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Article

# Genetic Mapping and Diversity of Indigenous and Exotic Rabbits: Adaptive and Conservation Strategies

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## Abstract

**Background:** Climate change threatens global food security, highlighting the need for adaptive traits in livestock to ensure sustainable production. Rabbits, known for their unique adaptability, require the preservation of genetic diversity to maintain resilience. The decline in genetic specificity among indigenous breeds underscores the urgency of conservation efforts to protect these critical resources. **Objectives:** This study investigates the genetic structure and diversity of indigenous rabbit populations, emphasizing genetic mapping as essential for sustaining adaptability. The findings aim to guide breeding programs that enhance biodiversity and support agricultural resilience. **Materials and Methods:** The study analyzed both native and exotic rabbit breeds. Native breeds included Black Baladi (BB), White Baladi (WB), Red Baladi (RB), and Jabali (JAB), while exotic breeds included New Zealand White (NZW), American Rex (AR), and Chinchilla (CH). Fourteen microsatellite loci were genotyped in 526 rabbits across all breeds. **Results:** A total of 467 alleles were identified, with an overall mean of 5.03. Expected heterozygote frequencies were medium to high. Polymorphism was high in BB, JAB, and NZW, and medium in WB, RB, AR, and CH.  $F_{IS}$  and  $F_{IT}$  values (-0.044 and 0.156) suggested possible non-intensive inbreeding.  $F_{ST}$  (0.220) showed breed differentiation and high within-breed variation. Gene flow averaged 1.872, indicating interbreed gene exchange. Neutrality and phylogenetic analyses revealed genetic reshaping; BB, WB, RB, AR, CH, and NZW showed overlap, while JAB retained high specificity. **Conclusion:** Urgent conservation strategies are essential to preserve native rabbit genetic diversity and unique traits, vital for sustaining biodiversity and livestock resilience globally.

**Keywords:** genetic diversity; conservation; microsatellites; phylogenetics; polymorphism

## 1. Introduction

Studying the genetic structure of livestock provides animal geneticists with invaluable information on population identification, polymorphism, uniqueness, and genetic diversity. This knowledge enables breeders to improve animal productivity and design conservation programs for genetically unique populations. Although native genetic resources are often lower in productivity, they harbor high genetic variation that can be harnessed to develop strains adapted to harsh environments and resistance to endemic diseases. Consequently, protecting local breeds from potential threats is a priority in sustainable management efforts [1].

As climates change and new ecological challenges emerge, conserving and identifying genetic diversity within rabbit populations becomes increasingly vital. Preserving such unique genetic resources is crucial globally, supporting adaptation to future ecological changes while ensuring food security and sustaining livestock operations across diverse climates and environments. This study provides a valuable framework for preserving essential genetic traits that contribute to biodiversity and sustainable livestock management. By mapping the genetic diversity and structure of indigenous and exotic rabbit breeds, it offers insights for breeding programs focused on developing resilient breeds that can adapt to specific environmental stressors, such as temperature fluctuations and disease pressures.

On a global scale, this research informs efforts to maintain and enhance the genetic health of rabbit populations, reducing the risks of genetic homogenization and inbreeding. It underscores the importance of genetic resource conservation in supporting resilient ecosystems and sustainable agricultural practices worldwide. By contributing to a broader understanding of genetic diversity in domesticated animals, this study offers essential insights for agricultural and ecological strategies aimed at fostering adaptation and resilience across diverse climates and regions.

The genetic structure of the rabbits in their native distribution range is the result of many factors, from geographical and ecological, to behavioral and molecular, that hierarchically interact through time and space [2]. The genetic studies so far have focused on a small number of rabbit breeds; therefore, patterns of population structure of domestic rabbits remain poorly characterized [3].

Microsatellite genotyping allows the estimation of genetic diversity in the breeds and provides additional information for the design and interpretation of the breeding programs [4,5]. Several microsatellite-specific markers have been used to genotype rabbits and to explain the population structures. A study reported 257 microsatellite alleles in 12 loci in three rabbit populations [6]. The number of alleles ranged from 5 to 16, and the mean  $H_o$  ranged from 0.040 to 0.402. Genotyping Soviet Chinchilla (SC) and Californian White (CW) rabbit breeds revealed 199 alleles in 12 microsatellite loci with high polymorphism, and the breeds were lowly differentiated ( $F_{ST} = 0.04$ ) [5]. Also, genotyping the local rabbit populations in Taiwan revealed a tendency to inbreeding [7].

