World Rabbit Science

World Rabbit Sci. 2017, 25: 9-16
doi:10.4995/wrs.2017.5298

DIVERSITY ASSESSMENT AMONG NATIVE MIDDLE EGYPT RABBIT POPULATIONS IN NORTH UPPER-EGYPT PROVINCE BY MICROSATELLITE POLYMORPHISM

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Abstract: Safeguarding biodiversity is an important goal for animal production in developed countries. This study investigated genetic diversity among native Middle-Egypt rabbit (NMER) populations in North Upper-Egypt province by using microsatellite polymorphism. Nineteen microsatellite loci were used in the study and an area of 231 km was surveyed, as native rabbits covered 14 points belonging to four Northern Upper Egypt governorates (South Giza, Fayoum, Beni Suef and Minya). Standard statistical parameters of genetic variability within and between populations confirmed that the highest genetic diversity was found towards the south. Among NMER populations, the mean number of alleles per locus was lowest in South Giza (5.32), while it was highest in Minya (6.00). This study found that NMER featured a high number of private alleles ranging between 7 and 11 (mean value was 10.5). Results also showed a high genetic diversity in NMER populations and that heterozygosity ranged between 0.384 and 0.445, strongly indicating extensive genetic variation in the NMER populations. The mean values of observed and expected heterozygosity were 0.405 and 0.612, respectively. Factorial correspondence analysis and neighbour joining trees (NJ) showed 2 main NMER rabbit groups: the Northern group (South Giza and Fayoum) and the Southern group (Beni Suef and Minya). All populations showed a high percentage of assignment in this study (0.913 to 0.946). The structure analysis showed that each population existed in separate clusters. This research provides an overview of genetic diversity of NMER populations in the Northern Upper Egypt province for the first time. In conclusion, results of this study could be used to designate priorities for conservation of NMER populations.

Key Words: rabbit, North Upper Egypt, genetic diversity, microsatellite.

INTRODUCTION

Animal Genetic Resources (AnGR) play a pivotal role in guaranteeing food security for over a billion people (Ligda and Zjalic, 2011). The increase in human population (especially in lesser-developed countries) should/will require an increase in livestock production with alternative strategies to avoid meat shortages and a protein gap (FAO, 2007).

Rabbit production by small-scale farmers plays an important role in solving the meat shortage problem in Egypt, (Galal and Khalil, 1994). Local Egyptian rabbit breeds were created by selecting lines of rabbits for meat production (Egyptian Red Baladi, ERB; Egyptian Black Baladi, EBB and Egyptian White Baladi, EBW). These breeds were produced by crossbreeding between native breeds and Flemish Giants (Badawy, 1975; Khalil, 2002). In addition, Egyptian Gabali Saini (EGS) are still raised in desert areas and recently in captivity (Ministry of Agriculture and Land Reclamation in Egypt, FAO2003). The Egyptian countryside is considered a valuable store of local breeds. The Native Middle-Egypt rabbit (NMER) is a famous breed, which belongs to North Upper Egypt (Abdel-Kafy *et al.,* 2011). This breed is required/wanted by consumers but is suffering from neglect in large-scale production (compared with foreign breeds).

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doi:10.4995/wrs.2017.5298

Assessment of genetic variability in rabbits is an important issue in preserving genetic resources and maintaining future breeding options (Galal *et al.,* 2013). Genetic variation is required to change the genetic make-up or genetic potential of domestic animal species to suit our needs (Ormandy *et al.,* 2011). Assessing genetic variability, as well as relationships within and among the population, parentage determination, possible bottlenecks, linkage disequilibrium and inbreeding coefficients are also essential for analysing complete population structure (El-Hentati *et al.,* 2013). The complete population structure helps to plan strategies for conservation and breed development (Martín Collado, 2013). Recent trends use molecular techniques for characterisation that detect genetic variation at the DNA level. Various markers have been used to assess the population structure and genetic variation both between and within breeds (Abdel-Mawgood, 2012).

