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# Microsatellite-Based Genetic Diversity among Egyptian Sheep Breeds

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## ABSTRACT

Sheep represent one of the most important domestic animals in Egypt, where meat and coarse wool production are the basic breeding objectives for Egyptian sheep. Therefore, evaluation of genetic diversity of sheep breeds is necessary for implementing the most suitable breed-region conservation programs. Animals from the three major Egyptian sheep breeds (Ossimi, Rahmani and Barki) were genotyped for 14 microsatellite markers. All loci tested were highly polymorphic. Various measures of genetic variation were calculated. The total observed number of alleles per microsatellite ranged from 6 to 12 for markers MAF65 and OarHH47, respectively. Estimates of effective number of alleles were between 3.11 for TGLA53 and 6.12 for OarHH47. The mean of polymorphism information content was 0.73. Overall gene diversity for all microsatellites analyzed was 0.66, 0.69 and 0.75 for Ossimi, Rahmani and Barki, respectively. For all breeds studied, estimates of observed heterozygosity were significantly lower than the expected heterozygosity. Average observed and expected heterozygosity estimates were 0.55 and 0.67, respectively. Significant departures from Hardy-Weinberg Equilibrium (HWE) due to heterozygote deficiency were observed for all the markers analyzed. The three breeds revealed significant deviation from HWE. The overall indicator of population subdivision ($F_{ST}$) was calculated to be 0.071; pointing out that about 7% of genetic diversity is due to genetic variation between breeds. Estimates of total inbreeding ($F_{IT}$) and within-breed inbreeding ($F_{IS}$) coefficients were 0.187 and 0.118, respectively. The estimates of pair-wise genetic differentiation were 0.039, 0.051 and 0.056 for Ossimi-Rahmani, Barki-Rahmani and Ossimi-Barki pairs, respectively. Measures of genetic distance between pairs of sheep breeds ranged from 0.423 to 0.615 for Rahmani-Ossimi and Barki-Ossimi sheep breed pairs. The results obtained in this study may be useful in sustainable breeding programs of Egyptian sheep breeds.

**Keywords:** microsatellites, genetic variation, inbreeding, domestic sheep, conservation

## INTRODUCTION

The major sheep breeds recognized in Egypt are Ossimi, Rahmani and Barki. In general, these breeds are characterized as fat-tailed, carpet-wool and medium-sized breeds. They are raised mainly for meat and wool production. Local genetic resources are very important due to their ecological adaptation, disease resistance, unique products and relatively high fertility.

Many animal genetic resources worldwide are at risk of extinction (FAO, 2011). Multiple factors seem to be involved in this issue. First, use of artificial insemination has decreased the number of breeding rams, resulting in a reduction in the effective population size of many breeds. Second, exchange from extensive to intensive production systems to meet the increased demand of the ever growing world population, leading to excluding lower-producing breeds and minority populations from today's sheep industry. Third, ease of transportation that facilitated the movement of genetic materials and made crossbreeding practice much easier. Therefore, the loss of genetic diversity in domestic animal species has important social, economic, environmental and scientific implications.

Progress realized in molecular biology over the past few decades has enabled studying genetic variation at the DNA level through genotyping animals for different molecular markers. Based on their particular characteristics in comparison to other genetic markers, it has become apparent that microsatellites are useful markers for genetic diversity studies (Baumung *et al.*, 2006). Over the past two decades, microsatellites have proven to be effective in genetic biodiversity studies in the ovine species (Moioli *et al.*, 2006; Lawson Handley *et al.*, 2007; Arora *et al.*, 2011; Azhar *et al.*, 2018).

Previous studies have been performed to characterize the genetic differentiation and distance among Egyptian sheep breeds by means of microsatellites (Hassan *et al.*, 2003; Elfawal *et al.*, 2008; El Nahas *et al.*, 2008; Ghazy *et al.*, 2013; Rushdi and Sabry, 2015). Nevertheless, most of them focused on particular breeds, near geographical regions, a limited sample of animals, and/or a small set of microsatellite loci.

The objective of the present study was to assess the genetic diversity between the most common breeds of Egyptian sheep (Ossimi, Rahmani and Barki) in order to get a clear insight into relationships within and between breeds through genotyping animals for a set of 14 microsatellite loci.

## MATERIALS AND METHODS

**Blood samples and DNA extraction:**

A total of 136 blood samples were randomly collected from Ossimi (48), Rahmani (45) and Barki (43) breeds, corresponding to 15, 13 and 12 flocks, respectively. Because of some problems related to the organizational structure of sheep breeding (e.g. non-availability of flock books and absence of breed registration societies), attention was given to collect blood samples from distinct sheep flocks showing breed-specific characteristics and also to sample genetically unrelated individuals. The maximum number of samples per flock didn't exceed 5 animals. Table 1 shows information about the biological samples collected in the present study. The three sheep breeds included in the present study exhibited long and coarse wool characteristics, and were mainly raised for meat and wool production purposes.

