Scientific Research Publishing

Open Journal of Applied Sciences, 2025, 15(8), 2319-2330
https://www.scirp.org/journal/ojapps
ISSN Online: 2165-3925
ISSN Print: 2165-3917

Genetic Diversity of Kuri Cattle Based on Microsatellite Analysis

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How to cite this paper: Souleymane, M.A., Zeuh, V., Ibrahim, M.A.M., Hanotte, O. and Adoum, I.Y. (2025) Genetic Diversity of Kuri Cattle Based on Microsatellite Analysis. *Open Journal of Applied Sciences*, **15**, 2319-2330.
https://doi.org/10.4236/ojapps.2025.158154

Received: May 5, 2025
Accepted: August 15, 2025
Published: August 18, 2025

Abstract

This study aimed to assess the genetic variation within Kuri cattle and to explore the utility of a *Y*-specific marker for sire selection. We utilized a panel of 15 bovine-specific microsatellite markers and one *Y*-specific marker, as recommended by the International Society of Animal Genetics (ISAG). A total of 213 Kuri cattle from the Center for Safeguard of the Kuri Cattle (CSK) in Lake Chad were sampled. All microsatellite loci were polymorphic, yielding 142 alleles with a range of 4 to 9 alleles per locus and a mean of 6.0 alleles. All markers were informative, with Polymorphism Information Content (PIC) values ranging from 0.471 (ETH152) to 0.844 (BM2113). Observed (Ho) and expected (He) heterozygosities ranged from 0.522 to 0.859 and 0.522 to 0.862, respectively. Among 15 bulls, only one indicine *Y*-specific allele (0.067%), classified as zebu type, was detected. Bayesian clustering analyses suggested the presence of three genetic clusters, although distinct differentiation among them was challenging. The results indicate a primary genetic division between Kuri cattle from the islands within Lake Chad and those from the mainland, a finding that warrants further validation with comprehensive sampling. These findings provide insights into the genetic structure and purity of the Kuri cattle breed, highlighting its importance as a conservation candidate due to its unique phenotypic, cultural, and historical value.

Keywords

Kuri Cattle, Genetic Diversity, Autosomal Y-Specific Microsatellite, Chad

1. Introduction

The Kuri breed, also known by several local names such as *White Lake Chad*,

Boudouma, *Bahari*, *Bare*, *Borrie*, *and Kuburi* [1] [2], represents the most significant humpless cattle breed inhabiting the Lake Chad basin. Historically, it has been classified as *Bos taurus* [3] [4], or alternatively as *Bos africanus* or Sanga [5]. Kuri cattle predominantly thrive on the shores and islands of Lake Chad, spanning between 12˚20' and 14˚20'N latitude and 13˚ and 15˚30'E longitude, an area bordering Cameroon, Chad, Niger, and Nigeria. Given their apparent restricted occurrence to the Lake Chad vicinity and its tributary rivers, Kuri cattle were initially hypothesized to be a relic of an early migration of long-horned cattle into West Africa, having evolved locally over time [6]. However, documented sightings of Kuri as far as the Central African Republic [7] and Ethiopia [8] challenge this hypothesis. It is important to note that these latter cases are likely instances of spontaneous introduction, as the Kuri breed does not appear on the FAO cattle data lists for these two countries [2].

Kuri cattle are well-adapted to the hot and humid climate of their environment. They are typically managed under traditional systems, grazing on the small islands of Lake Chad. Notably, they are excellent swimmers, capable of following their herdsmen through water as they traverse between islands, a feat aided by their distinctive bulbous horns, which are considered to assist in flotation. The primary utility of Kuri cattle is for milk production, with meat being a secondary, though limited, product [9]. Conversely, another source places greater emphasis on meat production [2]. The breed is predominantly white with pigmented mucous membranes, although individuals with red, grey, dun, dark, or black spots are also observed [9]. Morphometric characteristics include a body length ranging from 133.7 to 144 cm in females and 152 to 165 cm in males. Heart girth measures approximately 172 cm in females, while in males it ranges from 183 to 211 cm. A defining feature is their massive, bulbous-shaped horns, which can reach lengths of 70 to 150 cm and circumferences up to 100 cm [10] [11]. Daily milk production of Kuri cows is reported to fluctuate between 3 and 10 liters, with a fat content of 30 to 35 g per liter [12]-[15]. Lactation yields typically range from 975 to 1560 kg [1] [13], with an absolute record of 2240 kg reported by Epstein [10]. More recent data indicate a broader variation in daily production, from 1.2 to 13.40 liters, and an average lactation yield of 1628.70 kg over 305 days [16].

