Estimating the Genetic Situation of Native Upper Egypt Subpopulations of Rabbits Using Microsatellite Markers

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ABSTRAK

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Penelitian ini bertujuan untuk mengeksplorasi keragaman genetik pada empat subpopulasi kelinci asli Mesir Hulu menggunakan penanda mikrosatelit. Sebanyak 247 sampel biologis dikumpulkan dari individu subpopulasi kelinci asli Mesir Hulu (NUER) yang tidak berkerabat di 77 pedesaan dan dilakukan pencirian genotipe melalui 31 lokus mikrosatelit. Empat ratus sembilan puluh enam alel tercatat di antara 4 subpopulasi NUER dengan sekitar 43% tercatat sebagai alel privat. Subpopulasi Luxor menunjukkan nilai rata-rata jumlah alel terbesar adalah 19,012, kekayaan alel adalah 8,009, dan alel privat adalah 133. Nilai negatif dari koefisien *inbreeding* tercatat di Qena dan Luxor (masing-masing -0,084 dan -0,134) Tentang 45% lokus memberikan kandungan informasi polimorfik tinggi (PIC) dan 58% tidak signifikan dalam keseimbangan Hardy –Weinberg (HWE). Tumpang tindih antara Asyut dan Sohag tampak pada analisis diskriminan komponen utama (DAPC). Secara umum, kami menyimpulkan bahwa klasifikasi ditemukan menurut arah geografis pada subpopulasi selatan (Qena dan Luxor) dan utara (Asyut dan Sohag). Kecuali itu, subpopulasi selatan (Qena dan Luxor) menunjukkan variasi genetik yang tinggi. Penelitian ini dapat digunakan sebagai dokumen pendukung bagi para peneliti di bidang peternakan kelinci dan pertanian kelinci di tingkat nasional dan daerah.

Kata Kunci: Keragaman Genetik, Kelinci Lokal, Mesir Hulu, Mikrosatelit

ABSTRACT

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This study aimed to explore genetic diversity in four native upper Egypt subpopulations of rabbits using microsatellite markers. A total of 247 biological samples were collected from unrelated individuals of native Upper Egypt rabbit (NUER) subpopulations across 77 rural villages and were genotyped via 31 microsatellite loci. Four hundred ninety-six alleles were recorded among the 4 NUER subpopulations, with about 43% being private. Luxor's subpopulation exhibited the most significant values of the mean number of alleles, which was 19.012, allelic richness was 8.009, and private alleles were 133. The negative values of the inbreeding coefficient were recorded in Qena and Luxor ($^{-}$0.084 and $^{-}$0.134, respectively). About 45% of loci gave highly polymorphic information content (PIC), and 58% were insignificant in Hardy –Weinberg equilibrium (HWE). The overlapping between Asyut and Sohag has appeared in the discriminant analysis of principal components (DAPC). Generally, we concluded that the classification is based on geographical directions to southern subpopulations (Qena and Luxor) and northern (Asyut and Sohag). Except that, the southern subpopulations (Qena and Luxor) showed high genetic variation. This study could be used as supporting documents for researchers in rabbit breeding and agriculture at national and regional levels.

Key Words: Genetic Diversity, Native Rabbits, Upper Egypt, Microsatellite

INTRODUCTION

Local farm animal breeds play an essential role in sustainable agriculture for rural and fragile societies in developing countries (Datta et al. 2024). They have adapted throughout generations to abiotic stresses such as endemic illnesses and parasites, the capacity to survive extended periods of feed and water scarcity, and tolerance to heat stress (Mapiye et al. 2019). Egyptian native rabbits are one of the essential local farm animal breeds widely distributed in rural areas for self-sufficiency under the backyard familial system (Mostafa et al. 2020; Youssef et al. 2021). Genetic maintenance generating and amelioration strategies for Egyptian rabbit genetic resources may benefit from this data in the future (Allam et al. 2024). Limited studies were conducted to investigate genetic variability deeply for farm animals in the Upper Egypt strip, characterized by a hot and dry climate (Galal 2021).

Genetic markers are considered the best methods for genetic evaluation and structure differentiation in codominant and highly polymorphic livestock

(Loukovitis et al. 2023). Microsatellites, as genetic markers, are considered simple sequence repeats, short tandem repeats, and simple sequence length polymorphisms (Yadav et al. 2024). They are widespread and have become a requirement for farm animals' genetic evaluation, which could contribute to achieving food security and protein basket variability (Kasarda et al. 2020). Several factors are affected by genetic variation, like genetic drift, migration, mutation, and selection (Kardos et al. 2021).

