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Genetic variation amongst four rabbit populations in Nigeria using microsatellite marker

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***Target audience:** Animal Scientists, Breeders/Geneticists, Animal Biotechnologists and Molecular Biologists*

Abstract

Seven microsatellite markers were used with 100 genomic deoxyribonucleic acid (DNA) isolated from New Zealand White, New Zealand Red, Californian White and Chinchilla rabbit breeds in Nigeria to determine genetic variation amongst the breeds. Power of microsatellite markers i.e. combined exclusion probabilities (CEP) and polymorphism information content (PIC) of markers; were determined to ascertain the informativeness of the markers. Equal number of samples were obtained from each of the rabbit population. Polymerase chain reaction (PCR) was carried out using each marker, isolated DNA, double distilled water and PCR Master Mix. PCR products generated were subjected to polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. DNA bands were scored based on size of ladder with Gene Scan 3.1.2. Bands obtained were designated as alleles and prepared into Excel Worksheet with Microsatellite Analyzer version 4.05 software and allele frequencies were generated. Mean inbreeding coefficient across loci (F_{IS}) was - 0.0201 and average genetic differentiation (F_{ST}) among breeds was 0.0479. Seventy nine alleles of which 18.98% were rare and 81.02% represent fixed alleles across the rabbit populations were observed. PIC per marker across populations ranged from 0.6800 (SAT 8) to 0.8100 (SOL 28) indicating that the markers were informative (PIC ≥ 0.50). CEP across markers and populations was 0.999999, meaning that the selected microsatellite markers were suitable for parentage verification of these four rabbit breeds.

***Key words:** Genetic differentiation, informativeness, microsatellite markers, DNA, Electrophoresis, rabbits*

Description of Problem

Rabbits described as micro-livestock specie by [1] are ubiquitous, providing protein, fibre, experimental models and companionship. Rabbit reproductive potentials and other economically important traits in it have been reiterated by [2]. Genetic variation has been defined as the variation within and among breeds of given species. It is influenced by interaction of different forces such as selection, genetic drift, mutation, and migration [3]. Genetic variation allows breeders to develop new characteristics in response to changes in environmental and diseases outbreak [4]. Lukefahr [5] noted that a high degree of heterozygosity in the rabbit populations might be important for fitness-related characteristics such as fertility and survival which contributes to the final local adaptation. Microsatellites, otherwise known as simple sequence repeats (SSRs), are repeating sequences of 1-6 base pairs of DNA,

which have been used to evaluate genetic variation and relationship in various organisms. The advantages of the marker include its ability to detect polymorphisms in many loci, higher heterozygosity, relative ease of scoring and the co-dominant nature of generated markers [6, 7 and 8]. Population history of rabbit breeds kept in Nigeria till date is not available; thus, rabbit rearing could be speculated to have commenced in Nigeria at the advent of slave-trade and European invasion into Africa, when most exotic agriculturally important crops and animals were introduced [9]. Rabbit production in Nigeria is relatively at low level and the genetic characterization of the rabbit population has not yet been adequately carried out unlike the other animal genetic resources Hence, it is imperative that genetic variation parameters (heterozygosity, mean number of alleles, F-statistics, gene flow) be known in the populations; and the exclusion probability, combined exclusion probability and polymorphism information content of the microsatellite markers that can be used with rabbit population be documented. This would facilitate long time breeding strategies, formulation of conservation policies, provide ample genetic information and will ultimately lead to rapid improvement of this important genetic stock, thereby contribute some percentages of animal protein needs of man. The objectives of the study were to determine the genetic variations among the four rabbit populations in Nigeria and the effectiveness of the microsatellite markers.

Materials and Methods

Blood sampling, DNA extraction, quality and concentration verification

A total of one hundred blood samples were collected from four rabbit breeds (New Zealand White, Californian White, New Zealand Red and Chinchilla). Equal numbers of samples (25 samples) from each of the rabbit population were collected from reputable farms and Research Institutes across Nigeria for the study.

Approximately 1 ml of blood was collected from each rabbit through the Saphenous rear leg venial puncture aseptically into ethylene di-amine-tetracetic acid (EDTA) tubes using 1 ml sterilized syringe into 5 ml EDTA tubes, which were placed in an ice-box and later transported to the Biotechnology Laboratory, Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, where the samples were stored at -20^{o}C before the genetic material was isolated from the samples collected.