**Table 1. Information about the individuals analyzed in the study**

| Governorate | Number of flocks | | |
|---|---|---|---|
| | Ossimi | Rahmani | Barki |
| Alexandria | 1 | 1 | 2 |
| Beheira | 2 | 3 | 2 |
| Fayyum | 2 | 1 | - |
| Giza | 4 | 2 | 1 |
| Ismailia | 1 | 2 | 2 |
| Marsa Matruh | - | - | 5 |
| Menofya | 3 | 2 | - |
| Sharkia | 2 | 2 | - |
| Total | 15 | 13 | 12 |

Blood samples of 5 ml were collected from jugular vein into vacuum tubes containing 0.25% ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Samples were sent in dried ice to the laboratory for DNA isolation. Genomic DNA was isolated from white blood cells using genomic blood DNA purification kit (GeneJET Whole Blood Genomic DNA Purification Mini Kit, Thermo Fisher Scientific, Vilnius, Lithuania) and was kept frozen at -20°C till further analysis.

**Microsatellite genotyping:**

An overview on the analyzed loci is demonstrated in Table 2. The 14 microsatellite markers used in this study have been chosen according to the recommendations of joint ISAG/FAO standing committee (ISAG, 2004) and FAO's draft guidelines on molecular genetic characterization of animal genetic resources (FAO, 2011). Two exceptions regarding marker selection have been occurred. Microsatellite OarCP20 was selected from International Bovine Reference Population (Roslin Institute, Scotland, UK, https://www.ed.ac.uk/roslin). On the other hand, locus OarFCB11 was chosen from MARC97 (Meat Animal Research Center of United States Department of Agriculture, https://www.ars.usda.gov/plains-area/clay-center-ne/marc).

**Table 2. Characteristics of microsatellites analyzed in the three sheep breeds**

| Locus (Origin) | Chr | $N_a$ | $N_e$ | PIC | Size (bp) | $T_n$ | Ho | He | Primer A sequence<br>Primer B sequence | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| MAF65 | 15 | 6 | 3.58 | 0.64 | 117-145 | 55 | 0.64 | 0.67 | AAAGGCCAGAGTATGCAATTAGGAG<br>CCACTCCTCCTGAGAATATAACATG | Buchanan *et al.* (1992) |
| MAF70 | 4 | 11 | 5.88 | 0.77 | 124-166 | 60 | 0.82 | 0.82 | CACGGAGTCACAAAGAGTCAGACC<br>GCAGGACTCTACGGGGCCTTTGC | Buchanan & Crawford (1992a) |
| MAF214 | 16 | 10 | 5.03 | 0.78 | 183-221 | 58 | 0.65 | 0.69 | AATGCAGGAGATCTGAGGCAGGGACG<br>GGGTGATCTTAGGGAGGTTTTGGAGG | Buchanan & Crawford (1992b) |
| BM1329 | 6 | 8 | 4.66 | 0.76 | 160-186 | 63 | 0.76 | 0.72 | TTGTTTAGGCAAGTCCAAAGTC<br>AACACCGCAGCTTCATCC | Bishop *et al.* (1994) |
| BM8125 | 17 | 8 | 4.02 | 0.71 | 98-130 | 56 | 0.67 | 0.69 | CTCTATCTGTGGAAAAGGTGGG<br>GGGGGTTAGACTTCAACATACG | Bishop *et al.* (1994) |
| ILSTS005 | 7 | 7 | 4.24 | 0.70 | 160-216 | 55 | 0.55 | 0.61 | GGAAGCAATGAAATCTATAGCC<br>TGTTCTGTGAGTTTGTAAGC | Brezinsky *et al.* (1993) |
| TGLA53 | 9 | 8 | 3.11 | 0.58 | 146-158 | 52 | 0.48 | 0.59 | CAGCAGACAGCTGCAAGAGTTAGC<br>CTTTCAGAAATAGTTTGCATTCATGCAG | Georges and Massey (1992) |
| OarCP20 | 21 | 9 | 4.38 | 0.73 | 69-103 | 63 | 0.70 | 0.73 | GGCATTTCATGGCTTTAGCAGG<br>GTTTGATCCCCTGGAGGAGGAAACGG | Baumung *et al.* (2006) |
| OarFCB11 | 2 | 8 | 4.72 | 0.75 | 100-160 | 63 | 0.68 | 0.74 | GCAAGCAGGTTCTTTACCACTAGCACC<br>GGCCTGAACTCACAAGTTGATATATCTATCAC | Baumung *et al.* (2006) |
| OarFCB20 | 2 | 10 | 4.93 | 0.80 | 96-124 | 56 | 0.79 | 0.78 | GGAAAACCCCCATATATACCTATAC<br>AAATGTGTTTAAGATTCCATACATGTG | Buchanan *et al.* (1994) |
| OarFCB128 | 2 | 8 | 3.48 | 0.69 | 104-128 | 64 | 0.70 | 0.77 | ATTAAAGCATCTTCTCTTTATTTCCTCGC<br>CAGCTGAGCAACTAAGACATACATGCG | Buchanan & Crawford (1993) |
| OarHH35 | 4 | 9 | 5.44 | 0.79 | 107-133 | 59 | 0.75 | 0.78 | AATTGCATTCAGTATCTTTAAACATCTGGC<br>ATGAAAATATAAAGAGAATGAACCACACGG | Henry *et al.* (1993) |
| OarHH47 | 18 | 12 | 6.12 | 0.83 | 128-156 | 58 | 0.77 | 0.83 | TTTATTGACAAACTCTCTTCCTAACTCCACC<br>GTAGTTATTTAAAAAAATATCATACCTCTTAAGG | Henry *et al.* (1993) |
| OarJMP29 | 24 | 8 | 3.93 | 0.69 | 96-150 | 56 | 0.61 | 0.68 | GTATACACGTGGACACCGCTTTGTAC<br>GAAGTGGCAAGATTCAGAGGGGAAG | Lawson Handley *et al.* (2007) |

**Chr: chromosome number, $N_a$: total observed number of alleles, $N_e$: effective number of alleles, PIC: polymorphism information content, bp: base pairs, $T_n$: annealing temperature, Ho: observed heterozygosity, He: expected heterozygosity, Ref: reference**

Primer sequences and polymerase chain reaction (PCR) conditions of the selected microsatellites were those referred in literature. PCR amplifications were carried out for each marker in 25µl reactions with minor modifications when necessary to improve the quality of PCR products. Amplified PCR products were separated through an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Beijing, China). Genotypes were determined by using the software package GenoTyper 2.1 (Applied Biosystems).

