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Genetic diversity and phenotype characterization of native rabbit in Middle-Egypt

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Abstract-Survey of native rabbit populations were conducted in three governorates; Fayum (FY), Bani-Suef (BN) and Minya (MN) in Middle-Egypt region .The phenotype data and samples of Native Middle-Egypt rabbits (NMER) were collected randomly from 8 cities in these governorates. Phenotype pattern of NMER rabbit population was included body parts, coat color and body dimension in rabbit. Samples from the same city were considered as a population. Eight microsatellite loci were used to provide a comprehensive insight into the genetic status and relationship among 8 NMER rabbit populations. A total of 92 rabbits from 8 cities of Middle-Egypt region were studied. Standard statistics parameters of genetic variability within and between populations were calculated. The observed heterozygosity, unbiased expected heterozygosity and the effective number of alleles were used to assess the genetic variation of each indigenous breed. Results show a moderate genetic diversity and observed heterozygosity ranged between 0.062 and 0.428. The F_{ST} values between pairs of breeds, using all data; indicate a generally high level of genetic differentiation, ranging from 0.026 to 1.743 which indicated a little connection between FY and MN governorate result to the long geographical distance and nature barriers. We can conclude that Phenotype pattern of NMER populations had common model in body parts but their coat color follows different models. NMER populations are a small to medium-sized breed. The current study is the first detailed analysis of the genetic diversity of native rabbit populations. The data generated here provides valuable information about the genetic structure of the 8 rabbit populations and this can be used to designate priorities for their conservation. It is needed to increase numbers the samples and the microsatellite investigated and more genetic studies by using mitochondrial DNA and microsatellite data for rabbit in Egypt.

keys words - Survey, rabbit populations, Egypt, Native, phenotype.

1. Introduction

Rabbit production could be a good source of meat in Egypt since it is a prolific animal, fast growing and of high fecundity. In Egypt, an early attempt was made to develop selected lines of meat rabbits, beginning with getting of Giza White, (GW) breed in 1937 as reported by El-Khishin et al., (1951). Breeds selected for meat production, like Baladi Red (BR), Baladi Black (BB) and Baladi White (BW), were produced by crossbreeding between native rabbits and exotic Flemish Giant in Animal Production Research Institute (Badawy, 1975; Galal and Khalil, 1994). Although Native Egypt rabbits were used early to get BR, BB, BW, no studies have been published concerning these rabbits. Native Rabbit kept under smallholder-low input systems are considered important genetic resources that should be protected against production threats. Characterization of the genetic resource of rabbit is essential for the identification and improvement of management and to facilitate their conservation. Domestic rabbits are classified into breeds based on coat color, external body measurements and origin (Sandford, 1996). It is important to differentiate between these rabbit breeds using molecular markers. Characterization at the molecular level is undertaken mainly to explore genetic diversity within and between animal populations and to determine genetic relationships among populations (Rahimi et al., 2005). In the present study, we survey native rabbit populations in Middle-Egypt to pattern of phenotype and to elucidate the degree of genetic variability by using 8 microsatellite markers to explain the genetic relationships in 8 populations.

2. Materials ans methods

Data and Samples rabbit

Survey of native rabbit populations were conducted in Middle-Egypt region and included three governorates, Fayum (FY), Bani-Suef (BN) and Minya (MN). The phenotype data and samples of Native Middle-Egypt rabbits (NMER) were collected randomly from 8 cities in these governorates. Details about the governorates, the cities and its code are shown in Table 1. Geographical locations of these governorates and cities are shown in Figures 1 and 2 A and B.

Table 1: Rabbit samples used in this study

Governorate	City	Code	Sample Size (n)
Minya	Bani-Mazar	BM	9
n=33	Sumalot	SA	14
	Maghagha	MG	10
Fayum	Fayum	FY	11
n=24	Ebshawi	EB	13
Bani-Suef	Beba	BB	11
n=35	Bani-Suef	BN	14
	Fashn	FA	10

Pattern of phenotype of NMER rabbit population was recorded on 158 rabbits mature and the descriptive was based on model for rabbit genetic resources data bank (Khalil 1993) that included body parts, coat color and body dimension. Body dimension (cm) measurements included: Body length measured from atlas to the first coccygeal vertebra; Chest circumference measured behind the shoulder blades; Abdominal circumference taken at the level of the 7th lumbar vertebra; Ear width taken from the outside to another side of the ear; Ear length taken from the bottom to the top of the ear; Thigh circumference measured before hind legs. The population sizes of native Middle-Egypt rabbit (NMER) were estimated on the data collected from the markets in the 8 cities which were studied in Middle-Egypt area.

