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Genetic Variation and Bottleneck Tests in Iraqi Native Cows of Babylon Province by STR Markers

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Abstract: In the current study, we evaluated genetic variation and bottleneck analyses of Iraqi native cows in three locations of Babylon province. A total of 70 individuals of three cows' population were genotyped using ten the short tandem repeat markers (STR). The STR loci showed high variation of polymorphism. The average total number of alleles (TNA) and effective number of alleles (Ae) values were 5.30± 0.17 and 3.89± 0.11, respectively. The TNA at the marker level ranged from 3.00 (ETH3) to 8.00 (TGLA227), while the Ae ranged from 2.69 (ETH3) to 5.54 (TGLA227). The Shannon index (I) at the marker level ranged from 1.05 (ETH3) to 1.87 (TGLA227), with mean 1.48± 0.28. Also, the mean polymorphism information content (PIC) was 0.65±0.15, with a range of 0.54 (ETH3) to 0.81 (TGLA227). The average observed heterozygosity (Obs_Het) was 0.54 ± 0.18 and varied from 0.16 (ETH3) to 0.77 (TGLA53). The average expected heterozygosity (Exp_Het) was 0.75±0.25, with a range of 0.62 (ETH3) to 0.85 (TGLA227). The means HS unbiased gene diversity, and inbreeding coefficient (F_{IS}) were 0.73±0.23, and 0.24±0.09, respectively. The mean Fis, Fit and Fst, and gene flow (Nm) were 0.296 ± 0.065, 0.314 ± 0.090, 0.032 ± 0.002, and 7.259 ± 2.113 respectively. The native cow's population is not at bottleneck, and normal L-shaped distribution of allele frequencies, that it has not experienced any recent decline in effective population size and has remained in equilibrium between mutation and drift. The Bayesian analysis showed that all animals were heterogeneous and formed three distinct clusters.

Keywords: *Bottleneck, Genetic variation, Iraqi cows, STR loci.*

Introduction

Livestock farming is an important source of income for rural populations, and cattle are one of the most important species of livestock that played an important role in human culture and history, that have a noticeable impact on human society (Herrero *et al*., 2013). In addition, cattle are mainly primarily for meat and milk production and sometimes for the use of hides, horns, and dung in agriculture (Banda & Tanganyika, 2021).

In Iraq, there are four native breeds: Karadi, Sharabi, Restaki, and Janoubi. These breeds are distributed as follows: Restaki in the center and Janoubi in the southern regions, Karadi and Sharabi in the northern

regions (Al-Murrani *et al*., 2003). The uncontrolled crossing and random mating have led to the spread of hybrid breeds such that the genetic structure of native cattle has become largely unknown (Alshawi *et al*., 2019). This practice has led to growing concerns about the erosion of local genetic resources. In addition, due to wars, sieges, drought, animal smuggling, the use of traditional methods of livestock management, animal migration between cities, and the use of artificial insemination, this has led to a genetic mixture between Iraqi native and exotic cattle herds (Faraj *et al*., 2023).

Developing genetic conservation plans and breeding strategies for cattle breeds requires the genetic characterization of individuals, populations, and breeds in order to assess genetic variation (Toro & Caballero, 2005). The discovery of molecular markers has made it possible to evaluate and study genetic variation as well as genetic selection. (Hayes *et al*., 2009).

The short tandem repeat markers (STRs) method is one of the most common molecular markers, essentially due to the choice of blending its analysis with the polymerase chain reaction (PCR) (Fan & Chu, 2007). The use of STRs is a powerful tool for calculating genetic and gene variation, calculating gene variation, genetic distances, structure, and detecting bottleneck tests due to the random distribution in the genome, high degree of polymorphism, and co-dominance (Putman & Carbone, 2014).

In recent years, only a few similar studies have been conducted in Iraq on cattle breeds (Al-Jub & Riyadh Hamad senkal, 2023; Hadi & Alnajm, 2024). So, the present study is necessary to preserve genetic resources and is considered a genetic base for genetic improvement and maintaining genetic resources. The study aimed to determine genetic variation and bottleneck tests by identification of 10 STR markers in 70 animals from the Iraqi native cows raised in the region of Babylon province, Iraq.

Materials & Methods

Animals and Blood Sample Collection

The study involved 70 unrelated cows (males and females) selected from the South (24), Middle (23), and North (23) regions of populations Babylon Province (Fig. 1) were randomly collected from their areas of origin. Blood was collected between November 2023 and February 2024. Blood sample was taken from each individual from the jugular vein and were placed in Ethylenediaminetetraacetic acid (EDTA) tubes. The blood samples were centrifuged for 10 minutes at 5000 rpm in order to collect the buffy coat. Then, the buffy coat was then placed 1.5 ml tubes and kept −20 ˚C until the DNA was isolated.

