GENETIC DIVERSITY IN BARKI SHEEP BREED IN ITS NATIVE TRACT IN EGYPT

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SUMMARY

Nine microsatellite markers were used to investigate genetic variations among and within four subpopulations of Barki sheep breed from four different locations along the North-Western Coastal Zone (NWCZ) of Egypt. The selected locations were ElHammam, Matrouh, Negeila and Salloum. The nine microsatellites were BM757, BM8125, OarAE129, OarJMP29, OarVH72, ELT1, 320R, OB1 and OB2. All markers used showed polymorphism overall studied subpopulations. The total observed numbers of alleles in the four subpopulations were 61, 43, 30 and 56, respectively for all studied markers. Expected heterozygosity was the highest in Salloum subpopulation (0.771). The polymorphic information contents (PIC) were 0.696, 0.590, 0.516 and 0.744, respectively. All studied subpopulations were in Hardy-Weinberg equilibrium showing significant excess of heterozygosity in the state of Hardy-Weinberg equilibrium compared to heterozygosity in the state of mutation-drift equilibrium, indicating the presence of real genetic bottleneck. All subpopulations showed positive inbreeding (F_{IS}) when averaged across all loci. The subpopulation of Matrouh had the highest F_{IS} (0.470). Genetic distance analysis divided the four subpopulations into two clusters, the first included ElHammam and Negeila, while the other included Matrouh and Salloum. Differences in genetic distance and genetic identity between the studied subpopulations are indeed low, reflecting the great genetic similarity among them.

Keywords: Microsatellites, genetic variability, genetic bottleneck, biodiversity

INTRODUCTION

In view of the declining diversity in animal populations, awareness has arisen for the study of variation among and within animal genetic resources. In livestock, animal genetic resources diversity is expressed among and within breeds. Such diversity is of great importance in planning and implementing genetic improvement programs (Moazami-Goudarzi *et al.*, 1997; Hall and Bradley, 1995).

Microsatellites, or Simple Sequence Repeats (SSR), are segments of repetitive DNA sequences that occur in eukaryotic genomes and characterized by a variable number of copies (typically 5-50) of a sequence of around 5 or fewer bases called "a repeat unit" (Hamada and Kakunagu, 1982; Tautz and Renz, 1984). The presence of multiple alleles (high level of polymorphism) has enabled SSR to be a powerful marker in many different species. They are detected by PCR. Most microsatellites are codominant, inherited in Mendelian fashion, distributed widely in the genome and easily genotyped (Bruford and Wayne, 1993; Queller et al., 1993).

In Egypt, sheep are used mainly as a source of meat with wool as a secondary product.

Barki is the smallest among the three major Egyptian sheep breeds, Barki, Ossimi and Rahmani, with mature male body weight of 60 kg, 62 kg and 73 kg, respectively. The breed plays an important role in the livelihood of peoples in North-Western Coastal Zone (NWCZ) of Egypt which is the home tract of this breed in the country. It is named after the Libyan province Barka. It is considered a main contribute to livestock population in the southern Mediterranean coast. Its population in 2006 in Egypt was about 406,360 (Economic Section of The Ministry of Agriculture and Land Reclamation, 2007). The breed extends from the eastern provinces of Libya to the west of Alexandria in Egypt. The breed is well adapted to the desert conditions (Aboul-Naga and Afifi, 1982) and has a distinct mothering ability (Galal et al., 1972). Until now no study was performed on the pattern of biodiversity of Barki sheep subpopulations along NWCZ of Egypt from the Libyan borders in the west to Alexandria in the east. Very few studies were carried out on genetic variability in the breed at the molecular level, e.g. El Nahas et al. (2008) using fourteen microsatellites.

The objective of this study was to investigate the molecular genetic variability and the relationships among and within subpopulations of Barki sheep along the NWCZ in Egypt using microsatellite markers.

