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Original scientific article

MICROSATELLITE MARKERS APPLICATION IN THE GENETIC SURVEY OF NATIVE RABBITS IN THE EGYPTIAN DELTA

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The human interest in exotic animal breeds in the agricultural sector led to the deterioration of local breeds. The interest in national farm animal genetic studies is important for the agriculture ecosystems under climate change challenges. Microsatellite markers are important tools to determine the genetic status of breeds, populations, and subpopulations. In this study, 28 microsatellite loci were used to investigate the genetic situation among 274 biological samples collected from the native Delta Egypt rabbits (NDER) population in the north of Egypt. They belonged to eight subpopulations (Damietta, Dakahlia, Kafr El sheikh, Beheira, Gharbia, Menoufia, Sharqia, and Qalyubia). It was found that expected heterozygosity (H_e) values were greater than observed heterozygosity (H_o). A total of 184 alleles were identified, with a mean of 6.571 and 4.122 as effective alleles. About 89% of microsatellite markers expressed high informative values in the polymorphism information content (*PIC*). The comparison among 8 NDER subpopulations showed low genetic variability parameters with high inbreeding coefficient (F_{IS}) values in the north (Damietta, Dakahlia, Kafr El sheikh, Beheira, and Gharbia). However, values of genetic variables increased with decreasing FIS in the middle (Menoufia), east (Sharqia), and south (Qalyubia) Delta. Furthermore, the discriminant analysis principal components (DAPC) showed overlaying in the north. In the same context, the neighbor-joining tree (NJ) and heatmap showed the genetic convergence among the northern subpopulations. The analysis of STRUCTURE found 4 clusters (K= 8). The north subpopulations were in one cluster, while others in the middle,

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east, and south were a separate cluster for each subpopulation. Our findings show that the NDER suffers from genetic drift in the northern Delta subpopulations. On the contrary, the south, east, and middle subpopulations showed more genetic variability. A strategy of correct mating should be fostered to improve the genetic traits of rabbits.

Keywords: Native Rabbits, Delta, Subpopulation, Egypt, Microsatellite

INTRODUCTION

The world sustainable development agenda of 2030 is considered a road map that grants the adaptation to global warming, which has a devastating impact on the agriculture sector, disposing of poverty, hunger, and biodiversity loss (GRI, 2015). It is considered the cornerstone to ensuring human demands for food and livelihood security (FAO, 2022). Therefore, the encouragement of studying the biodiversity of the indigenous animal breeds that withstand climate change is one of the priority goals of the Egyptian strategy for climate change - 2050 (ENCCS, 2022). The investigation of genetic diversity is used to determine the stability of farm animal genetic resources (FAGRs) under several challenges that pressure on ecosystems (FAO, 2015). Researchers use genetic diversity to measure genetic differences among farm animal populations to evaluate genetic situations and maintenance by using suitable breeding programs (ROMANOV *et al.,* 2023). Taking into consideration that several indigenous breeds are considered historical and cultural heritage (MARSONER *et al.*, 2018).

Egypt is the first leading country in the Middle East and Africa in terms of rabbit production, at about 75 tons/year (HELG LIBRARY, 2023). The Nile Delta is the most important region for agriculture and livestock activities in Egypt (YAHIA *et al.,* 2023). According to AESS (2022), about 82% of rabbit production is concentrated in Delta. In Egypt, rural production depends on indigenous (native or Baladi) rabbits (MOSTAFA *et al*., 2020) that are found in different phenotypes according to ABDEL-KAFY *et al.* (2016) who described the same breed in middle Egypt. It is distinctive in being resistant to several abiotic stresses (YOUSSEF *et al.*, 2021). Native Delta Egypt rabbits (NDER) are widely produced on small scales and in backyard production systems (MOSTAFA *et al.,* 2020). Native rabbits are considered important local genetic resources in Egypt (MALR and FAO, 2003; 2013).