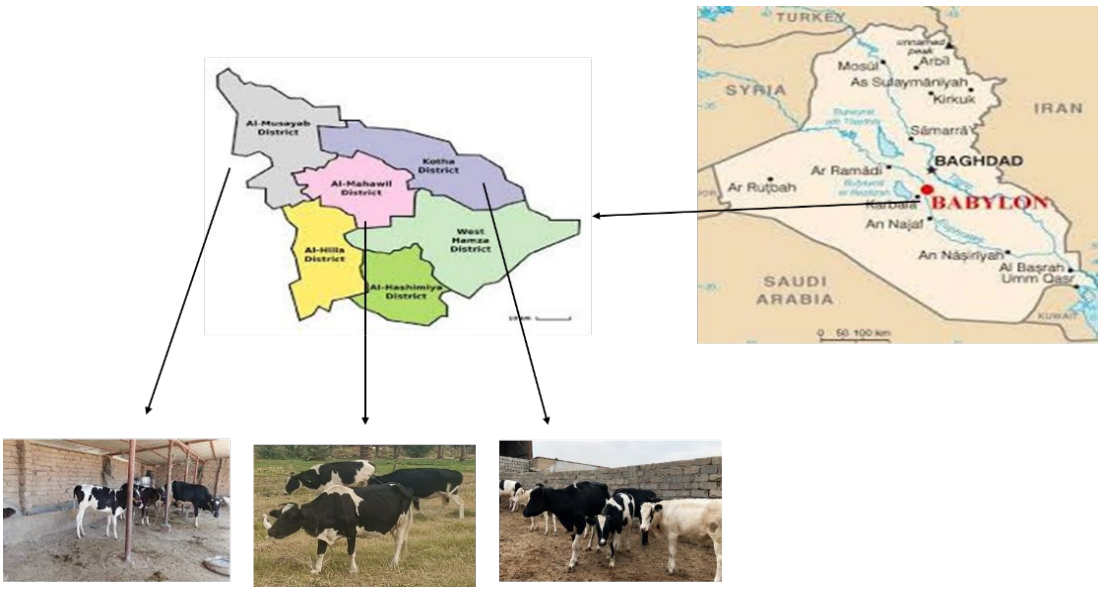

Fig. (1): - Native cows population samples collection distribution in Babylon Province/ Iraq.

DNA Isolation and Genotyping of Individuals

The genomic DNA was isolated from the buffy coats blood using a commercial kit (Geneaid, USA) by the manufacturer's recommended methodology. The DNA quantity and quality were measured using the electrophoresis method. The genome samples were loaded onto an agarose gel at a concentration of 1%, and the isolated DNA was stored at -20°C until genotyping analyses. The animals were genotyped using the 10 STR loci selected from the FAO (2011) recommended list (Table 1).

Table (1): STR markers, chromosome's location, sequences, allele size and annealing temperature.

Primers	Chr. location	Primer sequence (5' – 3')	Annealing (°C)	Allele size (bp)	GenBank accession number
ETH10	D5S3	F- GTTCAGGGACTGGCCCTGCTAACA R- CCTCCAGGCCCACTTTCTCTTCTC	65	206-222	Z22739
BM1818	D23S21	F- AGCTGGGAATATAACCAAAGG R- AGTGCTTTCAAGGTCCATGC	59	253-277	G18391
INRA023	D3S10	F- GAGTAGAGCTACAAGATAAACTTC R- TAACTACAGGTGTTAGATGAACTC	48	201-225	X67830
SPS115	D15	F- AAAGTGAACAACAGCTTCACCAG R- AACCGAGTGTCCTAGTTTGGCTGTG	57	247-261	FJ828565
CSSM66	D14S31	F- AATTTAATGCACTGAGGAGCTTGG R- ACACAAATCCTTTCTGCCAGCTGA	58	177-203	AF232764
TGLA227	D18S1	F- GGAATTCCAAATCTGTTAATTTGCT R- ACAGACAGAAACTCAATGAAAGCA	55	76-104	CM067176
ETH225	D9S2	F- GATCAACCTTGCCACTATTTCCT R- ACATGGACAGCCAGCTGCTACT	58	139-157	Z14043
BM1824	D1S34	F- GAGCCAAGGTGTTTTTCCAATC R- CATTCTCCCAACTGCTTCCTTG	50	176-188	G18394
ETH3	D19S2	F- GAACCTGGCCTCTCCTGCATTGG R- ACTCTGCCCTGTGGCCAAGTAGG	65	100-128	Z22744
TGLA53	D16S3	F- GCTTTCAGAAATAGTTTGCATTCA R- ATCTTCACATGATATTACAGCAGA	51	151-187	KP874730

PCR Amplification

The PCR amplification of STRs primer was made in a final volume of 15 µL containing 6 µL Green Master Mix ($MgCl_2$, dNTPs (dATP, dTTP, dGTP, and dCTP) (Cyntol, Rusia), 0.1 µL primer (forward and reverse), 9 µL nuclease-free $_{dd}H_2O$, and 1 µL DNA sample. All the PCR reactions were performed of STRs loci in a thermal cycler (Biometra, Germany) using cycling conditions as: initial denaturation at 96°C for 8 min, followed by 35 cycles of 30 sec at 94°C, 30 sec at annealing of 45, 50, 55 or 65°C (according to the STRs markers) and 45 sec extensions at 72°C, then final extension at 72°C for 15 min ended the reactions. The PCR amplified products were resolved on 3% denaturing metaphor agarose gels, and allele sizes were estimated using the 11 lines (50-1500 bp) ladder (Life Science Company), and all gels products were analyzed using UV-doc 2.0 software (UVI Tech, Cambridge, UK).

