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Genetic Diversity of Rabbit (*Oryctolagus cuniculus*) Population in South Eastern Nigeria Using Microsatellite Markers

A. I. Adeolu[a,*], M. Wheto[b], V. U. Oleforuh-Okoleh[c], R. N. Nwose[a], A. S. Adenaike[b], A. Yakubu[d], E. M. Abiola[a], & B. G. Mohammed[a]

[a]Department of Agriculture, Animal Science Programme, Alex Ekwueme Federal University, Ndufu-Alike, P.M. B. 1010, Abakaliki, Ebonyi State, Nigeria
[b]Department of Animal Breeding and Genetics, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, P. M. B. 2240 Ogun State, Nigeria
[c]Department of Animal Science, Rivers State University of Science and Technology, Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Rivers State, Nigeria
[d]Department of Animal Science, Faculty of Agriculture, Nasarawa State University, Keffi, Shabu-Lafia Campus, P.M.B. 135, Lafia, Nigeria
*Corresponding Author: adeolufunai2012@gmail.com
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ABSTRACT

A study was conducted to estimate the diversity that exists among three rabbit populations adapted to the South-Eastern part of Nigeria. Blood samples were collected from 75 matured, mixed-sex, and unrelated three rabbit breeds selected across the zone. Eight microsatellites (Sol30, Sol33, and Sol44, Sat3, Sat7, Sat8, Sat12, and INRA) markers were used for the study. These microsatellites were uniformly distributed among rabbit genomes for genotyping. Subsequently, genetic variability within and between breeds was calculated. Allelic frequencies and Hardy-Weinberg equilibriums as well as Analysis of Molecular Variance, were also estimated using GenAlEX 6.41 software. Discriminant Analysis of Principal Components (DAPC) for the population structure of the rabbit breeds was performed in R v.3.5.0 using the R package adegenet. All the 8 loci amplified in this study were found to be 100% polymorphic, the observed allele sizes and their frequencies for the microsatellite markers in every three breeds showed that the highest frequency was 0.330 for the allele with the size of 470bp at Sol33 locus in New Zealand White (NZW) rabbits. The Nei's genetic identities and distances between Chinchilla (CHI) and Dutch (DUT), CHI and NZW, DUT and NZW obtained in this study were [0.173, 0.185, and 0.189] and [1.753, 1.689, and 1.666] respectively. The dendrogram and biplot revealed that the three breeds were identified at two separate clusters. In addition, the admixture level of an individual rabbit among the three breeds indicated that the breeds were not pure and also the existence of more polymorphism within the breed than among the breed diversity.

Keywords: genetic diversity; rabbit; breeds; microsatellite markers

INTRODUCTION

Rabbit is a prolific, fast-growing, and high fecundity; thus, rabbit production is one of the animal protein sources in developing countries like Nigeria. Rabbit meat is rich in micronutrients such as iron, zinc, iodine, and vitamin B_{12} and low in cholesterol and sodium level (Adeolu *et al.*, 2020). Hence, its consumption was recommended for alleviating hidden hunger in women of reproductive age and infants within 1000 days (window period) of life. Despite some of the nutritional and health benefits of eating rabbit meat, rabbits exhibit exceptional phenotype diversity, which could serve great commercial benefits and also serve as important animal models in biomedical research (Carneiro *et al*., 2011).

These attributes have brought about increased clamor and excitement for upgrading available rabbit breeds for high reproductive and growth potentials (FAO, 2004). For upgrading to be effective, information concerning economic traits and their diversities needs to be examined and documented under the prevailing environmental conditions. Information on the degree of genetic variation and diversity changes of adapted rabbits over time will contribute to efficient conservation, maintenance, and rational utilization of germplasm resources that determine future breeding strategies for the assessment of useful genes from divergent germplasm.

Domestic rabbits are classified into different breeds based on the color, biometric traits, and origin (Sanford, 1996), but there is a need to differentiate between these rabbit breeds with the aid of molecular markers because they are more abundant, ubiquitous, hypervariable in nature, and evenly distributed along the chromosomes. Characterization at the molecular level is carried out to explore the genetic diversity between and within livestock populations and also to determine the genetic

relationship among the different populations (Rahimi *et al*., 2015). Studies (Chantry-Darmon *et al*., 2006; Grimal *et al*., 2012; El-Aksher *et al*., 2016) have shown the importance of microsatellites in generating the information necessary for planning crossbreeding and selection of genotypes in genetic breeding programs in rabbits.

