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Original Research Article

Genetic Diversity and Population Structure of Domestic Rabbit (O*ryctolagus cuniculus*) (L) Ecotypes from Eight Selected Kenyan Counties Using Microsatellite Markers

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Abstract

This study estimated the molecular diversity among Kenyan rabbit populations using microsatellites. Seven microsatellite loci were used in the study and eight counties were sampled purposively because they are the major rabbit rearing areas. Summary statistical parameters of genetic variability within and between populations confirmed that the highest genetic diversity was found in the Kenyan rabbits. The observed number of alleles for all the microsatellites was 2.0 while the mean effective number of alleles was 1.65. The mean values of Ho and He recorded in the study were 0.903 and 0.89 respectively. Nei's genetic diversity indices varied from 0.335 to 0.578 for markers SOL30 and SAT8 respectively with a mean of 0.353. All the microsatellite markers used in the study were polymorphic and the average polymorphic index was very high (0.808). The genetic differences

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measured among counties was 6%, while 94% of the genetic variation was attributed to within county genetic diversity indicating that population variation observed was genetic and not due to geographical differentiation. Factorial and polymorphic analyses revealed that the Kenyan rabbit population is clustered into three groups but failed to deduce geographical segregation. This research provides an overview of the genetic diversity of rabbit populations in Kenya. The findings of this study will be useful to guide future breeding programs for enhanced productivity and genetic conservation.

Keywords: Domesticated Rabbit, Genetic Diversity, Microsatellite Markers, Kenya

Introduction

In the face of climate change effects, the need to develop, and sustain resilient and diversified livelihood options is vital and urgent. Rabbits provide such an option to smallholder resource deficient farmers, especially in Sub-Saharan Africa. Being small livestock, rabbits are prolific, highly fecund, and fast-growing. Rabbit meat significantly contributes to household animal protein with additional nutritional benefits. It is rich in micronutrients such as iron, iodine zinc, and vitamin B and is low in sodium cholesterol (Adeolu *et al.,* 2020). Rabbit meat is thus recommended for alleviating hidden hunger in women and infants (Cullere and Zotte, 2018).

Rabbit enterprises are an emerging viable commercial venture due to their high profitability, exceptional phenotype diversity, and applicability as animal models in biomedical research (Adeolu et al., 2020, Carneiro et al., 2011). Rabbit farmers are therefore eager to improve available rabbit breeds for enhanced productivity and profitability (FAO, 2020).

All domestic and wild rabbit forms worldwide are thought to be descended from the European rabbit (*Oryctolagus cuniculus)* and the observed divergence is mainly attributed to domestication and breed formation. Domestic rabbit breeds are classified based on their origin, biometric traits, and color (Jamil et al., 2021). The use of phenotypic and biochemical markers for classification is archaic and inefficient especially in segregating close genetic relatives.

Data on the diversity of available rabbit ecotypes, rate of domestication and breed formation, and genetic erosion indices in Kenya are scarce and unreliable. Diversity data based on growth and production traits, adaptability, and economic viability under prevailing agroecological conditions is essential for domestication, improvement, commercialization, and genetic conservation. This information is vital in informing sustainable rabbit breeding strategies (Awuor et al., 2019).

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Molecular markers are the platform of choice in modern times because they are more, ubiquitous, abundant hypervariable, evenly distributed along the chromosomes, and have more resolution for segregating closely related individuals (Zhao et al., 2006). Molecular markers have been widely applied in animal diversity studies in different settings. Commonly used molecular markers include random amplified length polymorphisms (RAPDs), microsatellites (SSRs), Short Tandem Repeats (STRs), and mtDNA (mitochondrial DNA). Microsatellite markers have been widely applied in conservation, pedigree, and population structure studies because they are Mendelian inherited, codominant, polymorphic, easily typed, and automated (Al-Samarai, and Al-Kazaz, 2015). The study was designed to evaluate the genetic diversity of domestic rabbit ecotypes from eight selected Kenyan counties.

Materials and Methods

Experimental Animals

A total of 75 mature, unrelated, and mixed-sex rabbits of three common breeds Dutch, Chinchilla, New Zealand White, and respective cross breeds were sampled. Stratified purposive sampling was employed in the study, the study site was stratified into eight counties and the samples were purposively collected from farmers who reared rabbits. The animals were collected from farmers in eight select counties; Nandi, Elgeyo Marakwet, Kakamega, Vihiga, Busia, Bungoma, Baringo, and Trans-Nzoia.

