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# Phylogeny and Evolutionary Analysis of Goat Breeds in Jordan Based on DNA Sequencing

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**Abstract:** Phylogeny and evolutionary analyses were performed for Jordan goat breeds based on DNA sequencing. DNA segment of 0.5-kb from sixteen goat individuals of four breeds was sequenced. The DNA sequencing was analyzed by both Arlequin and MEGA softwares. The results showed a quite evolutionary differentiation found within goat breeds between. Furthermore, phylogeny tree was reconstructed providing evidences for a close phylogenetic alliance among breeds. The resulted evolutionary sequencing and phylogeny trees provide evidences that sequencing data were worthy to describe the evolutionary and phylogeny genetics in goats breeds in Jordan. On other hand the data were given scopes for possible sequences of gene(s) and identify polymorphisms, given possibility to identify Caprine genes from other close species genome like cattle. In the future we intend, in order to clearly identify the genetic polymorphisms, to detect further genetic variation and to develop tests particularly suitable for specific interested genes genotyping.

**Key words:** Phylogeny, DNA sequences, goat breeds, molecular diversity

## INTRODUCTION

Goat are important for the livelihood of farmers in general and in particular to those of Mediterranean area where they are an effective transforming low roughage quality into high quality animal protein (Boyazoglu and Flamant, 1990). Furthermore, goat of this area possess great adaptability to tropical environmental conditions and used as a dual purpose (Al-Tamimi, 2005). Goat in Jordan, on other hand, recently received high attention by researchers and other stakeholders as they only source of alleviation poverty in rural poverty stricken areas. So far identifying and characterizing several native breeds were accomplished. These breeds, Mountain Black, Dhaiwi, Desert and Damascus (Zaitoun *et al*., 2004, 2005). They vary in their own morphological characteristics, predominant geographical sources and production systems (Tabbaa and Al-Atiyat, 2009).

The general and common agreement that domesticated animals descended from a single ancestor, originating in Asia. It is also reported that all world's breeds originated from central Asia since stone ages (Crawford, 1995; Piper and Ruvinsky, 1997). At the present time, the Jordan goat breeds are closely related to each other based on morphological studies (Zaitoun *et al*., 2005). However, there is no published data on goat breeds' evolutionary relationships and phylogeny based on DNA techniques. For example, DNA sequencing technology has provided wide opportunities to analyze genetic variability at the DNA level in animal breeds (Karaca *et al*., 1999; Nidup *et al*., 2005). Furthermore, sequencing DNA of goat facilitates homology studies that compare the sequences among close species and breeds data base. These would help to find and understand some common shared genes of interest (Simon, 1990). The aim of this study is to report, hopefully for the first time, phylogeny and evolutionary analysis of goat breeds in Jordan based on of DNA sequencing with reference to sequence region of interested genes in cattle genome.

## MATERIALS AND METHODS

**Goat population:** Mountain Black, Dhaiwi, Desert and Damascus goat individuals were collected and sampled from Jordan for a total of four individuals per breed. Each individual came from unrelated and geographically separated goat population.

**DNA extraction and quantification:** DNA extraction was performed using a commercially available kit/protocol of E.Z.N.A® MicroElute Genomic DNA extraction Kit

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(OMEGA Bio-Teck, 2010, www.omegabiotek.com). Concentrations of DNA were estimated by Nano-DNA spectrophotometer (AlphaSpec®, 2010) in which the quality of DNA was evaluated using the ratio of A260/A280. The DNA concentration of each sample was made of 10 ng $\mu L^{-1}$.

**DNA sequencing:** DNA sequencing service was provided by Macrogen® Commercial Company (www.macrogen.com) incorporating universal primers, T7promoter (5'-TAATACGACTCACTATAGGG-3'), into a PCR product using high throughput Applied Biosystems 3730XL sequencer. General primers of T7 vector was designed and used to amplify the corresponding region of around 700 bp in animals DNA (Dunn and Studier, 1983). The primers of T7 considered as arbitrary primers that provides a sequencing region. The brief details of sequencing reaction were made of 2.5 μL, 2 μL dNTPs, 0.5 μL of forward and reverse primers of 50 pmoL $\mu L^{-1}$, 1 U Taq polymerase, 100 ng of DNA and $ddH_2O$ to all up of 25 μL. The PCR cycling program was 5 min at 95°C, then 1 min at 95°C for 30 cycles, then 1 min at 61°C and 2.5 at 72°C and then incubation at 72°C for 10 min, then storing at 5°C. The PCR products were cloned into the PCR T-vector and sequenced by ABI PRISMTM 377 DNA sequencer.

