Detection of genetic diversity of local Iraqi black goats by using D-loop Region

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Summary

This study was conducted at the College of Agriculture / University of Basrah for the period from (1/12/2020) to (30/11/2021). The study aims to characterize the phenotypic and genetics of local Iraqi black goats using mitochondrial DNA inheritance (D-Loop). 160 blood samples were collected from local Iraqi black goats aged (1-2) years in the governorates of Al-Muthanna, DhiQar, Basrah and Maysan(40 samples for each governorate). The mtDNA was extracted, the genes were multiplied, and the sequences were found. A segment of 980 bp of the D-loop region were amplified.The number of haplotypes of the D-loop area reached 10 haplotypes.The total genetic polymorphism (NH) reached 31, and more than half of the total number of polymorphisms was in the animals of Al-Muthanna (17). The least polymorphism recorded in Thi-Qar and Maysan (2 each). The total genetic polymorphism was 96 (24 forms for each province).The total number of transition mutations for the D-loop region was 101, distributed equally on different governorates.The total number of transversion mutations for the D-Loop was 16 (4 for each governorates).The total haplotype diversity (HD) of the the D-loop region, the total haplotype diversity value was 0.752, and the highest haplotype diversity was in Al-Muthanna (0.779). As for the nucleotide diversity (π) of the D-loop region revealed a value of 0.01314, and the highest value was in Thi-Qar (0.01344).The results of the analysis of the haplotypes pattern of the D-loop area, one haplotype pattern recorded for local goat, and other patterns appeared for each global breed included India, Iran, Ireland, Romania and Turkey, and Italy and Spain shared one pattern.

Introduction

Goats are characterized by the ability to survive and thrive under very harsh climatic conditions, such as high ambient temperatures, water shortages and scarcity of pastures. Goats have been widely used as a good source of meat, milk and hair, especially in areas with harsh climatic conditions that negatively affect the performance and production of other animals. Despite the large contribution of goats to food security in arid and semi-arid areas compared to other farm animals, studies on goat production systems and phenotypic and genetic characterization are few (Al-Araimi et. al., 2017).Phenotypic and genetic characterization to assess biological diversity and differences between the current Iraqi goat breeds is a prerequisite and an important condition to facilitate their conservation and use in breeding and improvement programs, as Iraqi goats are not classified and the information available about them is very limited. Therefore, the phenotypic characterization of animal genetic resources is the process of identifying the different groups and breeds and describing their external and productive characteristics in

a particular environment and under a certain administration, and the information provided by phenotypic studies is necessary in planning and managing animal genetic resources at the local, national, regional and global levels (Othman and Mahfouz, 2016). Determining the polymorphisms in the DNA sequence in the mitochondrial genome has unique applications in genetic studies of pets, as the mitochondrial genome is inherited from the mother, and it is haploid and its genes are not determined, and the D-loop region is a highly variable region in the mtDNA, which is It is of particular interest because, unlike protein-coding gene regions, it has a high level of sequence divergence (Aquadro and Greenberg, 1983).

Thus, D-loop sequence variation along with the recombination results in an informative tool for studying maternal relationships between animals (Mirol et al, 2002; Cozzi et al 2004; Priskin et al, 2010).(2020) Deniskova found in his study on the D-loop gene in goats to know the maternal origins and haplotype diversity of seven of the Russian goat breeds. About 59 haplotypes were identified in the Russian goat breeds, all of which differed from the haplotypes of the Saanen goats, and that all of these observed haplotypes were Specific, in addition to that, 56 out of 59 haplotypes belonged to Haplogroup A, which was consistent with the universal genotype of origin seen in most goats around the world, in addition to the detection of the presence of Haplogroup C and Haplogroup D and also found that the goat strains Existing in Russia are strains that were introduced through different migrations.

The present study was part of a project to recognize the genetic structure of Iraqi local camel (Ayied et al., 2018) and cattle (Faraj et al, 2020 and 2021).The aim of the present study was to detect D- loop polymorphisms and genetic diversity of local Iraqi Black goat.

Materials and Methods

160 samples were collected from the local Iraqi goat breed, 40 samples from each of the governorates of Muthanna, DhiQar, Basrah and Maysan, and the animals were not related to each other.

DNA extraction

The gSYNC™ DNA Extraction Kit was used for DNA extraction, manufactured by the Taiwanese Geneaid Company, and according to the instructions in the leaflet attached by the company.

