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Assessment of the Genetic Diversity, Breeds Structure and Genetic Relationships in Four Egyptian Camel Breeds using Microsatellite and Start Codon Targeted (SCoT) Markers

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Abstract

The genetic diversity, relationships and population structure of sixty Egyptian camels derived from four breeds (Baladi, Sudani, Somali, and Maghrabi) were investigated using 18 microsatellite (SSRs) loci. In addition, the four breeds were genotyped using 16 Start Codon Targeted (SCoT) primers. A total of 346 SSR alleles were detected across the four camel breeds with an overall mean of 9.3 ± 0.66 alleles / locus. The mean number of alleles (MNA) and effective number of alleles (Ne) ranged from 9.2 ± 1.45 in the Baladi to 9.5 ± 1.27 in the Maghrabi breeds and from 6.5 ± 0.82 in the Maghrabi to 7.1 ± 0.93 in the Somali breeds, respectively. The values of observed heterozygosity (HObs) and expected heterozygosity (HExp) per breed varied from 0.82 ± 0.07 in the Maghrabi to 0.87 ± 0.07 in the Sudani camel breeds, and from 0.75 ± 0.03 in the Sudani to 0.79 ± 0.03 in the Maghrabi breeds , respectively. The genetic diversity estimated as the Shannon's information index (I) revealed the highest value (1.88 ± 0.14) in the Maghrabi and the lowest value (1.78 ± 0.18) in the Sudani breed. The values for fixation indices (FIS, FST and FIT) were -0.07284, 0.12364 and 0.05981, respectively. Thus indicating a moderate level of differentiation among the four breeds and a random mating process within each breed. The genetic structure revealed that the three breeds (Baladi, Sudani and Maghrabi) were genetically distinct and look like pure breeds, while the Somali breed showed some degree of admixture. A total of 153 amplicons were generated by the 16 SCoT primers, with an average of 9.56 amplicon/ primer and a polymorphism rate of 49%. The phylogenetic tree based on microsatellite and SCoT markers revealed that Maghrabi was separated in one cluster while, the second cluster comprised two sub-clusters. Sudani and Somali formed one sub-cluster and Baladi was in the second sub-cluster. Thus, the closest phylogenetic relationship was between the Sudani and Somali breeds.

Keywords:

Genetic diversity; Population structure; Phylogenetic tree; Animal Genetic Resources (AnGR); Microsatellite; Start Codon Targeted (SCoT)

Introduction

The dromedary camel (*Camelus dromedarius*) is the most common of all camelus species and is easily distinguished from its congeners. Dromedaries are widespread throughout northern and eastern Africa, the Arabian Peninsula and southwest Asia, and a large feral population exists in Australia. Throughout their range, dromedaries are bred for a multitude of purposes, including meat, milk production, transportation, wool and sport [1].

Camel has unique physiological characteristics that enable it to adapt its desert environments, such as fluctuation of its body temperature, tolerance of water loss and capability of drinking more water in less time [2-4].

The total global number of camel population is 24.7 million heads and the largest population has been found in Somalia (7 million). Ninety-seven different breeds are currently listed on FAO DAD-IS database [3]. In Egypt, camels are economically important as they are considered dual purpose animals.

In the Nile Valley and Delta, they are mainly raised for meat production and for some agricultural labor. In the desert, they are raised equally for meat and milk productions, while some for labor and transport, and some are especially for camel racing. The main camel breeds reared in Egypt are Maghrabi, Falahy or Baladi, Sudani, Somali and Mowaled (hybrid between Maghrabi and Falahi) [5-7].

According to the FAO, the analysis of data from 182 countries by the Global Databank for farm animal genetic resources (FAnGR) revealed that 8% of all farm animals (local, regional trans-boundary and international trans-boundary) breeds could already be considered extinct, 22% are at varying degrees of extinction risk and 34% are of unknown risk status [6].

As a result, the loss of farm animal genetic diversity will reduce the range of opportunities available to confront the challenges of unpredictable future events, such as climate change, social society change, disease epidemics, selective failures and unexpected

catastrophic events and a final consequence of erosion of animal genetic resource [8-12].

Genetic characterization is the primary step in developing genetic resources protection strategies and prevention of animal germplasm erosion. Moreover, the assessment of genetic diversity both within and among breeds/population is the most important strategy for management of animal biodiversity, especially in identifying genetically unique structure [13-16].