**Analysis of DNA sequences:** Resulting DNA sequences were edited and assembled into contiguous sequences (470 to 560 bp) by use of the Arelquin (Excoffier and Lischer, 2010) and MEGA software programs (Tamura *et al*., 2011). The resulting DNA sequence information was analyzed for following basic evolutionary analyses (Kumar and Gadagkar, 2001), evolutionary distances and phylogenetic reconstructions (Nei and Kumar, 2000; Tamura *et al*., 2011). Sequencing data were analyzed using Arelquin software to estimate different genetic diversity parameters. In addition, sequencing data of sheep and goat with its counterpart of cattle (Bos Taurus) were compared using Gene Bank data through BLAST genetic software (BLAST, 2013).

## RESULTS AND DISCUSSION

**Molecular diversity parameters:** Sequencing data were extracted and then tabulated and then executed by Arelquin and BLAST softwares. The results show that there were low allele numbers and lower heterozygosity values using DNA sequence from those few individual goats (Table 1). The expected heterozygosity was 0.502 which considered moderate (Table 1). In addition, number of genes that expected to be found in the sequenced region was 19 genes as a number of gene copies only for polymorphic loci (Table 1). In general, the results showed high genetic diversity in studied goat breeds. On the other hand, relative frequencies in all individuals were found high frequent of more than 50% for haplotypes, whereas it was found low for second and fourth haplotypes (Table 2).

Table 1: No. of alleles, expected heterozygosity and no. of gene copies only for polymorphic loci

| Locus# no. of alleles | Expected heterozygosity | No. of gene copies |
|---|---|---|
| Mean 2.685 | 0. 50162 | 19.000 |
| S.D 0.637 | 0.159490 | 0.000 |

Table 2: Relative haplotype frequencies for four studied goat samples, one of each breed

| Haplotype | Relative frequency |
|---|---|
| 01 | 0.3160 |
| 02 | 0.1050 |
| 03 | 0.5260 |
| 04 | 0.0526 |

Table 2 shows number of transitions, transversions and substitution values with their probability (Theta) of that sequences have evolved with the same pattern of substitution, as judged from the extent of differences in base composition biases for each sequence pair of studied goat breeds. The best explanation for the results is that nucleotide structure comparisons are heterogeneous in which substitution patterns were high between each haplotype pair. Rates of different transitional substitutions and those of transversional substitutions are shown in the Table 2. However, the transversional substitutions rates were higher. Generally, the sums of nucleotides substitution pattern and of substitution rates among sites are makeable different as a result of computing methods (Kumar and Gadagkar, 2001). This summation is in agreement with Tamura *et al*. (2004) who reported that pairwise distances and the related substitution parameters are accurately estimated by maximizing the composite likelihood than any other methods. Table 3 shows Molecular diversity indexes for studied goat samples.

**Genetic distance:** Distance matrix constructed from sequencing data is shown in Table 4 for goat. The distance calculation methods used by Arelquin software is Inter-haplotypic distance matrix based on pairwise difference with no correction method. The results are comparable with distance matrices found earlier for goat using morphological characteristics (Zaitoun *et al*., 2005). Thus, utilizing these results of the distance matrix, cluster analysis was formulated as UPGMA phenogram (Fig. 1). The cluster shows three clusters where the first showed closeness of Dihawi and Black Mountain, then both are closer to Desert in the second cluster. Whereas the third

Table 3: Molecular diversity indexes for studied goat samples

| Statistics | Mean |
|---|---|
| No. of transitions | 420 |
| No. of transversions | 456 |
| No. of substitutions | 876 |
| No. of indels | 0 |
| No. of ts. sites | 283 |
| No. of tv. sites | 456 |
| No. of subst. sites | 510 |
| No. private subst. sites | 510 |
| No. of indel sites | 0 |
| Pi | 255.8 |
| Theta_k | 1.28 |
| Theta_k_lower | 0.408 |
| Theta_k_upper | 3.48 |
| Theta_H | 1.36 |
| S.D. Theta_H | 0.499 |
| Theta_S | 145.9 |
| S.D. Theta_S | 49.90 |
| Theta_pi | 255.82 |
| S.D. Theta_pi | 127.99 |

