MICROSATELLITE POLYMORPHISM IN THREE EGYPTIAN SHEEP BREEDS

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SUMMARY

Four microsatellite markers were used to investigate genetic variations among and within three Egyptian sheep breeds; Rahmani, Ossimi, and Saidi. The four microsatellites were OARAE101, BM1329, INRA63 and OARFCB20. All markers tested were found to be polymorphic. The total observed numbers of alleles in the three breeds were 5, 7, 12 and 12 for the four markers respectively, while polymorphism information content (PIC) was 0.660, 0.755, 0.574, and 0.847, respectively. The three breeds showed significant deviation from Hardy-Weinberg equilibrium. The genetic distance test showed that the three breeds had the same cluster of origin but Rahmani showed separate sub-cluster while Ossimi and Saidi are separated together from the other sub-cluster. The average gene diversity was 0.75 for all studied breeds.

Keywords: sheep, microsatellites, Egyptian breeds, Rahmani, Ossimi, Saidi, diversity

INTRODUCTION

Recent developments in molecular biology enable scientists to develop many useful types of DNA genetic markers to identify genetic variation among and within livestock breeds at the DNA level. Microsatellites are among such markers. They consist of tandem repeats of short sequence motifs, from 2-6bp up to 150bp in length, and were first discovered by Hamada and Kakunaga (1982). A large number of highly polymorphic microsatellites has been characterized and used in mapping quantitative trait loci (Ashwell *et al*., 2004) and studying the genetic diversity and phylogeny between and within animal breeds (Diez-Tascon *et al*. 2000; Arranz *et al*. 2001; Jia *et al*. 2003; Tapio *et al*. 2003)

In Egypt, sheep contribute 6% of the total red meat production (Galal *et al*., 2005) in addition to their production of wool and manure. Egyptian sheep breeds are fat tailed, their body covered with carpet wool, and are medium in size. Rahmani and Ossimi are among the major breeds in Egypt while Saidi is one of the minor breeds (Galal *et al*., 2005).

Reviewed literature showed only a single study by Hassan *et al*. (2003) that dealt with the genetic diversity of some microsatellite markers in Ossimi, Rahmani and Barki sheep breeds. The present study (2006) was conducted to investigate the genetic diversity for some sheep breeds using microsatellite markers.

MATERIALS AND METHODS

Animals

Hundred adult sheep of both sexes were sampled from two experimental stations, (El-Serw and Seds) belonging to the Animal Production Research Institute (APRI), Egyptian Ministry of Agriculture and Land Reclamation. The system of three matings (Juauary, September and May) per two years was followed where lambs were dropped in June, February and October. Ewes were assigned to the mating groups randomly with the avoidance of sib, ram-daughter and ewe-son matings (Shaat *et al*., 2004). The numbers within each breed were 35 Ossimi, 32 Rahmani and 33 Saidi. Animals were unrelated up to the third generation.

Blood sampling and DNA isolation

Ten milliliters of peripheral blood were collected from the animal jugular vein using vacutainer tubes having EDTA as anticoagulant. DNA was extracted using salting out procedure described by Miller *et al*. (1988).

Microsatellite information

The microsatellite markers investigated in this study were selected according to MoDAD (FAO, 2004) for the genetic diversity studies. Details of these markers are presented in Table 1.

Table 1. Forward and reverse primer sequences for the studied microsatelites

Marker name	Primer sequence	Reference
BM1329	TTGTTTAGGCAAGTCCAAAGTC AACACCGCAGCTTCATCC	Bishop *et al*. (1994)
OARAE101	TAAGAAATATATTTGAAAAAACTGTATCTCCC TTCTTATAGATGCACTCAAGCTAGG	Montgomery *et al*. (1993)
INRA063	ATTTGCACAAGCTAAATCTAACCAAACC ACAGAAATGCTTGGAAG	Vaiman *et al*. (1994)
OARFCB20	AAATGTGTTTAAGATTCCATACAGTG GGAAAACCCCCATATATACCTATAC	Buchanan *et al*. (1994)