The historical record of rabbit rearing in Nigeria shows that rabbits are not native to Nigeria and South Eastern Zone in particular. Many breeds were introduced into the country with the advent of the slave trade and European invasion into Africa, and hence, the genetic information of those adapted to various geopolitical zones is scanty or not available. In the present study, we sampled three populations of adapted rabbits in the South Eastern Zone of Nigeria to evaluate the degree of genetic variability over a period of adaptation using eight (8) microsatellite markers that exploit the genetic relationship among rabbit populations based on certain parameters.

MATERIALS AND METHODS

Experimental Animals

A total of 75 matured, mixed-sex and unrelated rabbits, Dutch (DUT), Chinchilla (CHI), and New Zealand White (NZW) from research and teaching farm of five (5) Universities within South Eastern Nigeria were drawn from the entire population to form the samples. These include 5 samples each per breed from Michael Okpala University of Agriculture, Umudike (MOUAU), Nnamdi Azikwe University, Akwa (UNIZIK), Ebonyi State University, Abakaliki (EBSU), University of Nigeria, Nsukka (UNN), and Federal University of Science and Technology, Owerri (FUTO). Animal ethics and welfare certificate/approval was obtained from the University Research Ethics Committee of Alex Ekwueme Federal University Ndufu-Alike [AE-FUNAI] with reference No.: FUNAI/SEN/EBC/17/VOL. 1/22.

Blood Collection and DNA Extraction

Approximately 1-2 mL of blood was collected aseptically from each experimental animal and put into EDTA bottle with the aid of a needle and syringe. Genomic DNA was extracted using Qiagen DNA extraction kits with strict compliance to the manufacturer protocols. Quantification of DNA yield and assessment of quality were done using a Nanodrop™ spectrophotometer and 1% agarose gel electrophoresis, respectively.

Genotyping of Microsatellite Markers

A total of eight (8) microsatellite markers (Sol30, Sol33, and Sol44, Sat3, Sat7, Sat8, Sat12, and INRA) uniformly distributed across the rabbit genomes as reported by El-Aksher *et al*. (2016) were used for this study. PCR amplification was carried out in a thermocycler. The 25 µL PCR reaction mix was prepared in PCR tubes containing 2 µL of DNA template, 1µL each of forward and reverse primers, 12.5µL of 2X PCR Master mix, and 8.5µL of Nuclease free water. The amplification reaction was as follows: Initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 1 minute, annealing at 53.2°C to 60.0°C for 1 minute, extension at 72°C for 1 minute, and followed by the final extension at 72°C for 10 minutes. The resulting amplicons were visualized using 1.5% agarose to separate the PCR products into different sizes at 100Volts for 1 hour using DNA ladder of 100bp. The gel was stained with ethidium bromide, the resulting band was viewed under transilluminator and genotyped using GelAnalyser.

Microsatellite data analysis. Genotypic, allelic frequencies, and Hard-Weinberg equilibriums as well as Analysis of Molecular variance, were estimated using GenAlEX 6.41 software. To investigate the population genomic structure of rabbit breeds, Discriminant Analysis of Principal Components (DAPC) was performed in R v.3.5.0 (R Development Core Team 2008), using the R package adegenet.

RESULTS

Genetic Differentiation Among and Within the Population

From the summary of the Analysis of Molecular Variance, AMOVA in Table 1, the genetic differentiation measured among population and individual of the total genetic variance were 1% and 4% respectively, while 96% of the genetic variation was attributed to within-population genetic diversity.

Allele Frequencies for the Microsatellite Loci Across the Three Breeds

The observed allele sizes and their frequencies for the microsatellite markers in each breed's population in Figure 1 show that the highest frequency was 0.330 for the allele with the size of 470bp at Sol33 locus in New Zealand White (NZW) rabbits while the lowest (0.083) was common to Sol44 in each breed within a population. Other allele frequency results obtained from the interpretation of Figure 1 for the microsatellite loci across the three breeds were mostly polymorphic.