MATERIALS AND METHODS

Animals:

Ninety four adult Barki sheep of both sexes were sampled from four regions along a trajectory in the NWCZ of Egypt extending from Alexandria to Salloum for 457 km, 24, 26, 20, 24 samples from ElHammam, Matrouh, Negeila and Salloum, from east to west, respectively. To avoid individuals with common ancestors, unrelatedness between animals was considered during collecting samples by collecting maximum of two samples from the same flock (ram and ewe) and also asking the breeders about the rams used in the mating of their ewes and if they exchange them with other breeders or not. The most eastward sample site, ElHammam, is 65 km west of the Nile delta. Distances between locations sampled are, ElHhammam- Matrouh: 235 km, ElHhammam- Negeila : 325 km, ElHhammam- Salloum: 457 km, Matrouh-Negeila: 90 km, Matrouh- Salloum: 222 km, and Negeila- Salloum: 132 km.

Blood sampling and DNA extraction:

Ninety four blood samples drawn from the jugular vein of animals of both sexes were sampled in a 10 ml tube containing 0.5 ml Ethylene Diamine Tetra-Acetic Acid EDTA, as an anticoagulant. DNA was isolated according to Phenol-chloroform extraction method (Sambrook *et al.*, 1989). The quantification of DNA samples was made using the spectrophotometer at 26° A°, the DNA was diluted to have final concentration of the DNA (20-50) ng/µl.

Microsatellite markers:

Nine microsatellites were used in the present study (Table 2). The first five were selected on the basis of the guidelines of FAO's DAD-IS (MoDAD) (FAO, 2004). The other four were chosen according to Mulsant *et al.* (2001) in their paper on FecB gene in different species.

PCR mastermix and amplification:

PCR products were performed according to Williams *et al.* (1990) in tubes containing a 12.5 µl of PCR mixture that consists of 2.5 µl 5 X Green buffer, 1.25 µl MgCl₂ solution (25 mM), 0.25 µl PCR Nucleotide mix, 10mM each (dNTPs), 1 µl (10 µM) Forward primer, 1 µl (10 µM) Reverse primer, 0.06 µl (5 U / µl) GoTaq[®] DNA polymerase, 2 µl Template DNA and 4.44 µl H₂O (D.W). Two types of PCR programs were performed; Touchdown PCR was used as recommended by FAO (2004) for primers BM757, OarAE129,

OarJMP29, OarVH72 and BM8125, and another PCR program was used to amplify the primers according to Mulsant et al. (2001) on FecB gene in different species as follows: the first cycle (initial denaturation) at 94°C for 2 minutes. The next 35 cycles at 94 °C for 15 sec. (denaturation) followed by 52° C for 45sec (annealing) and then 72° C for 2 minutes (extension), respectively in one cycle. The last cycle (final extension) was performed at 72° C for 5 minutes. At the end of PCR runs, an approximately 5 µl of PCR products were separated on 6% polyacrylamide gel to detect the polymorphism. After finishing the electrophoresis, the gels were gently elevated and immediately placed into ethidium bromide dye solution (100 ml distilled water containing 10 µl of 1% ethidium bromide stock) at a dark place for 10 - 20 min, then gels were immediately photographed at the end of the run using UV-based gel documentation system (Bio-Rad Gel Doc 2000 apparatus).

Statistical analysis:

PCR products were used to detect the allele size of the samples using LabImage program, version 2.7.0 (Jnied et al., 2009). Quantitative measurements of observed number of alleles in each locus, allele frequency, and exclusive alleles in each population, observed and expected heterozygosity were estimated using the program of GENETIX software (Belkhir et al., 2004). Polymorphism Information Content (PIC) value, the probability that one parent is a marker heterozygote (marker informative) and its mate has a different genotype which could be back crossing or families with full information (Lynch and Walsh, 1998), was estimated using the MStools analysis software (Excel microsatellite toolkit v. 3.1). Deviations from Hardy-Weinberg equilibrium were estimated using GenePop software (Raymond and Rousset, 1995). FSTAT software version 2.9.3 (Goudet, 2002) was used to calculate the observed and expected heterozygosity over all loci as defined by Nei (1978). The different subpopulations were tested for genetic bottleneck under the three SSR mutation models, the IAM (Infinite Allele Model), TPM (Two-Phase mutation Model) and the SMM (Stepwise Mutation Model) with the BOTTLENECK program software version 1.2.02 (Cornuet and Luikart, 1996). Neighborjoining diagram was constructed on genetic distances among subpopulations using Nei's distance (Saito and Nei, 1987) and among individuals using distance values based on the proportion of alleles shared by two individual averaged over loci (Bowcock et al., 1994) and calculated using POPGENE. TreeView32 application was used to draw Neighbor-joining diagram.

