Research in Zoology 2013, 3(1): 1-9
DOI: 10.5923/j.zoology.20130301.01

Genetic Diversity and Distances of Three Egyptian Local Sheep Breeds Using Microsatellite Markers

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Abstract An analysis of nineteen microsatellite loci in 79 unrelated individuals was performed to define the genetic structure and variability of three local sheep breeds: "Sinai, Rahmani and Ossimi". Allele diversity, observed heterozygosity, expected heterozygosity and genetic distance were calculated. Wright F-statistics, (F_{IS}, F_{IT} and F_{ST}) values were estimated. The average number of alleles per locus was 21.1 . Within breeds, the mean number of alleles ranged from 15.26 in Rahmani to 16.15 in Sinai. Mean expected heterozygosity (He) ranged from 0.923 in Sinai to 0.932 in Ossimi. Inbreeding coefficient for all populations is rather low F_{IS} = 0.097 ranging from -0.079 in Rahmani to 0.118 in Ossimi. The mean genetic differentiation F_{ST} was 0.02 and demonstrated that 98% of total genetic variation is due to genetic differentiation within each population. In the neighbor–joining tree based on genetic distance (D_S) Rahmani and Ossimi were grouped together, than Sinai. This study on sheep genetic diversity in Egypt provides valuable information on Sinai, Rahmani and Ossimi sheep genetic resources, and can assist in developing a national plan for the genetic improvement of indigenous sheep breeds in Egypt.

Keywords Genetic Distance, Genetic Diversity, Local Breeds, Microsatellite, Sheep

1. Introduction

Sheep play an important role in maintenance of rural populations and also has a particular cultural importance due to its traditional use in rites and celebrations. In Egypt, there are about 4 million sheep heads contribute 6% of the total red meet produced. Rahmani and Ossimi are the main sheep breeds in Egypt with a population of 990,000 and 514,000 heads, respectively[11]. Sinai sheep breed (Bedouin sheep) is about 135,649 heads[18]. Sinai sheep breed live under desert condition that characterized by a long hot summer with high solar radiation, along with poor and sparsely distributed pastures. Throughout their distribution area water is scarce and the pasture is meager and mostly of low quality-high in fiber and low in protein. An assessment of genetic variability in domestic sheep is a first step towards conservation of genetic resources for maintaining breeding options. Genetic characterization and determination of genetic differences between sheep breeds help in the genetic improvement programs. Molecular methods used molecular markers, such as RAPD, RFLP and microsatellites, are useful tools to study the genetic variations. Microsatellites have often been used for genetic diversity studies, because of their distribution throughout the genome, high level of polymorphism, co-dominant inheritance and easy to analyze[7]. Several studies had investigated the genetic diversity in sheep using microsatellites[1],[8-10],[13],[14]. In the present study, a set of nineteen microsatellite markers were used to evaluate the genetic diversity within and between three Egyptian sheep breeds (Sinai, Rahmani and Ossimi) and to measure the distance among these breeds.

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Published online at http://journal.sapub.org/zoology

2. Materials and Methods

2.1. Animals and Samples Collection

In this study, 79 randomly blood samples were collected from different individuals of three Egyptian sheep breeds (Sinai, 26; Rahmani, 29; and Ossimi, 24). 10 ml of blood was collected via the jugular vein in K3EDTA containing tubes for prevention of coagulation. DNA was extracted from blood using the Genomic DNA Purification Kit of (Fermentas Co.). DNA concentration was determined using NanoDrop (Spectrophotometer ND-1000).

2.2. Microsatellite Analysis

In this study, nineteen microsatellite markers across the ovine genome were used. Grading PCR thermal cycle was used to detect the suitable annealing temperature for each

marker. The PCR products were tested in agrose gel to estimate the best annealing temperature for each primer. Studied microsatellite markers, their primer sequences, detected annealing temperature and their allele size ranges are showed in Table1. The selected microsatellites were amplified with polymorphism chain reaction (PCR) using genomic DNA extracted from individual animals. The PCR was performed for each locus in 10µl reactions consisted of 2µl of Genomic DNA (20ng), 5µl 2X PCR AmpliTag gold PCR Master mix (applied biosystems), 0.4 µl primer mix (50 pmoles) and 2.6 µl DNase free water. The PCR program was carried out at95oC for 10 min, followed by 35 cycles of 95℃ for 30 sec., annealing temperature which was determined for each primer (Table1) for 30 sec. and 72℃ for 30 sec., and final extension at 72℃ for 10 min. Following the completion of the PCR cycles, 3µl of the reaction products was mixed with 1µl 6X gel loading dye and then loaded into each well of vertical 8% polyacrylamide gel mad with 1X TBE buffer at 100 V for 60 to 90 min and stained with Ethidium bromide (1%). A 50bp DNA ladder was used to estimate allele sizes in base pairs (bp).

