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RESEARCH ARTICLE

Analysis of the genetic diversity of three Egyptian sheep breeds using microsatellite markers

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Abstract

Genetic diversity of Barki, Rahmani and Ossimi Egyptian sheep breeds were assessed for ten microsatellite markers. Average number of alleles, effective number of alleles, polymorphism information content and allelic richness per marker within breeds were 8.0, 4.70, 0.71 and 6.25, respectively. Barki had the highest gene diversity over all considered loci (0.85), followed by Rahmani (0.79) and Ossimi (0.69). Average observed heterozygosity estimates were higher than their corresponding expected ones for all breeds. The average fixation indices (F_{it}, F_{st} and F_{is}) were 0.006, 0.036 and -0.033. Estimates of population subdivision (F_{st}) were consistently positive for all markers, and its overall value was 0.036, indicating that approximately 4% of genetic diversity can be attributed to genetic variation between breeds. Genetic distances between each pair of the studied breeds were 0.340, 0.369 and 0.442 between Barki and Rahmani, Barki and Ossimi as well as Ossimi and Rahmani, respectively, indicating a medium-high differentiation rate. Results of genetic distance studies might have a direct impact on future genetic conservation studies and breeding programs.

INTRODUCTION

In Egypt, three major sheep breeds are identified, known as Barki, Rahmani and Ossimi. These breeds are characterized as fat tailed, coarse wool fleece, small to medium size, and are mainly raised for meat production (Aboul-Naga, 1976). With some exceptions, the Egyptian sheep exploit marginal agricultural resources, since animals are usually kept by small holders, herd size is very small (1-3 heads), large farms are rare, herdbooks and breed registration are not in vogue, and species-wise census data are not available (Galal, 2007). Because artificial insemination with frozen semen is not practiced and lack of parentage control, this could consequently lead to a high inbreeding coefficient. Peter *et al.* (2007) suggested that a relative lack of breed purity and few herdbooks resulted in greater gene flow between sheep breeds in southeastern Europe and the Middle East, while herdbook-based breeding and intensive management have been associated with genetic isolation and a reduction in the effective population sizes of more northern and northwestern European breeds.

Advance achieved in molecular genetics techniques has provided an opportunity to study genetic variation at the DNA level using various molecular markers. Due to their advantageous characteristics compared to other genetic markers, microsatellites have been considered the marker of choice for the development of linkage maps and genetic diversity studies in sheep (Crawford *et al.*, 1995 and Baumung *et al.*, 2006). The genetic relationships of Egyptian sheep breeds have been previously studied in recent years using microsatellites (Hassan *et al.*, 2003; Elfawal *et al.* 2008; El Nahas *et al.* 2008; and Ghazy *et al.* 2013). However, most of these studies considered

particular breeds, a relatively small number of animals, a limited set of genetic markers, and mainly focusing on particular geographical regions.

The aim of this study was to estimate the genetic diversity between the three major Egyptian sheep breeds (Barki, Rahmani and Ossimi) with a view to obtaining a deeper insight into relationships within and between breeds based on individual genotypes at 10 microsatellite markers. This work provides a first step towards conservation and sustainable utilization of these native genetic resources.

Materials and Methods

Animals and samples

Three Egyptian sheep breeds, called as Ossimi, Barki and Rahmani, were analyzed for genetic variation at 10 microsatellite loci. All the sampled breeds had long and coarse wool, and were used for meat and wool production purposes. A more detailed description of morphological aspects of the investigated breeds is given by Aboul-Naga (1976). To overcome absence of reliable records and herdbooks, animals for genotyping were chosen in order to make sure, as much as possible, that they were a representative sample of each breed, originating from different flocks, and genetically unrelated. A total of 113 adult individuals, including males and females, corresponding to the three breeds were sampled. Breeds and, in brackets, number of individuals and herds sampled were as follows: Ossimi (40; 12), Barki (35; 8) and Rahmani (38; 11). The maximum number of sampled animals did not exceed 5 per flock. All breeds investigated are not considered endangered in Egypt.

