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Genetic diversity in Egyptian and Saudi goat breeds using microsatellite markers

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ABSTRACT

Objective: The genetic polymorphism within and among three indigenous goat breeds found in Egypt (*Barki* and Zaraibi) and in Saudi Arabia (*Ardi*) was detected by twelve microsatellites.

Methodology and results: A total of 95 blood samples were collected from the Egyptian and Saudi goat breeds. Genomic DNA was extracted from whole blood of each sample and microsatellites techniques were used for analysis of DNA. The results showed that, the total number of detected alleles varied from 2 (MMP9) to 16 (DRB2). The mean numbers of alleles per locus are 4.8, 4.3 and 6.2 in *Barki*, *Ardi* and *Zaraibi*, respectively. The mean of expected heterozygosity of the breeds ranged from 0.696 to 0.725. After corrections for multiple significance tests, deviations from Hardy-Weinberg equilibrium were statistically significant over all populations and loci, reflecting the deficiencies of heterozygotes (global FIS=0.053). Based on pairwise FST and Nm between different breeds, there was a great genetic differentiation between *Ardi* goat and the other two Egyptian breeds, UPGMA dendrogram based on Nei's genetic distance grouped the investigated goat breeds genotypes into two clusters. The first cluster includes Egyptian breeds (*Barki* and *Zaraibi*) where as the second cluster includes Saudi breed (*Ardi*) which appeared to be most distant from the other breeds.

Conclusions and application of findings. In conclusion, these results can be useful for the development of a rational breeding strategy for genetic improvement of goats in Egypt and Saudi Arabia**.** The studied Mediterranean breeds sampled from African and Asian populations seem to have differentiated from each other with only little genetic exchange between the geographically isolated populations.

Key words: Microsatellites, Goats, Genetic diversity, Genotype**.**

INTRODUCTION

The goat was the first animal domesticated production by humankind. Goats are the most widely spread domestic species in the world and play an important economical role in developing countries (Adriana *et al*., 2010). Goats have been important for food and economic securities for countless years and their contributions to economic returns in developed countries has been rising as well (Sahlu *et al*., 2005).

In Egypt, goats are an important source of meat. They are distributed across the country, especially dense in the Nile valley and delta region (Galal *et al*. 2005). Most of the livestock breeds in Egypt lack molecular characterization required for establishing

adequate utilization of genetic variation in developing animal production. Goats are spread over a wide range of habitats with a substantial concentration in the tropics and dry zones in developing countries (Galal 2005; FAOSTAT 2006). Therefore, they are expected to show a large amount of genetic diversity in adapting to the varying ecosystems. So far, the goat diversity studies based on microsatellites have been because of their high degree of polymorphism, random distribution across the genome and neutrality with respect to selection. Many bovine microsatellite markers have been used for genetic analysis in sheep and goats (Bruford & Wayne 1993). The Egyptian goats are classified into several breeds differing in colour, size and other morphological features, such as *Zaraibi*, *Baladi*, *Sinawi* or *Bedouin*, *Barki* and *Saidi*. However, in Saudi Arabia where the climate is suitable for goat, the number of goats is believed to exceed 7.5 million mainly of the *Masri* and Ardiin breeds. The *Ardi* goats are more adapted to the arid region than the *Masri* the region (Salah *et al*., 1989). The *Ardi* goats are more adapted to the arid region than *Masri*. Although, the latter produces more milk (Al Saidy *et al*., 2007), while the first produces milk steadily and therefore, is greatly appreciated by desert dwellers, where it is widely spread. The contribution of goat meat to the total meat income of Saudi Arabia is about 30%. Although, goats have a large and substantial contribution to the total meat income of Saudi Arabia, this species remain neglected and rearing is exclusively in the hand of the nomadic people. Little information is available about the phylogeny and genetic relations between Saudi Arabia goat breeds. In addition, few studies were conducted for genotyping of most important economic traits of Saudi Arabia goats and few steps were taken in the area of genetic improvement of these local adaptive genetic resources. Microsatellite markers are widely accepted as a choice marker for genetic characterization of populations as they are highly polymorphic single locus DNA sequences scattered throughout the genome and are readily adaptable to Polymerase Chain Reaction (PCR). Many authors have used microsatellites for molecular genetics characterization of goat (Ramljak *et al*., 2011). However, the genetic characterization for Egyptian and Saudi goat breeds has not been assessed and the present work will become the first study of the kind.

