

**Genetic Structure and Phylogeny of Three Goat Populations in the Middle East**

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**ABSTRACT:** This study aimed to analyze genetic structure and differentiation of Ardi, Black Bedouin and Damascus goats from Saudi Arabia, Jordan and Syria respectively. Therefore, four Microsatellite markers were genotyped onto a total of 99 individuals. The heterozygosity, intra-population differentiation and admixture coefficients were analyzed. All populations exhibit high average allele number and expected heterozygosity. The *F*-statistics coefficients indicating 6.0% of total genetic variation corresponded to differences between populations, whereas 94.0% was explained by difference between individuals. Damascus goat was more differentiated from Black Bedouin goat. Strong evidence of admixture between Ardi and Black Bedouin goat was found. The Genetic distances between Ardi and Black Bedouin goat was the shortest. Phylogeny relationship also revealed the expected degree of differentiation in all populations. Overall, the present study sheds new light on genetic differentiation and relationships of native goats in core countries of Middle East.

**Keywords:** Goat; Microsatellite Marker; Heterozygosity; Relationships

**Introduction**

Goat has historically been assigned to countries of Arabian Peninsula and Middle East (Mason 1984). Those countries have harsh tropical environmental conditions where goats express great adaptability (Galal et al. 2008). It is also reported that all world's breeds originated from central Asia since stone ages (Piper and Ruvinsky, 1997). At present time, the native goat breeds of most Arabian countries are expected to be closely related to each other based on morphological studies. For example, most common native breed in Saudi Arabia (SA) is Ardi goat; Similar breed is most common goat breed in Jordan which is Black Bedouin. On the other hand, the most known goat breed in Syria is Damascus goat.

In general, evidences indicate that goat breeds of tropical areas have a range of unique genetic resources of adaptive traits (Ajmone-Marsan *et al.*, 2010). As a consequence, they have low genetic potential for better production when compared to other exotic goat breeds (Tabbaa and Al-Atiyat, 2009). However, goat breeds have recently faced major threats to their genetic diversity which resulted from systemic drought and unplanned crossbreeding. Even though it is widely accepted that domesticated goats of this region are the most closely related to their common ancestors (Piper and Ruvinsky, 1997). There is no published reports stating so using molecular DNA markers. Microsatellites markers (MS) in particular have provided

wide opportunities to analyze biodiversity and genetic relationship between and within world goat breeds (Karaca *et al.*, 1999). The aim of this study is to report, hopefully for the first time genetic diversity, phylogeny and evolutionary analysis of most native goat breeds in KSA, Jordan and Syria based on DNA microsatellite markers.

**Materials And Methods**

The goat individuals were 30 Ardi from SA, 37 Black Bedouin from Jordan and 22 Damascus from Syria. DNA was extracted using E.Z.N.A<sup>®</sup> MicroElute Genomic DNA extraction Kit (OMEGA Bio-Teck, 2010). DNA Concentrations were evaluated using Nano-DNA spectrophotometer (AlphaSpec<sup>®</sup>, 2010). The DNA concentration was made to 10ng/μL for DNA genotyping process. Four Microsatellite DNA Markers (Table 1) were used. PCR reaction utilized 10 μl volume of DNA and master mix for genotyping the DNA samples. Thermal cycling was performed on a GeneAmp<sup>®</sup> PCR system 9700; Applied Biosystem. The Amplified PCR products were fragmented using 3130 Genetic Analyzer. The size of the microsatellite alleles was visualized, determined and scored into Excel Sheet files using Gene Mapper Software (Version 3.0). The microsatellite alleles of Excel Sheet file were analyzed by Arelquin<sup>®</sup> genetic Software (Excoffier *et al.* 2010).

**Table 1.** Genetic variation measures within each studied goat population at the studied four loci

Locus	Alleles No.	<i>H<sub>o</sub></i>	<i>H<sub>e</sub></i>	G
Ardi				
<i>CSRD247</i>	7	0.667	0.740	0
<i>OARAE54</i>	11	0.767	0.848	0
<i>ILSTS005</i>	5	0.700	0.588	0
<i>McM527</i>	10	0.767	0.825	0
Mean	8.25	0.725	0.750	0
Black Bedouin				
<i>CSRD247</i>	9	0.839	0.817	0
<i>OARAE54</i>	12	0.857	0.860	0
<i>ILSTS005</i>	5	0.821	0.689	0
<i>McM527</i>	10	0.893	0.849	0
Mean	9	0.853	0.804	0
Damascus				
<i>CSRD247</i>	9	0.773	0.817	0
<i>OARAE54</i>	8	0.591	0.836	0
<i>ILSTS005</i>	5	0.545	0.659	0
<i>McM527</i>	7	0.909	0.819	0
Mean	7.25	0.705	0.783	0
Overall Mean	8.17	0.761	0.779	0

