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Full Length Research Paper

Analysis of genetic variation in different sheep breeds using microsatellites

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Genetic variation in three Egyptian indigenous sheep breeds namely: Barki, Ossimi and Rahmani were investigated using fourteen microsatellite loci. The total number of alleles ranged from 6 in CSSM47 locus to 14 in TGLA 377 locus. The fourteen tested loci were all polymorphic in the three breeds. Major differences between the breeds were found at ten of the tested loci, where the alleles at the highest frequency are different in the three breeds. While, at loci OARCP20, OARVH72, CSSM47 and OARAE129, two of the tested breeds have similar alleles at the highest allele frequency. The average direct count of heterozygosity overall loci in each tested breed was less than the expected heterozygosity. Tests of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE), at each locus overall breeds, revealed significant departure from HWE due to heterozygote deficiency. A slightly high rate of inbreeding within the three breeds was noticed (global F_{IS} = 0.308). Low genetic differentiation was detected by estimation of F_{ST} index between all pairs of breeds. Cluster analysis revealed that Ossimi and Rahmani breeds clustered independently from Barki breed at 0.43 of genetic distance. The obtained results can be useful for the development of a rational breeding strategy for genetic improvement of sheep in Egypt.

Key words: Microsatellites, sheep, genetic, diversity.

INTRODUCTION

Species are the most recognized and protected units of biodiversity. Yet we tend to ignore the importance of genetic diversity that is fundamental to species survival, and to the continued evolution of new species (Crawford and Littlejohn, 1998). Genetic diversity is shaped by past population processes and affects the sustainability of species and populations in the future (Soule, 1987). The maintenance of genetic diversity is a key to the long-term survival of most species (Hall and Bardley, 1995). Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objective (Crawford and Littlejohn, 1998; Kumar et al., 2006).

Sheep contribute 6% of the total red meet produced in Egypt. The total sheep population in Egypt is 4,200,000 heads. Barki, Ossimi and Rahmani are of the main sheep breeds in Egypt with a population of 470,000, 514,000 and 990,000, respectively (Galal et al., 2005). Barki is found in the Mediterranean coastal strip west of Alexandria; Rahmani is found in the Northern Delta (middle of Nile Delta), whereas Ossimi is found in South of Nile Delta. Egyptian sheep breeds are fat tailed and their body covered with carpet wool. Each breed has its productive characteristics. The mature weight of Rahmani and Ossimi is higher than that of Barki. The fleece weight and quality are higher in Barki than that in Ossimi and Rahmani (Galal et al., 2005). Barki breed is well adapted to desert conditions (Aboul Naga, 1976), while Ossimi has a wider range of adaptability than Barki. Rahmani is believed to be more resistant/tolerant to internal parasites than other Egyptian breeds. Also, the twinning rate is relatively high in Rahmani breed. Although the lactation period is longer in Barki, total yield of milk is about the same in the three breeds (Aboul Naga and El Shobokshy, 1981).

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The genetic diversity of indigenous sheep in Egypt has not been sufficiently studied. Genetic characterization and determination of genetic differences between sheep breeds will help in the genetic improvement programs.

Molecular methods and molecular markers, such as microsatellites, are useful tools to study the genetic variations. Microsatellites are stable, polymorphic, easy to analyze and occur regularly throughout an animal genome. Microsatellites are co-dominant markers, so that all alleles can be scored. The availability of microsatellites markers has facilitated genetic linkage studies; including mapping and searching for genes affecting productive traits as well as estimating genetic diversity in farm animals (Crawford and Littlejohn, 1998; Jouquand et al., 2000; Moioli et al., 2001; Kumar et al., 2006). Several studies had investigated the genetic diversity in sheep using microsatellites (Diez-tascon et al., 2000; Hassan et al., 2003; Arora and Bhatia, 2004; Elfawal, 2006; Gutierrez-Gil et al., 2006).

In the present study, a set of thirteen microsatellite markers were chosen from the microsatellites list recommended by FAO (2004) to evaluate the genetic diversity within and between three sheep breeds (Barki, Ossimi, and Rahmani) reared in Egypt as a basis for development of rational breeding and for maintenance of adequate genetic diversity within indigenous sheep breeds. An additional microsatellite, TGLA377 was chosen from a set of microsatellites used for the construction of the sheep linkage map (Georges and Massey, 1992).

