GENOTYPE OF SOME SUDANESE CAMEL (TYPES AND SUBTYPES) USING MICROSATELLITE

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ABSTRACT

Twenty five polymorphic microsatellite out of 50 loci were used to genotype some Sudanese Camel (Camelus drome darius) types and subtypes (Naylawi, Shanapla, Lahawi, Kinani, Rashaydi, Bani- Amir, Bishari Shallagyi, Annafi and Bishari Arririt). The highest number of alleles were 23 in locus CVRL01 and lowest were 2 in YWLL59. The observed heterozygosity (Hobs) were 0.990 and 0.038 for CVRL01 and YWLL09, respectively while the expected heterozygosity (HExp) were 0.907 and 0.105 for locus CVRL01 and YWLL58 respectively and HExp mean was 0.6914. Polymorphic information content (PIC) ranged between 0.896-0.103 in locus CVRL01 and YWLL58 respectively and the PIC mean was 0.6459. The genetic distance ranged between 0.346 – 0.098 for Bishari Shallagyai and Shanabla and between Rashida- Annafi respectively. The don drogram shows that there is a relationship between the genetic makeup and geographical distributions and also between the genetic makeup and phenotypic characteristic.

Key words: Camel, genotype, polymorphic, microsatellite

INTRODUCTION

From about 24.730,730 Camels world (1). The dromedary account about 95%. The Near east, North Africa and the sahel region have about 70% of the worlds dromedary population Somalia and the Sudan together own more than have of this figure (2). Sudan has the second largest camel population in the world, estimated at nearly 4.7 million head (3), distributed as follows, Kordufan State 36.81%, Darfur State 23.70%, Gedaref State 5.18%, Kassala State 13.47%, Red Sea State 7.01%, Blue Nile State 4.48%, Sinnar State 2.45%, Gezzera State 2.59%, White Nile State 0.74%, Northern State 1.03%, River Nile State 2.40%, and Khartoum State 0.14% (4).

The camel has been a very important animal in many countries at arid and semiarid regions, because of its ability to provide milk, meat, and transport under the harsh dry environmental conditions (5). Camel export in some countries began to contribute substantially to national economy. Camel racing practiced in some Arab countries has furnished new and extra dimension in camel industry. In Sudan the ability of the camel to thrive in the arid and semi-arid areas (13^{o}N) western and eastern Sudan made it an important source of livelihood to nomadic people in these parts of the ountry.

Camels in Sudan are owned by tribes that inhabit the dry semi-desert areas, and because of its limited distribution and number, there has been no development in identification of afferent breeds as in case with other types of farm animals. Camels in Sudan and elsewhere are classified as pack (heavy) and riding (light) types according to their function. Recent studies has been made to classify the camels according to their performance like dairy camels, meat camels, dual purpose camels and racing camels (6)& (7).

This study to identify the different Sudanese camel types and subtypes by their DNA profiling using microsatellite technique, to measure the genetic distance between the different camel types and subtypes and to study the relationship between the phenotypic characteristics and the genetic makeup according to the DNA profiling.

Sudanese camel (Camels dromedaries) types and subtypes under study are Naylawi, Shanapla, Lahawi, Kinani, Rashaydi, AnnafiBani- Awir, Bishari Shallagyi and Bishari Arririt.

MATERIAL AND METHODS

Blood samples were collected in 4.5 ml vaccutainers with EDTA from each camel types and subtypes from all over the country, cross types and mutants were avoided. Regions were visited 1-3 times according to the nomad's seasonal movement nine Sudanese types and subtypes were included in this study. Regions of sampling and visits in Sudan were Kurdofan State, Gedarif State, Kassala State, Red sea State, Butana Region, Gazera State and River Nile State.

Isolation and Evaluation of Genomic DNA from Whole Blood:
Genomic DNA Isolated in the Commission of Genetic Engineering and Biotechnology, Ministry of Science and Technology and evaluated by Agarose Gel and UV – Spectrophotometer in the Virology lab, Department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum.