Statistical Analysis

The POPGENE software ver. 1.31(Yeh et al., 1997) was used to calculated the genotype, alleles frequency (A. F.), total number of alleles observed (TNA), effective allele number (Ae), observed (Obs_ Hom) and expected (Exp_ Hom) homozygosity observed (Ho) and expected (He) heterozygosity, HS unbiased gene diversity (Nei), genetic distances, and phylogenetic tree. Also, the GenAlEX software ver. 6.5 (Peakall & Smouse, 2012) was used to calculated Shannon index (I), polymorphic information content (PIC), coefficient of inbreeding (FIS), F-statistics (Fis, Fit and Fst), and gene flow (Nm).

To show the status of populations in terms of genetic bottlenecks, the dataset was tested using I.A.M (Infinite Allele Model), S.M.M (Stepwise Mutation Model) and T.P.M (Two Phase Mutation Model) in Bottleneck software ver. 1.2.02 (Cornuet and Luikart, 1996), Sign, Standardized differences and Wilcoxon tests by 1000 simulations (Piry *et al*., 1999).

STRUCTURE software ve. 2.3.4 (Pritchard *et al*., 2000) was used to identify the genetic structure of the given population and assign individuals to the populations. To estimate the optimal number of clusters (K), was run with K varying from 1 to 10, with ten runs for each K value to determine the real value of K, the case statistic DK was used. The method described by (Evanno *et al.,* 2005) to estimate the most probable value of K for the analyzed data, used the web tool Structure Harvester software ver. 0.6 (Earl and VonHoldt, 2012).

Results & Discussion

All bovine STRs loci in the native cow's population in Babylon province. The allelic frequencies of the 10 STRs markers in the Iraqi native cows are showed in Table 2. The FAO has stated that a minimum of four alleles is important in assessing genetic variation between native cow's populations (FAO, 2011). According to this criterion, all STR markers used showed good polymorphism for assessing genetic variation among local cow populations. The number of alleles were five alleles for ETH10, INRA023 and ETH225, four alleles for BM1818 and BM1824, six alleles for SPS115, and TGLA53, seven alleles for CSSM66, eight alleles for TGLA227, and three alleles for ETH3. Allele size 244 bp of BM1818, 206 bp of ETH10, 180 bp of BM1824, 193 bp of INRA023, 110 bp of ETH3, 151 bp of TGLA53, 252 bp of SPS115, 143 bp of ETH225, 0.285 bp of CSSM66 and 84 bp of TGLA227 had the highest frequency (0.514, 0.535, 0.485, 0.464,

0.414, 0.350, 0.335, 0.314, 0.285, and 0.243, respectively. The STR Markers (SPS115, CSSM66, TGLA227, and TGLA53) had high alleles frequencies information content in this study because their high polymorphism. These results are similar with those of Delgado *et al*., (2012) & Ladyka *et al*., (2019).

Table (2): The allele sizes (A.S), genotypes, alleles (A.), and alleles frequency (A. F.) at different STR loci.

Markers	Chr.	A. S. (bp)	Genotypes (Numbers)	A.	A. F.
			206/ 206 bp (21)	206	**0.514**
ETH10	5	206-222 bp	206/ 210 bp (18)	210	0.186
			210/ 210 bp (4)	216	0.036
			216/ 220 bp (5)	220	0.121
			206/220 bp (12)	222	0.143
			222/ 222bp (10)		
			244/244 bp (20)	244	**0.535**
			244/248bp (13)	248	0.240
BM1818	23	244-276 bp	248/ 248 bp (6)	260	0.150
			248/ 260 bp (9)	276	0.071
			244/260 bp (12)		
			244/276 bp (10)		
INRA023	3	193-227 bp	**193/193 bp (27)**	193	**0.464**
			193/197 bp (11)	197	0.364
			197/197 bp (15)	201	0.050
			201/211 bp (7)	211	0.050
			197/ 227 bp (10)	227	0.072
SPS115	15	246-262 bp	**246/246 bp (18)**	246	0.257
			250/252 bp (10)	250	0.072
			252/252 bp (13)	252	**0.335**
			252/256 bp (11)	256	0.108
			258/258 bp (9)	258	0.128
			256/262 bp (4)	262	0.100
			262/262 bp (5)		
CSSM66	14	177-203 bp	177/177 bp (10)	177	**0.285**
			177/181 bp (5)	181	0.035
			185/185 bp (13)	185	0.251
			177/185 bp (9)	191	0.100
			191/191 bp (7)	193	0.043
			177/193 bp (6)	199	0.171
			199/199 bp (11)	203	0.115
			199/203 bp (2)		
			203/ 203 bp (7)		