The Mean (N_a) and Effective (N_e) Number of Alleles at Various Loci Across Population

The mean number of alleles (N_a) observed in the overall population of three rabbit breeds in the present study was 10.208 (Table 2). In the subpopulation, Dutch (DUT) breed had the highest N_a of 10.375 when com-

Table 1. Summary AMOVA table

Source	Df	MS	Est. Var.	%
Among population	2	4.361	0.027	1%
Among individual	15	4.033	0.142	4%
Within individual	18	3.750	3.750	96%
Total	35		3.919	100%

Note: Df= Degree of freedom; MS= Mean square; Est.Var.= Estimated variance.

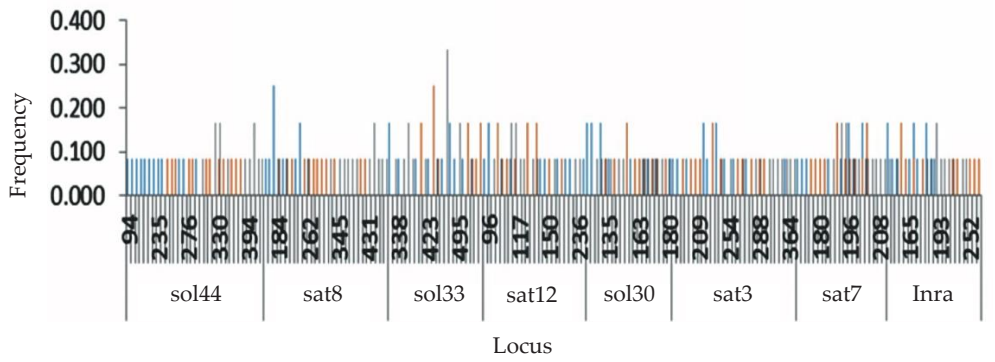

Figure 1. Allele frequencies for all the microsatellite markers across the three breeds. ■= Chinchilla; ■= Dutch ; ■= New Zealand.

Table 2. Results of parameters analyzed at various microsatellite loci across the three breeds

Population	Locus	N	N_a	N_e	I	H_o	H_e	uH_e	F
CHI									
	Sol30	6	9.000	8.000	2.138	0.833	0.875	0.955	0.048
	Sol33	6	10.000	9.000	2.254	0.667	0.889	0.970	0.250
	Sol44	6	12.000	12.000	2.485	1.000	0.917	1.000	-0.091
	Sat3	6	10.000	9.000	2.254	1.000	0.889	0.970	-0.125
	Sat7	6	10.000	9.000	2.254	1.000	0.889	0.970	-0.125
	Sat8	6	9.000	7.200	2.095	1.000	0.861	0.939	-0.161
	Sat12	6	11.000	10.286	2.369	0.833	0.903	0.985	0.077
	Inra	6	9.000	8.000	2.138	1.000	0.875	0.955	-0.143
	Mean ± SE	6.000±0.000	10.000±0.378	9.061±0.532	2.248±0.046	0.917±0.045	0.887±0.006	0.968±0.007	-0.034±0.051
DUT									
	Sol30	6	11.000	10.286	2.369	1.000	0.903	0.985	-0.108
	Sol33	6	7.000	6.000	1.864	1.000	0.833	0.909	-0.200
	Sol44	6	12.000	12.000	2.485	1.000	0.917	1.000	-0.091
	Sat3	6	11.000	10.286	2.369	1.000	0.903	0.985	-0.108
	Sat7	6	10.000	9.000	2.254	1.000	0.889	0.970	-0.125
	Sat8	6	12.000	12.000	2.485	1.000	0.917	1.000	-0.091
	Sat12	6	9.000	8.000	2.138	1.000	0.875	0.955	-0.143
	Inra	6	11.000	10.286	2.369	1.000	0.903	0.985	-0.108
	Mean ± SE	6.000±0.000	10.375±0.596	9.732±0.716	2.292±0.073	1.000±0.000	0.892±0.010	0.973±0.011	-0.122±0.013
NZW									
	Sol30	6	12.000	12.000	2.485	1.000	0.917	1.000	-0.091
	Sol33	6	7.000	5.143	1.792	0.333	0.806	0.879	0.586
	Sol44	6	9.000	8.000	2.138	0.833	0.875	0.955	0.048
	Sat3	6	12.000	12.000	2.485	1.000	0.917	1.000	-0.091
	Sat7	6	10.000	9.000	2.254	1.000	0.889	0.970	-0.125
	Sat8	6	11.000	10.286	2.369	1.000	0.903	0.985	-0.108
	Sat12	6	10.000	9.000	2.254	1.000	0.889	0.970	-0.125
	Inra	6	11.000	10.286	2.369	1.000	0.903	0.985	-0.108
	Mean ± SE	6.000±0.000	10.250±0.590	9.464±0.796	2.268±0.080	0.896±0.083	0.887±0.013	0.968±0.014	-0.002±0.086
	GrandMean ± SE	6.000±0.000	10.208±0.295	9.419±0.385	2.269±0.038	0.938±0.031	0.889±0.005	0.970±0.006	-0.052±0.034