A study genotyped 17 polymorphic microsatellite loci in the Egyptian native breeds, Black Baladi (BB), Jabali (JAB), Red Baladi (RB) and White Giza (WG) and the exotic line, Spanish-New Zealand White (NZW) [8]. The mean number of alleles per locus was 5.41, and NZW was the population most differentiated. It was reported there was a high homogeneity between the native rabbit breeds [9]. Also, the microsatellite genotyping of the native breeds in Egypt revealed moderate to high polymorphism in all loci [10–12].

In Egypt, the genetic resources of native and exotic breeds are maintained in state farms and research institutes, and most of the native rabbits are sustained by smallholders in the rural regions. Although the native rabbit breeds naturally adapt to the warm climate, they have not been protected by conservation programs. Accordingly, the total number of does and bucks has become quite small, and some breeds face the threats of losing genetic diversity. The objectives of this study were to explore the genetic structures of the native and exotic rabbit resources in Egypt, and to assess the genetic relationship between them.

## 2. Materials and Methods

### 2.1. Experimental Animals

This study was carried out in the Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt. Blood samples were collected, and microsatellite genotyping was subsequently performed.

The study included four most common native rabbit breeds and three exotic breeds in Egypt. The native breeds were Black Baladi (BB), White Baladi (WB), Red Baladi (RB) and Jabali (JAB), and the exotic breeds were New Zealand White (NZW), American Rex (AR) and Chinchilla (CH). The breeds BB, WB and RB have been formed during the 1970s in the Research Institute of Animal Production (RIAP), Ministry of Agriculture, by a crossbreeding program in three phases. In the first phase, the local random-bred Baladi does, that are spread in the rural regions along the Nile valley and Delta, have been mated by Giant Flemish bucks to form the crossbred population (C1). In the second phase, the heavy crossbred in C1 was mated by Giant Flander bucks to produce the crossbred population (C2). In the third phase, the individuals of C2 have been segregated into three genetic groups by color (black, white and red) and called BB, WB and RB, and several generations have been obtained in each group until the breeds have stabilized [13]. The JAB rabbits are spread in the western desert in Northwest of Egypt and Sinai Peninsula in East of Egypt, and they adapt the desert environment. The JAB has been maintained by the Desert Research Institute (DRI), Ministry of Agriculture, in a small population [14]. The breed NZW is maintained in RIAP, and the breeds AR and CH are maintained in small populations in RIAP. All native and exotic breeds are randomly bred in the respective research sites. The number of individuals used in this experiment was 127 (BB), 40 (WB), 40 (RB), 112 (JAB), 87 (NZW), 60 (AR) and 60 (CH).

### 2.2. Experiment Procedures

The blood samples (3mL/individual) were collected from the ear veins of the rabbits in sterilized tubes containing ethylene-di-amine-tetra-acetic-acid (EDTA), and immediately stored at -20°C. Upon use, the blood samples were thawed, and the genomic DNA was extracted from 200ul blood according to the protocol of phenol-chloroform extraction. Extracted DNA samples were first visualized on 1% agarose gel. The DNA concentration was estimated at 260 and 280 nm wavelength using spectrophotometer (PG instruments, Alma Park, wibtoft, Lutter worth LE 175BH, UK). The genome samples were genotyped by 14 microsatellite primers (Table 1) [15–18]. PCR was performed in the thermal cycler (Techne, TC3000, Barloworld Scientific Ltd., Beacon Road Stone, UK), using total volume 25µl of the reaction components. The reaction components included 4µl of genomic DNA (75ng), 2µl of each of the forward and reverse primers (25pmol), 12.5µl of master mix (Bio Basic Inc., Canada) and 4.5µl of PCR-grade water. The PCR program was set at initial denaturation (94°C/5 min), followed by 35 cycles (denaturation at 94°C/40sec, annealing at 54-70°C/40sec and extension at 72°C/40-120sec), and ended by final extension (72°C/10min) and final hold (10sec).