2.3. Statistical Analysis

Genotypes were assigned for each animal based on allele size data. Frequencies and number of alleles for each locus, observed and expected heterozygosity and F-statistics (F_{IS} is the inbreeding coefficient of an individual relative to the subpopulation, F_{IT} is the inbreeding coefficient of an individual relative to the total population and F_{ST} is the effect of subpopulation compared to the total population) were estimated using FSTAT (version 2.9.3.2)[12]. F_{ST} (measure of differentiation among populations) were also calculated between all pairs of the tested breeds. The polymorphic information content (PIC) value was calculated according to[5]. Nei's[19] standard genetic distances (DS) among populations were computed by POPGENE (version 1.31)[21]. The same program was used to construct the dendogram of un-weighted pair group with arithmetic mean (UPGMA).

Table 1. Sequences of microsatellite marker primers, Chromosome location, annealing temperatures and detected allele size range

Microsatellite Names	Primer sequences (5` 3`)	Chromosomal location	Annealing temp. ℃	Allelic size range (bp)
BM143	F: ACCTGGGAAGCCTCCATATC R: CTGCAGGCAGATTCTTTATCG	6	62	98-130
ETH225	F: GATCACCTTGCCACTATTTCCT R: ACATGACAGCCAGCTGCTACT	9	57	126-172
HSC	F: CTGCCAATGCAGAGACACAAGA R: GTCTGTCTCCTGTCTTGTCATC	20	58.2	260-294
ILSTS005	F: GGAAGCAATGAAATCTATAGCC R: TGTTCTGTGAGTTTGTAAGC	7	55	194-252
ILSTS008	F: GAATCATGGATTTTCTGGGG R: TAGCAGTGAGTGAGGTTGGC	9	51	156-210
ILSTS29	F: TGTTTTGATGGAACACAGCC R: TGGATTTAGACCAGGGTTGG	1	55	152-200
ILSTS30	F: CTGCAGTTCTGCATATGTGG R: CTTAGACAACAGGGGTTTGG	2	55	148-190
ILSTS49	F: CAATTTTCTTGTCTCTCCCC R: GCTGAATCTTGTCAAACAGG	3	51	150-190
ILSTS82	F: TTCGTTCCTCATAGTGCTGG R: AGAGGATTACACCAATCACC	2	55	92-146
ILSTS87	F: AGCAGACATGATGACTCAGC R: CTGCCTCTTTTCTTGAGAGC	6	54	150-190
MAF33	F: GATCTTTGTTTCAATCTATTCCAATTTC R: GATCATCTGAGTGTGAGTATATACAG	9	60	112-158
MAF65	F: AAAGGCCAGAGTATGCAATTAGGAG R: CCACTCCTCCTGAGAATATAACATG	15	60	122-160
MAF70	F: CACGGAGTCACAAAGAGTCAGACC R: GCAGGACTCTACGGGGCCTTTGC	4	63	130-174
MCM42	F: CATCTTTCAAAAGAACTCCGAAAGTG R: CTTGGAATCCTTCCTAACTTTCGG	9	55	66-120
OarCP34	F: GCTGAACAATGTGATATGTTCAGG R: GGGACAATACTGTCTTAGATGCTGC	3	53	110-142
OarFCB11	F:GGCCTGAACTCACAAGTTGATATATCTATCAC R: GCAAGCAGGTTCTTTACCACTAGCACC	2	62	128-168
OarFCB20	F: GGAAAACCCCCATATATACCTATAC R: AAATGTGTTTAAGATTCCATACATGTG	2	58	94-130
OarJMP29	F: GTATACACGTGGACACCGCTTTGTAC R: GAAGTGGCAAGATTCAGAGGGGAAG	24	52	152-200
RM004	F: CAGCAAAATATCAGCAAACCT R: CCACCTGGGAAGGCCTTTA	15	55	146-188

3. Results and Discussions

Figures 1 to figure 19 represent an example of the results of nineteen microsatellite markers PCR products on polyacrylamide gels (8%) (PAGE).

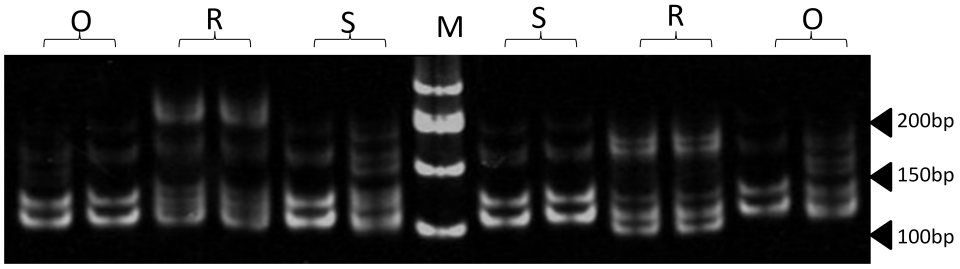

Figure 1. Polyacrylamide gel (8%) showing alleles concerning BM143 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