Several genetic markers were used in genetic studies of rabbits, such as Sequence-related amplified polymorphism (SRAP) (Mohamed & Abdelfattah 2018), mitochondrial DNA (mtDNA) (Emam et al. 2020); simple sequence repeat (Adeolu et al. 2021) and single-nucleotide polymorphisms (Ballan et al. 2022). Microsatellites have been efficiently used in the evaluation of the genetic status of commercial lines rabbits (Jochová et al. 2017; Omotoso et al. 2019; Adeolu et al. 2021), and native rabbit populations in North African countries (Ben Larbi et al. 2014; s

MATERIALS AND METHODS

Ethics approval

This study followed the guidelines set via The Institutional Animal Care & Use Committee, IACUC; Menoufia University (IACUC reference № is MUFAG/F/AP/04/23).

Samples collection

Seventy-seven rural villages belonging to Upper Egypt governorates (Asyut, Sohag, Qena, and Luxor) were surveyed to collect 247 biological samples (hair bulbs and tissues) of native rabbits (Figure 1; Table 1). Samples were collected from January 2022 to March 2023. Every governorate was regarded as a subpopulation. The rabbits in the research were unrelated; if they were, they came from just one parent (buck or dosage), and the offspring were only evaluated as one individual. Rabbit hair samples were plucked with a bulb and maintained in tiny plastic bags, while tissue samples were kept in Eppendorf tubes containing 95% ethanol.

Laboratory procedures

The DNA extraction was conducted using an alkaline lysis protocol for the hair bulb and tissue samples (Cinelli et al. 2007). Initially, the quality of DNA was assessed by 0.8% agarose gel. After passing the quality control step, 31 microsatellite loci (Invitrogen, France) for rabbit biodiversity were studied on the purified DNA. The PCR reaction was performed on 5 multiplexes according to the PanelPlex Software (Ann Arbor, MI, USA). The condition of PCR for each multiplex is shown in Table 2. The quality of PCR products was then checked using agarose gel (2%). The sizes of the fragments were determined using the genetic analyzer (ABI PRISM 3730 XL; Applied Biosystems, Foster City, CA, USA). Genotyping was read by GeneMapper® Software 5 (Applied Biosystems, Foster City, CA, USA).

Data analysis

The GENAIEX 6.4.1 program (Peakall & Smouse 2006) calculated the following: analysis for each subpopulation and locus; analysis for each subpopulation and locus; mean number of observed alleles (MNa) for each subpopulation; the number of alleles for each locus (Na); the number of private alleles (Pa) for each locus; and observed and expected heterozygosity (H_o and H_e) for each subpopulation and locus. In addition, the percentage of molecular variance was estimated by the previous program. The values of allelic richness (Ar), inbreeding coefficient among populations and loci (F_{IS}), pairwise genetic differentiation among populations (F_{ST}), and reduction in heterozygosity due to inbreeding for each locus (F_{IT}) were calculated using the FSTAT 2.9.3.2 program (Goudet 2002). The program of Cervus 3.0.6 was used to calculate the polymorphic information content (*PIC*) and Hardy-Weinberg equilibrium (HWE) for each locus (Kalinowski et al. 2007). The ape (version 3.5) was used to achieve the neighbor-joining (NJ) tree, and the Adegenet Package (version 1.3-5) was used to achieve the discriminant analysis of principal components (DAPC) via the R program (R Core Development Team 2008). The STRUCTURE program 2.3.4 (Pritchard et al. 2000) was used to estimate the population structure by using an analysis of Bayesian clustering. The estimation was based on independent runs with 100000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 50000 steps, adhering to the rule of $1 \leq K \leq 8$ (K= number of assumed clusters). The ΔK statisticas was calculated by Evanno et al. (2005) method.

RESULTS AND DISCUSSION

Genetic variability among 4 NUER subpopulations

The genetic variability among the 4 NUER subpopulations (Asyut, Sohag, Qena, and Luxor) is presented in Table 3. The results were characterized by a superior value of MNa (15.999). This value was higher than the MNa of the European domestic rabbits (MNa=3.136) (Alves et al. 2015) and some commercial rabbit lines in Egypt (MNa= 4.980) (El-Aksher et al. 2017). Moreover, more MNa was found in Luxor and Qena than in others. It is an essential indicator of genetic

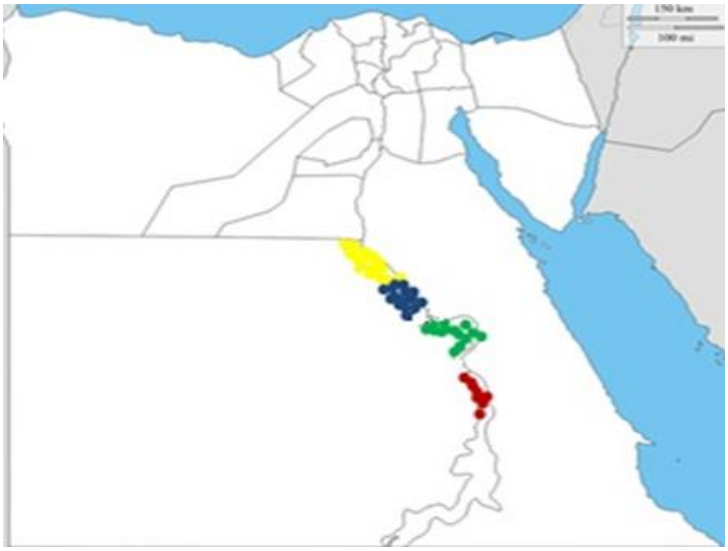

Figure 1. Locations of samples for the four subpopulations are Asyut (*yellow*), Sohag (*blue*), Qena (*green*), and Luxor (*red*)