DNA isolation was carried out using Norgen DNA isolation kit with strict adherence to the manufacturer's guidelines. The isolated DNA purity quantification process was carried out by spectrophotometry using the Nanodrop^{TM} Lite. The mean OD260/OD280 ratio for all purified DNA was ~1.810. Further examination was done using 1% agarose gel electrophoresis ran at 100 volts for 30 mins for visual quality determination of the extracted DNA (Plate 1).

Polymerase chain reaction and microsatellite genotyping

Seven microsatellite markers (SAT3, SAT8, SAT12, SOL 3, SOL 8, SOL 28 and SOL 30) used in this study are represented in Table 1. PCR carried out 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 primer (1.00 µL of each forward and reverse), 0.20 µL of *Taq* polymerase, 2.20 µL of 25 mm/Mol Mg2+ and 16.10 µL distilled water. Denaturing temperature of 94°C (1 minute) and annealing temperature for the seven microsatellite primers ranged from 52°C- 60°C (Table 1) The initial extension was at 72°C (1 minute)

followed by the final extension at 72°C (10 minutes). 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. Bands were designated as alleles and prepared into Excel Worksheet.

Table 1: Sequences and the annealing temperature of seven microsatellite markers used in this study

Locus	Primer Sequence	Annealing temperature (℃)
SAT3	F: 5'GGAGAGTGAATCAGTGGGTG3' R: 5'GAGGGAAAGAGAGAGACAGG3'	60
SAT8	F: 5'CTTGAGTTTTAAATTCGGGC3' R: 5'GTTTGGATGCTATCTCAGTCC3'	55
SAT12	F: 5'GGATTGGGCCCTTTGCTCACACTTG3' R: 5'ATCGCAGCCATATCTGAGAGAACTC3'	58
SOL3	F: 5'ATTGCGGCCCTGGGGAATGAACC3' R: 5'TTGGGGGGATATCTTCAATTTCAGA3'	58
SOL8	F: 5'CAGACCCGGCAGTTGCAGAG3' R: 5'GGGAGAGAGGGATGGAGGTATG3'	60
SOL28	F: 5'TACCGAGCACCAGATATTAGTTAC3' R: 5'GTTGCCTGTGTTTTGGAGTTCTTA3'	52
SOL30	F: 5'CCCGAGCCCCAGATATTGTTACCA3' R: 5'TGCAGCACTTCATAGTCTCAGGTC3'	52

Data analyses

Allele frequencies, observed heterozygosity (H_O), expected heterozygosity (H_E), mean number of alleles (MNA) and rare/unique alleles (R_A) were estimated for seven microsatellite markers using Microsatellite Analyser version 4.05 developed by [10]. F-statistics was obtained using the Genepop 4.1 program [11, 12]. Gene flow was calculated using the formula suggested by [13]. PIC for each marker and in each rabbit population was calculated using the formula long suggested by [14]. Combined exclusion probability (CEP) across markers and populations was calculated using multiple products of each marker exclusion probabilities defined as:

$CP_E = 1 - (1 - P_{E1})(1 - P_{E2})(1 - P_{E3}) \ldots\ldots\ldots\ldots\ldots (1 - P_{EN})$ by [15],

where $P_{E1}\ldots P_{EN}$ is exclusion probabilities of the seven microsatellite markers used and expressed as:

$P_{E1} = 1 - 2\sum P_i^2 + \sum P_i^3 + 2\sum P_i^4 - 3\sum P_i^5 - 2(\sum P_i^2)^2 + 3(\sum P_i^2)(\sum P_i^3)$ according to [15].

Results and Discussion

Number of alleles, rare alleles and heterozygosities are summarized in Table 2. The number of alleles observed across the seven microsatellite markers varied between 3 (SOL30) for New Zealand White and 15 (SOL28) for the Chinchilla rabbits. Mean

number of alleles identified in the rabbit population was 11.142±1.164, while for the four sub-populations, the values were 6.000±1.024 for the New Zealand White, 8.857±0.884 for Californian White, 8.143±0.738 for New Zealand Red and 9.000±1.069 for Chinchilla rabbit. The Chinchilla breed had the highest mean number of alleles of 9.000±1.069 compared to other breeds. The MNA observed over the seven loci for the four rabbit breeds are considered to be good indicators in defining the genetic variability within the population. The high overall MNA value recorded among the rabbit populations is indicative of great allelic diversity, which could have been influenced by crossbreeding or admixture among the rabbit populations.

The seven microsatellite markers used for this study had at least one rare allele across the rabbit breeds. Rare alleles are alleles unique to a particular breed and/or population, thus, it is only fitting for such alleles to be used in the genetic identification of such population. [16] emphasized the importance of high frequency of rare alleles in the genotyping and line identification of populations. Rare alleles observed in the different breeds for the markers used in this study were as follows: Californian White rabbit (6), New Zealand Red (4), New Zealand White (3) and Chinchilla (2).