Microsatellite Technique Application:
Fifty Microsatellite loci was chosen for this study from previous research articles, 50markers was screened with 12 samples, the most distinct camel types selected from all samples so as to realize the polymorphic Markers and so as to screen it to all samples, 5 primers *(CMS 121, CMS 13, CMS 15, CMS16 and CMS50*) is already known as a polymorphic in dromedary camels.

PCR amplifications were carried out in 25 µl reactions containing 50 ng DNA, 25 pmol each primer, 1.5 U *Taq* DNA polymerase, 0.2 mM each dNTP, 2.5 µl lOX*Taq* DNA polymerase buffer containing 10mM Tris-HCI (pH 9.0), 1.5 mM $MgCl_2$. The PCR amplification program consisted of an initial denaturation temperature of 95°C for 5 min, then. 29 - 39 cycles (depending on Locus) at 94°C for 45s, 50-62°C for 1min-depending on the primer pair used and 72°C for 1min, final extension was carried out at 72°C for 15 min. The microsatellite bands were observed in 1% agarose gel electrophoresis stained with ethidium bromide to determine the band size and then resolved by 6% urea polyacrylamide gel electrophoresis, stained with silver nitrate (8).

Twenty nine out of 45 markers were polymorphic then 25 polymorphic microsatellite markers chosen for this study fluorescently labelled with either by FAM, NED, HEX or VIC dyes (Table 1).

PCR Components and Program:-
Template DNA 50ng, PCR Buffer 1X, Primers (F & R) 5pmol, Taq 1U, $MgCl_2$ 2.0mM and the Total volume 15µl (AmpliTaq Gold Kit). PCR program performed as initial denaturing at 95°C for 5min, then 35 cycles at 94°C for 45sec, 55°C for 45sec, 72°Cfor 1min, final extension at 72°C for 10min and then finally holding at 4°C.

Microsatellite Loci Understudy:
LCA08, LCA18, LCA19, LCA22, LCA24, LCA30, LCA33, LCA36, LCA54, LCA65, LCA68, LCA70, LCA71, LCA90, VOLP03, VOLP08, VOLP10, VOLP67, VOLP77, YWLL08, YWLL09, YWLL29, YWLL36, YWLL38, YWLL40, YWLL44, YWLL58, YWLL59, LCA56, LCA63, LCA66, CVRL01, CVRL02, CVRL03, CVRL04, CVRL05, CVRL06, CVRL07, CVRL08, VOLP32, LCA05, LCA37, LCA77, YWLL43, YWLL46, CMS13, CMS15, CMS16, CMS50 and CMS121.