Polymerase Chain Reaction

The special materials were prepared using PCR technique, then they were placed in a container containing pieces of ice to protect them from heat. The work was done in a sterile and clean place in a special cabinet (PCR Cabinet), which contains ultraviolet (UV) rays, to sterilize the micropipettes, tubes and tubes. The PCR reaction mixture was prepared in a 100 µlEppendorf tube and the final volume of components was (50 µl) and then the tubes were placed in a microcentrifuge for 30 seconds (Table 1). The primer was 'R-5'-CTGATTAGTCATTAGTCCATC-3, F-5'-CGTGTATGCAAGTACATTAC-3' (مصدر).

Table (1) Quantities (µl) of materials used in PCR-mtDNA technique

Master mix	Template DNA	Primers		D. W.	Total volume
		Forward	Reverse		
25	6	2	2	15	50

Results and Discussion

Molecular diversity of Region D-loop

The results of the genetic diversity of the D-loop gene showed that the total number of its sequences reached 160 in all studied regions, 40 sequences were distributed in each region (Al-Muthanna, DhiQar, Basrah and Maysan).

The total number of genetic formations (NH) was 96, distributed among the governorates of Muthanna, DhiQar, Basra and Maysan for the strain studied, each of which had 24 formations. The total number of transitions was 101, distributed (24, 26, 25 and 26) between the governorates of Muthanna, DhiQar, Basrah and

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Maysan, respectively. While the total number of shifts showed 4 shifts distributed in all governorates. As for the content of nitrogenous bases, it was rich in nitrogen bases content of adenine A, thymine T and cytosine C (table 2).

Table (2) Molecular diversity of the D-loop region in the Iraqi local goat breed

Governorate	Mysan	Basrah	Dhi-Qar	Al-Muthana	Total*
Number of sequences (N)	40	40	40	40	160
Number of polymorphic (NH)	24	24	24	24	96
Number of transitions	26	25	26	24	101
Number of transversions	4	4	4	4	16
Nucleotide composition	A= 30.42% T= 30.80% C= 22.82% G= 15.96%	A= 29.87% T= 30.98% C= 23.01% G= 16.14%	A= 27.25% T= 29.87% C= 25.93% G= 16.95%	A= 30.24% T= 30.43% C= 23.37% G= 15.95%	

The presence of genetic formations, transitions and joint transformations

Genetic diversity of Region D-loop
The results of the genetic diversity of the D-loop region showed that the total haplotype diversity (HD) values were high for the studied strain, which amounted to 0.752, and the highest genetic diversity was for the animals of Al-Muthanna Governorate, which reached 0.779, followed by the animals of the provinces of Maysan and DhiQar (0.778 and 0.769), respectively, and the lowest diversity value Hereditary was for the group of Basra governorate animals, which amounted to (0.726), while the total values of nucleotide diversity (π) were low for the studied strain, which amounted to 0.01314, and the highest nucleotide diversity for the group of animals of DhiQar governorate was 0.01344, followed by the animals of Muthanna and Maysan governorates (0.01342 and 0.01334). Respectively, the lowest value of genetic diversity was for the group of animals in Basra Governorate, which amounted to (0.01315) (Table 3).

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Table (3) Genetic diversity of the D-loop gene of the Iraqi local goat breed

Governorate	Nucleotide diversity (π)	Haplotype diversity (HD)

Muthana	0.01342	0.779
ThiQar	0.01344	0.769
Basrah	0.01315	0.726
Mysan	0.01334	0.778
Total	0.01314	0.752

Haplotypes network of Region D-loop

The results showed that the total number of haplotypes for the D-loop area is equal to 10 haplotypes, of which 10 are in DhiQar governorate, 10 in Basra governorate, 9 in Muthanna governorate, and 8 in Maysan governorate. The governorates of Muthanna, DhiQar, and Maysan, and the H-10 haplotype is common to the governorates of DhiQar and Basra (Fig. 1).

Figure (1) The D-loop haplotype network of the Iraqi local goat breed among the governorates