The Kuri cattle population in Chad was estimated at 120,000 in 1991, representing approximately three-quarters of the total Kuri population [17]. Other estimates suggest a lower overall population figure of 110,000 [18]. Possible reasons for this declining trend include prolonged droughts, the receding waters of Lake Chad, and extensive crossbreeding with Zebu cattle when they graze on the shores [17] [19].

In the early 1980s, a project was initiated to evaluate, select, and distribute male animals based on milk yield performance in the region [19] [20]. This was followed by the establishment of a genetic evaluation program in 1994 [21], which subsequently led to the implementation of an *in situ* conservation program in Bol, the capital of the Lake Chad region, in 2003. The objective of the present study is

to assess the molecular genetic variation of Kuri cattle and to explore the potential for sire selection using a *Y*-specific marker.

2. Materials and Methods

2.1. Sample Collection

Samples were collected from Kuri cattle housed at the Centre of Safeguard of the Kuri Cattle (CSK), situated approximately 15 km southeast of Bol, Lake Chad Department, Chad. Animals were acquired from three distinct markets (A, B, and C) within the Lake Chad region, which served as the study sites (Figure 1). The geographical coordinates for each market are as follows: Zone A (Bol): 13˚28'13.99"N, 14˚42' 27.84"E. Zone B (Amerom): 13˚22'59.99'N, 15˚19'59.99"W. Zone C (Kalia): 13˚37'59.99"N, 14˚13'0.00"E. Cattle from Zone A primarily originated from islands within Lake Chad and were managed by Boudouma breeders. In contrast, animals from Zones B and C were sourced from both the lake and mainland areas, representing various ethnic groups within the region.

Figure 1. Map of Lake Chad showing sites from where cattle were purchased to build the station herd. Source: Map from http://www.weather-forecast.com/locations/Bol-Berim.

Blood samples were collected from a total of 213 Kuri cattle housed at the Centre of Safeguard of the Kuri Cattle (CSK). The sampled population comprised 168 adults acquired through field purchases (153 females, 15 males) and 45 individuals born within the CSK facility (37 males, 8 females). Each animal's unique identification number was integrated into the serial numbers for subsequent laboratory analyses. Of the 213 animals sampled, 90 originated from the Lake Chad islands (Zone A), while 54 and 69 animals were sourced from Zone B and Zone C, respectively. This distribution represented 42.3% of samples from the lake environment and 57.7% from the mainland. Genetic characterization was performed at the International Livestock Research Institute (ILRI) in Nairobi. Samples were genotyped using 15 autosomal microsatellite markers and the *Y*-chromosome specific

microsatellite marker, INRA124, to assess genetic variation.

2.2. Microsatellite Amplification and Genotyping

Genomic DNA was extracted from Peripheral Blood Lymphocytes (PBL), stored in a urea solution, using a standard salting-out protocol. High-quality DNA was successfully obtained from 207 samples, which were then subjected to microsatellite genotyping using 16 markers. The markers were selected from the FAO recommended panel (https://www.fao.org/4/i2413e/i2413e00.htm) and included fifteen dinucleotide microsatellites: AGLA293, BM2113, CSSM66, ETH225, ETH152, HEL01, ILSTS050, ILSTS033, ILSTS023, ILSTS006, ILSTS005, INRA032, INRA023, INRA005, and TGLA122, along with the Y-specific marker INRA124. Marker INRA035 was initially considered but subsequently excluded due to the presence of null allele(s) (non-amplifying alleles).

Polymerase Chain Reactions (PCR) were performed in a total volume of 15 µL. Each reaction contained 1X PCR buffer, 0.2 mM dNTPs, 1.0 - 3.0 mM $MgCl_2$_2$, 0.2 µM of each primer, 0.5 U of Taq polymerase (Thermo Fisher Scientific, Waltham, MA, USA), and 30 - 50 µg of template DNA. The thermal cycling program included an initial denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 94˚C for 30 seconds, primer annealing for 30 seconds (at temperatures between 50˚C and 65˚C), and extension at 72˚C for 1 minute. A final extension step was performed at 72˚C for 10 minutes. Primer sets with similar thermal profiles and $MgCl_2$ requirements were pooled and co-amplified in the same tube.

Amplified products were then analysed using semi-automatic scoring on an ABI 3730 capillary sequencer, with data processed using Gene mapper software.

