAD^{TM} PTC-200. The thermal cycling conditions for the PCR were as fallows Initial denaturation, at 94**°**C for 4 minutes, denaturation at 94**°**C for 1 minutes, annealing temperature (55-59**°**C) standardized for different SSR primers for 2 minutes then extension step at 72**°**C for 2 minutes and final extension at 72**°**C for 10 minutes.

RAPDs:

For RAPD analysis, primers used are described in table (1).

Microsatellite marker analysis:

Different microsatellite primers (YWLL-08F, YWLL-08R) (VOLP-IOF, VOLP-IOR) and (LCA-63F, (LCA-63R) used in present study are given in table 1.

RESULTS AND DISCUSSION

Camel morphological descriptions are shown in table 2. In general, the concentration of DNA was low for the most of the sample and it ranged from 7.4 to 134, with mean 18.56±15.6. This may be because of the low number of hair for each samples that 6 in average. Furthermore, most of them were with no follicle. Goossens *et al*. 1998 recommend that 10 hair should be used to obtained good quality DNA.

The PCR based RAPD profile of Alarabia, Almhare, Altebesty and Alsertawiya breeds, in our study reviled that the PCR-RAPD primer produced number of band with varying frequencies among the four breeds that are shown in Table 3. Our results presented in the figure 1 showed that the RAPD markers successfully amplified in the tested Libyan camel breeds. The primers GATC4 produced the highest number of polymorphic bands among the RAPD primers, which represented 43% preparation, these founding in the close agreement with the Mehta et al. 2006 and Al- Swailem 2007). RAPD profiles revealed high genetic similarity or different among indigenous camel breeds. Furthermore this information can be used to distinguish different camel breeds from one and other. Previously, no prior knowledge of differences in DNA sequence of these breeds was available.

Microsatellite markers were also used in this study to investigate the genetic variation in Libyan camel population (table 4). Result indicated the existence of enough genetic variation among the four Libyan breeds. In this study i successfully amplified the polymorphic DNA in the four different Libyan camels breeds, and used three primers of the microsatellite markers which considered as the most powerful genetic markers for

Table 1. Primers used for RAPD and microsattelite markers on Alarabian, Alsertawy, Altebestee and Almaharee Libyan camel breeds

Molecular marker	Primers	Sequence from 5 -3	Annealing Tem. (°C)	Reference
RAPD	GATC4	GATCGATCGATCGATC	49.2	Al-Swailem et al. 2007
	CACA4	GACAGACAGACAGACA	49.2	Al-Swailem et al. 2007
	CA8	TTATTCGATACCTACATGC	50.2	Al-Swailem et al. 2007
Microsattelite	LCA.-63F	TACCCAGTCCTTCGTGGG	58.05	Vijh et al.2007
	LCA-63R	GGAACCTCGTGGT6ATGGAA	58.05	Vijh et al.2007
	YWLL-08F	ATCAAGTGAGGTGCT6ATCC	57.9	Gautam et al. 2004
	YWLL-08R	CCATGGCATGTGT6GAAGAC	57.9	Gautam et al. 2004
	VOLP-IOF	CTTCTCCTTCCTCCCTACT	56.2	Mehta et al.2007
	VOLP-IOR	CGTCCACTCCTTCATTC	56.2	Mehta et al.2007

Table 2.The morphological phenotype of four Libyan camel breeds.

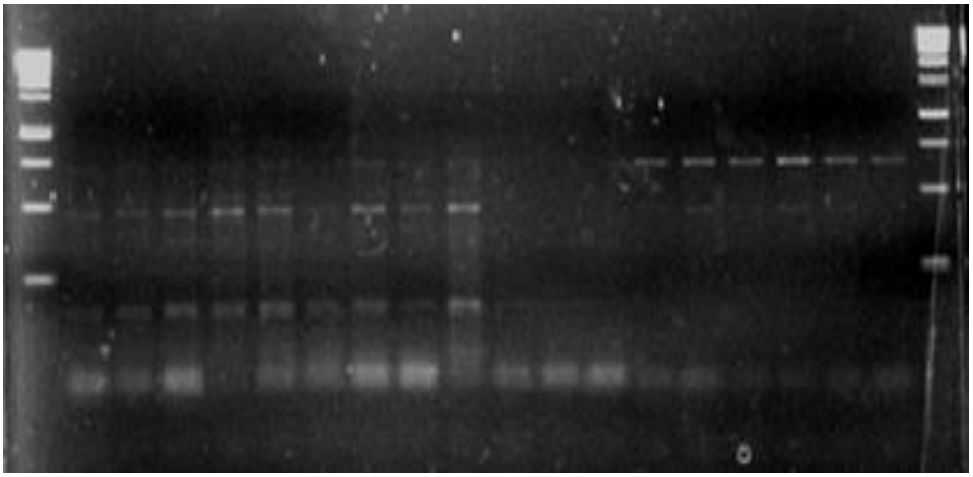
Breed	Region	Morphological description	No of camels	Code
Alarabia	Zwela (South Libya)	Characterize by medium size, large head, graceful and beautiful scenery, the ears straight, the hump is surround the body and legs are consistent, average tall,the colour is white, light brown and gray	9	A1, A2, A3, A4, A5,A6, A7, A8 & A9
Almaharee	Owbari South west Libya)	The phenotype characterize long high, light weight, small head size, long and thin limbs, quick of locomotion, the legs are straight and long, the distances between them are wide range, using for racing. The hump surround and at the middle of the animals, the colour is blue and yellow,.	7	M1, M2, M3, M4, M5, M6 & M7
Altebestee	Traghen (South Libya)	Morphological characterized much smaller size; most of her colour yellow and yellow tend to the sand colour, and gray colour. The hump is small and little bit back, the legs are long and the neck is tall and tiny, the ears straight and the huge	7	T1, T2, T3, T4, T5, T6, & T7
Alsertaweya	Sert (Middle of Libya)	Morphological characterized large size, including several colours mostly light brown, dark brown and blue, high produce of milk, abundant hyrax (hair) , the tail is wide, the tap is tights of meat	7	S1, S2, S3, S4, S5, S6 & S7