Table 1. Biological samples geographical location

Geographical coordinates	Village	Center	Governorate
Asyut	Dairut	El Matawaa	27°34'56"N 30°50'49"E
		El sharaqwaa Bahary	27°34'54"N 30°45'46"E
		Dashloot	27°33'51"N 30°42'18"E
	El Ghanayem	El Amry	26°56'66"N 31°47'21"E
		Al Azayzaa	26°54'51"N 31°32'20"E
		Deer El Janadlaa	26°56'57"N 31°35'17"E
	El Qusiaa	Tanaghaa	27°12'27"N 30°30'31"E
		Aramya El Khudary	27°30'45"N 30°31'27"E
		Deer El Qaseer	27°30'55"N 30°31'42"E
		El Saraqnaa	27°24'21"N 30°46'49"E
		Elshiekh Dawood	27°26'24"N 30°49'37"E
	Abnun	Al Sawalem	27°14'41"N 31°09'32"E
		Nazlet Qadaieh	27°20'19"N 31°09'36"E
		Alma'abda	27°19'48"N 31°02'24"E
		Bani Mahmediat	27°19'09"N 31°03'36"E
		Deer sho	27°17'18"N 31°08'49"E
	Manfalout	El Hawatka	27°15'31"N 31°06'11"E
		Hamma	27°17'38"N 30°56'09"E
		Bani Shaquier	27°20'51"N 30°44'11"E
		Bani Magraa	27°16'45"N 30°56'14"E
		Arab El Amaiem	27°17'28"N 30°53'35"E
	Asyut	Awlad Ebrahim	27°09'08"N 31°13'16"E
		El Hasanni	27°15'08"N 31°03'25"E
		Alnamayssah	27°09'02"N 31°14'56"E

Geographical coordinates	Village	Center	Governorate
		Manqabaad	27°12'09"N 30°06'40"E
		Alwaan	27°12'44"N 30°02'32"E
	El Fatteh	El Kalabaat	27°11'42"N 31°18'35"E
		Deer Basra	27°09'34"N 31°16'59"E
		Jazereet Alakraad	27°12'04"N 31°08'21"E
		Taal Awlaad Serraj	27°10'56"N 31°13'47"E
	Abu Tij	Abu Kherss	27°00'09"N 31°14'32"E
		Baquraa	27°06'02"N 31°17'30"E
		Dakraan	26°57'52"N 31°17'31"E
	Sahel Saleem	El Afardaa	27°02'39"N 31°22'39"E
		Deer Tassa	27°02'02"N 31°24'16"E
		El Zarabbii	26°58'49"N 31°16'02"E
	Badary	Hamameyaa	26°55'20"N 31°28'47"E
		Temmaa	26°56'34"N 31°26'08"E
	Sidfa	Koom Abu Hajer	26°55'01"N 31°31'21"E
		Magries	26°58'46"N 31°22'16"E
		Bani Feez	26°57'04"N 31°27'23"E
Sohag	Tima	El Aghanaa	26°51'02"N 31°20'31"E
		El Hadiqaa	26°51'41"N 31°23'45"E
		Mashtaa	26°51'57"N 31°28'39"E
	Tahta	Banjaa	26°48'22"N 31°28'51"E
		Nazlet Alqadii	26°45'10"N 31°24'52"E
	Juhaynah	El Tolayhat	26°44'16"N 31°27'43"E
		Nazzah	26°42'19"N 31°28'55"E
		El Harafshaa	26°43'57"N 31°28'41"E
		Nazlet Ali	26°43'17"N 31°25'05"E
	Saqultah	El Galawyah	26°45'34"N 31°27'23"E
		El Faraseyah	26°42'44"N 31°37'21"E
		Bani Wasel	26°39'16"N 31°41'56"E
		Na'ge Hammed	26°57'29"N 31°41'32"E
	Al Maraghah	Eqsas	26°40'52"N 31°37'04"E
		Al Hariedyah	26°42'21"N 31°34'27"E
		Shandawell	26°39'19"N 31°36'10"E
	Akhmim	Abar Malek	26°36'25"N 31°43'45"E
		Al Salamouney	26°37'31"N 31°45'36"E
		Pardiess	26°17'09"N 31°57'46"E
		Halafy	26°19'54"N 31°58'34"E
Qena	Abu Tesht	El Alimat	26°14'31"N 31°59'56"E
		El Qaleia	26°05'00"N 32°10'06"E

