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Asian Journal of Animal and Veterinary Advances

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Asian Journal of Animal and Veterinary Advances 7 (5): 376-389, 2012
ISSN 1683-9919 / DOI: 10.3923/ajava.2012.376.389

Analysis of Genetic Variation of Fat Tailed-sheep in Southern Region of Jordan

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ABSTRACT

Genetic diversity of fat-tailed sheep of Jordan was investigated using microsatellite markers (MS). Six ovine and bovine MS located on chromosomes 2 and 6 of sheep genome were employed for genotyping three flocks in southern region of Jordan. The genetic diversity was evaluated by measuring expected (H_e) and observed (H_o) heterozygosity, Polymorphic Information Content (PIC) and the number of alleles per locus. The latter indicator showed specific allele frequency profiles in which the level of genetic differentiation between flocks observed as differences in allele frequencies and allele sizes. Therefore, allele frequency profiles were utilized to explain the level of genetic differentiation between flocks. Overall H_o was 0.67 for all flocks, whereas H_e was 0.70. The average H_e for each flock was 0.667, 0.652 and 0.716, while PIC was 0.639, 0.643 and 0.714 for flocks 1, 2 and 3, respectively. On the other hand, genetic distances undoubtedly revealed the expected degree of differentiation among the three flocks. The finding showed closeness of flocks 1 and 2 to each other and Flock 3 to Flock 2 than to Flock 1. Despite the three flocks were small in size number, had effective selective mating and gene flow, they matched with the Hardy-Weinberg Equilibrium, strongly proving the usefulness of MS as a genetic tool in population genetics analyses of sheep. From a genetic conservation point of view, it is recommended to maintain genetic diversity of small flocks when genetic migration of good genetic resources is absence. The present study provides new information about the population genetics parameters of fat-tailed sheep in southern Jordan.

Key words: Genetic variation, fat-tailed sheep, microsatellite markers

INTRODUCTION

Small ruminants such as sheep and goat are important for the livelihood of farmers in the world. In particular, the Mediterranean area is an effective transformation area for thousands of marginal hectares into high quality animal protein mostly by sheep (Boyazoglu and Flamant, 1990). Sheep in Jordan is a fat-tailed breed known as the Awassi sheep (Hailat, 2005) which possesses great adaptability to tropical environmental conditions. Galal *et al.* (2008) stated that

Awassi sheep is often used as a triple purpose sheep, better for high milk production, in most of the countries of the Middle East.

On the other hand, Jordan has experienced diverse roles of sheep in poverty alleviation and as a result, farmers increasingly turn to sheep as their main source of life and income for its unique products of Jameed, fermented dried yoghurt (Al-Omari *et al*., 2008) and meat (Quasem *et al*., 2009). Unfortunately, fat-tailed sheep has recently faced major threats to their genetic diversity which resulted from systemic, regional and global economic crises as well as drought. A major threat was the sharp reduction in number as a result of persistent drought since 2007. Most of the sheep population is found in north region of Jordan (60%) while (26%) is found in center region and the rest (14%) is found in the south region (MOA, 2010) where drought has mostly been hit.

It is widely accepted that domesticated animals descended from a single ancestor, originating in Asia. It is also reported that all world's sheep breeds originated from central Asia since stone ages (Crawford, 1984). At the present time, the Mediterranean domesticated sheep breeds are the most closely related to their ancestors, which were longtime ago the animals brought from Fertile Crescent (Moiseeva *et al*., 1996) which includes Jordan. However, there is no published data on the fat-tailed sheep biodiversity or its ancestors in Jordan. The use of advanced molecular DNA technology has provided wide opportunities to analyze genetic variability at the DNA level in animal breeds of Jordan (Al-Atiyat, 2009). Such technology is microsatellites DNA markers that are widely used in livestock species since they are polymorphic and are randomly distributed in the organism's genome (Karaca *et al*., 1999; Ahmadi *et al*., 2007; Sulaiman *et al*., 2011; Ismoyowati and Purwantini, 2011). These markers have also been successfully used to study the biodiversity and genetic relationship between and within sheep breeds and/or populations (Romanov and Weigend, 2001; Rosenberg *et al*., 2001). They provide reliable information on allele profiles and allele frequencies for a single DNA sample that can be extracted from blood or tissue.

Limited information is available on the molecular genetic variation of Awassi sheep in Jordan (Galal *et al*., 2008). Therefore, the present study aimed to analyze the genetic diversity of fat-tailed sheep in the southern region of Jordan and to utilize the possible findings for future management conservation of genetic resources of fat-tailed sheep in Jordan.

MATERIALS AND METHODS

Sheep flocks: Three flocks of Awassi sheep were studied in southern region of Jordan in Maan governorate. Capital departments of Maan governorate include the city of Maan, Showbak, Husseiniya and Petra (Alhroot and Al-Alak, 2009) (Fig. 1). The three flocks were selected from Husseiniya and Petra and named as Flocks 1, 2 and 3, respectively. Flock 1 was 30 km away from Flock 2, whereas Flock 3 was 50 km away from Flock 2 within an area of oldest and most known historical places of Petra, Showbak Castle and Dana nature reserve (Fig. 1). The three flocks were managed by their own owners and have not been in connection to exchange animals into their flock from each other for the past 50 years.