Blood samples of 10 ml were collected under aseptic conditions by jugular venipuncture, using vacuum tubes treated with 0.25% ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Samples were transferred to the laboratory in icebox and were kept in refrigerator at 4ºC until DNA isolation.

DNA extraction and PCR amplification

Genomic DNA was extracted from the fresh blood samples, collected within 24 hours preceding isolation, using genomic blood DNA purification kit (Sigma Co., Cairo, Egypt). DNA concentration and purity were measured by Nano Drop ND-1000 Spectrophotometer, then DNA was diluted to a final concentration of 50 ng/ml in water and stored frozen at – 20ºC until analysis. Eight microsatellite markers (BM1329, BM8125, ILSTS005, MAF65, MAF70, OarFCB20, OarHH47 and OarJMP29) were analyzed on all the individuals. Selection of the investigated microsatellites was based mainly on the minimum requirements in recommendations of joint ISAG/FAO standing committee (ISAG, 2004) and FAO's draft guidelines on molecular genetic characterization of animal genetic resources (FAO, 2011). In addition, two loci (OarCP20 and OarFCB11) were chosen from International Bovine Reference Population (Roslin Institute, Scotland, UK) and MARC97 (Meat Animal Research Center of United States Department of Agriculture, http://www.marc.usda.gov/genome/genome.html), respectively.

A criterion of loci location on different chromosomes was also taken into consideration, resulting in that the selected markers were located on 9 different ovine chromosomes. Primer sequences and polymerase chain reaction (PCR) conditions of the selected loci were those referred in literature. An overview on the typed microsatellites is shown in Table 1. PCR amplifications were performed for each marker in 25µl reactions with minor modifications when necessary to improve the quality of PCR products. Amplified PCR products were separated using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Cairo, Egypt). Alleles were distinguished and genotypes were determined by the software packages GeneScan 2.0 and GenoTyper 2.1 (Applied Biosystems).

Statistical analysis

Statistical data analyses and graphical representations were carried out under R statistical environment (R Core Team, 2014), http://www.R-project.org/, along with various R packages. Exploratory data analysis (e.g. number of alleles, H_o and H_e) was carried out using summary command of "adegenet" package (Jombart, 2008). Allele frequencies, PIC and overall values of row disequilibrium (D), scaled disequilibrium (D') and correlation coefficient (r) were calculated using "genetics" package (Warnes *et al.*, 2013; http://CRAN.R-project.org/package=genetics). Deviation from Hardy–Weinberg equilibrium (HWE) using chi square test, and phylogenic analysis using Nei's distance (Nei, 1987) were carried out using "pegas" package (Paradis, 2010). Population subdivision was examined using Weir & Cockerham unbiased estimator of Wrights fixation index (Weir & Cockerham, 1984). All F-statistics (F_{it}, F_{st} and F_{is}) were computed using "pegas" package (Paradis, 2010). The null hypothesis was that the estimates were not significantly different from zero, and the level of significance ($P < 0.001$) was adjusted using Bonferroni correction. Allelic richness and effective number of alleles for each breed were estimated using hierfstat package (Goudet, 2014). Phylogenetic analysis was carried out using APE (Paradis *et al.*, 2004).

Results

A summary of results from marker analyses are given in Table 1. A total of 80 alleles were detected across the 10 investigated loci. MAF70 had the greatest number of alleles per locus (11), while MAF65 and OarJMP29 revealed the lowest (6). Ovine-derived loci had on average more alleles than loci derived from cattle genome (8.57 vs. 7.33 alleles). ENA per locus ranged from 4.19 for BM8125 to 5.52 for MAF70, while the overall mean per markers within breeds was 4.70. Estimates of PIC varied from 0.65 for BM8125, ILSTS005 and OarJMP29 to 0.81 for MAF70, with an average of 0.71.The average H_e per marker ranged from 0.71 for BM8125 to 0.84 for MAF70. While the corresponding estimates of the H_o varied between 0.67 and 0.86 for OarJMP29 and MAF70, respectively.