The aim of this study is attempt to identify the genetic variations within and between *Barki*, *Zaraibi* Egyptian goats and *Ardi* Saudi goats based on microsatellite analysis.

MATERIALS AND METHODS

Animal Material: Ninety Five **(**95) blood samples were collected from the three goat breeds, Where, *Barki* breed samples (n.35) obtained from the research farm of the Department of Animal Production, Faculty of Agriculture, Cairo University, while, *Zaraibi* breed samples (n.35) from the Agriculture research station (El-Serow, Domiatta) of the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture. Meanwhile, the sample of Saudi (25) *Ardi* breed was collected from different Farms of Faculty of Science, King-Abd El-Aziz University. The samples were collected from unrelated animals.

DNA extraction: Genomic DNA extraction was extracted from blood samples using a standard phenol: chloroform extraction method (Sambrook *et al*., 1989). Twelve microsatellite markers were studied: OarFCB128, TGLA53, D5S2, BP33, MAF65, DRB2, OarFCB20, INRA49, CSRD247, INRA172, MMP9, and BP28. Microsatellites amplification was carried out using fluorescent-labelled primers. The amplified products were analysed with a DNA capillary sequencer ABI Prism® 310 Genetic Analyzer (Applied Biosystems).

Statistical analysis: POPGENE software package (Yeh *et al*., 1999) was used to calculate allele frequencies, observed number of alleles, effective number of alleles (Kimura and Crow, 1964), observed (Ho) and expected (He) heterozygosity at each locus in the six populations under study. Polymorphism information content (PIC) value for each locus was calculated by using the method described by Bostein *et al*. (1980). Pair-wise allele sharing was calculated manually from the raw data. Using the variance-base method of Weir and Cockerham (1984), population differentiation by *F*-statistics was computed using FSTAT version 2.9.3.2 computer program (Goudet, 2002). Mean standard deviations of the *F*-statistics program that are analogue to Wright's (1951, 1978). FIS and FST were obtained across breeds by the Jackknifing procedure over loci (Weir, 1990). The extent

of global inbreeding was further studied with the same software by estimated FIS value. The effects of migration and gene flow on the genetic structure of the analyzed populations were estimated between pairs of populations according to an island model under neutrality and negligible mutation (Slatkin, 1985). Genetic distances among populations were estimated using (Ds) standard genetic distance of Nei (1972).

RESULTS AND DISCUSSION:

The result showed that all 12 markers successfully amplified in Egyptian and Saudi goat microsatellite loci. All marker microsatellite loci examined were polymorphic in all tested breeds. The number of alleles for each of the twelve microsatellite loci in each of the three breeds. The total number of detected alleles varied from 2 (MMP9) to 16 (DRB2). The mean numbers of alleles per locus are 4.8, 4.3 and 6.2 in *Barki*, *Ardi* and *Zaraibi*, respectively. The mean number of alleles shared between *Barki* and *Ardi* is 3.1, between *Barki* and *Zaraibi* is 4.0 and between *Ardi* and *Zaraibi* is 3.5, whereas the mean number of the alleles shared by the three breeds is 2.3. The most noticeable difference is found at MMP9 locus, where only 1 allele, out of a total of 13 alleles, is shared by the three breeds. On the other hand, 2 alleles, out of the total of 3 alleles, are shared by the three breeds at OarFCB128 and INRA49 loci. It is also noticed that all alleles representing the OarFCB128 and MMP9 loci are present in *Zaraibi* breed, while allele of the INRA49 Loci is present in *Ardi* breed (Table 1).

Table 1: Number of alleles at each microsatellite locus in the three breeds and the number of alleles shared between breeds.