Also, molecular marker studies for population/breeds are an important tool for biodiversity conservation, as they could unravel the genetic diversity that constitutes a repertoire of genes for the development of sustainable animal production [17-26].

Many different types of DNA molecular markers had been explored to evaluate the genetic diversity of animal genetic resources, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), single-strand conformation polymorphism (SSCP) and microsatellite DNA. In addition, with biotechnological and computer innovations, novel strategies such as whole genome SNP chips and DNA Barcoding have emerged.

However, for the genetic characterization of livestock, microsatellite markers have shown clear advantages over the other markers [15].

In camels microsatellite has been the first option for genetic diversity studies performed in different countries, e.g., India, Saudi Arabia, South Africa, Tunisia, Canary Islands, Kenya, Egypt, Australia and Sudan [27-30].

In recent years, due to the tremendous growth in public biological databases, the development of functional markers that are located in or near the candidate genes has become considerable easy. Start Codon Targeted (SCoT) polymorphism has been developed by Collard and Mackill as a novel functional marker system based on the short conserved region flanking the ATG start codon.

SCoT markers are generally reproducible, dominant markers and could be used for genetic diversity studies, quantitative trait loci (QTLs) mapping and bulk segregation analysis [31-34].

Therefore, this study was planned to investigate the genetic diversity, breed structure and relationships among four Egyptian camel breeds at the molecular level using two types of molecular markers, i.e., microsatellites and SCoT. The two marker types were chosen to target different parts of the genome, thus ensuring high genome coverage.

The microsatellite markers are targeting repetitive sequences, while the SCoT markers were employed to target the polymorphism in sequences near the genes [35-40].

Materials and Methods

DNA extraction

Sixty blood samples representing four camel breeds (Baladi, Sudani, Somali, and Maghrabi) were collected from 3 different regions in Egypt (Giza, Halaieb and Matrouh). Fifteen samples were taken from each breed. Whole blood samples were collected from Jugular vein in a tube containing 0.5 ml EDTA (0.5 M) as a coagulant reagent. The DNA was extracted using the Qiagen Blood and Tissue Kit (cat No. 69506) according to its manual instructions with minor modifications.

Microsatellite analysis

The PCR reaction was carried out in a total volume of 20 µl using 18 SSR markers. The thirteen microsatellite markers were chosen according to the recommendation of International Society of Animal Genetics (ISAG). In addition, 5 markers (Cd00824, Cd00829. Cd00833, Cd00852 and Cd00855) were selected from the microsatellite markers. These primers were synthesized by Eurofins Genomics (Munich, Germany).

The thermocycling profile was included: a primary denaturation at 94°C for 4 min for 1 cycle, then, 35 cycles as follows : 95°C for 60 sec, 55-65°C for 45-60 sec, and 72°C for 30 sec. A final extension step was at 72°C for 10 min., then the reaction was stored at 4°C. The PCR products were separated on 10% polyacrylamide gels, (Serva, Germany) according to the methodology described with some modifications [41-43].

SCoT analysis

SCoT analysis was conducted on the bulked genomic DNA representing the four camel breeds, generated by mixing equal concentration of the DNA from the fifteen individuals of each breed. Twenty one SCoT primers were initially used to screen the polymorphism among the four camel breeds. Only sixteen primers revealed discernible patterns. The nucleotide sequence of the sixteen primers.

The PCR reaction and nucleotide sequence of the primers were adopted. The total reaction volume was 25 µl containing 1X reaction buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.4 µM of a single primer, 50 mg bulked genomic DNA and 2U of Taq DNA polymerase (Qiagen Ltd., Germany).

The PCR temperature profile was as follows: an initial denaturation step at 94°C for 3 min followed by 36 cycles of a denaturation step at 94°C for 50 sec, an annealing step at 50°C for 1 min and an extension step at 72°C for 2 min. The final extension step was at 72°C for 5 min. The amplified products were resolved by electrophoresis in a 1.5% agarose gel in 1 x TBE buffer, containing ethidium bromide (0.5 mg/mL) and visualized under UV light [41-45].

Data analysis and statistical calculations

Gels were visualized and scored with the Alpha imager 2200 software Version 4.0.1. All scored microsatellite data were first adjusted using a Tandem Repeat Analyzer software package to estimate each allele size according to its number of repeats for each marker. Then, a spreadsheet program (Microsoft Excel) was used to arrange the data for each breed regarding each locus.