Locus heterozygosity over all markers and for the three breeds together averaged 0.78 (Table 2). The average gene diversity over all loci ranged from 0.69 in Ossimi to 0.85 in Barki, while it had an intermediate estimate (0.79) in Rahmani breed. The lowest and highest gene diversity within breed was found for ILSTS005 (0.53 and 0.95 in Ossimi and Rahmani).

The average allelic richness for marker over all breeds varied between 4.99 for OarJMP29 to 8.00 for OarHH47, with an average of 6.25 alleles per locus (Table 3). Considering breeds, allelic richness ranged from 5.78 in Ossimi to 6.90 in Barki.

Loci per breed that showed significant deviation from HWE are illustrated in (Table 4). Only one marker (MAF70) deviated significantly from HWE in all breeds studied. Six loci (BM1329, BM8125, OarCP20, OarFCB11, OarHH47 and OarJMP29) showed significant deviations from HWE in only one sheep breed. However, none of those loci were excluded from the final analysis.

Average heterozygosity estimates for the three Egyptian sheep breeds estimated through the Wrights *F* diversity indices are shown in Table 5. The average H_e over all loci varied from 0.68 in Ossimi to 0.77 in Barki, while H_o ranged between 0.74 in Ossimi and 0.85 in Barki; however, differences were not significant. Mean estimates of H_e and H_o over all loci and breeds were 0.76 and 0.80, respectively.

The indication for inbreeding was checked later by calculating fixation indices illustrated in Table 6. Means of all inbreeding indicators (F_{it}, F_{st} and F_{is}) over all loci were positive, except F_{is}. Estimates of genetic differentiation (F_{st}) were consistently positive for all markers, and ranged between 0.011 and 0.083 for BM8125 and OarCP20, respectively. Inbreeding coefficients within populations (F_{is}) ranged between -0.155 and 0.036 for ILSTS005 and OarJMP29, while the global F_{is} value was -0.033. The total inbreeding coefficient of an individual related to whole population (F_{it}) averaged 0.006, while values varied from -0.068 for ILSTS005 to 0.077 for OarJMP29.The pair-wise comparisons of differentiation among breeds (F_{st}) ranged from 0.027 between Barki and Rahmani to 0.049 between Ossimi and Rahmani (Table 7). The calculated genetic distance between each two sheep breeds varied from 0.340 to 0.442 (Table 7).

Phylogenetic tree of the three Egyptian sheep breeds created using Neighbor-Joining method is shown in Figure 1. The cluster analysis shows that each breed was clustered independently.

Table 1. Characteristics and summary statistics for microsatellite loci analyzed in 3 sheep breeds

Locus (Origin)	Genebank Accession Number	Chr	A_t	ENA	PIC	Size (bp)	T_n	Ho	He	Forward primer sequence Reverse primer sequence	Ref
BM1329 (Bovine)	G18422	6	8	4.50	0.69	160-186	63	0.77	0.75	TTGTTTAGGCAAGTCCAAAGTC AACACCGCAGCTTCATCC	Bishop *et al.*(1994)
BM8125 (Bovine)	G18475	17	7	4.19	0.65	96-128	56	0.71	0.71	CTCTATCTGTGGAAAAGGTGGG GGGGGTTAGACTTCAACATACG	Bishop *et al.*(1994)
ILSTS005 (Bovine)	L23481	7	7	4.28	0.65	160-218	55	0.80	0.72	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	Brezinsky*et al.* (1993)
MAF65 (Ovine)	M67437	15	6	4.45	0.68	119–143	55	0.78	0.74	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	Buchanan *et al.* (1992)
MAF70 (Ovine)	M77199	4	11	5.52	0.81	124-166	60	0.86	0.84	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	Buchanan &Crawford(1992)
OarCP20 (Ovine)	U15695	21	8	5.03	0.72	69-99	63	0.81	0.80	GGCATTTCATGGCTTTAGCAGG GTTTGATCCCCTGGAGGAGGAAACGG	Baumung*et al.* (2006)
OarFCB11 (Ovine)	L01531	2	8	5.03	0.74	100-160	63	0.78	0.80	GCAAGCAGGTTCTTTACCACTAGCACC GGCCTGAACTCACAAGTTGATATATCTATCAC	Baumung*et al.* (2006)
OarFCB20 (Ovine)	L20004	2	9	4.56	0.71	96-120	56	0.76	0.76	GGAAAACCCCCATATATACCTATAC AAATGTGTTTAAGATTCCATACATGTG	Buchanan *et al.* (1994)
OarHH47 (Ovine)	L12557	18	10	5.22	0.77	130-152	58	0.82	0.82	TTTATTGACAAACTCTCTTCCTAACTCCACC GTAGTTATTTAAAAAAATATCATACCTCTTAAGG	Henry*et al.*(1993
OarJMP29 (Ovine)	U30893	24	6	4.21	0.65	96-150	56	0.67	0.72	GTATACACGTGGACACCGCTTTGTAC GAAGTGGCAAGATTCAGAGGGGAAG	Crawford *et al.* (1995)