The hierarchical genetic structure of the samples and Analysis of molecular variance (AMOVA), F-statistics and pairwise difference ( $F_{st}$ ) were computed using ARLEQUIN (ver 3.11). The analyses were gene diversity, standard diversity indices and Intra-population differentiation level methods. Furthermore, Garza-Williamson ( $G-W$ ) index is used for measuring population bottleneck (Garza and Williamson, 2001). The population structure was analyzed using STRUCTURE software (Pritchard et al., 2000) considering an admixture model and correlated allele frequencies between studied populations. The length of the burn-in Monte Carlo Markov chain (MCMC) were 1000 and 10000 in 100 runs for possible number of clusters (K) from 2 to 6. For each K value, log probability of data (L[K]) and  $F_{st}$  values for each cluster were estimated. On the other hand, genetic distances matrix between the three breeds were measured based on Nei's genetic distances estimates (Nei's, 1987). Using genetic distance matrix, the neighbor-joining and UPGAM phylogeny tree was drawn using MEGA software program, version 5 (Tamura et al., 2011).

## Results and Discussion

The total number of alleles and allele frequencies for each locus in each population are presented in Table 1. The highest number of alleles among the polymorphic markers was for *OARAE54* in both Ardi (11) and in Black Bedouin (12). Locus *ILSTS005* exhibited the smallest number of alleles (5) in all populations. The average number of alleles varied from 8.25 in Ardi, 9 in Black Bedouin and 7.25 in Damascus goats. These results show that Damascus goat is less diverse than the other two goat populations. The overall mean of allele number in all populations was 8.17. Similar results were reported by independent studies on goat using different MS markers (Dixit et al. 2009). There are some similar observations where most frequent allele of *CSRD247* is 218 and of *McM527* is 154, whereas, the most common in *OARAE54* is 120 in Ardi and Black Mountain populations and 134 in Damascus goat population. However, the comparisons between three breeds showed that some alleles are not commonly shared between them. The possible inference might be geographical location, different selection forces, and non-common ancestor. Goat breed specific alleles were previously observed at different loci for different populations with low frequency. Similar observations were reported for Swiss goat breeds (Glowatzki-Mullis et al., 2008). This may indicate that the alleles did not get an advantage either due to random or selective forces.

The Averages of observed and expected heterozygosity were ranged from 0.545 to 0.909 and 0.588 to 0.860, respectively. The Black Bedouin goat showed the highest average gene diversity (0.804), while Ardi goat showed the lowest gene diversity (0.750). At most loci,  $H_o$  was lower than  $H_e$ , except *McM527*. In relation, number of allele at the locus in Damascus goat was 7.25 alleles, whereas it is 8.25 and 9 in Ardi and Black Bedouin goats, respectively. *ILSTS005* showed lowest  $H_o$  and  $H_e$  in all goat populations, whereas *OARAE54* showed highest value of  $H_e$ . Similar range of results was reported (Carmen et

al. 2010; Magdalena et al. 2009). In a study of 14 MS markers genotyped on Ardi goat, the expected heterozygosity was 0.675 (Aljumaah et al. 2012).

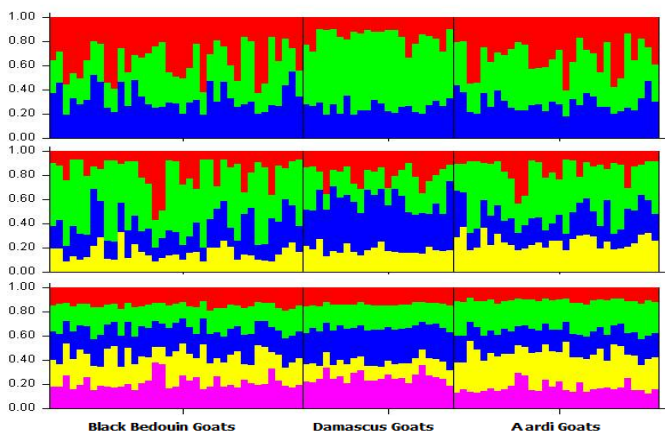
Garza and Williamson ( $G-W$ ) statistics show highest value (0.415) for Black Bedouin goats, then lower value (0.405) for Ardi goats and lowest value (0.363) for Damascus goat. Whenever  $G-W$  is low in a population, it is expected that the population to be sensitive to bottleneck (Garza and Williamson, 2001). Therefore, Damascus goat population was subjected to bottleneck.

