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Analysis of genetic diversity and differentiation of sheep populations in Jordan

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ABSTRACT

Background: Genetic diversity of sheep in Jordan was investigated using microsatellite markers (MS). Six ovine and bovine MS located on chromosomes 2 and 6 of sheep genome were genotyped on 294 individual from ten geographical regions.
Results: The number of alleles per locus (A), the expected heterozygosity (H_e) and observed heterozygosity (H_o) were measured. Overall A, H_e and H_o were 12.67, 0.820 and 0.684, respectively. On the other hand, genetic distances undoubtedly revealed the expected degree of differentiation among the studied populations. The finding showed closeness of three populations from south (Maan, Showbak and Tafeilah) to each other. Populations from the middle regions of Jordan (Karak, Madaba, Amman, AzZarqa and Mafraq) were found to be in one cluster. Only two populations of the middle region were an exception: AlSalt and Dead Sea. Finally, sheep populations from Irbid were located in separated cluster. It was clear that the studied predefined populations were subdivided from four populations and would be most probably accounted as ancestral populations. These results indicate that number of population is less than the predefined population as ten based on geographical sampling areas.
Conclusions: The possible inference might be that geographical location, genetic migration, similar selection forces, and common ancestor account for population admixture and subdivision of Awassi sheep breed in Jordan. Finally, the present study sheds new light on the molecular and population genetics of Awassi sheep from different regions of Jordan and to utilize the possible findings for future management of genetic conservation under conditions of climate changes and crossbreeding policy.

1. Introduction

Sheep is common and popular small ruminant livestock in Jordan. Sheep in Jordan is a fat-tailed breed known as the Awassi sheep [1] which possesses great adaptability to tropical environmental conditions. Awassi sheep is often used as a triple purpose sheep, better for high milk production, in most of the countries of the Middle East [1]. They have little variation in morphological characteristics and production and reproduction traits. As a consequence to geographical rearing area and rearing system, there are different Arabic names given such as Baladi (local), Naiemi, and Sahrawi (desert). The differentiation between these sheep strains or breeds has not yet been established on ground. A molecular genetic differentiation of sheep in Jordan was limited to using few populations or using arbitrary and/or limited DNA markers in genetic differentiation studies [2,3]. Advance markers such as microsatellite (MS) and single nucleotide polymorphism (SNP) have not been applied for sheep genetic diversity and differentiation. In particular, MS markers have provided wide opportunities to analyze genetic variability at DNA level in universe sheep breeds. Microsatellite DNA markers are widely used since they are polymorphic and are randomly distributed in the organism's genome [4]. These markers have also been successfully used to study the biodiversity and genetic relationship and differentiation between and within breeds [5,6].

On the other hand, Jordan has experienced sharp reduction in sheep numbers as a result of persistent drought since 2007 [7]. Most of the sheep population is found in the north region of Jordan where drought has mostly been hit. In addition, another major threat to their genetic diversity is from unplanned crossbreeding with exotic improved Awassi strain and other exotic breeds. Based on Galal et al.'s [1] recommendation that limited information is available on the molecular biodiversity of

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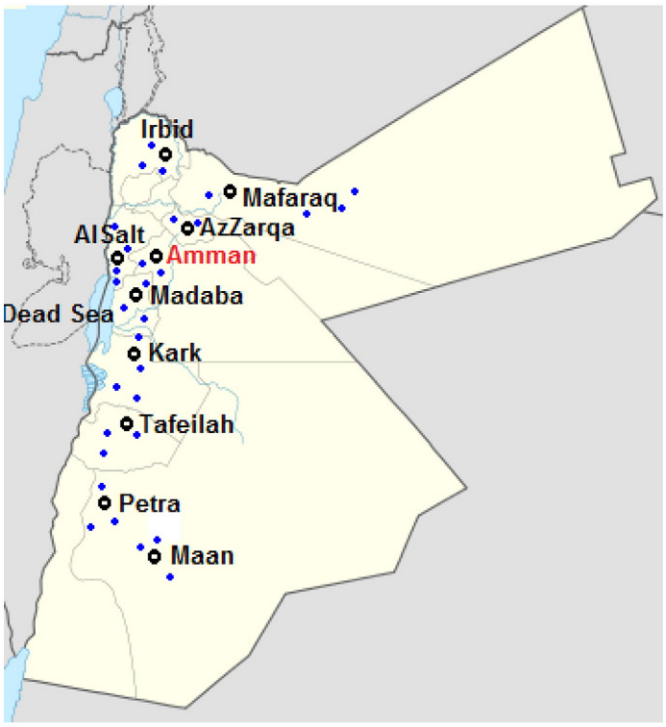