Additional information on markers can be found in the FAO's draft guidelines on molecular genetic characterization of animal genetic resources, (http://www.fao.org/documents/search/en, verified October 2014). chr, chromosome location; A_t, total number of alleles; ENA, effective number of Alleles; PIC, polymorphism information content; bp, base pairs; T_n, annealing temperature; Ho, observed heterozygosity; He, expected heterozygosity; primer sequences and Ref, reference.

Table 2. Number of alleles at each locus and average gene diversity overall and within each breed

	Overall		Ossimi		Barki		Rahmani	
	N alleles	Gene diversity	N alleles	Gene diversity	N alleles	Gene diversity	N alleles	Gene diversity
BM1329	8	0.78	5	0.68	5	0.89	5	0.76
BM8125	7	0.71	5	0.63	5	0.79	6	0.71
ILSTS005	7	0.81	5	0.53	7	0.94	6	0.95
MAF65	6	0.78	6	0.75	6	0.82	5	0.76
MAF70	11	0.86	8	0.85	8	0.91	7	0.82
OarCP20	8	0.81	5	0.68	8	0.91	6	0.84
OarFCB11	8	0.78	8	0.63	7	0.80	7	0.92
OarFCB20	9	0.77	7	0.78	8	0.89	6	0.63
OarHH47	10	0.83	7	0.75	8	0.86	9	0.87
OarJMP29	6	0.67	5	0.63	6	0.71	4	0.68
Mean/Locus	8	0.78	6.1	0.69	6.8	0.85	6.1	0.79

Table 3. Allelic richness for each breed

Marker	Ossimi	Barki	Rahmani	Mean
BM1329	5.00	5.00	5.00	5.00
BM8125	5.00	6.00	5.92	5.64
ILSTS005	4.97	7.00	6.00	5.99
MAF65	5.86	6.00	5.00	5.62
MAF70	8.00	8.00	6.92	7.64
OarCP20	5.00	8.00	5.92	6.31
OarFCB11	4.99	7.00	7.00	6.33
OarFCB20	6.98	8.00	6.00	6.99
OarHH47	6.99	8.00	9.00	8.00
OarJMP29	4.98	6.00	4.00	4.99
Mean	5.78	6.90	6.08	6.25

Table 4. P-values for Pearson's χ^2 test of Hardy-Weinberg equilibrium

Microsatellites	Ossimi	Barki	Rahmani
BM1329	0.10	0.02*	0.99
BM8125	0.97	0.03*	0.24
ILSTS005	0.38	0.38	0.20
MAF65	0.99	0.15	0.33
MAF70	0.01*	0.001**	<0.0001***
OarCP20	0.02*	0.64	0.07
OarFCB11	0.27	0.42	0.05*
OarFCB20	0.97	0.29	0.94
OarHH47	0.72	0.06	<0.0001***
OarJMP29	0.50	0.10	0.02*

*significant deviation from zero with P< 0.05.
**significant deviation from zero with P< 0.01.
***significant deviation from zero with P< 0.001.