Note: N_a= Mean number of alleles; Ne= Effective number of alleles; I= Shannon's Information Index; H_o= Observed Heterozygosity; H_e= Expected Heterozygosity; uH_e= Unbiased Expected Heterozygosity; F= Fixation Index; Mean H_e= Average H_e across the populations; Mean H_o= Average H_o across the populations; H_t= Total Expected Heterozygosity; CHI= Chinchilla; DUT= Dutch; NZW= New Zealand White.

pared to the Chinchilla (CHI), (N_a= 10.000), and NZW (N_a= 10.250).

The number of effective alleles observed (N_e) among eight loci within the three breeds ranged from 7-12. Sol33 locus produced the least number of alleles (N_e= 7) in DUT and NZW, while the highest (N_e= 12) was made by Sol44 locus (in CHI and DUT), Sol30, and Sat3 loci (in NZW), and Sat8 locus (in DUT).

The mean effective number of alleles (N_e) in population across various loci were 9.061, 9.732, and 9.464 in CHI, DUT, and NZW breeds, respectively, and ranged from 5.143 at Sol33 locus in NZW to 12.000 at Sol44 lo-

cus in CHI, Sol44 and Sat8 loci in DUT, and Sol30 and Sat3 in NZW.

The Observed (H_o), Expected (H_e), and Unbiased Expected (uH_e) Heterozygosity at Various Loci Across Population

The results of these parameters are presented in Table 2. The overall mean of observed heterozygosity (H_o) was 0.938 and ranged from 0.333 to 1.000. The expected heterozygosity (H_e) for all loci studied averaged 0.889, while the mean H_e recorded for CHI, DUT, and NZW breeds were 0.887, 0.892, and 0.887, respectively. The unbiased expected heterozygosity (uH_e) ranged from 0.939 to 1.000 in CHI, 0.909 to 1.000 in DUT, and 0.879 to 1.000 in NZW (Table 2).

Hardy-Weinberg Equilibrium (HWE) at Various Loci Across Population

Departure from Hardy-Weinberg equilibrium (HWE) was tested across the three rabbit breeds within the loci studied. No significant deviation was observed except for the Sol33 locus, which was found to be significantly deviating from the HWE in NZW rabbits (Table 3).

Genetic Differentiation by Reduction in Heterozygosity Due to Inbreeding

The F-statistics (F_{IS}, F_{IT}, and F_{ST}) presented in Table 4 showed the reduction in heterozygosity at various loci across the population studied. The mean values

Table 3. Chi-Square (χ2) values for testing Hardy-Weinberg Equilibrium (HWE) across the populations over each locus

Population	Locus	DF	χ2	Probability	Significance
CHI	Sol30	36	36.000	0.469	ns
	Sol33	45	54.000	0.168	ns
	Sol44	66	66.000	0.477	ns
	Sat3	45	54.000	0.168	ns
	Sat7	45	42.000	0.600	ns
	Sat8	36	42.000	0.227	ns
	Sat12	55	60.000	0.299	ns
	Inra	36	42.000	0.227	ns
DUT	Sol30	55	54.000	0.513	ns
	Sol33	21	30.000	0.092	ns
	Sol44	66	66.000	0.477	ns
	Sat3	55	54.000	0.513	ns
	Sat7	45	42.000	0.600	ns
	Sat8	66	66.000	0.477	ns
	Sat12	36	36.000	0.469	ns
	Inra	55	54.000	0.513	ns
NZW	Sol30	66	66.000	0.477	ns
	Sol33	21	36.000	0.022	*
	Sol44	36	48.000	0.087	ns
	Sat3	66	66.000	0.477	ns
	Sat7	45	42.000	0.600	ns
	Sat8	55	54.000	0.513	ns
	Sat12	45	54.000	0.168	ns
	Inra	55	54.000	0.513	ns

Note: CHI= Chinchilla; DUT= Dutch; NZW=New Zealand White; DF= degree of freedom, ns= not significant, *= significant at p<0.05.