Fig. 1. Geographical areas of Jordan in which Awassi sheep populations in this study were samples. The filled dotted line shows the spots where sampled individuals occurred in each region that were named and represented by an empty-core dot.

Awassi sheep in Arabian countries like Jordan, the present study aims to analyze their genetic biodiversity and differentiation.

2. Materials and methods

2.1. Sheep populations and sampling

Thirty one populations of Awassi sheep were studied in ten different regions of Jordan as described in Fig. 1. The ten targeted regions are Irbid, Mafaraq, AzZarqa, AlSalt, Madaba, Dead Sea, Kark, Petra, Tafeilah (Dana), and Maan. The populations were of small, medium, large size and few in numbers that were grazed on road sides or reared in backyards. A total of 294 samples of mature unrelated ewes and rams were samples. Sample of 0.5 cm tissue was taken from each animal's ear using an ear puncher. The collected samples were stored at -18°C until extracted for DNA.

Table 2
Number of alleles per locus, expected heterozygosity (H_e), observed heterozygosity (H_o) and fixation index for each region (population).

Population		H_e	H_o	F_{is}
Irbid	39	0.700	0.654	0.067
Mafaraq	31	0.702	0.667	0.051
AzZarqa	10	0.696	0.617	0.119
AlSalt	12	0.841	0.667	0.214
Madaba	46	0.751	0.696	0.074
Dead Sea	17	0.725	0.696	0.042
Kark	35	0.767	0.676	0.120
Petra	23	0.731	0.717	0.020
Tafeilah	47	0.712	0.734	-0.031
Maan	34	0.713	0.652	0.087
Average		0.734	0.678	0.078

2.2. Sampling and DNA extraction

DNA extraction was performed using a commercially available protocol of E.Z.N.A.® MicroElute Genomic DNA Extraction Kit [8]. Subsequently, DNA concentrations were estimated by a Nano-DNA spectrophotometer in which the quality of DNA was evaluated using the ratio of A260/A280.

2.3. DNA genotyping

Six ovine and bovine MS (Table 1), located on chromosomes 2 and 6, were employed for genotyping experiments using Silver Sequence™ DNA System of Promega® [9]. Selection of the markers was based upon their close linkage to each other on chromosomes 2 and 6. On the other hand, their primers were selected for ease of use in PCR reaction with special regard to the annealing temperature and $MgCl_2$ concentration in particular. Primer sequences were taken from the Australian Sheep Gene Mapping website [10] and synthesized by BioEngland® (Table 1). PCR reaction utilized a 10 μl volume of DNA and reagents for genotyping. DNA samples were liquated into a 48 well PCR plate. Thermal cycling was performed on an MJ Research PTC-100 thermal-cycler. The Amplified PCR products were resolved on a 5% polyacrylamide gel electrophoresis using a Sequi-Gen GT gel rig for Silver staining [9]. Sequencing ladders were prepared using a *fmol*® DNA Cycle Sequencing system [9] and 3 μl of each of the four reactions loaded onto the gel, so that the size of the MS alleles was determined. When the electrophoresis run was completed, the gel was recovered and developed. Then, the gel was dried and viewed by the APC Film Development method [9]. The film was developed as a photo picture to be ready for scoring the genotypes. Allele sizes were scored by visual comparison with the sequencing ladder; pGEM®-3Zf(-) Vector.