Samples from the same city were considered as a population. A total of 92 rabbits were tested using molecular markers and the number of animals per city is given in Table 1.

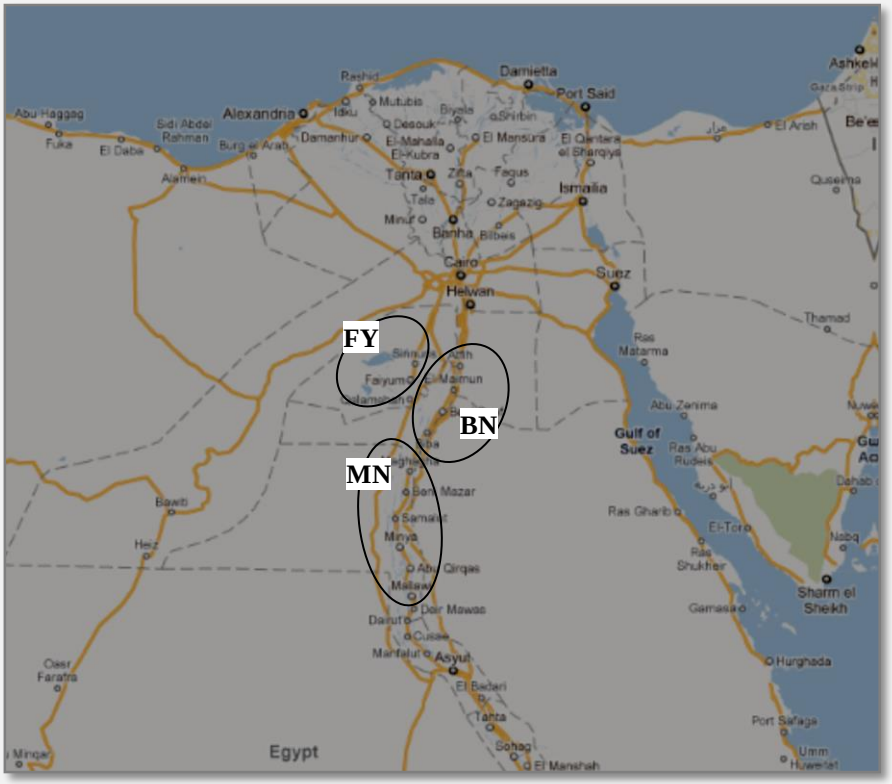

Figure 1: The geographical locations of Fayum (FY), Bani-Suef (BN) and Minya (MN) governorates in Egypt.

