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Egyptian Native Rabbits Along The Nile River: A Microsatellite Marker-Based Genetic Field Study

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ABSTRACT

The human interest in exotic animal breeds in the agriculture sector led to the deterioration of local breeds. Microsatellite markers are important tools to determine the genetic status of breeds and populations. This study aimed to detect the genetic situation among the Egyptian native rabbit (ENR) breed along the Nile River in rural areas. The survey covered 804 km (50 geographical points) to collect 410 biological samples from three populations: native Delta Egypt rabbits (NDER, n = 153), native Middle Egypt rabbits (NMER, n = 124), and native Upper Egypt rabbits (NUER, n = 133). Across 27 markers, a total of 284 alleles were observed in this study, and about 63% of them were highly polymorphism-formative with no significance for Hardy-Weinberg equilibrium (HWE). The highest values of the mean number of alleles (MNa), private alleles (Pa), and allelic richness (Ar) were recorded in the NUER population (13.519, 21, and 6.762, respectively). The observed heterozygosity (H_o)< expected (H_e) in two populations (NDER and NMER). In contrast, the NUER population (H_o >H_e). Values of genetic variability showed that population genetic variability increased in the south direction (NMER and NUER). In the north direction (NDER), the current study recorded a bottleneck with a high significant value of inbreeding coefficient (F_{IS}) of 0.418 vs. 0.145 for NUER in the south. This study found that the ENR situation is critical for increasing the north, while it characterized stability in the south direction.

1. INTRODUCTION

Rabbit (*Oryctolagus cuniculus*) is considered one of the cheap animal protein sources in Egyptian rural areas along the Nile River [1] . Its production is a good economic way to improve rural livelihoods [2-3]. It contributes to eliminating poverty and hunger, achieving the first and second goals of the Sustainable Development Agenda 2030 [4], and supporting the sustainable agriculture strategy development in Egypt 2030 [5]. The Egyptian native rabbit (ENR) breed is the most common indigenous rabbit breed found in Egyptian rural areas [6]. The ENR is a medium-sized breed with a body weight

range of 1550–1980 g [7]. Abdel-Kafy *et al*. [8] stated that the different fur colors are distinguished in ENR (white mixing with gray, brown, black, and red). Also, in white, gray, and black fur coats [7-8]. In addition, it is distinguished by disease tolerance, low nutritional requirements, and high adaptability to harsh local conditions [1]. Furthermore, it was considered the cornerstone of the Egyptian lines that were produced in the previous century. Whether through selection such as the white Giza rabbit [9] , or by crossing with the Flemish Giant rabbit breed to produce white, red, and black Baladi rabbits [10-11].

The documentation of genetic resources is one of the main areas of livestock conservation activities [12]. The livestock sector is a substantial component of agriculture output by producing high-quality food [13]. On the other hand, local farm animal breeds are considered important resources for smallholders under the low-input system, especially in developing countries [14-15]. Moreover, the encouragement of carrying out biodiversity studies for local animals that can withstand climate change is one of the goals of the Egyptian strategy for climate change 2050 [16]. In this regard, microsatellite loci could be used as a useful tool to explore the genetic situation of livestock [17]. It is used for estimating genetic variation, division, drift, gene flow, and structure among breeds, populations, and subpopulations [18]. Based on that, it qualifies to be an influential method for formulating effective scenarios for breeding strategies that prompt broad sustainability of farm animal genetic resources [19-21]. Consequently, the purpose of the current study is to investigate the genetic status of the ENR breed in three main agricultural areas in the Egypt Delta (in the north), Middle Egypt (in the middle), and Upper Egypt (in the south) along the Nile River by using microsatellite markers.