Sampling and DNA extraction: Tissue sample were collected from ewes and rams of each flock. The total number of sheep samples was 140 samples (Table 1) taken as 0.5 cm tissue samples from ears using an ear puncher. Samples were placed in Eppendorf microcentrifuge tubes and stored at -18°C until analyzed. DNA extraction was performed using a commercially available kit/protocol of E.Z.N.A® MicroElute Genomic DNA extraction Kit (OMEGA Bio-Teck Corporation, 2009).Subsequently, DNA concentrations were estimated by Nano-DNA spectrophotometer (AlphaSpec®) in which the quality of DNA was evaluated using the ratio of A260/A280.

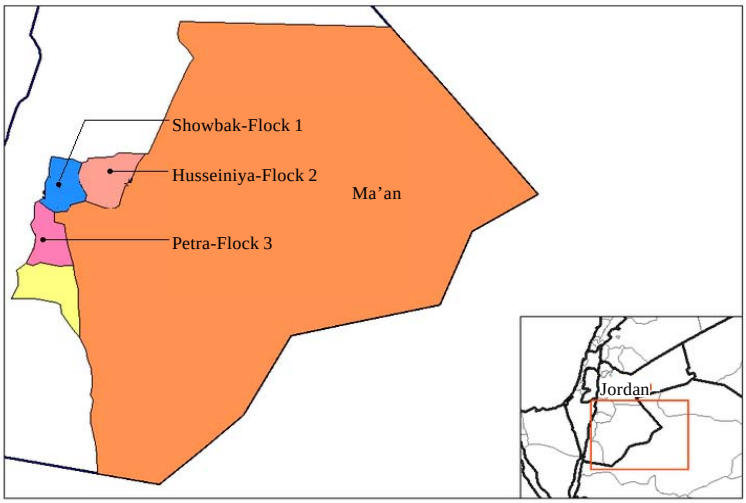

Fig. 1: Map showing regions of the three sampled sheep flocks

Table 1: Number of animals sampled collected from the three flocks

Flock name	No. of rams	No. of ewes	Flock total	Overall
Flock 1	3	49	52	140
Flock 2	2	34	36	140
Flock 3	3	49	52	140

Table 2: Microsatellite markers used for genotyping the three flocks

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

Ch: Chromosome no. in sheep genome; F: Forward primer; R: Reverse primer

DNA genotyping: Six ovine and bovine MS (Table 2), located on chromosomes 2 and 6, were employed for genotyping experiments using Silver Sequence™ DNA System of Promega® company (Promega Corporation, 1998). Selection of the markers was based upon their close linkage to each other. 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 (Maddox *et al*., 2001) and synthesized by BioEngland® (address) (Table 2).

PCR reaction utilized 10 μL volumes 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 of Promega® company. Sequencing ladders were prepared using fmol® DNA Cycle Sequencing system (Promega Corporation, 1998) and 3 μL of each of the four reactions loaded onto the gel, so that the size of the microsatellite alleles could be determined. When the electrophoresis run was completed, the gel was recovered and developed. Then, the gel was dried and viewed by APC Film Development method. 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.

Genetic analysis: Population genetics of the three studied flocks was investigated. Allele frequency and polymorphism under Hardy-Weinberg equilibrium (HWE) were analyzed using Cervus software package (Marshall *et al*., 1998). The first parameter was observed heterozygosity (Ho) which was calculated as:

$$\hat{H}_o = \frac{\sum N_{ij}}{N}$$

and the second was expected heterozygosity (He) at each locus calculated as (Nei, 1987):

$$\hat{H}_e = \frac{2N}{2N-1}\left(1-\sum_{i=1}^{n}\hat{p}_i^{\,2}\right)$$

where, N is the number of individuals in the sample, N_{ij} is the number of observed heterozygotes, n is the number of alleles and p_i is the frequency of the ith allele.

Furthermore, third parameter was polymorphic information content (PIC) which was estimated to describe the amount of polymorphism at a single locus using the following formula by CERVUS software (Botstein *et al*., 1980):

$$PIC = 2\sum_{i-1}^{n-1}\sum_{j=i+1}^{n} p_i p_j (1-p_i p_j)$$

where: p_i is the population frequency of the i-th allele and p_j is the frequency of the jth allele.

On the other hand genetic distances matrix between populations were measured using Genetic Data Analysis (GDA) software (Weir, 1996),which utilizes the most widely used measure of genetic distances proposed by Nei (1972) as:

$$Da = \frac{-\ln G_{ij}}{G_i\ G_j}$$

where, G_i, G_j and G_{ij} are frequencies of the ith and jth allele respectively drawn in populations i and j (Nei, 1972). Using genetic distance matrix, the neighbor-joining tree was formulated using MEGA software program, version 5 (Tamura *et al*., 2011).