Geographical coordinates	Village	Center	Governorate
		El Maharza	26°10'43"N 32°07'19"E
	Farshut	El Dahsaa	26°03'28"N 31°08'51"E
		Koom Al Bagaa	26°05'35"N 32°10'27"E
	Nagaa Hammadi	Salamiah	26°04'15"N 32°16'04"E
		Al Sha'aina	26°05'48"N 32°19'46"E
		Bahjourah	26°02'35"N 32°10'27"E
	Qus	El Harajiaa	25°65'47"N 32°46'32"E
Luxor	Al Zayteyah	El Madawed	25°44'25"N 32°42'37"E
	Luxor	El Qarnaa	25°43'05"N 32°38'37"E
		El Daeyah	25°39'10"N 32°35'36"E
	Armant	El Domeqrat	25°44'25"N 32°42'37"E
		El Rayanah	25°44'25"N 32°42'37"E
	Esna	El Adaymah	25°14'14"N 32°35'22"E
		El Dayabyah	25°38'01"N 32°50'16"E

Table 2. Multiplexes contain and PCR reaction conditions

Multiplex	Locus	Accession Number	Repeat Pattern	Temperature	Time	Cycles
1	INRACCDDV0101	AJ874443	$(TG)_{12}$	95	15 m	
	INRACCDDV0106	AJ874448	$(CA)_{14}$	95	30 s	8X
	INRACCDDV0108	AJ874450	$(CA)_{13}$	63 - 56 (↓10)	1 min	
	INRACCDDV0139	AJ874479	(TG)16	72	45 s	
	INRACCDDV0016	AJ874380	(CA)13	95	30 s	35X
	INRACCDDV0172	AJ874510	(AC)14	55	30 s	
	INRACCDDV0176	AJ874514	$(TC)_{11}(TG)_{13}AG(TG)_3$	72	45 s	
	INRACCDDV0203	AJ874540	$(GT)_{16}$	60	20 m	
	INRACCDDV0119	AJ874461	$(GT)_{16}$	95	15 m	
2	INRACCDDV0140	AJ874480	$(TG)_{14}$	95	30 s	7X
	INRACCDDV0157	AJ874497	$(GT)_{12}$	61 - 55 (↓10)	45 s	
	INRACCDDV0201	AJ874538	$(TG)_{14}(AG)_{10}$	72	45 s	
	INRACCDDV0087	AJ874430	$(TG)_{14}$	95	30 s	37X
	INRACCDDV0089	AJ874432	$(CA)_{14}$	55	45 s	
				72	1 min	
				60	20 m	
				10	∞	
3	SAT03	J03744	$(TC)_{22}$	95	15 min	
	SAT04	M33582	$(TC)_{13}(N)_2(TC)_2TG(TC)_7$	95	30 sec	32x
	SAT05	X99887	$(TC)_{23}TTT(CT)_5$	60	30 sec	
	SAT07	X99888	$(TG)_{14}$	72	45 sec	8x
	SAT08	X99889	$(CT)_{14(}GT)_8TT(GT)_5$	95	30 sec	

Multiplex	Locus	Accession Number	Repeat Pattern	Temperature	Time	Cycles
				53	30 sec	
				72	45 sec	
				60	20 min	
				12	∞	
4	INRACCDDV0102	AJ874444	$(AC)_{18}$	95	15 m	
	INRACCDDV0104	AJ874446	$(GT)_{14}$	95	30 s	9x
	INRACCDDV0169	AJ874508	$(CA)_{17}$	59 - 54 (↓10)	30 s	
	INRACCDDV0192	AJ874530	$(TG)_{11}$	72	30 s	
	INRACCDDV0205	AJ874542	$(TG)_{17}$	95	30 s	31x
	INRACCDDV0228	AJ874561	$(TG)_{12}$	54	45 s	
	SAT13	X99892	$(GT)_{13}$	72	45 s	
				95	30 s	9x
				53	30 s	
				72	45 s	
				60	20 m	
				10	∞	
5	INRACCDDV0182	AJ874520	$(TG)_{20}$	95	15 m	
	INRACCDDV0185	AJ874523	$(AC)_{13}$	95	30 s	8X
	INRACCDDV0259	AJ874589	$(GT)_{14}(GA)_{9}$	63 - 56 (↓10)	45 s	
	INRACCDDV0313	AJ874634	$(TG)_{7}(AC)_{10}GC(AC)_{6}GC(AC)_{8}$	72	45 s	
	INRACCDDV0040	AJ874400	$(TG)_{16}$	95	30 s	39X
				56	30 s	
				72	45 s	
				60	20 m	
				10	∞	