Table 4: Inter-haplotypic distance matrix based on pairwise difference

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | | 9.87 | 9.45 | 9.79 |
| 2 | 390 | 9.79 | 10.86 | |
| 3 | 407 | 393 | 10.23 | |
| 4 | 393 | 339 | 375 | |

SD above diagonal

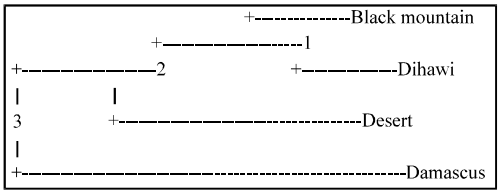


Fig. 1: Cluster analysis among the studied goat breeds according to the UPGMA phenogram

cluster group those three breeds together with Damascus. Therefore, these finding undoubtedly confirmed the breed similarity and differentiation among the studied goat breeds in term of morphology (Zaitoun *et al*., 2005) and production traits (Tabbaa and Al-Atiyat, 2009) and heat tolerance traits (Al-Tamimi *et al*., 2013). Such evolutionary distances would be fundamental for molecular evolution and are useful for phylogenetic reconstructions and the estimation of divergence times between studied populations (Nei and Kumar, 2000). The present study provides the first molecular genetics phylogenetic reconstructions and breed differentiation of goat breeds in Jordan. In particular, reconstruction of the evolutionary history of genes and species is currently one of the most important subjects in molecular evolution (Nei and Kumar, 2000). If reliable phylogenies are produced, they will shed light on the sequence of evolutionary events that generated the present day diversity of genes and species and help us to understand the mechanisms of evolution as well as the history of organisms. Another scope for benefiting the results is to be employed for suitable breeding objectives in national breeding program for each breed. This recommendation was made earlier to characterise each breed according to preferable breeding objectives by their own farmers (Tabbaa and Al-Atiyat, 2009). Figure 2 presented Alignment between the analyzed Caprine breed (Goat) and the sequence cattle genome from GenBank.

**Comparative sequencing study:** The amplified DNA sequences of goat were compared with mouse cattle genome to identify a possible recognition site for this

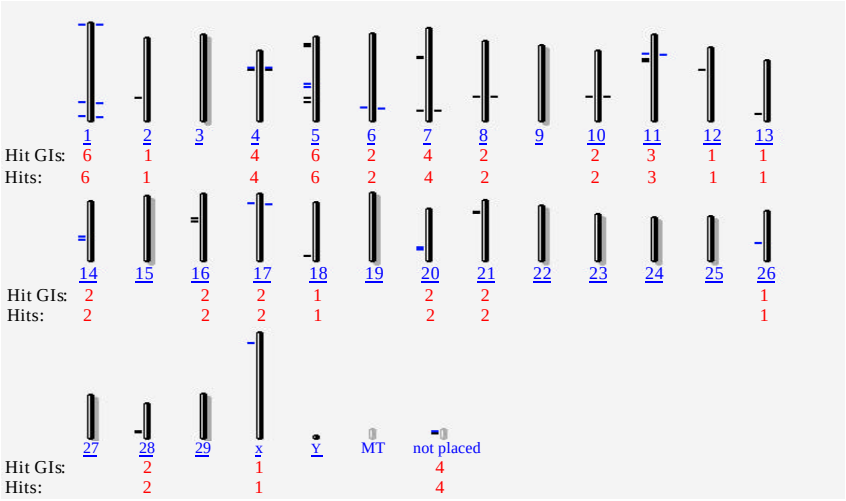


Fig. 2: Alignment between the analyzed caprine breed (Goat) and the sequence cattle genome from GenBank

fragment. A quite good long of base pair fragments is found for all chromosomes except chromosomes 3, 9, 15, 19, 22, 23, 24, 25, 27 and 29. The function of this fragment remain has potential sequences of a number of gene on cattle genome. Similar results revealed homologies and divergences between the subfamilies Bovin and Caprin (Iannuzzi *et al*., 2000).

## CONCLUSION

Sequencing data were worthy to describe the evolutionary and phylogeny genetics in goats breeds in Jordan. On other hand, the data were given scopes for possible sequences of gene(s) and identify the possible polymorphisms. Considering gene from GenBank, it is possible to identify caprine genes. In the future we intend, in order to clearly identify the genetic polymorphisms, to detect further genetic variation and to develop tests particularly suitable for specific interested genes genotyping.

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