PCR mastermix and run information

The PCR reaction volume was 20µl for each sample. The PCR mastermix formula contained 50ng/ µl from the DNA template, 10X PCR buffer that included 1.5 mM MgCl2 and 10 pmol from each forward and reverse primers and 200 µM final concentration from each dNTP.The initial PCR cycle was 95 °C for 3 min then 35 cycles at 95 °C for 15 sec., 55-60 °C for 30-60 sec., 72°C for 30 sec. and final extension: 72 °C for 5 min., storage at 15 °C. The PCR products were tested for success on 2% agarose in TAE buffer in a horizontal electrophoresis chamber and stained with ethidium bromide. The successful runs were subjected to the vertical

electrophoresis run on 12% polyacrylamide. The polyacrylamide gels were stained with ethidium bromide and the images were captured using gel documentation system. Allelic sizes were determined using free software Lab. Image V2.7 dispersed free from Proland company (Germany) at http://www.labimaging.com/servlet/engine/home/start.html. Preparations and staining of the polyacrylamide gels were done using the protocol described by Sambrook *et al*. 1998.

Statistical analysis

POPGENE Version 1.31 package was used for estimating allele frequencies, observed and expected numbers of alleles, observed and expected heterozygosity (Nei, 1978), average gene diversity, genetic identity, genetic distance (Nei, 1978), deviation from Hardy-Weinberg using chi square test, and phylogenic analysis using Nei's distance (Nei, 1987). Average gene diversity was calculated according to Nei (1987) for each breed and for all breeds together. The Polymorphism Information Content (PIC), i.e. the probability that one parent is a marker informative and its progeny has different genotype (Lynch and Walsh, 1998), was estimated according to Botstein *et al*. (1980) as

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n} \sum_{j=i+1}^{n-1} P_i^2 P_j^2 ,$$

F-statistics, FIS (the inbreeding coefficient of an individual related to the subpopulation), FIT (the inbreeding coefficient of an individual related to the whole population) and FST (genetic differentiation estimates or the average inbreeding of the subpopulation related to the whole population) were calculated using the updated version from FSTAT software, version 2.9.3.2 (Goudet, 1995). The Tree View 32 software (Page, 1996) was employed to draw the dendrogram showing the genetic distances and relationships between the breeds understudy.

RESULTS AND DISCUSSION

Observed number of alleles

All microsattelites used in the present study showed polymorphism in all the studied breeds. Table 2 presents the number of observed alleles for each of the four microsattelite loci. The total number of detected alleles was 5 for BM1329, 7 for OARAE101 and 12 for each of INRA063 and OARFCB20. Histograms 1 to 4 show the allele frequencies in the populations under study. In the present study the bovine microsattelite BM1329 showed 5 alleles ranging in size from 168 to186 bp, while this microsatellite showed 6 alleles in the Chinese sheep breeds (Chu *et al*., 2001) and 9 alleles in Blanca Andaluza goat (Martinz *et al*., 2004). Although this marker is from bovine origin, it cross-hybridized with sheep and goat DNA. FAO (2004) recommended it for the use in sheep and goat genetic diversity studies. The microsatellite OARAE101 showed 7 alleles ranging in size from 105 to123 bp, while it showed 9 alleles in the Chinese sheep breeds (Chu *et al*., 2001), and 4 alleles in Small Tail Han sheep (Chu *et al*., 2002). The microsatellite INRA063 showed 12 alleles with a size range 163~211 bp, while this microsatellite showed 19 alleles in the Swiss sheep breeds by Saitbekova *et al*. (2000) and 7 alleles in Swiss goat breeds (Saitbekova *et al*., 1999). Finally, the microsattelite OARFCB20 showed 12 alleles

ranging in size from 92 to 125 bp. This microsatellite showed 10 alleles in Rasa Aragonesa sheep of Spain (Arruga *et al.*, 2001).