variability (Vajed Ebrahimi et al. 2017; Hoban et al. 2022). Likewise, the mean value of Ar (7.036) was higher than the Algerian native rabbits (2.349) (Bouhali et al. 2023). The elevated levels of Ar were found in the southern subpopulations (7.501 in Qena and 8.009 in Luxor), which is a reliable marker of a population's capacity for evolution and conservation (Bora et al. 2023). In addition, The positive relationship between warm conditions and genetic variety contributes to the archetype of mutations, which are the inherent source of increasing genetic variety (Teixeira and Huber 2021; Bora et al. 2023). On the other hand, our results indicated that in the southern subpopulations (Qena and Luxor), the H_o was higher than H_e, with negative FIS values. In contrast, the northern subpopulations (Asyut and Sohag) have H_o smaller than H_e, while they have positive F_{IS} values. Consequently, current results agree with some findings in rabbit (Abdel-Kafy et al. 2018) and pig (Zorc et al. 2022) populations. It could be due to the prosperity of genetic variables in isolated breaking effects (Ismail et al. 2018).

Polymorphism of loci

Genetic variability for each locus in all subpopulations is presented in Table 4. A total of 496 alleles were recorded in this study. Previous studies of local rabbits found 119 alleles in Tunisia (Ben Larbi et al. 2014) and 120 alleles in Egypt (Emam et al. 2017). The INRA192 locus exhibited the most significant alleles (30), while INRA172 was the lowest (5). Our results on NUER subpopulations showed that 45 % of loci were highly formative of *PIC* (>0.5). Also, several studies reported a high percentage of formative *PIC* (El-Aksher et al. 2016; Emam et al. 2017; Lai et al. 2018). Moreover, 58% of the loci were not significant in terms

Table3. Parameters of genetic variation calculated for 4 NUER subpopulations

Subpopulation	N	MNa±SD	Ar±SD	H_o±SD	H_e±SD	F_{IS}±SD
Asyut	75	12.130±0.398	5.901±0.333	0.928±0.046	0.681±0.036	0.214[a]±0.027
Sohag	64	15.200±0.387	6.734±0.368	0.933±0.048	0.767±0.046	0.096[b]±0.019
Qena	59	17.633±0.371	7.501±0.356	0.992±0.028	0.770±0.039	-0.084[c]±0.020
Luxor	49	19.012±0.407	8.009±0.352	0.999±0.011	0.777±0.010	-0.134[c]±0.012
Mean values		15.995±0293	7.036±0.352	0.963±0.033	0.749±0.014	0.023±0.011

Number of samples (N). Mean number of observed alleles (MNa), Stander deviation (SD), number of private alleles (Pa), mean observed and expected heterozygosity (Ho and He), allelic richness (Ar), inbreeding coefficient (F_{IS}). Value followed by different superscripts (a, b, and c) within the last column are significantly different (P≤0.05)

Table 4. Parameters of genetic variation for each locus among 4 NUER subpopulations

Multiplex	Marker	Locus	Na	H_o ±SD	H_e±SD	F_{IS}	F_{IT}	F_{ST}	*PIC*	HWE
Multiplex-1		INRA101	7	0.931 ±0.016	0.816 ±0.015	0.796801	0.275193	0.087062	0.811	*
		INRA106	6	0.972 ±0.014	0.758±0.026	0.319937	0.231384	0.067089	0.655	**
		INRA108	14	0.981 ±0.023	0.954±0.020	0.032704	0.224597	0.077195	0.501	***
		INRA139	16	0.761 ±0.139	0.918±0.024	-0.195980	-0.481000	0.564966	0.400	NS
		INRA016	8	0.901 ±0.013	0.789±0.023	0.494116	0.471729	0.59179	0.727	*
		INRA172	5	0.750 ±0.116	0.922±0.031	0.528080	-0.381610	0.032544	0.666	**
		INRA176	18	0.688 ±0.123	0.862±0.033	-0.415720	-0.340160	0.053375	0.311	NS
		INRA203	18	0.975 ±0.020	0.769±0.035	-0.299580	-0.184580	0.088492	0.202	NS
Multiplex-2		INRA119	24	0.924 ±0.015	0.795±0.038	-0.095980	-0.153590	0.082622	0.050	NS
		INRA140	7	0.753±0.097	0.977±0.043	0.427974	-0.189930	0.103951	0.5675	**
		INRA157	23	0.927 ±0.037	0.867±0.025	0.022136	0.100973	0.09857	0.501	***
		INRA201	23	0.967 ±0.024	0.888±0.041	-0.26914	-0.133650	0.106755	0.112	NS
		INRA087	23	0.992 ±0.002	0.830±0.034	-0.20547	-0.131800	0.061119	0.109	NS

