Using of Microsatellites to Study the Genetic Polymorphisms of SRYm18 Region in Iraqi Sheep

Hawraa Al-Mohamadawi[1], Asaad Y. Ayied[2]
[1,2] *Animal Production Department, College of Agriculture, University of Basrah*
Corresponding Author: asaad.yheia@gmail.com

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Abstract

The origin of animals is usually determined by their paternal genotypes of the genes on the Y chromosome. In addition to the genes and their polymorphisms in the genome of mitochondria that are inherited through dams. In view of the lack of studies focusing on the genes of the Y chromosome in the world and their absence in Iraq. The aim of the present study was to identify the multiple genetic polymorphisms of the SRYM18 gene in the Arabi and Awassi sheep raised in Iraq. The study was conducted in the Genetic Engineering Laboratories - College of Agriculture, University of Basra, as well as in the Basra Genome Laboratory. The amplification of the SRYM18 gene showed genetic polymorphisms and gave a gene segment of (103-880) bp. The number of alleles of the SRYm18 gene was 13 alleles in the Arabi and 16 alleles in the Awassi breeds. The equilibrium test showed that the two breeds were under equilibrium. The two breeds were identical with nine alleles, while the number of special alleles for the Arabi breed was two, while the Awassi breed was distinguished by five. The number of rare alleles reached 20, of which seven were of the Arabi breed, and 13 of them were of the Awassi breed. Mean expected heterozygosity was 0.6386 with nonsignificant Fis for Arabi breed (0.1541) but significant for Awassi breed (0.2213). Mean neutrality was close to lower bound (0.1721) and (0.1270) for Arabi and Awassi breeds respectively.

** The research is quoted from the master's thesis of the first researcher.*

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Introduction

The genetic diversity of farm animals has long been a subject of concern and has important implications for genetic improvement, breeding management and genetic resource conservation programs. Microsatellite markers are the most common markers used in the genetic characterization of sheep breeds (Tapio et al, 2010; Calvo et al., 2011). Sheep were examined mostly by maternal analysis of mitochondrial DNA inherited in modern sheep breeds to determine the genetic history of domestication (Tapio et al, 2006), as well as using some genes on the Y chromosome.

The mammalian Y chromosome is one of the smallest chromosomes in the genome and contains the male pseudogenes through which, with the unique SSR technique of the Y chromosomes, it is possible to understand, develop and maintain sex chromosomes in mammals (Ginja et al, 2009). Y chromosome represents about 2% of the total DNA in the cells (Genomic Research, 2020). It was used to trace the paternal lineage of a population and to understand differences in migration and genetic variation between males and females with the addition of advantages of small size, effective population, and low mutation rate are sufficient markers for the distribution of the haplotype of a population (Jobling and Tyler-Smith, 1995; Jin and Su; 2000; Underhill et al, 2000). In comparison with other chromosomes, the y chromosome contains a limited number of genes. Perhaps the low genes number of Y is a result of the tendency of these genes to change during evolution since they are nowadays fragmenting of an ancient common ancestor with the X chromosome (Graves, 1995).
The Y chromosome is made up of five different types of non-heterochromatic, pseudo- (XTR) regions, X-transposed, X-degenerate and AMP

(Skaletsky et al., 2003). In addition to the central chromosomes, the Y chromosome contains a large segment of heterochromatin of 31 MB on an arm, which is more than half of its total length (Skaletsky et al, 2003). The four regions are all isochromatic in contrast to the heterochromatic (Hughes and Rozen, 2012; Skaletsky et al, 2003). The two pseudo regions of the x, y chromosome contribute to the correct pairing and segregation in mitotic cell division resulting in sperms originating in the XDG regions (Hughes and Rozen, 2012; Skaletsky et al, 2003).

In view of the lack of studies focusing on the genes of the Y chromosome in the world and their absence in Iraq, the aim of the present study was to identify the multiple genetic polymorphisms of the SRYm18 in the Arabi and Awassi sheep raised in Iraq.

Materials and Methods

Ethical Approval

Prior to sampling, the objectives of the study were explained to the animal owners in their local languages so that they could make an informed decision regarding giving consent to sample their animals. Government veterinary, animal welfare, and health regulations were observed during sampling of the populations analyzed here. The procedures involving animal sample collection also followed the recommendation of directive 2010/63/EU. Collection of blood samples was permitted by the Iraqi Ministry of Agriculture.

This study was conducted in the Genetic Engineering Laboratory, College of Agriculture, University of Basra, and the laboratories of the Iraqi company and the Basra National Genome Laboratory. Blood samples were collected from the Iraqi sheep breeds, 105 samples from the Awassi, and 75 and Arabi breeds. A 5 ml of blood samples from each animal were collected from both breeds from Maysan (Al-Majar Al-Kabir District) and Basra (Karma Ali District) southern Iraq.