Table 2. Observed number of alleles for different microsatelites and different breeds

Breed	Microsatellite allele				Total	Mean per breed
	BM1329	OARAE101	INRA063	OARFCB20		
Rahmani	5	3	10	8	26	6.5
Ossimi	5	6	11	9	31	7.75
Saidi	5	7	10	12	34	8.5

The data in Table 2 show that Saidi breed had the highest average number of alleles while Rahmani showed the lowest. Saidi sheep are named after Upper Egypt (Said) which extends from the south of Cairo to the border with Sudan. The breed having more alleles than the other two breeds could indicate that it is a mixture of populations. Phenotypically, both Rahmani and Ossimi sheep have uniform and distinct phenotypes from each other while Saidi have a mixture of colors, tail shapes and horns etc.

Allele frequencies

Details of the observed number of microsatellite alleles, their sizes and frequencies are presented in Tables from 3 to 6 and the Histograms from 1 to 4.

Table 3. Allele size and frequency for microsatellite BM1329

Allele number	Allele size (bp)	Allele frequency			
		Rahmani	Ossimi	Saidi	All breeds
1	166	0.437	0.343	0.410	0.396
2	174	0.203	0.057	0.060	0.104
3	176	0.016	0.143	0.091	0.084
4	182	0.016	0.414	0.167	0.203
5	186	0.328	0.043	0.273	0.213

Table 4. Allele size and frequency for microsatellite OARAE101

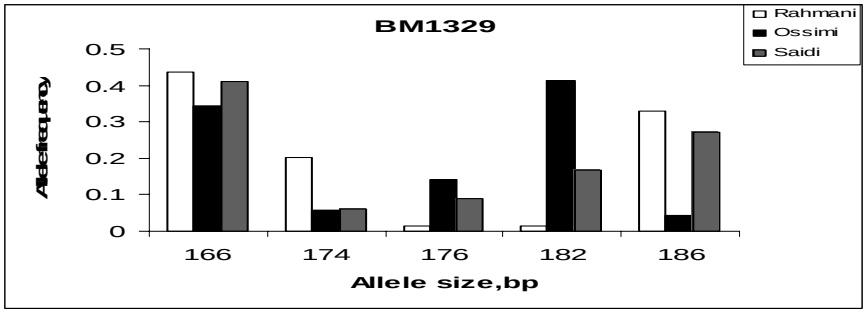
Allele number	Allele size (bp)	Allele frequency			
		Rahmani	Ossimi	Saidi	All breeds
1	105	0	0.028	0.030	0.020
2	108	0	0	0.136	0.046
3	111	0	0.023	0.182	0.071
4	114	0	0.040	0.091	0.047
5	117	0.445	0.310	0.140	0.306
6	120	0.055	0.030	0.060	0.036
7	123	0.500	0.580	0.361	0.474

Table 5. Allele size and frequency for microsatellite INRA63

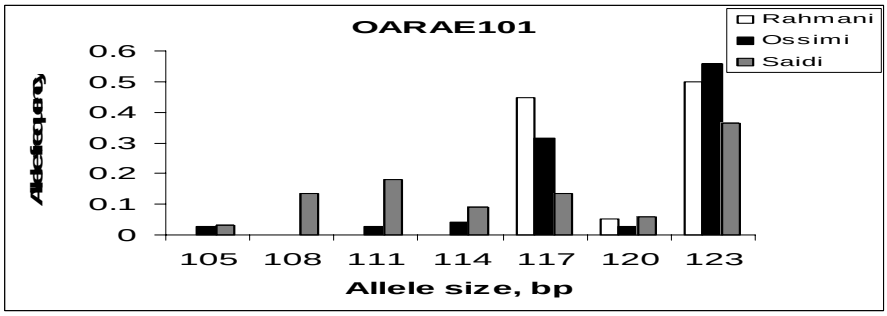
Allele no	Allele size (bp)	Allele frequency			
		Rahmani	Ossimi	Saidi	All breeds
1	163	0.094	0.143	0.136	0.119
2	165	0.016	0.086	0	0.038
3	169	0.016	0.043	0.015	0.025
4	175	0.313	0.257	0.106	0.228
5	179	0.063	0.029	0.015	0.030
6	185	0.078	0.157	0.060	0.109
7	189	0.125	0.014	0	0.055
8	195	0.172	0.1	0.242	0.163
9	197	0	0	0.030	0.010
10	199	0.094	0.086	0.136	0.104
11	201	0.031	0.029	0.030	0.030
12	211	0	0.057	0.227	0.094

