World Rabbit Science

World Rabbit Sci. 2019, 27: 135-141
doi:10.4995/wrs.2019.8923
© WRSA, UPV, 2003

MOLECULAR GENETIC DIVERSITY AND CONSERVATION PRIORITIES OF EGYPTIAN RABBIT BREEDS

BADR O.A.M.*, EL-SHAWAF I.I.S.*, KHALIL M.H.A.†, REFAAT M.H.*, RAMADAN S.I.A.‡

*Department of Genetics and Genetic Engineering, Faculty of Agriculture at Moshtohor, Benha University, Kalubia, Egypt.
†Department of Animal Production, Faculty of Agriculture at Moshtohor, Benha University, Kalubia, Egypt.
‡Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Kalubia, Egypt.

Abstract: The limited rabbit resources in Egypt are threatened by the danger of extinction, whereas genetic diversity studies of native breeds could play a vital role in conservation and improvement of these breeds. In this study, 3 native rabbit breeds: Gabali (G), Baladi Red (BR) and Baladi Black (BB), in addition to New Zealand White (NZW), were genotyped using 12 microsatellite markers. All the typed microsatellites were polymorphic by average number of alleles 5.25 per locus. Observed and expected heterozygosity per locus averaged 0.62 and 0.68, respectively. The average polymorphic information content was 0.71 and the highest polymorphic information content was recorded in locus SOL33 by 0.85. All the studied loci except SAT7 and SAT2 showed deviation from Hardy-Weinberg equilibrium with significant level. The inbreeding coefficient of the individuals relative to the total population was 0.07. The within-population heterozygote deficit averaged 0.07 and ranged from 0.141 in BR to 0.015 in BB breeds. The highest pairwise differentiation among the populations was recorded between BB and NZW (0.071), while the lowest value was recorded between BR and both of G (0.038) and BB (0.039). The lowest pairwise Nei's genetic distance was recorded between BR and BB (0.190), while the highest was recorded between NZW and BB breeds (0.409). BR and G populations were clustered together forming an admixed mosaic cluster. BR recorded the highest contribution in the aggregate genetic diversity based on the three prioritisation methods used.

Key Words: Egyptian rabbits, genetic diversity, microsatellite markers, prioritisation, conservation.

INTRODUCTION

In Egypt, the most common native breeds of rabbit were Baladi Red (BR), Baladi White (BW), Baladi Black (BB), Giza White (GW), and Gabali (G). These Egyptian rabbits are medium-sized breeds and they are mainly used for meat production (Khalil, 1999). However, 2 of these native breeds (Baladi White and Giza White) became extinct and another 2 (Baladi Red and Gabali) are endangered and under threat of extinction (Khalil and Baselga, 2002; Galal, 2007). Egyptian native rabbit breeds are highly adapted to harsh environmental conditions and thought to constitute genetic reservoirs. For instance, Gabali and Baladi rabbits are characterised by their high tolerance to climatic stress and their resistance to diseases in comparison with the exotic breeds raised in Egypt (Khalil, 1999; Khalil and Baselga, 2002).

Genetic diversity studies of the native breeds provide valuable information, which enables us to understand the domestication process and evolution history of these breeds. Such studies help us suggest correct breeding plans for the conservation and improvement of native breeds (Emam *et al.,* 2017). Moreover, appropriate genetic markers are able to generate the information necessary for the planning of crossing and selection of genotypes in the genetic breeding programmes (Bruford and Wayne, 1993; MacHugh *et al.,* 1997; Khalil *et al.,* 2008).