Table 4. F-Statistics and estimates of N_m over-all population for each locus

Locus	F_{IS}	F_{IT}	F_{ST}	N_m
Sol30	-0.052	-0.003	0.046	5.196
Sol33	0.209	0.294	0.108	2.068
Sol44	-0.046	0.021	0.064	3.656
Sat3	-0.108	-0.040	0.061	3.849
Sat7	-0.125	-0.071	0.048	4.966
Sat8	-0.119	-0.042	0.069	3.366
Sat12	-0.063	0.005	0.063	3.692
Inra	-0.119	-0.062	0.051	4.669
Mean ± SE	-0.053±0.039	0.013±0.042	0.064±0.007	3.933±0.357

Note: F_{IS}= reduction in heterozygosity due to inbreeding within each population; F_{IT}= reduction in heterozygosity due to total inbreeding for each locus; F_{ST}= Genetic differentiation among the population; N_m= Limited gene flow among the population.

for F_{IS} and F_{IT} were -0.053 and 0.013, respectively. F_{ST} value ranged from 0.046 at locus Sol30 and 0.108 at locus Sol33, and it averaged 0.064. The mean F_{ST} (0.064) can be translated to 6.4% and 93.6% for among/inter-population and within/intra-population variation respectively. The mean level of gene flow (Nm) among the population was estimated to be 3.933, ranging from 2.068 to 5.196 (Table 4). The pairwise F_{ST} values among the three populations were also ranged from 0.048 to 0.049 (Table 5).

Genetic Identity and Distance Among Populations

To further clarify the gene differentiation among different populations, Nei's pairwise (Nei, 1978) genetic similar (identity) and distance coefficients were assessed (Table 6). Genetic similar coefficients varied from 0.173 to 0.189 with an average of 0.182. The Nei's genetic distances between CHI and DUT, CHI and NZW, as well as DUT and NZW obtained in this study, were 1.753, 1.689, and 1.666, respectively.

In order to further illustrate the relationships among populations, a dendrogram based on Nei's genetic similarity, clustered the three populations into two major groups (Figure 2) with the CHI breed in a distinct cluster from the DUT and NZW breeds showing the considerable genetic distance between the chinchilla breed and other two breeds.

Discriminant analysis of Principal Components (DAPC) shows three clusters identified (Figure 4). Linear discriminant 1 separated the Dutch breed from the other two breeds, while linear discriminant 2 isolated New Zealand White from the other two breeds.

DISCUSSION

The higher value of within-population genetic diversity (Table 1) obtained in the present study is similar to the work of El-Aksher *et al.* (2016), and such results may largely be attributed to existing random breeding systems in the study area. This usually resulted in frequent gene flow among individuals and increases the chance of gene recombination.

In reference to Figure 1, all the 8 loci amplified in this study were found to be 100% polymorphic in all the three breeds, which was consistent with the find-

Table 5. Pairwise population of F_{ST} values

	Chinchilla (CHI)	Dutch (DUT)	New Zealand White (NZW)
Chinchilla (CHI)	0.000		
Dutch (DUT)	0.049	0.000	
New Zealand White (NZW)	0.049	0.048	0.000

Table 6. Pairwise population matrix of Nei's genetic distance and identity

	Chinchilla (CHI)	Dutch (DUT)	New Zealand White (NZW)
Chinchilla (CHI)	0.000	0.173	0.185
Dutch (DUT)	1.753	0.000	0.189
New Zealand White (NZW)	1.689	1.666	0.000