According to FAO (2019) statistics, about 15–17% of farm animal genetic resources (FAGRs) are at risk of extinction. In the same context, more than 80% of Middle Eastern local breeds have unknown genetic situations (FAO, 2015). In several species, DNA markers are used to evaluate the genetic situation among subpopulations (IBRAHIM *et al*, 2022) populations (CHO *et al.,* 2022.), and breeds (DEMIR *et al.,* 2023). Microsatellites are widely used in diversity studies for high polymorphic, Mendelian inheritance and low cost (CORTES *et al.,* 2022; CHEBO *et al.,* 2022; SARTIKA *et al.,* 2023). These benefits make them suitable for profiling genetic variation and structure, drawing the genetic map, pedigree analysis, and differentiation detection among subpopulations, populations, and breeds (EMAM *et al.,* 2017; LOUKOVITIS *et al.,* 2022; SUSILORINI *et al.,* 2022). Moreover, microsatellite markers are essential for the sustainability of FAGRs for selection requirements necessary for the future (HOQUE *et al*., 2022; HAPPY HARIYONO and ENDRAWATI, 2023). They facilitate breeding strategies and conservation policies, which will lead to improvements in the genetic stock found (TOMKA *et al*., 2023). It has also assisted in reducing the protein gap for human needs (OMOTOSO *et al.,* 2019; TENZIN *et al.*, 2023). Since 2014,

microsatellite markers have played an important role in the genetic situation investigation of local rabbit breeds in North Africa, such as Tunisia (BEN LARABI *et al.,* 2014), Algeria (BOUKABENE *et al.,* 2018; BOUHALI *et al.,* 2023) and Egypt (ABDEL- KAFY *et al.,* 2016 ; EMAM *et al.,* 2017).

Thus, the objective of the current study is to assess the genetic situation of eight subpopulations of NDER in Egypt using 28 microsatellite markers.

MATERIAL AND METHODS

Samples Collection

A total of 274 NDER biological samples (hair bulbs and tissues) of rabbits were collected from 42 geographical points belonging to 8 governorates in the north of Egypt (Table1 and Figure1). Each governorate was considered a subpopulation. It was taken into consideration that there weren't relations among rabbits under study when samples were collected. Rabbit hair samples were picked with a bulb and kept in small plastic bags, whereas tissue samples were kept in Eppendorf tubes containing 95% ethanol.

Table 1. The governorates with the selected cities, centers and villages where rabbits' hair and tissue samples were collected.

Subpopulation	Location of samples	Total number of samples
Damietta (DAM)	Damietta center, El Zarqa, Faraskur and Kafr Saad.	29
Dakahlia (DAK)	Agga, El Mansoura, Talkha, Bani Ebid, Shirbin and Bilqas.	24
Kafr El sheikh (KFR)	Desouk, Sakha, Metoubes, Fuwah, Qallin and El Riyadh.	23
Beheira (BEH)	Rashied, Abu Hummous, Shabrakhit, Kafr El Dawar, Koom Hammadah and Damanhour.	38
Gharbia (GHR)	El Mahala El Koubra, Basion, Tanta, Zefta and Qutour.	39
Menoufia (MEN)	Shebeen ElKoom, Menouf, Quesna, El Bagour, Talla, Birket El sab'a and Ashmoun.	50
Sharqia (SHR)	Faqos, Kafr Saqr, Abu Hammad and Abu Kbeir.	34
Qalyubia (QAL)	El Qanater El- Khayreya, Benha, Qaha and Shubra ElKheima.	37

Figure 1. A representative map of the Delta region at the Northern part of Egypt, showing the selected areas (cities, centers and villages) where the studied rabbit populations samples (hairs and tissues) were collected. Map source: https://4.bp.blogspot.com

Laboratory Work

The DNA extraction was carried out by alkaline lysis for the hair bulb and tissue samples according to a previous report (CINELLI *et al.*, 2007) following the standard instructions. Initially, the quality of DNA was assessed by 0.8% agarose gel. After passing the quality control step, a total of 28 microsatellite loci (Invitrogen, France) for rabbit biodiversity were studied on the purified DNA. The PCR reactions for 28 microsatellite markers were performed in 5 multiplexes as the suggestion of the PanelPlex PCR design. The PCR reaction was carried out by Verti TM thermal cycler machine (Applied Biosystems, USA). The condition of PCR for each multiplex is shown in supplemental Table S1. 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 5 software (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