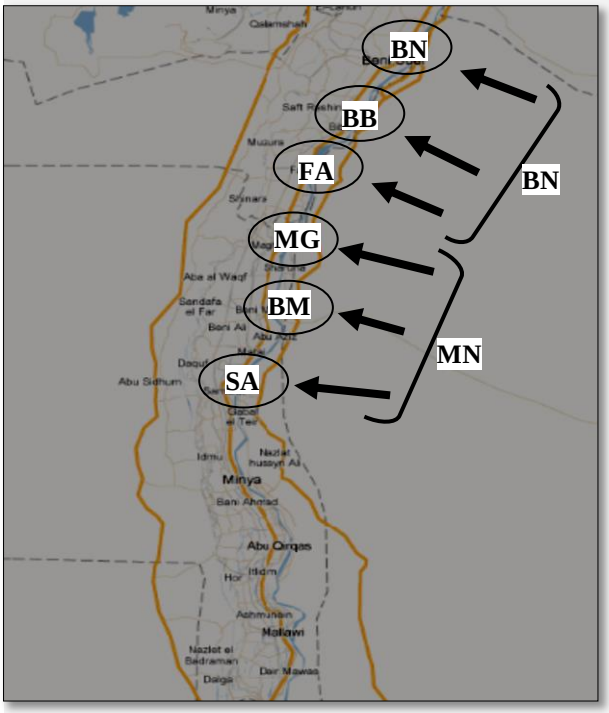

Figure 2 A: Geographical locations of cities in Bani-suef and Minya governorates

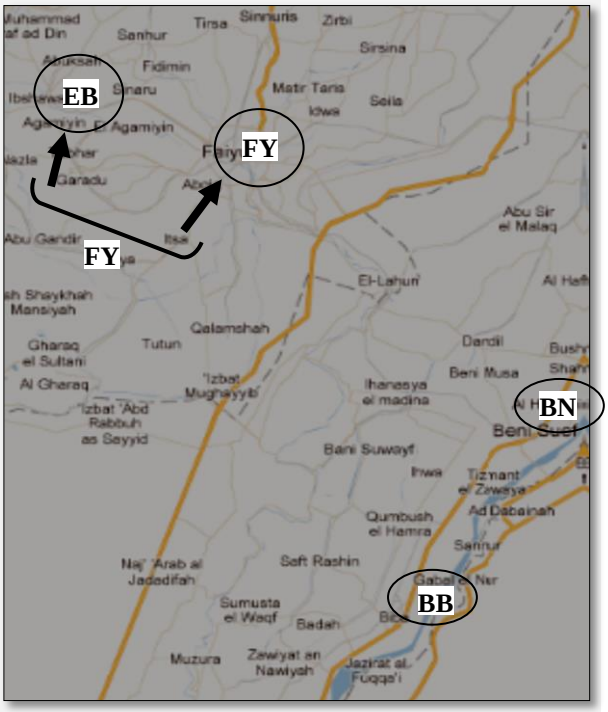

Figure 2 B: Geographical locations of cities in Fayum governorate.

Microsatellite genotyping

Blood samples were collected from the marginal ear vein into 5 mL vacutainer tubes containing EDTA as anticoagulant and stored at 4°C until molecular analyses were performed. To determine levels and patterns of genetic variation, a set of 8 microsatellites (*Sat3*, *Sat7*, *Sat12*, *Sat8*, *Sol 3*, *Sol 8*, *Sol33* and *Sol 44)* was used and chosen from previous studies (Mougel, 1997; Surridge *et al.*, 1997, Rico et al., 1994). The primers used for the amplification of these microsatellite loci were listed in Table 2. Genomic DNA samples were extracted from white blood cells.

The PCR reactions were carried out in a thermocycler in 25µl final volume consisting of 200 ng DNA, 10 pM forward primer; 10 pMeverse primer, and 10 µM Master Mix (HT Biotechnology). After an initial 5 min denaturation at 94ºC, 30 cycles were performed as follow: 30 s denaturation at 94ºC, 30 s annealing ranged from 52 to 60ºC (listed in Table 2) and 30 s extension at 72ºC. A final elongation was carried out for 10 min at 72ºC. PCR products were separated on denaturing electrophoresis in 3 % polyactylamide gels containing urea and bands were visualized by rapid silver staining (Sanguinetti et al., 1994).

Genetic data analysis:

Within population genetic diversity

To estimate genetic variation within populations, the total number of alleles, number of observed alleles per locus (Ao), expected heterozygosity (He) estimated following Nei (1987) and observed heterozygosity (Ho) were calculated using Genetix 4.05 software (Belkhir*et al.*, 2004). Reduction in heterozygosity (FIS) due to inbreeding per population was determined using GENEPOP 4.1 program (Raymond and Rousset 1995; Rousset, 2008). Significance of non-zero FIS values per population was established by permutation (1000 permutations per population).

Population subdivision and relationships among breeds.

The population differentiation pattern was described by a factorial correspondence analysis (FCA) of the individual multilocus genotypes. Pairwise distances between individuals were estimated from the proportion of shared alleles according to Bowcock *et al.* (1994).

Genetic differentiation among and within populations was estimated by the F-Statistics (Wright ,1951); they were calculated according to Weir and Cockerham (1984) using Genetix 4.0 and FSTAT

2.8: intrapopulation structure was investigated using the FIS parameter (observed individual heterozygosity compared to the theoretical one within breed; 0 means that the samples are from a panmictic population (at Hardy-Weinberg equilibrium). Genetic differentiation between populations was estimated from the FST parameter (breeds heterozygosity compared to the overall heterozygosity; 0 means no differentiation between breeds). Moreover, values of pairwise genetic differentiation (Fst) were computed for all pairs from the 8 populations. The Reynolds genetic distance (DR) was calculated for each pair of populations based on allele frequencies (Reynolds *et al.*, 1983) using the Genetix software, version 4.03 (Belkhir*et al.*, 2004). Phylogenetic tree were performed on locus. Neighbour-Joining Trees were edited by Mega 5 package (Tamura *et al*., 2007).