2. MATERIALS AND METHODS

2.1. Rabbit Samples and genotyping of microsatellite markers:

The geographical survey of ENR covered 804 km belonging to 50 central points along the Nile River in Egypt (**Figure 1 and Supplementary Table S1**) from March 2021 to February 2023. The overall hair and skin tissue samples (collected from slaughtered rabbits) were 153 for native Delta Egypt rabbits (NDER) in the north, 124 for native Middle Egypt rabbits (NMER) in the middle, and 133 for native Upper Egypt rabbits (NUER) in the south. Tissue samples were kept in an Eppendorf tube filled with 95% ethanol, while hair samples were kept in small sample bags. The Cinelli *et al.* [22] method was used to extract DNA.

In this study, 27 microsatellite loci (Invitrogen, France) were divided into five multiplexes (**Supplementary Table S2**) depending on PanelPlex Software (Ann Arbor, MI, USA). The multiplex PCR contained 5µl of master mix (Qiagen, 20614), 1 µl of multiplex microsatellite loci (forward 0.1 µl / primer reverse), 1 µl of DNA, and 3 µl of Deuterium-depleted water (dd H_2O). The multiplexes PCR products were examined on agarose gel 2% by a horizontal gel electrophoresis system and then genotyped on an automated ABI PRISM 3730 (Applied Biosystems).

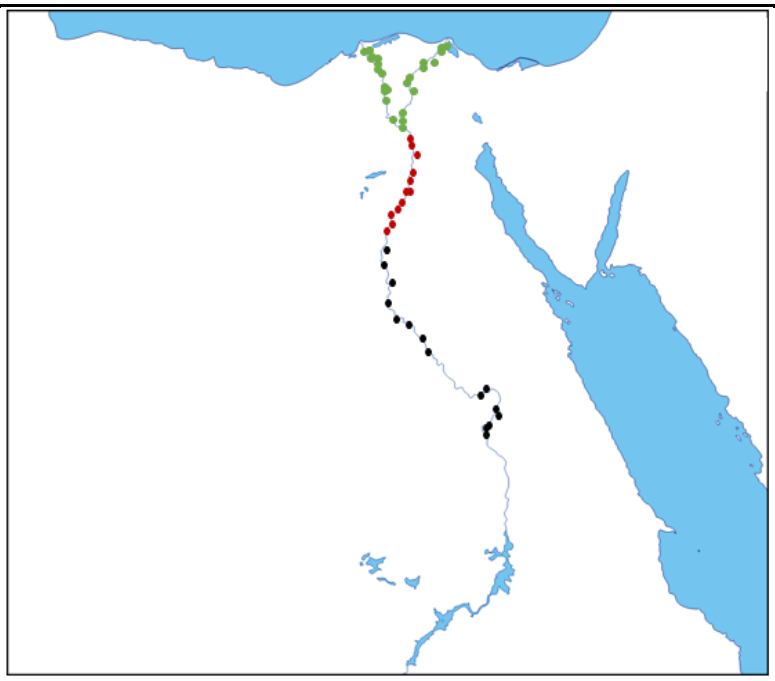

Figure 1. The distribution of geographical points of sampling strategy. The green points are or Delta populations, the red points are for middle Egypt population, and the black points are for Upper Egypt populations

2.2. Data Analysis:

Several software packages were used to calculate different genetic parameters during statistical analysis. The software GenAlEX 6.41 [23] was used to estimate the estimated number of observed alleles per locus (Na), the mean number of alleles (MNa), the private number of alleles (Pa), both observed and expected heterozygosity (H_o and H_e), and the analysis of molecular variance (AMOVA). The calculation of polymorphism information content (*PIC*) and the Hardy-Weinberg equilibrium (HWE) was estimated by Cervus 3.0.6 software [24]. The calculation of F statics values for each locus [pairwise genetic 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})], inbreeding coefficient among populations F_{IS}, and allelic richness (Ar) was conducted by FSTAT 2.9.3.2 [25]. The testing for bottlenecks was established using Wilcoxon ($P > 0.05$) signed-rank method 1000 simulations through the infinite allele model (IAM), two-phase model (TPM) and stepwise mutation model (SMM) by using BOTTLENECK 1.2.02 [26]. The graph of factorial correspondence analysis (FCA) was estimated using GENETIX 4.05 software [27]. The drawing of the neighbor-joining tree (NJ) was visualized by Mega tree explorer depending on the Reynolds matrix [28]. STRUCTURE 2.3.4 program was used to evaluate the population genomic structure of populations [29] by using 500000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 20000 steps following the rule of $1 \leq K \leq 6$ (K= number of assumed clusters). The statistical ΔK was computed to detect the highest rate of change in the log-likelihood between successive for a detailed graphic explanation [30].