			76/ 76 bp (6)	76	0.164
TGLA227	18	76 – 104 bp	76/ 80 bp (11)	80	0.143
			80/ 84 bp (9)	84	**0.243**
			84/ 84 bp (4)	86	0.086
			84/ 86 bp (12)	90	0.114
			90/ 90 bp (8)	94	0.036
			84/ 94 bp (5)	98	0.050
			98/ 104 bp (7)	104	0.164
			104/ 104 bp (8)		
ETH225	9	137-157 bp	**137/ 143 bp (16)**	137	0.200
			143/143 bp (9)	143	**0.314**
			147/147 bp (5)	147	0.157
			137/147 bp (12)	151	0.229
			151/151 bp (4)	157	0.100
			143/151 bp (10)		
			151/157 bp (14)		
BM1824	1	174-186 bp	174/174 bp (8)	174	0.229
			174/180 bp (11)	180	**0.485**
			180/180 bp (18)	184	0.086
			180/ 184 bp (7)	186	0.200
			174/ 184 bp (5)		
			180/186 bp (14)		
			186/ 186 bp (7)		
ETH3	19	98- 130 bp	**98/ 98 bp (15)**	98	0. 286
			98/ 110 bp (10)	100	0.079
			100/ 110 bp (11)	110	**0.414**
			110/ 110 bp (12)	130	0.221
			110/ 130 bp (13)		
			130/ 130 bp (9)		
TGLA53	16	151-189	**151/ 151 bp (18)**	151	**0.350**
			151/ 157 bp (13)	157	0.093
			163/ 163 bp (6)	163	0.143
			163/171 bp (8)	171	0.129
			171/171 bp (5)	183	0.171
			183/183 bp (4)	189	0.114
			183/189 bp (16)		

A total of 53 alleles were identified from 10 STR loci used in the present research. The molecular genetic statistical parameters obtained from the 10 STR loci used are given in Table. 3. The total number of alleles observed (TNA) ranged from 3.00 (ETH3) to 8.00 (TGLA227), and the mean number of alleles being 5.30± 0 .17, but the effective number of alleles (Ae) from 2.69 (ETH3) to 5 .54 (TGLA227) and a mean of 3.89± 0.11 across all STR loci, which showed high genetic polymorphism (Table 3). The means

TNA values obtained were similar to those obtained in Iraq (Al-Jub & Riyadh Hamad senkal, 2023) and in Niger (Grema *et al*., 2017; 7.86) but lower than those obtained in cows breeds from India (Elavarasan *et al*., 2023; 8.28 ± 0.47), in Iraq (Hadi & Alnajm, 2024; 7± 1.56), in Zimbabwe (Gororo *et al*., 2018; 5.167±0.182), and in northeast India (Sharma *et al*., 2013; 4.369±0.898). A higher mean Ae was previously reported for different genetic variation studies (Ndiaye *et al*., 2015; four native cows in Senegal at 6.088; Sharma *et al*., 2020; Indian native cows at 11.423). The Shannon index (I), which is an indicator of the genetic variation of the population or breeds, ranged from 1.05 in the ETH3 to 1.87 in the TGLA227 markers, with a mean of 1.48± 0.28. This result is greater than the value reported by (Bora *et al*., (2023; I= 1.28) using 16 STR loci, and Nwachukwu *et al*., (2022; I= 1.22) using 14 STR markers, and then that estimated by (Rahal *et al*., (2021; I= 1.540) using 3 STRs, and (Hadi & Alnajm, (2024; I= 1.75) using 10 STR markers. Polymorphic information content (PIC) has been described as a statistical assessment of the informativeness of a marker (Botstein *et al*., 1980). The PIC values result in the present research ranged from 0.54 (ETH3) to 0.81 (TGLA227), and a mean of 0.65± 0.15 for all 10 loci. The results show that all STR loci were highly polymorphic, since the STR markers displayed a PIC of more than 0.50. Mean PIC value was similar to the value reported by Ramesha *et al*., (2016; PIC = 0.642), and higher than that (Abdelmanova *et al*., (2020; PIC = 0.61), but lower than that Bigirwa *et al*., (2019; PIC = 0.723), and Bora *et al*., (2023; PIC= 0.70).

Table (3): Genetic parameters measured in the native cows using 10 S TRs loci.

Markers	TNA	Ae	I	PIC
ETH10	4.00	4.73	1.42	0.64
BM1818	4.00	3.39	1.26	0.59
INRA023	5.00	3.32	1.39	0.64
SPS115	6.00	5.46	1.68	0.69
CSSM65	7.00	5.26	1.83	0.73
TGLA227	5.00	5.54	1.87	0.81
ETH225	6.00	3.29	1.39	0.58
BM1824	4.00	3.72	1.31	0.65
ETH3	3.00	2.69	1.05	0.54
TGLA53	6.00	4.89	1.67	0.71
Mean	5.30	3.89	1.48	0.65
SD	0.17	0.11	0.28	0.15

SD: Standard Deviation.