Microsatellite	Total	*Barki*	*Ardi*	*Zaraibi*	Shared by *Barki-Ardi*	Shared by *Barki-Zaraibi*	Shared by *Ardi-Zaraibi*	Shared by the three
OarFCB128	3	2	2	3	2	2	2	2
TGLA53	11	6	4	9	4	5	4	3
D5S2	4	2	3	2	2	2	2	2
BP33	7	5	4	6	3	4	3	2
MAF65	10	7	5	10	4	7	4	3
DRB2	16	8	7	10	5	7	6	4
OarFCB20	8	6	5	7	4	4	4	3
INRA49	3	4	3	4	2	3	3	2
CSRD247	13	6	5	7	4	4	4	2
INRA172	9	5	4	6	2	4	4	2
MMP9	2	3	3	2	2	2	2	1
BP28	13	4	6	8	4	4	6	3
Mean	8.25	4.8	4.3	6.2	3.1	4.0	3.5	2.3

The mean number of alleles identified in this study is 8.25 and this value similar to the values obtained for the Croatian spotted goat and the Markhoz goat, which number of alleles of 8.1 (Ramljak *et al*., 2011; Mahmoudi *et al*., 2009), for Barbari goats from India was 6.3 (Ramamoorthi *et al*., 2009), Raeini goat from Iran was 7.8 (Sadeghi *et al*., 2010) and for Egyptian and Italian goat breeds was 6.5 (Agha *et al*., 2008). The average Observed and expected heterozygosity (*Ho* and *He*) are shown in Table 2 the highest observed heterozygosity (0.671) was shown in *Ardi* breed, while the lowest (0.611) was shown in *Barki* breed**.** The observed heterozygosity showed high genetic variability. This might be due to low selection pressure, large population size and immigration of new genetic materials. Average value of *Ho* of the three goat breeds were higher than some breeds such as Gohilwari (*Ho*=0.51; Kumar *et al*., 2009), Sirohi (*Ho*=0.50; Verma *et al*., 2007), Sub-Saharan breeds (*Ho*=0.56; Muema *et al*., 2009) Korian goat (*Ho*=0.36; Kim *et al*., 2002), Jamunapari of India (*Ho*=0.42; Gour *et al*., 2006) and Gohilwadi breed (*Ho*=0.63; Fatima *et al*., 2008), but lower than Spanish Guadarrama (*Ho*=0.78; Serrano *et al*., 2009) and Croatian spotted breed (*Ho*=0.76; Jelena *et al*., 2011).

Table 2: Number of animals (n), mean observed number (na) (and SD) of alleles, mean (and SE) of observed (Hobs) and expected heterozygosity (Hexp) and the exact test for Hardy-Weinberg equilibrium (HWE) in Egyptian and Saudi goat breeds

Breed	n	n_a	H_{obs}	H_{exp}	HWE test[1]
Barki	**35**	**4.8 (2.3)**	**0.611 (0.069)**	**0. 725 (0.083)**	**0.0566**
Ardi	**25**	**4.3 (1.98)**	**0.671 (0.035)**	**0.711 (0.033)**	**0.1170**
Zaraibi	**35**	**6.2 (2.89)**	**0.628 (0.072)**	**0.696 (0.089)**	**0.1246**