RESULTS AND DISCUSSION

DNA quantity and quality: The DNA concentration of the samples was determined for optimal MS genotyping and prior to the PCR amplification step in the genotyping process. The results of DNA concentration showed wide range of 50 to 400 ng 1 μL^{-1} (Fig. 2). This difference in concentration was due mainly to size of sheep ear tissue and to the amount and time of Proteinase-K enzyme that also used in the extraction process and the incubation period. In general, the best performance with PCR amplification of MS markers using commercially available kits occurred within a fairly narrow range of input DNA amount typically on the order of 5 to 2.5 ng per 1 µL (Sambrook *et al*., 1989; Rane and Barve, 2011). Therefore, based on DNA concentration data, every sample was diluted to a concentration of 10 ng per 1 µL as a workable sample for PCR reactions. This was in one hand regarding to quantification of DNA extracted. On the other hand, quality of DNA extracted was also evaluated using the ratio at A260/A280 (Fig. 2). As a general rule, the quality of DNA is established by the ratio of absorbance at 260 nm to the absorbance of 280 nm. For highly purified DNA, this ratio is about 1.8 (Sambrook *et al*., 1989). Figure 4 shows that all samples' absorbance ratios were averaged 1.83 and ranged from 1.6 to 2.2.

Genetic variation parameters

Allele frequency: Estimates of allelic frequency of every locus were calculated to describe the genetic variation within and between flocks. The described alleles and their frequencies in the six

Table 3: No. of alleles per locus, expected heterozygosity (H_e), observed heterozygosity (H_o) at the marker loci, polymorphic information content (PIC) and allele sizes for 6 MS loci in the three flocks under Hardy-Weinberg equilibrium (HWE)

Locus	Allele No. /locus	H_o	H_e	PIC	HWE
INRA40	6	0.673	0.703	0.649	NS
ORAHH30	5	0.472	0.553	0.501	NS
ILSTS30	6	0.611	0.636	0.592	NS
ORAE101	6	0.643	0.741	0.69	NS
ORAHH55	8	0.793	0.76	0.719	NS
BM143	6	0.839	0.805	0.772	NS
Mean	6.17	0.67	0.70	0.65	

NS: Not significant at $p<0.05$

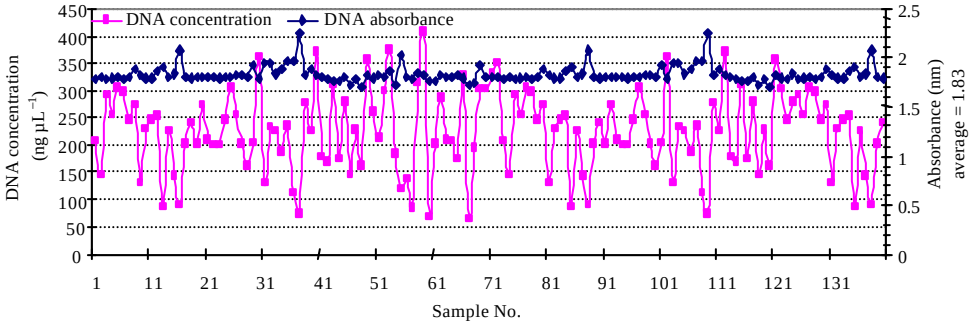

Fig. 2: DNA quantification of all samples using Nano-DNA spectrophotometer

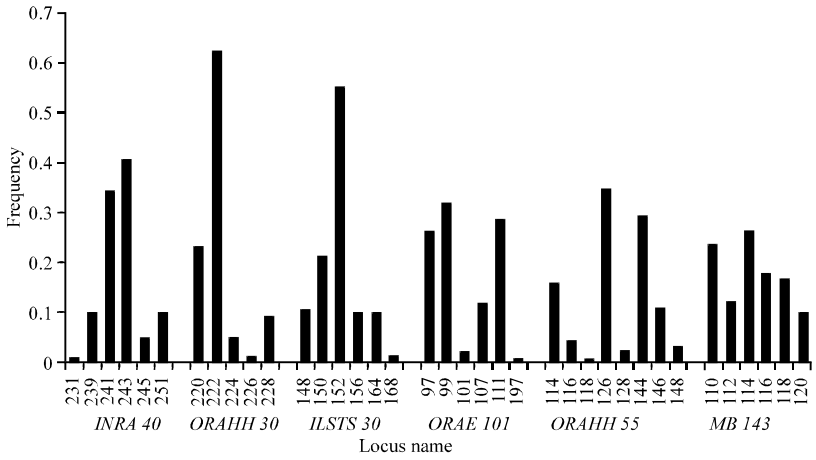

Fig. 3: Allele frequency of the six studied marker for the studied sheep population

studied loci for each flock, are presented in Fig. 3, while the average number of allele per locus, H_o, H_e and PIC are presented in Table 3.