Table 1: Polymorphic Microsatellite Locus

	Locus	Primer sequences (5'- 3')	Tag
1	VOLP 03	F: AGACGGTTGGGAAGGTGGTA - R: CGACAGCAAGGCACAGGA	FAM
2	VOLP08	F: CCATTCACCCCATCTCTC - R: TCGCCAGTGACCTTATTTAGA	FAM
3	VOLP10	F: CTTTCTCCTTTCCTCCCTACT - R: CGTCCACTTCCTTCATTTC	HEX
4	VOLP 67	F: TTAGAGGGTCTATCCAGTTTC – R: TGGACCTAAAAGAGTGGAG	HEX
5	YWLL 08	F: ATCAAGTTTGAGGTGCTTTCC – R: CCATGGCATTGTGTTGAAGAC	FAM
6	YWLL09	F: AAGTCTAGGAACCGGAATGC – R: AGTCAATCTACACTCCTTGC	FAM
7	YWLL38	F: GGCCTAAATCCTACTAGAC – R: CCTCTCACTCTTGTTCTCCTC	HEX
8	YWLL58	F: GGCATCTCTTCCTCATCAAT – R: GACATCTCCAACTTGGAATC	FAM
9	YWLL59	F: TGTGCAGGAGTTAGGTGTA – R: CCATGTCTCTGAAGCTCTGGA	FAM
10	LCA18	F:TCCACCCATTTAGACACAAGC– R:TAGGAAGCTCCAAGAAGAAAAGAC	FAM
11	LCA 22	F: TTAAGAGTCTAAAAGAGAAAGGCG – R:CAGATGACAGCTGGGATTGA	FAM
12	LCA33	F:GAGCACAGGGAAGGATATTCA– R:ACAGCAAAGTGATTCCATAATACA	NED
13	LCA 63	F: TTACCCAGTCCTTCGTGGG – R: GGAACCTCGTGGTTATGGAA	NED
14	LCA 66	F: GTGCAGCGTCCAAATAGTCA – R: CCAGCATCGTCCAGTATTCA	FAM
15	LCA90	F:TATAACCCTGGTCTCGCCAA – R: CCAAGTAGTATTCCATTATGCG	FAM
16	CVRL01	F: GAAGAGGTTGGGGCACTAC – R: CAGGCAGATATCCATTGAA	VIC
17	CVRL03	F: ATCTCCTCCCTCCCCAAAAT – R: AACCAACAGCATGCCTCAATA	HEX
18	CVRL04	F: CCCTACCTCTGGACTTTG – R: CCTTTTTGGGTATTTTCAG	FAM
19	CVRL05	F: CCTTGGACCTCCTTGCTCTG – R: GCCACTGGTCCCTGTCATT	NED
20	CVRL07	F:AATACCCTAGTTGAAGCTCTGTCCT–R: AGTGCCTTTATAAATATGGGTCTG	FAM
21	CMS13	F: TAGCCTGACTCTATCCATTTCTC – R: ATTATTTGGAATTCAACTGTAAGG	NED
22	CMS15	F: AAATACTTAAAGGTTCCCAGA – R: TTGTAAACTAAAGCCAGAAAG	FAM
23	CMS16	F: ATTTTGCAATTTGTTCGTTCTTTC – R: GGAGTTTATTTGCTTCCAACACTT	NED
24	CMS50	F: TTTATAGTCAGAGAGAGTGCTG – R: TGTAGGGTTCATTGTAACA	NED
25	CMS121	F: CAAGAGAACTGGTGAGGATTTTC – R: AGTTGATAAAAATACAGCTGGAAAG	NED

Multiplex Markers:
Gp1: YWLL08, YWLL38, LCA18 and CVRL04. **Gp2:** VOLP08, YWLL58, LCA66, CVRL01 and CVRL05. **Gp3:** VOLP03, CVRL03. **Gp4:** YWLL59, LCA90, YWLL09, CMS121, VOLP67 and CMS13. **Gp5:** CMS15, CVRL07, LCA33, VOLP10 and CMS50. **Gp6:** LCA22 and LCA63. **Gp7:** CMS16.

Data Analysis and Calculations:
Microsoft office Excel 2010 used for arranging and sorting up the data to makeup the input file for Gene Class 2.0.h (2005) and Genepop 4.0.11 software (2010), then the Genepop 4.0 input file converted to Cervus 3.0.3 (9) input file. Gene Class software applied to calculate the genetic distance between populations (10), individual assignment using Frequencies – based method (11) and Bayesian methods (12) (13). Gene Class also used to calculate the allele frequency and then the allele frequency results used in input file for PHLIP 3.69 software using the UPGMA method of clustering by DRAWGRAM program to obtain the phylogenetic tree (Dondrogram).

RESULTS AND DISCUSSION

The number of alleles that were obtained from 25 polymorphic microsatellite loci ranged between 23 in locus CVRL01 and 2 alleles in locus YWLL59, in all populations the mean number of alleles per locus is 8.640. The observed Heterozygosity (Hobs) ranged between 0.038-0.990 between YWLL09 and CVRL01 respectively, while the expected. Heterozygosity (HEXP) ranged between 0.105-0.907 in locus YWLL58 and CVRL01 respectively and mean expected heterozygosity 0.6914. The microsatellite statistics was obtained by using Gene class 2 software. Polymorphic information content (PIC) ranged between 0.896-0.103 in locus CVRL01 and locus YWLL58 and mean polymorphic information content (PIC) was 0.6459 (Table 2).

