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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.

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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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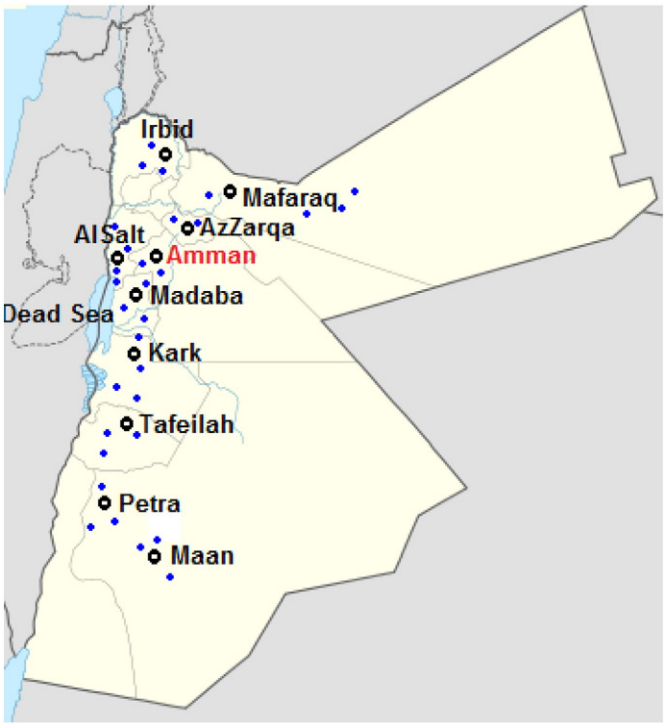

Fig. 1. Geographical areas of Jordan in which Awassi sheep populations in this study were samples. The filled dotted line shows the spots where sampled individuals occurred in each region that were named and represented by an empty-core dot.

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.

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