The average number of alleles per loci for all population was 6.17, whereas the number of alleles per each locus ranged from 5 to 8 (Table 3). The average allele number for each flock was 5.5 4.7 and 5.5 for Flock 1, Flock 2 and Flock 3, respectively (Table 4). This indicates a high number of MS alleles in studied sheep flocks. MS loci varied in number of described alleles from 7 (*OARHH55*) in Flock 1 to 3 (*OARAE101*) in Flock 3. It is worthy to make full description of allele frequency profile in which allele frequency and size profile are presented (Fig. 3). In particular, there is a high number of MS alleles as well as a wide range of allele sizes for each locus in the studied flocks. Similar observation was reported by Arranz *et al.* (1998) who estimated MS variation of Spanish Merino sheep. Furthermore, a specific profile of allele frequencies at each locus was observed, reflecting specific distributions of allele frequencies and sizes (Fig. 3). Such profiles are mostly reflecting a normal distribution and thus could be used to predict the level of the genetic variation and genetic differentiation within and between populations. Similar observations of population genetic variations using allele specific profiles were reported for sheep populations by Iovenko (2002) and Peter *et al.* (2007). Furthermore, a similar pattern of allele frequency profiles in sheep has been reported earlier by Arranz *et al.* (2001). On the other hand, Meadows *et al.* (2008) reported that analysis of genetic diversity within five sheep breeds showed merino contained the highest genetic diversity as average number of alleles observed per locus was 8.13 and He was 0.70, whereas Macarthur Merinos contained the lowest amount of diversity, with an average number of alleles of 3.03 and He of 0.4.

Furthermore, the MS loci are different in the allele frequencies in three flocks, considerably from locus to locus and from flock to flock (Fig. 4). Again, in the three flocks, the profile of allele frequencies appears to be similar to binomial distribution (for example *INRA040* and *ILSTS30* for the three flocks). Despite the significant variation of allele frequencies from locus to locus within and between flocks, there are some obvious similarities. For instance allele 222 (*ORAHH30*) is by far the most common in all three flocks and in each one (Fig. 4), followed by 152 (*ILSTS30*). In general, the majority of alleles were found in all three populations, except the alleles with low frequencies. However, the comparisons between three flocks showed that some alleles are not commonly shared between them. For example, allele 231 of *INRA40* was not found in Flock 2 but

Table 4: No. of alleles per locus, expected heterozygosity (H_e), observed heterozygosity (H_o) at the marker loci, polymorphic information content (PIC) and allele sizes for 6 MS loci in each sheep flock

	Husineh 1					Husineh 2					Al-Shoubak				
Locus	Allele No. /locus	No. of genotyped sheep	H_o	H_e	PIC	Allele No. /locus	No. of genotyped sheep	H_e	H_e	PIC	Allele No. /locus	No. of genotyped sheep	H_o	H_e	PIC
INRA40	6	34	0.618	0.686	0.62	5	29	0.448	0.632	0.549	6	50	0.84	0.736	0.689
ORAHH30	4	49	0.429	0.549	0.489	5	27	0.481	0.574	0.515	5	51	0.51	0.548	0.492
ILSTS30	5	37	0.486	0.556	0.509	4	25	0.64	0.543	0.483	6	51	0.686	0.697	0.639
ORAE101	5	48	0.583	0.711	0.648	3	29	0.655	0.678	0.592	5	49	0.694	0.779	0.732
ORAHH55	7	43	0.814	0.726	0.687	5	24	0.792	0.714	0.64	5	49	0.776	0.753	0.703
BM143	6	42	0.905	0.775	0.732	6	32	0.844	0.771	0.723	6	50	0.78	0.782	0.737
Mean	5.5		0.639	0.667	0.614	4.7		0.643	0.652	0.584	5.5		0.714	0.716	0.665

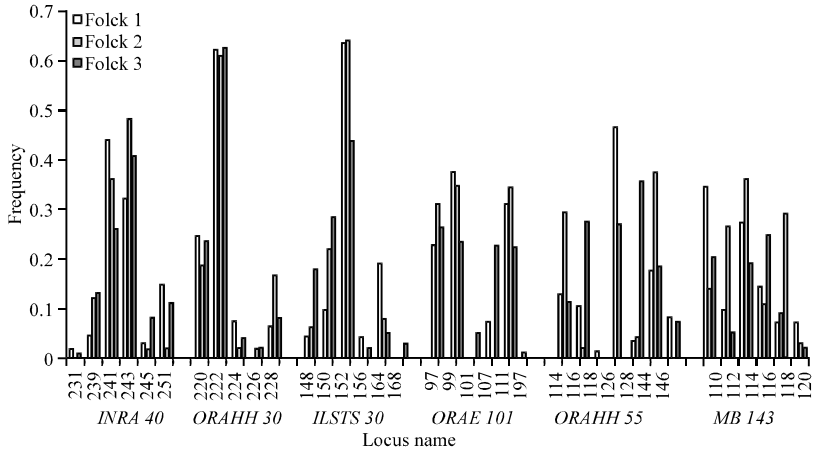

Fig. 4: Allele frequency of the six studied markers for of the three flocks

found in Flock 1 and Flock 3. Also, allele 118 of *ORAHH55* was only found in Flock 1 (Fig. 4). Two possible conclusions can be drawn. First, locus-specific profiles of allele frequencies, at least in some loci, were inherited from the common ancestor. Second there might be some forces that are maintaining such similarity.