2.3. Data Analysis

Pair-wise sharing alleles were calculated manually from the raw results using the variance-base method described by Weir and Cockerham (1980). The Hardy-Weinberg equilibrium (HWE), observed and expected heterozygosities (*Ho* and *He*, respectively), polymorphism information content (PIC) and Inbreeding coefficient (*F*is) were done using following packages: GenAlEx 6 [22], FSTAT 2.932 [23], TFPGA 1.3 [24], the PHYLIP 3.66package [25], Structure 2.1 [26] and BAPS 4.14 [27] [28]. Genetic relationships were also explored by Principal Component Analysis (PCA).

Genetic data derived from microsatellite genotyping were subjected to a comprehensive analysis using various specialized software packages.

Pair-wise allele sharing was calculated manually from the raw genotyping data, employing the variance-based method described by Weir and Cockerham (1980). To assess genetic diversity and population structure, the following parameters were calculated: Hardy-Weinberg Equilibrium (HWE), observed (Ho) and expected (He) heterozygosities, polymorphism information content (PIC), and the inbreeding coefficient (Fis). These analyses were performed using a combination

of software packages: GenAlEx 6 [22], FSTAT 2.932 [23], and TFPGA 1.3 [24].

Further investigations into genetic relationships and population structure included: Principal Component Analysis (PCA): This was utilized to explore genetic relationships among individualsm, Population structure analysis: Performed using Structure 2.1 [26] and BAPS 4.14 [27] [28] and Phylogenetic analysis: Conducted using the PHYLIP 3.66 package [25].

3. Results and Discussion

3.1. Genetic Diversity

A total of 142 alleles were detected across the 15 autosomal microsatellite markers, resulting in an average of 9.5 alleles per locus (**Table 1**). All markers demonstrated high informativeness, with Polymorphism Information Content (PIC) values ranging from 0.471 (ETH152) to 0.844 (BM2113). Observed heterozygosity (H_O) ranged from 0.522 to 0.859, while expected heterozygosity (H_e) varied from 0.522 to 0.862.

The level of genetic diversity observed in the Kuri cattle population in this study is comparable to or higher than values reported for other cattle breeds [29] [30]. For instance, a study on four Cameroonian indigenous cattle breeds (*Arab Shuwa*, *Ngaoundere Gudali*, *Namchi*, and *White Fulani*) using 13 autosomal microsatellite loci found an average of 10.69 alleles per locus, with mean PIC of 0.75 and expected mean heterozygosity ranging from 0.65 - 0.76 [31]. Similarly, Nguni cattle in South Africa, studied with 22 microsatellite markers, exhibited a mean of 9 alleles per locus and an average of 70% heterozygosity [32]. These findings highlight the robust genetic diversity present in the Kuri cattle. A comparison with the findings of Souvenir Zafindrajaona *et al.* [33] on Kuri cattle revealed an identical number of alleles for most shared microsatellites, with the exception of ETH225 (9 alleles in this study versus 14 alleles in Souvenir Zafindrajaona *et al.* [33]). These foundational analyses indicate that the genotyped markers are robust tools for discerning population structure within our study population, and that substantial genetic variation exists among the sampled individuals. Such high levels of genetic diversity could be attributed to extensive gene flow among the animals and/or historical introgression with zebu cattle, a phenomenon observed in other African cattle populations [34] [35].

For the *Y*-specific analysis, the INRA124 microsatellite marker was utilized, as taurine and zebu lineages can be readily distinguished by *Y*-chromosome loci [36]. Previous studies have reported that INRA124, along with BM861 and INRA189, possess taurine- and zebu-specific alleles [37] [38]. Specifically, two primary alleles (130 bp and 132 bp) have been identified for INRA124, corresponding to zebu and taurine cattle, respectively [37] [39]. An additional allele of 134 bp has also been reported [40]. In the present study, among the 15 genotyped bulls, only one individual (serial number: 164, animal herd number: 29) carried the shorter INRA124 allele (130 bp), suggesting a zebu-type *Y* chromosome for this animal. All other bulls exhibited the larger 132 bp allele, which is characteristic of taurine

lineage. This finding is consistent with the established utility of INRA124 for paternal lineage differentiation in cattle [40] [41], and further supports the presence of both taurine and zebu genetic contributions in African cattle populations, often reflecting complex historical movements and introgression events [42] [43].