MATERIALS AND METHODS

Blood sampling and DNA extraction

Fifty adult unrelated sheep of both sexes, representing three indigenous breeds namely: Barki (n = 18), Ossimi (n = 16) and Rahmani (n = 16) were selected from distant located experimental stations belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, where random-mating strategies are employed. To minimize the likelihood of any close genetic relationships, the number of the breed samples from each station was restricted deliberately.

Blood samples were collected from the jugular vein into ethylenediamine tetra-acetic acid (EDTA)-containing vacuutainer tubes. DNA was extracted from fresh blood according to established protocols (Blin and Stafford, 1976). DNA concentration was determined using a UV spectrophotometer (Pharmacia LKB-Ultraspec III) at optical density of 260 nm.

Microsatellite polymorphism detection

The selected fourteen microsatellites (Table 1) were amplified with polymerase chain reaction (PCR) using genomic DNA extracted from individual animals. The PCR was performed for each locus in 25 µl reaction mixture consisting of 0.2 mM dNTPs, 10 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 1 µM upper and lower primers, 0.125 units Taq polymerase and 100 ng DNA. The reaction mixture was overlaid with sterile mineral oil and was run in an MJ Research PTC-100 thermocycler. The reaction cycled for 1 min at 94ºC, 2 min at an optimized annealing temperature which was determined for each primer (www.marc.usda.gov/) (Table 1), and 2 min at 72ºC for 35 cycles. Following the completion of the PCR cycles, the reaction products and the appropriate DNA marker were subjected to electrophoresis in 10% non-denaturing polyacrylamide gel and stained with ethidium bromide (Crawford and Littlejohn, 1998). The gel was photographed after visualization using UV transilluminator (Bachofer D-7410). Patterns of the different genotypes for each microsatellite locus were analyzed using 'Gel – Pro analyzer, version 3.1 for windows™, which determine the alleles sizes in each animal.

Statistical analysis

Allele frequencies at each locus for each breed were calculated using a computer program FSTAT (version 2.9.3) (Goudet, 1995). FSTAT program was also used to calculate F-statistics that include three indices F_{IT}, F_{ST} and F_{IS}. F_{IT} is the inbreeding coefficient of an individual (I) relative to the total (T) population, F_{IS} is the inbreeding coefficient of an individual (I) relative to the subpopulation (S) and F_{ST} is the effect of subpopulation (S) compared to the total population (T). Weir and Cockerham (1984) estimation of F_{IT}, F_{IS} and F_{ST} were performed for every locus overall breeds. Per pair estimator of F_{ST} (measure of differentiation among populations) were also calculated between all pairs of the tested breeds.

GENPOP version 3.1 (Raymond and Rousset, 1995) was used for calculating the observed and expected heterozygosity overall loci in each of the three breeds (Levene, 1949; Nei, 1978) and for testing for deviation from HWE at each locus overall breeds. The genetic distance (D_A) was estimated according to the method of Nei (1978) and the unweighted pair group method with arithmetic mean (UPGMA) was used for dendogram construction using the same program.

RESULTS

The number of alleles for each of the fourteen microsatellite loci in each of the three breeds is presented in Table 2. The total number of detected alleles varied from 6 (CSSM47) to 14 (TGLA377). The mean numbers of alleles per locus are 8.2, 5.7 and 7.2 in Barki, Ossimi and Rahmani, respectively. The mean number of alleles shared between Barki and Ossimi is 4.3, between Barki and Rahmani is 6.0 and between Ossimi and Rahmani is 4.2, whereas the mean number of the alleles shared by the three breeds is 3.5. The most noticeable difference is found at BM827 locus, where only 1 allele, out of a total of 13 alleles, is shared by the three breeds. On the other hand, 5 alleles, out of the total of 8 alleles, are shared by the three breeds at OARCP20 and CSSM31 loci. It is also noticed that all alleles representing the OARCP20, OARCP34 and OARJHP8 loci are present in Barki breed. While all alleles of the CSSM47 and OARHH64 Loci are present in Rahmani breed.