RESULTS AND DISCUSSION

Observed number of alleles:

Observed numbers of alleles showed some differences between the studied subpopulations (Table 2).

All microsatellites used in the present study showed polymorphism in all studied subpopulations (Table 3).

BM8125 showed the highest number of polymorphic alleles (22 alleles), while BM757 and ETL1 showed the lowest of polymorphic alleles (8 alleles, each). El Nahas et al. (2008) reported 9 alleles in Barki sheep using OarVH72 as compared to 12 alleles found in the present study, and the same number of alleles (10 alleles) as that found in the present study for OarAE129. Arora and Bhatia (2004) used microsatellites BM757, BM8125, OarAE129, OarJMP29 and OarVH72 to study the genetic structure of Muzzafarnagri sheep breed in India and reported a lower number of alleles (4, 5, 4, 6 and 5, respectively) as compared to 8, 22, 10, 20 and 12 in the present study. OarJMP29 showed 20 alleles (100-205bp), while the same locus showed 6 alleles (125-135bp) in Marwari goats in India (Kumar et al., 2005). Mulsant et al. (2001) reported that the microsatellites OB1, OB2, 320R and ELT1 were related to the fecundity gene FecB in Boorola sheep (responsible for increasing the ovulation rate and litter size of the animal). These microsatellites were used in the present study and showed variable number of alleles (9, 12, 17 and 8, respectively).

Polymorphic information content (PIC):

PIC is used as a measure of polymorphism for a marker locus (Shete et al., 2000). PIC will be high when the locus has a high number of alleles or/ and allele frequency is distributed equally among alleles. The variance will be the highest if the allele frequency was in the middle, then, the heterozygosity, gene diversity and PIC will be the highest. PIC ranged between 0.293 for BM757 and ELT1, each, in the subpopulation of Negeila, and 0.906 for subpopulation OarJMP29 in the of ElHammam. All markers were informative in all studied subpopulations. Nine locationmarkers were reasonably informative overall studied subpopulations. These were OB2 in ElHammam subpopulation, BM757, OarVH72, OB1 and ELT1 in Matrouh subpopulation and BM757, BM8125, OB1 and ELT1 in Negeila subpopulation.

Observed and expected heterozygosity:

Mean expected heterozygosity was higher than the observed in all studied subpopulations (Table 4). Heterozygosity is considered to be a good estimator of the genetic variability present in a population (Kim et al., 2002). Genetic variability of the studied subpopulations is relatively high, as evidenced from the mean expected heterozygosity (0.680). Expected heterozygosity of the subpopulations of Salloum and ElHammam was the highest (0.771, 0.729 respectively). That may be explained by the high average number of alleles and alleles per locus in these subpopulations. Observed heterozygosity on the average for all loci for the studied subpopulations was always lower than the expected heterozygosity, the highest difference being in ElHammam (0.295), while the lowest in Negeila (0.1).

Hardy-Weinberg equilibrium (HWE):

Deviations from HWE reflect the strength of forces affecting allele frequency (genetic drift, selection, migration and mutation). All studied subpopulations were in Hardy-Weinberg equilibrium, which could be due to absence of intensive selection in these subpopulations; the size of these subpopulations is large enough to counter affect drift; and mutations are not large enough to have a great effect on the allele frequency. Although, there was a general heterozygosity deficit in the subpopulations, it was not large enough to produce significant deviation of HWE.