Table2: The sequence of microsatellite primers and annealing temperature (T_A,).

Locus	Primer sequence	fragment (bp)	T_A (°C)
Sat-3	Forward 5' GGAGAGTGAATCAGTGGGTG 3' Reverse 5' GAGGGAAAGAGAGAGACAGG 3'	146-162	60
Sat-7	Forward 5' GTAACCACCCATGCACACTC 3' Reverse 5' GCACAATACCTGGGATGTAG 3'	183-195	60
Sat-8	Forward 5' CAGACCCGGCAGTTGCAGAG 3' Reverse 5' GGGAGAGAGGGATGGAGGTATG 3'	136-158	60
Sat-12	Forward 5' CTTGAGTTTTAAATTCGGGC 3' Reverse 5' GTTTGGATGCTATCTCAGTCC 3'	122-138	55
Sol-33	Forward 5' GAAGGCTCTGAGATCTAGAT 3' Reverse 5' GGGCCAATAGGTACTGATCCATGT 3'	189–219	52
Sol-44	Forward 5' GGCCCTAGTCTGACTCTGATTG 3' Reverse 5' GGTGGGGCGGCGGGTCTGAAAC 3'	178–208	62
Sol-30	Forward 5' CCCGAGCCCCAGATATTGTTACCA 3' Reverse 5' TGCAGCACTTCATAGTCTCAGGTC 3'	143–165	54
Sol-8	Forward 5' GGATTGGGCCCTTTGCTCACACTTG 3' Reverse5' ATCGCAGCCATATCTGAGAGAACTC 3'	104–122	55

3. Results and discussion

I-Phenotype

Phenotype Pattern of Native Middle-Egypt rabbits (NMER) populations had common model in body parts but their coat color shows different models.

1- Body Parts

Profile of back starts comparatively low at the base of the neck and inclines slightly upward till it reaches the shoulders (Point 1, Photo 1).Here it bends to ascend and round nicely, yet in a gentle arch which peaks over the rump (Point 2, Photo 1). At this point it curves much more steeply to the base of the tail (Photo 1). The rump itself was wide and gives a rounded and full appearance an even when viewed from the back (Line 1, Photo 2). Shoulders apart were low and narrow (Line 2, Photo 2), but they were sturdy.

Head was between medium and small in size. It was long (Line 1, Photo 3) and narrow (Line 2, Photo 3) in proportion, with a profile that slopes from the base of the ears down to the nose in a line which was almost straight. The head of the buck was bolder than that of the doe. Ears were relatively long (Line 3, Photo 3) at average of about 8.5 cm (Table 1) which was closely to average of ear length (8 cm) in wild rabbit (Nowak, 1999). Ears were also somewhat thin but well covered with fur. Eyes were large and protruding (Point 1, Photo 4). Their color was black or dark gray or red in white fur only.

The neck was short but visible. A dewlap was quite common in the doe (Point 2, Photo 4), generally being small, neat and round (Photo 4). It was prohibited in the buck. Legs were strong, straight with a medium length. Rabbit phenotypic descriptions in NMER were very close to the wild in Malta (Tax-Xiber) as reported by Gauci-Mestre (1999).

2- Coat color

Fur was dense, flexible and relatively short. Also, fur was softer than it appears. It makes for a fast coat which resumes its position immediately when stroked in any direction. Length of fur was 2.5 cm. The coat colors include different models: grayish, brown and sometimes gray or red sprinkled throughout (Photo 1, 2 and 4). Another model has the underside of the body pale gray or black or brown and the upper side of the body white (Photo 3). These agree with Macdonald (1984) who reported that the coat of wild *rabbits* was generally grayish, with black and brown and sometimes red

sprinkled throughout. The wild rabbit of Malta has two varieties of color, the normal grey wild agouti and the red wild agouti (Gauci-Mestre, 1999).