The analysis of molecular variance (AMOVA), mean number of observed alleles (MNa) for each subpopulation, number of alleles for each loci (NA), number of effective alleles (Ne) for each locus, Shannon Index (SI) for each loci, observed and expected heterozygosity (H_o and H_e) for each subpopulation and locus and analysis for each subpopulation and locus were calculated by the GENAIEX 6.4.1 program (PEAKALL and SMOUSE, 2012). The software of FSTAT 2.9.3.2 was used for calculating the allelic richness (Ar) as a measure of the number of alleles independent of sample size, hence allowing comparison among different sample sizes (GOUDET, 2001). The same software was used to estimate the co-efficient for inbreeding within populations (F_{IS}) and for differentiation among populations (F_{ST}). The software of Genepop 4.0 was used to measure the deviation from Hardy-Weinberg equilibrium (dHWE) at each locus within subpopulations, following the method of ROUSSET (2008). The testing for bottlenecks was performed by BOTTLENECK v.1.2.02 (PIRY *et al.*, 1999). The test was established using Wilcoxon ($P > 0.05$) signed-rank method 1000 simulations under two models: the two-phase model (TPM) and the stepwise mutation model (SMM). The calculation of polymorphism information content (*PIC*) was carried out using Cervus 3.0.6 software (KALINOWSKI *et al.*, 2007). The neighbor- joining (NJ) tree according to the Reynolds matrix, discriminant analysis, principal components (DAPC) and heatmap were carried out by R package adegenet V.3.5.0 (R DEVELOPMENT CORE TEAM, 2008). The evaluation of population structure was performed according to Bayesian clustering analysis by employing the structure 2.3.4 program (PITCHARD *et al.*, 2000) and based upon independent runs using 100000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 50000 steps, when K values were changed from 1 to 10. The statistical ΔK was computed to detect the highest rate of change in the log-likelihood between successive for a detailed graphic explanation (EVANNO *et al.*, 2005).

RESULTS

Genetic Variability and Bottleneck for Tested Subpopulations

The values of MNa, H_o, H_e, AR, dHWE, and F_{IS} are presented in Table 2. The MNa and Ar ranged from 6.997 and 3.88 to 5.996 and 2.28 in QAL to DAM, respectively. The lowest

values of dHWE were recorded in QAL (1). Whilst, the highest were in DAM (24). Both H_e and H_o frequencies within populations across loci were 0.550 (ranging from 0.507 to 0.597) and 0.406 (ranging from 0.352 to 0.458), respectively. The F_{IS} was significant in all the subpopulations studied. It varied from 0.487 to 0.253 for DOM to QAL, respectively. The genetic bottleneck situation for 8 subpopulations is illustrated in Figure 2. The genetic bottleneck was observed in critical situations in the north (DAM, DAK, KFR, BEH and GHR), while, it disappeared in the middle (MEN), east (SHR) and the south (QAL).

Table 2. The genetic variability of 8 NDER subpopulations based on the 28 microsatellite markers

Subpopulation / parameter	DAM	DAK	KFR	BEH	GHR	MEN	SHR	QAL	Mean
MNa ±SD	5.996 ±0.274	6.191 ±0.250	6.397 ±0.277	6.496 ±0.225	6.635 ±0.340	6.888 ±0.291	6. 961 ±0.270	6.997 ±0.264	6.571 ±0.274
H_o ±SD	0.352 ±0.031	0.376 ±0.034	0.386 ±0.033	0.399 ±0.039	0.400 ±0.031	0.419 ±0.034	0.451 ±0.035	0.458 ±0.036	0.406 ±0.034
H_e ±SD	0.507 ±0.032	0.518 ±0.040	0.536 ±0.034	0.537 ±0.042	0.532 ±0.046	0.585 ±0.043	0.590 ±0.034	0.597 ±0.047	0.550 ± 0.040
Ar ±SD	2.280 ±0.141	2.310 ±0.149	2.891 ± 0.156	2.962 ±0.169	3.620 ±0.178	3.681 ±0.193	3.700 ±0.222	3.881 ±0.319	3.165 ± 0.191
dHWE	24	20	16	8	6	4	2	1	10.12
F_{Is}	0.487[a]	0.471[a]	0.400[b]	0.392[b]	0.338[c]	0.289[d]	0. 277[d]	0.253[d]	0.363

DAM: Damietta, DAK: Dakahlia, KFR: Kafr El Sheikh, GHA: Gharbia, BEH: Beheira, MEN: Menoufia, SHR: Sharqia, QAL: Qalyubia:. MNa: Mean Number of observed alleles, SD: Standard deviation, H_o: Mean observed of Heterozygosity, H_e: Mean expected of Heterozygosity, Ar: Mean allelic richness, dHWE: number of loci deviated from Hardy-Weinberg equilibrium and F_{IS}: inbreeding coefficient per breed