The conservation sufficient variation in cows breeds is necessary for their development and adaptation in the case of global climate change (Boettcher *et al*., 2015). The observed homozygosity (Obs_Hom) and expected homozygosity (Exp_Hom), observed heterozygosity (Obs_ Het) and expected heterozygosity (Exp_ Het), unbiased gene diversity (HS), and coefficient of inbreeding (F_{IS}) all over the individuals all 10 STR markers are presented in Tables 4. The observed homozygosity (Obs_ Hom) values in the native cows ranged from 0.13 (TGLA53) to 0.84 (ETH3) with a mean of 0.43±0.16, but the expected homozygosity (Exp_ Hom) varied from 0.16 (TGLA227) to 0.38 (ETH3), with a mean of 0.24± 0.11. This results a value that was higher to the value reported in (Al-Jub & Riyadh Hamad senkal, 2023; Obs_ Hom = 0.26, and Exp_ Hom =

0.24). The observed heterozygosity (Obs_Het) varied from 0.16 (ETH3) to 0.77 (TGLA53) with a mean of 0.54 ± 0.18, while the expected heterozygosity (Exp_Het) varied from 0.62 (ETH3) to 0.85 (TGLA227), with a mean of 0.75± 0.25. In all populations, the values of Exp_Het did not exceed Obs_ Het. The means heterozygosity (Obs_Het) and (Exp_Het) values were similar to the values reported by (Al-Jub & Riyadh Hamad senkal, (2023; Obs_ Het = 0.054, Exp_Het = 0.75), and higher than that reported by Mukherjee *et al*., (2022; Obs_ Het = 0.54, Exp_Het = 0.74) using 19 STR loci, and Nwachukwu *et al*., (2022; Obs_ Het = 0.352, Exp_Het = 0.605) using 14 STR markers, and lower than Rahal *et al*., (2021; Obs_ Het = 0.78, Exp_Het = 0.84) using 22 STRs. The unbiased gene diversity (H_S) values ranged from 0.60 (ETH3) to 0.83 (TGLA227) with a mean of 0.73±0.23. This results a value that was similar to the value reported in (Bora *et al.,* 2023; HS = 0.73), and higher than (Abdelmanova *et al.,* 2020; HS = 0.71), but lower than (Gororo *et al*., 2018; Nei = 0.756). The observed heterozygosity (Obs_ Het) values in all cows were significantly ($p < 0.05$) small than the unbiased gene diversity (HS), this showed of overall medium heterozygosity and medium to high genetic variation in Iraqi native cows in Babylon city. The medium heterozygosity may be because the non-random mating in native cows with a few larger, better-quality bulls having greater opportunities to mate with females, and a low population size. Results from this research indicate that the native cows have a good level of genetic variation which confirms the observation by Hadi & Alnajm, (2024) that the cows native breeds located in the Middle Al-Furat zone tend to display medium to high values of allelic variation. The F_{IS} ranged from -0.02 (TGLA53) to 0.42 (BM1818) with mean 0.24±0.12. The F_{IS} was negative only in two STR loci, while it was positive in the rest of the markers. All three regions native cows of Babylon province showed significantly positive mean of F_{IS} indicating an increase in inbreeding and low heterozygosity. The F_{IS} positive values obtained were similar to those obtained in Niger (Grema *et al*., 2017), and in India (Peixoto *et al*., 2021; Sharma *et al*., 2023).

Table (4): The homozygosity, heterozygosity, unbiased gene diversity, and coefficient of inbreeding in the studied 10 STR loci of native cows' population.

Markers	Obs_Hom	Exp_Hom	Obs_Het	Exp_Het	HS	FIS
ETH10	0 .44	0 .21	0 .56	0 .78	0 .77	0 .31
BM1818	0 .27	0 .28	0 .73	0 .72	0 .71	0 .42
INRA023	0 .52	0 .25	0 .48	0 .75	0 .73	0 .32
SPS115	0 .39	0 .19	0 .61	0 .80	0 .78	-0 .06
CSSM65	0 .19	0 .19	0 .76	0 .82	0 .79	0 .37
TGLA227	0 .36	0 .16	0 .64	0 .85	0 .83	0 .23
ETH225	0 .41	0 .29	0 .59	0 .71	0 .70	0 .19
BM1824	0 .73	0 .30	0 .27	0 .70	0 .68	0 .40
ETH3	0 .84	0 .38	0 .16	0 .62	0 .60	0 .13
TGLA53	0 .23	0 .20	0 .77	0 .80	0 .79	-0 .02
Mean	0 .43	0 .25	0 .54	0 .75	0 .73	0 .24
SD	0 .16	0 .13	0 .18	0 .25	0 .23	0 .09

SD: Standard Deviation.

Table (5) represent the inbreeding coefficient within populations (Fis), overall inbreeding coefficient (Fit), genetic differentiation coefficient within populations

(Fst), and gene flow (Nm). The Fis values of all marker loci varied from 0.124 (INRA023) to 0.575 (ETH3), with an average value was 0.296 ± 0.065. The Fit varied from 0 .152 (BM1818) to 0 .447 (ETH3), with an average of 0 .314 ± 0 .090 indicating a decrease in heterozygosity across the population (Table. 5). These results indicate that inbreeding is low in the native cow's herds in Babylon city because the nature of mating. The Fst values varied from 0 .012 (BM1824) to 0.056 (SPS115), and the average Fst was 0.032 ± 0.002, indicating decreased genetic differentiation between the subpopulations. This Fst value indicated that 3.2% of the total variance was because unique allelic differentiation between the subpopulation, while the remaining 96.8% corresponded to differences between individuals within the group across the 10 STR loci. The genetic differentiation estimated through Fst revealed low differentiation among the three native cow's populations with a variation of 1.1%. Since the average Fst value obtained is less than 0.05, reflecting low genetic variation in the population. The Fst from 0.05 to 0.3 is typical for differentiating cows breeds. The low value of genetic differentiation native cow's populations in three regions of Babil in Iraq may be attributed to the low selection as compared to cows breeds of developed countries. Therefore, the value obtained for Fst within the range reported by Frankham *et al*. (2002). Obtained Fst values were lower than Vietnamese indigenous cow's populations (Pham *et al*., 2013); Algerian cows breeds (Rahal *et al.,* 2021), and Iraqi cows breeds (Hadi & Alnajm, 2024), while was higher than French cow's populations (Amigues *et al*., 2011), and Zimbabwean Sanga cows breeds (Gororo *et al*., 2018).