**Statistical analysis:**

Observed number of alleles ($N_a$), effective number of alleles ($N_e$), allele frequencies, observed heterozygosity (Ho) and expected heterozygosity (He) were estimated using POPGENE program (Yeh *et al*., 1999). Polymorphism information content (PIC) was calculated using the formula proposed by Botstein *et al*. (1980). The software package GENEPOP version 1.2 (Raymond and Rousset, 1995) was applied to assess the deviations from Hardy-Weinberg Equilibrium (HWE) for all locus-population combinations and linkage disequilibrium between pairs of microsatellite loci.

In order to examine genetic structure of the investigated populations composed of three Egyptian sheep breeds, F-statistics parameters, such as total inbreeding ($F_{IT}$), population differentiation ($F_{ST}$) and within-population inbreeding estimate ($F_{IS}$) were computed by FSTAT version 2.9.3.2 software package (Goudet, 2002). The null hypothesis was that the estimates were not significantly different from zero. The significance level ($P<0.05$) was assigned using Bonferroni corrections performed over all markers analyzed.

Allele frequencies were used to compute estimates of genetic distance based on Nei's genetic distance procedure (Nei *et al*., 1983). Subsequently, phylogenetic tree was built using the neighbor-joining (NJ) method included in the POPULATIONS 1.2.28 (Langella, 2002) software package. Genetic diversity between sheep breeds

was measured based on analysis of molecular variance (AMOVA) proposed by Excoffier *et al*. (2005).

## RESULTS

**Genetic variability:**

Table 2 illustrates the indices of genetic variability of loci analyzed in the current study. One hundred and twenty two alleles were identified across the 14 microsatellite markers spread over 11 ovine chromosomes in the 3 breeds investigated. The most polymorphic locus was OarHH47, with 12 alleles; while MAF65 exhibited the lowest observed number of alleles per locus (6).

The $N_e$ per locus varied from 3.11 for TGLA53 to 6.12 for OarHH47, while the overall mean for markers within breeds was 4.54. Estimates of PIC varied from 0.58 for TGLA53 to 0.83 for OarHH47, with an average of 0.73. The average Ho per marker varied between 0.48 and 0.82 for TGLA53 and MAF70, respectively. While the corresponding estimates of the He ranged from 0.59 to 0.83 for microsatellites TGLA53 and OarHH47, respectively.

For all the microsatellites genotyped in the three breeds investigated, the overall locus heterozygosity averaged 0.70 (Table 3). The average gene diversity over all loci ranged between 0.66 in Ossimi and 0.75 in Barki, while it had an intermediate estimate (0.69) in Rahmani breed. The lowest gene diversity within breed was observed for OarHH35 in Ossimi (0.51), while the highest estimate was recorded for MAF70 in Barki (0.85). Ossimi sheep breed had the lowest $N_a$ per locus (4) for MAF65, with a gene diversity value of 0.54. On the other hand, the highest $N_a$ per locus (10) has been recorded three times for two different markers. Firstly, locus MAF70 in Barki breed, with a gene diversity value of 0.85. Secondly, microsatellite OarHH47 in both Barki and Rahmani breeds, with gene diversity values of 0.82 and 0.79, respectively.

**Table 3. Observed number of alleles 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 |
| MAF65 | 6 | 0.59 | 4 | 0.54 | 5 | 0.63 | 5 | 0.60 |
| MAF70 | 11 | 0.81 | 7 | 0.75 | 10 | 0.85 | 8 | 0.82 |
| MAF214 | 10 | 0.72 | 5 | 0.66 | 9 | 0.74 | 7 | 0.76 |
| BM1329 | 8 | 0.69 | 7 | 0.73 | 5 | 0.64 | 7 | 0.70 |
| BM8125 | 8 | 0.71 | 7 | 0.68 | 5 | 0.78 | 6 | 0.67 |
| ILSTS005 | 7 | 0.66 | 6 | 0.64 | 6 | 0.73 | 5 | 0.62 |
| TGLA53 | 8 | 0.62 | 6 | 0.68 | 8 | 0.66 | 7 | 0.52 |
| OarCP20 | 9 | 0.75 | 7 | 0.69 | 8 | 0.82 | 6 | 0.75 |
| OarFCB11 | 8 | 0.72 | 7 | 0.62 | 6 | 0.76 | 7 | 0.79 |
| OarFCB20 | 10 | 0.73 | 8 | 0.72 | 9 | 0.81 | 6 | 0.66 |
| OarFCB128 | 8 | 0.73 | 7 | 0.75 | 6 | 0.72 | 6 | 0.71 |
| OarHH35 | 9 | 0.63 | 5 | 0.51 | 9 | 0.77 | 6 | 0.62 |
| OarHH47 | 12 | 0.78 | 7 | 0.72 | 10 | 0.82 | 10 | 0.79 |
| OarJMP29 | 8 | 0.68 | 5 | 0.61 | 8 | 0.75 | 6 | 0.67 |
| Mean/Locus | 8.7 | 0.70 | 6.3 | 0.66 | 7.4 | 0.75 | 6.6 | 0.69 |