On the other hand, it is notable that the most frequent alleles in one population were quite often frequent in the others. Some exceptions are there where the most frequent alleles in a flock were not most frequent in others like allele 126 of *ORAHH55* in Flock1. In some cases however it is vice versa. Generally, alleles of extreme size in all flocks and loci occur at very low frequencies. Similar observations were reported for human populations by Zapata *et al.* (2001) and for sheep breeds by Peter *et al.* (2007). This may indicate that the alleles did not get an advantage either due to random or selective forces. There are little variations in their allele frequencies profile between flocks. This probably reflects that random drift in Flock 1 and Flock 2 and gene flow into Flock 3 and may contribute to this genetic variation. Allelic frequencies and sizes of alleles for Flock 3 were found to be highly variable from those in Flock 1 and Flock 2.

Heterozygosity and polymorphic information content (PIC): The commonly used measures of genetic diversity at a single locus, expected and observed heterozygosity and PIC, are shown in Table 3 for all flocks and in Table 4 for each flock. Overall H_o was 0.67 for all flocks. In most of the cases, H_o was lower than H_e and resulted overall in slightly higher average H_e of 0.7 for all flocks (Table 3, 4). The average H_e for each flock was 0.667, 0.652 and 0.716 for Flocks 1, 2 and 3, respectively (Table 4). These results showed high H_e in all flocks and for most of studied loci except *OARHH30* in which H_o was 0.47 and H_e is 0.55. In regards, each MS locus, *OARHH30* showed lowest in Flock 1 and Flock 3, whereas *INRA40* showed the lowest H_o and H_e in Flock 2. While, the highest values of both H_o and H_e were for BM143 locus. In a similar study, based on three microsatelhte loci, the heterozygoties of Awassi, Kivircik, Akkaraman breeds of Turkey as well as two of their crossbreeds were high and ranged from 0.667 to 0.782 (Soysal *et al.*, 2005). The average expected heterozygosity was 0.72 and the average PIC was 0.67 in Afshari sheep breed of Iran (Qanbari *et al.*, 2007). Arora *et al.* (2011) 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. The results also show that PIC values were high for all studied loci. It seems that high values of either H_o or H_e, were highly relevant to higher values of PIC.

The PIC average was 0.65 for overall flocks (Table 4). They were 0.639, 0.643 and 0.714 for Flock 1, Flock 2 Flock 3, respectively (Table 4). Similar range of results was reported by Arora *et al.* (2011) in which PIC varied from 0.543 to 0.929 and averaged 0.775. In the current study, the lowest value (0.483) was for *ILSTS30* in Flock 2 and the highest value (0.737) of PIC was for BM143 in Flock 3 (Table 4). In general, estimates of H_e and H_o and PIC at the 6 studied loci were high and close to each other. These results were expected for microsatellite loci, which demonstrated high polymorphism in all species studied so far. In addition, it reflects the well-known relationship between high number of alleles per MS locus and as a consequence high heterozygosity and high PIC. These results closely match those of Uzun *et al.* (2006) who stated that the mean number of alleles per locus ranged from 9.3 to 10.4, except for Hemsin sheep (7.8) and for Spanish Churra sheep (9.2). They also reported that the gene diversities (H_e) varied from 0.694 in Hemsin to 0.738 in all studied Turkish sheep. The overall level of microsatellite loci variation observed in this study is within the recently reported range observed for different worldwide sheep breeds (Zahedi- Zahra *et al.*, 2007; Alvarez *et al.*, 2009; Ligda *et al.*, 2009; Zhong *et al.*, 2010; Arora *et al.*, 2011).

The three studied flocks with small sheep numbers revealed high level of genetic variation seen from high number of alleles per locus, H_e, H_o and PIC. These results were similar to those reported in other studies of microsatellite markers in sheep populations such as Bancroft *et al.* (1995) and Coltman *et al.* (2003). The most genetically variable flock was found to be Flock 3, which had more alleles per locus, higher H_e, H_o and PIC. This might be a result of gene flow into this flock, whereas the other two were using more of a self-replacing strategy. This observation was explained as when genetic material, in form of male gametes mainly, migrates from one flock to another (Alvarez *et al.*, 2004) statement is vague. However, the high level of genetic variation in both Flock 1 and Flock 2 could be due to several possible factors such as management and breeding practices under which selection took place. The latter is more likely to explain the high genetic variation that could be a result of selection in these flocks as explained by Woolaston and Piper (1996). This explanation may receive relative attention whether variation can be explained by selection. Different types of selection and their effects were extensively studied and then reported in this regard. For example balancing selection as an example might act directly or indirectly on the trait by maintaining variation at loci. Direct selection can maintain variation by inducing heterozygote advantage (Barton and Slatkin, 1986). The major type of selection in the studied flocks was selecting sires from within-family dam of good reputation. The consequences of family selection on the viability of small populations both in the short and in the long term was studied using simulation model by Theodorou and Couvet (2003). They suggested that family selection could be proposed for use in management programs of small flocks since it increases genetic variabihty and short-term viability. Generally, the conditions assumed in this simulation (small size, well managed and family selection) were typically the same conditioned applied for the three studied sheep flocks. On the other hand, Kijas *et al.* (2009) revealed that the genetic structure of sheep in the world was a result of selection. Therefore, it is plausible to suggest that selection of within-family was the cause of high genetic variation within the sheep flocks. From genetic conservation point of view, this management could be recommended to maintain biodiversity of small flocks in case of genetic migration of good genetic resources is absence.