Blood Sample Collection and Genomic DNA Extraction

A total of 2ml of whole blood was collected by Saphenous rear leg venial puncture of the rabbits and transferred into serum tubes containing 1 ml ethylene di-amine-tetraacetic acid (EDTA) tubes using a 1 ml sterilized syringe and stored at - 40^0C.

Genomic DNA extraction was done using a Quick-gDNA MiniPrep kit (Catalog NO: D3025) from Qiagen Limited following manufacturers' recommendations. A total of 400ul of genomic lysis buffer was added to 100ul of whole blood in a microcentrifuge tube. This was mixed completely by vortexing for 6s and then let to stand for 10 min at room temperature. The mixture was transferred to mini-spin columns in a collection tube and then centrifuged at 10000g for 1 min. The collection tube with the flow-through was discarded.

The mini spin column was then transferred to a new collection tube and 200ul of DNA pre-wash buffer was added to the Spin column and then centrifuged at 10,000g for 1 min. The Spin column was transferred to a clean collection tube and 500ul of gDNA wash buffer was added to the spin column and centrifuged at 10,000g for 1 min. The spin column was transferred to a

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clean microcentrifuge tube and 50ul of DNA elution buffer was added to the spin column, incubated at room temperature for 5 min, and then centrifuged at top speed for 30 seconds to elute the genomic DNA. The genomic DNA was then stored at -20^{0}C for further molecular-based applications.

DNA Quantification

The purity and concentration of the isolated DNA were determined using NanoDrop 2000c spectrophotometer (Thermo Scientific) and Agarose gel electrophoresis. Nanodrop spectrophotometry involved the determination of the concentration of DNA from the absorbance of DNA at 260 nm (1OD (A260) = 50 µg for double-stranded DNA/µl). The purity of the DNA sample was determined by the A260:A280 ratio (1.6±1.8 for pure DNA). Agarose gel electrophoresis involved running the extracts in a 1% agarose gel (1g agarose and 100ml TBE buffer) pre-stained with Ethidium bromide staining dye at a voltage of 100 Volts and a current of 400mA for 30 minutes. The extracts were visualized on a UV Transilluminator. The presence of DNA in the sample is indicated by the presence of a band while DNA quantity in the sample is shown by the brightness of the band. The sharpness of the bands indicates the quality of the isolated DNA (Sharp bands indicate good quality, while smears indicated sheered DNA).

Polymerase Chain Reaction (PCR) and Microsatellite Genotyping

Seven microsatellite markers (SAT3, SAT8, SAT12, SOL 3, SOL 8, SOL 28, and SOL 30) used in the study are tabulated (El-Aksher *et al.* (2016) (Table 1). The markers were selected because they are uniformly distributed across the rabbit genomes and have been associated with growth and meat yield traits. Selected Rabbit genetic diversity SSR Markers used in the study (El-Aksher *et al.*, 2016). PCR amplification of isolated DNA was carried out to amplify the selected loci. The PCR mix was prepared in a 25.0µl volume contained 1.0µl of DNA template, 2.0µl of 10 × DNA amplification buffer, 6.0µl Master mix, and 16.0µl distilled water. Denaturing temperature of 94°C for one minute and annealing temperature for the seven microsatellite primers ranged from 52°C - 60°C (Table 1) The initial extension was at 72°C for one minute.

Data Analysis

Standard genetic diversity statistics; allelic frequencies, observed heterozygosity (Ho), expected heterozygosity (HE), the mean number of alleles (MNA) and Hard-Weinberg equilibrium were estimated using Popgen version 2.03 software. Inter and intrapopulation diversity were estimated by Analysis of Molecular Variance (AMOVA) executed in GenAlEX 6.41 software.

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Rabbit population structure was determined using Discriminant Analysis of Principal Components (DAPC) computed in DARwin 6.021 software (Perrier, 2006).