3. RESULTS

3.1.Genetic variability among and within NER populations:

The ascending order of the genetic variability values from the north (NDER) to the south (NUER) was characterized in this study (**Table 1**). The lowest and highest values in terms of MNa, Ar, and Pa were recorded in NDER (6.270, 4.569, and 8) and NUER (13.519, 6.7622, and 21), respectively. The values of H_o and H_e varied between NUER (0.367 and 0.679) and NDER (0.828 and 0.811). The inbreeding coefficient (F_{IS}) significantly decreased towards the south. It was recorded as the lowest value in NUER (0.145), while it was the highest in NMER (0.418).

Table 1. Genetic variability records in NER populations Native Delta Egypt rabbit (NDER), Native Middle Egypt rabbits (NMER), Native Upper Egypt rabbits (NUER), Mean number of observed alleles (MNa), standard deviation (SD), number of private alleles (Pa), mean observed and expected heterozygosity (H_o and H_e), allelic richness (Ar), and inbreeding coefficient (F_{IS}). Value followed by different superscripts, (a, b and c), within the last column are significantly different ($P \leq 0.05$).

Population	N	MNa± SD	Pa	Ar± SD	H_o ± SD	H_e ± SD	F_{IS} ± SD
NDER	153	6.270 ± 0.363	8	4.569 ±0.367	0.367 ±0.025	0.679 ±0.028	0.418^{a} ±0.037
NMER	124	11.858 ±0.760	11	5.818 ±0.292	0.525 ±0.029	0.868 ±0.021	0.323^{b}±0.028
NUER	133	13.519 ±0.868	21	6.762 ±0.309	0.828 ±0.021	0.811 ±0.014	0.145^{c} ±0.030
Mean values		**10.518 ±0.523**	**13.3**	**5.716 ±0.323**	**0.573 ±0.025**	**0.786 ±0.021**	**0.295 ±0.021**

According to the Wilcoxon results **Figure 2**, the bottleneck was observed in the northern population (NDER). On the contrary, it was absent in the middle and south (NMER and NUER).

In **Table 2**, a total of 284 alleles were detected across 27 microsatellite loci in three ENR populations. Furthermore, the values of Na varied from 17 to 3 (INRA 205 and 140, respectively). The average *PIC* value is 0.649, with a range from 0.327 to 0.832. It is noteworthy that 17/27 loci were highly polymorphism-formative (*PIC > 0.5*) and not significant in HWE. The mean of $F_{IS,}$ $F_{IT,}$ *and* F_{ST} were calculated in microsatellite loci (0.295, 0.352, and 0.065, respectively). One locus only (INRA 087) determined the negative value of $F_{IS.}$

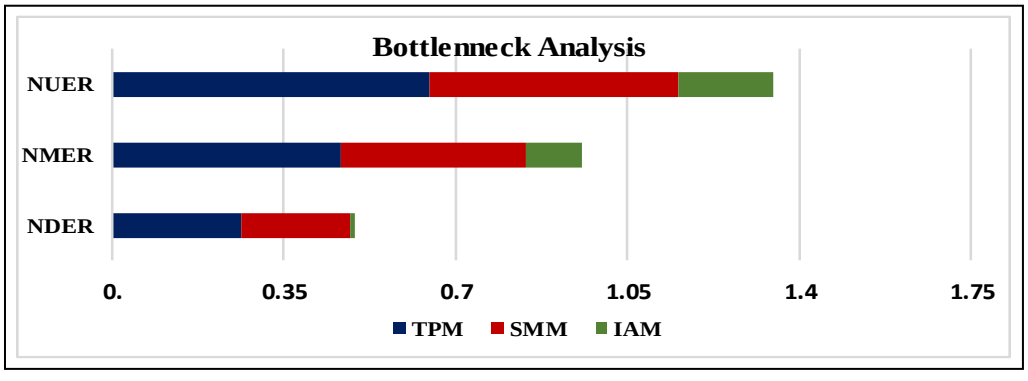

Figure 2. The Bottleneck analysis results for native Egyptian rabbits. Native Delta Egypt rabbit (NDER), Native Middle Egypt rabbits (NMER), Native Upper Egypt rabbits (NUER).