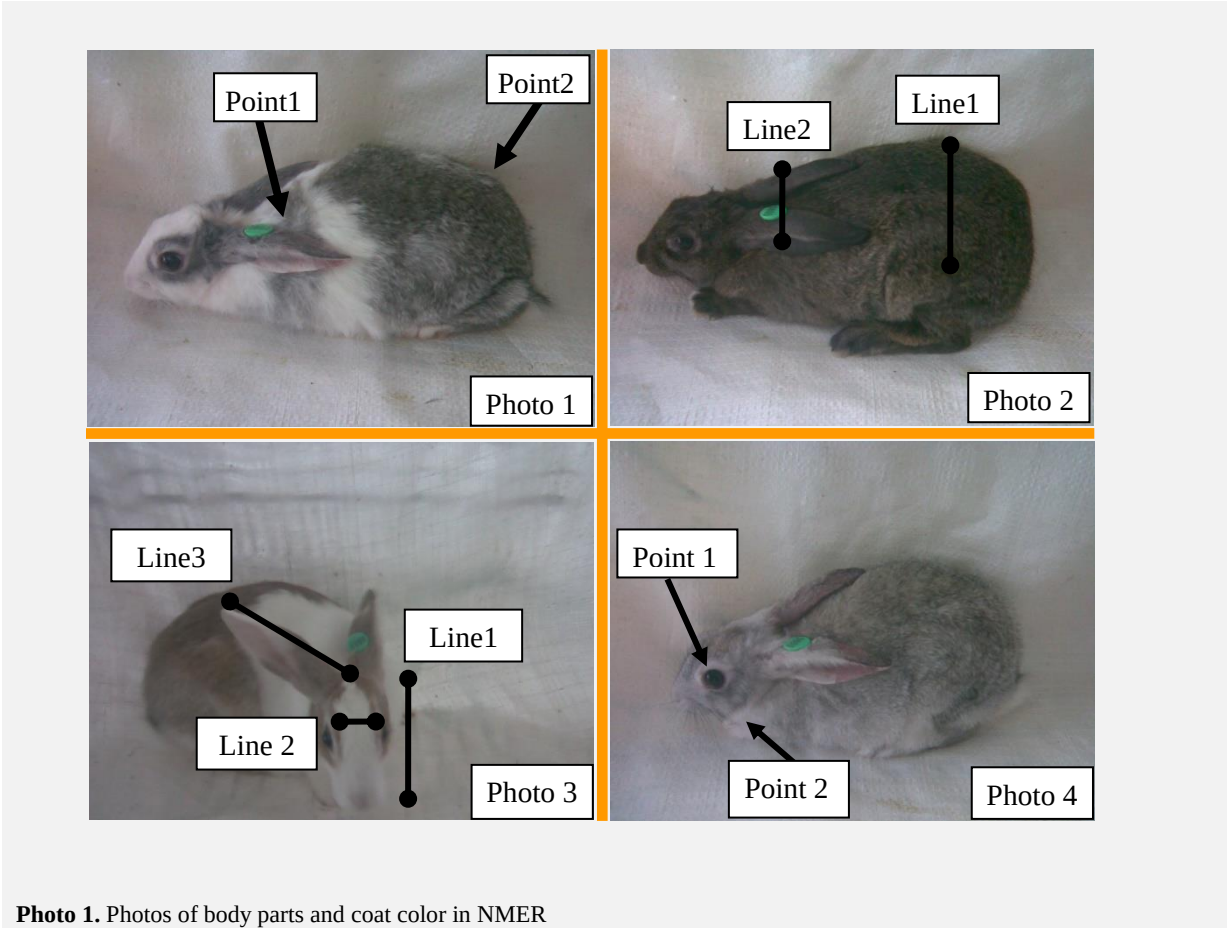

Photo 1. Photos of body parts and coat color in NMER

3- Body weight and body dimensions

Native Middle-Egypt rabbits (NMER) are a small to medium-sized breed. The average weight of mature NMER was 1995g for bucks and does with a range of 1372 and 2562g and the average length for them was 33.2cm with a range of 29 and 37.5cm (Table 3). These weights were within the range of 1.5-2.5 kg given for the wild rabbits (Macdonald, 1984). These weights were close to Dwarf rabbit in Italy; female and male at 45 week of age were 1953 and 1850 g, respectively (Dalle-Zotte et al., 2013). Data reported in Table 3 indicate that NMER have medium body length, relatively similar to that of the local breed in Lebanon (Hajj *et al*., 2002). This length was shorter than for wild rabbits which ranged from 38 to 50 cm (Macdonald, 1984). Chest circumference was relatively similar to local breeds in Morocco rabbits (Bouzekraoui, 2002; Barkok and Jaouzi 2002), but it appears narrower (12.6 Vs 15 and 16.1cm) which may impair meat production. Body length and ear length at week 12 (Table 3) were relatively similar to that of local breeds in Egypt (Baladi Red and Baladi White) while body length was shorter than in BB (Khalil, 2002).