In comparison with some countries of the world, the total number of haplotypes for the D-loop region was 7 haplotypes, including 1 and 1 in each of Iraq, India, Iran, Ireland, Romania and Turkey, and Italy and Spain shared one pattern, which is the H-5 pattern. The H-5 haplotype was shared by Italy and Spain, while the rest of the haplotypes were independent of the other (Fig. 2).
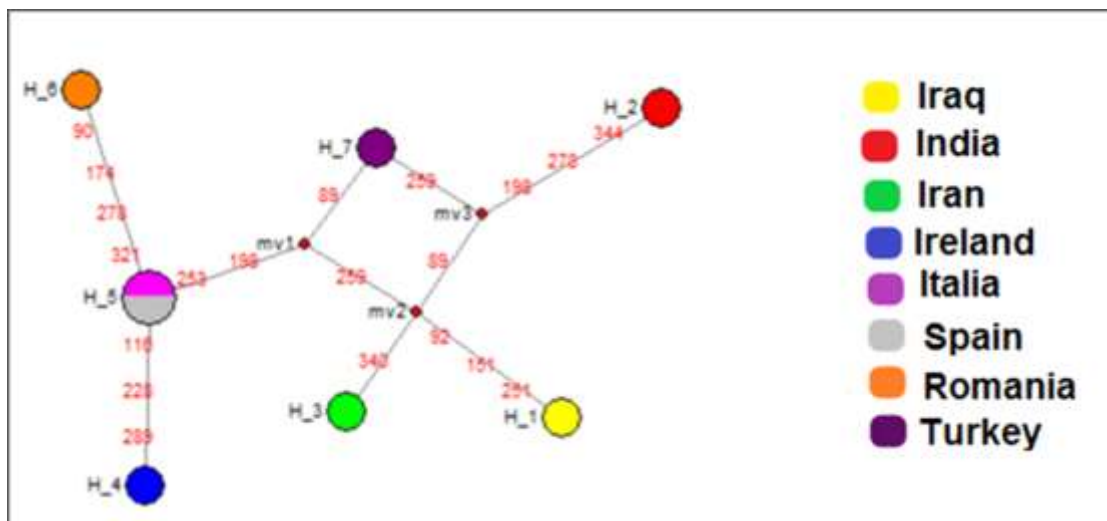
Figure (2) The network of haplotypes of the D-loop region between the local Iraqi goat breed and the goat breeds in some countries

Phylogenetic tree of Region D-loop

The results of the phylogenetic tree for the D-loop region showed the presence of two main branches, the first branch included the animals of DhiQar governorate, while the second branch included two secondary branches, the first of which included the animals of the Muthanna and Basra governorates, while the second branch included the animals of Maysan governorate (Fig. 11).

Genetic distance of Region D-loop

The fixation index Fst was used to express the genetic distance of the D-loop region of the Iraqi local goat breed in the studied areas of Muthanna, DhiQar, Basra and Maysan. The genetic distance of the dynasty was 0.748 between the provinces of DhiQar and Basra. Because of the high values in the genetic distance of the study gene, it indicates the presence of very high genetic differences in different provinces (Wright 1978; Hanti and Clark, 1997) Table (4).

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Table (4) The genetic distance of the D-loop region of the Iraqi domestic goat breed

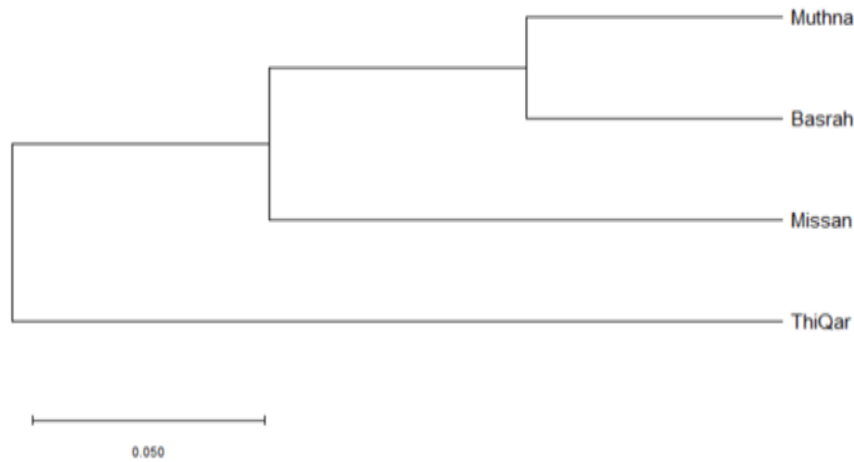| Zone	Muthana	ThiQar	Basrah	Mysan
Muthana	-			
ThiQar	0,774	-		
Basrah	0,753	0,748	-	
Mysan	0,778	0,773	0,752	-