Note: Upper diagonal - Genetic Identity, Lower diagonal - Genetic Distance

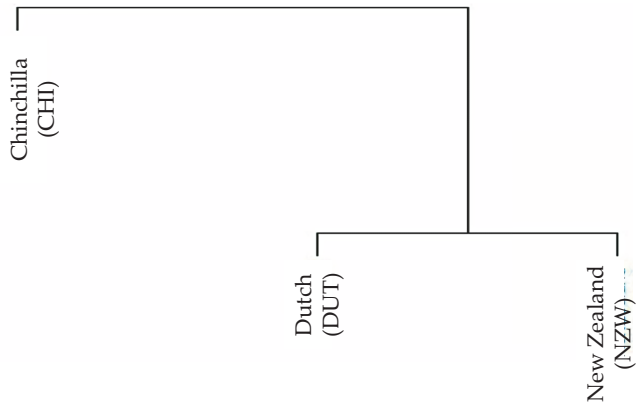

Figure 2. Dendrogram showing genetic relationships among the three rabbit breeds based on Fixation indices.

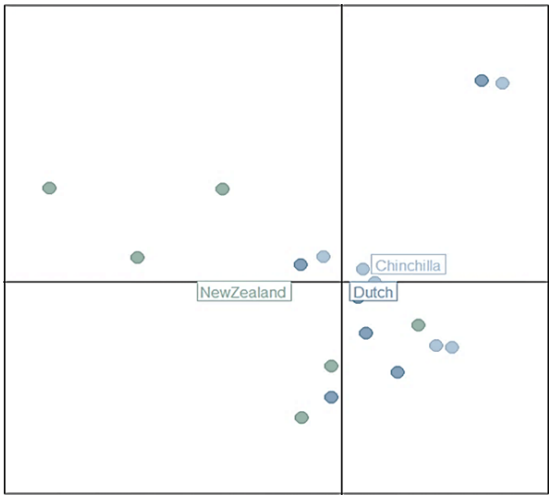

Figure 3. Biplot showing genetic relationships among the three rabbit breeds.

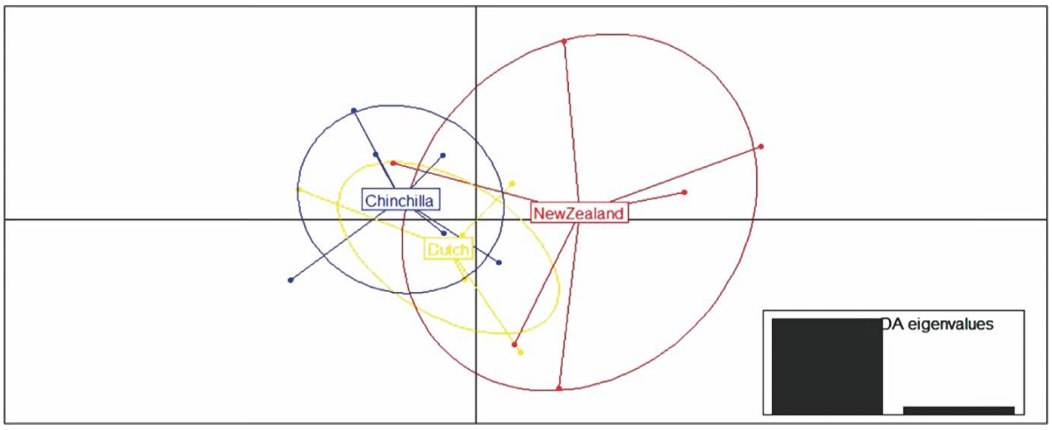

Figure 4. Discriminant analysis of Principal Components (DAPC) where the horizontal axis represents the first linear discriminant, the vertical axis represents the second linear discriminant.

ings of Grimal *et al.* (2012) in Egyptian and Spanish rabbits, Thimmayya & Buskirk (2012) in pygmy rabbits, Ben-Larbi *et al.* (2014) in Tunisian indigenous rabbits, El-Aksher *et al.* (2016) in Egypt Moshtohor line rabbits, and Kannegundla *et al.* (2018) in India rabbits. However, this result was higher than 81% polymorphic of the utilized microsatellites in European rabbits, as reported by Chantry-Darmon *et al.* (2006). El-Aksher *et al.* (2016) recommended high polymorphic loci (microsatellites) in the determination of the genetic structure and assessment of the gene pattern flow in any population.