This study depended on location and phenotypic characteristics that classified types and subtypes with known names that related to location, tribal ownership or a distinguished characteristics, same like the studies that were done by (14) when he used 23 microsatellite loci to genotype the four Indian camel breeds Bikaneri, Jaisalweri, Kutchi, and Mewari, and (15) also for Indian camel populations and by (16) for Kenyan dromedary breeds. Sudan hold very rich genetic variations between camel types and even between subtypes but with small differences.

The Genetic Distance Calculated according to (10) by using Gene class software (INRA/ CIRAD). Gene class 2.0.h-2-Aug- 2005), (Table 3) between 9 populations of Sudanese camel types and subtypes. The Genetic distance was found 0.346 and 0.098 between Shallagyai (Bishari subtype)-Shanapla type and between Annafi- Rashyda type respectively (Table 3).

Phylip 3.6 was utilized for phylogentic tree (Dendrogram) using the UPGMA method of clustering by DRAWGRAM program of PHY LLIP package. The dendrogram demonstrated that there's a relation between the geographical distribution or phenotypic characteristics and genetic makeup depending on this 25 microsatellite markers (Figure 1). We found that Rashyda and Annafi type more closely, although they are different in their phenotypes, both found in one cluster with Lahawi, Kinani and Bani- Aamir camel types, all of them found in the Great Butana Area (GBA) and Kassala State.

In other cluster we found Shanapla, (Kordofan State) and Naylawi (Darfur State) in on cluster same like their state that accommodate them and according to the tree Shanapla more closely to GBA and Kassala State types than Naylawi type.

The Dendrogram for Sudanese types and subtype showed that Rashayda and Annafi comes closer and also Kinani, BaniAamir and Lahawi in one cluster but Kinani and BaniAamir more closer in this cluster.

We found also Shanapla and Nylawi in a cluster while Shallagyai comes closer to arririt in different cluster when companed with other Sudanese types and subtypes. The Dendrogram shows that the North East camels Shallagyi and Arririt (Bishari subtypes) comes in one cluster and all types and subtypes included in this study from Kurdofan (Shanapla), Darfur (Naylawi), Great Butant Area (GBA) (Kinani, BaniAamir, Lahawi, Rashydi and Annafi) in other cluster.

(17) in Saudi Arabia they used sixteen microsatellite markers to investigate the genetic polymorphism in Majaheem population of camels in the kingdom of Saudia Arabia. Genemic DNA was extracted from hair roots of 40 unrelated Majaheem camels. Out of these 16 markers, only microsatellite VOLP67 did not produce 3 any PCR amplification from all individuals studied. The result showed that 102 allele were generated by the 15 microsatellites loci with a mean of 6.8 and a range of 3to 14 alleles per locus. The mean expected it Helerozygosity (He) was (0.652) ranged from 0.422 to 0.807 and the mean observed Heterozygosity (Ho) was 0.665 with a range of 0.275 to 0.900. The polymorphic

information content (PIC) values ranged from 0.340 to 0.768 with a mean of 0.590.

(18) investigated 16 microsatellite loci for studing the genetic polymorphism in Bikaneri (India camels), the results indicated existence of enough genetic variation among dromedary individuals and the potential use of microsatellite markers for further genetic diversity analysis and production enhancement. He showed that the genetic distance between the three Indian breeds was estimated using the alleles frequency at 15 polymerphic microsatellite loci in the three breeds using PHYLIP 3.6 software. The consensus arrived from observed data indicated close phylogenetic relationship between the dromedary breeds.