Correspondence: O.A.M Badr, *omnia.badr@fagr.bu.edu.eg.* Received *November 2017* - Accepted *July 2019.*
https://doi.org/10.4995/wrs.2019.8923

Assessment of genetic diversity using microsatellite markers is the most common important tool in conservation and cross-breeding programmes of animal herds (Ramadan *et al.,* 2012; Crispim *et al.,* 2014; Lai *et al.*, 2018). As the resources for conservation of rabbit breeds are limited, prioritisation is often necessary (Marsjan and Oldenbroek, 2007). Microsatellite markers were involved in a few recent studies to assess the genetic diversity within and among Egyptian rabbit populations (Grimal *et al.,* 2012; El-Aksher *et al.,* 2017; Abdel-Kafy *et al.,* 2018). The main objectives of the present study were: first, to investigate the genetic diversity among 3 Egyptian rabbit breeds (BR, BB and G) in addition to NZW rabbits as an out-group breed using a set of 12 microsatellite loci; and second, to set the priorities for conservation of these breeds based on data on genetic diversity assessed using microsatellites

MATERIALS AND METHODS

Blood sampling and DNA extraction

Blood samples were obtained from a total of 120 individuals from 3 Egyptian rabbit breeds named Baladi Black, Baladi Red and Gabali, along with the New Zealand White rabbits as an out-group breed, taking 30 samples from each breed. The animals used in the present study were chosen from 4 farms; the rabbitry of the Animal Production department, Faculty of Agriculture, Benha University, and the rabbitries of Inshas, Gimmeza and Sakha, which belong to the Animal Production Research Institute (APRI), Agriculture Research Center, Ministry of Agriculture, Egypt. The samples were taken randomly from pedigreed animals with the least relationship (avoiding full-sibs and half-sibs) to decrease the genetic similarity between the genotyped animals. Approximately 3-5 mL of the venous blood sample per animal was collected from the rabbit ear vein by 2-gauge 1.5-injection needle into tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Genomic DNA was extracted from leukocytes using the Promega Wizard Genomic DNA Purification Kit.

Microsatellite genotyping

Genotyping of the individuals was carried out with a set of 12 microsatellite loci (SAT2, SAT4, SAT5, SAT7, SAT8, SAT12, SAT13, SAT16, SOL30, SOL33, SOL44 and INRACCDDV0003). Loci were selected depending on their polymorphism and conditions of amplifications. PCR reactions were carried out for all loci separately in a total volume of 25 µL containing 2 µL of 50 ng genomic DNA as a template, 30 pmol of primers, 2 mM of dNTP mix (dATP, dCTP, dTTP and dGTP; ABgene, Surrey, UK), 5X PCR buffer, 25 mM $MgCl_2$, 1 unit Taq DNA polymerase and 8.3 µL d.d H_2O. Each PCR amplification cycle consisted of 3 steps: a denaturation step at 94°C for 40 s, an annealing step at 60°C for SAT4, SAT5, SAT7, SAT8, SOL30 and INRACCDDV0003 for 1 min, and at 55°C for SAT2, SAT12, SAT13, SAT16 and SOL33 for 1 min, and at 58°C for SOL44 for 1 min, and an elongation step at 72°C for 1 min for all markers. After the last cycle, the marker extension segment was extended to 10 min at 72°C in the final extending cycle, then followed by soaking at 4°C until reaction and removed from the PCR thermocycler. Polyacrylamide gel electrophoresis (PAGE) was used for resolving microsatellite PCR amplicons. Non-denaturing polyacrylamide gel at 12% was prepared. The stock solution was prepared for 80 mL of 12% polyacrylamide gel and contained 32 mL of 30% acrylamide: bisacrylamide (29:1) (% w/v), 640 µL of ammonium persulfate (10% w/v), 8 mL of 80% glycerol, 8 mL of 10× TBE electrophoresis buffer, 40 µL tetramethylethylenediamine (TEMED) and 32 mL of d.d H_2O. After migration, gels were completely submerged in staining solution (250 µL ethidium bromide in 1 litre). Staining lasted for 10-15 min at room temperature; gels were then destained in d.d H_2O for 5 min. Images were captured using Gel Documentation System (Gel-Doc 2000 with Diversity Database software Ver. 2.1, Bio-Rad Laboratories, Hercules, California, USA). A 100 bp DNA ladder (Thermo Scientific) was applied to determine the allele fragment size using a TotalLab™ Quant v13 supplied by Nonlinear Dynamics Company.