Table 6. Allele size and frequency for microsatellite OARFCB20

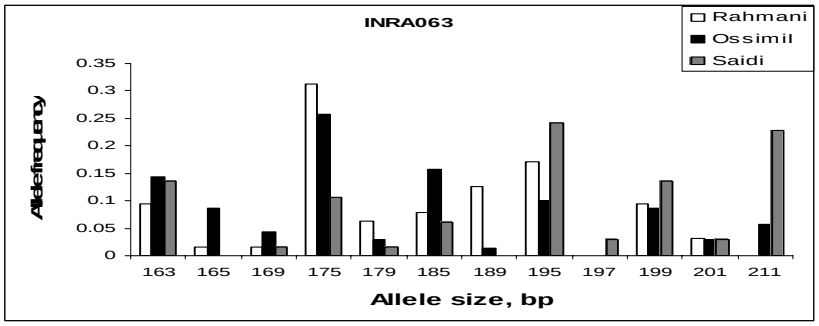
Allele number	Allele size (bp)	Allele frequency			
		Rahmani	Ossimi	Saidi	All
1	92	0	0	0.136	0.045
2	95	0	0.071	0.015	0.030
3	98	0	0.286	0.182	0.161
4	101	0.016	0.143	0.045	0.071
5	104	0.226	0.029	0.015	0.086
6	107	0.193	0.071	0.076	0.111
7	110	0.032	0.229	0.197	0.157
8	113	0.145	0.114	0.136	0.131
9	116	0.097	0.014	0.061	0.056
10	119	0.161	0	0.076	0.076
11	122	0.129	0.043	0.015	0.061
12	125	0	0	0.045	0.015



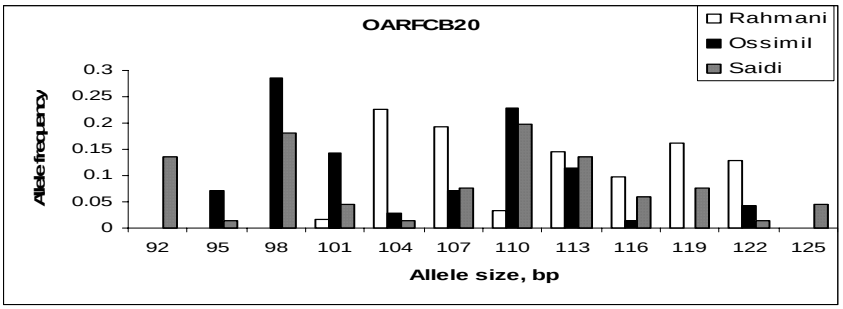
Histogram 1. Allele frequency for microsatellite BM1329 in Rahmani,Ossimi and Saidi breeds



Histogram 2. Allele frequency for microsatellite OARAE101 in Rahmani, Ossimi and Saidi breeds



Histogram 3. Allele frequency for microsatellite INRA063 in Rahmani, Ossimi and Saidi breeds



Histogram 4. Allele frequency for microsatellite OARFCB20 in Rahmani, Ossimi and Saidi breeds