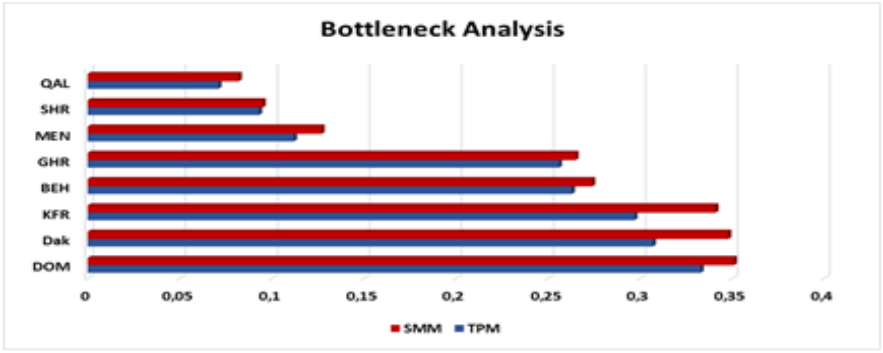

Figure 2. The bottleneck analysis for NDER. TPM: two-phase model and SMM: stepwise mutation model (). DAM: Damietta, DAK: Dakahlia, Kafr El Sheikh: KFR, Gharbia: GHA, Beheira: BEH, Menoufia: MEN, Sharqia: SHR, Qalyubia: QAL.

Loci Polymorphism

A total of 184 alleles were detected across 28 loci (Table 3). The current study reported that the mean Ne and Na were 4.122 and 6.571. The Na and Ne were varied from 16 and 1 to 9.875 and 0.575 (INRACCDDV0205 and INRACCDDV0176, respectively). The 28 loci analyzed, reached a H_o higher than 0.2 and stretched from 0.261 (SAT12) to 0.599 (INRACCDDV0102) with a mean 0.404. The H_e per locus is ranged from 0.362 (INRACCDDV0101) to 0.700 (INRACCDDV0087) and the mean was moderated (0.551). The values of the *PIC* were varied from 0.912 to 0.215 (INRACCDDV0205 and INRACCDDV0176, respectively). The average of

SI among loci was 0.989, while it ranged from 0.281 to 1.556 for INRACCDDV0176 and INRACCDDV0087, respectively.

Table 3. Genetic variability for each locus in all subpopulations

Multiplex	Locus	Na	Ne	H_o	± SD	H_e	± SD	*PIC*	SI
1	SAT03	9	5.750	0.514	± 0.042	0.582	± 0.043	0.679	1.076
	SAT04	10	6.375	0.488	± 0.041	0.664	± 0.040	0.802	1.311
	SAT05	9	3.125	0.264	± 0.040	0.393	± 0.074	0.703	0.718
	SAT07	7	4.750	0.449	± 0.037	0.618	± 0.055	0.682	1.207
	SAT08	4	3.125	0.410	± 0.031	0.542	± 0.048	0.505	0.910
2	INRACCDDV0101	5	2.750	0.351	± 0.048	0.362	± 0.046	0.523	0.589
	INRACCDDV0106	6	4.000	0.470	± 0.041	0.589	± 0.063	0.589	1.048
	INRACCDDV0108	10	7.500	0.562	± 0.043	0.740	± 0.016	0.721	1.492
	INRACCDDV0176	1	0.575	0.386	± 0.020	0.418	± 0.042	0.215	0.218
	INRACCDDV0203	6	3.375	0.292	± 0.035	0.603	± 0.050	0.552	1.138
3	INRACCDDV0102	6	4.750	0.599	± 0.041	0.647	± 0.028	0.561	1.235
	INRACCDDV0169	6	3.750	0.488	± 0.052	0.593	± 0.040	0.559	1.051
	INRACCDDV0192	7	4.500	0.578	± 0.040	0.636	± 0.035	0.601	1.165
	INRACCDDV0205	16	9.875	0.390	± 0.042	0.579	± 0.035	0.912	1.124
	INRACCDDV0228	4	2.750	0.465	± 0.044	0.533	± 0.065	0.502	0.924
	SAT13	4	2.250	0.298	±0.028	0.621	± 00.36	0.498	0.702
4	INRACCDDV0004	6	3.125	0.410	± 0.047	0.536	± 0.055	0.512	1.044
	INRACCDDV0182	5	3.125	0.294	± 0.049	0.469	± 0.040	0.505	0.770
	INRACCDDV0185	4	3.000	0.362	± 0.011	0.497	± 0.044	0.496	0.660
	INRACCDDV0259	8	5.800	0.433	± 0.040	0.521	± 0.041	0.663	0.999
	INRACCDDV0313	6	4.500	0.414	± 0.034	0.599	± 0.044	0.615	1.112
5	INRACCDDV0087	10	4.925	0.645	± 0.029	0.700	± 0.036	0.808	1.556
	INRACCDDV0119	6	3.500	0.362	± 0.020	0.554	± 0.049	0.586	0.959
	INRACCDDV0140	6	3.750	0.404	± 0.029	0.464	± 0.048	0.600	0.830
	INRACCDDV0157	7	4.375	0.267	± 0.030	0.563	± 0.053	0.635	1.039
	INRACCDDV0190	3	2.750	0.325	± 0.012	0.463	± 0.046	0.308	0.758
	INRACCDDV0201	7	3.250	0.362	± 0.032	0.508	± 0.054	0.645	0.940
	SAT12	6	4.125	0.261	± 0.036	0.621	± 0.035	0.573	1.132
	Mean	6.571	4.122	0.404	± 0.035	0.551	0.0460	0.591	0.989