**Table 1.** The molecular information of microsatellite primers.

Microsatellite	Length, bp	Primer sequence	A°	Reference
Sat3	F, 21	5' AAGCAAGTGCTGGCTGTGCTC 3'	60	Mougel <i>et al.</i> (1997) [14] Korstanje <i>et al.</i> (2001) [15]
	R, 20	5' TCCTGCCCTTAGCTACGCAC 3'		
Sol33	F, 20	5'GAAGGCTCTGAGATCTAGAT 3'	55	Surridge <i>et al.</i> (1997) [16] Korstanje <i>et al.</i> (2001) [15]
	R, 24	5'GGGCAATAGGTACTGATCCATGT 3'		
Sol44	F, 20	5'AGGAAGTGAGGGGAGGTGTT 3'	58	Surridge <i>et al.</i> (1997) [16]
	R, 24	5'ATAATGTGCTGCCAAAATAGAAAT 3'		
Sat5	F, 20	5'GCTTCTGGCTTCAACCTGAC 3'	56	Mougel <i>et al.</i> (1997) [14]
	R, 23	5'CTTAGGGTGCAGAATTATAAGAG 3'		
D5Utr4a	F, 24	5'AAAGTGAGCCTGCAGATGAGAGCA 3'	65	Korstanje <i>et al.</i> (2003) [17]
	R, 18	5'GGCGGGGCGGTACAGT 3'		
D5Utr4b	F, 20	5'CAGCGGTAAGAGTGAGAAAC 3'	60	Korstanje <i>et al.</i> (2003) [17]
	R, 19	5'TCCCCATAACAAAAGAGG 3'		

D5Utr4c	F, 19 R, 21	5' GCTCTGGCTCCTGGTTTC 3' 5' AGAGTTCTCCGTCCCTGATGG 3'	60	Korstanje <i>et al.</i> (2003) [17]
D5Utr4d	F, 22 R, 22	5' GCTGCTTGGCTCCTAATGTGT 3' 5' CTTACCGGGAAATCTCTGACCT 3'	60	Korstanje <i>et al.</i> (2003) [17]
D5Utr4e	F, 17 R, 18	5' AGGTGGGTGAGGAGACC 3' 5' TTGTAATCGGCTCACTAT 3'	65	Korstanje <i>et al.</i> (2003) [17]
D5Utr4f	F, 20 R, 19	5' CCAGCTGGTAATAGTAGAGA 3' 5' AAGGCATTTGTGGAGTGAA 3'	60	Korstanje <i>et al.</i> (2003) [17]
D7Utr4a	F, 22 R, 20	5' TGCTAATGTGCCCAGAAAGGTA 3' 5' GGCATCCCAAAGGCAGTAT 3'	60	Korstanje <i>et al.</i> (2003) [17]
D7Utr4b	F, 20 R, 17	5' TAGGCATTTAGGGAGTGAAC 3' 5' GGAGGGGGATGGTAGAG 3'	60	Korstanje <i>et al.</i> (2003) [17]
D19Utr4a	F, 22 R, 23	5' CGACCGTGGGCTCAGAAGAA 3' 5' TGTATGTGGGTGTGGGTGTAGAG 3'	70	Korstanje <i>et al.</i> (2003) [17]
D19Utr4b	F, 23 R, 23	5' TGTATGTGGGTGTGGGTGTAGAG 3' 5' TACTGTGTCTGCTGGGATTTTA 3'	60	Korstanje <i>et al.</i> (2003) [17]

A<sup>\*</sup> = annealing temperature.

The amplified PCR-products were separated using 8% non-denatured PAGE. The polyacrylamide gel was prepared (12.8ml of 30% acrylamide solution, 25.6ml of ddH<sub>2</sub>O, 800µl of 10%APS, 40µl of TEMED, 9.6ml of 5X-TBE), and 10ul of each PCR product was loaded into wells. A 50-bp ladder (GeneDireX, 50-bp DNA ladder RTU) was also loaded to determine the lengths (bp) of the amplified fragments. Electrophoresis was run at 100v for one hour or until the lower dye escaped from the gel. The gel was submerged in ethidium bromide (Ei.Br) staining solution (0.5mg ml Ei.Br in 100ml dH<sub>2</sub>O) for 5-10 min at room temperature. The PCR products of the breeds were visualized and photographed using the WGD-30 WiseDoc Gel Documentation (Daihan Scientific, Co, Ltd., 244, Sangwolgok-Dong, Sungbuk-Kit, Seoul, Korea).