Figure (3) Phylogenetic tree of the D-loop region of the Iraqi local goat breed

In comparison with some countries, the results for the D-loop region showed the presence of two main branches, the first branch included the Iraqi goat breed and the Iranian goat and the second branch included Turkish goats and another branch contained two branches, the first included Indian goats and the second included two branches, the first included the Romanian goat breed and the second included all of the goat breeds in Ireland, Italy and Spain. This corresponded to a network of haplotypes that included four haplogroups. (Fig. 4)
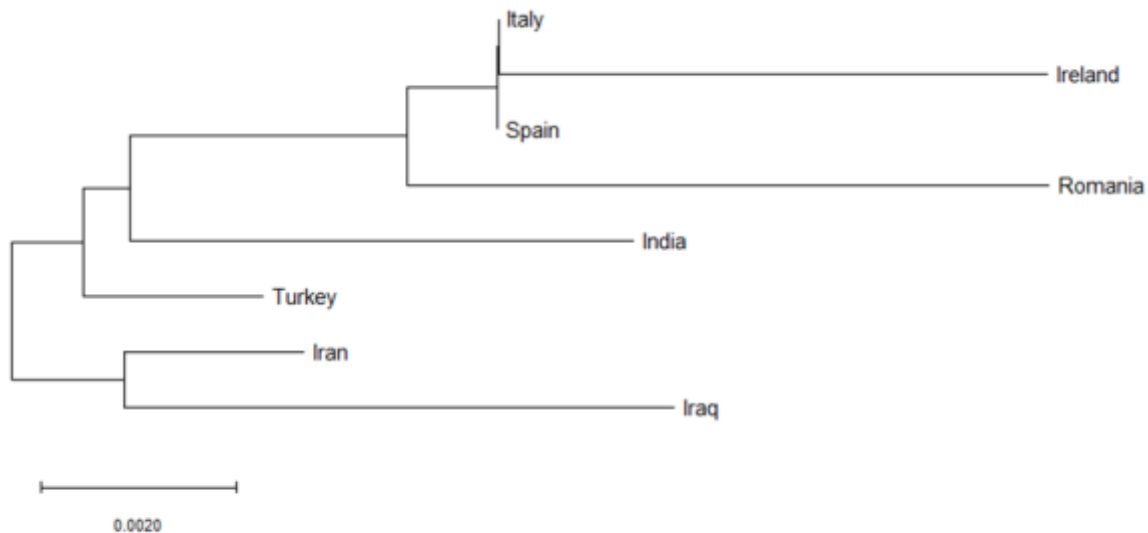
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Figure (4) The phylogenetic tree of the D-loop gene between the local goat breed and the countries of the world

Mismatch distribution of Region D-loop
The results of the mismatch distribution for the D-loop region showed the absence of a unimodal distribution in the studied animals for all governorates and the presence of two-peak distributions with differences peaks (7.190, 7.179, 7.136 and 7.033) for the governorates of DhiQar, Muthanna, Maysan and Basra, respectively, and the values of the index (R) for animals The same two governorates (0.222, 0.188, 0.187 and 0.183) for the governorates of

DhiQar, Maysan, Basra and Muthanna, respectively.

The unimodel form indicates that the populations are subject to demographic expansion, where the results of the studied gene in all governorates indicated that they are monomorphic, which indicates that they are in a state of demographic equilibrium (Hudson and Slatkin, 1991; Rogers and Harpending, 1992; Jobling et al., 2004). (Table 5 and Figure 5).

Table (5) mismatch distribution for the D-loop region of the Iraqi domestic goat breed