Na: Allele number of each locus, Ne: effective number of alleles, H_o: mean observed and H_e: expected heterozygosity with SD: standard deviation, *PIC*: polymorphic information content, and SI: Shannon Index.

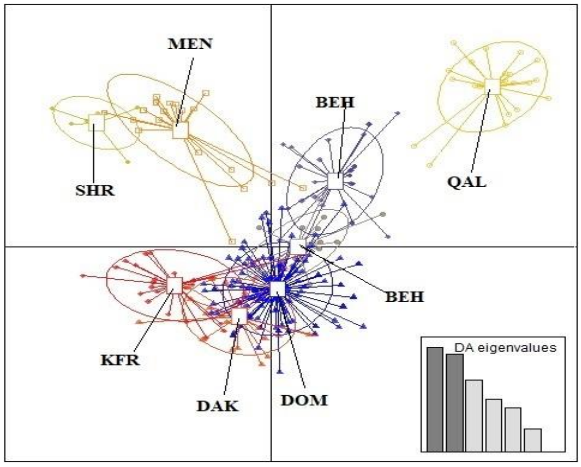

Figure 3. Discriminant analysis principal components (DAPC) for NDER subpopulations. DAM: Damietta, DAK: Dakahlia, KFR: Kafr El Sheikh, GHA: Gharbia, BEH: Beheira, MEN: Menoufia, SHR:Sharqia, QAL:Qalyubia.

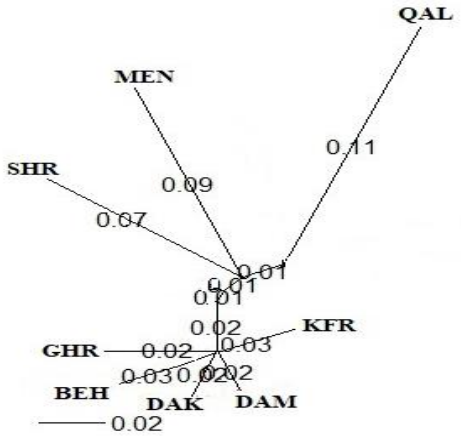

Figure 4. The neighbour-Joining tree among native NDER subpopulations. DAM: Damietta, DAK: Dakahlia, KFR: Kafr El Sheikh, GHA: Gharbia, BEH: Beheira, MEN: Menoufia, SHR:Sharqia, QAL:Qalyubia.

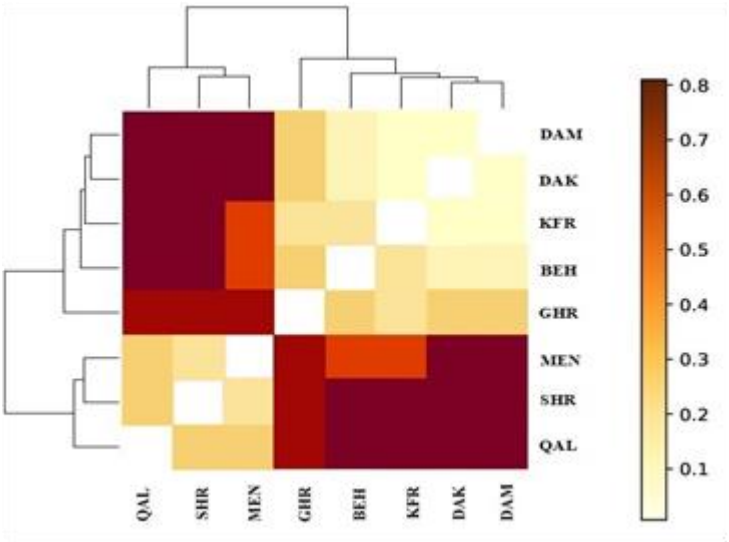

Figure 5. Heatmap and dendogram calculated among the NDER subpopulations. The color palette ranged from Yellow (low values) to red (high value). DAM: Damietta, DAK: Dakahlia, KFR: Kafr El Sheikh, GHA: Gharbia, BEH: Beheira, MEN: Menoufia, SHR:Sharqia, QAL:Qalyubia.