After data conversion using convert program the data were analyzed to estimate the allele frequencies, total number of alleles (TNA), mean observed heterozygosity (HObs) and expected heterozygosity (HExp) and mean polymorphism information content (PIC) per locus and breed using the Cervus version 3.03 software.

The allelic pattern showing a number of alleles at various frequencies and the the Shannon's information index were generated using the GENALEX version 6.4 software. The hierarchical analysis of molecular variance (AMOVA) was performed using the Arlequin software version 3.5 (Excoffier and Lischer 2010). The three dimension of a multivariate factorial correspondence analysis (FCA) were computed using the GENETIX version 4.05 [45-48].

The genetic structure and degree of admixture of the four Egyptian breeds were investigated using the Bayesian clustering procedure of the software structure version 2.3. The structure harvester was employed to identify the most probable groups (K) that best fit the data. POPGENE software package version 1.31 was used to compute the genetic distance using the Nei index.

The banding patterns generated by SCoT markers were compared to determine the genetic relationship of the four Egyptian camel breeds. Clear and distinct amplification products were scored as (1) for present and (0) for absent bands for all samples. Bands of the same mobility were scored as identical.

The unweighted Pair-group method with arithmetic average (UPGMA) was employed to measure the genetic similarity and construct a phylogenetic tree (dendrogram) using the Non-Linear Dynamics software [49-54].

Results and Discussion

Genetic diversity among and within the four Egyptian camel breeds as detected by microsatellite markers analysis

Microsatellites are considered the markers of choice for livestock genetic characterization. All the microsatellite primer pairs were successful to generate discernible amplicons.

The mean number of alleles (MNA), the expected (HExp) and observed (HObs) heterozygosity, the polymorphism information content (PIC) and the deviation from the Hardy-Weinberg equilibrium for the 18 loci are presented. The eighteen microsatellite loci generated a total of 346 alleles across the four Egyptian camel breeds.

The mean number of alleles per locus ranged from 2 in VOLP32 to 20.8 in CVRL01 with an overall mean of 9.3 ± 0.66 alleles per locus. This could be an indicator of high genetic variation. The mean number of alleles detected in the present study is higher than that reported in previous studies for different camel populations such as Saudi camel populations and Indian camel breeds.

However, the mean number of alleles detected in Australian dromedary camel was 10.3 ± 0.9 revealed 18.8 alleles per locus in Indian camel population (Figures 1-7).

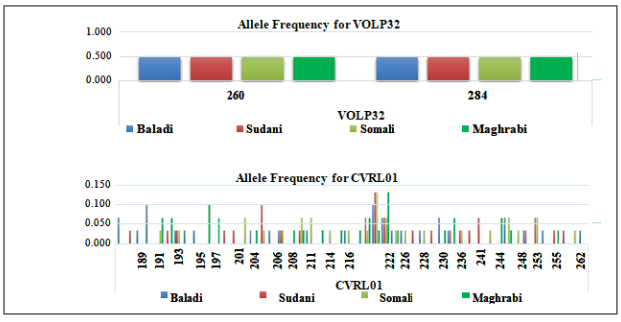

Figure 1: Allele frequency distributions for VOLP32 and CVRL01 micro satellite markers in the four Egyptian camel breeds. The horizontal axes denote allele length and vertical axes show the frequencies found for each allele in the breed samples.

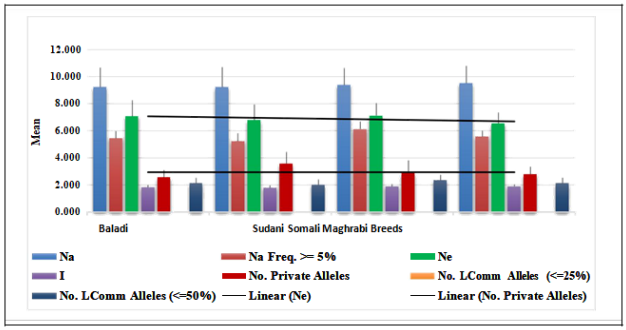

Figure 2: Mean of allelic patterns for the four Egyptian camel breeds. Number of alleles (Na), Effective number of alleles (Ne), Number of private alleles (NPV), Shannon information index (I), Number of common alleles.