Data analysis

Genetic diversity was assessed by calculating the observed (N_o) and effective (N_e) number of alleles and the observed heterozygosity (H_o) and expected heterozygosity (H_e) using GENALEX version 6.0 (Peakall and Smouse, 2006). Hardy-Weinberg equilibrium (HWE) over the loci within each breed was tested using the GENEPOP program (Raymond, 1995). Polymorphism information content (*PIC*) was calculated using CERVUS version 3 software (Weir and Cockerham,

1984; Kalinowski *et al.,* 2007). The F-statistics of pairwise genetic differentiation among populations (F_{ST}), reduction in heterozygosity due to inbreeding for each locus (F_{IT}) and the reduction in heterozygosity due to inbreeding within each breed (F_{IS}) across the studied populations were calculated using GENEPOP version 3.4 (Raymond, 1995).

Nei's genetic distance and the pairwise F_{ST} were estimated among the 4 rabbit populations across the 12 microsatellite studied loci (Nei *et al.,* 1983). A phylogenetic tree was constructed based on the Nei's genetic distance, using the neighbour-joining method (Saitou and Nei, 1987). The robustness of tree topologies was evaluated with a bootstrap test of 1000 resamplings across loci. These processes were conducted using POPULATIONS version 1.2.30 software (http://bioinformatics.org/~tryphon/populations/). The genetic structure of the sampled populations was investigated using a Bayesian clustering procedure implemented in STRUCTURE software with the admixture method (Rosenberg, 2004). Fifty runs were used for each value of K ($2 \leq K \leq 4$), with 60 000 iterations following a burn-in period of 10 000. Pairwise comparisons of the 50 solutions of each K value were run along with 50 permutations using CLUMPP software (Jakobsson and Rosenberg, 2007). Finally, the clustering pattern was graphically displayed for the selected K value using DISTRUCT software (Rosenberg, 2004).

Three prioritisation methods were utilised, measuring the breed contribution in the aggregate genetic diversity by the following methods: (1) according to (Petit *et al.,* 1998); (2) according to (Caballero and Toro, 2002); and (3) according to (Ollivier and Foulley, 2005) The detailed information and calculations of these 3 methods were described previously by (Ramadan *et al.,* 2012).

RESULTS AND DISCUSSION

Polymorphism of microsatellite loci in the breeds studied

The observed (N_o) and effective (N_e) number of alleles, observed (H_o) and expected (H_e) heterozygosity, polymorphic information content (*PIC*) and Hardy-Weinberg Equilibrium (HWE) for each locus are shown in Table 1. All the studied microsatellite loci were polymorphic across the 4 studied rabbit breeds.

A total of 81 alleles were observed across the 4 rabbit populations. The average number of alleles per locus was 5.250 and the highest number of observed alleles was recorded for loci SAT4 and SAT16 (9 alleles), while the lowest number was recorded for marker SAT2 (4 alleles). Similar results were obtained by El-Aksher *et al.* (2017) who evaluated 16 microsatellite loci across 4 rabbits populations (V-line, M-line, Gabali and French Giant Papillon), as well as Abdel-Kafy *et al.* (2018), who studied 8 loci across 3 Egyptian populations collected from 3 different Egyptian

Table 1: The observed (N_o) and effective (N_e) numbers of alleles, observed (H_o) and expected (H_e) heterozygosity, polymorphic information content (*PIC*) and Hardy-Weinberg equilibrium (HWE), F-statistics (F_{ST}, F_{IT} and F_{IS}) per microsatellite marker across the breeds studied.