Multiplex	Marker	Locus	Na	H_o ±SD	H_e±SD	F_{IS}	F_{IT}	F_{ST}	*PIC*	HWE
Multiplex-3		INRA089	7	0.898 ±0.031	0.745±0.041	0.694213	0.19397400	0.110386	0.719	*
		Sat3	17	0.969 ±0.001	0.824±0.027	-0.21352	-0.071910	0.116693	0.185	NS
		Sat4	17	0.94 ±0.035	0.825±0.037	-0.21087	-0.080910	0.107322	0.182	NS
		Sat5	15	0.988 ±0.038	0.759±0.025	-0.21087	0.119284	0.1508	0.502	NS
		Sat7	15	0.961 ±0.040	0.842±0.030	-0.18753	-0.070230	0.098775	0.145	NS
		Sat8	8	0.948 ±0.011	0.896±0.017	0.399282	0.115500	0.112063	0.521	***
Multiplex-4		INRA102	13	0.942 ±0.035	0.812±0.029	0.133165	0.148562	0.067462	0.502	***
		INRA104	10	0.973 ±0.025	0.794±0.028	0.208797	0.263542	0.034577	0.508	***
		INRA169	25	0.954 ±0.043	0.798±0.041	-0.253130	-0.184170	0.055028	0.109	NS
		INRA192	30	0.992 ±0.021	0.797±0.022	-0.255490	-0.115080	0.111834	0.104	NS
		INRA205	26	0.682±0.154	0.945±0.041	-0.384830	-0.209360	0.126708	0.090	NS
		INRA228	8	0.747±0.169	0.983±0.035	0.446278	0.132374	0.142971	0.703	*
		Sat13	19	0.978±0.014	0.767±0.051	-0.210870	0.190921	0.133732	0.107	NS
Multiplex-5		INRA182	8	0.978±0.021	0.837 ±0.054	0.489048	0.185160	0.080593	0.711	*
		INRA185	19	0.948±0.017	0.903±0.0547	-0.309260	-0.165210	0.110023	0.111	NS
		INRA259	18	0.952±0.014	0.842±0.026	-0.186870	-0.136010	0.042851	0.103	NS
		INRA313	26	0.827±0.111	0.941±0.033	-0.25421	-0.149480	0.083503	0.126	NS
		INRA040	23	0.777±0.101	0.969±0.040	-0.11421	-0.169480	0.083503	0.100	NS
Mean Values			16	0.902±0.049	0.852±0.033	0.023194	-0.02242	0.122076	0.359	

Na= number of observed alleles; H_o and H_e= mean observed and expected heterozygosity standard deviation (SD); PIC= polymorphic information content per locus; HWE= Hardy-Weinberg Equilibrium. Differentiation among populations (F_{ST}), reduction in heterozygosity due to inbreeding for each locus (F_{IT}), reduction in heterozygosity within each breed due to inbreeding . (F_{IS});*P≤0.05; **P≤0.01;*** P≤0.001; NS= Non-Significant

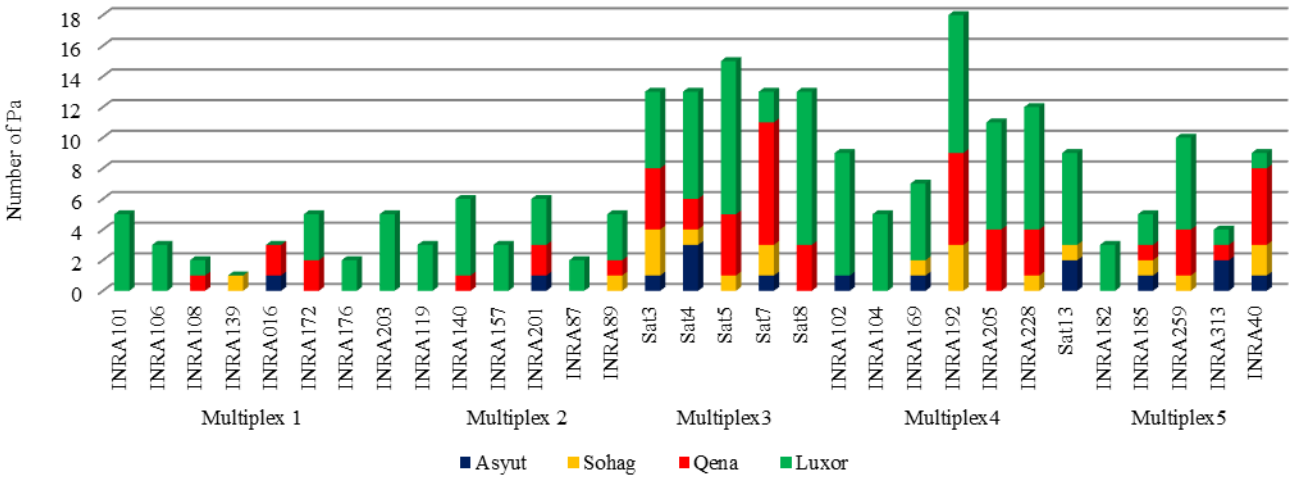

Figure 2. Distribution of private alleles (Pa) among subpopulations and microsatellite markers