The Mean number of alleles (N_a) obtained in the subpopulation for CHI, DUT, and NZW were higher than the findings of Omotoso *et al.* (2019) in four rabbit breeds reared in South Western Nigeria. Authors deduced high N_a as an indication of great allelic diversity which could have been influenced by crossbreeding or admixture among the population studied.

The marker(s) associated with range (7-12) of the mean effective number of alleles (N_e) across various loci reported in the present study are usually referred to least and most polymorphic marker(s), respectively. This range also showed that the markers used for the study were appropriate because their polymorphisms were higher than the minimum of 5 alleles (FAO, 2011) required for estimation of genetic diversity within an animal population through microsatellite markers. Therefore, a minimum of 7 alleles per locus indicated amplification and confirmed enough alleles to estimate the genetic diversity among the three genetic groups studied (Kannegundla *et al.*, 2018). The range of N_e recorded in the present study was higher than the range of 3-7 reported by Wu *et al.* (2010). However, El-Aksher *et al.* (2016) and Kannegundla *et al.* (2018) reported a closed similar range of 4-10 and 5-11, respectively. According to Frankham *et al.* (2002), the N_e observed over a range of loci for different breeds are also known as the allelic diversity, and it is an important parameter of genetic variation. The overall means N_e across genotypes are relatively higher than the mean N_e of 3.89 reported by El-Aksher *et al.* (2016) in Moshtohor line rabbits of Egypt, 4.156 reported by Abdel-Kafy *et al.* (2018) in native rabbits in Middle Egypt, as well as 6.728 and 6.874 reported by Kannegundla *et al.* (2018) in Soviet Chinchilla and Californian White rabbits, respectively.

The values obtained for expected heterozygosity (H_e) are comparable and consistent to the findings of Kannegundla *et al.* (2018) in Indian rabbits (0.842-0.849), Wu *et al.* (2010) in American Rex Rabbit (0.675-0.820), and El-Aksher *et al.* (2016) in Egypt Moshtohor line rabbits (0.66-0.88), but higher than the findings of Thimmayya & Buskirk (2012) in Pygmy rabbits (0.54-0.60), Ben-Larbi *et al.* (2014) in indigenous Tunisian rabbit (0.39-0.58), and Rabie *et al.* (2020) in five Egyptian rabbit breeds (0.20-0.65). Generally, the observed heterozygosity (H_o) in all loci were higher than H_e (except at Sol30, Sol33, and Sat12 in CHI and Sol44 and Sol33 in NZW). This result was contrary to the findings of El-Aksher *et al.* (2016) and Grimal *et al.* (2012) who respectively, reported H_o lower than H_e in 14 loci out of 16 used and in four Egyptian rabbit breeds and NZW. The authors conclusively affirmed H_e as the most parameters widely used to measure the genetic diversity across and within the populations.

Similarly, the results obtained for unbiased expected (uH_e) heterozygosity at various loci across populations were also higher than the ranges of 0.747 to 0.913 in Soviet Chinchilla and 0.809 to 0.915 in Californian White as reported by Kannegundla *et al.* (2018).

Chi-Square tests showed that the allele frequencies are in Hardy-Weinberg equilibrium ($p>0.05$) across populations studied over each locus**.** The implication is that the populations of rabbits used may be randomly bred due to the uncontrolled breeding system usually practiced by farmers in the study area. Zenger *et al.* (2003) used seven SAT microsatellite loci in 252 wild rabbits from five populations across Australia as against data from Europe and discovered that deviations ($p>0.05$) from HWE were non-significant in any of the Australian data. Non-significant deviations from HWE showed the existence of random breeding among three breeds' rabbit population, and allelic frequencies might remain the same except the population is influenced by evolutionary forces. Exception observed at locus Sol33 in NZW rabbits was an indication of selection at this locus, as observed by Kannegundla *et al*. (2018).