Marker (Locus)	N_o	N_e	H_o	H_e	*PIC*	HWE	F_{IS}	F_{ST}	F_{IT}
SAT8	6	3.326	0.67	0.68	0.67	***	0.02	0.03	0.04
INRA	6	3.519	0.39	0.70	0.73	***	0.44	0.08	0.49
SOL30	7	2.905	0.74	0.63	0.65	**	–0.20	0.06	–0.10
SAT7	7	2.121	0.60	0.52	0.48	NS	–0.15	0.03	–0.12
SAT5	6	4.696	0.68	0.78	0.78	***	0.13	0.04	0.17
SAT4	9	6.184	0.68	0.84	0.83	***	0.19	0.02	0.22
SOL33	8	4.460	0.73	0.76	0.85	***	0.05	0.11	0.16
SOL44	6	4.319	0.74	0.77	0.77	***	0.03	0.04	0.07
SAT2	4	1.542	0.42	0.35	0.35	NS	–0.18	0.05	–0.12
SAT12	5	2.875	0.53	0.64	0.73	***	0.16	0.17	0.31
SAT16	9	4.824	0.57	0.78	0.85	***	0.27	0.01	0.34
SAT13	8	4.068	0.66	0.75	0.83	***	0.12	0.11	0.22
Overall mean	5.25	3.74	0.62	0.68	0.71		0.075	0.138	0.07
±standard error	±0.23	±0.21	±0.02	±0.02	±0.01		±0.050	±0.060	±0.01

provinces; they recorded the highest number of observed alleles (10 alleles) at SAT16 and the lowest number at SAT2 (4 and 3 alleles respectively), with averages of 6.75 and 6.13 alleles, respectively.

In our study, for all studied microsatellite loci (except SOL30, SAT7 and SAT2), the H_O were lower than the H_e values across the studied breeds (Table 1). The H_O ranged from 0.39 in INRA to 0.74 in SOL30 with an average of 0.62, while the H_e ranged from 0.35 in SAT2 to 0.84 in SAT4 with an average of 0.68.

The values of polymorphic information content (*PIC*) of the studied markers showed high values as shown in Table 1. The *PIC* values in all studied loci ranged from 0.351 at locus SAT2 to 0.846 at locus SOL33 with an average of 0.710. The average *PIC* value of our study was higher (0.689) than that recorded by Abdel-Kafy *et al.* (2018), who evaluated 8 loci across 3 Egyptian rabbit populations, and lower than (0.760) that recorded by El-Aksher *et al.* (2017), who evaluated 16 microsatellite loci across 4 rabbits populations. The values of our study could suggest their usefulness for genetic diversity studies and linkage mapping programmes in Egyptian rabbits. The *PIC* values will be high when loci have a high number of alleles with allele frequency distributed equally among the alleles. In our study, all microsatellite loci (except SAT2 and SAT7) showed higher *PIC* values (*PIC*>0.50), and these loci can therefore be considered highly informative markers.

All the loci studied except SAT7 and SAT2 showed deviations from the Hardy-Weinberg equilibrium, with highly significant levels (Table 1). This result might be attributed to disequilibrium created by non-random mating and selection practised on the studied rabbit populations.

The F-statistics (F_{IS}, F_{IT}, and F_{ST}) for each locus across the 4 investigated breeds are presented in Table 1. The highest F_{IS} was observed for the locus INRA (0.44) and the lowest value was found for locus SOL30 (–0.20). The mean F_{IS} value across all loci and populations was moderately positive (0.075), indicating that there is a moderate level of inbreeding. However, the high inbreeding values can be attributed to non-random mating and some loci might be linked to some economically selected traits. Our results were consistent with those of (El-Aksher *et al.,* 2017; Abdel-Kafy *et al.,* 2018), who reported moderate positive values for F_{IS} (0.083 and 0.073 respectively).

The F_{ST} value of each microsatellite locus across the breeds studied ranged from 0.175 (SAT12) to 0.016 (SAT4) with a moderately high mean (0.138), indicating that there was a genetic differentiation among the studied populations. Our F_{ST} value was higher than the 0.107 recorded by El-Aksher *et al.* (2017) and 0.034 recorded by Abdel-Kafy *et al.* (2018).

The mean value for the inbreeding coefficient of individuals relative to the total population (F_{IT}) was 0.071 (Table 1). The highest value of F_{IT} was recorded for INRA marker (0.486), while the lowest value was recorded for SAT2 marker (–0.124).

Genetic diversity within and among populations

Within each studied population, the mean observed (N_O) and effective (N_e) numbers of alleles, observed (H_o) and expected (H_e) heterozygosity and the fixation coefficient of an individual within a subpopulation (F_{IS}) are presented in Table 2.