Genetic bottleneck:

Genetic bottlenecks are the changes in allele frequency of the population, which change the heterozygosity and the state of the mutation-drift equilibrium. In other words, it implies the changes in the heterozygosity of the population that changes the mutation-drift equilibrium. It can result from the isolation of small populations that are subject to the effect of genetic drift, which can lead to decreasing the effective population size. It can also occur due to mutation and inbreeding. The population bottleneck is a significant reduction in the size of the population and decrease in the heterozygosity and the genetic diversity. The bottleneck can increase the risk of population extinction in case of decreasing heterozygosity and should be avoided (Glowatzki-Mullis et al., 2008). The genetic bottleneck was computed for all studied subpopulations (Tables 5). Wilcoxon test is a statistical test for assessing the significance in the case of two related or repeated samples or repeated measurements on the same sample. The test was performed for each mutation model to determine whether a subpopulation exhibits a significant number of loci with heterozygosity excess or deficit (Luikart et al., 1998).

In the subpopulations of both ElHammam and Matrouh, there was a significant heterozygosity excess under the IAM and the TPM (P<0.05), while under the SMM it was neither heterozygosity excess nor deficit (P>0.05) (i.e. stable population). The modeshift graphical test for allele frequency distribution (a qualitative descriptor of the allele frequency distribution in the population) indicated an approximately L-shaped (as expected under mutation-drift equilibrium) for ElHammam samples (Figure not shown) reflecting the absence of a genetic bottleneck in its subpopulation (Gour et al., 2005). In both Negeila and Salloum subpopulations, there was a significant excess of heterozygosity (P<0.05) under the three mutation models (IAM, TPM and SSM). Mode-shift graphical test for allele frequency distribution showed a shifted mode (from the stable population) indicating that this subpopulation was modified from stable population (mutation-drift equilibrium) to the bottlenecked population (excess of heterozygosity). The different results of SMM model in the subpopulations of ElHammam and Matrouh may be due to that the SMM model is very sensitive to the mutational changes in the SSR, and the IAM model is less sensitive to the mutational changes in the SSR, while the TPM model is intermediate between SMM and IAM (Cornuet and Luikart, 1996). The genetic bottlenecks that were observed in these subpopulations could not be factually explained due to the absence of historical background and the breeding practices of the flocks in these locations.

Inbreeding measurements:

Inbreeding within a subpopulation (F_{IS}) refers to the inbreeding coefficient of an individual related to its population (Falconer and Mackay, 1996). F_{IS} was positive in all subpopulations when averaged across all loci. Inbreeding within subpopulations may be caused by nonrandom mating of closely related individuals of the population (Murray, 1996). Subpopulation of Matrouh showed the highest F_{IS} (0.443). Total inbreeding (F_{IT}) refers to the inbreeding coefficient of an individual related to the whole population (Falconer and Mackay, 1996). F_{IT} (Table 6) ranged from -0.002 in 320R to 0.928 in OB2. All studied loci showed positive F_{IT} except 320R, which indicates that all studied subpopulations have heterozygosity deficit except 320R which has heterozygosity excess.

Genetic differentiation estimates (F_{ST}) refer to the average inbreeding of population related to the whole population (Falconer and Mackay, 1996). F_{ST} (Table 7) ranges between zero to one. When F_{ST} equals zero this means that the populations have the same allele frequency (absence of genetic structure), while, when F_{ST} equals one this assumes that every population has unique different allele frequency (maximal structure) (McDonald, 1996).

Estimated pair-wise F_{ST} (Table 7) showed that the genetic differentiation was the highest between subpopulations of ElHammam and of Negeila (0.306) while it was the lowest between subpopulations of ElHammam and Salloum (0.162). The genetic differentiation between the other studied subpopulations was higher (ranging from 0.239 to 0.272).