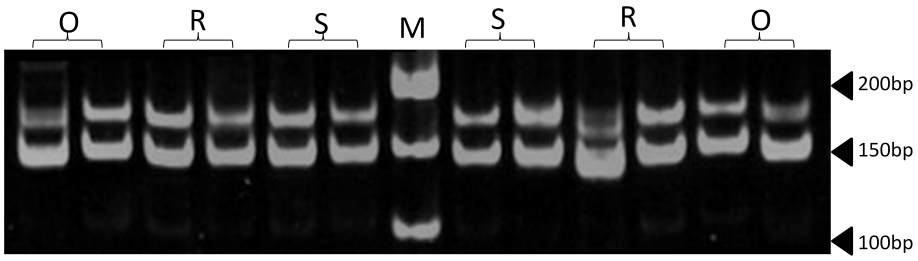

Figure 2. Polyacrylamide gel (8%) showing alleles concerning ETH225 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

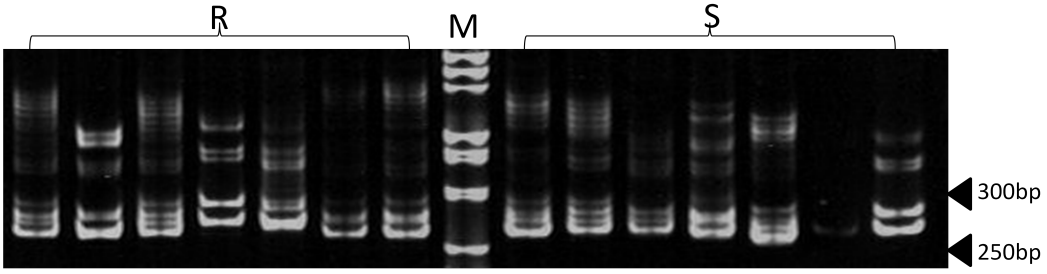

Figure 3. Polyacrylamide gel (8%) showing alleles concerning HSC marker. DNA ladder is on well M, Sinai sheep (S) and Rahmani (R)

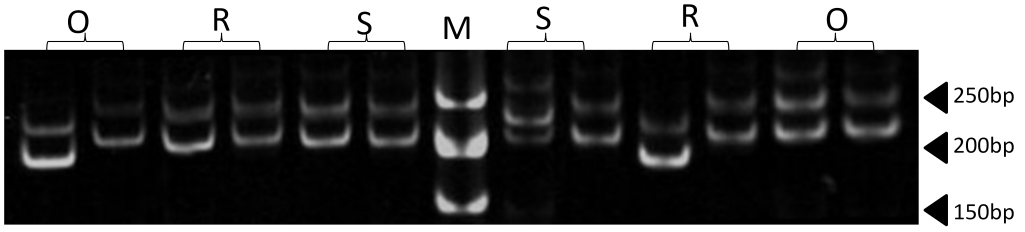

Figure 4. Polyacrylamide gel (8%) showing alleles concerning ILSTS005 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

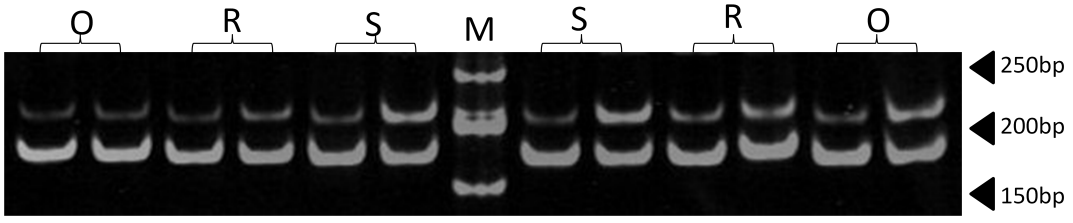

Figure 5. Polyacrylamide gel (8%) showing alleles concerning ILSTS008 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

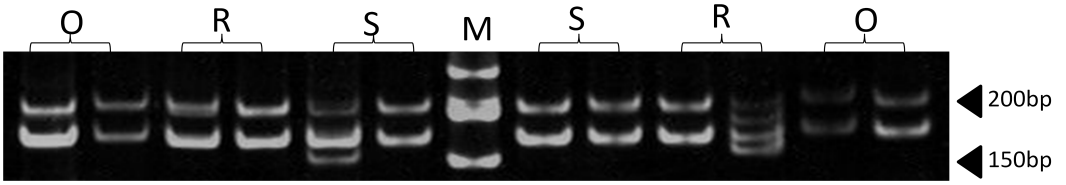

Figure 6. Polyacrylamide gel (8%) showing alleles concerning ILSTS29 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

