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Using Molecular Markers for Evaluate the Uniformity among the Awassi Flocks in South Kirkuk Province

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Annotation: The objective of this study was to assess the genetic diversity and population structure of the indigenous Awassi sheep in Southern Kirkuk (North of Iraq). Four flocks were randomly chosen from different geographical regions and 10 rams and 10 ewes were randomly selected in each flock. Five polymorphic microsatellite markers SSR characterised by their high polymorphism in sheep were analysed on blood-derived genomic DNA samples. PCR amplification and genetic analysis were based on established molecular and statistical procedures. The genetic diversity was estimated by diversity indices such as observed (Na) and effective number of alleles (Ne), Nei's gene diversity (h), Shannon's index (I), and percentage of polymorphic loci. The results showed that, in both sexes, females showed higher allactic richness (Na = 1.4000), gene diversity (h = 0.1500) and Shannon's index (I = 0.2249) than males. Ht was twice as large in females (0.1500) compared to males (0.0750), but Hs was equal to zero in both, revealing that genetic Structure was high. The strong

genetic differentiation (Gst = 1.0000) and low gene flow (Nm = 0.0000 in females, 1.0000 in males) might indicate reproductive isolation, most likely among the females. A dendrogram based on pairwise genetic identity matrices confirmed this, showing comparatively greater variation among females than among males, which were more homogeneous in their genetic differentiation. Significance These results underline the necessity of sex-influenced genetic manager of conservation purpose and the need of broader genetic repertoire of the genome for maintain genetic diversity not to be lowered in Awassi sheep populations.

Keywords: Awassi, sheep, Molecular, Markers.

Introduction

The Awassi is the most famous native sheep breed in the Middle East, including Iraq, Syria, Jordan, and Turkey [1]. By virtue of its adaptability to harsh environmental conditions and climate change, disease resistance and high milk and meat yield, Awassi is an important economic breed in the region [2]. In addition, its genetic diversity is necessary to keep adaptive features, robustness, disease resistance, and performance in agriculture and production [3]. In addition, this diversity, although a great asset, makes management of breeding programs for its obtaining and dissemination more difficult [4]. This breed has been historically spread in several areas and its animals are classically kept in different flocks between governorates and villages.

Assessing the genetic diversity both within and between Awassi sheep flocks is therefore crucial for conservation and maximizing the sustainability of productive flock management [5]. In order to measure the genetic diversity, structure, stature and homogeneity of wild and domestic livestock populations, these molecular markers are now found to be of great value and importance in contemporary genetics [6]. Such markers are polymorphic, DNA-based sequences that are polymorphic and differ among individuals, and thus can also be used to study genetic relationship, calculate inbreeding coefficients, and assess breeding programmes [7]. Molecular markers have application in the assessment of herd-level uniformity or heterogeneity, gene dispersal, selection and adaptation to different environments [8]; as for the Awassi sheep, the past decades have shown an increase in the number of molecular markers applicable to assess genetic uniformity in livestock stocks brought about by biotechnological developments coupled with the decreasing cost of high-throughput sequencing technologies [9].

Molecular markers for the same purpose, such as microsatellites [10], single nucleotide polymorphisms (SNPs) [11], and mtDNA [12], are commonly used to estimate genetic diversity between and within herds [13]. Molecular marker-based research is essential [14] for detecting genetic variation that may exist between geographically or administratively separated sheep flocks (for example, any Awassi sheep flocks available within an academic and agricultural research institute are classified according to scientific production practice methods that need to be

confirmed). Knowing the extent of genetic variation within each population, as well as between populations, is crucial for decision-making regarding breeding schemes, such as whether to introduce genetic material from other populations from within and different regions to reduce inbreeding or maintain genetic distinctiveness for specific traits of interest [15]. Previous and documented research using molecular markers on large and small livestock populations has indicated that they can be a powerful tool for estimating within- and between-farm genetic diversity and for regulating breeding and productivity schemes [16]. A common example of this is previous studies on the Awassi sheep breed, which have shown significant genetic diversity within the breed itself, both between regional populations and within regions, despite their morphological similarity and some production traits [17]. This might partly reflect the substantial influence of the local environment, ecology, and climate on the genetic structure of the Awassi sheep, which is important for breed management and the conservation of genetic variation [18]. Ensuring genetic diversity between flocks, for instance, might be crucial to ensure that populations can respond to sudden climate shifts, disease outbreaks and reduced productivity (Booman et al., 2022). In contrast, chronic inbreeding can deplete this adaptive capacity and facilitate its genetic assimilation [19]. Molecular marker techniques are powerful tools for allele discovery (associated with traits of biological and/or agronomic significance) including disease resistance, milk production and growth rate [20]), thus marker based approaches are also very beneficial for identifying the genomic region and loci of interest. These advances will enable breeders to select individuals for good genes that could be useful for enhancement of breeding and production schemes [21]. These resources can likewise be of great help to reduce the detrimental and deleterious effect of genetic drift, inbreeding and genetic variation and thus, ensuring the long term and short term viability of Awassi sheep industry [22]. Based on the above, we conclude that: Background: Several molecular markers have been utilized to understand the genetic and epigenetic relationship among the Awassi sheep reproductive flocks through their genetic resource and productivity. Awassi sheep is an important breed highly produces milk with well-adapted, resistant to diseases and sustainable for sheep farming in the Middle East and the surrounding area; therefore, its quality is crucial and basic genetic information needed.