Table 1
Microsatellite markers used for genotyping the three populations.

No.	Marker	Primer (5′–3′)	Ch[a]	Position (cM)	Size (bp)	Species
1	*INRA40*	*F*: *TCAGTCTGGAGGAGAGAAAAC* *R*: *CTCTGCCCTGGGGATGATTG*	2	149.9	205–257	Bovine
2	*OARHH30*	*F*: *CTCAGTCTCAACTTTGTTCCTCTATAGC* *R*: *GAAAGCTAAGGCTGAACATTGTGCCC*	2	167.4	103–117	Ovine
3	*ILSTS030*	*F*: *CTGCAGTTCTGCATATGTGG* *R*: *CTTAGACAACAGGGGTTTGG*	2	180.5	140–164	Bovine
4	*OARAE101*	*F*: *TAAGAAATATATTTGAAAAAACTGATC* *R*: *CTTCTTATAGATGCACTCAAGCTAGG*	6	49.8	99–123	Ovine
5	*OARHH55*	*F*: *GTTATTCCATATTCTTTCCTCCATCATAA* *R*: *GCCACACAGAGCAACTAAAACCCAGC*	6	54.6	117–155	Ovine
6	*BM143*	*F*: *ACCTGGGAAGCCTCCATATC* *R*: *CTGCAGGCAGATTCTTTATCG*	6	59.0	102–128	Bovine

[a] Ch: chromosome number in sheep genome. *F*: forward primer; *R*: reverse primer.

Table 3
Number of alleles per locus, expected heterozygosity (H_e), observed heterozygosity (H_o) and fixation index for each studied locus.

Locus	n	A	H_e	H_o	F_{is}
INRA40	294	9	0.745	0.500	0.329
OARHH30	294	8	0.759	0.524	0.310
ILSTS30	294	18	0.844	0.741	0.122
OARAE101	294	16	0.873	0.793	0.093
OARHH55	294	13	0.840	0.786	0.065
BM143	294	12	0.857	0.759	0.115
Average	294	12.7	0.820	0.684	0.166

2.4. Genetic analysis

A total of 294 samples were fully informative for the six studied MS loci without any missing genotypes (Table 1). Population genetics of the studied populations was then investigated. Allele frequency and polymorphism under Hardy–Weinberg equilibrium were measured using Genetic Data Analysis (GDA) software [11] and by CERVUS software [12]. The parameters were observed heterozygosity (H_o) and expected heterozygosity (H_e) at each locus [13], and polymorphic information content (*PIC*) [14].

On the other hand genetic distance matrix between populations was measured using GDA software which utilizes the most widely used measure of genetic distances [15]. The phylogeny tree was drawn using MEGA software [16]. The population structure was analyzed using STRUCTURE software [17] considering an admixture model and correlated allele frequencies between studied populations. The lengths of the burn-in Monte Carlo Markov chain (MCMC) were 1000 and 10 000 in 100 runs for possible number of clusters (breeds/population) (K) from 2 to 5. For each K value, logarithmic likelihood probability of data (L[K] = ln Pr(X|K)) and F_{st} values for each cluster were estimated. In addition, GENETIX (ver 4.05) software was used to predefine and identify the studied goat breeds [18].