4- Origin and population sizes

The rabbit populationswere founded in Middle-Egypt. As far as we know, no studies have been published concerning to the Native rabbit population in Middle-Egypt. Baladi or Native Middle-Egypt rabbit population (NMER) is bred in rustic areas and small-scale farms. NMER is used mainly for meat production. The Population size was estimated with ≈ 6600 (5280 ♀&1320♂) and were produced ≈ 15840 young animals, yearly.

Table 3: Means of weight and body dimensions of NMER

Traits	Mean	Min.-Max.
Weight (g)	1995	1372-2562
Body length (cm)	33.2	29.0-37.5
Chest circumference(cm)	24.0	20.0-32.5
Abdominal girth (cm)	28.4	22.0-34.6
Ear width (cm)	4.4	4.0-5.8
Ear length (cm)	8.5	7.0-10.0
Thigh circumference (cm)	12.6	11.0-15.0

II-Genetic diversity

II-1- Within-population diversity

The 8 loci surveyed across the rabbit populations. The rabbits in Bani-Suef (BN) population were showed the highest mean effective allele number (Ao=1.428), while Fayum (FY) population was the lowest locus variability (Ao=1.125). Levels of expected heterozygosity (He) within study ranged between 0.046 (in FY) and 0.214 (in the BN) as shown in Table 4.

The observed heterozygosity (Ho) in Native Middle-Egypt rabbit (NMER) populations (Table 4) were exhibited the same compared to Tunisian (Ho ranging from 0. 365 to 0.482) and Egyptian and Spanish breeds (Ho ranging from 0.36 to 0.48) as reported by Ben Larbi*et al*. 2014 and Grimal*et al.*, 2012, respectively. On other hand, the number of alleles observed in these 8 populations was lower than that observed in European rabbit breeds (from 3 to 4.2) using microsatellite DNA markers (Bolet*et al.*, 2000). It was probably due to the reason that the allelic number of these microsatellite loci in the documents came from the European wild rabbit populations living in open(Zhu et al.,2004). While the domestic rabbit populations in this study were fed in enclosed environment which may have lead to the loss of some alleles. Overall, the observed heterozygosity (Ho) as a genetic diversity was found in BN population (0.428), while the lowest genetic diversity was shown in FY population (0.062) as shown in Table 4.

Table 4: Within-population summary statistics: number of data (N), of effective alleles (Ao), observed heterozygosity (Ho) and expected heterozygosity (He) of local Middle-Egypt rabbit populations

Population	N	Ao	He	Ho	F_{IS}
Bani-Mazar (BM)	9	1.375	0.156	0.250	**0.20**
Sumalot (SA)	14	1.375	0.158	0.236	**0.54**
Maghagha (MA)	10	1.375	0.149	0.125	**0.04**
Fayum (FY)	11	1.125	0.046	0.062	**0.27**
Ebshawi (EB)	13	1.142	0.071	0.142	**0.33**
Beba (BB)	11	1.250	0.121	0.156	**0.40**
Bani-Suef (BN)	14	1.428	0.214	0.428	**0.20**
Fashn (FA)	10	1.375	0.088	0.152	**0.69**

Mean number of observed alleles (Ao), observed heterozygosity (Ho), expected heterozygosity (He) and heterozygote deficit (F_{IS}).

The overall within-population heterozygote deficit (F_{IS}) was 0.365. Fashn (FA) population had highest value in F_{IS} and Maghagha (MG) had lowest heterozygosity (Table 4). The results inducted that genetic variation was greater in Fashn (FA), Beba (BB) and Sumalot (SA) populations than in the other rabbit populations. Fashn (FA) is very nearly to Beba (BB) as shown in Figure 2 A. So it is logical…. heterozygote deficit (F_{IS}) was close and the genetic variation within-population was high (all values were positives). Also, Maghagha (MG) and Bani-Mazar (BM) are near (Figure 2 A) and they had heterozygote deficit (F_{IS}) low as shown in Table 4.