Finally, deviations from HWE in this study were estimated utilizing genotypic data in the three flocks (Table 4) and using Bonferroni correction test. There was no significant deviation from HWE (Table 4) for all studied loci in three flocks. Corresponding observations were found for no consistent

Table 5: Pairwise genetic distances between the three flocks

Population	Flock 2	Flock 3
Flock 1	0.026	0.035
Flock 2		0.033

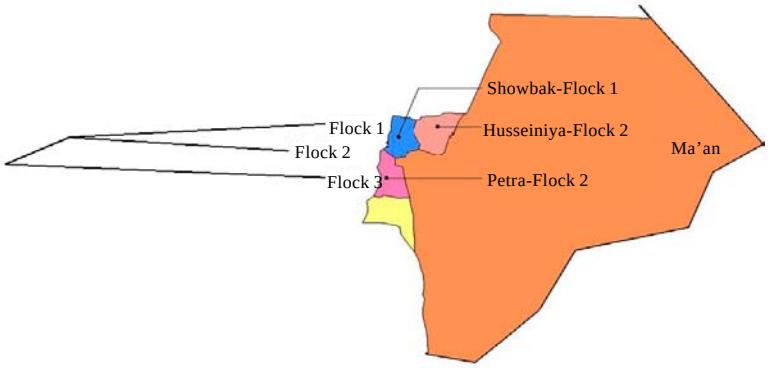

Fig. 5: Genetic relationship among the three sheep flocks according to the neighbour-joining phenogram with its location

HWE deviations across five Turkish sheep breeds (Uzun *et al*., 2006) and for any of the MS loci analyzed. In contrast, Zahedi-Zahra *et al*. (2007) reported that twelve loci were at Hardy-Weinberg equilibrium ($p<0.005$) in Iranian sheep population. However, analysis of deviations from HWE for each locus was then executed in each population revealing that only one MS locus, INRA40, in Flock 3 was significantly deviated from HWE (Chi-square = 8.23, $p<0.004$). The significant deviations from HWE are usually observed when there was an excess of heterozygotes or deficiency of heterozygotes (Wahlund effect) (Guo and Thompson, 1992). In this case of INRA40, Flock3 showed heterozygote excess in the locus but without significant deviation caused by heterozygote deficit in this flock. A conclusion can be drawn that the small population size, limited number of used sires, which leads to nonrandom mating and selection pressures were sufficient factors to significantly shift the genetic structure of Flock3 from the Hardy-Weinberg proportions in this locus. It is worthy to note that Hardy-Weinberg principle states that single-locus genotypic frequencies after one generation of random mating can be represented by a binomial (two alleles) or multinomial (with multiple alleles) function of allelic frequency (Hedrick, 2000). Overall, the studied sheep flocks were in HW equilibrium, even though they were small in size and non-randomly mating took place of a few sires.

Genetic distances: Genetic distances were estimated as pairwise genetic distances using the GDA® software (Weir, 1996) from the allele differences between two flocks as 0.026 between Flock 1 and Flock 2, 0.035 between Flock 1 and Flock 3 and finally 0.033 between Flock 2 and Flock 3. This was based on common alleles per locus that should be used to estimate genetic distances, as suggested by Kalinowski (2002). For genetic distances, it is unity ? when they have no common alleles and zero if the alleles are identical. As expected, the pairwise genetic distance between Flock 1 and Flocks 2 was relatively short; 0.026 (Table 5). The distances between these flocks were described by phylogenetic tree which is shown in Fig. 5 as neighbor-Joining phenogram (Tamura *et al*., 2011). Figure 5 revealed a considerable degree of differentiation between flocks. The finding

firstly supports closeness of Flock 1 and Flock 2 to each other. It also shows that Flock 3 is slightly closer to the Flock 2 than to Flock 1. These results are in agreement with known history of the three flocks in regards to their location and thus possible gene flow and their common ancestors.

CONCLUSION

The three studied flocks with small sheep numbers revealed a high level of genetic variation. The genetic variation was revealed by measuring H_e and H_o, PIC and the number of alleles per locus. The latter showed specific allele frequency profiles which can be used for different genetic and evolutionary studies, in particular for genetic conservation of livestock in hand of their owners. Furthermore, allele frequency profiles were useful tools to explain the level of genetic differentiation between populations using the differences in allele frequencies and allele sizes. In general, estimates of H_e and H_o and PIC at the six studied loci were high and close to each other. These results reflected the well known relationship between high number of alleles per MS locus and as a consequence high heterozygosity and PIC values. In addition, the results also showed whenever PIC values were high for studied loci, the values of either H_o or H_e was high. These results were expected for microsatellite loci, which demonstrated high polymorphism in all species studied so far.