Tests for deviations from HWE for all locus-breed combinations are shown in Table 4. Four loci (MAF65, MAF214, TGLA53, OarFCB20) demonstrated significant deviation from HWE in the three breeds studied. Three markers (BM1329, OarHH35, OarHH47) showed significant deviation from HWE solely in two breeds. Seven loci (MAF70, BM8125, ILSTS005, OarCP20, OarFCB11, OarFCB128, OarJMP29) deviated significantly from HWE only in one sheep breed.

**Table 4. P-values for Pearson's $\chi^2$ test of Hardy-Weinberg equilibrium**

| Microsatellites | Ossimi | Barki | Rahmani |
|---|---|---|---|
| MAF65 | 0.261* | 0.118* | 0.215* |
| MAF70 | 0.057 | -0.053 | 0.128* |
| MAF214 | 0.216* | 0.139* | 0.314* |
| BM1329 | 0.152* | -0.037 | 0.208* |
| BM8125 | 0.117* | -0.016 | -0.023 |
| ILSTS005 | 0.235* | 0.183 | 0.031 |
| TGLA53 | 0.204* | 0.165* | 0.273* |
| OarCP20 | 0.077 | 0.262* | 0.112 |
| OarFCB11 | 0.197 | 0.234* | 0.039 |
| OarFCB20 | 0.276* | 0.132* | 0.183* |
| OarFCB128 | -0.028 | 0.116* | -0.041 |
| OarHH35 | 0.144* | 0.082 | 0.235* |
| OarHH47 | 0.153* | 0.056 | 0.213* |
| OarJMP29 | 0.122 | 0.187* | 0.092 |
| Overall mean | 0.156 | 0.112 | 0.141 |

***Significant deviation from zero with P<0.05**

Average heterozygosity estimates for the studied sheep breeds assessed through the Wright's F diversity indices are given in Table 5. The average Ho over all loci ranged between 0.50 and 0.61 in Ossimi and Barki, respectively. Estimates of He varied from 0.59 in Ossimi to 0.75 in Barki. Differences between He and Ho were significant (P<0.05). Mean estimates of Ho and He over all markers and breeds were 0.55 and 0.67, respectively.

**Table 5. Sample size and heterozygosity averaged over 14 microsatellite markers**

| Breed | No. animals | Average heterozygosity | |
|---|---|---|---|
| | | Observed | Expected |
| Ossimi | 48 | 0.50±0.11 | 0.59±0.06 |
| Rahmani | 45 | 0.55±0.05 | 0.67±0.08 |
| Barki | 43 | 0.61±0.09 | 0.75±0.16 |
| Total | 136 | 0.55±0.08 | 0.67±0.10 |

**Genetic differentiation and distance:**

Calculations of the three inbreeding indicators are demonstrated in Table 6. Means of all inbreeding parameters ($F_{IT}$, $F_{ST}$ and $F_{IS}$) over all loci were positive and significant (P<0.05). Estimates of $F_{ST}$ were consistently positive for all markers. The overall high value of $F_{ST}$ (0.071) calculated across all the markers reflects a relatively high degree of sheep breed differentiation. Inbreeding coefficients of $F_{IS}$ varied from -0.027 to 0.297 for BM1329 and OarHH35, respectively. Only three

markers gave negative $F_{IS}$ estimates (BM1329, OarCP20, OarFCB20). The total inbreeding coefficient of an individual related to whole population ($F_{IT}$) averaged 0.187, while extreme values ranged between 0.017 for BM1329 and 0.371 for OarHH35.

The pair-wise comparisons of differentiation among breeds ($F_{ST}$) varied from 0.039 between Ossimi and Rahmani to 0.056 between Ossimi and Barki (Table 7). The shortest genetic distance was 0.423 between Ossimi and Rahmani. On the other hand, the longest one was 0.615 recorded between Barki-Ossimi breed pair. In addition, the cluster analysis using NJ approach was carried out with the entire data set. The results indicated that Ossimi-Rahmani breed pair clustered in one group, while Barki breed formed an independent cluster.

**Table 6. Estimators of F statistics at each locus across the three sheep breeds**

| Locus | $F_{ST}$ | $F_{IS}$ | $F_{IT}$ |
|---|---|---|---|
| MAF65 | 0.115* | 0.132* | 0.243* |
| MAF70 | 0.079 | 0.066* | 0.144* |
| MAF214 | 0.065* | 0.168* | 0.221* |
| BM1329 | 0.045* | -0.027* | 0.017* |
| BM8125 | 0.058 | 0.154 | 0.212 |
| ILSTS005 | 0.147 | 0.123 | 0.265* |
| TGLA53 | 0.038 | 0.216 | 0.272 |
| OarCP20 | 0.053* | -0.014* | 0.038* |
| OarFCB11 | 0.113 | 0.108 | 0.217 |
| OarFCB20 | 0.046* | -0.015 | 0.029* |
| OarFCB128 | 0.064 | 0.193* | 0.257* |
| OarHH35 | 0.078* | 0.297* | 0.371* |
| OarHH47 | 0.027* | 0.098* | 0.124 |
| OarJMP29 | 0.066 | 0.146* | 0.208* |
| Overall mean | 0.071* | 0.118* | 0.187* |

