NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 53, 2020 (2): 59–66

ISSN 1337-9984
E-ISSN 1338-0095

POPULATION STRUCTURE AND PHYLOGENETIC RELATIONSHIP AMONG FOUR BREEDS OF RABBIT IN NIGERIA BASED ON MICROSATELLITE MARKERS

Adewunmi O. OMOTOSO[1,5]*, Olajide OLOWOFESO[1], Olajide M. SOGUNLE[2], Adewale O. TALABI[3], Noah E. T. TOR[1,4]

[1]Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria
[2]Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, Nigeria
[3]Department of Veterinary Medicine and Surgery, Federal University of Agriculture, Abeokuta, Nigeria
[4]Department of Animal Breeding and Physiology, Federal University of Agriculture, Makurdi, Nigeria
[5]Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany

ABSTRACT

This study defined the population structure and phylogenetic relationship among the commonest breeds of rabbit in Nigeria. A total of 100 genomic DNA isolated from New Zealand White, Californian White, New Zealand Red and Chinchilla rabbit breeds in Nigeria were used with seven microsatellite markers in order to assess the genetic diversity, phylogenetic relationships and population structure of these rabbit breeds. Genomic DNA was extracted, amplification was done using PCR with each microsatellite marker, the products generated were subjected to 12 % polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. Bands on gels were scored based on size of ladder with Gene Scan 3.1.2. and designated as alleles on an Excel Worksheet. Allele frequencies were generated with Microsatellite Analyzer version 4.05 and further used in determining other population genetic parameters such as the genetic differentiation and genetic distance. The genetic sub-structuring carried out basing on the Evanno's method revealed 2 clusters, which are the New Zealand White (cluster 1) and the New Zealand Red, Chinchilla and Californian White (cluster 2) having a high degree of admixture and interbreeding with no significant gene pool variation between rabbit individual in cluster 2. Conclusively, these findings show that, in spite of the high level of genetic variation among the rabbit breeds, the breeding strategies adopted could have led to genetic admixture among the breeds as a result of relatively high gene-flow among the breeds.

Key words: rabbit; admixture; structure; genetic differentiation; bottleneck; microsatellite

INTRODUCTION

Rabbit production in Nigeria at present is subsistent, non-commercial and smallholder-type. Provision of suitable breeding stocks well adapted to backyard systems is a key requirement to the sustainability of Animal Genetic Resources (AnGR) systems as far as rabbit production is concerned in Nigeria. Stocks of rabbits used in many parts of the developing world, including Nigeria, are highly heterogeneous and heterozygous, on account of their multiple breeds of origin (Lebas *et al*., 1998; Lukefahr, 1998a). Attempts to produce adapted genotypes that can perform under the conditions in smallholder units have not received much attention and do not appear to be a priority (Lukefahr, 1998a).

***Correspondence:** E-mail: adewunmi.omotoso@outlook.com
Adewunmi O. Omotoso, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria; Institute of Genome Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany

Received: September 30, 2019
Accepted: December 17, 2019

As a potential solution, local breeds of rabbits, as well as heterogeneous stocks described as "non-standard breeds" have to attract increased attention. These stocks are predominant in many backyard systems in Africa including Nigeria.

Lukefahr, (1998a), citing Falconer and Mackay (1996), argued that the maintenance of heterogeneous populations that are locally-adapted may have real merit in adverse environments. Lukefahr (1998a) noted that a high degree of genetic variation and/or heterosis might be important for fitness-related characteristics (fertility and survival) as a means for eventual local adaptation.

Zerrouki *et al*. (2004) noted that the utilization of local genetic resources first requires characterization of the population existing in the country, which would be effectively achieved by the application of a resourceful genetic appraisal tool, such as a microsatellite. Hence, this study, which focuses on the determination of the population structure, phylogenetic relationship among the four commonest rabbit breeds in Nigeria based on a microsatellite marker with an aim to engender an improvement of the existing Nigerian rabbit populations and provide a preliminary investigation into rabbit genetic resource in Nigeria.