Despite the three flocks were small in number and both selective mating and possible gene flow were effective, they were in HWE. Therefore, this is a proof of MS usefulness as a genetic tool in genetic population analysis of sheep. The results clarified that for a population of small size, policy can be very effective for preserving significant level of genetic variation. Genetic distances also undoubtedly revealed the expected degree of differentiation in the three flocks. From genetic conservation point of view, it is recommended to maintain biodiversity of small flocks in case of genetic migration of good genetic resources is absence. Overall, the present study sheds new light on the molecular and population genetics of fat-tailed sheep in Jordan.

ACKNOWLEDGMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Food and Agriculture Sciences, Research Center.

REFERENCES

Ahmadi, A.K., G. Rahimi, A. Vafaei and H. Sayyazadeh, 2007. Microsatellite analysis of genetic diversity in pekin (*Anas platyrhynchos*) and muscovy (*Cairina moschata*) duck populations. Int. J. Poult. Sci., 6: 378-382.

Al-Atiyat, R.M., 2009. DNA polymorphism of indigenous chickens in Jordan. Asian J. Anim. Vet. Adv., 4: 237-244.

Al-Omari, A., J.M. Quasem and A.S. Mazahreh, 2008. Microbiological analysis of solar and freeze-dried Jameed produced from cow and sheep milk with the addition of carrageenan mix to the Jameed paste. Pak. J. Nutr., 7: 726-729.

Alhroot, A.H.H. and B.A.M. Al-Alak, 2009. Tourism roots and destination marketing bridging civilizations, cultural heritage and tourism theories. Asian J. Market., 3: 108-116.

Alvarez, I., A. Traore, H.H. Tamboura, A. Kabore and L.J. Royo *et al.*, 2009. Microsatellite analysis characterizes Burkina Faso as a genetic contact zone between Sahelian and Djallonke sheep. Anim. Biotechnol., 20: 47-57.

Alvarez, I., L.J. Royo, I. Fernandez, J.P. Gutierrez, E. Gomez and F. Goyache, 2004. Genetic relationships and admixture among sheep breeds from Northern Spain assessed using microsatellites. J. Anim. Sci., 82: 2246-2252.

Arora, R.J., S. Bhatia, B.P. Mishra, A. Jain and B. Prakash, 2011. Diversity analysis of sheep breeds from Southern peninsular and Eastern regions of India. Trop. Anim. Health Prod., 43: 401-408.

Arranz, J.J., Y. Bayon and F. San Primitivo, 1998. Genetic relationships among Spanish sheep using microsatellites. Anim. Genet., 29: 435-440.

Arranz, J.J., Y. Bayon and F. San-Primitivo, 2001. Genetic variation at microsatellite loci in Spanish sheep. Small Ruminant Res., 39: 3-10.

Bancroft, D.R., J.M. Pemberton and P. King, 1995. Extensive protein and microsatellite variability in an isolated, cyclic ungulate population. Heredity, 74: 326-336.

Barton, N.H. and M. Slatkin, 1986. A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity, 56: 409-415.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Boyazoglu, J. and J.C. Flamant, 1990. Mediterranean Systems of Animal Production. In: The World of Pastoralism: Herding Systems in Comparative Perspective, Galaty, J.G. and D.L. Johnson (Eds.). Guilford Press, New York, USA, pp: 353.

Coltman, D.W., J.G. Pilkington and J.M. Pemberton, 2003. Fine-scale genetic structure in a free-living ungulate population. Mol. Ecol., 12: 733-742.

Crawford, R.D., 1984. Domestic Fowl. In: Evolution of Domesticated Animals, Mason, I.L. (Ed.). Longman, London, UK., pp: 298-311.

Galal, S., O. Gursoy and I. Shaat, 2008. Awassi sheep as a genetic resource and efforts for their genetic improvement - A review. Small Rumin. Res., 79: 99-108.

Guo, S.W. and E.A. Thompson, 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 48: 361-372.

Hailat, N., 2005. Small Ruminant Breeds of Jordan. In: Characterization of Small Ruminant Breeds in West Asia and North Africa, Iniguez, L. (Ed.). Vol. 1. ICARDA, Aleppo, Syria, pp: 30-61.

Hedrick, P.W., 2000. Genetics of Populations. 2nd Edn., Jones and Bartlett Publishers International, London, UK., ISBN-13: 978-0763710767, Pages: 553.

Iovenko, V.N., 2002. Genetic diversity of protein markers in sheep population from Ukraine. Genetika, 38: 1669-1676.

Ismoyowati and D. Purwantini, 2011. Genetic Variability of Bali and Alabio ducks on the basis of phenotypic and microsatellites. Asian J. Poult. Sci., 5: 107-115.

Kalinowski, S.T., 2002. How many alleles per locus should be used to estimate genetic distances. Heredity, 88: 62-65.

Karaca, M., F.G. Karaca, C. Patel and M.G. Emara, 1999. Preliminary analysis of microsatelhite loci in commercial broiler chickens. Proceedings of the Plant and Animal Genome 7th Conference, January 17-21, 1999, San Diego, CA. USA., pp: 155.