Blood Sample Collection

Blood samples (5 ml/animal) were withdrawn from the jugular vein using a 10 ml medical syringe after the jugular vein area was cleaned and sterilized with ethyl alcohol. Blood samples were injected into tubes containing an anticoagulant (Tetra Acetic-Acid-EDTA Ethylene Diamine). The samples were freeze-preserved at -4° C until the DNA extraction process was performed.

DNA Extraction

DNA was extracted from blood samples of Iraqi sheep breeds, in the genetic engineering laboratory, using the kit provided by the American company Invitrogen and according to the instructions provided in the kit.

PCR Product

A fragment (106–145 bp) of the SRYm18 was amplified using two primers: Forward primer: F 5'-GGC ATC ACA AAC AGG ATC AGC AAT -3' and Reverse primer: R 5'- GTG ATG GCA GTT CTC ACA ATC TCC T -3 (Meadows et al, 2006). The PCR amplifications were conducted in a 50 µl volume containing 20 ng genomic DNA, 25 µl of Master Mix, 2 µl each primer, 15 µl free water. The amplification conditions were as follows: initial denaturation at 95 C for 5 min followed by 35 cycles of denaturation at 95 C for 1 min, annealing at 56 C for 1min, and extension at 72 C for 1.5 min, and then the final extension at 72 C for 10 min. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success.

Preparation of Agarose Gel

After preparing the gel basin, washing and attaching the comb at one end and placing the plastic pieces on the edges of the basin, the transfer is done on the agarose gel at a concentration of 1%, i. e. dissolving 0.25 g of the agarose in 25 ml of 10x TBE solution and put it in a beaker and then heating it by microwave for 3 minutes until the clear color of the mixture. After that, 1 µL of Ethidium bromide dye is added, taking into account the well-shaking of the beaker for the purpose of homogeneity of the dye with the mixture. Then the gel is poured into the transfer basin and left for the purpose of hardening (Williams et al, 1990).

Electrophoresis

The success of DNA extraction is determined by the use of the agarose gel electrophoresis technique by submerging the migration gel basin in the main basin containing the 10x TBE transfer solution. Then the mixture is injected into the drill and after the completion of the injection process, the electrodes are connected to the power supply and the electric current is fixed at 80 volts and 56 milliamps. The gel is left until the Bromophenol blue dye flows from the drill to the other side and

after the migration process is finished, the gel is checked in the data documentation device (Gel documentation) for observing DNA bands interfering with Ethidium bromide

Statistical Analysis

For SRYm18 microsatellites, allele frequency, private alleles, and locus diversity parameters (expected heterozygosity (He), observed heterozygosity (Ho), Hardy–Weinberg equilibrium (HWE), allelic richness (AR), and neutrality test were calculated using the POPGENE 1.32 software program (Yeh et al, 1999).

Results and Discussion

Number of Alleles and their Frequencies

Table 1 shows the number of alleles and their frequencies of the SRYM18 microsatellite in the Arabi and Awassi sheep breeds. Arabi breed exhibited 13 alleles and those of Awassi were 16. Allele frequency of Arabi and Awassi breeds ranged from 0.017-0.600 and 0.007-0.573 respectively. Allele frequencies in both breeds were 0.009-0.581. This study is the first study that uses a marker from Y chromosome of Iraqi sheep. Therefore, all discussion will depend on different microsatellites other that of Y chromosome. The aim of researching microsatellites is to use their markers to measure animal genetic diversity in order to aid maintenance and management strategies, improve mating processes, and reduce inbreeding rates (Crispima et al, 2014).

Similar, special and Rare Alleles

Table 2 shows the number of similar and specific alleles for the Arabi and Al-Awassi breeds of the microsatellite sequences of the SRYm18 microsatellites. The number of shared alleles was 9. and the number of special alleles was 2 and 5 for the Arabi and Awassi breed respectively. In light of the current study, it is evident that there is a genetic variation between the two breeds, and that the genetic variation that was found in the Awassi and Arabi breeds is expressed by the presence of special alleles.

Table 1. Allele number and frequencies of SRYM18 microsatellites of Arabi and Awassi breeds

Allele number	Frequency (number)		
	Arabi	Awassi	Both breeds
103	-	0.020 (2)	0.011 (2)
110	0.017 (1)	0.029 (3)	0.022 (4)
128	0.035 (3)	-	0.017 (3)
143	0.050 (4)	0.020 (2)	0.033 (6)
157	0.035 (3)	-	0.017 (3)
173	0.035 (3)	0.020 (2)	0.028 (5)
188	0.100 (8)	0.080 (8)	0.089 (16)
194	-	0.020 (2)	0.011 (2)
201	-	0.013 (1)	0.006 (1)
215	0.017 (1)	0.013 (1)	0.011 (2)
242	-	0.013 (1)	0.006 (1)
254	0.040 (3)	0.013 (1)	0.028 (4)
591	0.017 (1)	0.133 (14)	0.083 (15)
610	0.017 (1)	0.013 (1)	0.011 (2)
643	0.017 (1)	0.020 (2)	0.017 (3)
764	0.017 (1)	0.020 (2)	0.017 (3)
800	0.600 (45)	0.573 (60)	0.581 (105)
880	-	0.0267 (3)	0.017 (3)