3. Results and discussion

3.1. Genetic variation within populations

AMOVA analysis indicated that the genetic variation percentage among individuals within population was 5.55%, whereas it was 12.26% among populations. Estimates of allelic number at every locus were calculated to describe the genetic variation within and between populations (Table 2). The average number of alleles per loci for all population and at studied loci was 12.67. At a single locus, the number of allele was lowest (9) at *INRA40* and highest (18) at *ILSTS30*. This result indicates a high number of MS alleles in studied sheep populations and thus could be used to predict the level of the genetic variation and genetic differentiation within and between populations. Similar observation was reported by Arranz et al. [19,20] who estimated MS variation of Spanish Merino sheep. Furthermore, Iovenko [21] and Peter et al. [22] reported that high sheep population genetic variations were due to high allele variation profiles. On the other hand, Meadows et al. [23] reported that the analysis of genetic diversity within five sheep breeds showed that Merino contained the highest genetic diversity as average number of alleles observed per locus was 8.13, whereas Macarthur Merinos contained the lowest amount of diversity, with an average number of alleles of 3.03.

The H_e and H_o as measures of genetic diversity at a single locus, are shown in Table 2 for all populations and in Table 3 for each locus. Overall H_e and H_o values were 0.678 and 0.734 for all populations. In most of the cases, H_o was lower than H_e, except that for Tafeilah sheep population and resulted overall in a slightly higher average H_e of 0.734 for all populations (Table 2). In particular, sheep population of AlSalt has the highest variation value of 0.841, whereas sheep of AzZarqa has the lowest value of 0.696 (Fig. 2). Fig. 2 shows the heterogeneity level of each population at each studied locus. The average H_e and H_o for all loci were 0.820 and 0.684, respectively