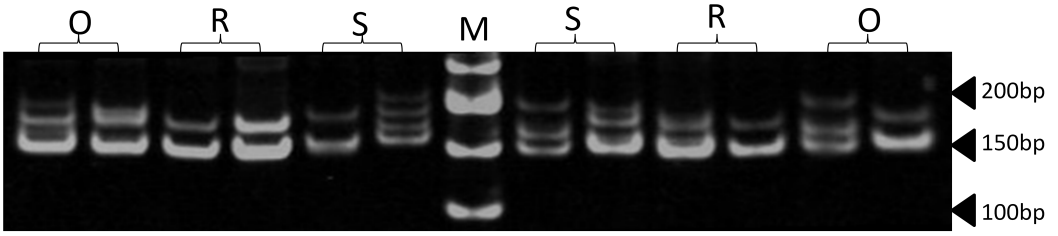

Figure 7. Polyacrylamide gel (8%) showing alleles concerning ILSTS30 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

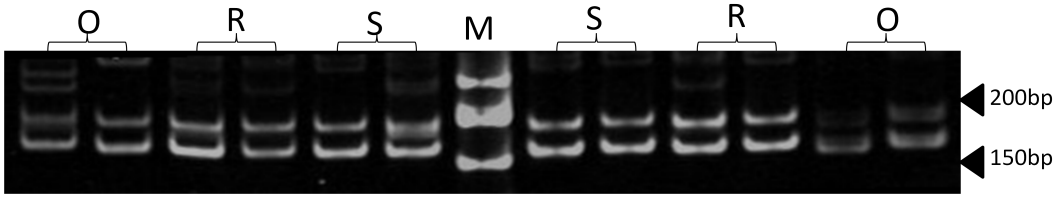

Figure 8. Polyacrylamide gel (8%) showing alleles concerning ILSTS49 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

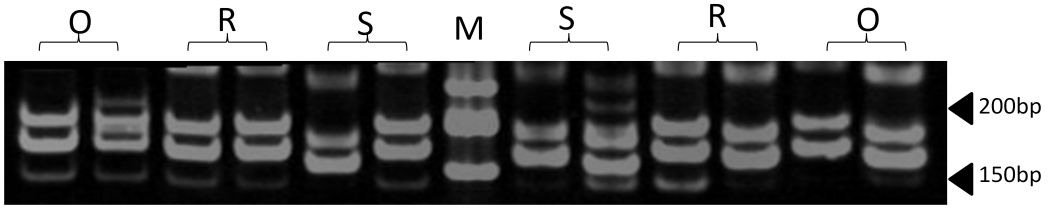

Figure 9. Polyacrylamide gel (8%) showing alleles concerning ILSTS87 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

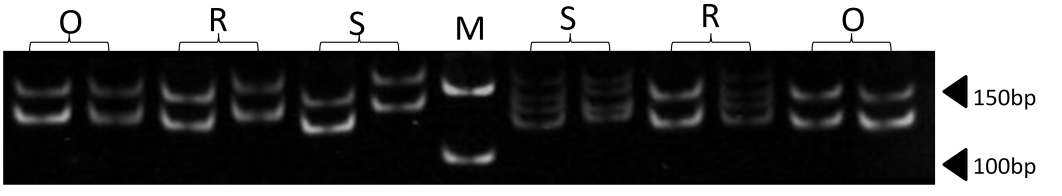

Figure 10. Polyacrylamide gel (8%) showing alleles concerning MAF33 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

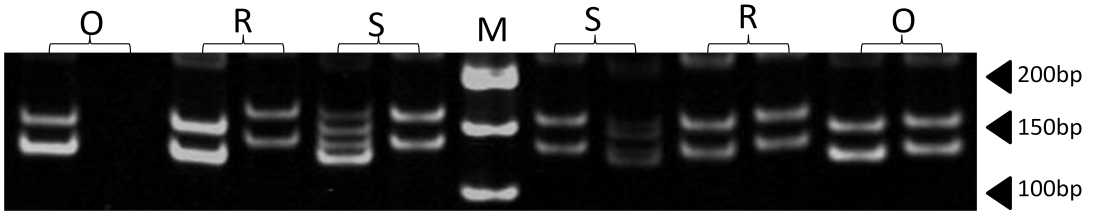

Figure 11. Polyacrylamide gel (8%) showing alleles concerning MAF65 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

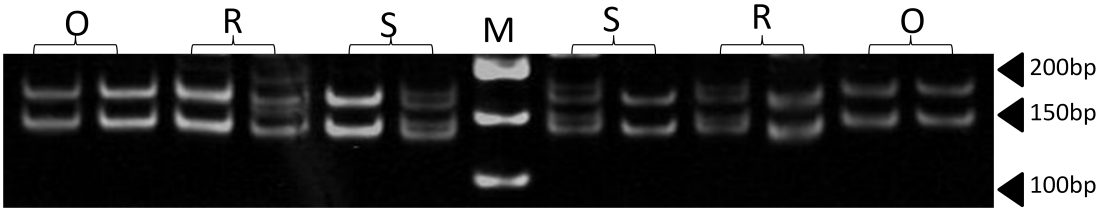

Figure 12. Polyacrylamide gel (8%) showing alleles concerning MAF70 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