Finally, gene flow (Nm) value ranged from 2.834 (ETH10) to 13.113 (ETH225), with a mean of 7.259 ± 2.113. The values implied low gene flow between native cow's populations, it was noted the Obs_ Het was far lower than the Exp_ Het, this is supported by the low gene flow value (Nm) due to inbreeding, the Walunde effect and natural mating. This result is higher than that of Madilindi *et al*., (2019; Nm= 4.37; Mukherjee *et a*l., (2022; Nm= 2.429); Bora *et al*., (2023; Nm= 3.01), but lower than that of Duan *et al*., (2023; Nm= 31.289).

Table (5): F-statistics and gene flow used 10 STR loci in the three cow's populations.

Markers	Fis	Fit	Fst	Nm
ETH10	0.357	0.405	0.059	2.834
BM1818	0.132	0.152	0.031	6.456
INRA023	0.124	0.259	0.016	12.123
SPS115	0.471	0.409	0.056	3.731
CSSM65	0.277	0.343	0.049	4.649
TGLA227	0.421	0.430	0.029	6.758
ETH225	0.249	0.264	0.014	13.113
BM1824	0.175	0.260	0.012	12.113
ETH3	0.575	0.447	0.018	5.908
TGLA53	0.179	0.173	0.045	4.913
Mean	0.296	0.314	0.032	7.259
SD	0.065	0.090	0.002	2.113

SD: Standard Deviation.

The genetic distance obtained in the current study was from 0.780 to 0.882 (Table, 6). The highest distance was observed among cattle populations in the South and North

regions of Babylon. However, the lowest distance was observed among the Middle and North regions (Table. 6). Genetic distance differentiated the farthest South and North, indicating a high degree of differentiation within cows' population because geographical distance. This result is similar with that of Grema *et al*., (2017); using three Nigerian cattle breeds with 27 STR loci); Bora *et al.,* (2023); using three Ethiopian indigenous cattle with 16 STR markers.

Table (6): The genetic distances within cows' population in the Babylon province.

Regions	South	Middle	North
South	0.00	0.862	0.780
Middle	0.174	0.00	0.882
North	0.223	0.105	0.00

Above Diagonal: Genetic Distance, and Below Diagonal: Genetic Identity.

Seventy cows (South, Middle, and North regions) were classified into three clusters by neighbor-joining group analysis based on an unweighted method used the alleles frequencies data of the 10 STR markers (Fig.2). The middle (POP2) and north (POP3) native cows' population tended to cluster together. The South population (POP1) appeared to be relatively distinct from them. The result was expected because the animals are in the same province and there is overlap between the groups. However, it was groups analysis revealed a great deal of genetic variation among the cattle herds because farmers use artificial insemination and different males. Populations collected from South (POP1), Middle (POP2) and North (POP3) in Babylon province had a strong relationship. The Middle (POP2) and North (POP3) cows' population were close to each other and the existence of gene low among the neighboring populations seems possible. These results are similar with those (Grema *et al.,* 2017; Weiwei *et al*., 2018; Ozsensoy *et al.,* 2019; Radhika *et al.,* 2023).

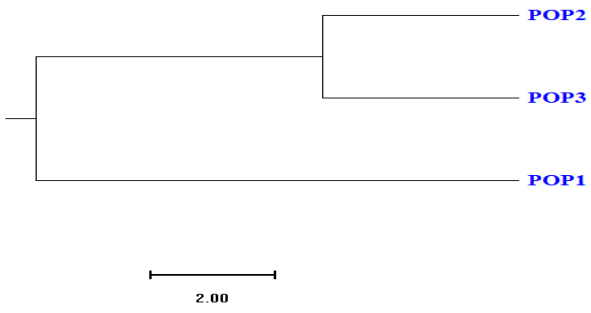

Fig. (2): Neighbors-joining tree between South (POP1), Middle (POP2) and North (POP3) cows' population in Babylon province**.**