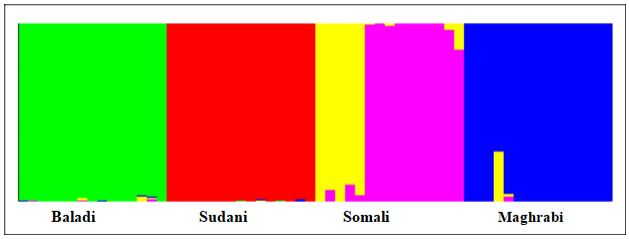

Figure 3: Population Structure of the analyzed four Egyptian camel breeds without prior population affiliation using a model based clustering method implemented in structure software. The graph is showing the estimated population structures of the breeds at K=5. Three of the breeds (Baladi, Sudani and Maghrabi) were genetically distinct with lower degree of admixture (looks pure breed). While, Somali breed showed some degree of admixture.

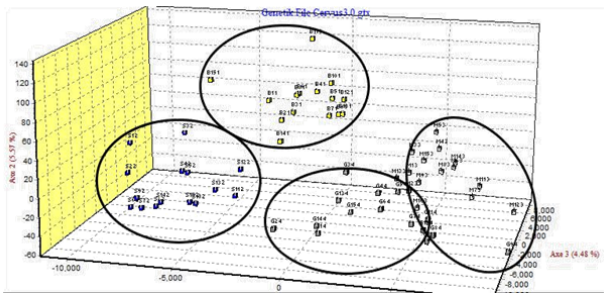

Figure 4: Factorial Correspondence Analysis of individual camel microsatellite genotypes calculated using GENETIX software.

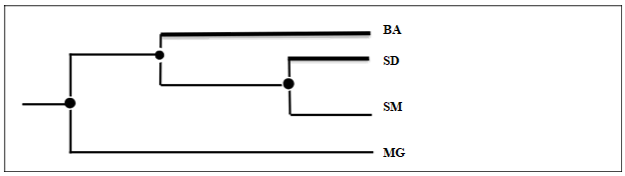

Figure 5: Phylogenetic tree showing the genetic relationships among the four Egyptian camel breeds, using Nei's (1972) genetic distance on the basis of allele frequencies from the 18 microsatellite markers.

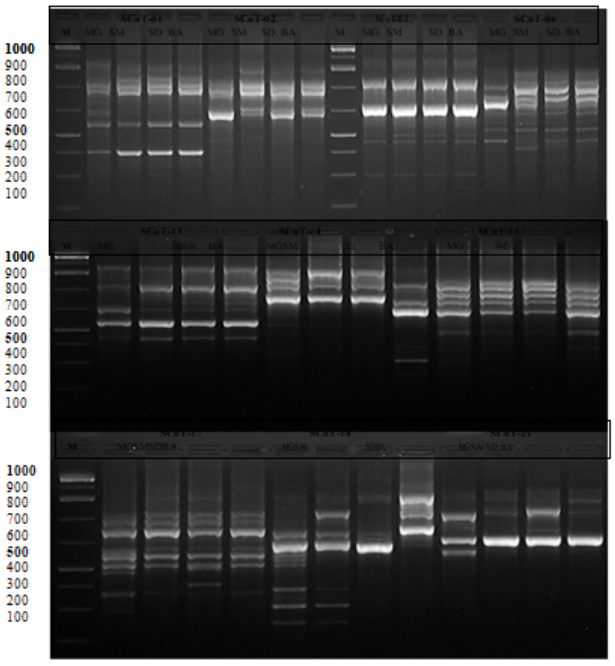

Figure 6: SCoT banding profiles generated from the amplification of the bulked genomic DNA of the four Egyptian camel breeds (Maghrabi, MG; Somali, SM; Sudani, SD and Baladi, BA) using 12 different SCoT primers.

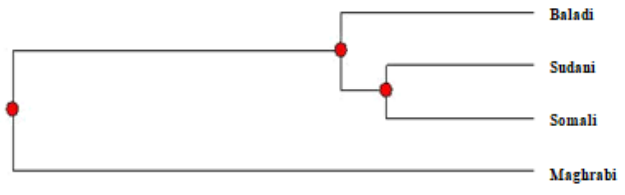

Figure 7: Dendrogram based on genetic similarity computed from the SCoT data using the UPGMA algorithm for the four Egyptian camel breeds.