Table 5.Sample size and heterozygosity averaged over 10 microsatellite loci

Breed	No. animals	Average heterozygosity

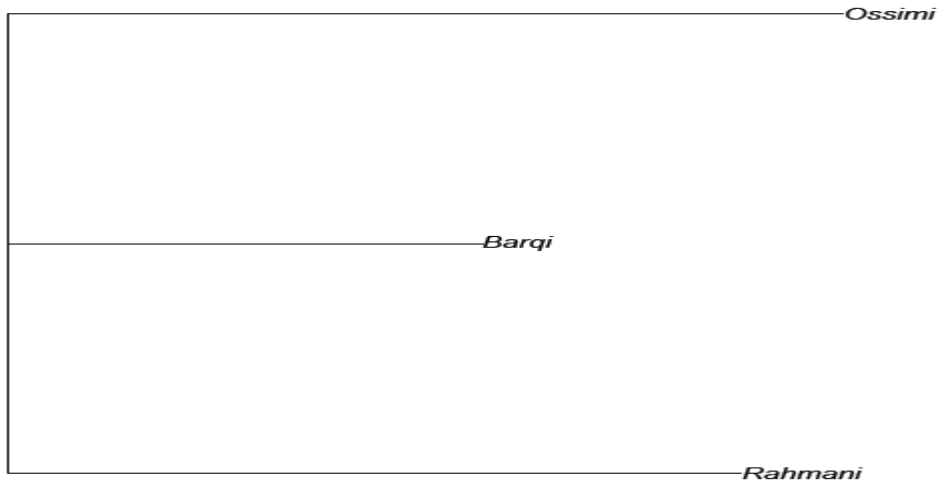
		Observed	Expected
Ossimi	40	0.74±0.15	0.68±0.10
Barki	35	0.85±0.07	0.77±0.05
Rahmani	38	0.80±0.10	0.76±0.05
Total	113	0.80±0.10	0.76±0.05

Table 6. Fixation indices (F_{it}, F_{st} and F_{is}) according to Weir & Cockerham (1984)

Locus	F_{it}	F_{st}	F_{is}
BM1329	-0.014	0.022	-0.037
BM8125	0.015	0.011	0.003
ILSTS005	-0.068	0.075	-0.155
MAF65	-0.030	0.031	-0.064
MAF70	-0.010	0.012	-0.023
OarCP20	0.028	0.083	-0.059
OarFCB11	0.047	0.041	0.005
OarFCB20	0.004	0.018	-0.014
OarHH47	0.008	0.026	-0.019
OarJMP29	0.077	0.042	0.036
Overall mean	0.006	0.036	-0.033

Table 7. Fixation index (F_{st}) as a measure of the genetic differentiation (upper diagonal) and genetic distance (lower diagonal) between each pair of Egyptian sheep breeds

	Ossimi	Barki	Rahmani
Ossimi	-	0.038	0.049
Barki	0.369	-	0.027
Rahmani	0.442	0.340	-



Discussion

All the microsatellite markers were successfully amplified and were found to be highly polymorphic in the three Egyptian sheep breeds included in the present study. The range of alleles per locus was between 6 and 11 (Table 1). Detected alleles per locus averaged eight (Table 2). Comparable results were found by Elfawal *et al*. (2008) and El

Nahas *et al.* (2008). The NEA obtained (4.19) is close to that of 4.77 mentioned by Elfawal *et al.* (2008) and markedly lower than that of 14.13 reported by Ghazy *et al.* (2013).

The average PIC per microsatellite (0.71) was within the range for the Egyptian breeds mentioned by Elfawal *et al.* (2008) and Ghazy *et al.* (2013). According to Botstein *et al.* (1980), loci with PIC value of 1 or close to 1 with many alleles are normally desired for genetic diversity studies. Therefore, PIC values obtained in this study can be considered somewhat high and markers are highly polymorphic, possessing total PIC values greater than 0.5. These results suggest the applicability of those microsatellites for posterior genetic studies in Egyptian sheep populations.