**$F_{ST}$: measurement of population differentiation, $F_{IS}$: within-population inbreeding, $F_{IT}$: total inbreeding estimate, *: P<0.05**

**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 | Rahmani | Barki |
|---|---|---|---|
| Ossimi | - | 0.039 | 0.056 |
| Rahmani | 0.423 | - | 0.051 |
| Barki | 0.615 | 0.569 | - |

## DISCUSSION

**Genetic variability:**

The whole set of the microsatellites analyzed were successfully amplified in the native Egyptian sheep included in the current study. The $N_a$ ranged from 6 to 12, as shown in Table 2. Detected alleles per locus averaged 8.7 (Table 3). This finding is in accordance with preceding studies carried out by Elfawal *et al*. (2008), El Nahas *et al*. (2008) and Rushdi and Sabry (2015) in Egyptian; Baumung *et al*. (2006) in Austrian; Moioli *et al*. (2006) in Italian and Mukesh *et al*. (2006) and Arora *et al*. (2011) in Indian sheep. However, it was much higher than that reported in Pakistani sheep (Ibrahim *et al*., 2010). Because the observed number of alleles in a given sample depends largely on sample size, the set of alleles identified in the present study was greater than that observed in the earlier study of Rushdi and Sabry (2015; 122 *vs* 80 alleles, respectively), due to the increment in number of the animals and markers genotyped. Nevertheless, Ghazy *et al.* (2013) found $N_a$ for Ossimi and Rahmani higher than that reported in this study.

Generally speaking, large differences in $N_e$ between loci analyzed were observed. The estimates of $N_e$ obtained were moderate, ranging from 3.11 to 6.12 (Table 2). The average $N_e$ calculated in the present study (4.54) is close to that of 4.77 reported by Elfawal *et al.* (2008) and much lower compared to that of 14.13 mentioned by Ghazy *et al.* (2013) in Egyptian sheep breeds. In Indian sheep, Mukesh *et al*. (2006) reported that the estimates of $N_e$ were 3.05, 3.49 and 3.24 for Chokla, Nali and Garole breeds, respectively.

Regarding to PIC, the average PIC per locus (0.73) was within the range for the Egyptian sheep reported by Elfawal *et al.* (2008) and Ghazy *et al*. (2013). The analysis of the three Egyptian sheep breeds with 14 microsatellites demonstrated that all the loci analyzed were highly polymorphic, having a PIC value of more than 0.5 (Botstein *et al*., 1980). The authors indicated that loci with PIC value of approximately 1 and also with many alleles are recommended for genetic biodiversity studies. Since PIC estimates ranged between 0.58 for TGLA53 and 0.83 for OarHH47, PIC values obtained in the present study can be generally considered relatively high, and markers are highly polymorphic. This result supports the utilization and application of that set of microsatellites in assessing biodiversity of indigenous Egyptian sheep populations. Comparable findings for PIC were found (Mukesh *et al*., 2006; Elfawal *et al*., 2008; Arora *et al*., 2011; Ghazy *et al*., 2013; Rushdi and Sabry, 2015).

All estimates of He were higher than their Ho counterparts for the entire set of markers analyzed with exception of BM1329 and OarFCB20 (Table 2). Also, microsatellite MAF70 had equal Ho and He estimates. The overall heterozygosity for all markers over breeds averaged 0.70 (Table 3), indicating a high variability, which is in harmony with the preceding studies carried out by Baumung *et al.* (2006), Mukesh *et al*. (2006), Elfawal *et al.* (2008) and Rushdi and Sabry (2015). Out of the three studied breeds, Barki had the greatest gene diversity over all considered markers followed by Rahmani and Ossimi. Similar result in Egyptian sheep was reported by El Nahas *et al.* (2008).

According to Buchanan *et al.* (1994), $N_a$ reflects genetic diversity at a particular locus and subsequently affects within-breed diversity degree in terms of heterozygosity estimates. Average Ho per breed estimated from 14 microsatellites indicates a substantial variability among the three breeds (Table 5). The highest average Ho and He values were registered for Barki (Ho = 0.61, He = 0.75) followed by Rahmani (Ho = 0.55, He = 0.67) and Ossimi (Ho = 0.50, He = 0.59). Not surprisingly, Barki breed showed the highest average $N_a$ (7.4) while; the lowest estimate was calculated for Ossimi (6.3).

Obviously, all estimates of the He between breeds were greater than the Ho ones. Estimates of Ho and He differed significantly (P<0.05) from each other. These findings mirror other results reported by Moioli *et al.* (2006), Peter *et al.* (2007), El Nahas *et al.* (2008) and Ghazy *et al.* (2013). Shortage of heterozygosity due to

inbreeding and/or segregation of null alleles seem to be the foremost rationale for this result. In contrast, Elfawal *et al.* (2008) found that estimates of Ho were higher than their corresponding He values in Rahmani, Ossimi and Saidi sheep breeds.

Heterozygote deficiency analysis showed that the three sheep breeds displayed significant deviations from HWE ($P<0.05$) at many microsatellites. It is not easy to determine exactly the basis of this departure; however, the segregation of null alleles with low frequency at these marker loci could be a possible cause, as previously explained by Peter *et al*. (2007). Different reasons have been proposed as potential factors responsible for the widespread heterozygote deficit in sheep breeds, including the presence of null alleles, subdivision among flocks, small sample size and nonrandom mating due to inbreeding (Lawson Handley *et al*., 2007).