The highest values of N_O (5.750) and N_e (3.989) were recorded for BR breed, while the lowest ones N_O (5.000) N_e (3.511) were recorded for BB.

Table 2: The observed (N_o) and effective (N_e) numbers of alleles, the observed (H_O) and expected (H_E) heterozygosities, and fixation coefficient of an individual within a subpopulation (F_{IS}) per each breed of rabbits.

Breed	N	N_O±SE	N_e±SE	H_O±SE	H_E±SE	F_{IS}±SE
BB	30	5.00±0.55	3.51±0.46	0.63±0.04	0.66±0.05	0.015±0.084
BR	30	5.75±0.43	3.99±0.46	0.59±0.05	0.70±0.04	0.141±0.068
G	30	5.08±0.43	3.62±0.39	0.61±0.05	0.68±0.04	0.085±0.074
NZW	30	5.17±0.46	3.83±0.41	0.63±0.04	0.69±0.04	0.063±0.055
mean±SE	120	5.25±0.23	3.74±0.21	0.62±0.02	0.68±0.02	0.068±0.035

Gabali: G, Baladi Red: BR, Baladi Black: BB, New Zealand White: NZW. SE: standard error.

The highest value of H_e (0.700) was recorded for BR while the lowest (0.66) was recorded for BB populations with an average of 0.68. The F_{IS} ranged from 0.015 (BB) to 0.141 (BR) with an average of (0.68), as shown in Table 2. These differences in population genetic diversity indices between BR and BB might be attributed to differences in the breeding programme applied for each breed. Detected F_{IS} value was relatively lower than that of (Grimal *et al.,* 2012), who reported a value of 0.147 among the same studied Egyptian rabbit breeds (BB, BR and G), in addition to White Giza and NZW by 16 loci.

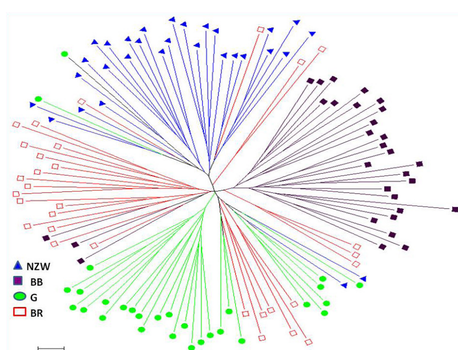

Figure 1: Neigbour-Joining phylogenetic tree among 120 rabbit individuals using allele shared distance. NZW: New Zealand; BB: Baladi Black; G: Giza White; BR: Baladi White.

Phylogenetic relationships and population structure

The lowest pairwise Nei's distance and F_{ST} were recorded between BR and BB breeds (0.190 and 0.038 respectively), followed by G rabbits (0.199 and 0.039), while the highest values were recorded between BB and NZW (0.409 and 0.71, respectively) as shown in Table 3. The values of pairwise F_{ST} and genetic distances among native rabbit breeds (BB, BR and G) were low, which reflects high genetic similarity among these breeds, and this was supported by the clustering pattern in the neighbour-joining phylogenetic tree (Figure 1). The tree topology showed a close relationship between BR and both of BB and G breeds; this close relationship might be explained on the basis that BR and BB breeds might have common ancestors. BR and BB were developed by crossing local Baladi with Flemish Giant rabbits for several generations, then selection took place based on fur colours into red (BR) and black (BB). The close relationship between BR and G was in agreement with previous studies (Grimal *et al.,* 2012) and might be attributed to introgression or gene flow that occurred between these 2 breeds. The local Baladi rabbits are a mongrel population originated from hybridisation among different native rabbits that might include G individuals. The G rabbits were raised in Sinai and in the northern coast of the western desert and were considered as native Egyptian rabbits that have a degree of fur colour similarity with BR. The 3 Egyptian breeds (BR, BB and G) were genetically separated from the NZW breed, and this was consistent with (Grimal *et al.,* 2012), who reported that the four Egyptian breeds (BB, RB, GW and G) were structurally separated from the Spanish NZW line.