Table 4. The pairwise genetic differentiation (F_{ST}) is above the diagonal and genetic distance of Nei below the diagonal.

Subpopulation	DAM	DAK	KFR	BEH	GHR	MEN	SHR	QAL
DAM	-	0.019	0.023	0.020	0.089	1.236	2.085	2.136
DAK	0.018	-	0.029	0.027	0.100	0.902	1.992	2.089
KFR	0.026	0.072	-	0.039	0.123	0.752	1.986	1.945
BEH	0.108	0.096	0.031	-	0.256	0.666	1.856	1.733
GHR	0.394	0.068	0.051	0.043	-	0.631	1.606	1.441
MEN	1.112	0.983	0.949	0.901	0.422	-	0.301	0.091
SHR	1.120	1.106	1.094	1.005	0.621	0.356	-	0.071
QAL	1.391	1.295	1.168	1.068	0.566	0.268	0.089	-

DAM: Damietta, DAK: Dakahlia, Kafr El Sheikh: KFR, Gharbia: GHA, Beheira: BEH, Menoufia: MEN, Sharqia: SHR, Qalyubia: QAL.

Structure Analysis

According to STRUCTURE analysis (Figure 6, a and b), the most likely value of ΔK was obtained when K=8, including 8 subpopulations that were assigned to four clusters (each color represented in the cluster). We noticed that the north subpopulations (Dom, DAK, KFR, BEH, and GHR) are clustered together. Other subpopulations were clustered separately for each one. The black line separates the individuals of the eight NDER subpopulations.

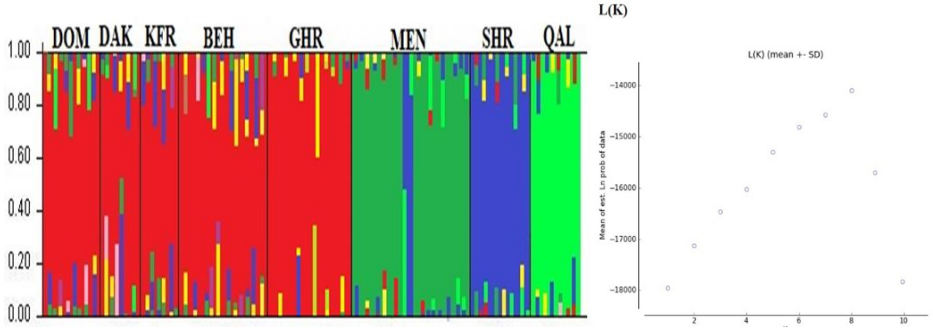

Figure 6. The result of STRUCTUR analysis. a) the estimation of structure for NDER subpopulations. In each K, the colors represent the percentage of each cluster that is present in each rabbit population. b) K: number of assumed clusters in this study. DAM: Damietta, DAM: Damietta, DAK: Dakahlia, KFR: Kafr El Sheikh, GHA: Gharbia, BEH: Beheira, MEN: Menoufia, SHR:Sharqia, QAL:Qalyubia.

AMOVA Analysis

The analysis of AMOVA results (Table 5) demonstrates that the variation was within individuals (58.843%), 28.9115 among individuals, and 12.246% among subpopulations that were compatible with the F_{ST} results.

Table 5. AMOVA summery result of NDER subpopulations

Source	df's	SS	Est. Var.	%
Among subpopulations	7	616.884	1.135	12.246%
Among Individuals	266	2877.523	2.681	28.911%
Within Individuals	274	1495.000	5.456	58.843%
Total	547	4989.407	9.272	100%

Degree of freedom (df); Sum of square (SS); Estimated variance (Est.Var). Components have significant differences from 0 at *p<0.0001*

DISCUSSION

This study reported that low genetic values were recorded across 8 subpopulations in NDER. The same observation was reported in Amami Japanese rabbits (OHNISHI *et al.,* 2017) and indigenous Chinese rabbits (REN *et al*., 2019). It was recorded that the mean of H_e (0.550) > H_o (0.406) that are in agreement with ADEOLU *et al.* (2021), BADR *et al*. (2019), and EMAM *et al.* (2016). It indicated a little variability according to LI *et al.* (2020); KÖVÉR *et al.* (2023). In addition, the mean of F_{IS} (0.363) with high dHWE (10.12 ± 0.611) could imply inbreeding for the most subpopulations.