II-2- Among populations diversity:

The overall genetic differentiation among populations (F_{ST}) value in using all data was 0.439. This level of differentiation is within the range reported in the literature for F_{ST} values in rabbit (Bolet*et al.*, 2002). The overall FST in Egyptian and Spanish breeds was 0.137 (Grimal*et al.*, 2012) while in Tunisian populations was 1.1(Ben Larbi*et al*. 2014). This implies that 96.6 % of the total genetic variation was explained by individual variability. The genetic drift and geographical isolation of the

study area may have contributed to the moderate level of differentiation among the native Middle-Egypt rabbit populations investigated. The F_{ST} values between pairs of breeds, using all data; indicate a generally high level of genetic differentiation, ranging from 0.026[between Maghagha (MG) and Bani-Mazar (BM)] to 1.743 [between Ebshawi (EB) and Fashn (FA)] as shown in Table 5.This indicated a little connection between the two rabbit in EB and FA result to the long geographical distance and nature barriers between Ebshawi and Fashn (Figures 2A and 2B). On constant that a short geographical distance may be caused a high connection between Maghagha and Bani-Mazar (Figures 2A).

Table 5: Pair-wise Reynolds's genetic distance (D_R) between pairs of rabbit populations (above the diagonal) and genetic differentiation (F_{ST}) between pairs of rabbit populations (below diagonal) as observed in this study.

	BM	SA	MG	FY	EB	BB	BN	FA
BM	-	0.091	0.026	0.529	1.218	0.361	0.712	1.127
SA	0.091	-	0.158	0.578	0.896	0.190	0.713	1.088
MG	0.026	0.158	-	0.371	1.304	0.354	0.722	1.098
FY	0.529	0.578	0.371	-	1.356	0.281	1.080	0.989
EB	1.218	0.896	1.304	1.356	-	0.806	1.724	1.743
BB	0.361	0.190	0.354	0.281	0.806	-	0.867	0.905
BN	0.712	0.713	0.722	1.080	1.724	0.867	-	1.743
FA	1.127	1.088	1.098	0.989	1.743	0.905	1.743	-

Correspondence Analysis (FCA) was performed including all animals and loci to summarize individual relationships. In FCA, the first three principal factors (PCs) explained 23.4% of the total variation, respectively (Figure 3). The three first dimensions of the FCA analysis showed a clear separation between 3 groups representing MG, BM and SA (in Minya governorate) as a one population, Beba (BB) and all other populations (Figure 3).

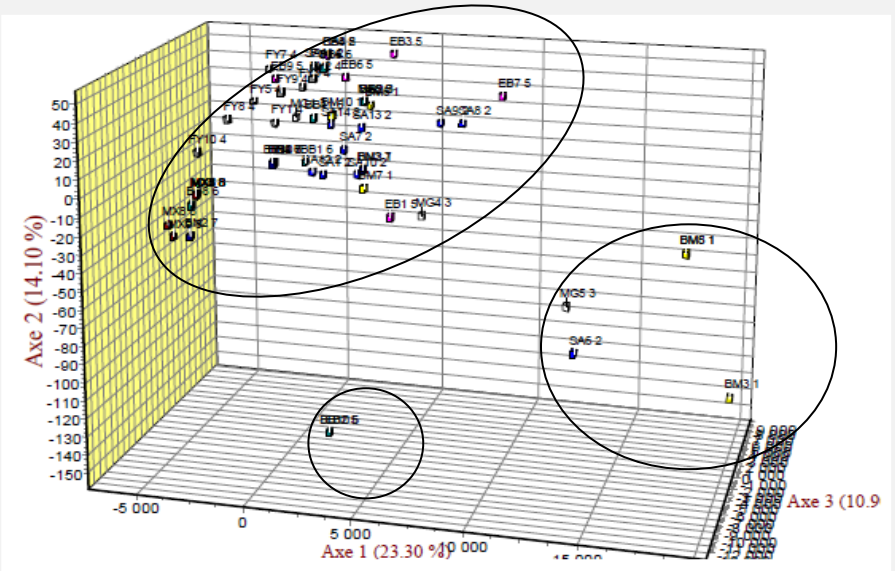

Figure 3: Factorial correspondence analysis based on the allele frequencies from 8 microsatellites loci genotyped in 8 rabbit populations. Population abbreviations defined in table 1. The number between parentheses represents the percentage of variability explained by each axis.