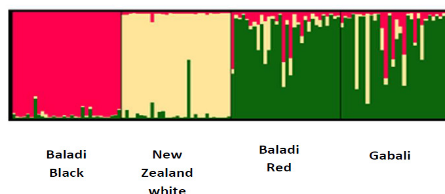

Figure 2: STRUCTURE clustering of the 4 rabbit breeds obtained for K=3.

Table 3: The estimates of Nei's genetic distance (above the diagonals) and pairwise F_{ST} (below the diagonals) among the 4 breeds.

Breed	BB	NZW	BR	G
BB		0.409	0.190	0.241
NZW	0.071		0.246	0.268
BR	0.039	0.043		0.199
G	0.048	0.051	0.038	

Gabali: G, Baladi Red: BR, Baladi Black: BB, New Zealand White: NZW.

The most probable structure clustering of the four studied populations was at K=3 (Figure 2). The NZW breed was assigned independently into its respective cluster, while BR and G were clustered together, forming an admixed mosaic cluster. The close relationship and the admixed mosaic cluster between BR and G were in agreement with the lower values for both of Nei's genetic distance and pairwise F_{ST}.

Table 4: The contributions of each breed to the aggregate genetic diversity.

Genetic diversity item	BB	BR	G
Among populations genetic diversity (Weitzman, 1993)	54.930	45.070	51.810
Within-population genetic diversity	–1.711	1.522	0.275
Aggregate genetic diversity by Petit *et al (*1998)	3.283	7.596	2.437
Aggregate genetic diversity by Caballero and Toro (2002)	-0.131	–2.134	–1.975
Aggregate genetic diversity by Ollivier and Foulley (2005)	2.363	4.660	3.986

Gabali: G, Baladi Red: BR, Baladi Black: BB, New Zealand White: NZW.

Conservation priorities of the Egyptian rabbits

The breed contributions to the aggregate genetic diversity using the 3 prioritisation methods for the conservation of the studied breeds are presented in Table 4. The BR breed recorded the highest contribution in the aggregate genetic diversity according to (Petit *et al.,* 1998; Caballero and Toro, 2002; Ollivier and Foulley, 2005), with values of –2.134, 7.596 and 4.660, respectively. Therefore, BR could be ranked the first, with the highest priorities for conservation purposes. This highest contribution of BR could be explained on the basis that BR showed the highest values for the expected heterozygosity and the highest observed and effective number of alleles per loci.

CONCLUSIONS

In conclusion, the results of our study confirmed the applicability and efficiency of this microsatellite panel for assessing genetic diversity and setting the conservation priorities for Egyptian local rabbits. This information could be used as an initial guide to design further investigations for the development of genetic improvement and conservation programmes for Egyptian rabbit genetic resources.

Conflict of interest: The authors declare that there is no conflict of interest.

Acknowledgement: Support from the Animal Production and Genetics Departments, Faculty of Agriculture, Benha University, Moshtohor, Qalyubia, Egypt, is gratefully acknowledged.

REFERENCES

Abdel-Kafy E.S.M., Ahmed S.S.E.D., El-Keredy A., Ali N.I., Ramadan S. , Farid A. 2018. Genetic and phenotypic characterization of the native rabbits in Middle Egypt. *Vet. World, 11: 1120. https://doi.org/10.14202/vetworld.2018.1120-1126*

Bruford M.W., Wayne R.K. 1993. Microsatellites and their application to population genetic studies. *Curr. Opin. Genet. Dev., 3: 939-943. https://doi.org/10.1016/0959-437X(93)90017-J*

Caballero A., Toro M.A. 2002. Analysis of genetic diversity for the management of conserved subdivided populations. *Conserv. Genet., 3: 289-299.*

Crispim B.d.A., Seno L.d.O., Egito A.A.d., Vargas Jr F.M.d., Grisolia A.B. 2014. Application of microsatellite markers for breeding and genetic conservation of herds of Pantaneiro sheep. *Electron. J. Biotechnol., 17: 317-321. https://doi.org/10.1016/j.ejbt.2014.09.007*