Genetic distance and identity:

Genetic distances between the studied subpopulations are indeed low, reflecting the similarity among great genetic these subpopulations and that is because of their belonging to the same breed (Table 8). GI between pairs of subpopulations are all relatively high being > 0.65 while the GD's are all <0.50.The detected genetic distances between the subpopulations may be due to the geographical distribution of the subpopulations. The subpopulation of Matrouh is slightly less distant and has slightly more genetic identity with that of Salloum than other studied subpopulations. The genetic identity between the subpopulations of Matrouh and ElHammam, and between Negeila and ElHammam, between Salloum and and ElHammam were high. The greatest genetic distance and the lowest genetic identity were between the subpopulations of Negeila and Salloum, despite them being geographically adjacent to each other.

Relationship Dendogram:

Standard genetic distance estimates (D_A) and the Neighbor Joining (NJ) trees (Saitou and Nei, 1987) were used to obtain the relationship dendogram (Figure 1). It divides the studied subpopulation into two clusters, which put the subpopulations of ElHammam and Negeila in one cluster (1), and the subpopulations of Matrouh and Salloum in another (2).

CONCLUSION

The results showed that there is a degree of genetic variability among the studied subpopulations which may be due to the difference in the geographical distribution. Although these variations are important for the breed diversity and conservation, they are not high enough to differentiate the population into four distinct subpopulations. There is a good amount of genetic variability that warrants establishing genetic improvement programs.

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| Primer | Chr | Sequence | Reference |
|----------|-----|----------------------------------|--------------------------------|
| BM757 | 0 | TGGAAACAATGTAAACCTGGG | Bishop at al. (1004) |
| DIVI/J/ | 7 | TTGAGCCACCAAGGAACC | Dishop <i>et al</i> . (1994) |
| BM8125 | 17 | CTCTATCTGTGGAAAAGGTGGG | Bishop <i>et al</i> (1994) |
| DW10123 | 17 | GGGGGTTAGACTTCAACATACG | Dishop <i>et ut</i> . (1994) |
| OarAE129 | 5 | AATCCAGTGTGTGAAAGACTAATCCAG | Points at al (1003) |
| | 5 | GTAGATCAAGATATAGAATATTTTTCAACACC | 1 city <i>et al</i> . (1995) |
| OarJMP29 | 24 | GTATACACGTGGACACCGCTTTGTAC | Penty $at al (1993)$ |
| | 27 | GAAGTAACAAGATTCAGAGGGGAAG | 1 city <i>et al</i> . (1995) |
| OarVU72 | 25 | CTCTAGAGGATCTGGAATGCAAAGCT | Pierson et al. (1993) |
| 0411172 | 23 | GGCCTCTCAAGGGGCAAGAGCAGG | 1 leison <i>ei ui</i> . (1993) |
| FI T1 | 6 | CATCAATGGGCCTGTGAAAT | Mulsant et al. (2001) |
| LLII | 0 | CAAACAATCACCAACTAATCC | Mulsunt <i>et ut</i> . (2001) |
| 320R | 6 | TCACCTTGAGTCATTTCTTTAG | Mulsant et al. (2001) |
| 520K | 0 | GTGGTGTTTGGAACTTTGTAA | Whitsant <i>et al.</i> (2001) |
| OB1 | 6 | CAGAACGGAATGAATGTAATAA | Mulsant et al. (2001) |
| ODI | 0 | AGAACAGGGTAAGGAAGTAAA | Mulsunt <i>et ut</i> . (2001) |
| OB2 | 6 | CTACAATAAATAATGAGGTGAAA | Mulsant et al. (2001) |
| OB2 | 0 | TGAGTAGAGACAAAGCTATAAA | Wuisant <i>et ut</i> . (2001) |