Also, Neighbour-Joining tree analyses among populations were obtained using the Reynolds genetic distances (Figure 4). Neighbour-Joining tree analyses of the Reynolds genetic distances (D_R) among populations shows a clear structurally separation of MG, BM and SA, in addition to Beba (BB) populations from other population (Figure 4). The largest branch length was seen for the Ebshawi (EB) and Fayum (FY) populations in side and MG, BM and SA (in Minya governorate) in another side as shown in Figure 4. The rabbit populations in Fayoum and in Minya governorate difficult access result to the long geographical distance and nature barriers between them cities. This explains its difference as shown in Figure 3. On other hand, these isolated populations do not show great

variability. Ebshawi (EB) may be population had migrated from Fayoum area to EB. Fayum (FY) population has moderate heterozygosity and near to EB. Bani-Suef (BN) is an intermediate in geographical distance between the populations of the governorate of Fayoum and Minya (Figures 2A and 2B). This probably due to BN population shows a high heterozygosity. BB is surprised as separated population.

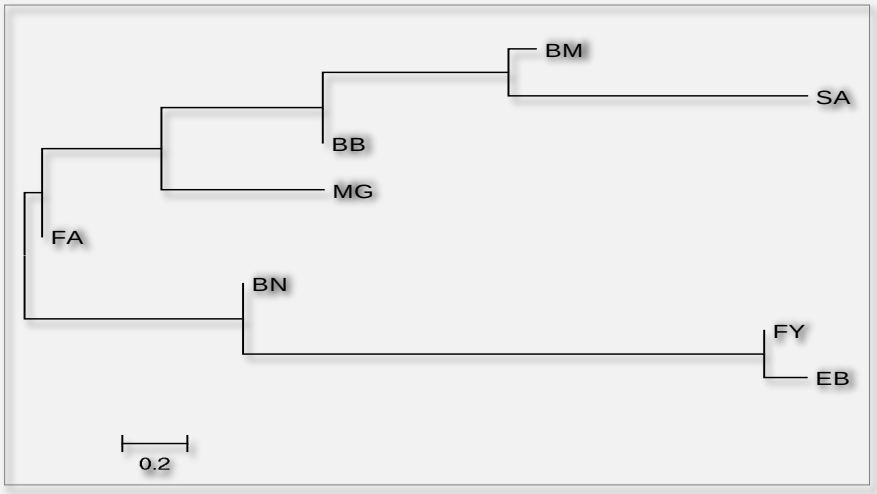

Figure 4: Neighbour-joining tree for the 8 rabbit populations in Middle-Egypt. Figures at nodes represent the bootstrap values over 1000 samples. Population abbreviations defined in table 1.

These molecular data show that Native Middle-Egypt rabbit (NMER) populations represent a low variability. This possible was due to absence of bottleneck effect or genetic drift.
Geographic isolation is an important factor of differentiation between populations which may explain the clearly identified structure between local rabbit in Fayoum and Minya governorate, despite the F_{ST} values observed between them. Also, the cities are nearly distance the genetic differentiation between native rabbits is low and moderate and this may be related to migration though the socio-cultural activities.

4. Conclusion

Results show that Phenotype Pattern of Native Middle-Egypt rabbits (NMER) populations had a common model in body parts but their coat color had different models. NMER populations are a small to medium-sized breed. The current study is the first detailed analysis of the genetic diversity of native rabbit populations in Egypt. This work discussed the genetic variation within and among the populations which can contribute to choose methods for protecting and using rabbit resource. This study revealed all microsatellite markers used were low polymorphic with native Middle-Egypt rabbits (NMER) comparing with European rabbit. The genetic distance among populations shows a clear structurally separation of Minya (MG, BM and SA), in addition to Beba (BB) population from other populations. It is needed to increase numbers the samples and the microsatellite investigated and more genetic studies by using mitochondrial DNA and microsatellite data for rabbit in Egypt.

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