Table 2: Shows the No. of Alleles (k) Alleles size range bp, Heterozygosity observed (Hobs), Expected Heterozygosity (HEXP) and Polumorphic Information Content (PIC)

Locus	k	Size bp	HObs	HExp	PIC
CVRL04	9	158-188	0.774	0.718	0.666
CVRL05	11	157-185	0.953	0.803	0.772
LCA18	3	222-234	0.538	0.607	0.536
VOLP03	8	144-176	0.660	0.786	0.748
YWLL38	4	180-188	0.456	0.608	0.527
CVRL01	23	188-240	0.990	0.907	0.896
LCA66	7	224-242	0.829	0.785	0.747
YWLL08	10	130-162	0.638	0.752	0.716
CVRL03	6	157-207	0.461	0.454	0.390
VOLP08	8	131-153	0.971	0.772	0.737
YWLL58	6	156-174	0.040	0.105	0.103
CMS121	9	148-166	0.623	0.728	0.675
CMS13	8	121-252	0.632	0.754	0.718
CMS15	6	121-144	0.864	0.740	0.700
CMS50	7	170-188	0.539	0.834	0.806
CVRL07	9	270-300	0.129	0.657	0.620
LCA22	3	170-180	0.794	0.622	0.544
LCA33	12	110-140	0.952	0.750	0.714
LCA63	13	198-232	0.717	0.833	0.808
LCA90	8	222-244	0.509	0.648	0.576
VOLP10	9	236-264	0.600	0.783	0.753
VOLP67	18	145-213	0.906	0.898	0.884
YWLL09	5	156-166	0.038	0.529	0.460
YWLL59	2	105-107	0.448	0.499	0.374
CMS16	12	172-214	0.594	0.713	0.677

Table 3: Genetic Distance between the Sudanese populations (9 camel types and subtypes).

	015 PopNailawi	005 PopShanabla	014 PopALahawi	014 PopKinani	015 PopBaniAamir	015 PopRashaida	015 PopAnnafi	004 PopShalagyai	009 PopAririt
Assigned sample	distance	distance	distance	distance	distance	distance	distance	distance	distance
015 PopNailawi	0.000	0.148	0.128	0.149	0.139	0.226	0.229	0.285	0.264
005 PopShanabla	0.148	0.000	0.166	0.162	0.164	0.244	0.186	0.346	0.243
014 PopALahawi	0.128	0.166	0.000	0.165	0.130	0.128	0.149	0.273	0.227
014 PopKinani	0.149	0.162	0.165	0.000	0.131	0.167	0.162	0.257	0.194
015 PopBaniAamir	0.139	0.164	0.130	0.131	0.000	0.124	0.110	0.204	0.177
015 PopRashaida	0.226	0.244	0.128	0.167	0.124	0.000	0.098	0.210	0.183
015 PopAnnafi	0.229	0.186	0.149	0.162	0.110	0.098	0.000	0.212	0.166
004 PopShalagyai	0.285	0.346	0.273	0.257	0.204	0.210	0.212	0.000	0.194
009 PopAririt	0.264	0.243	0.227	0.194	0.177	0.183	0.166	0.194	0.000