This study is aimed at the characterisation of genetic diversity of NMER populations in Northern Upper Egypt governorates at 14 geographical points. Native Middle-Egypt rabbit samples were collected randomly. Nineteen microsatellite markers were used to elucidate genetic variability degree and pattern, in addition to investigating the genetic relationship between NMER populations.

MATERIALS AND METHODS

Sampling and genotyping of microsatellite markers

A total of 120 rabbit samples belonging to NMER populations in the Northern Upper Egypt governorate (Figure 1) were used to carry out this study according to Abdel-Kafy *et al.* (2011). The survey covered all the provinces (231 km) in rural areas. Random hair and ear tissue samples were collected from 14 central points belonging to 4 governorates (Giza, Fayoum, Beni- Suef and Minya). In this study, we considered each governorate as a single population. The geographical central points (Figure 2) were south of Giza (Al Badrashine, Umm khenan and Ayyat), Fayoum (Fayoum city, Ibsheway, Itsaa and Sonoras), Beni Suef (Somsta, Markaz Naser, Elfashn, Beba and Seds) and Minya (Maghagha, Beni Mazar and Abo Qorqas). Each central point contained 3-6 branch points. Samples of DNA were extracted using DNeasy Blood & Tissue Kit (Qiaamp, Qiagen, GmbH, Hilden, Germany).

Microsatellite DNA Markers Selection and polymerase chain reaction (PCR)

Nineteen microsatellite loci (Table 1) were used in this study, namely (INRACCDDV 0087, 0089, 0102, 0104, 0119, 0140, 0157, 0169, 190 0192, 0201, 205, 288, Sat3, 4, 5, 7, 8 and 13). All loci were obtained from Invitrogen (France). The PCR was carried out using 5µL master mix (Qiagen 20614), 1 µL multiplex microsatellite loci (forward 0.1/primer reverse), 1 µL DNA and 3 µL distilled water. Amplification of PCR was carried out using a Bio rad–T100 Thermal. The standard PCR cycle was usually run as follows: primary denaturation: 95°C for 15 min. then: 30 cycles at 95°C for 30 s; 58-58.7°C for 60 s and 72°C for 45 s; a final extension of 20 min at 60°C, then storage at 4°C. The presence of PCR products was analysed by horizontal gel electrophoresis system (mini gel, Biometra®EU), using agarose gel 2% and staining with ethidium bromide. The Quantity one® software was used to measure PCR product bands with reference to the Ladder.

a b c d

Figure 1: Native Middle-Egypt rabbits from a, South Giza; b, Fayoum; c, Beni Suef and d, Menya.

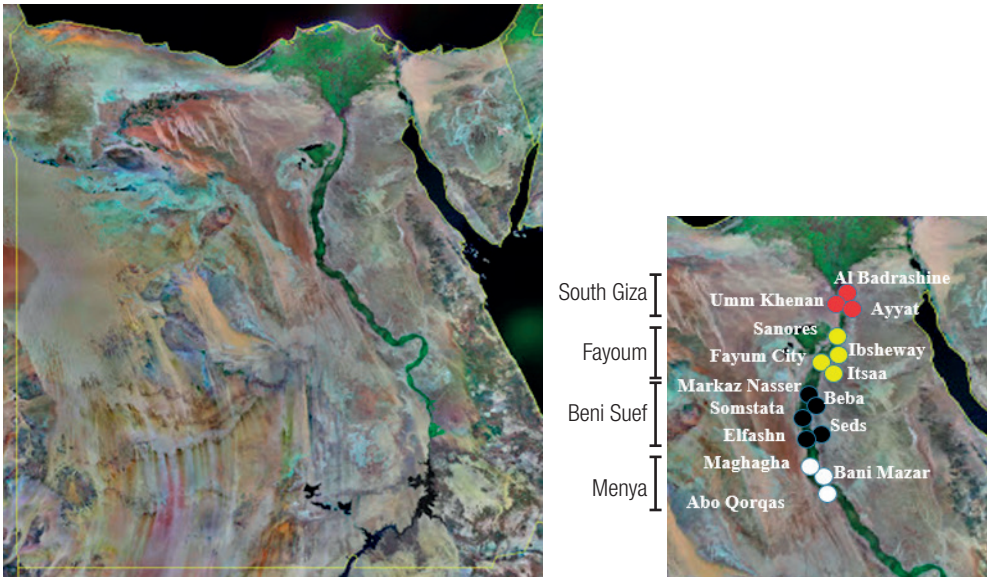

Figure 2: Geographical sampling locations. Red Points for South Giza, yellow points for Fayoum, Black points for Beni Suef and White points for Menya.