The probability values obtained under the three models using three different statistical tests are showed in Table. 7. The expected numbers of STR loci with heterozygosity excess were 6.05, 6.10 and 5.90 in I.A.M, T.P.M and S.M.M, respectively. In analyze of standardized difference test, the hypothesis of mutation-drift equilibrium was rejected for IAM ($p = 0.003$) and TPM ($p = 0.004$) models; under SMM ($p = 0.203$), the results showed no genetic bottleneck effect. In the Sign and Wilcoxon tests, the hypothesis was

not rejected and did not indicate a recent genetic bottleneck. A normal L-shaped distribution of allele frequencies was observed in the indigenous Iraqi cow's herds in Babylon, most probably due to the absence of recent bottleneck effects (Fig.3). Many factors make it difficult to identify bottlenecks, including migration, the length and timing of the bottleneck, the rate at which population size is declining, and the degree of genetic diversity before the bottleneck, all of which can obscure genetic signals of population decline (Garza & Williamson, 2001; Williamson-Natesan, 2005). It is evident that there is a decrease in the population sizes of native cows in Babylon province. However, the results showed that there is no loss of alleles and heterozygotes. We can explain this scenario by the presence of gene flow due to the high used of artificial insemination in these areas (South, Middle and North), which led to the random cross of native cows and thus increased the genetic admixture and led to an increase in the supposed genetic variation before the occurrence of the bottleneck. Among the results that are similar to our results and did not experience any genetic bottleneck test in the recent past are Grema *et al*., (2017); Demir & Balcioglu, (2019); Sharma *et al*., (2020); Manomohan *et al*., (2022).

Table (7): Test results according to three different mutation models for bottleneck analysis in local cows.

Models	Sign test	Standardized differences test	Wilcoxon test
I.A.M	Hee: 6.05 Hd: 2 He: 7 p : 0.263ns	T2 : 2.412 P: 0.003	P: (one tail for H deficiency): 0.829 P: (one tail for H excess): 0.002 P: (two tails for H excess and deficiency): 0.004
T.P.M	Hee: 6.10 Hd: 3 He: 9 p : 0.312ns	T2 : 3.632 p : 0.004	P: (one tail for H deficiency): 0.972 P: (one tail for H excess): 0.006 P: (two tails for H excess and deficiency): 0.003
S.M.M	Hee: 5.90 Hd: 5 He: 6 p: 0.192ns	T2 : 1.320 p : 0.203	P: (one tail for H deficiency): 0.762 P: (one tail for H excess): 0.270 P: (two tails for H excess and deficiency): 0.541

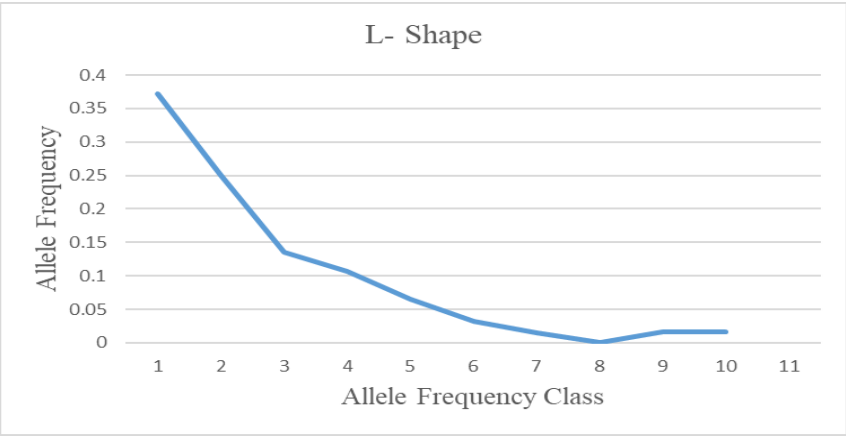

Fig. (3): L-shaped mode-shift showed non-bottleneck test in the Iraqi native cows.

Using a correlated allele frequencies model included into the STRUCTURE software, the genetic population structure of each region was established based on the level of admixture for each individual cows (Fig. 4). The genetic admixture among the cow's population of the three regions was estimated by using the method by Evanno *et al*. (2005). According to Delta K data, K = 3 was the ideal number of genetic clusters to represent the majority of similar all animals (Fig. 4 (A and B)). The Fig. (4. C and D) cluster analysis of 70 animals of three cow's populations, and it was found that there is a high overlap between the study animals because the animals are located in the same area. The results of Bayesian clustering analysis showed that native cows have low admixture. Although the cattle live in the same geographic area, levels of gene flow between the individuals are low. Also, the clustering model showed a relationship between patterns of genetic variation and the geographic origins of the groups.