Another measure of genetic variability is expected heterozygosity where, maximum expected heterozygosity (0.725) was showed in *Barki* goat and the minimum (0.696) was shown in *Zaraibi* goat. This study results indicate the three tested goat breeds have substantial amount of genetic diversity, when compared to some other goat breeds around the world where, Sri Lanka South (*He*=0.48), Sri Lanka N-Central (*He*=0.49) and Australian goat (*He*=0.45; Barker *et al*., 2001), some Indian goat breeds such as, Jamunapari (*He*=0.54), Marwari (*He*=0.63), Zalawadi (*He*=0.58), Gohilwadi (*He*=0.67), and Surti (*He*=0.64) (Fatima *et al*., 2008; Kumar *et al*., 2005; Gour *et al*., 2006), Swiss goat breeds (*He*=0.66; Glowatzki-Mullis *et al*., 2008), Canary Island goats (*He*=0.62; Martínez *et al*., 2004), Kalahari Red goats (*He*=0.63; Kotze *et al*., 2004), Sub-Saharan breeds (*He*=0.54; Muema *et al*., 2009) and some Korean goats (*He*=0.38; Kim *et al*., 2002). On the other hand, the goat breeds tested in our study showed less genetic diversity when compared to some Indian goat breeds such as, Kutchi (*He*=0.80), Sirohi (*He*=0.79) and Chegu (*He*=0.81), some Iranian goat breeds (*He*=0.74-0.80), Sardinian goat breed of Italy (*He*=0.74), Spanish Guadrrama (*He*=0.81), Croatian spotted breed (*He*=0.77) and Chinese goat breeds (*He*=0.78-82) (Behl *et al*., 2003; Dixit *et al*., 2008; Guo-hong *et al*., 2010; Jelena *et al*., 2011; Mahmoudi *et al*., 2010; Sechi *et al*., 2005; Serrano *et al*., 2009; Verma *et al*., 2007). While, the value of Observed and expected heterozygosity was similar to those reported for Portuguese goat breeds (HO=0.636, HE=0.702) (Bruno *et al*., 2011), meanwhile Croatian spotted goat with means for expected gene diversity of 0.771 and observed heterozygosity of 0.759 (Ramljak *et al*, 2011). Mean observed allele for all loci found to be 4.8, 4.3 and 6.2 in *Barki*, *Ardi* and *Zaraibi* respectively, which explains variation level of polymorphism of the studied microsatellites. The average values was lower than observed numbers of alleles were reported for Barbari goat from India (*na*=6.33; Ramamoorthi *et al*., 2009), Italian goat breeds (*na*=6.5; Agha *et al*., 2008) and Taleshi from Iran (n_a=6.7 Mahmoudi and Babayev, 2009). However, the Croatian spotted breed (n_a=8.1; Jelena *et al*., 2011) and the average value of seven Indian goat breeds (n_a=8.1-9.7; Rout *et al*., 2008).

Table 3: Fit, Fst and Fis values and chi-square test for HWE for each locus over all breeds.

Locus	Fit	Fst	Fis	Gene flow (Nm)	X^2 (Degrees of freedom)
	0.1370	**0.0741**	**0.2200**	**3.1250**	**10.36 (4)**
	0.0289	**0.01121**	**0.4391**	**1.9916**	**47.7 *** (18)**
	0.3778	**0.0800**	**0.3237**	**2.8750**	**8.0 (2)**
	0.6861	**0.1277**	**0.6402**	**1.7074**	**45.8 *** (12)**
	-0.0113	**0.0621**	**0.3783**	**3.7734**	**22.3 (20)**
	0.0723	**0.0793**	**0.4176**	**2.9019**	**32.9 * (20)**
	0.0726	**0.1246**	**0.2595**	**1.7563**	**34.3** (16)**
	0.0805	**0.0102**	**0.3642**	**0.9395**	**28.8*** (6)**
	0.0434	**0.0731**	**0.6321**	**3.1696**	**25.7* (14)**
	0.0630	**0.0547**	**0.3586**	**1.3657**	**39.9*** (12)**
	0.7290	**0.1097**	**0.6957**	**2.0294**	**12.8 (4)**
	0.1662	**0.1693**	**-0.0413**	**1.0046**	**60.8 *** (16)**

***p < 0.05; *** p < 0.001.**

The mean number of alleles and expected heterozygosities were very accurate indicators of the genetic polymorphism within the breed. Normally the average number of alleles depends on sample size and generally, number of observed alleles tends to increase with increase in population size. Tests of genotype frequencies for deviation from HWE, at each locus overall breeds, reveal significant departure from HWE (P>0.05 and P>0.001) (Table 3).The tested microsatellite markers showed significant inbreeding coefficients *Fis* were positive for 11 loci and negative for BP28 of the studied loci which ranged from 0.041 showed by BP28 locus to 0.696 showed by MMP9 locus.This level of inbreeding may be a result of high levels of mating between closely related individuals under field conditions. On the other hand inbreeding values in comparison with Mehsana (*Fis*=0.16; Aggarwal *et al*., 2007) and Jamunapari (*Fis*=0.19; Gour *et al*., 2006) breeds of India, Low inbreeding values were also reported within 45 rare breeds of 15 European and Middle Eastern countries (Caňón *et al*., 2006). Meanwhile some of the Indian breeds showed significant inbreeding such as Marwari (*Fis*=0.26; Kumar *et al*., 2005) and Kutchi (*Fis*=0.23; Dixit *et al*., 2008) breeds. Genetic differentiations quantified by Fst estimates ranged from 0.0102 showed by locus INRA49 to 0.169 showed by locus BP28 with an average of 0.12 showed in table 4. According to Hartl (1980), per pair FST value equals to 0.05 is indicative for moderate differentiation between populations. The pair FST values reported in the present investigation in all tested loci varied than 0.05, which may indicate a high differentiation between populations under investigation. The global Fit is 0.333 while, in this study Fit ranged from 0.0113 showed by MAF65 locus to 0.7290 showed by MMP9, which, indicated different degrees of genetic differentiation. This may be due to the high geographic distance between the areas in Egyptian and Saudi countries. Migration has a great effect on the reduction of genetic differentiation between populations (Laval *et al*., 2000). A great genetic differentiation between Saudi and the other two Egyptian goat breeds was found. From indirect estimates (Nm) of gene flow, where, the lowest Nm value was 0.9395 for the locus INRA49, while the highest value was 3.7734 for the locus MAF65.