Table 2. Genetic variability for microsatellite markers

Multiplex	Marker	Na	H_o ± SD	H_e ± SD	F_{IS}	F_{IT}	F_{ST}	Mean *PIC*	HWE
1	**INRA102**	9	0.731 ±0.088	0.982 ±0.048	0.140	0.117	0.025	0.463	NS
	SAT13	12	0.628±0.075	0.820 ±0.084	0.256	0.294	0.137	0.719	*
	INRA169	10	0.600 ±0.072	0.842 ±0.066	0.272	0.300	0.039	0.327	NS
	INRA205	17	0.555 ±0.094	0.814 ±0.075	0.317	0.355	0.055	0.411	NS
	INRA228	13	0.522 ±0.065	0.669 ±0.083	0.228	0.294	0.087	0.748	*
2	**INRA313**	12	0.561 ±0.074	0.779 ±0.069	0.344	0.378	0.046	0.684	*
	INRA342	8	0.658 ±0.096	0.834 ±0.057	0.218	0.252	0.044	0.416	NS
	INRA040	13	0.712 ±0.096	0.970 ±0.043	0.361	0.414	0.063	0.353	NS
	INRA185	10	0.405 ±0.076	0.766 ±0.075	0.272	0.437	0.227	0.587	*
	INRA259	10	0.607 ±0.076	0.928 ±0.045	0.209	0.272	0.079	0.607	*
3	**INRA108**	11	0.543 ±0.071	0.693 ±0.021	0.224	0.287	0.081	0.701	*
	INRA176	8	0.259 ±0.030	0.546 ±0.089	0.528	0.570	0.090	0.493	NS
	INRA106	10	0.535 ±0.073	0.798 ±0.028	0.337	0.375	0.056	0.598	*
	INRA101	11	0.517 ±0.049	0.779 ±0.071	0.307	0.354	0.067	0.466	NS
	INRA203	11	0.688 ±0.072	0.888 ±0.044	0.424	0.468	0.077	0.710	*
4	**INRA140**	3	0.495 ±0.074	0.746 ±0.043	0.190	0.239	0.061	0.371	NS
	INRA087	15	0.745 ±0.095	0942 ±0.087	-0.120	0.193	-0.083	0.832	*
	INRA157	11	0.521 ±0.074	0.712 ±0.046	0.407	0.450	0.073	0.681	*
	INRA190	4	0.238 ±0.089	0.538 ±0.056	0.654	0.671	0.050	0.483	NS
	SAT12	14	0.633 ±0.022	0.944 ±0.027	0.531	0.539	0.017	0.808	*
	INRA119	13	0.516 ±0.084	0.736 ±0.078	0.307	0.335	0.040	0.796	*
	INRA201	10	0.551 ±0.044	0.748 ±0.078	0.312	0.372	0.088	0.604	*
5	**SAT 03**	15	0.642 ±0.069	0.723 ±0.072	0.204	0.243	0.049	0.780	*
	SAT 04	11	0.653 ±0.108	0.769 ±0.059	0.169	0.225	0.068	0.687	*
	SAT 05	10	0.569 ±0.096	0.709 ±0.087	0.401	0.491	0.106	0.587	*
	SAT 08	4	0.532 ±0.069	0.677 ±0.058	0.223	0.272	0.062	0.455	NS
	SAT 07	9	0.769 ±0.017	0.881 ±0.135	0.260	0.295	0.046	0.555	**
Mean values		**10.518**	**0.573 ±0.072**	**0.786±0.068**	**0.295**	**0.352**	**0.065**	**0.649**	