Table 1. Microsatellite markers used in the present study

| Table 2. | Observed | number | of alleles in | each of th | e studied | subpopulations |
|----------|----------|--------|---------------|------------|-----------|----------------|
|----------|----------|--------|---------------|------------|-----------|----------------|

| Locus | ElHammam | Matrouh | Negeila | Salloum |
|----------|----------|---------|---------|---------|
| BM757 | 3 | 3 | 2 | 6 |
| BM8125 | 15 | 5 | 3 | 7 |
| OarVH72 | 5 | 3 | 4 | 6 |
| OARjmp29 | 14 | 8 | 5 | 10 |
| OarAE129 | 6 | 5 | 3 | 6 |
| 320R | 6 | 7 | 5 | 6 |
| OB2 | 5 | 6 | 3 | 5 |
| OB1 | 4 | 3 | 3 | 4 |
| ETL1 | 3 | 3 | 2 | 6 |
| Total | 61 | 43 | 30 | 56 |

Table 3. Observed number of alleles and allele size (in base-pairs) detected in the studied subpopulations

| Locus No. <u>Allele size of allele number</u> | | | | | | | | | | | | | | | | | | | | | | | |
|---|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Locus | alleles | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| BM757 | 8 | 210 | 222 | 232 | 246 | 264 | 286 | 310 | 316 | | | | | | | | | | | | | | |
| BM8125 | 22 | 92 | 94 | 98 | 100 | 104 | 108 | 110 | 114 | 118 | 122 | 126 | 128 | 134 | 140 | 142 | 146 | 152 | 156 | 158 | 162 | 168 | 170 |
| OarVH72 | 12 | 130 | 134 | 140 | 142 | 148 | 150 | 152 | 154 | 160 | 164 | 166 | 176 | | | | | | | | | | |
| OARjmp29 | 20 | 100 | 108 | 115 | 118 | 122 | 124 | 126 | 132 | 134 | 136 | 138 | 140 | 142 | 144 | 146 | 150 | 156 | 158 | 190 | 204 | | |
| OarAE129 | 10 | 96 | 100 | 124 | 128 | 132 | 138 | 140 | 144 | 152 | 176 | | | | | | | | | | | | |
| 320R | 17 | 194 | 202 | 218 | 220 | 232 | 236 | 250 | 254 | 266 | 270 | 278 | 318 | 390 | 404 | 412 | 440 | 472 | | | | | |
| OB2 | 12 | 128 | 146 | 168 | 172 | 174 | 176 | 178 | 192 | 210 | 238 | 276 | 328 | | | | | | | | | | |
| OB1 | 9 | 268 | 277 | 304 | 328 | 344 | 356 | 364 | 388 | 404 | | | | | | | | | | | | | |
| ETL1 | 8 | 210 | ٤22 | 232 | 244 | 264 | 284 | 310 | 318 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | |

| Table 4. Observed and expected heterozygosity for loci across the stud | ied subpopulations |
|--|--------------------|
|--|--------------------|

| Loona | | ElHamman | ı | | Matrouh | |
|------------------|------------------|------------------|------------------|-------|------------------|------------------|
| Locus – | No. ¹ | H _{obs} | H _{exp} | No. | H _{obs} | H _{exp} |
| BM757 | 46 | 0 | • 7815 | 46 | • | • • • • • • • |
| BM8125 | 42 | ٩.٤٨ | • • • • ٢ | 42 | . 1101 | •. ٧٢٦٨ |
| OarVH72 | 46 | • 1797 | . 1012 | 46 | • 7077 | . 0017 |
| OARjmp29 | 46 | | • . ٨٨ • ٩ | 44 | •_^٦٣٦ | • . ٨ £ 9 ٢ |
| OarAE129 | 38 | | | 48 | • 1917 | · VY £A |
| 320R | 48 | •_9177 | . VOAV | 52 | ١ | • ٧ • ٩٣ |
| OB2 | 48 | . 170. | • . 0779 | 50 | • 17 | |
| OB1 | 46 | • 77.9 | • 7750 | 48 | . 170 | . 3975 |
| ETL1 | 46 | ٠ | • . 7812 | 46 | • | . 0771 |
| Un weighted mean | 45 | 0.4346 | 0.7293 | 46.88 | 0.3709 | 0.6447 |
| Locus | | Negeila | | | Salloum | |
| | No. | H _{obs} | H _{exp} | No. | H _{obs} | H _{exp} |
| BM757 | 30 | •_£77V | | 40 | ۰.٤ | • ^ 1 |
| BM8125 | 38 | • | • 0719 | 44 | •. ٧٧٧٧ | •. ٧٧ ١٧ |
| OarVH72 | 40 | ١ | • • • • | 46 | ١ | . 1001 |
| OARjmp29 | 36 | •_9555 | | 40 | • 10 | •. 1740 |
| OarAE129 | 34 | . 7079 | . 0770 | 42 | • • ٤٧٦ | • 105 |
| 320R | 40 | ١ | | 46 | ١ | •_٧٨٣٦ |
| OB2 | 38 | • | • 110 | 44 | • | • . ٧٢٧٣ |
| OB1 | 40 | • | •.000 | 46 | • | • 1907 |
| ETL1 | 30 | •_£777 | | 40 | ۰.٤ | • ^ 1 |
| Un weighted mean | 36.22 | 0.47 | 0.5745 | 43 | 0.4967 | 0.7707 |