The number of alleles observed ranged from 3-15, with the lowest number (3) produced by SOL30 being the least polymorphic marker and the highest number of alleles (15) produced by SOL28 being the most polymorphic loci. The range observed in this study is similar to 2-18 earlier reported in Tunisian rabbit populations [17]; (2-12) in the Egyptian and Spanish line rabbit populations [18], (4-12) in the pygmy rabbit breeds [19], (4-10) in the Egyptian rabbit populations [20]. The markers used for this study were appropriate since their polymorphisms were higher than the minimum of 4 alleles required for microsatellite markers to be used in the estimation of genetic diversity.

Gene diversity, migrant rate, F-statistics, polymorphism information content, exclusion probabilities of marker and combined exclusion probabilities (Table 3). The PIC which takes into account the allele frequency per marker at a specific locus is a good indicator of genetic diversity evaluation.

The average PIC values were 0.7206, 0.8115, 0.7217 and 0.8214 for New Zealand White, Californian White, New Zealand Red and Chinchilla rabbit populations respectively. The PIC values were similar to those reported in earlier works on rabbit diversity studies using Simple tandem Repeats (STRs), (0.625 - 0.796) in Asian rabbits [21] and (0.60 – 0.86) in Egyptian rabbit [20]. The values of the PIC recorded for this study showed that the microsatellite markers used were highly polymorphic and informative for genetic diversity studies, since all loci PIC value in this study was greater than the threshold value of 0.5 (i.e. the value of at which the microsatellite marker can be regarded as being informative) [14].

Average heterozygosity is an appropriate measure of genetic variability within a population because it takes into account all levels of genetic variation rather than just classify into 2 categories (monomorphic or polymorphic). Table 2 shows the observed heterozygosity which ranged from 0.7567 in New Zealand White to 0.8508 in Chinchilla rabbit breeds, while the expected heterozygosity or the gene diversity ranged from 0.7726 in New Zealand White to 0.8529 in Chinchilla. Heterozygosity range in this study was wider compared to that reported, (0.53-0.62) by [22], (H_O, 0.61-0.63 and H_E, 0.63-0.65) [23]; (H_O, 0.39-0.58 and H_E, 0.30-0.56) [17]. High level of heterozygosity recorded in this study could be attributed to the mixed nature of the breeds. Furthermore, similarities in range were observed with

reported studies of (H_O 0.37-0.79 and H_E 0.66-0.88) [20], (0.63-0.72) [21]. The observed heterozygosity obtained in this study was lesser when compare to the expected heterozygosity in most population. This is attributed to the segregation of null alleles [22].

Table 2: Observed and expected heterozygosities, number of alleles, rare alleles, in each marker across rabbit populations*

Marker	Population	N_A	R_A	H_O	H_E
SAT3	NZW	4	0	0.8261	0.7402
	CAL	6	0	0.7619	0.8890
	NZR	6	1	0.8421	0.8852
	CHIN	7	0	0.8433	0.8901
SAT8	NZW	8	0	0.7368	0.7282
	CAL	8	0	0.8653	0.8150
	NZR	10	2	0.6111	0.7486
	CHIN	9	1	0.7539	0.8335
SAT12	NZW	5	0	0.7224	0.8194
	CAL	8	1	0.8529	0.8731
	NZR	8	0	0.7436	0.7714
	CHIN	9	0	0.8454	0.8429
SOL3	NZW	5	0	0.8571	0.7956
	CAL	8	1	0.7495	0.8635
	NZR	7	0	0.6523	0.8358
	CHIN	7	0	0.8888	0.8620
SOL8	NZW	3	0	0.7236	0.7809
	CAL	11	2	0.8952	0.8340
	NZR	9	1	0.7912	0.8111
	CHIN	9	0	0.8636	0.7780
SOL28	NZW	11	0	0.7047	0.8425
	CAL	13	1	0.8947	0.8546
	NZR	11	1	0.8235	0.8397
	CHIN	15	2	0.8947	0.8834
SOL30	NZW	6	3	0.7826	0.6714
	CAL	8	1	0.7591	0.8036
	NZR	6	0	0.8334	0.8804
	CHIN	7	1	0.8662	0.8808

**New Zealand white (NZW =25), Californian white (CAL =25), New Zealand red (NZR=25), Chinchilla (CHIN =25), N_A = Number of Alleles, R_A = rare alleles, H_O = observed heterozygosity, H_E = expected heterozygosity.*