In the investigated sheep populations shown in Table 4, $F_{IS}$ values greater than zero reflect a decrease in heterozygotes and an increase in homozygous genotypes. These findings may be due to a number of factors, e.g. presence of null alleles, sample relatedness, population heterogeneity and/or linkage with specific loci under selection (Mukesh *et al*., 2006; Peter *et al*., 2007; Elfawal *et al*., 2008). The limited sample size associated with relatedness of few samples under field conditions are probably the most important factors involved in the significant $F_{IS}$ values obtained in the current study. Several studies have documented the departure from HWE in sheep (Hassan *et al*., 2003; Baumung *et al*., 2006; Ghazy *et al*., 2013; Rushdi and Sabry, 2015; Azhar *et al*., 2018).

In general, the average mean values for all genetic diversity measures calculated in the current study suggested that the three sheep breeds displayed a relatively high level of genetic variation. All the sheep breeds investigated were genetically diverse at the 14 loci genotyped, as evident from the high values obtained for the observed number of alleles (≥6) and gene diversity (>0.6).

**Genetic differentiation and distance:**

Examination of population differentiation were performed by three fixation indices ($F_{IT}$, $F_{ST}$ and $F_{IS}$) for each of the 14 microsatellites analyzed across the three Egyptian sheep breeds (Table 6). The overall estimates for F-statistics differed significantly ($P<0.05$) from zero. The average genetic differentiation between all sheep breeds ($F_{ST}$) was 0.071, indicating moderate genetic discrimination between the three indigenous sheep breeds analyzed in this study. The multi-locus $F_{ST}$ values of breed differentiation pointed out that approximately 7.1% of the whole genetic variation was due to population/breed differences, with the remaining 92.9 % refers to differences among animals within the three breeds (intra-population genetic diversity) across the 14 microsatellite loci genotyped. Although the overall $F_{ST}$ value accounted to 7.1%, remarkable genetic diversity was demonstrated in terms of $N_a$, $N_e$ and PIC, as mentioned above. These results may present a good chance for genetic improvement of the native sheep breeds of Egypt by a means of within-breed selection.

Lack of specific selection schemes, genetic drift and founder effects may have contributed to the moderate level of differentiation among the studied breeds of ovine species. The levels of between-breed diversity given in Table 6 are in accordance with preceding studies (Baumung *et al*., 2006; Elfawal *et al*., 2008; El Nahas *et al*., 2008; Ibrahim *et al*., 2010; Arora *et al*., 2011; Rushdi and Sabry, 2015). Mukesh *et al.* (2006) concluded a substantial degree of differentiation between three Indian sheep breeds (Chokla, Nali and Garole) based on the obtained $F_{ST}$ estimate of 0.183 across all the 11 ovine-specific microsatellite loci genotyped. In the current study, caution has been taken for sampling genetically unrelated animals and only those individuals expressing breed-specific characteristics. Moreover, the maximum number of samples per flock didn't exceed five animals. Nevertheless, the possibility of mixing blood with non-descript animals or other breeds into the studied populations cannot be entirely avoided.

Inbreeding coefficients within breeds ($F_{IS}$) demonstrated in Table 6 point out that the genotyped animals were generally inbred. Values of $F_{IS}$ averaged 0.118, indicating the existence of a low degree of inbreeding in the populations analyzed. This finding may justify the relatively moderate Ho and He values obtained in the three breeds and the deviation from HWE that were observed in all markers overall breeds studied. This result is in agreement with that reported by Ghazy *et al*. (2013). Compared to other studies in Egyptian sheep, Hassan *et al*. (2003) obtained greater $F_{IS}$ estimate (0.302) that is similar to the positive estimate (0.308) reported by El Nahas *et al.* (2008). However, Elfawal *et al.* (2008) found negatively lower $F_{IS}$ estimate (-0.277) compared to the current study. Differences in sampling methods and experimental designs among those genetic diversity studies reflected on the results obtained.

Microsatellites BM1329, OarCP20 and OarFCB20 presented negative $F_{IS}$ estimates. In fact, the negative $F_{IS}$ values indicate that mating of more closely related animals over the mean population is limited. Approximately 88% of the variation is shown within rather than among breeds. This finding emphasizes the role of within-breed selection for genetic improvement of Egyptian sheep breeds.

Lawson Handley *et al*. (2007) concluded that when subdivision among flocks is present, sampling different flocks will result in positive $F_{IS}$ estimates. The sampling strategy followed in the current study was suitable to represent the whole breed where sampled individuals were genetically unrelated and the number of samples collected from each flock was ≤ 5. Consequently, it was proposed that subdivision among flocks affected deviations from HWE and inbreeding values obtained. As mentioned by Mukesh *et al*. (2006), fairly high positive $F_{IS}$ values could also be linked to deviations from HWE observed in Indian sheep populations. Nevertheless, the certain effect of null alleles on the observed deficit of heterozygotes and also on significant $F_{IS}$ values obtained in the present study cannot be disregard.

The global inbreeding coefficient for all loci was 0.187, indicating a weak level of inbreeding in the population investigated. The $F_{IT}$ estimate calculated in the present study is comparable with previous studies carried out in Egyptian sheep breeds (Hassan *et al*., 2003; Elfawal *et al*., 2008; El Nahas *et al*., 2008; Ghazy *et al*., 2013).