Exclusive and prevailing alleles

Exclusive alleles refer to unique alleles or breed specific alleles while prevailing alleles refer to common alleles with the highest frequency among the populations. The exclusive alleles always have extreme value small or large frequency (Arranz *et al.* 2001). Exclusive alleles were reported in different sheep breeds. Hassan *et al.* (2003) reported that 6 alleles out of 40 were exclusive in some Egyptian sheep

breeds. Buchanan *et al*. (1994) reported exclusive alleles in Bighorn sheep, Forbes *et al*. (1995) found unique alleles in domestic and Bighorn sheep and Arranz *et al.* (2001) found breed specific alleles in Spanish Merino. Farid *et al.* (1999) found 17 out of 93 alleles in one breed with low frequency in some Canadian sheep breeds. Grigaliunaite *et al.* (2003) reported that from 175 alleles, there were 36 unique alleles with no correlation reported between the mean number of alleles and the exclusive alleles in Baltic sheep breeds. Li *et al.*(2002) reported unique alleles with high frequency 0.74 in some Chinese sheep breeds, while Yang *et al.*(1999) reported that 3 alleles of 57 were found as breed specific.

In the present study, Saidi breed showed exclusive alleles in markers OARAE101, INRA063, and OARFCB20 with allele frequency (0.1364), (0.030), and (0.045-0.1364), respectively (Tables 4,5,6). This result may be due to the wide distribution of Saidi breed which might have imported the unique alleles from unknown breeds during a gene flow process. The prevailing and exclusive alleles are shown in Histograms 1 to 4.

Observed and expected heterozygosity

Heterozygosity is a recognized parameter that reflects genetic variability within breed (Arranz *et al.* 2000). Observed and expected heterozygosity for the different markers in the studied breeds are presented in Table (7). The high genetic heterozygosity could be a normal results of mixed generations, mixed populations from different territories, genetic drift, and natural selection (Grisez-Duranton *et al.,* 2002). The Ossimi and Rahmani breeds showed similar result of an average expected heterozygosity 7.22 and 7.49 respectively (Table 7). The average gene diversity of the Rahmani, Ossimi, Saidi was 0.751. Saidi breed showed the highest value of expected heterozygosity (0.815), which could be explained by the possibilty of the breed being a mix of many populations in Upper Egypt.

Table 7. Microsatellite alleles (No, observed number of alleles Ne, effective number of alleles), heterozygosity (Hto, observed; Hte, expected) and Polymorphism Information Content (PIC) at each locus in the different breeds

Breed			Locus				
			BM1329	OARAE101	INRA063	OARFCB20	Mean
Rahmani	Allele	No	5	3	10	8	6.5
		Ne	2.93	2.2	5.82	6.14	4.25
	Het	Hto	0.8	1.000	0.9	1.000	0.914
		Hte	0.67	0.556	0.841	0.851	0.722
	PIC		0.626	0.495	0.291	0.827	------
Ossimi	Allele	No	5	6	11	9	7.8
		Ne	3.177	2.41	6.93	5.623	4.552
	Het	Hto	0.943	0.778	0.833	0.944	0.875
		Hte	0.695	0.594	0.868	0.834	0.75
	PIC		0.658	0.991	0.607	0.811	--------
Saidi	Allele	No	5	7	10	12	8.500
		Ne	3.553	4.644	6.084	7.751	5.508
	Het	Hto	0.909	0.939	0.849	0.758	0.864
		Hte	0.730	0.797	0.849	0.884	0.815
	PIC		0.696	0.781	0.826	0.906	-------

Hardy-Weinberg equilibrium(HWE)

All studied breeds showed a highly significant deviation from HWE in all studied loci. This result could be due to a disequlibrium created by selection practiced in the stations and that the flocks were sampled immediately after the scarificial season of Eid al-Adha when many animals were sold out from the flocks.

Polymorphism information content (PIC)

PIC indicates the genetic variation, markers with high PIC value are considered highly informative markers (Arora *et al.,* 2004). All studied markers were highly informative, PIC was 0.626, 0.658, and 0.696 for BM1329 marker, 0.495, 0.99, and 0.781 for OARAE101 marker, 0.29, 0.6 and 0.826 for INRA063 marker and 0.826, 0.811and 0.90 for OARFCB20 marker in Rahmani, Ossimi and Saidi breeds, respectively (Table 7).