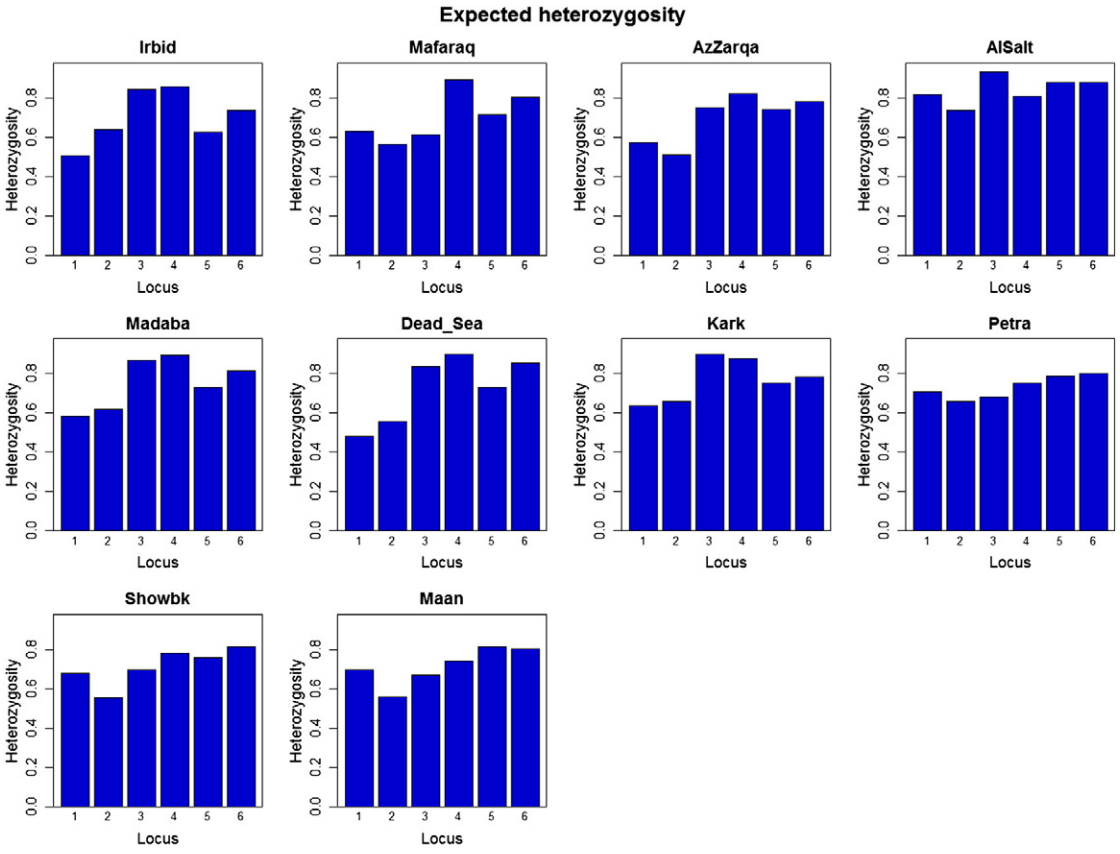

Fig. 2. Heterozygosity profile of the six microsatellite loci in the studied population.

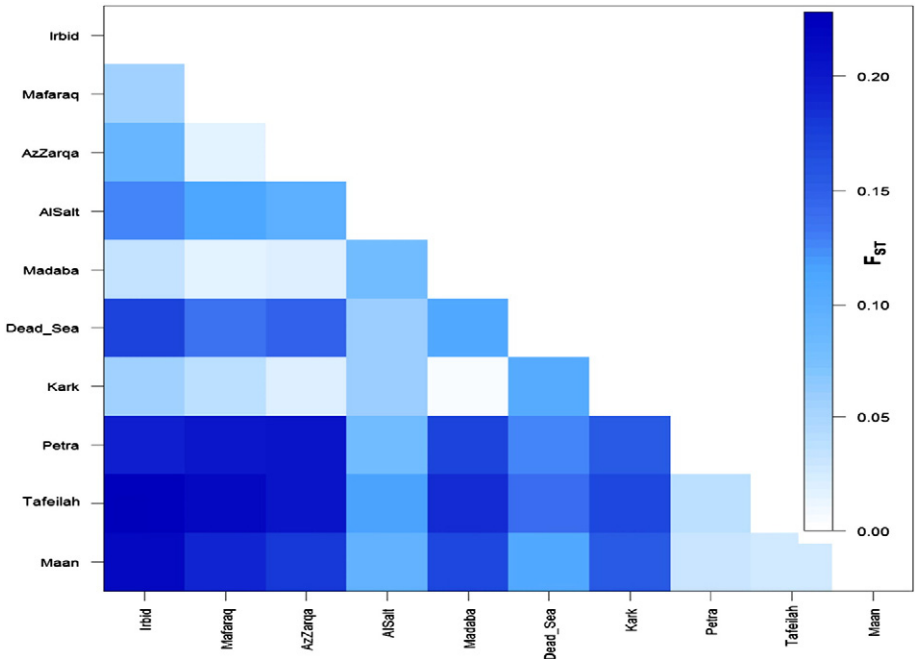

Fig. 3. Population average pairwise differences between populations.

(Table 3). These results showed high H_e in all populations and for most of studied loci except *INRA40* whose H_e and H_o showed the lowest values. In relation, H_e ranged from 0.745 (*INRA40*) to 0.873 (*OARAE101*), while H_o ranged from 0.500 (*INRA40*) to 0.793 (*OARAE101*) (Table 3). At all loci, H_o was lower than H_e. It is good to mention that, in this study, all loci had significant deviation from *HWE*, showing heterozygote advantage. These results mean that Jordan sheep are genetically subdivided. In general, deviation from *HWE* is probably a result of inbreeding, selection, and/or migration, then populations can be considered structured.