Analysis of microsatellite data

POPGENE software (version 3.2, Yeh *et al.,* 1999) was used to estimate the number of alleles per locus (NA) observed at each locus and the mean number of alleles (MNA) per breed. Fixation index per population (F_{IS}) was estimated, based on 1000 bootstraps, using GENETIX 4.05 software (Belkhir *et al.,* 1996-2004), as was observed and expected heterozygosity (H_0 and H_E) per locus for all populations. In addition, the same software was used to draw factorial correspondence analysis (FCA) based on allele frequency, which gave the chance to show the results using a graphic model with a considerable descriptive value. Reynolds distance was calculated among different populations using the POWER MARKER (Liu and Muse, 2005). The neighbour joining tree (NJ) was visualised in Mega tree explorer (Tamura *et al.,* 2013) according to the Reynolds matrix. The number of private alleles (PVT) was calculated through a direct count of allelic frequencies calculated by the software CONVERT (Glaubitz, 2004). Cervus 3.0.6 software (Kalinowski *et al.,* 2007) was used to calculate polymorphism information content (PIC) and the Hardy-Weinberg equilibrium (HWE) for test significance. The population structure evaluation was based on a Bayesian clustering analysis by employing the structure 2.3.4 program (Pritchard *et al.,* 2000). This method uses multi locus genotypes to infer the fraction of genetic ancestry for all individuals and tested breeds. The analysis carried out was based upon independent runs using 500000 Markov Chain Monte Carlo (MCMC) iterations and a burn-in of 20000 steps and was also performed for $2 \leq K \leq 6$ (K=number of assumed clusters). The statistic ΔK was computed, which detected the highest rate of change in the log-likelihood between successive runs for a detailed graphic explanation of ΔK calculations by using Evanno *et al.* (2005).

RESULTS

Marker polymorphism and Genetic diversity

The genetic variability of the studied populations is presented in Table 1. A total of 151 alleles were detected across 19 microsatellite loci in the 4 NMER populations. The NA ranged between 3 and 17 (INRACCDV089 and 205, respectively). The values of PIC ranged from 0.212 to 0.797 (INRACCDV089 and 87, respectively). The majority of the markers were characterised with high values per locus (>0.5), except for 3, namely INRACCDV089, 140 and 228. The MNA and PVT values (Table 2) ranged in ascending order from the South Giza population (5316±0.351 and 7)

Table 1: Genetic variability for each locus in all populations.

Locus	Allele range	NA	H_0±SE	H_E±SE	Mean PIC	HWE
INRACCDV087	190-214	9	0.653±0.012	0.823±0.008	0.797	**
INRACCDV089	94-98	3	0.220±0.101	0.238±0.077	0.212	NS
INRACCDV102	128-136	8	0.551±0.035	0.770±0.025	0.732	**
INRACCDV104	119-127	9	0.364±0.071	0.649±0.031	0.594	**
INRACCDV119	229-243	5	0.450±0.006	0.607±0.018	0.528	*
INRACCDV140	183-187	5	0.409±0.025	0.549±0.050	0.497	NS
INRACCDV157	138-144	6	0.286±0.045	0.733±0.025	0.681	*
INRACCDV169	174-180	8	0.483±0.054	0.771±0.039	0.732	**
INRACCDV190	200-212	6	0.240±0.056	0.582±0.049	0.520	**
INRACCDV192	114-130	9	0.517±0.050	0.770±0.069	0.652	*
INRACCDV201	133-143	8	0.325±0.036	0.695±0.056	0.652	**
INRACCDV205	176-190	17	0.370±0.107	0.724±0.069	0.705	**
INRACCDV228	228-232	4	0.293±0.028	0.329±0.038	0.307	NS
SAT03	146–162	10	0.438±0.071	0.675±0.021	0.625	**
SAT04	195–240	14	0.536±0.073	0.809±0.025	0.784	***
SAT05	206–234	9	0.367±0.072	0.623±0.083	0.568	*
SAT07	184–195	7	0.382±0.071	0.620±0.068	0.582	**
SAT08	136–158	7	0.420±0.043	0.714±0.040	0.661	**
SAT13	114–128	7	0.387±0.033	0.545±0.062	0.505	*