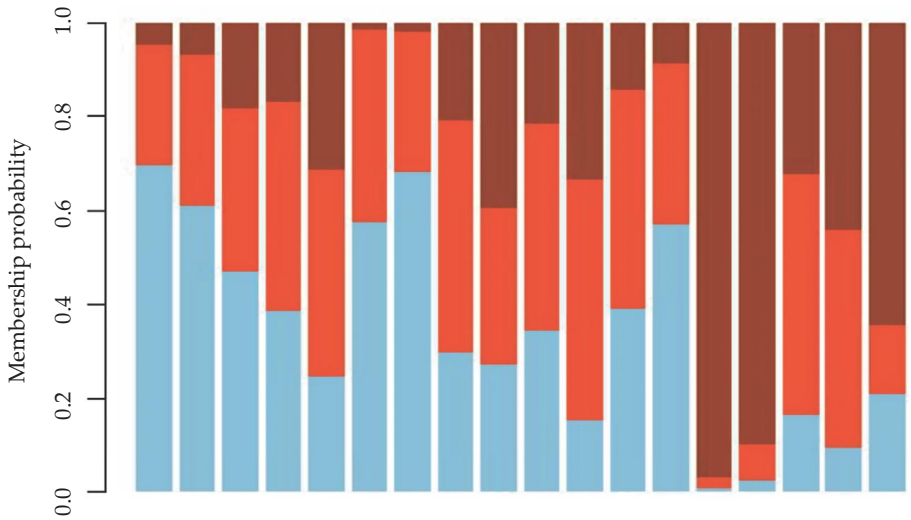

Figure 5. Admixture level of individual rabbit among the three breeds, ■= Chinchilla; ■= Dutch ; ■= New Zealand.

The reduction in heterozygosity due to inbreeding (F_{IS}, F_{IT}, and F_{ST}) was developed by Wright (1951) for testing the genetic differentiation among populations and also to summarize the genetic structure of a population and its subpopulations. The negative values for F_{IS} and F_{IT} at Sat8, Sol30, Sat3, Sat7, and Inra loci indicated the occurrence of heterozygote genotypes at a proportion higher than the homozygous genotypes at all loci. Such a negative value for F_{IS} corroborated -0.114 obtained by Wu *et al.* (2010).

The mean F_{ST} (0.064) obtained in the present study was higher than 0.04 and 0.0479, respectively, reported by Kannegundla *et al.* (2018) and Omotoso *et al.* (2019) but lower than 0.137 and 0.107 recorded by Grimal *et al.* (2012) and El-Aksher *et al.* (2017). Nevertheless, the range in values obtained for F_{ST} indicates moderate to high genetic differentiation among the population studied (El-Aksher *et al.*, 2017). This result also indicated that among/inter-population and within/intra-population variation contributed 6.40% and 93.60%, respectively. This relative level of variation was further supported by the Analysis of Molecular Variance, AMOVA (Table 1) of the present study. The mean level of gene flow (Nm) among the population (3.933) implied that a low gene flow would not prevent genetic drift, thus enabling the gene differentiation between populations.

The low pairwise F_{ST} values among breeds studied inferred that 95.2% to 95.1% of the total genetic variation was explained by the individual variability. These values also corroborated AMOVA and F-statistics results obtained in the present study and were in agreement with the report of El-Aksher *et al.* (2016).

Nei's genetic identity and distances showed lower levels of genetic differentiation partitioned among rabbit populations of South-Eastern Nigeria and suggested the three populations being closely related and had a common ancestor (Abdel-Kafy *et al.*, 2018). This can be verified from Figure 5, which shows the high admixture level of the three breeds and, therefore, pronouncing intra- and inter-breeding among the populations.

Discriminant analysis of Principal Components (DAPC) showed that the admixture level of an individual rabbit among the three breeds was not pure (Figure 5), and this may imply random mating among the three breeds population over time.

The differences obtained in the present study may be attributed to markers sampling error and/or the level of diversity detected, reinforcing against the importance of the number of loci and their coverages of the overall genomes in obtaining reliable characterization of genetic relationship among the population studied.

CONCLUSION

The Hardy-Weinberg equilibrium (HWE), pairwise genetic differentiation among the population (F_{ST}), Nei's genetic identity, and distances results showed levels of genetic differentiation partitioned among this population and put forward that the three populations study are closely related and had a common ancestor. Also, the polymorphic (100%) nature of the 8 microsatellite markers used indicated the suitability of these microsatellites for genetic diversity studies in the rabbit. Hence, a polymorphism that exists among the population studied is more within the breed than among the breed diversity.

CONFLICT OF INTEREST

The authors wish to declare that no conflict of interest exists.

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