Broadly speaking, the overall $F_{IT}$ value obtained indicates that the three sheep breeds studied suffer from a mild level of inbreeding. This result may explain the significant deviations ($P<0.05$) from HWE at all marker loci tested in Ossimi, Rahmani and Barki sheep.

Concerning genetic differentiation values based on two-breed comparisons ($F_{ST}$), the values ranged between 0.039 and 0.056 for Ossimi-Rahmani and Ossimi-Barki pairs, respectively. Barki and Rahmani showed an intermediate estimate of 0.051. In general, the estimates of $F_{ST}$ between each two breeds reflect relatively low genetic differentiation between the three sheep breeds (Table 7). Nevertheless, the results obtained indicated appreciable genetic differentiation between Barki sheep historically originated from Libya and the other two breeds of Egyptian origin. Similar findings were reported by Hassan *et al.* (2003) and Elfawal *et al.* (2008). The geographical proximity of Ossimi and Rahmani may explain the lowest estimate of genetic differentiation reported in the current study. Migration and gene flow have been stated as reasons of the reduced genetic differentiation among populations by many researchers (Lawson Handley *et al.*, 2007; Peter *et al.*, 2007; El Nahas *et al.*, 2008; Arora *et al*., 2011; Rushdi and Sabry, 2015).

According to Hartl and Clark (1997), pair-wise values of $F_{ST}$ near to 0.05 reflect moderate differentiation between breeds, while the values higher than 0.1 point out a high rate of identity. All $F_{ST}$ values reported in this study are generally proximate to 0.05, which may indicate a moderate degree of genetic diversity between the breeds investigated. These differences among Egyptian sheep breeds should be maintained using sustainable breeding programs of the local genetic resources. In fact, no organized breeding schemes for economically and ecologically important traits have been historically 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 intermediate degrees of genetic differentiation calculated between Ossimi and Barki followed by Barki and Rahmani, and finally Ossimi and Rahmani were later supported by a relatively low value of gene flow expressed as a short genetic distance between the two breeds originated historically from Nile Delta region (Ossimi and Rahmani) in comparison with that breed previously introduced to Egypt from Libya (Barki). The considerably noticeable level of gene flow between Barki and Ossimi (0.615) and between Barki and Rahmani (0.569) compared to Rahmani and Ossimi (0.423) supported the genetic relatedness of Rahmani and Ossimi sheep breeds. Also, the cluster analysis demonstrated a clear variation between Rahmani, Ossimi and Barki breeds. The Barki animals were clustered exclusively in a separate group, while grouping of both Rahmani and Ossimi individuals together supported their close genetic proximity. The results obtained in the present study are in harmony with the findings reported by El Nahas *et al.* (2008). The authors showed that Ossimi and Rahmani breeds were grouped exclusively in one cluster separate from Barki at genetic distance of 0.43. Similar findings were reported by Ghazy *et al.* (2013). On the other hand, Elfawal *et al.* (2008) mentioned that Ossimi and Rahmani were located in distinct clusters.

The estimates of genetic distance obtained in the current study between Barki and Ossimi as well as Barki and Rahmani were higher than those estimated by El Nahas *et al.* (2008) and Rushdi and Sabry (2015). On the same trend, the genetic distance between Ossimi and Rahmani was greater compared to that reported by Elfawal *et al.* (2008), El Nahas *et al.* (2008) and Ghazy *et al.* (2013). However, it was lower than that obtained by Rushdi and Sabry (2015). These estimates of genetic distance between Egyptian sheep are comparable to those reported for Indian (Mukesh *et al.*, 2006) and European and Middle Eastern (Peter *et al.*, 2007) sheep, but much higher than Italian (Moioli *et al*., 2006) and Pakistani (Ibrahim *et al.*, 2010) sheep breeds.

Presence of a considerable differentiation between Barki-Rahmani and Barki-Ossimi pairs may be due to the historically geographical isolation of Barki – the meat sheep breed of western region near to Libya – compared to Rahmani and Ossimi – the double-purpose sheep (meat and carpet wool) – that are the most dominant breeds in north and middle of Egypt. The genetic closeness of Rahmani-Ossimi breed pair was also supported by the low genetic distance between them, which lies in geographical proximity to each other in comparison to Barki breed. Nevertheless, important factors such as high gene flow and intermixing of gene pool between Rahmani and Ossimi breeds have to be taken into consideration. The closeness between the Egyptian sheep breeds may be attributed to sharing common breeding practices, which could have resulted in genetic exchange between the three breeds studied.

In addition to geographical proximity of the breeds investigated, the fact that sheep breeders of Egypt prefer the rams of heavier breeds like Rahmani for breeding in order to get heavier body weight of lambs may account for the results obtained. Supported by the estimates of gene diversity obtained in the present study, Rahmani could be classified as an intermediate breed between Ossimi and Barki sheep. The genetic drift within breeds affects the genetic variability among them when both genetic differentiation and distance are calculated using the allelic frequency data (Arora *et al*., 2011).

The hypothesis of an independent genetic origin for Barki breed based on the morphological traits has to be considered. This is because geographical origin tends to influence levels of genetic variation (Peter *et al.*, 2007; Arora *et al*., 2011; de Simoni Gouveia *et al*.; 2017). Curiously, Barki exhibited the greatest overall mean of gene diversity calculated along 14 loci and also had the highest Ho and He estimates in comparison with Ossimi and Rahmani breeds.