The Expected frequency of heterozygosity (HExp) and observed frequency of heterozygosity (HObs) are important parameters in representing the genetic diversity. The observed heterozygosity represents the direct count of heterozygotes in the population. While, the expected heterozygosity is estimated based on the allele frequency of the sampled individuals given that the population is in Hardy-Weinberg Equilibrium (HWE).

The relationship between HObs and HExp is very important in determining the breeding system. When HObs=HExp, the population are more likely to be in random mating. When, HObs<HExp the mode of mating system in a population is said to inbreeding and when HExp<HObs, the population may not be under inbreeding.

In the present investigation, the expected heterozygosity for the 18 loci ranged from 0.500 at VOLP32 to 0.939 at CVRL01 with a mean of 0.76 ± 0.022. In turn, the observed heterozygosity varied from 0.767 at CVRL05 to 1.000 at (LCA66, VOLP03, VOLP08, VOLP32, YWLL08, Cd00824, Cd00829, Cd00833, Cd00852 and Cd00855) with a mean of 0.846 ± 0.03. In this respect, different estimates for the HObs and HExp have been reported in camels.

The expected heterozygosity in Jaisalmeri camel reported by Gautam et al. (2004) was lower than the present findings and ranged from 0.32 to 0.651. Spencer and Woolnough (2010) recorded an expected heterozygosity ranging from 0.29 to 0.900 with a mean of 0.544 ± 0.069 in Australian camel population.

Also, studies reported that the mean observed heterozygosity of Indian Malvi camel was 0.604 ± 0.325, while, the mean expected heterozygosity was 0.597 ± 0.198. In addition, the expected heterozygosity estimate among three different regions of Tunisian dromedary camel ranged from 0.76 to 0.84.

Moreover, it is found that the expected heterozygosity for the two major ecotypes (Butana and Darfur) of Sudan was 0.72 ± 0.04. The results of the present study revealed that the overall mean of the observed heterozygosity was slightly higher than the expected heterozygosity (0.846 vs 0.760) for the four Egyptian camel breeds. This could suggest that the individuals within each of these breeds are more likely to be in random mating and may not be under inbreeding.

The mean polymorphism information content (PIC) is another important measure of DNA polymorphism. Besides being a measure of genetic variation, it is also used in the context of gene mapping. In addition, the PIC is a measure of the informativeness of the marker, ranging from 0 to 1 and loci with PIC value close to 1 with many alleles are desirable for genetic diversity studies.

Generally, the marker showing PIC value lower than 0.5, implies a locus moderately informative (0.5>PIC>0.25) and the rest of them were highly informative (PIC>0.5). As shown, our results revealed that all the microsatellite loci exhibited a PIC value higher than 0.5 (ranging from 0.511 to 0.905) except VOLP32 which showed a PIC of 0.375. The mean value of PIC for all loci was 0.777 ± 0.03.

Therefore, all the microsatellite loci included in the present study were highly informative (PIC>0.5) except VOLP32 locus. Thus, these microsatellite markers are suitable for the assessment of genetic diversity in Egyptian camels. Moreover, the present study indicated that the PIC values showed a positive correlation with the expected heterozygosity and the mean number of alleles at each locus.

The latter could be clearly deduced from the histograms in Figure 1 illustrating the frequencies of the different alleles for VOLP32

(exhibiting the lowest PIC) and CVRL01 (exhibiting the higher PIC). Similarly, the PIC values reported in New World Camelids were relatively high due to the high number of alleles at each locus.

The Hardy-Weinberg Equilibrium (HWE) is a supplementing parameter to investigate the population variation. The Hardy-Weinberg law defines a direct relationship between allele and genotypic proportions in a population.

This law states that for all loci except for sex chromosomes in diploid organisms, HWE is attained from one generation to the next with the underlying conditions that the population in not under any gene force (non- random mating, selection for genotype, mutations and migration), infinite population size and equal fertility of parent genotypes.

Our results showed that 55.6 % of all microsatellite loci were significantly deviated ($p<0.05$) from Hardy-Weinberg equilibrium. Thus, providing additional evidence for random mating within each breed.

Within each breed, the different microsatellite loci revealed variable levels of genetic diversity. As shown, in the Baladi breed, the total number of alleles (Na) and effective number of alleles (Ne) per locus ranged from 1 (Cd00824) to 22 (CVRL01) and from 1 (Cd00824) to 18.0 (CVRL01), respectively.