These results were similar to a study used the same MS markers on three flocks of Awassi sheep in south Jordan, and H_o and H_e were 0.67 and 0.70, respectively [3]. The heterozygoties of Awassi, Kivircik, and Akkaraman breeds and two of their crossbreeds of Turkey were high and ranged from 0.667 to 0.782 [24]. Arora et al. [25] reported that both H_o and H_e averaged 0.665 and 0.786, respectively and ranged from 6.40 to 7.92 in six Indian sheep breeds. In addition, Meadows et al. [23] reported that the analysis of genetic diversity within five sheep breeds showed that Merino contained the highest genetic diversity as average H_e was 0.70, even though it was as low as 0.4 for Macarthur Merinos. The studied populations revealed a high level of genetic variation seen from a high number of alleles per locus, H_e, and H_o. These results were similar to those reported in other studies of MS markers in sheep populations such as those of Bancroft et al. [26] and Coltman et al. [27]. The most genetically variable population was found to be AlSalt, which had more alleles per locus, higher H_e and H_o. This might be a result of gene flow into this population as well as due to sampling variation. This observation was explained as when genetic material, in the form of male gametes mainly, migrates from one population to another [28] as cases of introducing breeding rams from other flocks. However, the high level of genetic variation in populations could be due to several possible factors such as management and breeding practices under which selection took place [29,30,31].

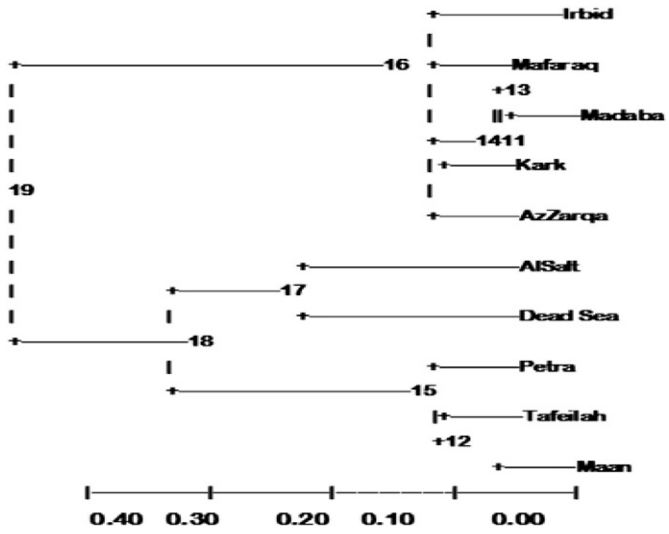

Fig. 4. UPGMA dendrogram showing the genetic relationship among the studied sheep populations. The numbers below each branch indicate the branch length in units of genetic distance.

3.2. Genetic variation between populations

Table 2 and Table 3 show inbreeding coefficients (F_{is}) for each population and at each locus respectively. F_{is} was positive in all

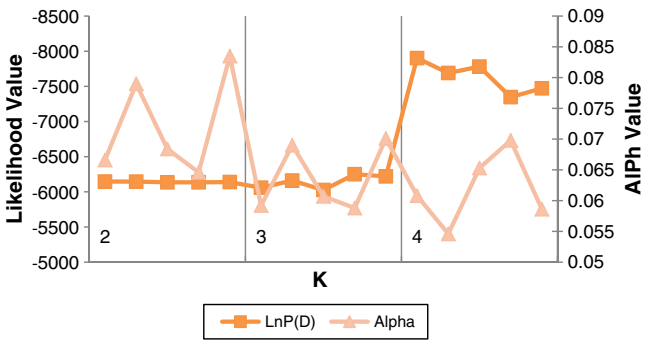

Fig. 5. Plot of data likelihoods and alpha values for several values of population number K (from 2 to 4) of ten independent runs.