### 2.3. Statistical Analysis

The images were analyzed using TotalLab [19] and the observed number of alleles (N<sub>o</sub>), effective number of alleles (N<sub>e</sub>), observed heterozygosity (H<sub>o</sub>), expected heterozygosity (H<sub>e</sub>) and polymorphic information content (PIC) were obtained [20,21]. The F-statistics analysis and the neutrality D value were estimated [22,23]. The images of loci Sat3, Sol33, D5Utr4c and D7Utr4a were randomly chosen to draw the phylogenetic charts using TotalLab [19]. The phylogenetic analysis is constructed on a locus basis, so that it can be used with neutrality results to explore the causes of the genetic changes in the breeds during evolution.

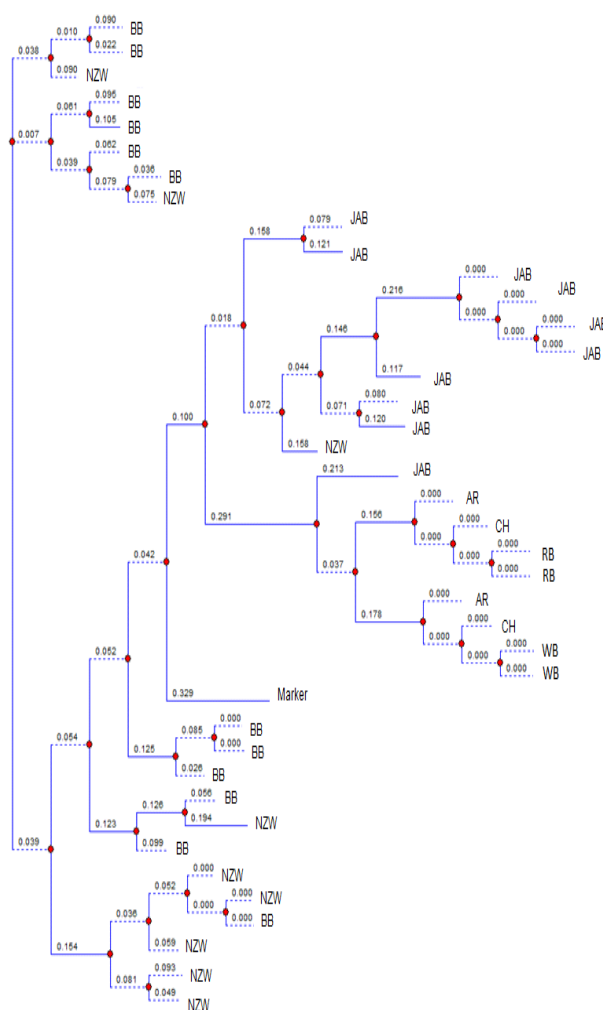
## 3. Results

Most of the microsatellite loci were multi-allelic. A total of 467 alleles were recognized in all breeds, and the overall mean was 5.03 alleles /locus/breed. Table 2 presents a summary for the allele information in different microsatellite loci. Mean N<sub>o</sub> ranged from 2.4 in AR to 9.7 in BB and NZW, and the N<sub>e</sub> ranged from 2.3 in AR to 7.7 in NZW. The mean difference between N<sub>o</sub> and N<sub>e</sub> was large in BB (2.3), JAB (1.1) and NZW (2.0) and small in WB (0.2), RB (0.1), AR (0.1) and CH (0.2). The mean H<sub>o</sub> ranged from 0.52 in WB and RB to 0.83 in NZW, and the mean H<sub>e</sub> ranged from 0.46 in AR to 0.82 in NZW. The difference between H<sub>o</sub> and H<sub>e</sub> ranged from 0.01 in WB, RB and NZW to 0.26 in AR. The polymorphism was high (PIC > 0.50) in NZW, BB and JAB, and was medium (0.25 < PIC < 0.50) in WB, RB, AR and CH. The mean inbreeding coefficients (F) were nearly zero in BB (-0.004), WB (+0.030), RB (+0.015), JAB (+0.005) and NZW (-0.044), respectively. The inbreeding was evident in AR (F = -0.442) and CH (F = -0.284).

The inbreeding index (F<sub>IS</sub>) in different loci was mostly negative (Table 3), and the mean F<sub>IS</sub> was nearly zero (-0.044). The variation index (F<sub>IT</sub>) ranged from -0.173 to 0.908. The mean F<sub>IT</sub> was 0.156 and indicated that the individual variation in rabbit populations accounted for approximately 16%. The mean differentiation index (F<sub>ST</sub>) was 0.220 and indicated high diversification (22%) between the



**Figure 1.** The phylogenetic analysis of the breeds at the microsatellite locus Sat3. BB, WB, RB, JAB, NZW, AR and CH express the breeds Black Baladi, White Baladi, Red Baladi, Jabali, New Zealand White, American Rex and Chinchilla.