The observed heterozygosity (HObs) and expected heterozygosity (HExp) ranged from 0.00 (Cd00824 and Cd00852) to 1.00 (CMS121, CVRL06, LCA66, VOLP03, VOLP08, VOLP32, YWLL08, YWLL38, YWLL44, Cd00829, Cd00833 and Cd00855), and 0.00 (Cd00824) to 0.94 (CVRL01), respectively.

The Shannon information index (I) in Baladi breed ranged from 0.0 (Cd00824) to 2.9 (CVRL01). While, in the Sudani breed the total number of alleles (Na) and effective number of alleles (Ne) per locus ranged from 2 (CVRL06, VOLP08 and VOLP32) to 21 (CVRL01) and ranged from 2 (CVRL06, VOLP08 and VOLP32) to 16.0 (CVRL01), respectively.

The observed heterozygosity (HObs) and expected heterozygosity (HExp) ranged from 0.00 to 1.00 and from 0.50 to 0.93, respectively. The Shannon information index (I) ranged from 0.6 to 2.9. In the Somali breed, the total number of alleles (Na) and effective number of alleles (Ne) per locus ranged from 1 to 21 and from 1 to 16.6. The observed heterozygosity (HObs) and expected heterozygosity (HExp) ranged from 0.00 to 1.00 and from 0.5 to 0.94.

The Shannon information index (I) ranged from 0.00 to 2.9. In the Maghrabi breed the total number of alleles (Na) and effective number of alleles (Ne) per locus ranged from 2 to 19 and from 2 to 15.0. The observed heterozygosity (HObs) and expected heterozygosity (HExp) ranged from 0.00 to 1.00 and from 0. 5 to 0.93, respectively, and the Shannon information index (I) ranged from 0.6 to 2.8.

Across the four camel breeds, the mean number of alleles (MNA) and effective number of alleles (Ne) ranged from 9.2 ± 1.45 for the Baladi to 9.5 ± 1.27 for the Maghrabi breed and from 6.5 ± 0.82 for the Maghrabi to 7.1 ± 0.93 for the Somali breeds, respectively (Figure 2). The present value of MNA is greater than that reported among five Egyptian camel breeds using 3 microsatellite markers. This lower MNA than our results may be attributed to the lower number of loci employed in their study.

The values of observed and expected heterozygosity per breed varied from 0.82 ± 0.07 for the Maghrabi to 0.87 ± 0.07 for the Sudani camel breeds, and from 0.75 ± 0.03 for the Sudani to 0.79 ± 0.03 for the Maghrabi breeds, respectively. The results revealed that the Maghrabi breed had the highest value of the mean number of alleles, and as a consequence, relatively higher expected heterozygosity (0.79 ± 0.03) than the other breeds. This was followed by Somali, Baladi and then Sudani, with a mean value of HExp of 0.77 ± 0.05, 0.76 ± 0.05 and 0.75 ± 0.03, respectively.

Our results also showed a considerable higher level of heterozygosity than the heterozygosity reported in Kenyan camel, Indian camels, Tunisian camels, Chinese, Mongolian camels, Saudi Arabian camels and Sudanese camels. In addition, the observed heterozygosity values were higher than those of expected heterozygosity for each breed.

These values were 0.85 ± 0.07 vs 0.76 ± 0.05, 0.87 ± 0.07 vs 0.75 ± 0.03, 0.84 ± 0.07 vs 0.77 ± 0.05 and 0.82 ± 0.07 vs 0.79 ± 0.07 for Baladi, Sudani, Somali and Maghrabi, respectively. This indicates that the four Egyptian camel breeds did not exhibit inbreeding mating, but the mating within each breed has been rather randomly performed.

The values of genetic diversity for each breed as estimated by the mean of Shannon's information index (I) revealed that the highest value (1.88 ± 0.14) was in the Maghrabi breed and the lowest value (1.78 ± 0.18) in the Sudani breed. Also a positive correlation between Shannon's information index and the mean number of alleles (MNA) and expected heterozygosity (HExp) was observed. A similar correlation was reported.

However, the present data revealed that the values of genetic diversity for the four Egyptian camel breeds (Baladi, Sudani, Somali and Maghrabi breeds) were higher than those reported on the same breeds (1.80, 1.78, 1.87, 1.88 vs 1.48, 1.37, 1.44, 1.38 respectively).