With exception of two loci (OarFCB11 and OarJMP29), all estimates of H_e were ≤ their H_o counterparts for each marker/breeds (Table 1). The overall locus heterozygosity averaged 0.78 over breeds (Table 2), reflecting a notably high variability, which is in agreement with earlier studies (Arranz *et al.*, 2001; Baumung *et al.*, 2006 and Elfawal *et al.*, 2008). Out of the three studied breeds, Barki had the highest gene diversity over all considered loci followed by Rahmani and Ossimi.

Because the observed number of alleles in a sample depends largely on sample size, allelic richness (R_t) was estimated over all samples for each locus and breed. With exception of MAF65 and OarJMP29, markers derived from sheep showed higher overall allelic richness than cattle loci. The estimates of allelic richness within breeds were moderate, ranging from 5.78 to 6.90. These estimates are in accordance to preceding studies (Lawson Handley *et al.*, 2007 and Peter *et al.*, 2007).

Although significant deviations from HWE have been observed for some loci at least in one breed, none of them were excluded from further calculations. Because of null alleles were very rare in this study, subsequently these markers were included in the genetic differentiation analyses. Departure from HWE at microsatellites has been documented by Arranz *et al.* (2001), Hassan *et al.* (2003), Baumung *et al.* (2006) and Ghazy *et al.* (2013). Lawson Handley *et al.* (2007) suggested three possible reasons for the widespread heterozygote deficit in sheep breeds, which are null alleles, subdivision among flocks and nonrandom mating due to inbreeding.

The observed number of alleles per each locus reflects genetic diversity at that locus and subsequently has a direct effect on levels of within-breed diversity in terms of heterozygosity estimates (Buchanan *et al.*, 1994). Overall mean H_o estimated from 10 microsatellites reflects a considerably high variability among the three breeds (0.80), as shown in Table 5. All estimates of the H_e between breeds were slightly lower than the H_o ones, without any significant differences. Similar results on Egyptian sheep were reported by Elfawal *et al.* (2008). On the other hand, Moioli *et al.* (2006), Peter *et al.* (2007), El Nahas *et al.* (2008) and Ghazy *et al.* (2013) found that H_e estimates were higher than their H_o counterparts. The authors refereed their results to lack of heterozygosity due to inbreeding and/or segregation of null alleles.

Considering fixation indices, although the overall F_{st} value only accounted to 3.6%, remarkable genetic diversity was demonstrated in terms of allelic number, allelic richness and PIC, as shown earlier. These findings may present a good opportunity for genetic improvement of indigenous sheep breeds in Egypt through selection within breeds. The levels of within-breed diversity given in Table 6 are comparable to those from preceding studies (Arranz *et al.*, 2001; Baumung *et al.*, 2006; Elfawal *et al.*, 2008 and El Nahas *et al.*, 2008).

Values of F_{is} presented in Table 6 reveal that the genotyped individuals were generally outbred. The negative F_{is} values indicate that mating of more closely related individuals over the mean population is not common. Moreover, about 96% of the variation is presented within rather than among sheep breeds. These results mirror other findings obtained by Hassan *et al.* (2003), Elfawal *et al.* (2008) and El Nahas *et al.* (2008). Lawson Handley *et al.* (2007) reported that if there is subdivision among flocks, sampling various flocks would result in positive estimates of F_{is}. Our sampling strategy applied was adequate to represent the whole breed where animals were genetically unrelated and samples collected from each flock were ≤ 5. It was suggested; therefore, that subdivision among flocks account for deviations from HWE and subsequently inbreeding values obtained.

The overall F_{it} value for all markers was 0.006, indicating a slight degree of inbreeding, which lies in the range of earlier studies (Hassan *et al.*, 2003, Elfawal *et al.*, 2008 and Ghazy *et al.*, 2013). In general, the global inbreeding coefficients point out that Barki, Ossimi and Rahmani breeds are far away from severe inbreeding. This finding could explain the deviation from HWE observed in seven loci analyzed in these breeds.