3.2. Sub-Structuring Data Analysis

Basic analyses indicated that sub-structuring within the Kuri cattle sample was weak. Observed heterozygosity (H_O) and expected heterozygosity (H_e) values were very close, suggesting a lack of significant genetic differentiation. Only one marker, ILSTS023, showed a statistically significant positive departure from Hardy-Weinberg Equilibrium (HWE) ($P < 0.05$) (Table 1). However, three other loci (AGLA293, INRA005, and INRA032) exhibited indicatively positive F_{IS} estimates ($P < 0.1$). Despite these minor deviations, the joint analysis utilizing all markers indicated only a slight, but statistically significant, positive departure from HWE across the population.

Table 1. Number of alleles (A#): polymorphism information content (PIC), H_O: heterozygosity observed; (H_e): heterozygosity expected, F_{IS} (= 1 − H_O/H_E).

	A#	PIC	H_o	H_e	F_{IS}
AGLA293	13	0.818	0.801	0.840	0.047
BM2113	11	0.844	0.859	0.862	0.004
CSSM66	11	0.708	0.745	0.745	0.000
ETH152	5	0.471	0.522	0.522	0.001
ETH225	9	0.692	0.706	0.732	0.035
HEL01	10	0.768	0.786	0.798	0.014
ILSTS023	9	0.589	0.576	0.641	0.101*
ILSTS005	5	0.636	0.731	0.69	-0.059
ILSTS006	9	0.679	0.700	0.714	0.018
ILSTS033	7	0.585	0.671	0.655	-0.025
ILSTS050	12	0.499	0.527	0.537	0.019
INRA005	6	0.590	0.597	0.64	0.067
INRA023	11	0.732	0.739	0.763	0.031
INRA032	11	0.772	0.763	0.802	0.048
TGLA122	13	0.702	0.701	0.731	0.041
Mean	**9.5**	**0.672**	**0.695**	**0.711**	**0.023*[a]**

*One tailed P value testing for value being significantly larger than zero < 0.05. [a]Overall F_{IS} estimate, not the mean of locus wise estimates.

Within-population substructure was further investigated through dendrograms illustrating tree hierarchy and by employing Bayesian clustering methods. A UPGMA (Unweighted Pair Group Method with Arithmetic Averages) dendrogram

was constructed using Nei's minimum distance between individuals. This distance, based on molecular coancestry corrected by mean self-coancestries, inversely reflects their relatedness. The dendrogram revealed three pairs of individuals forming very tight clusters (Table 2): KURI 13 and KURI 15; KURI 14 and KURI 16; and KURI 211 and KURI 212. Notably, KURI 211 and KURI 212 were fraternal twins with a 100% matching score. The other two pairs also exhibited high matching scores (96.67% and 89.29%, respectively). While a few other well-supported clusters were observed, the bootstrap resampling support for the nodes was generally weak. This finding is characteristic of a population lacking strong substructure.

Similarly, Principal Coordinates Analysis (PCoA) indicated that the first three dimensions accounted for 56% of the between-individual differences. Although this is a relatively large proportion, no clear clusters were detected (data not shown). This observation further supports the conclusion of weak genetic structuring within the Kuri cattle samples.

Table 2. Matching samples.

Sample 1 (Animal number)	Sample 2 (Animal number)	Matching Score	No. Alleles	No. Matched
KURI 211	KURI 212	100.00%	24	24
KURI 14	KURI 16	96.67%	30	29
KURI 13	KURI 15	89.29%	28	25

Score = the percentage of alleles matching; No. Alleles = the number of alleles compared; No. Matched = the number of matching alleles.

Further exploration using two Bayesian clustering methods, STRUCTURE [26] and BAPS [27], both indicated the presence of three genetic clusters. These model-based clustering approaches are generally more powerful than traditional methods as they analyze individuals as full or partial members of a genetic population, even when assessing each individual separately. However, the model-based clustering had significant difficulty distinguishing between two of the three inferred clusters. This outcome is often characteristic of a continuous geographical cline (isolation by distance) rather than clearly defined, discrete clusters.

The results suggest that the most pronounced genetic division lies between the Kuri cattle from the Lake Chad islands and those from the mainland. Individuals primarily associated with the island cluster are highlighted in red in the bar graphs (Figure 2). Of the 14 animals strongly representative of this "red" cluster, only two were purchased in Zone C (mainland), while the remaining 12 were acquired from purchasing area A (islands). This translates to 13.34% of Zone A animals and only 1.63% of mainland animals belonging to this distinct island cluster. This finding implies that Lake Chad may act as a geographical barrier, contributing to the preservation of the Kuri breed's genetic purity within the island populations. This observation, however, warrants further corroboration with more comprehensive sampling data.