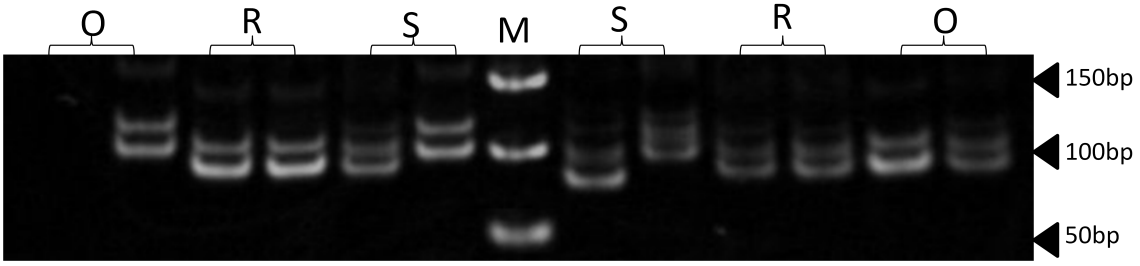

Figure 13. Polyacrylamide gel (8%) showing alleles concerning MCM42 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

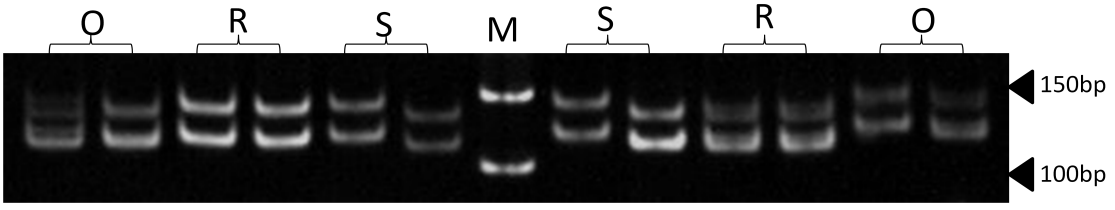

Figure 14. Polyacrylamide gel (8%) showing alleles concerning OarCP34 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

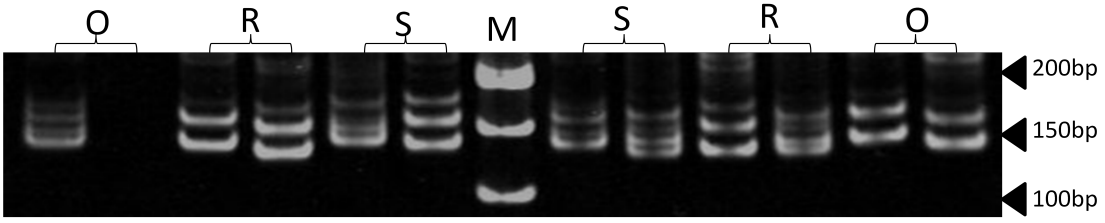

Figure 15. Polyacrylamide gel (8%) showing alleles concerning OarFCB11 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

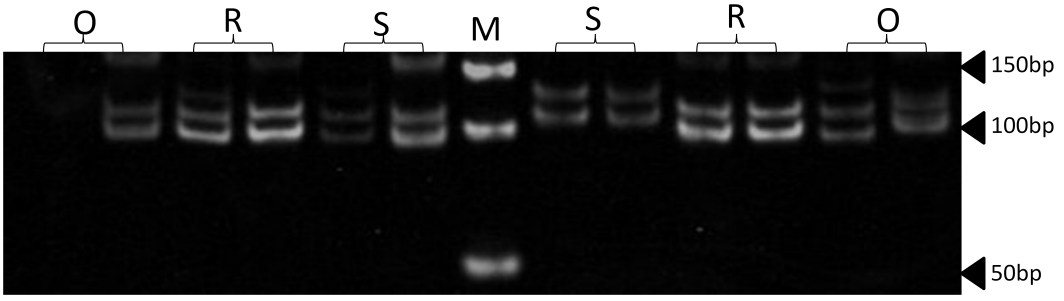

Figure 16. Polyacrylamide gel (8%) showing alleles concerning OarFCB20 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

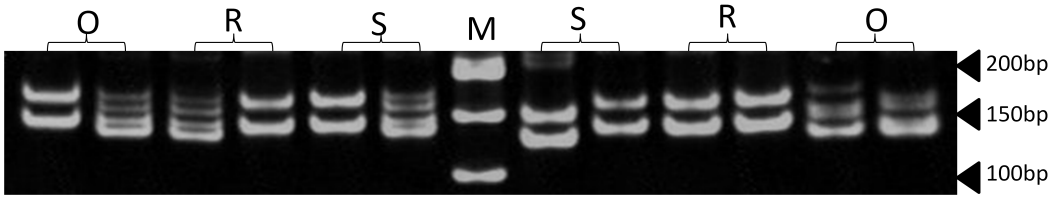

Figure 17. Polyacrylamide gel (8%) showing alleles concerning OarJMP29 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