MATERIAL AND METHODS

Blood sampling and DNA extraction

A total of hundred rabbit blood samples were collected from four rabbit breeds (New Zealand White n = 25, Californian White n = 25, New Zealand Red n = 25 and the Chinchilla n = 25), randomly selected among rabbit breeds available on different farms and animal research institutes across Nigeria. The random sampling of rabbits was strictly restricted to breed specific phenotypic representations and morphological features as presented by various standard rabbit breeder associations. Approximately 1 ml of blood was aseptically collected from each rabbit into ethylene di-amine-tetracetic acid (EDTA) tubes through the Saphenous rear leg venial puncture using sterilized 1 ml-syringes into 5 ml EDTA tubes, which were placed into an icebox and transported to the Biotechnology laboratory of the Department of Animal breeding and genetics, Federal University of Agriculture, Abeokuta, and were stored at -20 °C until DNA extraction. DNA extraction was carried out using Norgen DNA extraction kit with strict adherence to the manufacturer's guidelines. All animal samplings and handlings were done with strict adherence to the Nigerian animal welfare ethical legislation.

Quantification of DNA

The purity quantification of isolated DNA was carried out by spectrophotometry using Nanodroplite. The mean OD_{260}/OD_{280} ratio for all purified DNA was ~1.810. Further examination for visual quality determination of the extracted DNA was done using 1 % agarose gel electrophoresis ran at 100 volts for 30 mins (Figure 1).

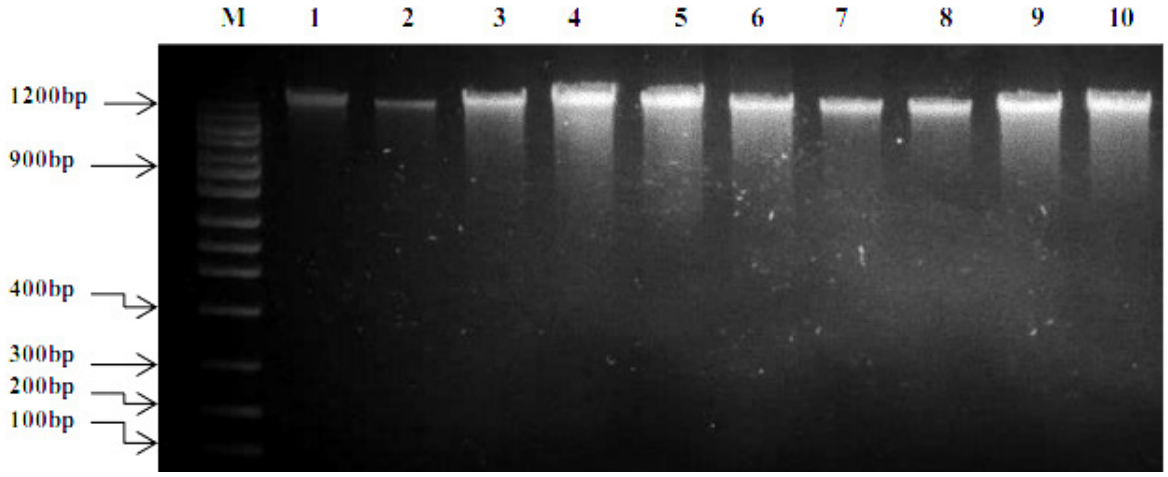

Figure 1. Electrophoretogram of purified DNA for quality verification

Polymerase chain reaction and microsatellite genotyping

Seven microsatellite markers (SAT3, SAT8, SAT12, SOL 3, SOL 8, SOL 28 and SOL 30) used for this study (Table 1). The polymerase chain reaction for the amplification of isolated DNA was prepared in a 25.00 µL cocktail mixture, which contained 1.00 µL of DNA, 2.50 µL of 10 × buffer, 1.00 µL of 25 mM dNTPs, 2.00 µL of primer (1.00 µL of each forward and reverse), 0.20 µL of Taq polymerase, 2.20 µL of 25 mm/Mol Mg^{2+} and 16.10 µL of distilled water. Denaturing temperature of 94 °C (1 min), annealing temperature for the seven microsatellite primers ranged from 52 °C – 60 °C, the initial extension done at 72 °C (1 min) followed by the final extension at 72 °C (10 min) (Table 1). The generated products were subjected to 12 % polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. Bands on gels were scored basing on size of ladder with Gene Scan 3.1.2. Bands were designated as alleles and prepared into Excel Worksheet.