Inbreeding measures

Inbreeding coefficient is defined as the correlation between uniting gametes also called coefficient of consanguinity (Lush, 1948). The inbreeding within populations (F_{IS}) is presented in Table 8. The average of F_{IS} in all studied loci showed that the individuals were generally outbred. Rahmani sheep were outbred in all the studied loci, Ossimi showed inbreeding in one locus (INRA063) with very small value (0.026) while Saidi showed inbreeding in two loci (INRA063 and OARFCB20). Hassan *et al.* (2003) reported higher estimates of inbreeding in Ossimi and Rahmani from private farms (0.315 and 0.289, respectively) as compared to -0.204 and -0.316, respectively in the present study. This result could be due to the practice by the experimental station to buy rams from other flocks to avoid inbreeding

Table 8. F_{IS} estimates (within population heterozygosity deficit or inbreeding) for each of the studied loci

Locus	Breed		
	Rahmani	**Ossimi**	**Saidi**
BM1329	-0.185	-0.365	-0.265
OARAE101	-0.830	-0.329	-0.197
INRA063	-0.056	0.026	0.020
OARFCB20	-0.194	-0.149	0.130
Average	-0.316	-0.204	-0.311

The total inbreeding coefficient of an individual related to whole population (F_{IT}) is shown in Table 9. All breeds under study showed high amount of heterozygosity (negative F_{IT}). All studied loci showed high heterozygosity excess, F_{IT} ranging from -0.339 (OARAE101) to -0.008 (OARFCB20). The means of all the studied loci showed an excess of heterozygosity and outbreeding for all studied markers. In contrast to this study, Hassan *et al.* (2003) reported a total inbreeding of 0.255.

F_{ST} is the average inbreeding of the breed related to the whole population (Falconer and Mackay, 1996) and it is a measure of differentiation among populations. F_{ST} estimates indicate that the most informative marker as far as genetic differentiation is concerned was BM1329 (0.0693) and the least was INRA063 (0.0326) for the studied breeds (Table 9)

Table 9. F statistics for the studied populations

Locus	F_{IT}	F_{ST}
BM1329	-0.188	0.069
OARAE101	-0.340	0.052
INRA063	-0.321	0.032
OARFCB20	-0.008	0.056
Mean	-0.108	0.052

The pair-wise comparisons of breed differentiation shown in Table 10 (upper diagonal) indicate low genetic differentiation between the investigated breeds. Hassan *et al.* (2003) reported that F_{ST} was 0.024 between Ossimi and Rahmani from the private farms as compared to 0.048 in the present study.

Table 10. Estimated pair-wise F_{ST} as a measure of the genetic differentiation between breeds (upper diagonal) and gene flow (lower diagonal) between pairs of the studied sheep breeds

Breed	Rahmani	Ossimi	Saidi
Rahmani	---------	0.048	0.040
Ossimi	4.954	---------	0.030
Saidi	5.912	8.028	--------

Gene flow

Gene flow was estimated according to Nei (1987) as Nm indicating the ratio of the migrants exchanged from one generation to another. The lowest value of genetic differentiation between the breeds is confirmed by the high level of gene flow between each two breeds (Table 10). The highest value of the gene flow was observed between Ossimi and Saidi breeds which also have the lowest value of F_{ST}, as a genetic differentiation. This could be explained by the geographical proximity of the two breeds.

Genetic distance and identity

Genetic distance and genetic identity showed small differences and high genetic similarity between each two breeds (Table 11) Ossimi and Saidi showed the smallest genetic distance (0.218) and the highest genetic similarity (0.804). This agrees with the close geographical origin of the two breeds (Figure 1).

Table 11. Nei's genetic identity (upper diagonal) and genetic distance (lower diagonal)

Breed	Rahmani	Ossimi	Saidi
Rahmani	-----	0.738	0.737
Ossimi	0.303	-----	0.804
Saidi	0.304	0.217	-----

Genetic divergence

Genetic divergence is another measure of how breeds divert from each other in terms of time. Nei (1978) calculated genetic divergence (Ds) as

$$Ds = 2\alpha t,$$
$$t = Ds * (2\alpha)\text{-}1$$

where α is the microsatellite mutation rate and t is the number of generations.