Table 3 presents the alleles frequency distribution at the analyzed loci in the three breeds. At loci OARCP20, OARVH72 and OARAE129, the same alleles are at the highest frequency in Ossimi and Rahmani breeds. While, Barki and Ossimi breeds have similar alleles at the highest allele frequency at locus CSSM47. For the other ten loci, alleles at the highest frequency are different in the three breeds.

The average direct count of heterozygosity (observed

Table 1. Characteristics of the microsatellites under investigation.

Name	Primer sequence	Accession no.	Type of repeat	Allelic range	Annealing Temp.	References
TGLA137	gttgacttgttaatcactgacagcc / ccttagacacacgtgaagtcc ac	Not available	Not available	132 -148	55°C	Georges and Massey, 1992
OARCP20	gatcccctggaggaggaaacgg / ggcatctcatggctttagcagg	U15695	gt	Not available	63°C	Ede et al., 1995
OARHH35	aattgcattcagtatctttaaacatctggc / atgaaaatataaagagaatgaaccacacgg	L12554	gt	121-137	54°C	Henry et al., 1993
OARCP34	gctgaacaatgtgatatgttcagg / gggacaatactgtcttagatgctgc	U15699	gt	Not available	52°C	Kappes et al., 1997
OARVH72	ggcctctcaaggggcaagagcagg / ctctagaggatctggaatgcaaagctc	L12548	gt	Not available	56°C	Pierson et al., 1993
OARJMP8	cgggatgatcttctgtccaaatatgc / catttgctttggcttcagaaccagag	U35059	gt	not available	58°C	Kappes et al., 1997
CSSM47	tctctgtctctatcactatatggc / cagggcacctgaaactatcatcat	U03821	gt	130-182	58 °C	Kappes et al., 1997
OARHH64	cgttccctcactatggaaagttatatatgc / cactctattgtaagaatttgaatgagagc	L12558	gt	Not available	Not available	Henry et al., 1993
CSSM31	ccaagtttagtacttgtaagtaga / gactctctagcactttatctgtgt	U03838	ac	not available	58°C	Moore et al., 1994
TGLA377	gactgtcattatcttccagcggag / agactttggatctctggttgaaatg	Not available	gt	Not available	54°C	Georges and Massey, 1992
BM827	gggctggtcgtatgctgag / gttggacttgctgaagtgacc	U06763	ac	212-224	58°C	Bishop et al., 1994
OARAE129	aatccagtgtgtgaaagactaatccag / gtagatcaagatatagaatatttttcaacacc	L11051	ac	Not available	56°C	Penty et al., 1993
OARHH47	tttattgacaaactctcttcctaactccacc / gtagttatttaaaaaaatatcatacctcttaagg	L12557	ac	130-152	56°C	Henry et al., 1993
BM6526	catgccaaacaatatccagc / tgaaggtagagagcaagcagc	G18454	ac	161-175	56°C	Bishop et al., 1994

Table 2. Number of alleles at each microsatellite locus in the three breeds and the number of alleles shared between breeds.

Locus	Number of alleles							
	Total	Barki	Ossimi	Rahmani	Shared by Barki-Ossimi	Shared by Barki- Rahmani	Shared by Ossimi- Rahmani	Shared by all
TGLA137	13	10	7	10	4	8	6	4
OARCP20	8	8	6	7	6	7	5	5
OARHH35	13	12	9	7	9	6	6	6
OARCP34	10	10	7	6	7	6	4	4
OARVH72	9	6	5	6	4	4	3	3
OARJMP8	7	7	3	6	3	6	5	3
CSSM47	6	5	4	6	3	5	4	3
OARHH64	8	7	4	8	3	7	4	3
CSSM31	8	7	7	6	6	5	6	5
TGLA377	14	6	7	8	2	4	3	2
BM827	13	10	4	4	2	3	1	1
OARAE129	10	9	4	7	3	7	3	3
OARHH47	13	9	7	9	3	7	4	2
BM6526	12	9	6	11	5	9	5	5
Mean	10.3	8.2	5.7	7.2	4.3	6	4.2	3.5

Table 3. Allele frequencies at each microsatellite locus in the three sheep breeds.