NA: number of observed alleles. H_0 and H_E: mean observed and expected heterozygosity. Mean PIC: mean polymorphism information content per locus, HWE: Hardy-Weinberg Equilibrium. SE: standard error.
*P<0.05; **P<0.01,*** P<0.001, NS: non-significant.

to Minya (6000±0.587 and 15). Overall, the highest heterozygosity within population H_0 was recorded in the Minya population (0.445), while the lowest value was recorded in South Giza (0.384). In contrast, the H_E across all the populations varied between 0.558 in Minya and 0.619 in South Giza. It was found that F_{IS} per population was significantly higher in the Minya and Beni Suef populations (0.387 and 0.374) than in Fayoum and the South Giza populations (0.297 and 0.279), respectively (Table 2).

Population Classifications and Relationships among NMER

FCA (Figure 3) further assesses the differentiation between individuals within each population with a three-dimension construction plot. There was a 9.07% variation in the first axis and different populations were separated from each other. Meanwhile, the second and third axis explained the total variation of 6.88% and 9.05%, respectively. The pairwise genetic distance indices of Reynolds genetic distances were used to construct an NJ tree (Figure 4). As shown in Figures 3 and 4, the 4 populations were separated in different groups except for the overlap between populations from Beni Suef and Minya (Figure 3). In addition, there were 2 main groups inside the NMER. The

Table 2: Within-population summary statics.

Population	N	MNA±SE	PVT	H_0±SE	H_E±SE	F_{IS}
South Giza	29	5.316±0.351	7	0.384±0.028	0.619±0.040	0.279b
Fayoum	25	5.421±0.473	9	0.390±0.042	0.598±0.042	0.297b
Beni Suef	35	5.632±0.436	11	0.401±0.035	0.601±0.028	0.374a
Menya	31	6.000±0.587	15	0.445±0.027	0.558±0.043	0.386a
Mean value		5.592±0.462	10.5	0.405±0.033	0.612±0.038	0.334

N: Number of samples. MNA: Mean number of observed alleles. SE: standar error. PVT: number of private alleles. H_0 and H_E: mean observed and expected heterozygosity. F_{IS}: intra breed structure.

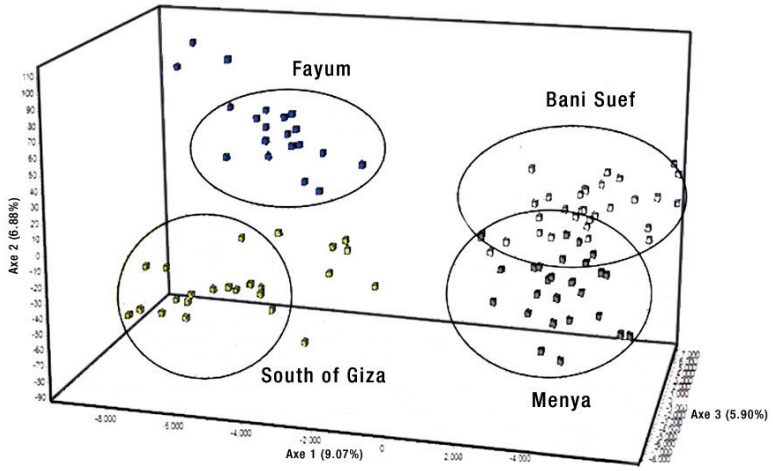

Figure 3: Factorial correspondence analysis for 120 rabbits based on the allele frequencies from microsatellites loci.

first group was expressed as the Northern group (South Giza and Fayoum), while the second was classified as the Southern group (Beni Suef and Minya).