Materials and methods

The present study was performed in Kirkuk city as an agricultural and pastoral area itself in Iraq. In order to measure the level of genetic uniformity and diversity in local Awassi sheep flocks, 4 flocks were randomly chosen from different geographical locations in the province, which reflects different flock management systems, flock size and location to represent the region. From each flock, blood or tissue samples were collected from 10 rams and 10 ewes (approximately 20 sheep/flock). To avoid confounding, this process was randomly performed and no animals with any signs of disease and/or physiological stress were included. The genomic DNA was isolated from faecal samples as recommended by the manufacturer (Geneaid). Homogenization of samples, lysis with buffer, and DNA precipitation with ethanol were performed for the extraction. The purity and concentration of DNA were evaluated by NanoDrop spectrophotometer (Thermo Fisher Scientific) for an A260/A280 nm reading between 1.8 and 2.0 in addition to agarose gel electrophoresis to confirm DNA integrity. Only DNA with good quality was sequenced for further genetic analysis. In order to evaluate genetic diversity and population structure, a set of 5 SSR molecular markers was used, which showed polymorphism and efficient utility in sheep genetics research. Microsatellite markers table 1, which have a high degree of polymorphism and ability to detect individual and population level variation, were selected. Microsatellite loci were amplified using polymerase chain reaction (PCR) with a final volume of 20 µL per reaction that contained [concentration of primer] of the forward and reverse primers, a PCR buffer, and [cycles] for the amplification. The PCR conditions optimized per previous study to each marker, such as the number of cycles, annealing temperature, and the cycle time are given in Table one. Genetic diversity was analyzed by using genetic diversity parameters including allelic richness, number of alleles per locus, observed and expected heterozygosity and fixation index of genetic

differentiation, the Fst Nei's genetic distance, hierarchical cluster analysis for individual and flock similarity. A pairwise Fst were used for assessing the genetic differentiation and analysis of the molecular variance was done using GenAlEx software version 6.4. The significance level was 0.01. These analyses served to evaluate intral and inter flock similarity and variability for awassi sheep.

Table 1: The information of the SSR markers were used

Marker Name	Primer	Position (cM)	Size	Chr.
INRA40	F = 5` TCAGTCTGGAGGAGAGAAAAC 3` R = 5` CTCTGCCCTGGGGATGATTG 3`	149.9	205-257	2
OARHH30	F = 5` CTCAGTCTCAACTTTGTTCCTCTATAGC 3` R = 5` GAAAGCTAAGGCTGAACATTGTGCCC 3`	167.4	103-117	2
ILSTS030	F = 5` CTGCAGTTCTGCATATGTGG 3` R = 5` CTTAGACAACAGGGGTTTGG 3`	180.5	140-164	2
OARAE101	F = 5` TAAGAAATATATTTGAAAAAACTGATC 3` R = 5` CTTCTTATAGATGCACTCAAGCTAGG 3`	49.8	99-123	6
BM143	F = 5` ACCTGGGAAGCCTCCATATC 3` R = 5` CTGCAGGCAGATTCTTTATCG 3`	59	102-128	6