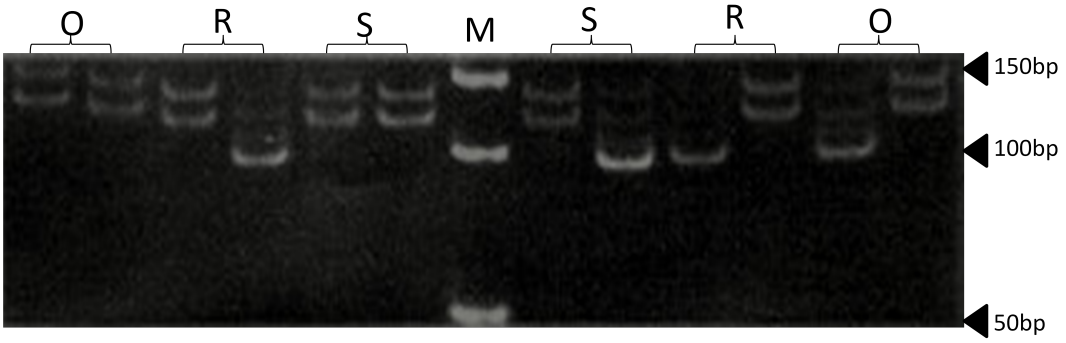

Figure 18. Polyacrylamide gel (8%) showing alleles concerning ILSTS82marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

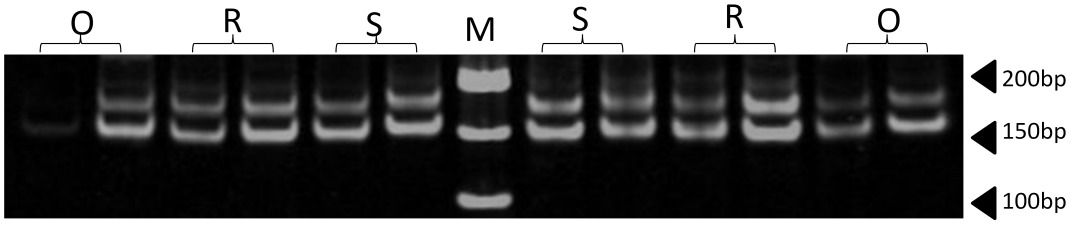

Figure 19. Polyacrylamide gel (8%) showing alleles concerning RM004 marker. DNA ladder is on well M, Sinai sheep (S), Rahmani (R), and Ossimi (O)

All 19 loci were successfully amplified and a total of 401 alleles were detected, MCM42 showed the highest number of alleles per locus (25) while BM143 and Oar FCB11 showed the lowest (17) (Table2) with a mean of 21.11 allele. The level of variation depicted by the number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within the populations[2]. The number of alleles and effective alleles for each of the nineteen microsatellite markers in each of the three breeds is presented in Table 2. The mean numbers of allele per locus are 16.2, 15.3 and 15.8 in Sinai, Rahmani and Ossimi, respectively. The highest mean number of alleles in Sinai sheep could suggest the existence of heterozygous genotypes in this population.

Generally the mean number of alleles is highly dependent on the sample size because of the unique alleles in populations, which occur in low frequencies and also because the number of observed alleles tends to increase depending on the population size. All 79 individuals in the three populations were considered in this study.

Effective number of alleles is a measure of allelic evenness. The effective number of alleles is also an index used to reveal the genetic diversity of the population. In this study, the results showed that the average effective number of alleles for the Ossimi sheep breed was higher than the corresponding number of alleles in the Sinai and Rahmani sheep populations at the majority of the microsatellite markers. The total number of effective alleles ranged from 10.23 for ILSTS30 to 17.39 for ILSTS87. The average effective number of alleles ranged from 14.4 for ILSTS49 in the Ossimi to 7.59 for BM143 in the Sinai sheep breed (Table 2). The mean of Polymorphic Information (PIC) in all breeds varied from 0.88 (ILSTS30 and OarCP34) to 0.92 (ILSTS005, MAF70 and OarJMP29). The mean PIC value of 0.9 reflected the high level of polymorphisms of the used set of microsatellites and heterogeneity in the three sheep populations. However, the high estimates of PIC further substantiated the suitability of the used set of markers to applications such as parentage control, linkage-mapping programs in addition to genetic studies in Egyptian sheep. All of the markers were highly polymorphic, having the PIC values of >0.5[5] as shown in Table 2.