The AMOVA revealed that percentage of variation between populations was 1.49% and within populations was 98.51%. Variance components among populations were highly significant for all the studied loci, indicating 97.68, 98.05, 99.345 and 99.15% for *CSRD247*, *OARAE54*, *ILSTS005* and *McM527*, respectively. While *CSRD247* and *OARAE54* contributed 2.32% and 1.94% of variability within populations, respectively, and *ILSTS005* and *McM527* showed the lowest variability of 0.66% and 0.85%, respectively.

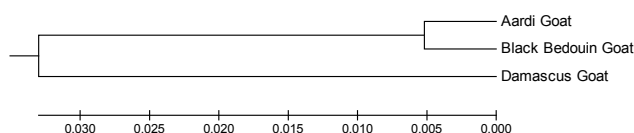
$F_{st}$  values for each pair of populations varied from 0.004 to 0.069. The  $F_{st}$  values showed more differentiation between the Damascus goat and Ardi goats ( $F_{st}=0.0220-0.069$ ) compared to differentiation among pairs of Ardi and Black Bedouin ( $F_{st}=0.004-0.010$ ). The pairwise differences between Black Bedouin and Damascus goats were ranged from 0.020 to 0.063. Overall results indicate evolutionary distinction of Damascus goat as an external group.

The average genetic distance values over all loci was 0.060, indicating that a 6.0% of total genetic variation corresponded to differences among populations, whereas 94.0% was explained by difference among individuals within all populations. The within population indices over the loci was 3.10. Mean pairwise comparisons between breeds showed that values were 3.12–3.22 a little higher than within population values. These results are indicating that amount of variation between Ardi and Damascus is around 3.1. The genetic distance between Ardi and Black Bedouin goat was short; 0.010. The notable difference was between Ardi and Damascus of 0.068 and Black Mountain and Damascus of 0.063.

Goat population Genetic structure was underlined using STRUCTURE software with K ranging from K=3 to K= 5 assuming admixture and correlated allele frequencies models. It stated that there would be two populations accounted as possible ancestral populations for current studied populations. As a consequence, individual admixture proportions showed that one cluster corresponded to both Ardi and Black Bedouin which could not be separated because of low genetic differentiation and the other cluster to Damascus goat. There is strong evidence of admixture between majority of Ardi and Black Bedouin goat individuals with individual admixture coefficients of more than 25%, (Figure 1).



**Figure 1.** Estimated population structure for each individual represented by a single vertical line broken into K colored segments, with lengths proportional to each predefined populations of K from 3 to 5.



**Figure 2.** Neighbour-Joining dendrogram showing the genetic relationship among the studied goat populations. The numbers below each branch indicate the branch length in units of genetic distance.

Figure 2 show evolutionary relationships of the three populations using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length equal to 0.071 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The NJ tree revealed two different clusters. The first cluster consisted of the Ardi and Black Bedouin goat breeds, and the second cluster consisted of the Damascus goat breed alone. The resulted Clusters reflected the outcomes from  $F_{st}$  estimates. There is long evolutionary distance separation between Ardi and Black Bedouin goat as one similar group from Damascus goat population. These results in accordance with the  $F_{st}$  data showing that Ardi and Black Bedouin goats belong to same evolutionary ancestor. Furthermore, they are from same evolutionary group and less related to Damascus goat.

### Conclusion

The three studied populations expressed a high level of genetic variation revealed by high  $H_e$  and  $H_o$ , the number of alleles per locus with significant deviation from HWE. Allele frequency results based on F-statistics estimates, clarified that differentiation between and within populations were reported, indicating that a 6.0% of total genetic variation corresponded to differences among populations, whereas 94.0% was explained by difference among individuals within all populations. There was strong evidence of admixture between Ardi and Black Bedouin goat individuals indicating possible migration or

shared ancestry. Genetic distances between Ardi and Black Bedouin goat was the shortest, whereas it was longest between Ardi and Damascus goats. From genetic conservation point of view, it is recommended to maintain biodiversity of those populations in case of genetic migration of good genetic resources is absence.

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