Table 1: Rabbit microsatellite (SSR) markers

Locus	Primer Sequence	Temp (°)	PIC
SAT3	F: 5′GGAGAGTGAATCAGTGGGTG3′ R: 5' GAGGGAAAGAGAGAGACAGG3′	60	0.72
SAT8	F: 5′CTTGAGTTTTAAATTCGGGC3′ R: 5′GTTTGGATGCTATCTCAGTCC3′	55	0.68
SAT12	F: 5′GGATTGGGCCCTTTGCTCACACTTG3′ R: 5′ATCGCAGCCATATCTGAGAGAACTC3′	58	0.8
SOL3	F: 5′ATTGCGGCCCTGGGGAATGAACC3′ R: 5′TTGGGGGGATATCTTCAATTTCAGA3′	58	0.78
SOL8	F: 5′CAGACCCGGCAGTTGCAGAG3′ R: 5′GGGAGAGAGGGATGGAGGTATG3′	60	0.77
SOL28	F: 5′TACCGAGCACCAGATATTAGTTAC3′ R: 5′GTTGCCTGTGTTTTGGAGTTCTTA3′	52	0.81
SOL30	F: 5′CCCGAGCCCCAGATATTGTTACCA3′ R: 5′TGCAGCACTTCATAGTCTCAGGTC3′	52	0.78

Results and Discussion

Marker Genotyping

Summary statistics of genetic diversity are presented in Table 2. The observed number of alleles for all the microsatellites was 2.0 while the effective number of alleles ranged between 0.357 and 1.916 for markers SOL30 and SAT3 respectively with a mean value of 1.65. The mean values of Ho and He recorded in the study were 0.903 and 0.89 respectively. Ho and He values above 50 percent, highlight higher genetic diversity across the studied rabbit ecotypes, and inform that the genetic fidelity of the rabbit populations is well managed and the rate of genetic erosion is low. Nei's genetic diversity indices varied from 0.335 to 0.578 for markers SOL30 and SAT8 respectively with a mean of 0.353. The high allelic richness reported in the study informs that the rabbit populations in the selected counties are genetically diverse and is also indicative of the population's long-term adaptability and resilience (Ozdemir and Cassandro 2018). All the microsatellite markers used in the study were polymorphic and polymorphic indices ranging between 0.651 and 0.98 for markers SAT3 and SAT8 respectively with a mean of 0.808. These high

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PIC values are an indicator that the markers used in the study have a higher resolution for segregating closely related ecotypes.

Table 2: Summary statistics of genetic diversity and Microsatellite polymorphism

Locus	Sample size	na*	ne*	h*	I*	Ho	He	PIC
SAT3	64	2	1.733	0.523	0.614	0.824	0.865	0.651
SAT8	64	2	1.916	0.578	0.671	0.633	0.898	0.982
SAT12	64	2	1.496	0.283	0.457	1.000	0.971	0.678
SOL3	64	2	1.882	0.469	0.661	1.000	0.893	0.816
SOL8	64	2	1.544	0.508	0.486	1.000	0.892	0.915
SOL28	64	2	1.600	0.375	0.562	1.000	0.836	0.784
SOL30	64	2	1.357	0.335	0.261	0.867	0.913	0.832
Mean	***64***	***2***	***1.647***	***0.439***	***0.530***	***0.903***	***0.890***	***0.808***
Std dev	***0***	***0***	***0.178***	***0.094***	***0.124***	***0.121***	***0.036***	***0.102***

**na = Observed number of alleles; * ne = Effective number of alleles (Kimura and Crow (1964); * h = Nei's (1973) gene diversity; * I = Shannon's Information index (Lewontin (1972), Ho = Observed Heterozygosity; He = Expected Heterozygosity; PIC = Polymorphic information content.*

Genetic Differentiation Among and Within the Population

Results of the Analysis of Molecular Variance (AMOVA) are summarized in Table 3. The genetic differentiation measured among counties was 6%, while 96% of the genetic variation was attributed to within county genetic diversity.

Table 3. Genetic Differentiation Among and Within the Population

Source	Df	MS	Est. Var	%
Among counties	10	4.361	0.027	4
Within counties	10	4.033	0.142	96
Total	20		3.919	100

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

Factorial Analysis

Genetic segregation of the individuals and population structure of the rabbit ecotypes within and between the counties was analyzed using factorial analysis. Factorial coordinates of the individuals were derived from the dissimilarity matrix that was calculated from raw SSR gel scores "1" and "0" matrix. Factorial analysis segregated the samples into three main groups without any pattern.