Na: number of observed alleles. H_o and H_e : mean observed and expected heterozygosity standard deviation (SD). Mean *PIC*: mean polymorphism informationcontent 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

3.2. The genetic differentiation among NER populations:

The factorial correspondence analysis (FCA) assessed the differentiation among individuals in each population (**Figure 3**). The variations for each axe were 41.54, 56.46, and 9.84%, respectively. Moreover, the separation was observed in the NDER population. Instead, there was overlap in some individuals between the NUER and NMER populations. In addition, the neighbor-joining (NJ) tree (**Figure 4**) showed two main clusters: the first cluster included the NUER and NMER, while the second was NDER.

Figure 5 (a and b) illustrates the analysis of STRUCTURE and ΔK value of NER populations. The highest ΔK value (46.18) was obtained at K = 4 (**Figure 5b**). Where K=1 is defined as one cluster. Likewise, the start of cluster separation was observed at K = 2 in two clusters. The first was for NDER in the north, while the second was for NMER and NUER in the middle and south. Moreover, in case K = 3 to K = 6, all population groups were detected in a separation cluster.

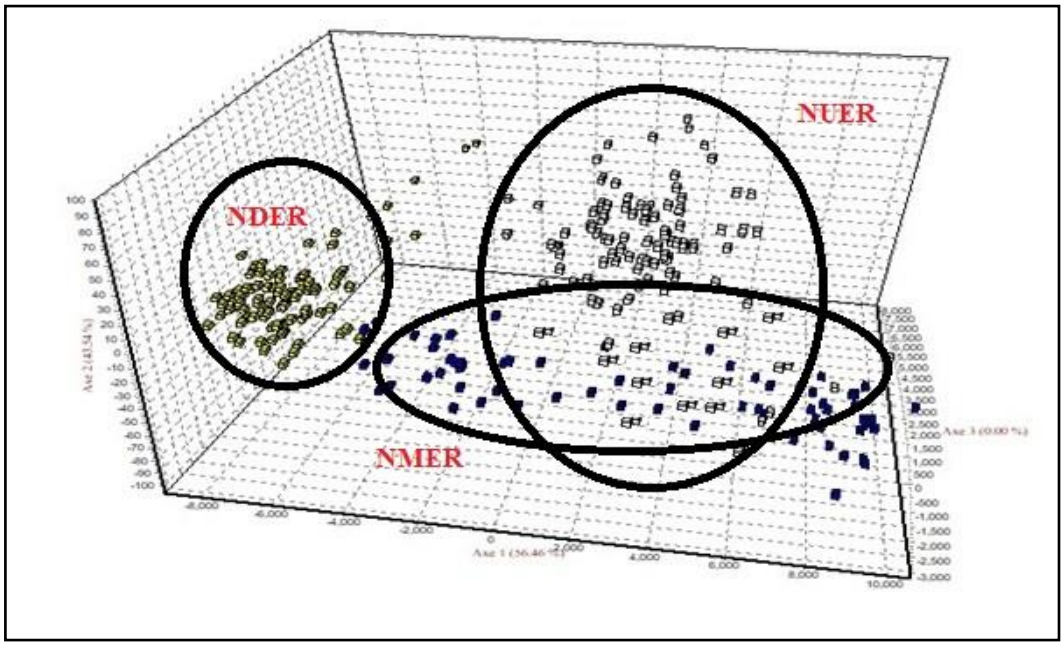

Figure 3. Factorial correspondence analysis (FCA) for native Egyptian rabbits based on the allele frequencies from microsatellites. Native Delta Egypt rabbit (NDER), Native Middle Egypt rabbits (NMER), Native Upper Egypt rabbits (NUER).