Table 2. Number of shared, special and rare alleles for ASRYm18 microsatellites in both

Breeds	No. of		
	shared alleles	special alleles	rare alleles
Arabi	11	2	7
Awassi		5	13

Heterozygosity and Homozygosity

Table 3 indicates the genetic diversity of both studied breeds expressed as observed and expected heterozygotes. Heterozygotic genetic percentage of Arabi is greater than in Awassi (0.5333 and 0.5048 respectively). With regard to the expected genotypes, Arabi breed recorded nearly similar to that of Awassi either calculated (0.6305 and 0.6533 respectively) or by Nei's method (0.620 and 0.6522 respectively). Although the observed heterozygosity is nearly 0.50 (0.5333 and 0.5048 respectively , the inbreeding coefficient for Awassi breed is significant (p= 0.027) and highly positive (0.2913). the value recorded by Arabi breed is positive but not significant (0.1212). the positive value indicate that the population is under inbreeding status as expected heterozygosity is higher than observed. Negative Fis values indicate heterozygote defects (Dixit et al., 2009; Ojo et al , 2018) as the observed heterozygosity is higher than expected. Heterozygote deficiency may result from the admixture of two breeds or more, which resulted in population sub-structure (Cerda-Flores et al, 2002; Muema et al, 2009). Population subdivision due to genetic drift, null alleles, and inbreeding are also factors to this deficiency (Hoarau et al , 2005). The results indicated that there is very low admixture between the two breeds. The change in the proportions of heterozygosity between the two breeds is due to the random mixing between them and the lack of selection for these groups. These results are similar to what was reached by Al-Barzinji et al (2011), who used different microsatellites of Iraqi sheep.

Table 3. Genetic diversity of Arabi and Awassi breeds for the SRYM18 microsatellites

Breed	Ho	He	He (Nei)	Mean He	Fis	P-value
Arabi	0.5333	0.6305	0.620	0.6386	0.1541	0.100
Awassi	0.5048	0.6533	0.6522	0.6386	0.2213	0.027

Ho: observed heterozygosity; He: expected heterozygosity, Fis: inbreeding Coefficient.

Neutrality Test

The mean of the neutral F test for Arabi and Awassi breeds was 0.1721 and 0.1270 respectively (table 4), The average of the neutrality indicates an expansion in the size of the population, that the values for the two breeds are close, and that these values confirm the balance of the two breeds as shown by the Hardy equilibrium test. The mean values for both strains are closer to the lower bound than to the upper bound, reflecting the low frequency of most alleles in the two strains. The decrease in frequency is an indication of an approaching loss of these alleles and may lead to a decrease in the genetic variation between individuals resulting from the method of breeding that depends on the presence of small-sized flocks and an increase in the effectiveness of genetic drift with an increase in the effect of some mutations.

The observed alleles richness was 13 and 16 alleles for Arabi and Awassi breeds respectively. The lowest and the highest bound of F for Arabi is higher than that for the Awassi breed (0.077 and 0.0455; 0.680 and 0.7592 respectively). As for the standard error of Arabi breed is less than that found in Awassi breed (0.0016, and 0.00025 respectively), and these values reflect the similarity of the allele frequencies in the Arabi breed more than in the Awassi breed. And that the variation in the frequencies of genotypes is a genetic variation among individuals of the same breed.

Table 4. Neutrality test for Arabi and Awassi breeds

Breed	**No. of alleles**	**Observed F**	**Lowest value of F**	**Highest value of F**	**Mean of F**	**Standard Error**	**Confidence interval (95%)**	
							Lower	**Higher**
Arabi	13	0.3800	0.0770	0.6800	0.1721	0.0025	0.1117	0.2956
Awassi	16	0.3428	0.0455	0.7592	0.1270	0.0016	0.0795	0.2299

The neutrality test uses a measure of the likelihood that the breeds have undergone demographic events such as genetic drift or expansion of population size. The results of the study showed that the values are positive for all tests in most cases. This explains the partial signs of the sudden expansion as a widespread population increases within the boundaries of the region in an expanding geographical area (Bruford et al, 2003).

Conclusion

In conclusion, the studied breeds shared more than half the alleles (9 alleles) of the SRYM18. Most the alleles existed with very low frequency (less than 0.05), which may explain the neutrality mean is close the lower bound as a result of genetic drift specially there is no systematic selection.

Conflict of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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