Result and Discussion

The dataset in Table 2 expresses sex differences in genetic diversity across five loci in Awassi sheep as sign evident that male values for all parameters measured (Na, Ne, h, and I) are lower than for females. Such divergences can benefit the breeding, conservation as well as population genetics of this autochthonous breed. The Na was higher for females (1.4000) than for males (1.2000), indicating a larger allelic gene pool. A higher allelic richness and standard deviation (0.5477) indicates more variability in genetic contribution from females. Such patterns can be attributable to either historical introgression (from the maternal lineage) or to selection maintaining diversity among females [23]. Likewise, Ne was also slightly higher for females (1.2400 vs. 1.1200), which indicates a less deviation in allele distribution. Which is crucial because rare allele frequency correlates with lowers heterozygosity and low adaptation to the environmental pressure [24]. RQ3: s.d. values for Ne in both sexes suggest similar variability, however females show a greater mean diversity. Similar trends were also observed for other genetic indexes, such as gene diversity (h) on Nei (1987) (0.1500 in females and 0.0750 in males). This implies that the among-female component of variation within populations is higher, which in turn corroborates studies that report an increased genetic complexity through female-mediated gene flow [25]. Wide S.D. values further suggest significant individual differences in both men and women. The Shannon Information Index I for richness and evenness was nearly 2-fold higher in females (0.2249) than males (0.1125), further suggesting that the allele distribution is better balanced among females (and thus suggesting more balanced sexes in females). Previous findings have thus also supported the notion that maternal traits, such as fertility and milk yield, remain targets of selection in local sheep breeds, and this is reflected by the generally higher levels of both sex-complex and autosomal diversity in females [26]. On the diversity front, the fact that most diversity is female is benecial considering its conservation genetics perspective. More diversity translates to a more resilient population among females, making them ideal candidates for breeding and conservation programs. Due to the clear need to maintain genetic diversity in smallholder and pastoral systems, recent genomic selection models have started to incorporate genetic diversity and female performance traits to maintain productivity while minimizing inbreeding risk [27]. Further, greater standard deviations in the previously indicated parental sex for all parameters also point to mixed heritable phenotypes due to unequal mating access, reproductive success, or population bottlenecking. This variability is required so that genetic erosion does not occur, hence careful monitoring should be encouraged to maintain a balanced gene pool [28].

Table 2: Genetic variation mean, and standard deviation for the five loci of the Awassi populations

Parameters	Male		Female	
	Mean	S.D.	Mean	S.D.
Na	1.2000	0.2683	1.4000	0.5477
Ne	1.1200	0.2683	1.2400	0.3286
h	0.0750	0.1677	0.1500	0.2054
I	0.1125	0.2515	0.2249	0.3080

Na = Observed number of alleles; Ne = Effective number of alleles [Kimura and Crow (1964)]; h = Nei's (1973) gene diversity; I = Shannon's Information index [Lewontin (1972)]

Table 3 also showed the clear sex-related differences in genetic diversity of the Awassi sheep. Overall gene diversity (Ht = 0.1500) was higher in females than in males (Ht = 0.0750), representing a wider spectrum of alleles and higher contribution in total genetic diversity. The increased female diversity possibly shows the diversity of maternal lines, long-term reproductive stability or selection on maternal characters that have been reported for local sheep [23, 29]. The within-population gene diversity (Hs), however, was zero for females and males, indicating high genetic homogeneity among individuals within a population. Potential explanations are inbreeding, small sample size or restricted flock management with sex groups under reproductive isolation [26]. The Gst (Gst = 1.0000) did not differ between the sexes, consistent with genetically isolated male and female subpopulations. Those results could nevertheless be indicative of sex-biased breeding or geographic discontinuity, or just the sampling of genetically distinct flocks [24], the latter being unlikely given that all wild populations are highly integrated over ecological and evolutionary time scales. This should be confirmed with higher resolution markers such as SNP arrays.

Gene flow (Nm) also confirmed this difference: males (Nm = 1.0000) presented moderate migration of genes between groups, with possible erection migration between flocks that is a common breeding management. By contrast, zero gene flow (Nm = 0.0000) in the form of direct gene exchange shown from females indicates there is absolute reproductive isolation, a population with the maternal line being kept in livestock system i.e. ewes kept in stable matriline [27, 28]. Second, the proportion of polymorphic loci was significantly larger in females (40%) than in males (20%), which is in line with the greater allelic richness in females, possibly resulting from adaptive selection for reproductive success or disease resistance [30].

Table 3: Gene diversity mean and standard deviation for the Awassi for populations

Parameters	Male		Female	
	Mean	S.D.	Mean	S.D.
Ht	0.0750	0.0281	0.1500	0.0422
Hs	0.0000	0.0000	0.0000	0.0000
Gst	1.0000	-	1.0000	-
Nm	1.0000	-	0.0000	-
Polymorphic loci %	20 %		40 %	

Nm= gene flow estimation

The comparative data for the genetic identity proportion and genetic distance in the four Awassi sheep populations, examined independently according to gender (male and female populations), are presented in Table 4. This pattern is characterized by a different sex-biased genetic structure, in which males are more homogeneous and females more diverged [51]. Such patterns have immediate relevance with respect to breeding strategies, and demographic and genetic conservation. We found relatively high (0.8000–1.0000) genetic identity values between the male populations that demonstrated similarity across populations. Population 1 is exactly the same as populations 2 and 4 (!) roughly similar to population 2 (¥0.1 ¥0.1 99.9% JAPAN), with moderate

similarity to population 3 (¥0.8 1234 this pages). Population 2 also perfectly mirrors population 1 in structure, with populations 3 and 4 with whom it shares 0.8000 and 0.8000 respectively, suggesting an arguably high degree of male-mediated geneflow and centre-periphery breeding. These results are consistent with common livestock management practices where herders manage male animals (rams) across their flocks for mating, reducing population differentiation in male animals and maintaining genetic diversity of male-typical traits [27, 28].