Allele no.	TGLA137			Allele no.	OARCP20		
	Barki	Ossimi	Rahmani		Barki	Ossimi	Rahmani
1	0.136	0.000	0.042	1	0.087	0.000	0.167
2	0.045	0.063	0.167	2	0.013	**0.313**	**0.250**
3	0.091	**0.250**	0.083	3	**0.239**	0.250	0.000
4	0.000	0.125	0.000	4	0.065	0.063	0.167
5	0.000	0.125	0.083	5	0.065	0.125	0.167
6	0.045	0.000	**0.167**	6	0.152	0.188	0.125
7	0.091	0.188	**0.167**	7	0.196	0.063	0.083
8	0.136	0.000	0.000	8	0.065	0.000	0.042
9	**0.273**	0.000	0.000				
10	0.045	0.000	0.083				
11	0.091	0.063	0.083				
12	0.000	0.188	0.083				
13	0.045	0.000	0.042				
Allele no.	**OARHH35**			**Allele no.**	**OARCP34**		
	Barki	**Ossimi**	**Rahmani**		**Barki**	**Ossimi**	**Rahmani**
1	0.053	0.056	0.167	1	0.083	0.000	0.100
2	0.105	0.111	0.000	2	0.167	0.083	0.100
3	0.026	0.167	0.083	3	0.083	**0.250**	0.100
4	0.105	0.056	**0.250**	4	0.111	0.167	0.100
5	**0.237**	0.056	0.000	5	0.028	0.000	0.000
6	0.053	0.000	0.000	6	0.056	0.000	0.150
7	0.026	0.056	0.083	7	0.083	0.083	0.000
8	0.026	**0.278**	**0.250**	8	0.083	0.167	**0.450**
9	0.079	0.167	0.000	9	0.111	0.083	0.000
10	0.000	0.000	0.083	10	**0.194**	0.167	0.000
11	0.184	0.056	0.083				
12	0.053	0.000	0.000				
13	0.053	0.000	0.000				
Allele no.	**OARVH72**			**Allele no.**	**OARJMP8**		
	Barki	**Ossimi**	**Rahmani**		**Barki**	**Ossimi**	**Rahmani**
1	0.083	0.000	0.150	1	0.147	0.250	0.000
2	0.167	0.150	0.150	2	0.118	0.000	0.071
3	0.000	0.000	0.100	3	0.178	0.000	0.071
4	0.278	**0.450**	**0.350**	4	0.029	0.000	0.357
5	**0.306**	0.100	0.100	5	0.176	**0.625**	**0.357**
6	0.000	0.250	0.000	**6**	**0.206**	0.125	0.071
7	0.000	0.000	0.150	7	0.147	0.000	0.071
8	0.111	0.000	0.000				
9	0.056	0.050	0.000				
Allele no.	**CSSM47**			**Allele no.**	**OARHH64**		
	Barki	**Ossimi**	**Rahmani**		**Barki**	**Ossimi**	**Rahmani**
1	0.238	0.250	**0.273**	1	0.119	0.125	0.188
2	**0.286**	**0.375**	0.136	2	0.071	0.000	0.313
3	0.238	0.000	0.091	3	0.190	0.125	0.063
4	0.119	0.250	0.136	4	0.190	0.375	0.063
5	0.119	0.000	0.182	5	**0.238**	0.000	**0.125**
6	0.000	0.125	0.182	6	0.119	0.000	0.063
				7	0.071	0.000	**0.125**
				8	0.000	**0.375**	0.063

Table 3. Continued.