Molecular variation and genetic divergence of the breeds based on the microsatellite marker analysis

Analyses of the molecular variance were performed to examine the partitioning of genetic variation and differentiation among and within the four Egyptian camel breeds. The molecular variance (AMOVA) analysis was carried out on three datasets according to studies using the program Arlequin. The first dataset consisted of the Egyptian camels grouped as the four breed types (among breeds).

The second analysis included data for the source of variation among individuals within the breed. While, the third analysis was performed to evaluate the genetic variations among individuals across all the breeds sampled (within individuals).

The results revealed that the highest (94%) molecular genetic variance was observed within individuals, followed by among breeds (12.4%) and was lowest (-6.3%) among individuals within breeds.

Therefore, the majority of the genetic variation was present within individuals across all the breeds sampled, which suggested that the heterogeneity was high within individuals. This is an unsurprising finding since this value represents the variation among the 60 camel individuals belonging to the four breeds.

To determine the genetic divergence or differentiation among the four Egyptian camel breeds based on microsatellite DNA variation, Fixation indices (FIS, FST and FIT) were computed according to Weir and Cockerham (1984) using the same program. The significance of fixation indices were determined using the permutation tests (1000 permutations). The values of fixation indices (FIS, FST and FIT) give

an idea about the degree of genetic differentiation in terms of inbreeding coefficient. The calculated fixation index of individuals within the population (FIT) was 0.05981.

While, among individuals within the breed's difference versus total variance was the lower (FST=0.12) indicating a moderate level of differentiation according to Wright (1978). He divided the value of (FST) into four intervals: (1) from 0 to 0.05, indicating little genetic differentiation, (2) from 0.05 to 0.15, indicating moderate genetic differentiation, (3) from 0.15 to 0.25, indicating great genetic differentiation, and (4) from 0.25 to 1, indicating very great genetic differentiation.

The pairwise difference between the four Egyptian camel breeds was (FIS=-0.07284). This negative FIS value may be explained by the random mating and the absence of inbreeding within the breeds under study and the high level of heterozygosity. This, in turn, could be attributed to two main reasons. The first is the sampling procedure as according to the regulations of FAO (1998) for such studies, the samples should be taken from unrelated animals.

The second, is the breeding system, as the farmer is usually, mating his camel with different sires from the area. This system minimizes the breeding of bull to their daughters and may be their mothers. In this respect, it is reported that the camel population in Tunisia has a moderate differentiation. They found that the mean values of FIT, FIS and FST were 0.27, 0.19 and 0.09, respectively. In addition, the fixation genetic index (FST) among the four Saudi camel populations was very low, ranging from 0.006 to 0.017, indicating low population differentiation between them.

Breeds structure and individual's assignment based on the microsatellite marker analysis

The genetic structure of the breeds was studied using the Bayesian clustering tool implemented in the structure software with values of K ranging from 1 to 6, thus assigning individuals into one or more breed probabilistically based on the allele frequencies detected at the eighteen micro satellite loci. The structure program was used to determine the unbiased structure without prior knowledge of the number of breeds. The log likelihood assignment revealed that the highest delta-K {L(K)} occurred at K=5 (Figure 3).

Therefore, the assumed best number of the studied breeds would be five clusters. Also, the population structure illustrated in Figure 4 indicated that the three Egyptian camel breeds were genetically distinct (they look like pure breeds). While, the Somali breed showed some degree of admixture. These results suggested that the individuals of the Somali breed were probably mixed with exotic breeds.

In order to add more insight in the degree of differentiation between all the individuals of the camel breeds, the Factorial Correspondence Analysis (FCA) was conducted using the GENETIX software as illustrated in Figure 5.

The (FCA) was used to visualize the individuals in three-dimensional space and to discover the relationships within and among the breeds. As shown in Figure 5 the individuals of each breed were clustered in the group they belonged to, rather than being mixed with each other. Individuals of Baladi, Sudani, Somali and Maghrabi breeds usually formed their own groups.