The average mutation rate of 28 human loci ($1.2 * 10^{-3}$) as reported by Weber and Wong (1993) and generation interval in these Egyptian sheep breeds of 4.35 years (Shaat *et al*., 2004) were used in the present study to calculate the number of years separating any two breeds. Genetic divergence analysis shows that the divergence time was 549 years between Rahmani and Ossimi, 394 between Ossimi and Saidi and 552 between Rahmani and Saidi (Table12).

Table 12. Estimated divergence time of the breeds under study on the basis of the 4 microsatellite loci studied.

		Divergence time	
Breed	**Ds**	**t, generations**	**Year**
Rahmani & Ossimi	0.3031	126.29	549
Rahman i& Saidi	0.3047	126.95	552
Ossimi & Saidi	0.2176	90.66	394

Relationship Dendrogram

Relationship dendrogram showed that Ossimi and Saidi were separated together in one sub cluster while Rahmani was located in the other sub cluster (Figure 1).

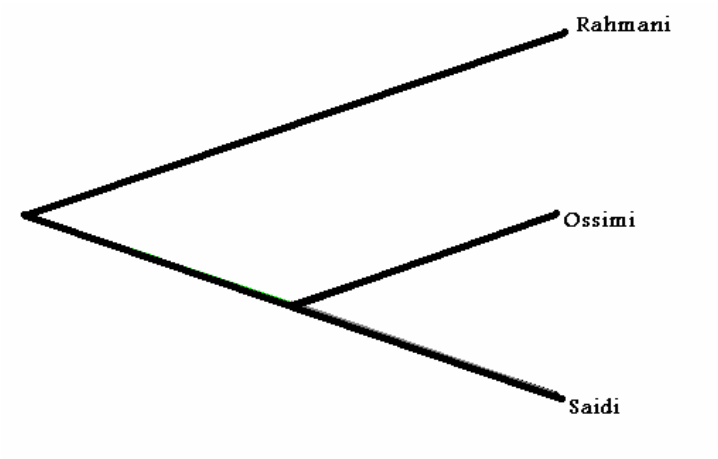


Figure 1. Phylogenetic tree representing relationship among the breeds under study using genetic distance based on four microsatellite loci

CONCLUSION

It could be concluded that while the three breeds are generally closely related, the Ossimi and Saidi are related to each other more than to the Rahmani, and that Saidi sheep are the most diverse among the three Egyptian breeds.

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التعدد الشكلى فى التوابع الدقيقة لثلاثة سلالات أغنام مصرية

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أستخدمت أربعة واسمات للتوابع الدقيقة لتوضيح الاختلافات الوراثية بين وداخل ثلاثة سلالات من الأغنام المصرية وهى الرحمانى الاوسيمى والصعيدى. كانت التوابع الدقيقة الأربعة هى (***OARAE101, BM1329, INRA63 and OARFCB20***) . أظهرت كل الواسمات موضع الدراسة تعددا أليليا فى كل السلالات حيث كان عدد الأليلات 5 ،7، 12و،12على الترتيب. بينما تراوح مقياس معلومات التعدد الأليلى (PIC) 0.660 ، 0.755 ، 0.574 و 0.847 على الترتيب. أوضحت الثلاث سلالات موضع الدراسة حيودا معنويا عن اتزان هاردى ووانبرج. أوضح اختبار المسافات الوراثية أن الثلاث سلالات لها نفس العنقود الوراثى الرئيسى ولكن انفصلت سلالة الرحمانى فى عنقود وراثى فرعى بينما انفصلت سلالة الأوسيمى والصعيدى فى العنقود الوراثى الفرعى الآخر. كان متوسط التنوع الجينى فى كل السلالات المدروسة 0.75 .