Allele no.	CSSM31			Allele no.	TGLA377		
	Barki	Ossimi	Rahmani		Barki	Ossimi	Rahmani
1	0.000	0.214	0.222	1	0.000	0.375	0.000
2	0.211	0.000	0.000	2	0.000	0.071	0.000
3	0.184	**0.286**	**0.222**	3	0.000	0.000	**0.278**
4	0.026	0.071	0.167	4	0.000	0.000	0.167
5	0.184	0.143	0.111	5	0.000	0.143	0.000
6	0.053	0.071	0.000	6	0.000	0.071	0.056
7	**0.289**	0.143	0.167	7	0.000	**0.214**	0.000
8	0.053	0.071	0.111	8	0.125	0.000	0.000
				9	**0.250**	0.071	0.111
				10	0.125	0.071	0.056
				11	**0.250**	0.000	0.000
				12	0.125	0.000	0.167
				13	0.000	0.000	0.111
				14	0.125	0.000	0.056

Allele no.	BM827			Allele no.	OARAE129		
	Barki	Ossimi	Rahmani		Barki	Ossimi	Rahmani
1	0.083	0.000	0.000	1	0.083	0.000	0.000
2	0.042	0.000	0.000	2	0.000	0.125	0.000
3	0.167	0.000	0.000	3	**0.417**	0.000	0.100
4	0.167	0.000	0.000	4	0.042	**0.500**	0.350
5	0.083	0.000	0.000	5	0.042	0.125	0.100
6	0.000	0.214	0.000	6	0.042	0.000	0.000
7	0.000	0.143	0.000	7	0.083	0.000	0.100
8	**0.208**	0.000	0.000	8	0.208	0.250	0.050
9	0.083	0.000	0.100	9	0.042	0.000	**0.250**
10	0.042	**0.500**	0.300	10	0.042	0.000	0.050
11	0.083	0.143	0.000				
12	0.000	0.000	0.200				
13	0.042	0.000	**0.400**				

Allele no.	OARHH47			Allele no.	BM6526		
	Barki	Ossimi	Rahmani		Barki	Ossimi	Rahmani
1	0.154	0.000	0.071	1	0.167	0.125	0.150
2	0.038	0.063	**0.143**	2	0.042	0.000	0.150
3	0.115	0.000	**0.143**	3	0.000	0.000	0.050
4	0.000	0.125	0.071	4	0.042	0.000	0.050
5	0.077	0.000	0.071	5	0.000	0.125	0.000
6	0.000	0.125	0.000	6	0.083	0.125	0.100
7	0.077	0.188	**0.143**	7	0.042	**0.250**	0.050
8	0.115	0.188	0.000	8	**0.250**	**0.250**	0.050
9	0.154	0.000	0.000	9	0.000	0.000	0.050
10	**0.192**	0.000	0.071	10	0.167	0.000	**0.200**
11	0.077	0.000	**0.143**	11	0.083	0.125	0.100
12	0.000	0.063	**0.143**	12	0.125	0.000	0.050
13	0.000	**0.250**	0.000				

The highest allele frequency / breed is in bold typeface

heterozygosity) overall loci in Barki, Ossimi and Rahmani breeds are 0.590, 0.547 and 0.615, respectively. Whereas the average expected heterozygosity overall loci in the three breeds are 0.860, 0.811 and 0.855, respectively (Table 4). These results show less hetrozygosity than expected in each breed.

Table 4. Mean heterozygosity in the three sheep breeds.

Heterozygosity	Barki	Ossimi	Rahmani
Mean observed heterozygosity ± SD	0.5905 ± 0.2906	0.5471 ± 0.2238	0.6157 ± 0.2284
Mean expected heterozygosity ± SD	0.8600 ± 0.0417	0.8111 ± 0.0899	0.8551 ± 0.0607

Table 5. Fit, Fst and Fis values and chi-square test for HWE for each locus over all breeds.

Locus	Fit	Fst	Fis	x^2 (Degrees of freedom)
TGLA137	0.379	0.018	0.368	245.7*** (78)
OARCP20	0.148	0.012	0.137	61.0*** (28)
OARHH35	0.110	0.033	0.080	157.6*** (78)
OARCP34	0.249	0.028	0.227	88.5*** (45)
OARVH72	0.552	0.015	0.546	129.5*** (36)
OARJMP8	0.223	0.064	0.169	69.6*** (21)
CSSM47	- 0.115	0.014	- 0.130	30.2* (15)
OARHH64	0.556	0.016	0.549	94.1*** (28)
CSSM31	0.203	0.011	0.194	94.1*** (28)
TGLA337	0.568	0.068	0.536	244.0*** (91)
BM827	0.780	0.112	0.753	336.2*** (78)
OARAE129	0.022	0.127	- 0.121	64.1* (45)
OARHH47	0.470	0.011	0.464	190.4*** (78)
BM6526	0.446	- 0.015	0.454	135.6*** (66)
Over all loci	0.333	0.037	0.308	

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

Table 6. Per pair Fst values between all pairs of the tested breeds.