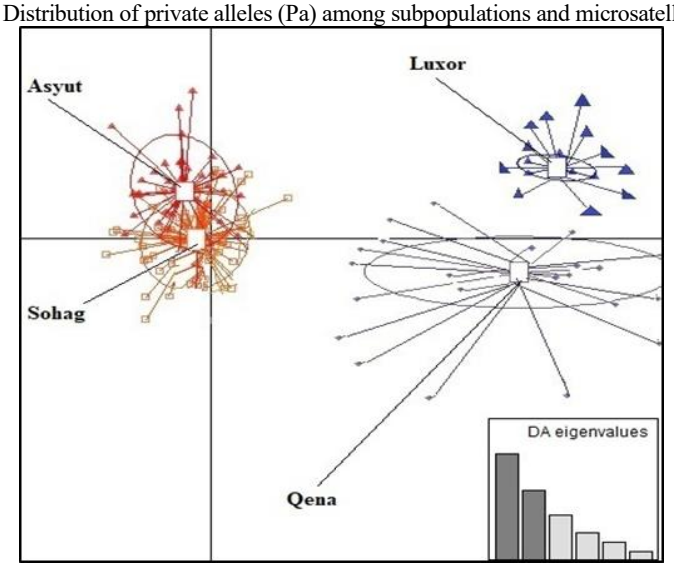

Figure 3. Analysis of discriminant analysis of principal components (DAPC) for NUER Subpopulations

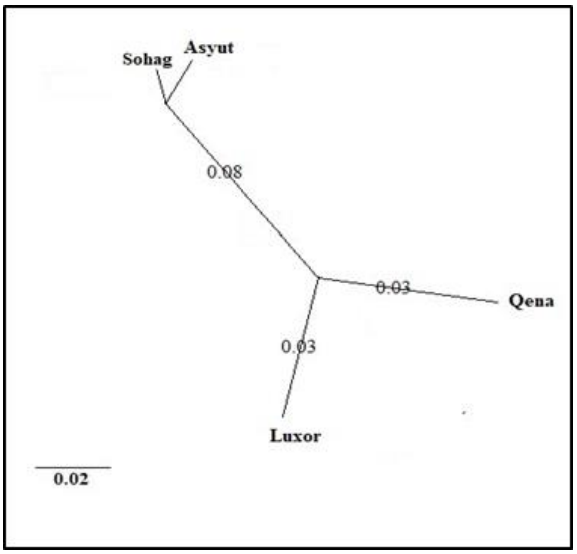

Figure 4. Neighbor-joining tree (NJ) for NUER Subpopulations

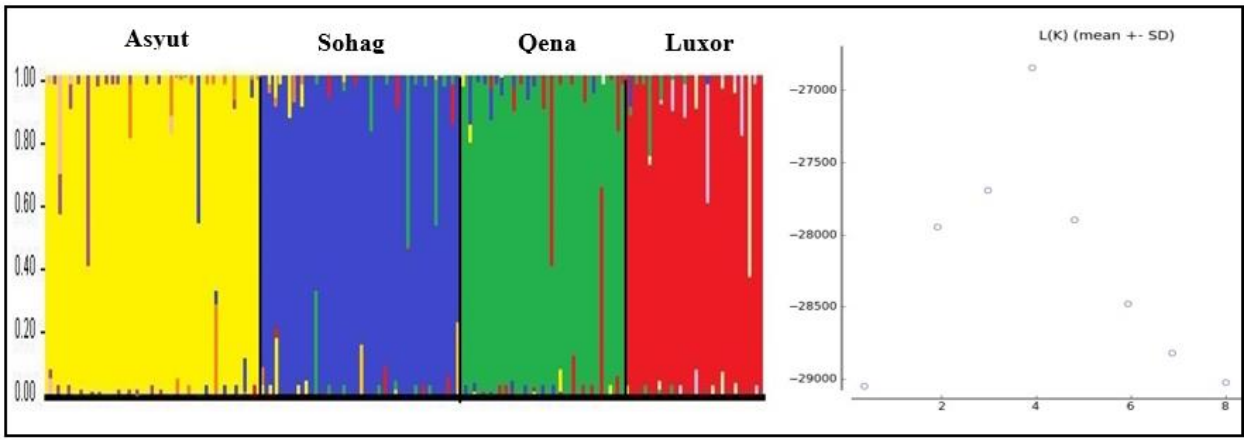

Figure 5. Estimated native Upper Egypt rabbits (NUER) subpopulation structure. In each K, the colors represent the percentage of each cluster in each rabbit population. ΔK calculated from K=1 to K=8