The results obtained probably point out to higher identity within Barki compared to other breeds, which may be due to historical background of the founder effects revealing greater isolation than other Egyptian sheep during evolution. It can be concluded that the higher genetic variation of Barki is a result of uncontrolled mating systems, including crossbreeding. Nevertheless, taking into account that the individuals sampled were considered pure-bred animals, according to the judgment of sheep farmers

and official experts, it can be thought that such variability is due partially to the essential genetic diversity of Barki breed that is historically originated from Libya.

In spite of the biological samples have been collected from different geographical areas, considerably higher level of gene flow was observed between pairs of sheep breeds. In fact, absence of specific geographical locations for each breed, and non-availability of breed associations as well as flock books are common characteristics of sheep breeding in Egypt. These factors can facilitate gene flow between breeds. The relatively low to moderate estimates of genetic distance between the three sheep breeds investigated strongly support this hypothesis. Measuring and maintaining genetic diversity is essential for survival and sustainability of local sheep breeds in order to fulfill the future production requirement in different environments (de Simoni Gouveia *et al*.; 2017; Azhar *et al*., 2018). The results reported in the present study provide complementary information to that reported in previous studies on the Egyptian sheep breeds.

## CONCLUSION

Genotyping animals belonging to three Egyptian sheep breeds for 14 microsatellites demonstrated that larger percentage of genetic variation was due to within-breed rather than between-breed genetic diversity. Measures of genetic variation applied in this study indicate that Egyptian sheep breeds maintain a considerable diversity, where the estimated inbreeding coefficients are relatively low. The present study further proposed that Barki breed is the most genetically distinct of the three sheep breeds and may be given priority in the future conservation programs. This work offers an important step towards conservation and sustainable use of these native genetic resources. However, more studies are required to design suitable breeding programs for sustainable use of the indigenous Egyptian sheep breeds in the future.

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## التنوع الوراثى القائم على الواسمات الوراثية الدقيقة بين سلالات الأغنام المصرية

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تمثل الأغنام واحدة من الحيوانات الداجنة الأكثر أهمية فى مصر، حيث يشكل إنتاج اللحم والصوف الخشن الأهداف الأساسية لتربية الأغنام المصرية. ولذلك، يعد تقييم التنوع الوراثى لسلالات الأغنام ضروريًا لتنفيذ برامج المحافظة على المصادر الوراثية الأكثر مناسبة لكل سلالة وفقاً للظروف المتاحة بكل إقليم. إشتملت الدراسة على حيوانات تنتمى إلى سلالات الأغنام المصرية الرئيسة الثلاثة (أوسيمى، رحمانى، برقى)، وتم تحديد التركيب الوراثى لها بالنسبة إلى 14 من الواسمات الوراثية الدقيقة (microsatellite). أظهرت جميع الواسمات درجة عالية من تعدد الأليلات. تم حساب مقاييس مختلفة للتنوع الوراثى فى العشيرة. تراوح العدد الكلى الملاحظ من الأليلات لكل واسم وراثى دقيق من 6 إلى 12 لكل من MAF65 و OarHH47، على الترتيب. كانت تقديرات العدد الكلى الفعال من الأليلات بين 3.11 للواسم TGLA53 و 6.12 للواسم OarHH47. كان متوسط المحتوى المعلوماتى عن تعدد الصور الأليلية (PIC) هو 0.73. كان المتوسط الشامل للتنوع الجينى للواسمات الوراثية الدقيقة التي تم تحليلها هو 0.66 و 0.69 و 0.75 فى كل من أغنام الأوسيمى والرحمانى والبرقى، على الترتيب. لجميع السلالات موضع الدراسة، كانت تقديرات التراكيب الوراثية الخليطة المُلاحظة أقل معنوياً مقارنة بالتراكيب الوراثية الخليطة المُتوقعة. كانت المتوسطات العامة لتقديرات التراكيب الوراثية الخليطة المُلاحظة والمُتوقعة 0.55 و 0.67 ، على التوالى. لوحظت إنحرافات معنوية عن توازن هاردى-فاينبرج (HWE) بسبب نقص التراكيب الوراثية الخليطة وذلك لكل الواسمات الوراثية الدقيقة محل الدراسة. أظهرت السلالات الثلاثة إنحرافاً معنوياً عن HWE. قيمة المؤشر العام لتقسيم العشيرة ($F_{ST}$) التى تم حسابها هى 0.071 ، مما يشير إلى أن حوالى 7 ٪ من التنوع الوراثى يرجع إلى الإختلاف الوراثى بين السلالات. كانت تقديرات معاملات التربية الداخلية الكلية ($F_{IT}$) والتربية الداخلية فى السلالة ($F_{IS}$) هى 0.187 و 0.118 ، على الترتيب. كانت تقديرات التباين الوراثى بين كل زوج من السلالات كالتالى: 0.039 ، 0.051 ، 0.056 لكل من الأوسيمى والرحمانى، البرقى والرحمانى، الأوسيمى والبرقى، على الترتيب. تراوحت مقاييس المسافة الوراثية بين أزواج سلالات الأغنام من 0.423 إلى 0.615 لزوجى الأغنام الرحمانى والأوسيمى، لزوجى البرقى والأوسيمى، على الترتيب. النتائج التي تم الحصول عليها فى هذه الدراسة قد تكون مفيدة فى برامج التربية المستدامة لسلالات الأغنام المصرية.