The estimates of F_{st} between two breeds indicate low genetic differentiation between the three sheep breeds (Table 7). Similar results were reported by Hassan *et al.* (2003) and Elfawal *et al.* (2008). Reduction in genetic differentiation between populations has been largely influenced by migration (Lawson Handley *et al.*, 2007; Peter *et al.*, 2007 and El Nahas *et al.*, 2008).

In general, two items point out that the three breeds analyzed maintain a random mating structure. First, no significant differences are found between H_e and H_o estimates. Second, all inbreeding measures are considered low. In fact, no organized breeding schemes for any economically important traits have been applied in the three

Egyptian sheep breeds. These findings can be considered as a good starting point for conservation and improvement programs, as suggested by Moioli *et al.* (2006).

The genetic distance between Ossimi and Rahmani was higher than that reported by Elfawal *et al.* (2008) and El Nahas *et al.* (2008). However, the estimates of genetic distance obtained in the current study between Barki and Ossimi as well as Barki and Rahmani were lower than those reported by El Nahas *et al.* (2008). According to Hartl & Clark (1997), pair-wise values of F_{st} approaching 0.05 point out to moderate differentiation between populations, while the values greater than 0.1 are considered of high rate. All F_{st} values obtained in the present study are superior to 0.1 which may indicate a high differentiation between breeds. These differences among Egyptian sheep breeds should be maintained through well-planned breeding programs for sustainable use of the indigenous genetic resources. The three breeds were clustered independently (Figure 1). Ossimi, which had the lowest H_o and H_e estimates, tended to be slightly distant from Rahmani and Barki. Similar result was obtained by Peter *et al.* (2007) who found that breeds with low heterozygosities tended to separate from the other breeds in the second principal component analysis (PCA). In contrast, El Nahas *et al.* (2008) indicated that Ossimi and Rahmani breeds were grouped together in one cluster separate from Barki at genetic distance of 0.43. Similar results were reported by Ghazy *et al.* (2013). However, Elfawal *et al.* (2008) indicated that Ossimi and Rahmani were located in different clusters.

The hypothesis of an independent genetic origin for Barki breed on the basis of morphological traits should be considered, because geographical origin seems to affect levels of genetic variation, as suggested by Peter *et al.* (2007). Interestingly, this breed, which is originated from Libya, showed the largest gene diversity over all considered loci as well as the highest H_o and H_e estimates compared with Ossimi and Rahmani breeds. These results probably indicate to greater uniformity within the Barki in comparison with other breeds, which may be a result of the particular historical background of the former revealing higher isolation than other Egyptian sheep during the evolutionary process. It can be inferred that the higher genetic variability of Barki is the consequence of uncontrolled crossbreeding practices. Nevertheless, because the animals analyzed were considered purebred individuals, based on the judgment of official experts, we believe that such variability is simply a reflection of the essential genetic diversity of Barki breed, maintained thanks to the random-mating structure.

Collection of biological samples was extensive; however, there are still involuntary gaps in the geographic coverage (i.e. missing populations). In addition, absence of specific breed borders and no use of herdbook breeding make gene flow between the breeds is probable. This hypothesis is supported by the current genetic distances between Egyptian sheep breeds. Maintaining genetic diversity is a key to the long-term survival of Egyptian sheep breeds and to meet current and future production needs in various environments, to allow sustainable genetic improvement**.**

Conclusion

Our survey of 10 microsatellite markers in Egyptian sheep breeds revealed that within-breed differences account for larger genetic variation than between-breed differences. Values of genetic variation measures obtained in the present study indicate that Egyptian sheep breeds harbor varied and estimable reservoirs of diversity. Inbreeding coefficients are low enough to encourage the implementation of rational conservation programs. It can be derived from the analysis that breed descriptions are often due to effective phenotypic and genetic differences as well as breeding history. The results obtained in the present study provide complementary information to that reported in previous studies on sheep breeds. Additional studies are required to design suitable breeding programs for sound conservation and sustainable utilization of the indigenous sheep breeds available in Egypt.

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