The values of MNa and Ar increased in QAL (6.997 and 3.88), SHR (6.961 and 3.70), and MEN (6.888 and 3.68) than other subpopulations. The high number of MNa and Ar among comparative populations and breeds contribute to its adaptability to more agro-ecological zones (AGAVIEZOR *et al.,* 2012; REHMAN *et al.,* 2021; CANALES *et al.,* 2023). Moreover, the high F_{IS}

values that recorded as significant in the DAM (0.487), DAK (0.471), KFR (0.400), BEH (0.392), and GHR (0.338). There is strong evidence for inbreeding that could be leading to a bottleneck situation in the north. According to KURU *et al.* (2022) and CANALES *et al*. (2023)**,** the positive and significant values of F_{IS} increase could be explained by the imbalance of the dHWE and low herd size that we observed in the north (DAM, DAK, KFR, BEH, and GHR). In the same context, the low values of MNa and Ar recorded in the northern subpopulations could be a sign of genetic drift (RAHAL *et al.,* 2020; GAO *et al.,* 2023; CANALES *et al.,* 2023). In contrast, the low F_{IS} values that were recorded in the south, east, and middle subpopulations (QAL, SHR, and MEN by 0.253, 0.277, and 0.289, respectively) might be strong evidence for random meetings and large herd size (RAHAL *et al*., 2021).

In Figure 2, two bottleneck detections of Wilcoxon models (TMP and SSM) were consistent with the bottleneck scenario found in the north subpopulations (DAM, DAK, KFR, BEH, and GHR) according to the results of the sign rank test, sign test, and standard difference test (TPM: 0.334, 0.307, 0.297, 0.263, and 0.256, respectively; SMM: 0.351, 0.348, 0.341, 0.274, and 0.265, respectively). The bottleneck was previously recorded in Helsinki rabbit populations (LAIHO, 2021). On the other hand, the same models observed that the South subpopulations (MEN, SHR, and QAL) were in a suitable position (TPM: 0.112, 0.093, and 0.071, respectively; SMM: 0.127, 0.095, and 0.082, respectively). Previous values are concurring with ZIEGE *et al.* (2020) in European rabbits. The limitation of subpopulation size could be confirmed by the bottleneck found (MACHOVÁ *et al.,* 2023).

In the current study, we reported 184 total alleles across 28 microsatellite loci in native rabbits. EMAM *et al.* (2017) reported that 151 alleles were found for 19 microsatellite loci in native rabbits in Middle Egypt (NRME), while 119 alleles of 36 microsatellite loci were found in 12 Tunisian local rabbit populations (BEN LARABI *et al.,* 2014). In commercial rabbits, EL-AKSHER *et al.* (2017) found 108 alleles for 16 microsatellite loci in Egypt. In Nigeria, OMOSOTO *et al.* (2019) detected 224 alleles across 6 microsatellite loci in 4 commercial rabbit lines. We found about 89% of the microsatellite loci in high content of *PIC* > 0.50, while 7% and 4% of the microsatellite loci showed formative (0.25 < *PIC* <0.5) and low formative values (*PIC* < 0.25), respectively. Several studies recorded high formative *PIC* values in rabbits. About 84% and 75% were highly informative in the middle Egypt native rabbit population (EMAM *et al.,* 2017; ABDEL-KAFY *et al.,* 2018). BADR *et al.* (2019) found 83% of loci expressed high informative values of *PIC* in 4 populations of commercial rabbits in Egypt. Both Na and *PIC* values were high. It could be due to a high number of subpopulation samples and an increased number of microsatellite loci (SHERIFF and ALEMAYEHU, 2018; JIN *et al.,* 2023; BORA *et al.,* 2023). The current study recorded a 6.571 for the Na value. It was not in agreement with ABDEL-KAFY *et al*. (2018) and BADR *et al*. (2019), when they found values were 4.156 and 3.740, respectively. The variation in MNa recorded might be explained by several sampling points of subpopulation when carrying out this study. The mean value of SI was less than 1 (0.989), which could indicate a limitation of genetic variability (BORA et al., 2023).

The DAPC showed that the QAL, SHR, and most of the MEN subpopulations (in the south, east, and middle Delta) are separated, while the overlapping is noticeable among others (DAM, DAK, KFR, BEH, and GHR) in the north of the Delta (Figure 3). Over and above, the same results were found in the NJ tree (Figure 4), heatmap (Figure 5), and STRUCTURE (Figure

6). All the north subpopulations clustered together, possibly for open borders among sample collection points (27 points in less than 270 km). The overlapping interpretation might be to the gene flow for a long time that reached to genetic swamping (ÁLVAREZ *et al.*, 2021; KOLTER *et al*., 2021). In addition, it depresses the outbreeding possibility inside subpopulations (DOGLIN *et al*., 2007; POOK *et al*., 2020). The South, Middle, and East (QAL, MEN, and SHR) were grouped separately in each cluster.