Kijas, J.W., D. Townley, B.P. Dalrymple, M.P. Heaton and J.F. Maddox *et al.*, 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. PloS ONE, Vol. 4, 10.1371/journal.pone.0004668

Ligda, C., J. Altarayrah and A. Georgoudis, 2009. Genetic analysis of Greek sheep breeds using microsatellite markers for setting conservation priorities. Small Rumin. Res., 83: 42-48.

MOA, 2010. Livestock annual statistics in Jordan. Ministry of Agriculture, Jordan.

Maddox, J.F., K.P. Davies, A.M. Crawford, D.J. Hulme and E.P. Cribiu *et al.*, 2001. An enhanced linkage map of the sheep genome comprising more than 1000 loci. Genome Res., 11: 1275-1289.

Marshall, T.C., J. Slate, L. Kruuk and J.M. Pemberton, 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol., 7: 639-655.

Meadows, J.R.S., E.K.F. Chan and J.W. Kijas, 2008. Linkage disequilibrium compared between five populations of domestic sheep. BMC Genet., Vol. 9, 10.1186/1471-2156-9-61

Moiseeva, I.G., I.G. Chzhan, I.A. Zakharov and A.A. Nikiforov, 1996. Comparative analysis of morphological traits in Mediterranean and Chinese chicken breeds. The problem of the origin of the domestic chicken. Genetika, 32: 1553-1561.

Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.

Nei, M., 1987. Molecular Evolutionary Genetics. 1st Edn., Columbia University Press, New York, USA.

OMEGA Bio-Teck Corporation, 2009. Micro-elite genomic DNA kit. OMEGA Bio-Teck Corporation, British Columbia, Canada.

Peter, C., M. Bruford, T. Perez, S. Dalamitra, G. Hewitt, G. Erhardt and ECONOGENE consortium, 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. Ani. Genet., 38: 37-44.

Promega Corporation, 1998. The Silver Sequence™ DNA sequencing system of Promega. Woods Hollow Road, Madison, USA.

Qanbari, S., R. Osfoori and M.P. Eskandari Nasab, 2007. A preliminary study of marker data applicability in gene introgression program for Afshari sheep breed. Biotechnology, 6: 513-519.

Quasem, J.M., A.S. Mazahreh and A.F. Al-Shawabkeh, 2009. Nutritive value of seven varieties of meat products (Sausage) produced in Jordan. Pak. J. Nutr., 8: 332-334.

Rane, P.P. and S.S. Barve, 2011. Standardization and optimization of mtDNA isolation and molecular genetic analysis of D-loop region in animal natural fibres. Int. J. Zool. Res., 7: 190-200.

Romanov, M.N. and S. Weigend, 2001. Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. Poult. Sci., 80: 1057-1063.

Rosenberg, N.A., T. Burke, K. Elo, M.W. Feldman and P.J. Freidlin *et al.*, 2001. Empirical evaluation of genetic clustering methods using multilocus genotypes from twenty chicken breeds. Genetics, 159: 699-713.

Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, USA., ISBN-13: 9780879695774, Pages: 3.

Soysal, M.I., E. Koban, E. Ozkan, V. Altunok, Z. Bulut, M. Nizamlioglu and I. Togan, 2005. Evolutionary relationship among three native, two crossbreed sheep breeds of Turkey: Preliminary results. Rev. Med. Vet., 156: 289-293.

Sulaiman, Y., C. Wu and C. Zhao, 2011. Phylogeny of 19 indigenous sheep populations in northwestern china inferred from mitochondrial DNA control region. Asian J. Anim. Vet. Adv., 6: 71-79.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likehhood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28: 2731-2739.

Theodorou, K. and D. Couvet, 2003. Familial versus mass selection in small populations. Genet. Sel. Evol., 35: 425-444.

Uzun, M., B. Gutierrez-Gil, J.J. Arranz, F. San Primitivo, M. Saatci, M. Kaya and Y. Bayon, 2006. Genetic relationships among Turkish sheep. Genet. Sel. Evol., 38: 513-524.

Weir, B.S., 1996. Genetic Data Analysis 2: Methods for Discrete Population Genetic Data. 2nd Edn., Sinauer Associates Inc., Sunderland, MA., USA., ISBN-13: 978-0878939022, Pages: 445.

Woolaston, R.R. and L.R. Piper, 1996. Selection of merino sheep for resistance to *Haemonchus contortus*: Genetic variation. J. Anim. Sci., 62: 451-460.

Zahedi-Zahra, S. Esmaeelkhanian and R.V. Torshizi, 2007. Microsatellite variation in one breed of Iranian sheep with 12 markers. Pak. J. Biol. Sci., 10: 4455-4460.

Zapata, C., C. Carollo and S. Rodriguez, 2001. Sampling variance and distribution of the D measure of overall gametic disequilibrium between multiallelic loci. Ann. Hum. Genet., 65: 395-406.

Zhong, T., J. Han, J. Guo, Q. Zhao and B. Fu *et al.*, 2010. Genetic diversity of Chinese indigenous sheep breeds inferred from microsatellite markers. Small Rumin. Res., 90: 88-94.