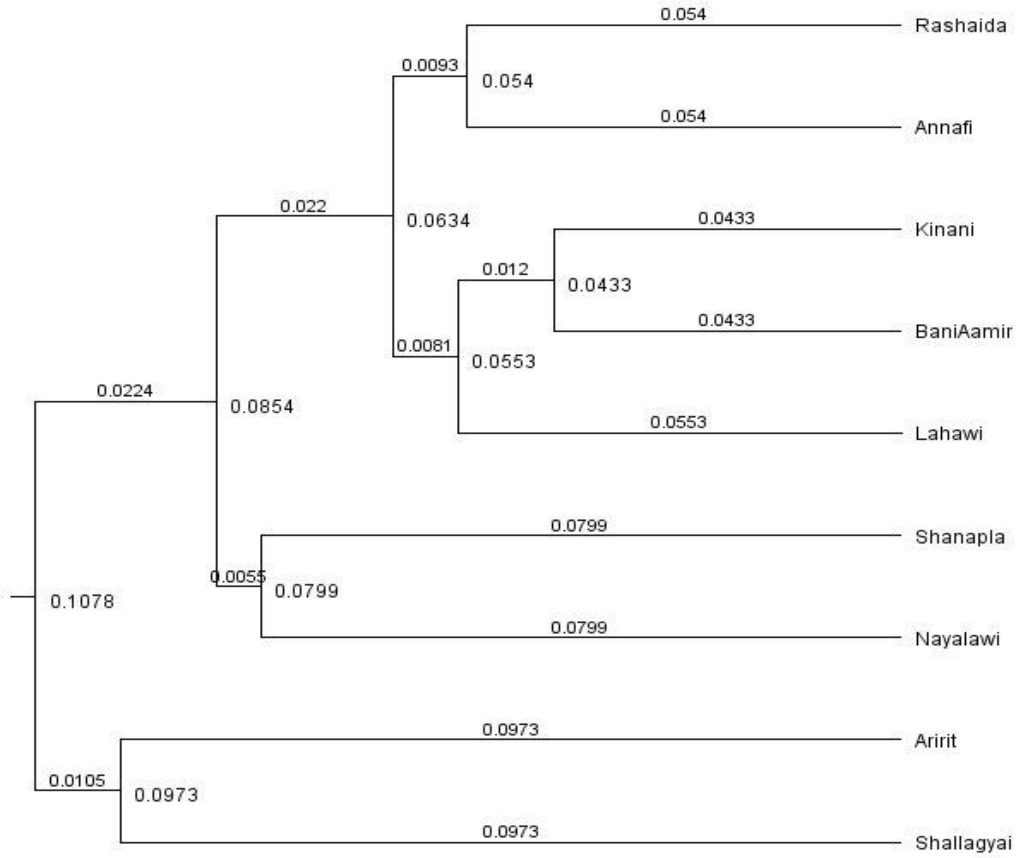

Figure 1: Showing rooted genetic relationship tree between 9 Sudanese types and subtypes with the tip, node, branch labels and scale bar.

RECOMMENDATION

- The available pedigreed populations of the camel farms can be utilized for linkage analysis.
- Camel Research Institutions should have a separate camel farm for pure types, subtypes and also for famous hybrid camels that takes its own line of breeding.
- Collaboration must be between camel research scientists all over the world.

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التصنيف الوراثي الجزيئي لبعض أنواع وتحت أنواع الإبل السودانية باستخدام تقنية المايكروستالايت

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تم إستخدام خمسة وعشرون مواضع مصغرة متعددة الأشكال من مجمل خمسين موضعاً للتصنيف بناءً على التركيب الوراثي لبعض أنواع وتحت أنواع الإبل وحيدة السنام في السودان (النيالاوي، الشنابلة ، اللحوي ، الكناني ، الرشايدي ، البني عامر، العنافي ، البشاري الشالاقاي والبشاري الأريري). أعلى عدد للأليلات كان ٢٣ مع البادئ (CVRL01) وأقل عدد كان ٢ أليل مع البادئ YWLL59، وأيضاً تغاير الزايجوت (Hobs) تراوح مابين ٠.٩٩٠ و ٠.٠٣٨ مع البادئين CVRL01 و YWLL09على التوالي في حين تغاير الزايجوت المتوقع مابين ٠.٩٠٧ و ٠.١٠٥ مع CVRL01 و YWLL58 على التوالي وقد كان متوسط تغاير الزايجوت المتوقع ٠.٦٩١٤. محتوى المعلومات المتعدد الأشكال (PIC) تراوح ما بين ٠.٨٩٦ – ٠.١٠٣ في البادئين CVRL01 و YWLL58على التوالي ، ومتوسط ٠.٦٤٥٩ ، وتراوحت المسافة الوراثية بين ٠.٣٤٦ – ٠.٠٩٨ لكل من البشاري شالاقاي والشنابلة وبين الرشايدي والعنافي على التوالي ، في حين الـ Dondrogram دل على وجود علاقة بين التركيبة الجينية والتوزيعات الجغرافية وكذلك بين التركيبة الجينية والصفات الظاهرية.