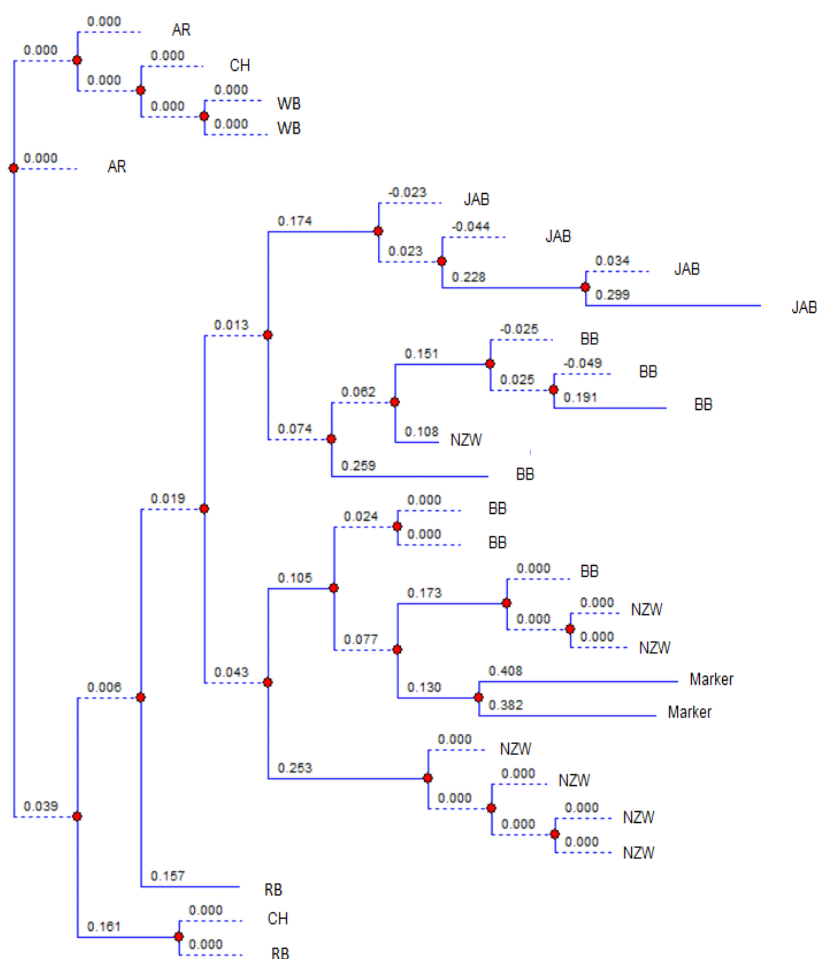


**Figure 2.** The phylogenetic analysis of the breeds at the microsatellite locus Sol33. BB, WB, RB, JAB, NZW, AR and CH express the breeds Black Baladi, White Baladi, Red Baladi, Jabali, New Zealand White, American Rex and Chinchilla.

**Table 2.** Summary of the microsatellite allele information (mean  $\pm$  SE) within rabbit breeds.

Breed	Allele number		Heterozygosity		PIC	F
	No	Ne	Ho	He		
Black Baladi	9.7 $\pm$ 1.2	7.4 $\pm$ 1.0	0.82 $\pm$ 0.08	0.80 $\pm$ 0.04	0.69 $\pm$ 0.06	- 0.004
White Baladi	2.9 $\pm$ 0.6	2.7 $\pm$ 0.5	0.52 $\pm$ 0.08	0.53 $\pm$ 0.06	0.45 $\pm$ 0.06	+ 0.030
Red Baladi	2.6 $\pm$ 0.4	2.5 $\pm$ 0.3	0.52 $\pm$ 0.12	0.51 $\pm$ 0.07	0.43 $\pm$ 0.06	+ 0.015
Jabali	5.3 $\pm$ 1.1	4.2 $\pm$ 0.9	0.75 $\pm$ 0.10	0.69 $\pm$ 0.05	0.63 $\pm$ 0.06	+ 0.005
New Zealand White	9.7 $\pm$ 1.3	7.7 $\pm$ 1.0	0.83 $\pm$ 0.06	0.82 $\pm$ 0.04	0.73 $\pm$ 0.06	- 0.044
American Rex	2.4 $\pm$ 0.3	2.3 $\pm$ 0.3	0.72 $\pm$ 0.11	0.46 $\pm$ 0.07	0.39 $\pm$ 0.07	- 0.442
Chinchilla	2.6 $\pm$ 0.4	2.4 $\pm$ 0.3	0.61 $\pm$ 0.11	0.47 $\pm$ 0.07	0.40 $\pm$ 0.07	- 0.284

No = number of observed alleles, Ne = effective number of alleles, Ho and He = observed and expected heterozygosity, PIC = polymorphic information content, F = inbreeding coefficient.