(1) Number of loci = 2 times number of sampled animals

Table 5. Testing the probability of genetic bottleneck for the studied populations under three possible models, IAM^1 , TPM^2 and SMM^3 for all studied loci using Wilcoxon test Location

| | | | | | | Loca | luon | | | | | |
|------------------------|--------------------|--------------------|--------------------|------------------|--------------------|--------------------|------------------|-------------|------------------|------------------|-------------|------------------|
| Probability | ElHammam | | | | Matrouh Negeila | | | | Salloum | | | |
| | IAM ¹ | TPM^2 | SMM ³ | IAM ¹ | TPM^2 | SMM ³ | IAM ¹ | TPM^2 | SMM ³ | IAM ¹ | TPM^2 | SMM ³ |
| He ⁴ excess | 0.001^{5} | 0.004^{6} | 0.150^{7} | 0.001^{5} | 0.004^{6} | 0.125 ⁷ | 0.002^{5} | 0.002^{6} | 0.002^{7} | 0.002^{5} | 0.002^{6} | 0.002^{7} |
| He deficiency | 0.999 ⁵ | 0.997 ⁶ | 0.875 ⁷ | 1^{5} | 0.997 ⁶ | 0.898 ⁷ | 1^{5} | 1^{6} | 1^7 | 1^{5} | 1^{6} | 1^7 |

¹Infinite Allele Mutation Model ² Two Phase Mutation Model ³ Step-wise Mutation Model ⁴ Expected heterozygosity under Hardy-Weinberg equilibrium ⁵ Assumptions: all loci fit IAM, mutation-drift equilibrium. ⁶ Assumptions: all loci fit TPM, mutation-drift equilibrium.

⁷ Assumptions: all loci fit IAM, mutation-drift equilibrium.

Table 6. $F_{\mbox{\scriptsize IT}}$ for studied loci in the studied subpopulations

| | Locus | | | | | | | | | | |
|------------------|--|--------|---------|----------|----------|--------|-------|-------|-------|--------|--|
| | BM757 | BM8125 | OarVH72 | OARjmp29 | OarAE129 | 320R | OB2 | OB1 | ETL1 | Mean | |
| No. ¹ | 162 | 162 | 178 | 166 | 162 | 186 | 180 | 180 | 162 | 170.88 | |
| F _{IT} | 0.801 | 0.481 | 0.089 | 0.108 | 0.805 | -0.002 | 0.928 | 0.890 | 0.801 | 0.544 | |
| Number | Number of loci = 2 times number of sampled animals | | | | | | | | | | |

| or population differentiation | | | | | | | | |
|-------------------------------|---------|---------|---------|--|--|--|--|--|
| Subpopulation | Matrouh | Negeila | Salloum | | | | | |
| ElHammam | 0.253 | 0.306 | 0.162 | | | | | |
| Matrouh | | 0.239 | 0.255 | | | | | |
| Negeila | | | 0.272 | | | | | |