The results of AMOVA (Table 5) with a high percentage of genetic variation among individuals (28.91%) could be a sign of environmental conditions (PAVLOVA *et al*., 2017; MA *et al.*, 2020). The percentage among populations variation was 12.25%, which is strong evidence of the closing inbreeding system and a direct result of genetic swamping (NÚÑEZ-TORRES and ALMEIDA-SECAIRA, 2022). In contrast, ADEOLU *et al*. (2021) reported that random breeding systems in Nigeria had lower percentages among populations 1 and 4%.

CONCLUSION

Our findings demonstrate low genetic variability in the Delta native rabbits. The northern subpopulations showed low and high values of inbreeding and expressed a bottleneck threat (DAM, DAK, KFR, BEH, and GHR). In parallel, the results of DAPC and structure confirmed overlap in the northern subpopulations. The south (QAL), middle (MEN), and east (SHR) showed higher genetic variability and found themselves in a stable position. The current study could be used as a genetic profile for native rabbits in the Delta region of Egypt. Also, it provides valuable information for decision-makers in the regional and national livestock institutions concerning planning and implementing conservation strategies for native rabbits in the Egyptian Delta which could contribute to achieve the UN sustainable goals number 2 (zero hunger), 12 (ensure sustainable consumption and production patterns), and 15 (stop biodiversity loss).

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ETHICS STATEMENT

All protocols of animal experiments were reviewed and approved by the Institutional of animal care and use committee (IACUC), faculty of science, Menoufia University, Egypt (MUFA/F/AP/3/23).

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MIKROSATELITSKI MARKERI U GENETIČKOM ISTRAŽIVANJU DOMAĆIH ZEČEVA U EGIPATSKOJ DELTI

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Izvod

Ljudski interes za egzotičnim rasama životinja u poljoprivrednom sektoru doveo je do propadanja lokalnih rasa. Biodiverzitet je važan indikator za merenje raznovrsosti genetičkih resursa domaćih životinja unutar poljoprivrednih ekosistema. Mikrosatelitski markeri su važni alati za određivanje genetskog statusa rasa, populacija i subpopulacija. Dvadeset osam mikrosatelitskih lokusa korišćeno je za istraživanje 274 bioloških uzoraka prikupljenih iz domaće populacije zečeva Delta Egipta (NDER) na severu Egipta, kojoj pripada osam subpopulacija (Damietta, Dakahlia, Kafr El Sheikh, Beheira, Gharbia, Menoufia, Sharqia i Qalyubia). Ovo istraživanje je pokazalo da su očekivane vrednosti heterozigotnosti (H_e) veće od uočene heterozigotnosti (H_o). Identifikovano je ukupno 184 alela, sa srednjim vrednostima od 6.571 i 4.122 kao efektivni aleli. Oko 89% mikrosatelitskih markera ispoljilo je visoke informativne vrednosti u sadržaju informacija o polimorfizmu (*PIC*). Poređenje između 8 NDER subpopulacija pokazalo je niske parametre genetske varijabilnosti sa visokim vrednostima koeficijenta inbreedinga (F_{IS}) na severu (Damietta, Dakahlia, Kafr El Sheikh, Beheira i Gharbia). Međutim, vrednosti genetskih varijabli su se povećavale sa smanjenjem F_{IS}-a u srednjoj (Menoufia), istočnoj (Sharqia) i južnoj (Qalyubia) delti. Nadalje, u diskriminantnoj analizi, glavne komponente (DAPC) su pokazale preklapanje na severu. U istom kontekstu, stablo spajanja suseda (NJ) i toplotna karta pokazali su genetsku konvergenciju među severnom subpopulacijom. Analizom STRUKTURE pronađena su 4 klastera (K= 8). Severne podpopulacije bile su izražene u jednom klasteru, dok su ostale u srednjem, istočnom i južnom delu bile zaseban klaster za svaku subpopulaciju. Naši nalazi pokazuju da NDER populacija pati od genetskog drifta u subpopulacijama severne Delte. Naprotiv, južne, istočne i srednje subpopulacije pokazale su veću genetsku varijabilnost. Strategiju ispravnog parenja treba negovati kako bi se poboljšale genetske osobine kunića.

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