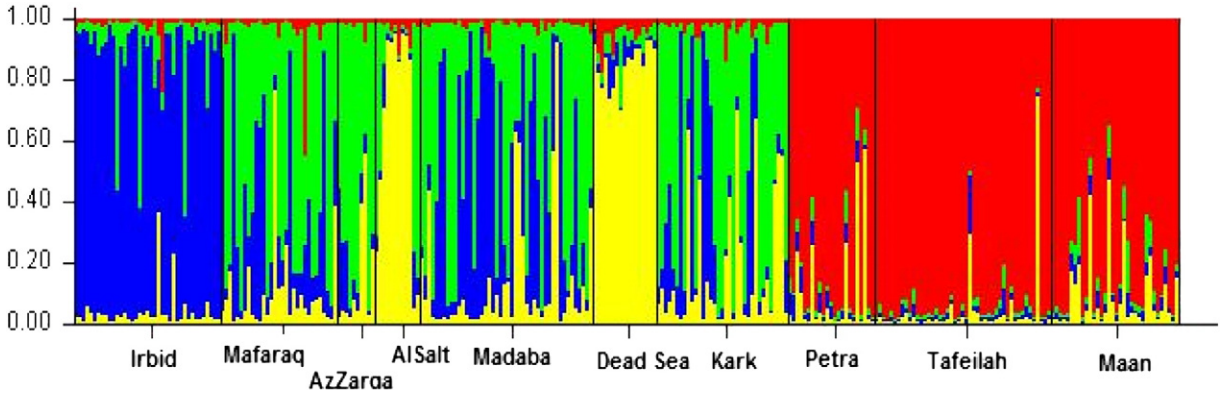

Fig. 6. Estimated population structure for each individual represented by a single vertical line broken into K colored segments, with lengths proportional to each predefined populations of K from 2 to 4.

population except Tafeilah, indicating that more inbreeding was found than it was expected in all sheep populations (Table 3). The average F_{is} for all populations was estimated at 0.078. The resulted inbreeding values at each locus were noticeably varying from 0.065 at *OARHH55* to 0.329 at *INRA40*. On the other hand, Fig. 3 shows population average pairwise differences (F_{st}) based on the distance method of different allele numbers. The overall F_{st} value for all populations was 0.123. The F_{st} values for each pair of populations varied from 0.0057 to 0.228. The F_{st} values showed more differentiation of Irbid sheep from and both Tafeilah ($F_{st} = 0.228$) and Maan ($F_{st} = 0.215$) sheep. In a comparison point of view, sheep of Tafeilah, Maan and Petra as well as Dead sea were highly differentiated from other studied populations ($F_{st} \sim 0.2$) (Fig. 3). Overall results indicate an evolutionary distinction of sheep of the south regions from those of the north regions of Jordan, whereas sheep of the middle regions was less differentiated. For example, Madaba sheep had least genetic differentiation from Karak sheep (Fig. 3). Similar results were found in literature indicating that convergence and divergence between studied populations. Overall F_{st} value was low for the North Spain sheep populations (0.061) [28]. In addition, F_{st} estimates in Spanish sheep reached similar (0.073) and an estimation was also obtained including were slightly greater (0.092) [20].

3.3. Genetic distances and phylogenetic tree

The pairwise genetic distance between populations was relatively short for population such as Maan, Tafeilah and Showbak but long for population of Kark with Mafraq, AzZarqa, Irbid, Dead Sea and AlSalt. The distances between these populations were described by drawn the UPGMA evolutionary phylogenetic tree which is shown in Fig. 4. In details, the cluster analysis based genetic distances revealed relatively short distances between (Node 11 = 0.057) Karak sheep and Madaba sheep. Fig. 4 revealed a very clear degree of differentiation of three clusters. Northern populations (Irbid, Mafraq) and some middle region populations (Madaba, Kark; Node 12 = 0.103115) and AzZarqa (Node 13 = 0.110) were grouped in one cluster. Other sheep populations of the middle region (AlSalt and Dead Sea; Node 17 = 0.348) were in a separated second cluster. The third cluster was comprised of sheep populations of the south region (Petra, Tafeilah and Maan; Node 18 = 0.526). In general, this result was similar to previous finding of closeness three native Awassi sheep populations of southern Jordan [3]. In addition, it is indicating a long evolutionary distance separation between southern sheep populations and populations of other geographical areas. In addition, the notable result was that populations of the middle region were located in the middle of the phylogenic tree. In general, this finding firstly supports the closeness of populations geographically close to each other. These results are in agreement with the known history of the populations in regard to their location and thus possible gene flow and their common ancestors. Overall, the clustering of populations in consensus UPGMA trees followed previously mentioned genetic differentiations between studied sheep populations (Fig. 3). Another scope for benefiting the results is that it might shed light on the traits of interest of closely related populations to understand the mechanisms of evolution considering the interaction with their own habitat on one hand. On the other hand, considering closer phylogenic populations for introgression and crossbreeding programs is hope of benefiting.