Breed	Barki	Ossimi
Barki		
Ossimi	0.0462	
Rahmani	0.0352	0.0215

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 5).

F-statistics for each locus overall breeds are given in Table 5. The highest within population inbreeding coefficient (F_{IS}) value is observed at BM827 locus while the highest F_{ST} and F_{IT} values are observed at TGLA377 and BM827 loci. The global F_{IS}, F_{ST} and F_{IT} are 0.308, 0.037 and 0.333, respectively.

Per pair estimator of F_{ST}, which is the measure of differentiation among population, is 0.046 between Barki and Ossimi, 0.035 between Barki and Rahmani and 0.021 between Ossimi and Rahmani (Table 6).

The calculated genetic distance matrix is shown in Table 7. The distance between Ossimi and Rahmani is smaller than the distance between Barki and Ossimi and between Barki and Rahmani.

UPGMA dendogram generated from Nei's genetic distance of the three sheep breeds is illustrated in Figure 1. The cluster analysis shows that Ossimi and Rahmani breeds cluster independently from Barki breed at 0.43 of genetic distance.

DISCUSSION

The study of genetic variation plays an important role in developing rational breeding strategies for economical animal species (Maudet et al., 2002). The advantage of the use of microsatellites for estimating genetic variations among breeds and among closely related populations has been investigated in farm animals such as: water buffalo (Barker et al., 1997; Moioli et al., 2001; Kumar et al., 2006), goat (Barker et al., 2001, Maudet et al., 2002; de Araujo et al., 2006) and cattle (MacHugh et al., 1997). Microsatellites have also been used to investigate sheep breeds. Study of genetic variation among Turkish sheep breeds using microsatellite loci indicated a high level of variation in the tested breeds (Gutierrez-Gil et al., 2006). Genetic characterization of Muzzafarnagri, the heaviest sheep breed in India, was established on the basis of individual genotypes at microsatellite loci (Arora and

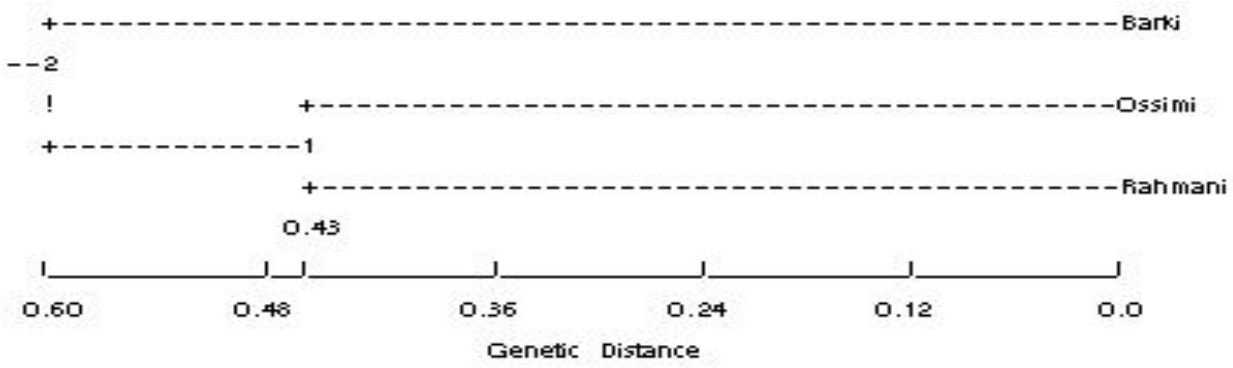

Figure 1. UPGMA dendogram generated from Nei's genetic distances of the three sheep breeds.

Table 7. Nei's genetic distances between the studied breeds.