El-Aksher S.H., Sherif H., Khalil M., El-Garhy H.A., Ramadan S. 2017. Molecular analysis of a new synthetic rabbit line and their parental populations using microsatellite and SNP markers. *Gene Reports, 8: 17-23. https://doi.org/10.1016/j.genrep.2017.05.001*

Emam A., Azoz A., Mehaisen G., Ferrand N. , Ahmed N. 2017. Diversity assessment among native Middle Egypt rabbit populations in North Upper-Egypt province by microsatellite polymorphism. *World rabbit Sci., 25: 9-16. https://doi.org/10.4995/wrs.2017.5298*

Galal S. 2007. Farm animal genetic resources in Egypt: fact sheet. *Egypt. J. Anim. Prod., 44: 1-23.*

Grimal A., Safaa H., Saenz-de-Juano M., Viudes-de-Castro M., Mehaisen G., Elsayed D., Lavara R., Marco-Jiménez F., Vicente J. 2012. Phylogenetic relationship among four Egyptian and one Spanish rabbit populations based on microsatellite markers. *In Proc.: 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 3-6 September, 2012. 3-6.*

Jakobsson M., Rosenberg N. A. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics, 23: 1801-1806. https://doi.org/10.1093/bioinformatics/btm233*

Kalinowski S.T., Taper M.L. , Marshall T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol., 16: 1099-1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x*

Khalil M. 1999. Rabbit genetic resources of Egypt. *Anim. Genet. Resour., 26: 95-111. https://doi.org/10.1017/S101423390000122X*

Khalil M., Baselga M. 2002. *Rabbit genetic resources in Mediterranean countries*, CIHEAM-IAMZ.

Khalil M., Motawei M., Al-Saef A., Al-Sobayil K., El-Zarei M. 2008. RAPD markers linked to litter, lactation and growth traits in rabbits. *In Proc.: 9th World Rabbit Congress, Verona, Italy, 10-13 June, 2008, 143-148*.

Lai F.Y., Ding S.T., Tu P.A., Chen R.S., Lin D.Y., Lin E.C., Wang P.H. Population structure and phylogenetic analysis of laboratory rabbits in Taiwan based on microsatellite markers. *World Rabbit Sci., 26: 57-70. https://doi.org/10.4995/wrs.2018.7362*

MacHugh D.E., Shriver M.D., Loftus R.T., Cunningham P., Bradley D.G. 1997. Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics, 146: 1071-1086.*

Marsjan P., Oldenbroek J. 2007. Molecular markers, a tool for exploring genetic diversity (Section C in part 4). *The State of the World's Animal Genetic Resources for Food and Agriculture. FAO.*

Nei M., Tajima F., Tateno Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol., 19: 153-170. https://doi.org/10.1007/BF02300753*

Ollivier L., Foulley J.L. 2005. Aggregate diversity: new approach combining within-and between-breed genetic diversity. *Livest. Prod. Sci., 95: 247-254. https://doi.org/10.1016/j.livprodsci.2005.01.005*

Peakall R., Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes., 6: 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x*

Petit R.J., El Mousadik A., Pons O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol., 12: 844-855. https://doi.org/10.1046/j.1523-1739.1998.96489.x*

Ramadan S., Kayang B.B., Inoue E., Nirasawa K., Hayakawa H., Ito S.I. , Inoue-Murayama M. 2012. Evaluation of genetic diversity and conservation priorities for Egyptian chickens. *O.J.A.S., 2: 183. https://doi.org/10.4236/ojas.2012.23025*

Raymond M. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered., 86: 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573*

Rosenberg N.A. 2004. DISTRUCT: a program for the graphical display of population structure. *Mol. Ecol. Notes, 4: 137-138. https://doi.org/10.1046/j.1471-8286.2003.00566.x*

Saitou N., Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol., 4: 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454*

Weir B.S., Cockerham C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution, 38: 1358-1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x*