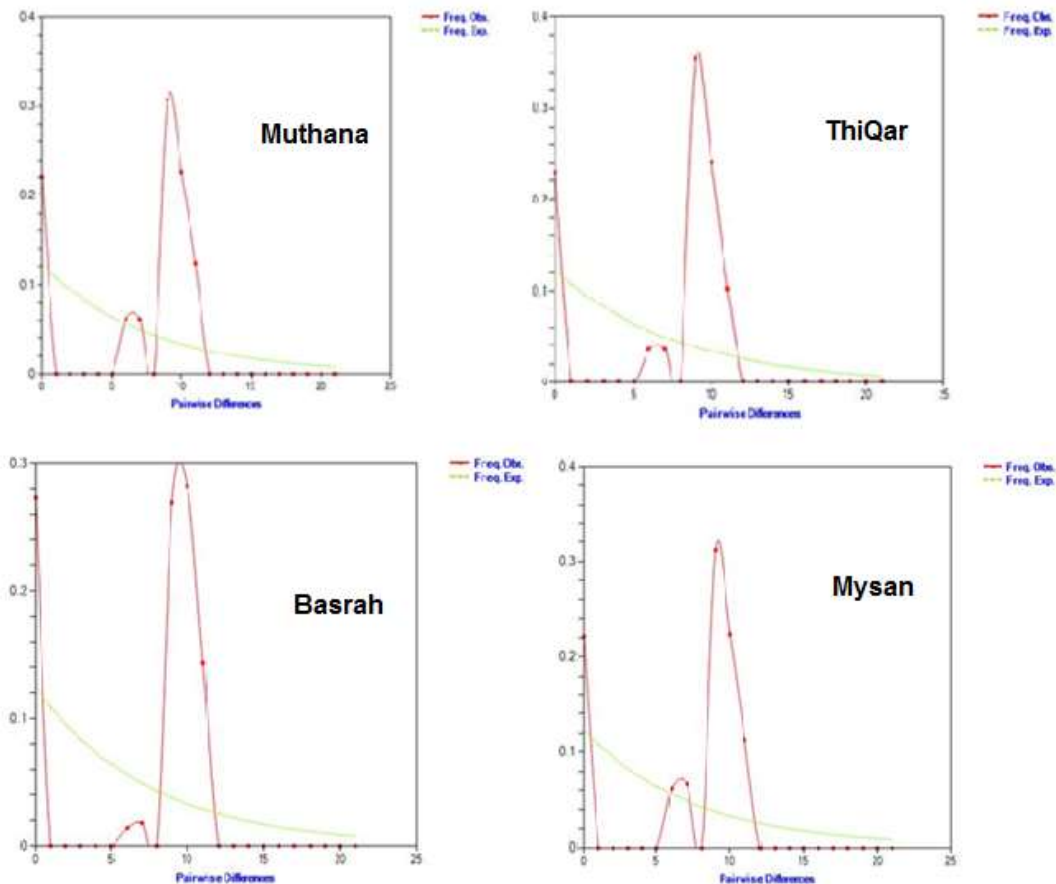| Governorate	Raggedness Index(R)	Mean Pairwise Difference (SD)
Muthana	0,183	7.179
ThiQar	0.223	7.190
Basrah	0.187	7.033
Mysan	0.188	7.136

Figure (5) Mismatch analysis of the D_loop region of the Iraqi local goat breed using the program DNASP

Neutrality Test of Region D-loop

The results showed that there was no negative value for the local Iraqi goat breed studied and in all governorates for the results of the Neutrality Test, where the average value of Tajima's D for all governorates was (1.379) for the D-loop gene, and the highest values for Tajima's D in Muthanna governorate were

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1.534) Followed by Maysan and DhiQar, which amounted to (1,430 and 1,310), respectively, and the lowest value was in Basra Governorate, which amounted to (1,241). And with values (significant $p < 0.05$), as for Fu's Fs, it averaged (5.886), where the animals of Al-Muthanna Governorate recorded the highest value (7.594), followed by Al-Muthanna and DhiQar, which recorded (6.399 and 4.831).), respectively, and the lowest value recorded for the animals of Maysan Governorate was (4,721) and all of them were significant at the level of $p<0.05$. The small size of the herd for the breeders, or genetic drift and the fixation of some genetic structures and genes, in addition to the loss of many rare alleles, which have an effect on many traits, which reach the clan to the so-called bottleneck, i.e. a significant deterioration in genetic diversity, and may be the reason for the Positive values for TajimaThe test for the study gene results from the absence of organized scientific cross-breeding programs or the fact that cross-breeding among animals is random, which may cause the loss of some genotypes (Tajima, 1989; Fu, 1997; Aris-Brosou and Excoffier, 1996) (Table 6).

Table (6) Neutrality test for D-loop region in Iraqi domestic goat breed

Governorate	Fu's Fs Statistic	Tajima's D Statistic
Muthana	6.399	1.53476
ThiQar	4.831	1.31017
Basrah	4.721	1.24112
Mysan	7.594	1.43086
Mean	5.886	1.37923

*there is a significant difference $p<0.05$

Analysis of molecular variation AMOVA of Region D-loop

The results of AMOVA analysis of the D-loop region between and within the provinces of the Iraqi local goat breed showed that the percentage of genetic variance among the governorates was 0.17% and the percentage of variance within the governorate was 99.83 (Table7).

Table (7) Molecular Variation Analysis of D-loop Region in Iraqi Domestic Goat Breed

The source of the difference	degrees of freedom	sum of squares	Contrast components	Variance %
Inter provinces	3	15.693	0.00827	0.17
Intra province	156	764.55	4.900	99.83
total	159	769.781	4.908	100

* There are significant differences between governorates

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