Breed	Barki	Ossimi
Barki		
Ossimi	0.5674	
Rahmani	0.5059	0.4058

Bhatia, 2004). Also, Diez-tascon and colleagues (2000) distinguished between related populations of Merino sheep breed on the basis of microsatellite analysis. In Egypt, preliminary studies were performed to evaluate the genetic diversity of Egyptian sheep breeds. Hassan et al. (2003) used four microsatellites to study the genetic diversity of Barki, Ossimi and Rahmani breebs. Also, Elfawal (2006) studied the diversity of Ossimi, Rahmani and saidi breeds using other four microsatellites.

In the present study fourteen microsatellite loci were used to evaluate the genetic diversity within and between Barki, Ossimi and Rahmani sheep breeds reared in Egypt. The fourteen microsatellites are all polymorphic in the three breeds. Major differences between the three breeds were observed. Ten of the tested loci have different alleles at the highest allele frequency. The rest of the loci used in the present study, OARCP20, OARVH72, CSSM47 and OARAE129, have similar alleles at the highest allele frequency in only two of the tested breeds. The use of microsatellites to evaluate the genetic diversity on the basis of allele frequency distribution has also been employed to differentiate between Italian, Greek and Egyptian buffalo populations (Moioli et al., 2001).

The average expected heterozygosity overall loci in Barki, Ossimi and Rahmani are 0.860, 0.811and 0.855, respectively. High value of average expected heterozygosity within the breed could be attributed to the large allele numbers detected in the tested loci (Kalinwski, 2002). The average direct count of heterozygosity overall loci in each of the three sheep breeds is less than the expected heterozygosity. This finding is an evidence for the presence of overall loss in heterozygosity within the three tested breeds (allele fixation) (de Araujo et al., 2006).

Tests of genotype frequencies for deviation from HWE at each locus over all breeds, revealed significant departure from HWE. Deviation from HWE at microsatellites loci have, also been reported in various studies (Barker et al., 2001; Laval et al., 2000; LuiKart et al., 1999; Hassan et al., 2003; Elfawal et al., 2006). It is known that a population is considered to be within HWE only when it is able to maintain its relative allele frequencies. Heterozygosis deficiency is one of the parameters underlying departure from HWE. Heterozygosis deficiency may result from one or more of the following reasons: i) the presence of a null allele which is the allele that fails to multiply during PCR using a given microsatellite primer due to a mutation at the primer site (Callen et al., 1993; Pemberton et al., 1995); ii) small sample size, where rare genotypes are likely to be included in the samples; iii) the Wahlund effect, i.e. presence of fewer heterozygotes in a population than predicted on account of population subdivision; iv) the decrease in heterozygosity due to increased consanguinity (inbreeding) (Kumar, 2006).

The global inbreeding coefficients F_{IS} (0.308) and F_{IT} (0.333) observed in the present study indicate that the three breeds (Barki, Ossimi and Rahmani) are slightly heading towards inbreeding. This result may explain the observed low value of direct count of heterozygosity in each breed and the deviation from HWE which were detected in all loci overall breeds.

According to Hartl (1980), per pair F_{ST} value equals to 0.05 is indicative for moderate differentiation between populations. The per pair F_{ST} values reported in the present investigation between all pairs of the tested breeds are less than 0.05 which may indicate a low differentiation between populations under investigation. However, the obtained F_{ST} value between Ossimi and Rahmani is found to be lower than that between Barki and Ossimi and Barki and Rahmani. A possible cross migration between Ossimi and Rahmani may occur due to the short geographic distance between the areas in

which these two breeds are distributed. Migration has a great effect on the reduction of genetic differentiation between populations (Laval et al., 2000). Also, the cluster analysis obtained from Nei's dendogram confirmed the closeness of Ossimi and Rahmani; both clustered independently from Barki breed at 0.43 of genetic distance.

In conclusion, the tested microsatellites, being all polymorphic in the three breeds, could be fruitfully used for the differentiation between breeds and for further research for the detection of quantitative trait loci. The evaluation of genetic variations within and between Barki, Ossimi and Rahmani sheep breeds may be used as basis for the development of rational breeding strategy for genetic improvement of these breeds. Results of the present investigation also suggests that the three breeds (Barki, Ossimi and Rahmani) are slightly heading towards inbreeding. This in its turn may raise concern for adopting more sound strategies for breeding sheep in Egypt.

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