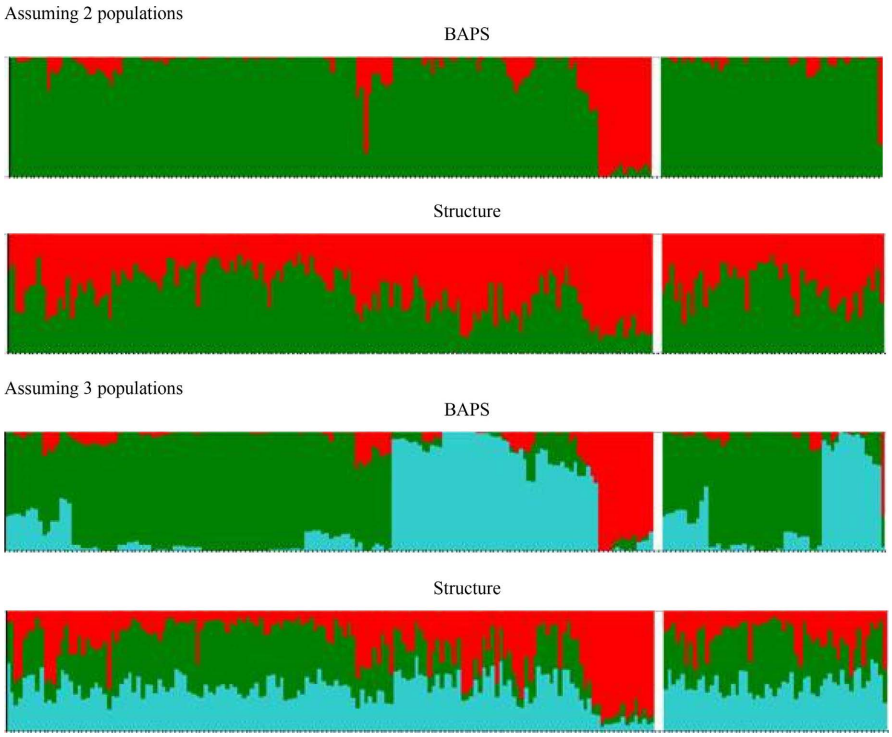

Figure 2. Bar graphs illustrate the inferred proportions of ancestry (y-axis) for each individual (x-axis) across 2 or 3 genetic populations. The individuals on the right, separated by a gap from the main cohort, represent the 53 animals identified as "best representatives" based on phenotypic traits of Kuri cattle.

3.3. Genetic Characterization and Environmental/Phenotypic Parameters

Detailed analysis and the inference of meaning from molecular genetic observations would significantly benefit from comprehensive background information on the sampled animals. Such information would ideally include:

Phenotypic indications of crossbreeding: Whether any animals exhibited observable signs of crossbreeding. Geographic coordinates of sample origins: Precise locations from where each sample was collected. Environmental data: Information regarding the animals' grazing habits, such as time spent on lake pastures. Unfortunately, none of these crucial environmental or phenotypic parameters were available for the current study, which limits the depth of interpretation regarding the observed genetic patterns.

4. Conclusion

Even with the limited number of animals analyzed relative to the total Kuri breed population, our findings indicate the presence of genetically distinct individuals within the studied sample. Specifically, the "red zone" identified by Bayesian clustering methods is strongly associated with animals from the Lake Chad islands (Zone A). This suggests that the island population could represent a genetic reservoir less impacted by introgression from mainland zebu cattle. Implementing

selective breeding programs focused on these island individuals would be crucial for preserving the distinctive traits and unique adaptations of the Kuri breed, while simultaneously minimizing the impact of hybridization. Concurrently, efforts should be directed towards a more precise characterization of mainland Kuri populations. This will help us understand the full extent of zebu introgression and identify any potential pockets of genetic purity that warrant conservation efforts.

Acknowledgements

The authors extend their sincere gratitude to the African Development Bank (AfDB) for providing financial support through agreement N° F/TCH/DEV-RUR/99/30, signed between the Republic of Chad and the African Development Fund (ADF). This agreement was instrumental in the establishment of the program for the Safeguard of the Kuri cattle breed and facilitated the data analyses presented in this study. We also wish to thank SODELAC (Lake Chad Development Company) for its multifaceted technical support throughout the project.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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