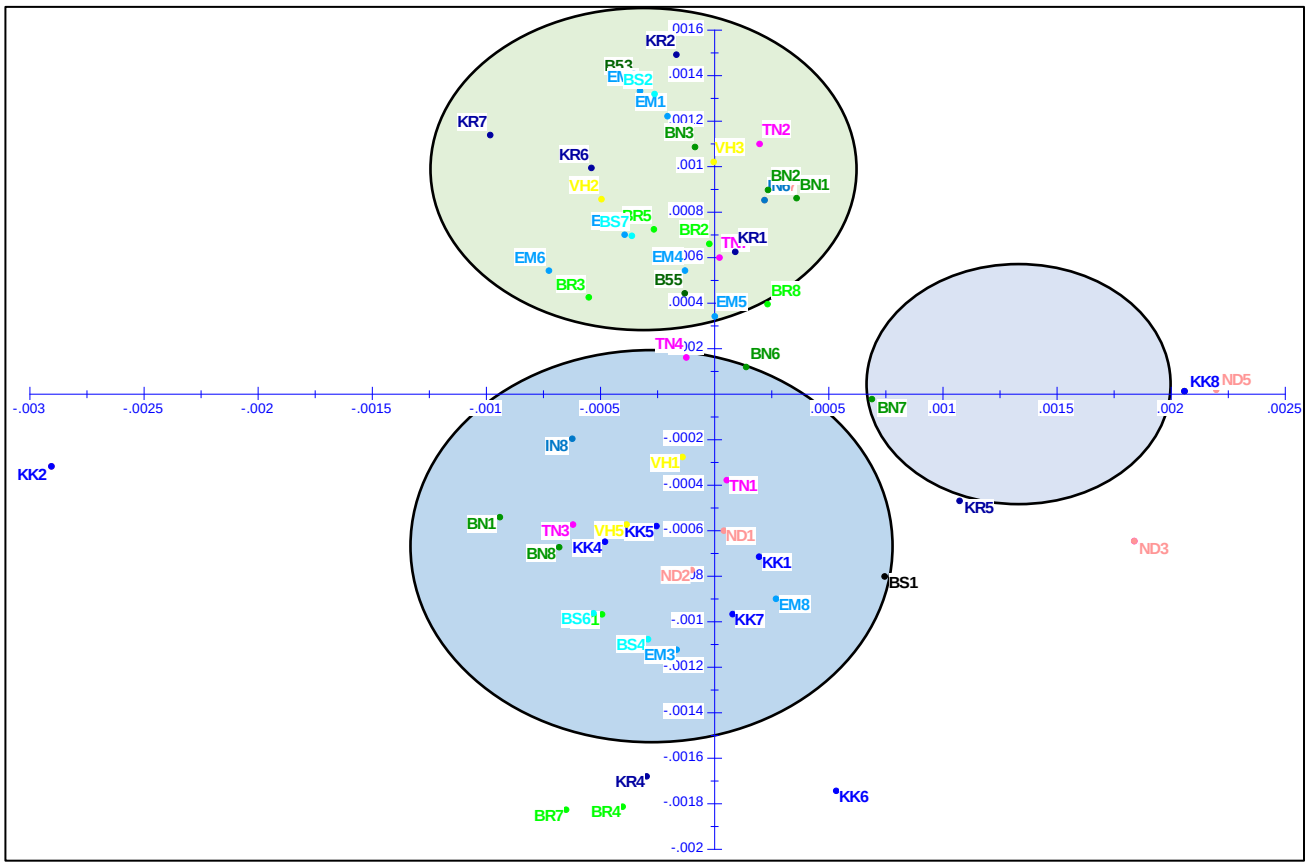

Figure 1: Factorial analysis showing segregation of the Kenyan rabbit ecotypes based on the SSR markers *(BN; Bungoma, ND; Nandi, TR; Trans Nzoia, BS; Busia, KK; Kakamega, VH; Vihiga, BR; Baringo and EM; Elgeyo Marakwet). Numbers = sample number.*