Data Analyses

Allele frequencies, observed heterozygosity, (HO) and expected heterozygosity (HE) were estimated for seven microsatellite markers using microsatellite analyzer (version 4.05), which is a platform independent analysis tool for large microsatellite dataset (Dieringer and Schlotterer, 2003). F-statistics was obtained using the Genepop 4.1 program (Raymond and Rousset, 1995; Rousset, 2008), the gene flow/migrant rate (NM) was calculated using the relation (Weir and Cockerham, 1984). Polymorphism information content (PIC) for each marker in each rabbit population was calculated using the formula suggested by (Botstein *et al*., 1980).

$$N_M = (0.25)\,(1 - F_{ST}) / F_{ST} \quad \text{..... (1)}$$

$$H_T = 1 - \sum Pi^2 \quad \text{..... (2)}$$

$$PIC = H_T - 2 \sum \sum Pi^2 Pj^2 \quad \text{..... (3)}$$

The genetic distance (D_A) (Nei *et al*., 1983) was calculated with MSA (Dieringer and Schlötterer, 2003). Genetic sub-structuring of rabbit breeds was investigated using the Bayesian clustering procedure of STRUCTURE ver. 2. (Pritchard *et al*., 2000).

Ten independent runs were performed for each K between 1 and 5, with a burn-in period of 300,000 iterations followed by 1,000,000 iterations of the Markov chain Monte Carlo algorithm. To identify the most probable groups (K) that best fit the data, the STRUCTURE HARVESTER (Earl and von Holdt, 2012), which implements the Evanno method (Evanno *et al*., 2005) was used.

Table 1. Sequences and the annealing temperature of seven microsatellite markers used

Locus	Primer Sequence	Annealing temperature (°C)
SAT 3	F: 5'GGAGAGTGAATCAGTGGGTG3' R: 5'GAGGGAAAGAGAGAGACAGG3'	60
SAT 8	F: 5'CTTGAGTTTTAAATTCGGGC3' R: 5'GTTTGGATGCTATCTCAGTCC3'	55
SAT 12	F: 5'GGATTGGGCCCTTTGCTCACACTTG3' R: 5'ATCGCAGCCATATCTGAGAGAACTC3'	58
SOL 3	F: 5'ATTGCGGCCCTGGGGAATGAACC3' R: 5'TTGGGGGGATATCTTCAATTTCAGA3'	58
SOL 8	F: 5'CAGACCCGGCAGTTGCAGAG3' R: 5'GGGAGAGAGGGATGGAGGTATG3'	60
SOL 28	F: 5'TACCGAGCACCAGATATTAGTTAC3' R: 5'GTTGCCTGTGTTTTGGAGTTCTTA3'	52
SOL 30	F: 5'CCCGAGCCCCAGATATTGTTACCA3' R: 5'TGCAGCACTTCATAGTCTCAGGTC3'	52

RESULTS

The average genetic differentiation among breeds (0.0479), which implies 96.6 % of the total genetic variation, was explained by individual variability and only 4.7 % which is attributable to differences among breeds (Table 2). Nei's genetic distance was used to ascertain the degree of relationship among the four rabbit populations in Nigeria.

The Californian White and the Chinchilla rabbit populations revealed the closest relationship (0.1005), while the farthest relationship was recorded between the New Zealand White and Chinchilla breeds of rabbit (0.3509) (Table 3). The genetic distance data were used in the construction of a consensus neighbor joining tree (Figure 2).

The genetic sub-structuring was further determined using STRUCTURE (Pritchard *et al*., 2000), which was run

Table 2. Gene diversity, migrant rate and F-statistics across markers within and among four rabbit breeds in Nigeria

Marker	GD	F_{IS}	F_{IT}	F_{ST}	N_M
SAT 3	0.8511	-0.0662	0.0360	0.0958	2.3696
SAT 8	0.7813	-0.0387	0.0407	0.0764	3.0222
SAT 12	0.8267	0.0788	0.1044	0.0278	8.7428
SOL 3	0.8392	-0.0681	-0.0395	0.0268	9.0783
SOL 8	0.7989	-0.0041	0.0056	0.0097	25.5231
SOL 28	0.8551	-0.0584	-0.0511	0.0069	35.9818
SOL 30	0.8091	0.0154	0.1064	0.0924	2.4551
Mean	0.8230	-0.0201	0.0289	0.0479	12.4532

GD = gene diversity, N_m = gene flow, F_{IS}, F_{IT} and F_{ST} are fixation indices.