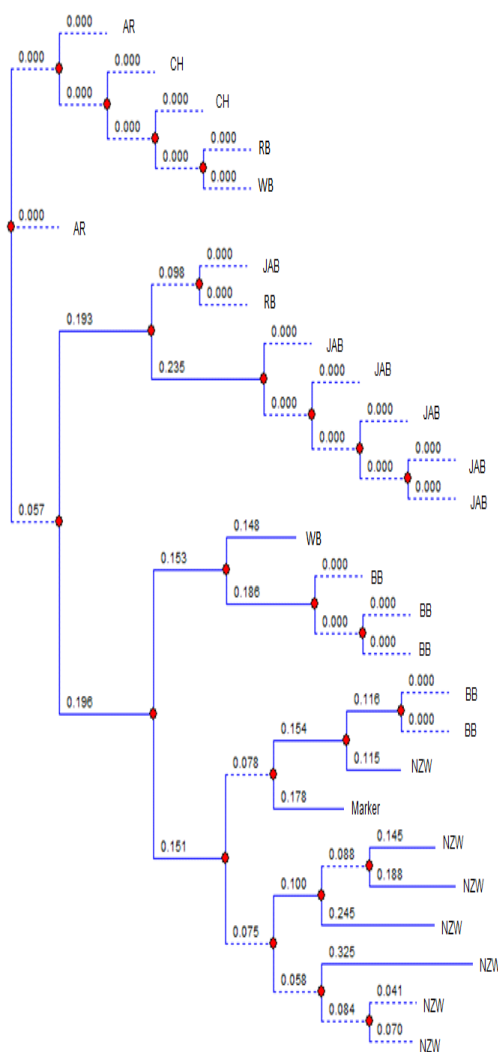


**Figure 3.** The phylogenetic analysis of the breeds at the microsatellite locus D5Utr4c. BB, WB, RB, JAB, NZW, AR and CH express the breeds Black Baladi, White Baladi, Red Baladi, Jabali, New Zealand White, American Rex and Chinchilla.

**Table 3.** The F-Statistics Analysis for the variation within and between rabbit breeds and the rate of gene flow ( $N_m$ ).

Microsatellite	$F_{is}$	$F_{IT}$	$F_{ST}$	$N_m$
Sat3	0.132	0.337	0.237	0.805
Sol33	0.018	0.232	0.217	0.902
Sol44	-0.079	-0.027	0.048	4.958
Sat5	0.027	0.090	0.065	3.596
D5Utr4a	-0.420	-0.103	0.223	0.871
D5Utr4b	0.665	0.908	0.725	0.095
D5Utr4c	-0.155	0.166	0.278	0.649
D5Utr4d	-0.693	-0.116	0.341	0.483
D5Utr4e	-0.135	-0.095	0.035	6.893
D5Utr4f	-0.042	0.054	0.092	2.467
D7Utr4a	0.048	0.299	0.264	0.697
D7Utr4b	-0.176	-0.173	0.002	---
D19Utr4a	-0.191	-0.026	0.138	1.562
D19Utr4b	0.383	0.635	0.408	0.363

Mean ± SE	- 0.044±0.086	0.156±0.083	0.220±0.051	1.872±0.574
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**Figure 4.** The phylogenetic analysis of the breeds at the microsatellite locus D7Utr4a. BB, WB, RB, JAB, NZW, AR and CH express the breeds Black Baladi, White Baladi, Red Baladi, Jabali, New Zealand White, American Rex and Chinchilla.