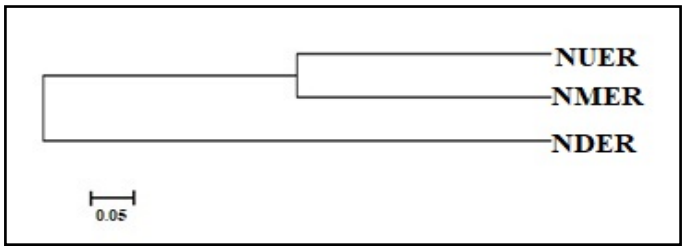

Figure 4. Neighbor joining tree for native Egyptian rabbits. Native Delta Egypt rabbit (NDER), Native Middle Egypt rabbits (NMER), Native Upper Egypt rabbits (NUER).

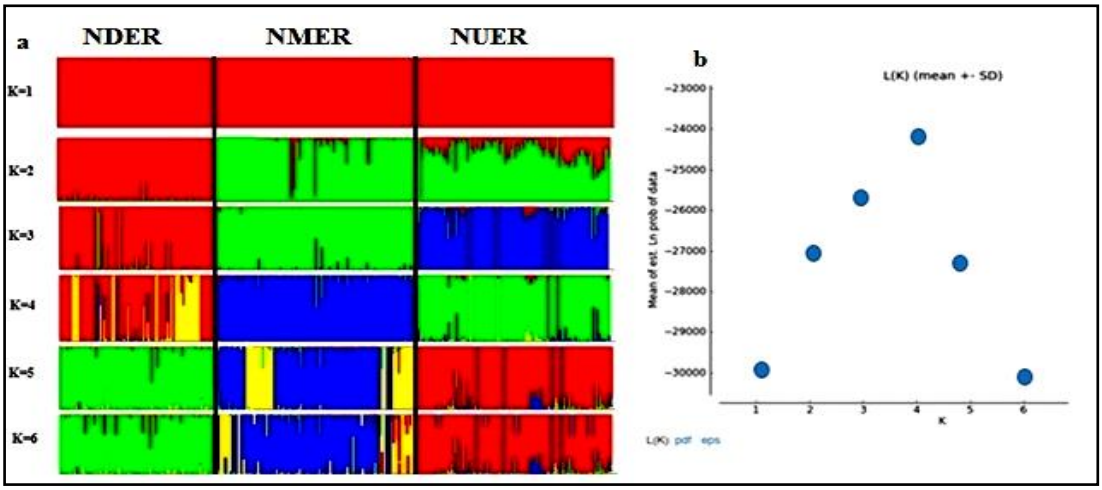

Figure 5. a. Estimated population structure for native Egyptian rabbits. In each K, the colors represent the percentage of each cluster that is present in each rabbit population. b. ΔK calculated from K=1 to K=6. Relation between populations. K: number of assumed cluster. Native Delta Egypt rabbit (NDER), Native Middle Egypt rabbits (NMER), Native Upper Egypt rabbits (NUER)

3.3. Analysis of molecular variance for populations

The summary of AMOVA (analysis of molecular variance) in Table 3 showed that the differentiation of genetics measured among populations and among individuals of the total genetic variance were 4% and 23%, respectively. However, the genetic variation within individuals was 73%.

Table 3. Summary analysis of molecular variance

Source	df's	SS	Est. Var.	%Var
Among Populations	2	411.207	3.261	4%
Among Individuals	286	3772.762	0.999	23%
Within Individuals	289	1927.500	6.670	73%
Total	**577**	**6111.469**	**10.930**	**100%**

Degrees of freedom (df's), sum of squares (SS), mean square (MS), estimated variance (Est.var.), and percentage of variation (% var.)

4. DISCUSSION

This study is the first genetic evaluation study among native Egyptian rabbit populations covering more than 800 km along the Nile River in the main agriculture zones (Delta, Middle, and Upper Egypt) using microsatellite markers.

As shown in **Table 1**, the mean value of MNa in the ENR was 10.518, this result is nearby similar to the Nigerian commercial rabbits (10.208) recorded by Adeolu *et al.* [31]. In contrast, the lowest value recorded for MNa in NMER rabbits was 6.125 [7]. The mean number of Pa was higher than Emam *et al.* [32] result, 10.5 *vs*. 13.3 in this study.