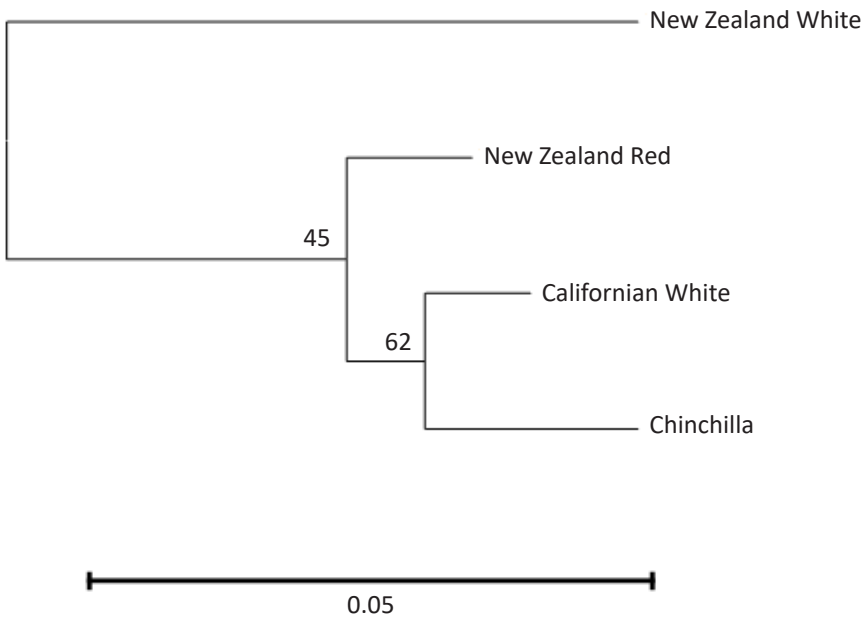

Figure 2. Phylogenetic tree constructed from DA by the neighbour-joining method showing the genetic relationships among the four rabbit breeds. The values at the nodes are the percentages of bootstrap values from 1,000 replications of re-samplings

10 times independently with K ranging from 1 to 5, in order to choose the appropriate value of K. The Evanno method (Evanno *et al*., 2005) implemented in the Structure Harvester software v0.6.8 (Earl and vonHoldt 2012), which uses an ad hoc statistic based on the second order rate of change of the likelihood function with respect to K, was used to determine the value for K. The Evanno method showed that the modal value for Δk was at k = 2, i.e. the statistic peak at K = 2 indicating strong support for 2 groups (Figure 3).

DISCUSSION

Genetic variation can be defined as the totality of differences exhibited by individuals in a population attributable to their genes and their distribution. It is essential for selection, because it can only increase or decrease frequency of alleles that already exist in the population. Population differentiation was examined by the fixation indices FIS, FIT and the FST for each locus and across all loci. The average genetic differentiation among breeds was 0.0479, which

Table 3. Nei's genetic distance (below diagonal) and the proportion of shared alleles (above diagonal) of rabbit populations examined

Population	New Zealand White	Californian White	New Zealand Red	Chinchilla
New Zealand White	00000	0.2863	0.3219	0.2722
Californian White	0.3258	00000	0.5139	0.5508
New Zealand Red	0.3108	0.1276	00000	0.5259
Chinchilla	0.3509	0.1005	0.1095	00000