Genetic distance and phylogenetic relationships based on microsatellite markers analysis

To verify the genetic relationships among the four Egyptian camel breeds based on the microsatellite allele frequencies, the Nei's (1972) genetic distance (DA) and pairwise FST statistic were calculated and the phylogenetic tree was constructed (Figure 7). The closest pairwise Nei's genetic distance (0.747) was recorded between the Sudani and Somali breeds. Similarly, the lowest pairwise FST value (0.078) was recorded between Sudani and Somali breeds. While, the highest genetic distance (1.005) was between Baladi and Maghrabi breeds.

Also, the genetic relationship between Baladi and Sudani was relatively close (0.750). These results were supported by the phylogenetic tree which demonstrated that the four Egyptian camel breeds have been clustered into two main clusters (Figure 7). Maghrabi was separated in one cluster. While, the second cluster comprised two sub-clusters. Sudani and Somali formed one sub-cluster, and Baladi was in the second sub-cluster.

Therefore, the phylogenetic tree based on the microsatellite markers revealed that the closest relationship was between Sudani and Somali breeds. This is in agreement with what they reported that the phylogenetic relationship between the five Egyptian camel breeds showed two groups based on micro-satelite and RAPD markers. The first group includes Baladi, Maghrabi and Mowallad, while the second group includes Somali and Sudani.

Genetic diversity analysis of the four Egyptian camel breeds using the SCoT markers

The Start Codon Targeted (SCoT) polymorphism is a novel, simple, and reliable gene-targeted markers technique based on the translation start codon. The SCoT technique is sensitive to the low level of genetic variations and provides very useful tools for analyzing population genetics on wide range of plants as well as identifying species or population in the species. The primer for SCoT marker analysis was designed based on the conserved region surrounding the translation initiation codon, ATG.

Although the SCoT marker technique was developed to be employed with plant species, however, it was applied in the present study to assess its efficiency in animal species. Based on the results of the microsatellite analysis which revealed very low genetic variation among the individuals of each breed, we decided to perform the SCoT analysis on bulked DNA samples representing the four camel breeds. Four bulked DNA samples were generated by mixing equal concentrations of the DNA from the fifteen individuals of each breed. Twenty one SCoT primers were initially examined, but only sixteen primers revealed discernible patterns. The nucleotide sequence of the primers was adopted.

The banding profiles obtained by SCoT markers were compiled into a data binary matrix based on the presence (1) and absence (0) of the bands. Figure 7 Illustrates the banding profile generated by 12 of the 16 SCoT primers and the bulked genomic DNA for the four camel breeds. As shown, a total of 153 amplicons were generated with an average of 9.56 bands per primer. Out of the 153 bands, 75 were polymorphic, producing a polymorphism rate of 49%. The number of polymorphic amplicons varied from zero (SCoT-08) to 12 (SCoT-15) with an average of 4.6 bands per primer across the four Egyptian camel breeds. The SCoT-15 marker exhibited the highest percentage of

polymorphism (92.3%). In contrast, the SCoT-08 marker exhibited (0%) polymorphic amplicons with 100 % monomorphic bands.

Identification of the four camel breeds by unique SCoT markers

The SCoT analysis was successful to identify each of the four camel breeds with positive and/ or negative markers. The Maghrabi camel breed expressed the largest number of unique positive markers and unique negative markers (28 and 18 bands, respectively). This was followed by the Baladi breed which showed 6 and 5 bands as unique positive and negative markers, respectively. While, the Sudani and Somali breeds revealed equal number of unique positive markers (only 2 band). In addition, the Sudani breed showed one unique negative marker. These unique bands are useful fingerprints characterizing each breed.

Genetic distance and phylogenetic relationships among the four camel breeds based on SCoT analysis

The results of the SCoT markers indicated that the genetic similarity among the four Egyptian camel breeds ranged from 0.70 (between Baladi and Maghrabi) to 0.93 (between Sudani and Somali). Based on the UPGMA (unweighted pair group method with arithmetic average) clustering algorithm generated from the obtained SCoT dataset, the four Egyptian camel breeds were clustered into 2 main clusters (Figure 7). Maghrabi was separated from the other breeds in a separate cluster.

While, the second cluster was comprised of two groups, Baladi was in one group while, Sudani and Somali formed the second group. This result indicated that the closest phylogenetic relationship based on SCoT marker was between Sudani and Somali breeds. This is in accordance with the phylogenetic relationship deduced from the microsatellite analysis. Therefore, the results of genetic relationship based on both markers (microsatellites and SCoT) confirmed the close relationship between the Sudani and Somali breeds.

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