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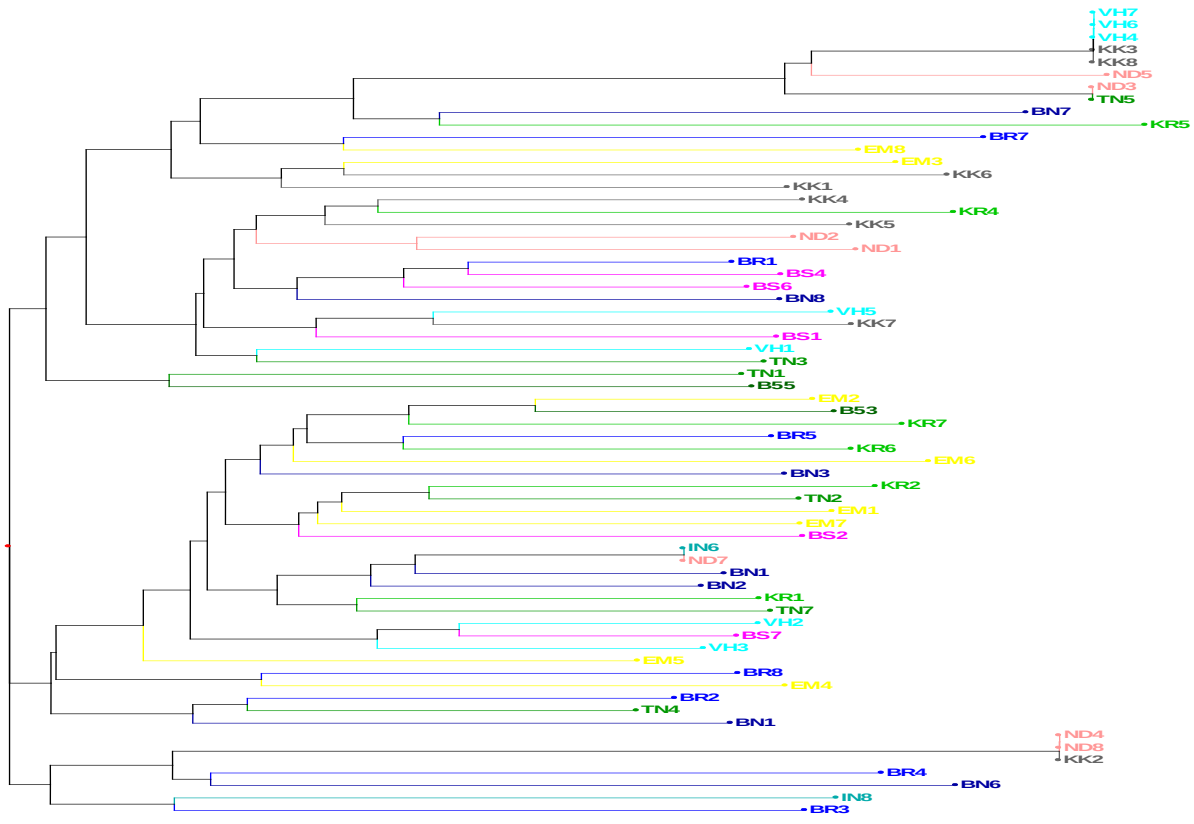

Figure 2: Phylogenetic tree showing clustering of Kenyan rabbit ecotypes (BN; Bungoma, ND; Nandi, TR; Trans Nzoia, BS; Busia, KK; Kakamega, VH; Vihiga, BR; Baringo and EM; Elgeyo Marakwet). Numbers = sample number

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Phylogenetic Analysis

A distance phylogram was constructed using the Unweighted Neighbor-Joining method with 1000 bootstrap replicates in Darwin 6.0.21. The minimum dissimilarity value recorded in the study was 0.06 while the maximum value was 1(Figure 2). Phylogenetic analysis grouped the 64 ecotypes into three major clusters without regard to the county of collection. This may be explained by inbreeding and transfer of the rabbits across counties. This finding is in agreement with the findings of Awuor *et al.,* 2019 who failed to deduce any geographical clustering of individual Kenyan rabbit haplotypes using *mt*DNA profiling.

Both factorial and phylogenetic population analyses showed that the genetic background of the rabbits in Kenya is mixed (Figure 2). Using the PCA approach, it was not possible to segregate the ecotypes analyzed into distinct geographical groups.

Conclusion and Recommendations

Results presented highlighted high genetic variability within and between Kenyan local rabbits. Microsatellites revealed clear sub-structuring between studied local populations, substantiating their local adaptation to respective agro-ecological zones.

Genetic erosion in the Kenyan rabbit population is still minimum and unnoticeable thus future breeding programs should seek to maintain the status and control possible genetic dilution. Microsatellite profiling highlighted that the Kenyan rabbits can be assigned to three major clusters with a single potential linage.

The results of the study have improved the knowledge on the genetic structure of Kenyan rabbit populations and has enhanced information on future breeding and conservation programs. Moreover, the findings play a role in enhancing the productivity of local breeds of rabbits in the study areas and in Kenya.

Based on the findings of the study, it is recommended that future research to involve use of more modern genetic diversity platforms such as Genome Wide Association Studies (GWAS) and Genotyping by Sequencing (GBS) which have higher resolution for segregating between individuals.

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