**Table 4.** Levels of neutrality in the rabbit breeds.

	BB	WB	RB	JAB	NZW	AR	CH
Sat3	0.842	- 0.333	- 1.000	- 0.453	1.894	.364	0.000
Sol33	1.770	- 0.333	- 1.000	.119	.494	0.000	0.667
Sol44	.059	- 0.636	1.080	- 0.791	0.273	- 1.636	- 1.636
Sat5	.970	- 1.636	- 0.448	- 0.227	1.207	- 1.636	- 1.636
D5Utr4a	0.794	- 1.333	- 1.333	- 1.386	.767	- 1.333	- 1.333
D5Utr4b	- 0.847	- 1.000	- 1.000	---	- 0.229	- 1.000	- 1.000
D5Utr4c	.755	- 1.333	- 1.636	- 1.333	- 0.099	- 1.333	- 0.636
D5Utr4d	- 1.006	- 1.333	- 1.333	---	- 0.606	- 1.333	- 1.333
D5Utr4e	2.413	- 0.636	- 0.636	---	2.545	- 1.333	- 0.636
D5Utr4f	.896	- 2.181	---	---	2.335	- 2.944	1.551
D7Utr4a	- 0.144	- 0.333	.667	- 1.152	0.351	- 1.333	- 0.333
D7Utr4b	0.807	- 1.449	- 0.190	1.054	0.028	- 2.190	.810
D19Utr4a	0.912	- 1.333	- 1.333	0.571	1.912	0.667	- 1.333
D19Utr4b	- 0.301	0.667	0.000	---	- 0.053	- 1.000	- 1.000
Mean	0.709	- 0.943	- 0.628	- 0.400	0.844	- 1.074	- 0.489

SE	0.267	0.193	.228	0.270	0.278	0.295	0.300
BB, WB, RB, JAB, NZW, AR and CH express the breeds Black Baladi, White Baladi, Red Baladi, Jabali, New Zealand White, American Rex and Chinchilla.							

#### 4. Discussion

The number of alleles expresses the allele diversity in the breeds. The  $N_o$  and  $N_e$  and the difference between them were high in BB, JAB and NZW, and were small in WB, RB, AR and CH. The results reveal the allele richness in BB, JAB and NZW. The values of  $H_e$  and PIC tended to have similar patterns in the breeds, being medium to high, and were higher in BB, JAB and NZW than in the other breeds. These results signify the relationship between population size and allele diversity. In BB and NZW, the difference between  $N_o$  and  $N_e$  was high and denoted possible changes in the genetic composition. In WB, RB, AR and CH, low allele diversity was possible since they have been retained in small sizes and restocked by random breeding.

The results are in close agreement with the results obtained in different native and exotic breeds in Egypt and elsewhere. In China, seven native and exotic breeds were genotyped and resulted in 151 alleles in 15 microsatellite loci [24]. The mean  $N_o$  and  $N_e$  were 10.07 and 6.625 alleles per locus, respectively. The mean  $H_e$  ranged from 0.161 in a native breed to 0.889 in an exotic breed, and the mean PIC ranged from 0.625 in a native breed to 0.796 in an exotic breed and indicated high genetic diversity. Number of 17 microsatellites were used to genotype the Egyptian native breeds BB, JAB, RB and White Giza (WG) and the exotic line Spanish-New Zealand White (NZW) [8]. The mean number of alleles per locus was 5.41. The mean  $H_o$  was 0.527 and ranged from 0.477 in NZW to 0.581 in WG. A study reported moderate polymorphism ( $0.25 < \text{PIC} < 0.50$ ) in European and NZW breeds [25]. In earlier study [11], the mean  $N_o$  was 2.71, 2.29, 2.64, 2.43 and 2.00 in WB, RB, AR, CH and NZW, respectively. Also, genotyping 12 microsatellite loci in Soviet Chinchilla (SC) and Californian White (CW) breeds yielded 199 alleles [5]. The number of alleles ranged from 4 to 11 in SC and 6 to 10 in CW. In Taiwan, five rabbit populations representing different regions were genotyped by 18 microsatellites [7]. The average values of  $N_o$ ,  $N_e$ ,  $H_o$  and  $H_e$  were 5.50, 2.437, 0.442 and 0.568 respectively, and revealed the inbreeding impact on the local rabbits in Taiwan. Our results also coincide with earlier research on Tunisian indigenous rabbit populations, which reported high genetic diversity ( $H_o = 0.3 - 0.5$ ), and abundant genetic variation in Tunisian rabbits (Ben Larbi et al., 2014). Additionally, our findings align with prior research such as [26], who described similar genetic structuring in Egyptian Delta rabbits.