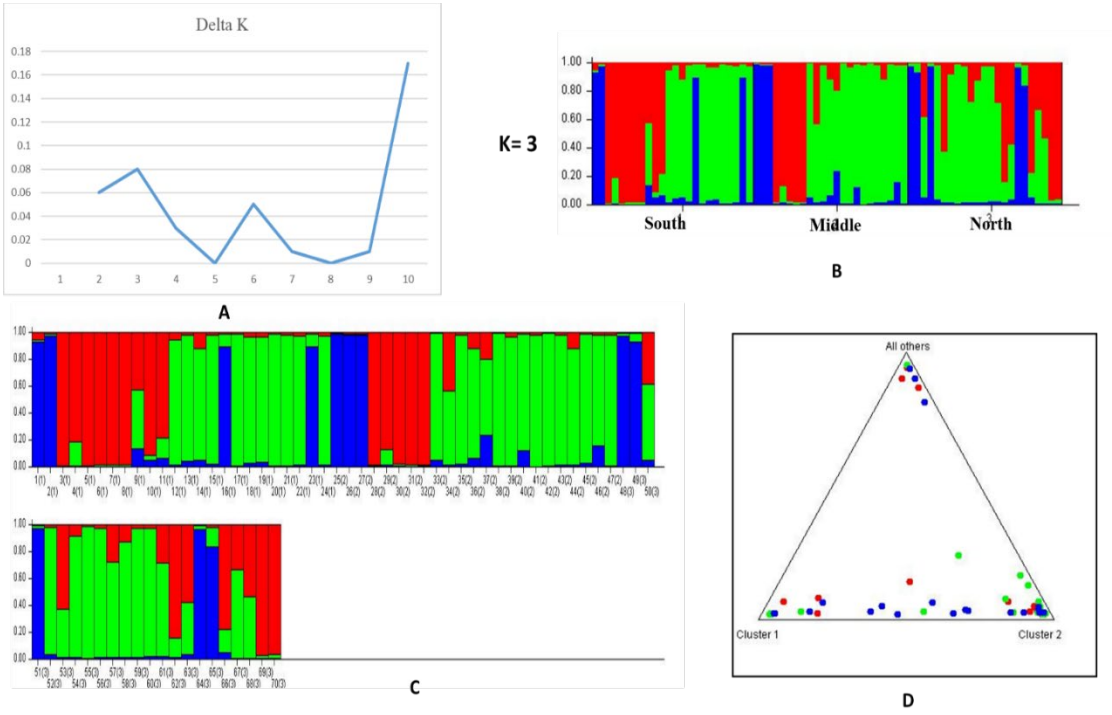

Fig. (4): A. The graph of delta (K) values of Iraqi native cows. **B.** The genetic admixture of three areas. **C.** The cluster analysis of 70 individuals of 3 native cow's populations. **D.** Tree cluster analysis.

Conclusion

The main findings of this study were a high genetic variation within animals, which implied a moderate genetic differentiation between Iraqi native cows in Babylon province. Thus, from the demographic analysis of the bottleneck, we can conclude, conservatively based on three mutation models and a qualitative test for mode shift in Iraqi native cow's populations, that it has not deviated from the mutation-drift equilibrium, indicating no bottleneck test recently. Controlling the pastoral movement and mating system of native cows breeds through effective management and breeding will make it possible to maintain and improve the special economic traits of important breeds in the context of global climate change.

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H.A.: The laboratory work, and statistical analysis of data.

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Conflicts of interest

We as researchers declare that there is no conflict of interest.

Ethical approval

The research was conducted with the permission of the Al-Furat Al-Awsat Technical University Animal Experiments Native Ethics Committee, dated 2/6/2024 and numbered 3480/37/7.

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اختبارات التباين الوراثي وعنق الزجاجة في الأبقار العراقية المحلية في مدينة بابل باستخدام واسمات مكررات المترادفة القصيرة الجسمية (STRs)

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المستخلص:

في الدراسة الحالية، تم تقييم التباين الوراثي وتحليل عنق الزجاجة للأبقار العراقية المحلية في ثلاث مناطق من مدينة بابل. تم تحديد التركيب الوراثي لـ 70 فردًا من ثلاث مجموعات من الابقار باستخدام 10 واسمات ذات تكرار مترادفة قصيرة (STRs) و53 أليلًا مختلفًا. أظهرت مواقع واسمات ذات تكرار مترادفة قصيرة (STRs) المستخدمة تغيرات عديدة من تعدد الأشكال. كانت قيم متوسط العدد المشاهد للأليلات (TNA) والعدد المؤثر للأليلات 0.17 ± 5.30 (Ae) و3.89 ± 0.11 على التوالي. تراوحت TNA على مستوى الواسم من 3.00 (ETH3) إلى 8.00 (TGLA227)، بينما تراوحت Ae من 2.69 (ETH3) إلى 5.54 (TGLA227). تراوح مؤشر شانون (I) على مستوى الواسم من 1.05 (ETH3) إلى 1.87 (TGLA227)، بمتوسط 1.48± 0.28. كما تراوح محتوى معلومات تعدد الأشكال (PIC) من 0.54 (ETH3) إلى 0.81 (TGLA227)، بمتوسط 0.65± 0.15. تراوح الخلط الأليلي المشاهد (Obs_Het) من 0.16 (ETH3) إلى 0.77 (TGLA53) وبلغ متوسطها 0.55 ± 0.11. تراوح الخلط الأليلي المتوقع (Exp_Het) من 0.62 (ETH3) إلى 0.84 (TGLA227)؛ وبلغ متوسطها 0.75± 0.25. بلغ متوسط التنوع الجيني غير المتحيز لـ HS ومعامل التربية الداخلية 0.23±0.73 (FIS) و0.24±0.09 على التوالي. وكان متوسط Fis وFit وFst ومتوسط الهجرة الجينية 0.065±0.296 (Nm) و0.314±0.090 و0.032±0.002 و7.259±2.113 على التوالي. إن تعداد الابقار المحلية ليس به عنق زجاجة، وتوزيع الترددات الأليلية على شكل حرف L طبيعي، ولم يشهد أي انخفاض حديث في حجم السكان الفعال وظل في حالة توازن بين الطفرة والانجراف. وأظهر التحليل البايزي أن جميع الحيوانات كانت غير متجانسة وشكلت ثلاث مجموعات مميزة.

الكلمات المفتاحية: *التباين الوراثي، عنق الزجاجة، الابقار العراقية، واسمات مكررات المترادفة القصيرة.*