Table (3) Number and size range of bands by RAPD primers.

Primers	No. of band	Total Range(Kb)	Size (Kb	Polymorphic bands and there frequency Alarabia	Almaharee	Altebestee	Alsertawea
GATC4	7	0.3-2	2	1.00	0	0	0.8
			1	1	1	0.8	2
			0.6	1	0.6	1	0.5
CACA4	5	0.3-1.8	1	0	1	0.6	0
			2	1	0	0.2	0.8
			0.4	1	0	0.8	1
			0.3	0	1	0.3	0
CA8	4	0.1-1.4	1	0.5	0.2	0.7	1.4
				0.8	1	0	1

Figure 1. Representative electrophorogram showing RAPD profiles of Altebesty and Almahare Libya camel breeds with primer GATC4.

L1 A1 A2 A3 A4 A5 A6 A7 A8 A9 T1 T2 T3 S1 S2 S3 S4 S5 S6 S7 L2



Note: Alarabia A1, A2,A3,A7, Alsertaweya S1, S2, S3 and AlsertaweyaS4, A4,A5,A6, represents Alarabia, and T1, T2, T3 represented Altebestee and Alsertaweya S3,S4, represented Alsertaweya breeds.

Table 4. Amplification of microsatellite loci in four Libyan camel breeds.

Locus	Primer (5-3)	Alleles (no)	Size (bp)	Temp	H0	He	PIC
LCA-63F	TACCCAGTCCTTCGTGGG	1	1082	58.8	0.44	0.30	0.20
LCA-63R	GGAACCTCGTGGTATGGAA	1	761	57.3	0.55	0.60	0.67
YWLL-08F	ATCAAGTGAGGTGCTATCC	2	326	55.3	0.17	0.32	0.51
YWLL-08R	CCATGGCATGTGTGAAGAC	1	136	57.9	0.32	0.19	0.42
VOLP-IOF	CTTCTCCTTCCTCCCTACT	1	555	57.9	0.81	0.21	0.10
VOLP-IOR	CGTCCACTCCTTCATTC	2	843	54.5	0.13	0.18	0.23

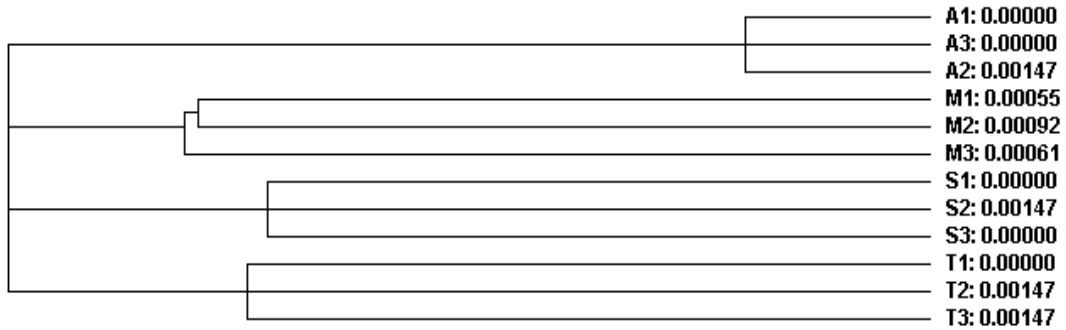


Figure 2. Dendrogram showing the genetic relationships among four Libyan camel breeds

characterisation of animal genetics resource. Results showed that how close are these four Libya camel breeds and the relationship between them by using the microsatellite markers (Figure 2). The A1, A3 and A2 (Arabian breeds) belong to the same group and this was expected based on phenotype descriptions. These result showed that based on microsatellite study, Libyan camel belong to deferent breeds are genetically different and can be easily identified using this toll. However, there is still need to confirm this information using large number of camels and primers.

Conclusions:

Our study revealed existing genetic variation among the four Libyan camel breeds and, I recommend using more sample and more microsatellite markers for

further genetic investigation, genetic diversity analysis, and conservation of genetic diversity which is important for long term genetic improvement.

ACKNOWLEDGEMENT

I would like to thank professor Tom Hayden for giving me the chance to do this research in his lab and Dr. James Carolan for helping me in analysing the data. Furthermore I acknowledge the help of my colleagues at the mammals lab at school of biological and environmental science. Furthermore, I acknowledge the owner of camels, many thanks to Dr. John O'Brian, Sebastien, Alan and Shahin for the review of this article. I also thank the people Abolgasem Bakory, Abdusalam , Alshtate and Hassan Albadree who works with me at field work in Libya.

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