It was reported that if the  $F_{ST}$  exceeds 0.15, it suggests a high degree of differentiation [27]. The results in our study provided evidence for breed differentiation ( $F_{ST} = 0.220$ ). It also indicated that most of the variation is within breeds. The results of  $F_{IS}$  and  $F_{IT}$  denoted to possible inbreeding history of the breeds. The  $F$  values suggested that WB, RB and JAB have experienced non-intensive inbreeding. The rate of gene flow ( $N_m$ ) between the breeds revealed that they have possibly practiced gene exchange by disassortative mating. A significant differentiation ( $F_{ST} = 0.11$ ) between Tunisian indigenous rabbit populations was previously reported [28]. In China, the native and exotic rabbit breeds had mean  $F_{ST}$  of 0.099 indicating barely fair differentiation between the breeds (10%), and  $N_m$  was high (0.818 to 6.031) denoting to possible gene exchange between the breeds [28]. In Taiwan, the  $F_{ST}$  between the native rabbit populations was 0.232, and indicated remarkable genetic diversity between the breeds [7]. The Egyptian native breeds BB, JAB, RB and White Giza (WG), and the exotic breed NZW had inbreeding coefficient ( $F_{IT}$ ) of 0.279, and the heterozygote deficit within populations ( $F$ ) ranged from 0.045 in NZW to 0.266 in BB [8]. The  $F_{ST}$  values indicated that NZW was the most differentiated population ( $F = 0.194$ ). Galal *et al.* reported low genetic variation in Egyptian native breeds [29]. In a study of the history of rabbit domestication, the rabbits have been diversified into well-defined breeds with differentiation coefficient ( $F_{ST}$ ) of 22% [3].

The neutrality test signifies the past and current genetic state of a population, in term of random versus non-random evolution. The distribution of  $D$  values is naturally conservative, and so the great deviation from the mean  $D$  expresses the power of  $D$  values in response to the genetic changes in a

population [22,30]. In this study, the great deviations of D values, in some loci, from the breed overall means reflect significant evolution events in the breeds. The breed BB had been split from a crossbred population and had not experienced selection. Similarly, NZW is randomly bred over subsequent generations, with no evidence of a breeding history. Therefore, the D values in BB and NZW denote to the allele richness, with intermediate frequencies for most of the alleles and the overlapping between BB and NZW was due to disassortative mating. The breeds WB, RB, AR and CH exhibited negative mean D values, denoting that they have possibly endured genetic drift to evolve to the modern breeds and the overlapping between them was due to disassortative mating. The breed JAB was initiated from small population, and it had possibly undergone founder effect to evolve in a separate genetic branch, and therefore it is highly conserved.

The analysis of the D values and the phylogenetic trees reveals the extent of the breed specificity. The JAB rabbits formed a unique and stable breed. There was overlapping between all the breeds, except JAB. The BB, WB, RB, NZW, AR and CH lacked genetic specificity, due to the gene exchange between them. Earlier studies reported genetic mixtures among the native and exotic rabbit breeds in Egypt [8,31]. Also, the genetic distances between WB and RB, NZW, AR and CH were estimated [11]. The shortest distance was between WB and RB (1.77), and the farthest was between NZW and AR (2.13). The results revealed that CH was genetically the closest to WB and RB.

## 5. Conclusions

The native rabbit breeds possess high genetic variation, making them highly diverse. However, the retention of these breeds in small flocks has led to inbreeding and gene exchange, resulting in a decline in their genetic specificity. This study underscores the urgent need for sustainable conservation effort to preserve these valuable rabbit resources, which are important for maintaining genetic diversity and adaptability—qualities that hold global significance in fostering resilient ecosystems, biodiversity, and sustainable agricultural practices worldwide.

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**Data Availability:** Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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## Abbreviations

The following abbreviations are used in this manuscript:

BB	Black Baladi
WB	White Baladi
RB	Red Baladi
JAB	Jabali
NZW	New Zealand White
AR	American Rex
CH	Chinchilla
RIAP	Research Institute of Animal Production
DRI	Desert Research Institute
H <sub>o</sub>	Observed Heterozygosity
H <sub>e</sub>	Expected heterozygosity
PIC	Polymorphic information content
F <sub>IS</sub>	Inbreeding index
F <sub>IT</sub>	Variation index
F <sub>ST</sub>	Differentiation index
C1	First Crossbred Population (Baladi × Giant Flemish)
C2	Second Crossbred Population (C1 × Giant Flander)

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