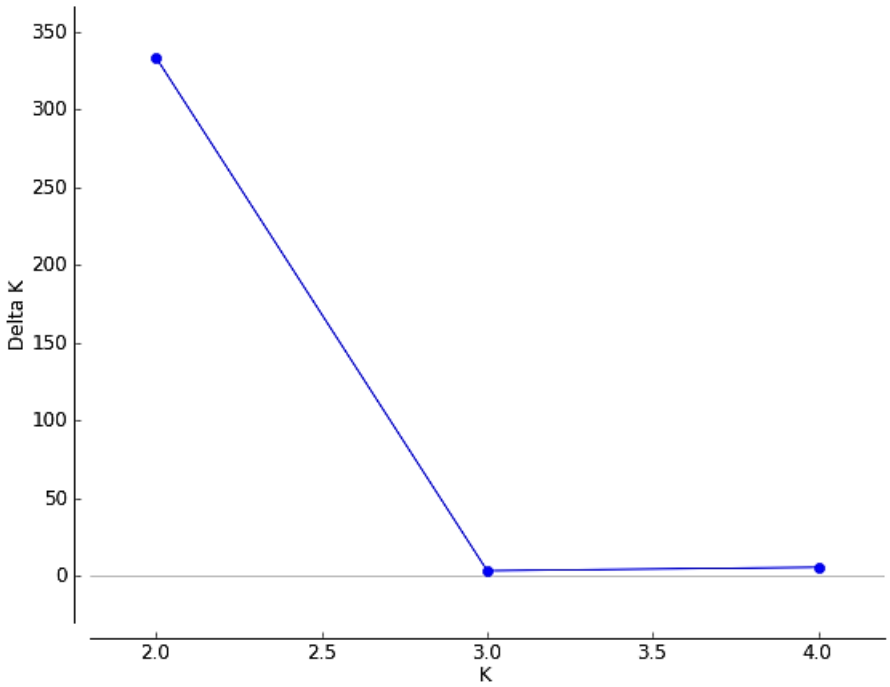

Figure 3. The optimum number of clusters as derived from the structure harvester

Table 4. Proportion of membership of each of the four rabbit population in the 2 inferred clusters

Cluster			
Population	1	2	N
New Zealand White	**23.468**	1.532	25
Californian White	3.811	**21.612**	25
New Zealand Red	3.979	**21.021**	25
Chinchilla	2.636	**22.364**	25
F_{ST}	0.1805	0.0030	

F_{ST}: Fixation index N: population size.

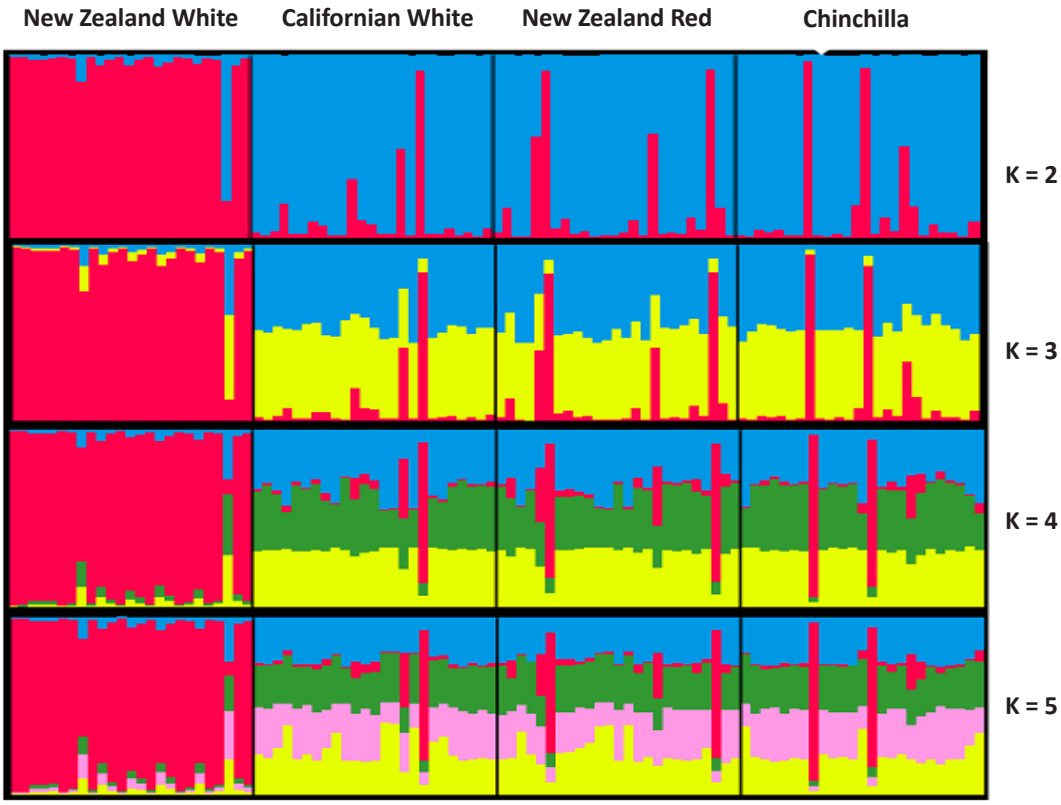

Figure 3. Genetic sub-structuring of four rabbit populations in Nigeria using STRUCTURE

implies that 96.6 % of the total genetic variation can be explained by individual variability and only 4.7 % is attributable to differences among breeds.