Population Structure

The investigation of population structure was carried out using the Bayesian approach and the number of clusters (K) are illustrated in Figure 5a. The highest ΔK values were obtained for K=4 (Figure 5b). When K=2, two clusters were defined (South Giza and Fayoum in the first cluster, Beni Suef and Minya in the second cluster). In the case of K=4 to K=7, all population groups were detected in separate clusters.

The analysis of the percentage of correctly assigned individuals (q>0.90) for K=4 is shown in Table 3. Results showed that the highest values of rabbit populations were correctly assigned to Beni Suef and Minya (100%). The proportion of membership in the different clusters (Table 3) was totally comparable among the breeds. All breeds displayed a very high assignment percentage (0.922, 0.936, 0.946 and 0.913 for South Giza, Fayoum, Beni Suef and Minya, respectively).

DISCUSSION

As shown in Table 2, the highest diversity values in the current study were expressed towards the south (Table 2). The mean values of MNA in the NMER breed was 5.59. This result is consistent with Alves *et al.* (2015), who found that the MNA was 6.35 in French domestic rabbits. On the other hand, the MNA of NMER in the current study was greater than that of the commercial European rabbit breeds shown in the studies of Bolet *et al.,* 2000; Alves *et al.,*

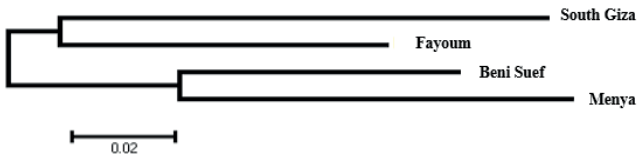

Figure 4: Neighbor-joining tree for the rabbit populations. Figures at nodes represent the bootstrap values over 1000 samples. Abbreviations as in Table 2.

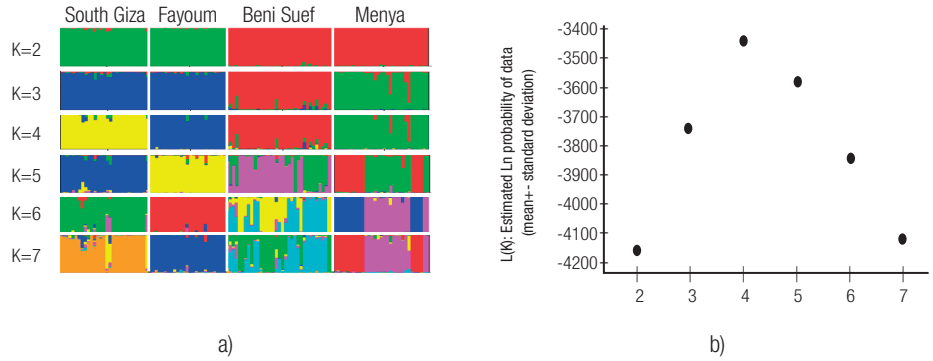

Figure 5: a) Estimated population structure for native Middle-Egypt rabbit in current study. In each K, the colors represent the percentage of each cluster that is present in each rabbit population. b) ΔK calculated from K=2 to K=7 Relation between populations. K: number of assumed cluster.

2015 (3.6 and 3.13, respectively). Grimal *et al.* (2012) used 17 microsatellites to investigate the genetic diversity in local Egyptian rabbit breeds in depth. They reported a lower MNA and PVT (ranging from 3.65 to 3.94 and 1 to 8) than those observed in our study in Table 2. These variations might be due to a genetic bottleneck position in other Egyptian local rabbit breeds (Emam *et al.,* 2016). Positive values $F_{IS.}$ were also observed in the current study. This might mean that individuals in a population are more related than expected under a model of random mating (Pellegrino *et al.,* 2015). These results agreed with Ben Larabi *et al.* (2012) in local Tunisian rabbit populations and European rabbits (Alda and Doadrio, 2014).