3.4. Genetic structure and admixture

Genetic structure analysis of studied sheep population was performed using STRUCTURE software with population number (K) ranging from K = 2 to K = 5 assuming admixture and correlated allele frequencies models. The likelihood values of the bootstrap samples were for choosing the optimal K value leading to the most reliable results membership coefficients to four clusters (K = 4; average likelihood = -7638.84 and alpha value = 0.062) (Fig. 5). It was clear that four populations would be most probably accounted as ancestral populations for current studied populations. This resulted case indicates that the number of population groups is less than predefined population as ten based on geographical sampling areas. As a consequence, individuals of all studied populations are subdivided into a group of four populations as presented in Fig. 6. Fig. 6 represents that all individuals of Irbid sheep population were assigned by more than 80% to the first cluster, except few individual of less than 80% assignment probability. On the other hand, most individuals of southern sheep populations (Petra, Tafeilah, Maan) were solely assigned to the fourth population. The second cluster has individuals of Mafaraq sheep with different assignment probabilities (~60%) (Fig. 6). On the other hand, the third cluster was formed from AlSalt and Dead Sea sheep individuals, mostly by ~90% admixture proportion (Fig. 5). The notable result was that admixture evidence was found in majority of Mafaraq, AzZarqa, Madaba and Kark sheep individuals. This result is probably shared ancestry between those studied populations. On the other hand, it might be due to the migration of individuals that usually occurred in the regions. In fact, Mafaraq and AzZarqa regions were considered as livestock rearing and trading regions where sheep from other regions are gathered for meat production and marketing. Structure and admixture analyses were performed on different sheep populations. For example, Álvarez et al. [32] reported that admixture analysis performed on the parental role of Burkina-Sahel and Djallonké sheep breeds from Africa and Mossi sheep breeds was a hybrid population nearer to the Djallonké breed. Furthermore, Ligda et al. [33] provided the genetic structure of ancestral populations of Greek sheep breeds. Turkish Awassi sheep as a fat-tail sheep was separated from other Turkish sheep breeds based on correspondence analysis [34].

In conclusion, the studied populations of Jordan Awassi sheep revealed a high level of genetic variation expressed by the number of

alleles, H_e and H_o measurements at population and loci levels. The latter showed a high allele number which reflected in high estimates of H_e and H_o at the six studied loci. At population level, these values were high and close to each other and reflected in explaining the level of genetic differentiation between the studied sheep populations. Genetic distances and phylogeny also undoubtedly revealed the degree of differentiation in the populations. It was clear that the studied predefined populations were subdivided from four populations and would be most probably accounted as ancestral populations. This resulted case indicates that the number of population groups is less than predefined population as ten based on geographical sampling areas. The possible inference might be that geographical location, genetic migration, similar selection forces, and common ancestor account for population admixture and subdivision of Awassi sheep breed in Jordan. Finally, the present study sheds new light on the molecular and population genetics of Awassi sheep from different regions of Jordan and to utilize the possible findings for future management of genetic conservation under conditions of climate changes and crossbreeding policy.

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Author contributions

Proposed theoretical frame: RMA; Conceived and designed the experiments: RMA, NMS, MJT; Software development: RMA, MJT; Contributed reagents/materials/analysis tools: RMA, NMS; Wrote the paper: RMA, NMS, MJT; Performed the experiments: RMA, NMS, MJT; Analyzed the data: RMA.

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