Table 7. Estimated pair-wise F_{ST} as a measure of between subpopulations heterozygosity deficit

| Table 8. Nei's unbiased measures of genetic identity (GI) (above diagonal) and genetic distant | aces |
|--|------|
| (GD) (below diagonal) | |

| subpopulation | ElHammam | Matrouh | Negeila | Salloum |
|---------------|----------|---------|---------|---------|
| ElHammam | | 0.7249 | 0.7374 | 0.7067 |
| Matrouh | 0.3217 | | 0.6833 | 0.7380 |
| Negeila | 0.3047 | 0.3809 | | 0.6508 |
| Salloum | 0.3471 | 0.3038 | 0.4296 | |



Figure 1. Phylogenetic tree representing relationship between the studied subpopulations using genetic distance based on 9 microsatellite loci.

التنوع الوراثى فى سلالة أغنام البرقى فى موطنها الأصلى فى مصر

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استخدمت تسعة واسمات للتوابع الور اثية الدقيقة لدراسة الإختلافات الور اثية بين أربعة عشائر من سلالة أغنام البرقى فى أربعة مواقع مختلفة بطول الساحل الشمالى الغربى فى مصر وهى الحمام ومطروح والنجيلة والسلوم. أظهرت الـتوابع الور اثية الدقيقة المستخدمه وهى (BM757, BM8125, OarAE129, OarJMP29, OarVH72 ELT1, 320R, OB1 and OB2). تعدد أليليا فى كل العشائر المدروسة حيث كان العدد الكلى للأليلات فى العشائر الاربعة ٢، ٣٤، ٣٤، ٣٤، على الترتيب. كان المتوسط المتوقع لتكرار الأفراد الخليطة لعشيرة السلوم الأعلى بين العشائر المدروسة (٧٠٧٠). وكان مقياس معلومات التعدد الأليلى (PIC) العشائر المدروسة حيث كان العدد الكلى للأليلات فى العشائر الاربعة ٦، ٣٤، ٣٠، على الترتيب. كان المتوسط المتوقع لتكرار الأفراد الخليطة لعشيرة السلوم الأعلى بين العشائر المدروسة (٧٠٧٠). وكان مقياس معلومات التعدد الأليلى (PIC) العشائر المدروسة زيادة معنوية فى الخليط فى حالة التران هادى والعشائر محل الدراسة حالة اتران هاردى وينبرج. أظهرت جميع العشائر المدروسة زيادة معنوية فى الخليط فى حالة التران هاردى والينبرج مقارنة بحالة اتزان الجنوح العشوائى مع الطفرة فى العشائر العشائر المدروسة زيادة معنوية فى الخليط فى حالة التران هاردى واينبرج مقارنة بحالة اتزان الجنوح العشوائى مع الطفرة فى العشائر المشائر المدروسة زيادة معنوية فى الخليط فى حالة التران هاردى واينبرج مقارنة بحالة اتزان الجنوح العشوائى مع الطفرة فى العشائر المدروسة. أظهرت جميع العشائر المدروسة قدرا من التربية الداخلية، أظهرت عشيرة مطروح أعلى قدر من التربية الداخلية بين المشائر المدروسة (٢٠٤٠). أظهر تحليل المسافات الور اثية للعشائر المدروسة انفصال عشيرتى الحمام و النجلية فى عنقود ، بينما انفصلت عشيرتا مطروح و السلوم فى عنقود آخر. كانت الاختلافات فى التباعد الور أتي والتبايق الوراثي والتطابق والتية الي المدروسة الفرال عشيرة المدروسة قلي المرور عن والم المدروسة قليلة المدروسة المدروسة والم الموروش والنجية والمالير والم والتطابق الوراثى والنطية والم المدروسة قليم والتباي معنور المدروسة قلي المشري ولائيسان عشيرتا ملور و والسلوم فى عنقود آخر. كانت الاختلافات فى التباعد الوراثى والتطابق الوراثى بين المداوسة والم والي والي والي علي والموالي علي والم الي والمرا