The mean genetic differentiation in this study was dissimilar to those reported in the Chinese rabbit 9.9 % by Tian-wein *et al*., 2010, Egyptian rabbits 7 % and 7.2 % in studies of El-Aksher *et al*. (2016) and Grimal *et al*. (2012), respectively. The low level of genetic differentiation (0.0479), which was supported by the high level gene flow rate NM (Nm, number of migrants per generation), suggested possible admixture within the rabbit population and lack of isolation (reproductively not geographically) between the breeding rabbit population in Nigeria.

The mean inbreeding coefficient of the individual relative to the sub-population (FIS) -0.0201 indicated the existence of outbreeding (heterozygosity excess) within the rabbit population, which might be as a result of indiscriminate negative assortative mating among rabbit breeds. The negative FIS for loci SAT3, SAT8, SOL3, SOL8 and SOL28 indicated that there is a homozygosity deficiency and/or heterozygosity excess.

Genetic distance is a population genetic parameter used to ascertain the degree of relatedness between sub-groups in a population. Values for the genetic distance measures range from 0 to 1, in which values tending towards 0 indicate closely related individuals, while values that tend towards 1 indicate distant related individuals. The close genetic relatedness between the Chinchilla and the Californian White in Nigeria is indicative of a higher level of intermixing between both breeds, while the distant related New Zealand White indicated possible isolation from other breeds. The neighbour joining tree is a distance based method which does not require the data to be ultra-metric i.e. does not require that all lineages have diverged by equal amounts.

Three distinct clusters were observed. The first cluster revealed the New Zealand White populations of rabbit relative divergence from all other rabbit populations, while the New Zealand Red rabbit formed the second cluster and thirdly, Californian White rabbit populations and Chinchilla were clustered together, thus, enhancing the reliability of the relationship. Additionally, Bootstrapping tested the robustness of the tree, revealing a 62 % support for the Chinchilla/Californian White node and 45 % for the New Zealand Red node; however, the low bootstrap values in this study might be as a result of high genetic similarity between the sampled rabbit breeds.

Grimal *et al*. (2012) showed a fine scale phylogeny among four Egyptian rabbits, which were clustered closely, and a diverging Spanish line rabbit (New Zealand White). Thus, diversity within a community of species, or between different communities, is affected by the phylogenetic relations among species (Brocchieri, 2015). Furthermore, a population consisting of more distantly related individuals would be more diverse, since distantly related individuals are likely to exhibit a greater number of unique features.

The genetic sub-structuring analysis shows a high level of breed admixture, as revealed by the Bayesian clustering, which indicated a weak subdivision between the four rabbit populations. Although the Bayesian analysis revealed the highest probability of forming 2 clusters, it needs to be interpreted with caution, as some of the clusters were not well defined. Furthermore, proportion of membership for each individual within the rabbit sub-population assignment to clusters was generated (Table 4). Cluster 1 showed a higher fraction of the New Zealand White rabbit, compared to lower range values of the Californian White, New Zealand Red and the Chinchilla and vice versa in the cluster 2 between the rabbit populations.

The variation between breeds is small and not in interaction with the geographic distribution of population. The investigated genetic differentiation between the rabbit breeds in Nigeria is, therefore, mainly not discernible among regions or geographical origin.

CONCLUSION

Molecular characterization, as revealed by the microsatellite markers, showed that the four rabbit breeds have more within-breed variation than between-breed variation as a result of the low values of genetic differentiation with regard to the inbreeding estimates, which indicated a relatively high out-breeding among the four rabbit breeds; thus high gene flow rate between rabbit populations. The genetic distance values indicated the closest genetic relationship between the Chinchilla and the Californian White rabbit in Nigeria, while the farthest relationship was recorded between the Chinchilla and New Zealand rabbit breeds in Nigeria.