Table 2. Number of observed alleles and number of effective alleles for each locus in each breed

Locus	Allelic Number				Effective allelic number				PIC mean
	Sinai Sheep	Rahmani	Ossimi	Total	Sinai Sheep	Rahmani	Ossimi	Total	
BM143	12	12	16	17	7.60	10.13	12.16	11.95	0.89
ETH225	18	18	13	23	12.29	13.14	10.69	15.23	0.91
HSC	11	13	13	18	8.50	10.58	10.89	12.10	0.89
ILSTS005	19	16	20	24	12.40	12.19	14.05	14.81	0.92
ILSTS008	17	17	19	24	9.20	12.37	13.92	15.99	0.91
ILSTS29	13	16	20	24	10.24	12.65	14.05	13.75	0.91
ILSTS30	19	12	15	21	10.24	8.57	8.00	10.23	0.88
ILSTS49	13	14	19	21	8.67	10.26	14.40	12.71	0.9
ILSTS82	18	12	16	21	11.68	9.24	12.80	14.52	0.9
ILSTS87	17	15	16	21	11.76	12.19	12.00	17.38	0.91
MAF33	17	14	16	21	11.86	8.57	12.30	13.21	0.9
MAF65	14	16	13	19	9.92	12.84	10.20	13.91	0.9
MAF70	19	17	15	23	14.38	11.76	12.66	16.04	0.92
MCM42	20	14	17	25	14.08	9.92	12.30	16.07	0.91
OarCP34	17	13	11	17	9.87	9.24	8.29	11.34	0.88
OarFCB11	17	16	17	20	12.88	13.02	11.50	16.89	0.91
OarFCB20	15	15	15	19	8.24	12.65	12.26	13.66	0.9
OarJMP29	17	20	18	22	12.64	14.02	14.05	16.60	0.92
RM004	14	20	12	21	9.66	12.90	7.85	12.10	0.89
Mean	16.16	15.26	15.84	21.11	10.85	11.38	11.81	14.13	0.903

The average direct count of heterozygosity (observed heterozygosity) overall loci in Sinai, Rahmani and Ossimi breeds are 0.847, 0.859 and 0.825, respectively. Whereas the average expected heterozygosity overall loci in the three breeds are 0.923, 0.926 and 0.932, respectively (Table 3). The average observed heterozygosity was less than expected for all populations and this could be due to segregation of non-amplifying (null) alleles and/or selection against heterozygotes or inbreeding[6]. These results show less observed than expected heterozygosity in each breed. It is of a similar magnitude as the average direct count of heterozygosity overall loci within Egyptian sheep breeds being less than the expected heterozygosity as reported by[9]. High value of average expected heterozygosity within a breed could be attributed to the large allele numbers detected in the tested loci[15]. In a comprehensive study involving 57 breeds from 15 European and Middle East countries,[20] reported that South-eastern European and Middle-Eastern sheep breeds were significantly more variable than northwestern and western European breeds. In Indian sheep the observed heterozygosity varied from 0.087 to 1.000 and expected genetic heterozygosity ranged from 0.083 to 0.828as reported by[4]. While the expected heterozygosity in Chinese sheep ranged from 0.593 to 0.893,[22].

Heterozygosity deficit within a population is measured by Wright's F_{IS}. The F_{IS} values for each population are given in Table 4. The mean values of inbreeding coefficient (F_{IS}) for Sinai, Rahmani and Ossimi were 0.098, 0.074 and 0.118, respectively. The highest F_{IS} within -populations was observed for the loci MAF33 in Sinai, MAF70 in Rahmani and OarCP34 in Ossimi sheep populations, whereas the lowest values were found for loci ILSTS49 (-0.111) in Sinai, MCM42 (-0.093) in Rahmani and ETH225 (-0.081) in Ossimi (Table 4). The negative values of F_{IS} for some loci indicated that the mates were less related to each other in comparison with the average population. The F_{IS} values which are very close to zero indicated low level of inbreeding within the populations. In order to test genotype frequencies for deviation from Hardy-Weinberg equilibrium (HWE), at each locus within and overall breeds, results in Table 4 revealed significant departure from HWE ($p<0.05$ and $p<0.001$). When the Hardy-Weinberg test was performed for the loci, deviations from the Hardy-Weinberg equilibrium were found to be highly significant ($p<0.001$) in all markers except ILSTS005, ILSTS49 and MCM42 markers being significant ($p<0.05$). Deviation from HWE at microsatellite loci have, also been reported in various studies[3],[9],[10],[14],[16],[17]. The variation at the microsatellite markers indicated deviations from random mating in the three sampled Egyptian breeds, although measures are practiced to avoid inbreeding, like restricting the use of same sires, exchanging sires between locations and adding new 'blood' from outside the population. The mean F_{IT} and F_{ST} values over all populations were 0.103 and 0.02, respectively.

The low F_{IT} and F_{ST} values which are very close to zero indicated low level of inbreeding in the populations and also refer to low genetic differentiation between the populations. The low inbreeding values can be attributed to random mating under field conditions.