The standard genetic distance and the UPGMA dendrogram of the populations are shown in Table 4 and in Figure 1, respectively. The highest genetic distance was found between *Barki* and *Ardi* breeds (0. 3825). The lowest value for genetic distance was found between *Zaraibi* and *Ardi* (0.2075) (Table4). High values for genetic identity means low values for genetic distance and vice versa.

Table 4: Genetic Identity and Genetic Distance (Nei 1972) for all loci and all breeds. Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

pop	*Barki*	*Ardi*	*Zaraibi*
Barki	****	**0.6821**	**0.8126**
Ardi	**0.3825**	****	**0.8280**
Zaraibi	**0.2075**	**0.1887**	****

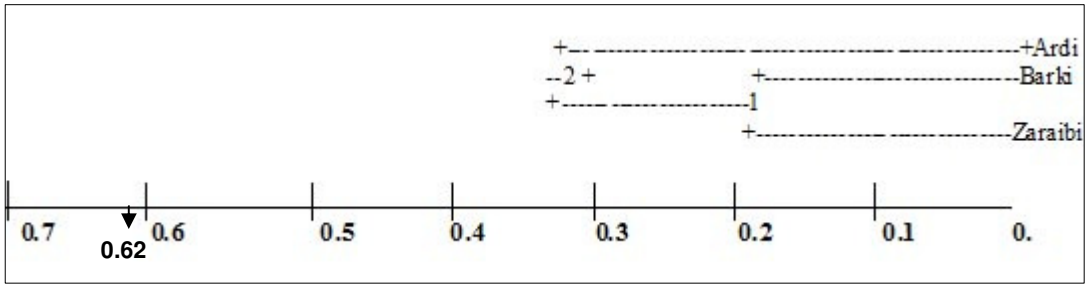

Fig 1. UPGMA dendogram generated from Nei s genetic distances of the three goat breeds

This study revealed the genetic distance matrix of the Egyptian *Barki* and *Zaraibi* goats is near or close areas while *Ardi* goats so far geographically (Saudi) from the Egyptian region Finally, the genetic relationship dendrogram showed that the *Barki* and *Zaraibi* goats are from a common ancestor (the same cluster) while the *Ardi* came from another ancestor or cluster. In addition, the cluster analysis obtained from Nei's

dendogram confirmed the closeness of *Zaraibi* and *Barki*; both clustered independently from *Ardi* breed at 0.62 of genetic distance.

CONCLUSION

This work has demonstrated that 12 microsatellite markers used in the present study were shown to be polymorphic. The *Ardi* goat showed high levels of genetic diversity from the Egyptian goats (*Barki*, *Zaraibi*). The markers used in this study are useful for the molecular characterization of Egyptian and Saudi goats. The information elucidated through the present study would be useful for the formulation of effective conservation strategies. This study is the first report using microsatellite analysis to understand the genetic diversity of the Saudi and Egyptian goat and different between them, this information is very important for meeting the demands of future breeding programs as well as for formulating effective conservation strategies for genetic diversity within breeds. Although we have used only three representative goat breeds to understand the genetic backgrounds of domestic goats in the two countries the present study contributes to the knowledge and genetic characterization of Egyptian and Saudi goat breeds.

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