The genetic sub-structuring using STRUCTURE analysis revealed 2 clusters, which are the New Zealand White (cluster 1) and the New Zealand Red, Chinchilla, and Californian White (cluster 2) revealing a high degree of admixture and interbreeding with no significant gene pool variation between individuals of cluster 2. Furthermore, it is noteworthy that geographic isolation is a crucial factor for differentiation between populations, and no clear geographic pattern was observed in this study, since animals from different Nigerian geographic settlement were considered as a single population based on the STRUCTURE analysis. The indiscriminate

breeding and mating patterns practiced by Nigerian subsistent rabbit farmers have significantly eroded the genetic integrity of the rabbit populations, as shown by the weak structure between the rabbit breeds in Nigeria.

REFERENCES

Botstein, D., White, R. L., Skolnick, M. & Davis, R. W. (1980). Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphism. *The American Journal of Human Genetics*, 32(3), 314–331.

Brocchieri, J. (2015). Phylogenetic Diversity and the Evolution of Molecular Sequences. *Journal of Phylogenetics and Evolution Biology*, 3:1. Doi:10.4172/2329-9002.1000e109

Dieringer, D. & Schlotterer, C. (2003). Two distinct modes of microsatellite mutation processes; evidence from the complete genomic sequences of nine species. *Genome Research*, 13(10): 2242–2251.

Earl, D. A. & vonHoldt, B. M. (2012). Structure Harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4(2), 359–361. Doi: 10.1007/s12686-011-9548-7

El-Aksher, S. H., Sherif, H. S., Khalil, M. H., El-Garhy, H. A. S. & Ramadan, S. (2016). Comparative genetic analysis among Moshtohor line rabbits and their parental lines using microsatellite markers. *3rd International Conference on Biotechnology Applications in Agriculture (ICBAA)*, Benha University, 5-9 April 2016, Egypt.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, 14, 2611–2620. Doi: 10.1111/j.1365-294X.2005.02553.x

Grimal, A., Safaa, H. M., Saenz-de-Juano, M. D., Viudes-de-Castro, M. P., Mehaisen, G. M. K., Elsayed, D. A. A., Lavara, R., Marco-Jiménez, F. & Vicente, J. S. (2012). Phylogenetic Relationship among Four Egyptian and One Spanish Rabbit Populations Based On Microsatellite Markers. World Rabbit Science Association in *Proceedings of the 10th World Rabbit Congress*, September 3-6, Sharm El-Sheikh, Egypt, 177–181.

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.

Lebas, F., Coudert, P., de Rochambeau, H. & Thébault, R. G. (1998). The Rabbit: Husbandry, Health and Production. FAO, Rome-Italy. ISBN: 92-5-103441-9. (http://www.fao.org/docrep/t1690E/t1690E00.HTM).

Lukefahr, S. D. (1998a). Review of global rabbit genetic resources: special emphasis on breeding programmes and practices in lesser developed countries. *Journal of Animal Genetic Resources Information*, 23, 49–67. Doi:10.1017/S1014233900001073

Lukefahr, S. D. (1998b). Rabbit production in Uganda: Potential vs. opportunity. *World Rabbit Science*, 6, 331–340. Doi:10.4995/wrs.1998.365

Nei, M. (1983). *Molecular Evolutionary Genetics*.Columbia University Press, New York.

Pritchard, K. J., Wen, X. & Falush, D. (2000). *Documentation of Structure software: version 2.3.* Department of Human genetics, University of Chicago. http//pritch.bsd.uchicago.edu/structure.html

Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248–249.

Rosenberg, N. A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology*, 4, 137–138.

Rousset, F. (2008). Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources*, 8, 103–106.

Tian-Wen, W., Gui-Jiang, X., Yu-Lai, P., Xi-Ping, X., Bi-Chun, L. & Xin-Sheng, W. (2010). Study on genetic diversity of seven rabbit populations evidenced by microsatellite makers. *Journal of Animal and Veterinary Advances*, 9, 359–365.

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Journal of Evolution*, 38(6), 1358–1370.

Zerrouki, N., Bolet, G., Berchiche, M. & Lebas, F. (2004). Breeding performance of a local Kabylian rabbit does in Algeria. *Proceedings of the 8th World Rabbit Congress*, Puebla, Mexico, 7-10 September, 371–377.