Table 3. Mean of heterozygosity in the three sheep breeds

Heterozygosity	Sinai	Rahmani	Ossimi	Mean
Mean observed heterozygosity ±SD	0.847±0.218	0.859±0.198	0.825±0.209	0.844±0.208
Mean expected heterozygosity ±SD	0.923±0.018	0.926±0.017	0.932±0.018	0.927±0.017

Table 4. F_{IS}, F_{IT} and F_{ST} values and Chi-square test for HWE for each locus within and over all tested populations

Locus	F_{IS}				F_{IT}	F_{ST}	χ^2 (degree of freedom).
	Sinai Sheep	Rahmani	Ossimi	Over all breeds			
BM143	0.045	0.061	-0.020	0.029	0.032	0.024	266.4** (136)
ETH225	-0.027	0.159	-0.081	0.017	0.017	0.019	943.1** (253)
HSC	0.293	0.255	0.185	0.244	0.240	0.020	406.1** (153)
ILSTS005	0.016	-0.034	-0.055	-0.020	-0.032	0.012	548.6* (276)
ILSTS008	-0.059	-0.033	0.039	-0.020	-0.010	0.027	460.5** (276)
ILSTS29	-0.046	0.007	-0.055	-0.030	-0.040	0.011	676.1** (276)
ILSTS30	0.082	0.007	0.116	0.068	0.065	0.017	422.4** (210)
ILSTS49	-0.111	-0.091	-0.009	-0.070	-0.069	0.018	440.9* (210)
ILSTS82	0.058	0.051	0.252	0.120	0.124	0.024	774.4** (210)
ILSTS87	0.262	0.041	0.292	0.198	0.202	0.028	534.1** (210)
MAF33	0.511	0.249	0.264	0.341	0.339	0.021	723.8** (210)
MAF65	0.042	0.045	0.051	0.046	0.047	0.021	548.9** (171)
MAF70	0.192	0.375	0.206	0.258	0.252	0.017	621.5** (253)
MCM42	-0.057	-0.093	0.028	-0.040	-0.035	0.024	437.5* (300)
OarCP34	0.248	0.243	0.449	0.313	0.312	0.024	425.9** (136)
OarFCB11	0.102	0.016	0.117	0.078	0.079	0.023	387.4** (190)
OarFCB20	0.100	0.044	0.068	0.071	0.073	0.023	280** (171)
OarJMP29	0.225	0.089	0.213	0.176	0.169	0.015	661.7** (231)
RM004	-0.010	0.015	0.182	0.062	0.058	0.019	309.2** (210)
Mean	0.098	0.074	0.118	0.097	0.103	0.020	

* $p<0.05$ and ** $p<0.001$

Pairwise F_{ST} estimates and Nei's[19] standard genetic distance between all pairs of the tested breeds are presented in Table 5. Pairwise genetic differentiations quantified by F_{ST} estimates indicated that genetic differentiation between Rahmani and Ossimi(0.0070) is rather lower than genetic differentiation between Sinai and Rahmani(0.0123) and Sinai and Ossimi (0.0086) (Table 5). Similarly, Nei's[19] standard genetic distance between Rahmani and Ossimi (0.2542) is smaller than the distance between Sinai and Rahmani (0.3023) and between Sinai and Ossimi (0.2875) (Table 5).

Table 5. Pairwise F_{ST} estimates (above diagonal) and Nei's (1987) standard genetic distance (below diagonal) between all pairs of the tested breeds

Population	Sinai	Rahmani	Ossimi
Sinai	--------	0.0123	0.0086
Rahmani	0.3023	--------	0.0070
Ossimi	0.2875	0.2542	--------

Also, the UPGMA tree showed that the Sinai forms the most distinct breed. The Rahmani and Ossimi clustered together and Sinai was clearly differentiated from the other two breeds (Figure 20). El-Nahas[9] reported similar results and suggested that 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 resulted in small genetic distance between Rahmani and Ossimi breeds.

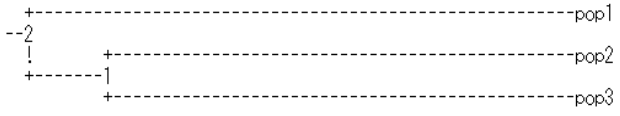

Figure 20. UPGMA dendogram generated from Nei's genetic distances of the three sheep breeds. (Pop1: Sinai, Pop2: Rahmani and Pop3: Ossimi)

4. Conclusions

This study presents an initial step in investigation of variability at the DNA level within and between some Egyptian sheep breeds. The results indicated that all studied breeds exhibited considerable genetic variation, based on their high mean number of alleles and gene diversity. The results also suggest that the three breeds (Sinai, Rahmani and Ossimi) are slightly heading towards inbreeding. Sinai breed distributed far away than the other Egyptian sheep breeds. The results indicated also that genetic differentiation between Sinai and the other two breeds is larger than between both breeds.

This study represents one of the preliminary studies designed to characterize the molecular genetic variability of the Egyptian sheep breeds. There is really a need to more thorough analysis of the genetic diversity of local sheep breeds from other regions to include more breeds, large sample sizes and additional molecular markers. The evaluation of genetic variations within and between

Egyptian sheep breeds may be used as basis for the development of a national breeding strategy.

ACKNOWLEDGEMENTS

The practical work of this study was prepared through a scholarship from Egyptian government and conducted at the Laboratory of Physiology; Department of Veterinary of Faculty of Agriculture, Iwate University- Japan.

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