Table 3: Gene diversity, polymorphism information content, fixation indices, migrant rate, exclusion probabilities of marker and combined exclusion probabilities across markers and among four rabbit populations in Nigeria as revealed by seven microsatellite markers

Marker	GD	PIC	F_{IS}	F_{IT}	F_{ST}	N_M	P_{EI}
SAT3	0.85	0.72	-0.0662	0.0360	0.0958	2.369	0.940
SAT8	0.78	0.68	-0.0387	0.0407	0.0764	3.022	0.926
SAT12	0.82	0.80	0.0788	0.1044	0.0278	8.743	0.930
SOL3	0.83	0.78	-0.0681	-0.0395	0.0268	9.078	0.931
SOL8	0.79	0.77	-0.0041	0.0056	0.0097	25.523	0.924
SOL28	0.85	0.81	-0.0584	-0.0511	0.0069	35.981	0.907
SOL30	0.80	0.78	0.0154	0.1064	0.0924	2.4551	0.924
MEAN	0.82	0.76	-0.0201	0.0289	0.0479	12.453	
CP_E							0.999999

GD = gene diversity, Nm = gene flow, F_{IS}, F_{IT} and F_{ST} are fixation indices, PIC = polymorphism information content, PE1 is an exclusion probabilities of N-number of markers for one parent and both parents excluded and CPE are combined exclusion probabilities for one and both parents excluded.

Population differentiation which was examined by the fixation indices viz: F_{IS}, F_{IT} and F_{ST} for each locus and across loci (Table 3). Average genetic differentiation among breeds was 0.0479, which implies 96.60 % of the total genetic variation was explained by individual variability. Mean inbreeding coefficient of the individual relative to the sub-population (F_{IS}) was - 0.0201, which indicate the existence of heterozygosity excess within the rabbit populations, however, this may be tested in further research. The low level of genetic differentiation (0.0479) was supported by the high level of gene flow (Nm), which suggests possible admixture among the rabbit populations. Exclusion probabilities of marker across populations (P_{EI}) ranged from 0.9000 (SOL28) to 0.9400 (SAT3), while the combined exclusion probabilities CEP across markers used was 9.99999 x 10^{-1} when one parent was excluded.

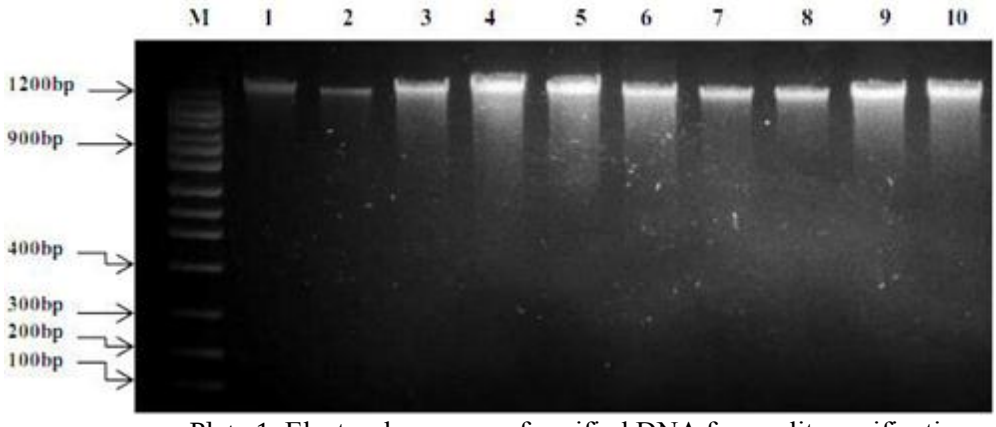

Plate 1. Electrophoregram of purified DNA for quality verification

Conclusion and Application

1. Microsatellite markers used in this study were found to be highly polymorphic and informative based on PIC > 0.5. The genetic characterization as revealed by the microsatellite markers showed that the four rabbit breeds has more within breed variation than between breed variation. Similarly, the low values of genetic differentiation with regard to the inbreeding estimates indicate relatively high outbreeding among the four rabbit breeds.
2. High values of mean number of alleles across loci and the expected heterozygosity recorded across loci in the rabbit populations indicates high genetic diversity among the rabbit populations in Nigeria.
3. Conclusively, the present study has documented the genetic variation parameters within and among the rabbit breeds in Nigeria based on microsatellite markers analysis. The combined power of the seven microsatellite markers used was 0.999999, thus signalling the informativeness and effectiveness of the markers in parentage verification of common rabbit breeds in Nigeria.

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