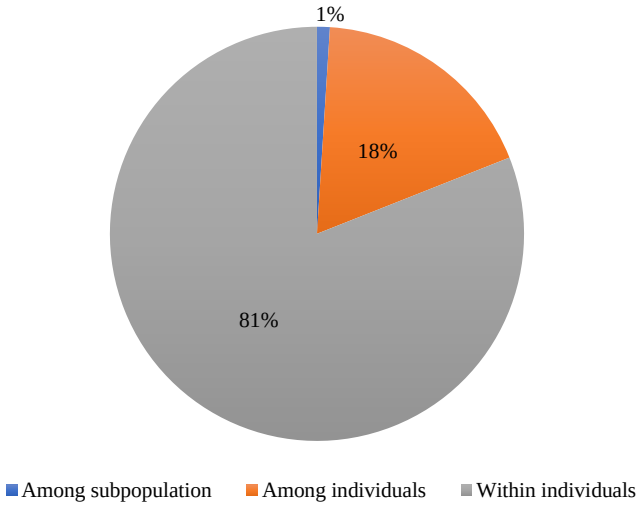

Figure 6. Percentage of molecular variations for NUER subpopulations

of HWE, which is characteristic of the absence of inbreeding situations in the majority of subpopulations, according to (Roden et al. 2023) and natural genetic selection (Demiray et al. 2024).

This study recorded about 43% of alleles (220) as Pa (Figure 4). The highest Pa is recorded in Luxor (133), while the lowest is in Asyut (15). The affluence of Pa was characterized in the NUER subpopulations. The highest values of private alleles were recorded by the subpopulation of Luxor (133) and SAT5 locus (15). The variety of private alleles strongly indicates the absence of a genetic bottleneck position (Holmes et al. 2023). studies reported a high percentage of formative PIC (El-Aksher et al. 2016; Emam et al. 2017; Lai et al. 2018). Moreover, 58% of the loci were not significant in terms of HWE, which is characteristic of the absence of inbreeding situations in the majority of subpopulations, according to (Roden et al. 2023) and natural genetic selection (Demiray et al. 2024).

This study recorded about 43% of alleles (220) as Pa (Figure 2). The highest Pa is recorded in Luxor (133), while the lowest is in Asyut (15). The affluence of Pa was characterized in the NUER subpopulations. The highest values of private alleles were recorded by the subpopulation of Luxor (133) and SAT5 locus (15). The variety of private alleles strongly indicates the absence of a genetic bottleneck position (Holmes et al. 2023).

Genetic differentiation and structure of NUER subpopulations

In Figure 3 and Figure 4, the NUER subpopulation was classified into two main groups: north (Asyut and Sohag) and south (Qena and Luxor). Genetic overlapping was observed in the northern subpopulation due to geographical proximity between the last and first points (less than 9 km). In contrast, the southern subpopulations expressed far greater separation for separation between the last point in the north of Qena and the first point of Luxor in the south (about 50 km). The categorization of rabbits according to geographical direction was reported in several studies on rabbits (Alda

& Doadrio 2014; Ben Larbi et al. 2014; Emam et al. 2017; Jochová et al. 2017; Iannella et al. 2019; Cheptanui 2022). The highest K values and ΔK for different clustering when K=4. Likewise, the approved value of ΔK was equal to the population number for previous studies (Emam et al. 2016; Dudu et al. 2020).

NUER subpopulation's molecular variance percentage

The estimation of molecular variance percentage for the NUER subpopulations is shown in Figure 6. The result revealed that the percentage of variance among subpopulations was only 1%, whereas 18% and 81% among and within individuals, respectively. The low variation among subpopulations (1%) is convincing evidence for random mating among populations and the limitation of inbreeding (Adeolu et al. 2021). It was a good sign for allowing a population to adapt and survive in shifting environmental conditions (Pavlova et al. 2017; Ma et al. 2020). Previous results agree with El-Aksher et al. (2016) and Adeolu et al. (2021) but do not match with Bouhali et al. (2023).

CONCLUSION

This study demonstrated the current genetic situation of the NUER population through a deep investigation of four subpopulations for the first time. The subpopulations of NUER shed light on the rich genetic variability with the absence of inbreeding and bottleneck positions. Towards the south in Qena and Luxor, genetic variability parameters increased more than in the north (Asyut and Sohag). The overlapping was observed in the northern subpopulations. The current study could be used to document rabbit genetic resources in the Upper Egypt strip in national and international reports. It could reflect the state's interest in local farm animal interest according to the climate change plan. Moreover, it opens the field of interest in local farm animals in rural and fragile areas as efforts to guarantee food security and improve livelihoods there. Additionally, similar studies should be replicated on distinct species in this vital area from Egypt's hot-dry regions.

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