Female populations, however, present values of genetic identity auguring for a wider range of taxonomic value from 0.0000 to 1.0000 with higher genetic differentiation than males, and possibly isolation. For instance, population 1 displays complete demographic identity with populations 3 and 4 but with only 0.6000 with 2, suggesting selective genetic sharing. Populations 3 and 4 are completely differentiated from population 1 (0.0000) but completely identical with each other (1.0000), indicating substructuring, which is probably attributed to localized maternal lineages or founder effects 23, 30]. The great divergence among female populations may be the result of lower mobility among females, well documented in traditional sheep farming where ewes tend to spend several generations within a flock [26].

The existence of some 0.0000 values for identity by descent in both sexes (in particular in the females) can also be the result of founder effects (that is; selective cohorts that were artificially made at start of the breeding line, genetic drift, or from a sampling of different breeding lines. In population genetics, it is well known that large divergence can often correspond to either a historical separation or else strong reproductive isolation, and that these two factors can influence a population's ability to adapt and respond to selection [24, 25]. Second, the moderate identity values of female population 2 with all other ones (all <0.5100–0.6000) indicate a central or admixed genetic background that might have occasionally had gene crossover with multiple flocks. This restricted gene flow in females causes the accumulation of private alleles and the rise of intra-group diversity, as indicated by the greater spread of the genetic id values. These trends are consistent with findings from other studies which stressed on the role of management systems, nutritional management and environmental variation as sources of heterogeneity and differentiation between indigenous sheep populations such as Awassi and Kurdi breed [31],[32],[33] . This may be done by individual animals or through management practices, if they are not genetically “burning bridges” with individuals from the isolated population [34].

Table 4: Genetic identity and genetic distance for the males and females.

Sex	Populations	1	2	3	4
Male	1	****	1.0000	0.8000	1.0000
	2	0.0000	****	0.8000	1.0000
	3	0.2231	0.2231	****	0.8000
	4	0.0000	0.0000	0.2231	****
Female	1	****	0.6000	1.0000	1.0000
	2	0.5108	****	0.6000	0.6000
	3	0.0000	0.5108	****	1.0000
	4	0.0000	0.5108	0.0000	****

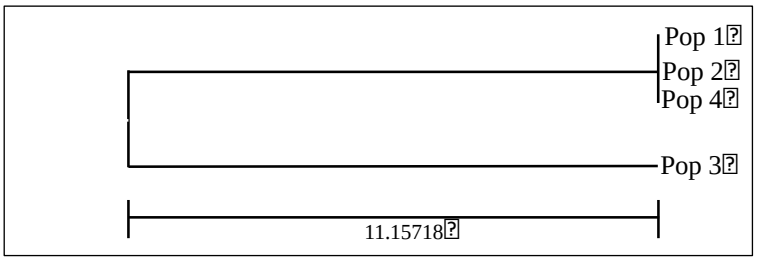

Fig 3: Dendogram for the males of the four populations of Awassi sheep based on the Nei’s (1978)

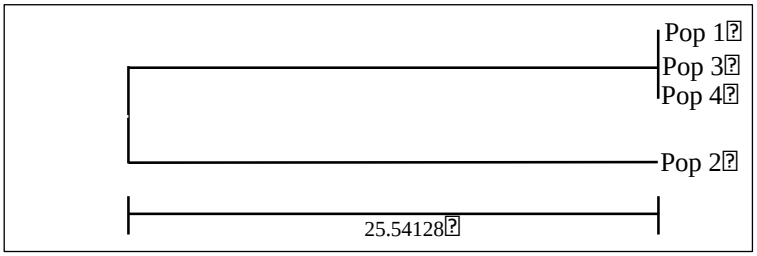

Fig 4: Dendogram for the females of the four populations of Awassi sheep based on the Nei's (1978)

Conclusion

In conclusion, the higher genetic diversity and heterogeneity of females than males provides support for the idea that females play a fundamental role in maintaining breed diversity of the Awassi sheep. The presence of both strong genetic structuring and low gene flow between the two male clusters stresses the necessity for sex-specific conservation measures and more sampling to ensure the genetic viability, adaptation and sustainable breeding of Awassi flocks in the long term.

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