In the current study, it was found that the highest genetic variability was increasing in the south direction (NMER and NUER). The population of NUER expressed the highest values of Pa (21), MNa (13.519), and Ar (6.762). This result is on the same side as Bouhali *et al.* [33] on the south of Algerian native rabbit populations. The superiority

of genetic variability values in the south direction is likely to be attributed to the high temperature is increased level of mutations [34-37]. It is considered an important factor in the superiority of genetic variability [38]. In addition, the increase of Ar in the south could indicate the population's adaptation to future environmental change [39].

The decrease of F_{IS} was recorded in the southern population (NUER), which gives a strong indicator for absence and strong inbreeding [40]. In contrast, the increase of F_{IS} in the NDER population is likely due to genetic drift caused by the small size of subpopulations [41-45]. In **Tables 1 and 2**, the mean of $H_o < H_e$ (0.573 *vs.* 0.786, respectively) agrees with some diversity studies on some north African local rabbit breeds in Tunisia [46], Algeria [33,47], and Egypt [6, 7, 48]. In **Table 1**, it was also found in the NMER and NDER populations. In contrast, it was recorded that $H_o > H_e$ (0.828 *vs.* 0.811, respectively) in the NUER population. It is suspected of having an isolation-breaking effect [49-50]. The superior population in genetic variability values could be widely used as the head of selection for the breed [51-52]. This matches with on NUER population. The test of Bottleneck (**Figure 2**) confirmed the deterioration of ENR in the north (NDER). It might be due to an increase in the expression of deleterious recessive mutations [45]. The rise of inbreeding rates is causing the random genetic drift probability [21]. In contrast, the bottleneck is absent in the middle south (NMER and NUER, respectively). The absence of bottleneck could be due to the random mating that directly causes genetic stability [53].

Table 2 shows that 17/27 loci were highly formative (*PIC >0.5*) and weren't significant in HWE. In agreement with Abdel-Kafy *et al.* [7], they found that 6/8 loci were highly formative and insignificant in HWE. It could be inasmuch as crossing absence [54-55]. In addition, about 75% of loci gave ≥ 10 alleles, which was approved by Adeolu *et al.* [31].

Both the FCA (**Figure 3**) and NJ tree (**Figure 4**) results illustrated that there is genetic overleaping between the NMER and NUER populations in the middle and south. It could be due to geographical proximity and touching boundaries (less than 40 km). The same results were recorded in Indian native cattle populations. In contrast, the northern population (NDER) was expressed as being separated from others (350 km). The overlapping was observed in Algeria [33]. In the same context, genetic overlapping in the south was shown in **Figure 5** when k = 2.

Otherwise, the percentages of AMOVA (**Table 3**) are nearby similar to the finding by Bouhali *et al*. [33], 20, and 77% (among populations, individuals, and within individuals variances, respectively). In addition, the high value of population genetic variation (4%) is a strong indicator of a high degree of geographical structuring and nonrandom mating in the tested population [56-57]. On the other hand, the high percentage of among individuals genetic variance (23%) is an indicator of the population's adaptability to environmental circumstances [58].

CONCLUSION

This study shed light on the genetic evaluation of NER populations along more than 800 km in Egyptian rural areas by using microsatellite markers for the first time. According to this study's results, high genetic variability and stability were characterized in the southern population (NUER). The situation of the northern population (NDER) is suffering from a high inbreeding coefficient and bottleneck, which is considered strong evidence of genetic drift. In addition, overlapping between the middle and southern

populations (NMER and NUER) was observed. The results of this study could be used as an illustrated document to formulate strategies or action plans for the conservation or improvement of the ENR breed. We recommended continuing the efforts of researchers to investigate subpopulations NDER and NUER by using microsatellites. In addition, studying genetic sequences for NER. Overall, this work could contribute for achieving goals numbers 1 (no poverty), 2 (zero hunger), and 15 (to halt biodiversity loss) of the United Nations' sustainable development agenda for 2030.

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