Moreover, results in Table 1 showed that 16/19 loci were highly formative in PIC values. These results agree with Shawartz *et al.* (2007), who recorded PIC values ranging from 0.27 to 0.77 by using 14 microsatellites. Moreover, our results in the Sat group agree with those of Wu *et al.* (2008), who found values ranging from 0.505 to 0.684 compared with 0.559 to 0.692 in our study. We also observed that 84% of loci were significant in HWE values (Table 1). Three levels of significance were shown, represented as $P<0.05$; $P<0.01$ and $P<0.001$. Accordingly, this null hypothesis approach allows only the identification of markers with the most severe deviations from HWE, so that the basic issues are large sample sizes, random sample collection and the tested population did not show mutation drift equilibrium (Morin *et al.,* 2009; Welleke, 2010).

According to molecular data analysis in Table 2, NMER in this study showed a high variability. These results are consistent with the lack of selection programmes and the totally absence of bottleneck or genetic drift (Nei *et al.,* 1975; Queney *et al.,* 2000).

The analysis of FCA (Figure 3) and NJ (Figure 4) classified data into 2 main groups according to the geographical location; (Northern and Southern groups). The geographical integration between the south of Giza and the Fayoum points (less than 100 km) may explain why the population in these regions is classified as one group. And the distance between the Beni Suef and the Minya points (less than 70 km) may also explain their classification in the

Table 3: Percentage of correctly assigned animals with q>0.90 and proportion of membership of the 4 rabbit populations for K=4.

	% corr. Assign	Clusters			
Population	(q>0.90)	1	2	3	4
South Giza	79.8	0.028	0.028	0.022	0.922*
Fayoum	88.0	0.028	0.017	0.936*	0.019
Beni Swif	100%	0.023	0.946*	0.013	0.018
Menya	100%	0.913*	0.041	0.038	0.009

% corr. Assign (q>0.90). Percentage of correctly assigned animals with q>0.90. *Clusters Contributions higher than 0.400.

same group. Geographical isolation was found for wild rabbits (Fuller *et al.,* 1997; Carneiro *et al.,* 2013; Alda and Doadrio, 2014). In this study, we confirmed the spatial representation of the genetic inter-individual distances using the FCA analysis (Figure 3). Generally, FCA recorded pattern of spacing of individuals out of populations range in Northern group (24.1 and 12%, in South of Giza and Fayoum respectively). In addition, Table 3 shows that 79.8 and 88% were correctly assigned in South Giza and Fayum, respectively. This might be due to the permanent movement or departure/removal of individuals in the Northern group, which regularly occurs for marketing/selling. The same concept agrees with Lowe and Allendorf (2010). There was only a narrow conjunction/overlap in the Southern group (Beni Suef and Minya). Figure 5 (a and b) shows the clustering pattern arising from the analyses. At K=2 nearby areas, surprisingly clustered together (South of Giza and Fayoum in first cluster while, Beni Suef and Minya in the second), which confirmed our results in Figures 3 and 4. The genetic differentiations among 4 NMER populations (K=4) were mainly discernible according to the geographic regions. This result disagrees with Ben Larabi *et al.* (2014) in North Africa, who reported that differentiation among 12 local Tunisian rabbit populations could not be classified by geographic regions.

CONCLUSION

In the present study, more light was shed on NMER diversity in North Upper Egypt. According to microsatellite polymorphism analyses, high diversity was recorded in the South. In addition, 2 main groups were discovered, corresponding to the geographical points North and South. Generally, the NMER breed requires more attention in Egyptian production systems to attain genetic benefits than confirmed by the current study. This study provides the documentation information for the NMER situation in the area under study. It can also be used as an illustrated guide for genetic improvement and conservation design programmes for Native Middle-Egypt rabbits (NMER).

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