

# Assessment of Genetic Diversity and Relationship of the Two Sanga Type Cattle of Botswana Based on Microsatellite Markers

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## Research Article

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# Abstract

The study was performed to evaluate genetic variation on two Sanga type cattle found in Botswana; Tswana and Tuli using twelve microsatellite markers. All amplified loci were polymorphic with 75 and 77 alleles genotyped in the Tswana and Tuli populations, respectively. The total number of alleles per locus ranged from 2 (BM1818) to 10 (TGLA227) with total mean of 6.25 for Tswana and 6.43 for Tuli population. Almost all the markers showed high polymorphic information content (PIC) apart from BM1818 (0.375) and INTRA23 (0.393) which were moderately informative in Tswana population. Most of the markers were in Hardy-Weinberg equilibrium except for CSSRM60 and CSSM66 loci in Tswana population and ETH10, ETH225 and CSSM66 loci in Tuli population. A total of 103 unique alleles were genotyped across the two breeds with 49 shared, and 26 and 28 were unique to Tswana and Tuli populations, respectively. The expected heterozygosity ( $H_e$ ) values were higher than the observed heterozygosity ( $H_o$ ) in both populations; Tswana ( $H_e=0.7895\pm 0.033$  vs  $H_o=0.631\pm 0.091$ ) and Tuli ( $H_e=0.8123\pm 0.033$  vs  $H_o=0.556\pm 0.021$ ). The inbreeding coefficient was  $0.200\pm 0.002$  and  $0.332\pm 0.001$  in Tswana and Tuli populations, respectively. Analysis of molecular variance revealed 6.8% of the total genetic variation corresponding to differences between the two breeds and 93.2% within populations. The genetic identity between the two breeds was 56% and there were similar levels of multilocus heterozygosity and allelic diversity in the two breeds. The use of Tswana and Tuli breeds in a crossbreeding program is likely to result in minimal heterosis and therefore not recommended.

# Introduction

The most common Sanga type breeds found in Botswana are the Tswana and Tuli. These types of cattle have evolved over the years under low levels of selection and have acquired peculiar adaptive traits that are relevant to local and regional climatic and production environments. They can withstand drought and tolerate high heat, poor quality forage, diseases, water inadequacy and low input management (Mapiye et al., 2019). They are often kept under low input extensive production systems and play a pivotal role in sustainable livestock production of smallholder farmers in support of food security and livelihoods (Podisi, 2000). Tswana cattle are indigenous to Botswana and can also be found in South-western Zimbabwe and in the Northern Cape of Transvaal (Rege and Tawah, 1999). The Tuli cattle is indigenous to Zimbabwe and was developed from a base population of Tswana-type cattle in Gwanda district of Zimbabwe at Tuli Research Station by Len Harvey 80 years ago (Scholtz, 2010). Apart from similar adaptive traits, the two breeds are renowned for high fertility, longevity, easy calving and excellent mothering ability. However, the Tuli breed has superiority for milk production, carcass and meat quality traits and is characterised by early maturity and high docility (Mpofu, 2002). Previous comparative studies indicated that the Tuli cattle has a high calving percentage (85%) as compared to Tswana (79%) and mortality rate of 7.1% as compared to 8.3% of Tswana cattle (Trail, 1977). It has been noted that due to its superiority, the Tuli breed offers high hybrid vigour hence suitable for crossbreeding programmes (Trail, 1977). It is, however, not clear if crossbreeding programs involving the Tswana and Tuli cattle breeds yield any substantial degree of heterosis owing to the common origin and recent isolation of the two breeds as well as the striking phenotypic similarities between the two breeds. The degree of genetic differentiation and genetic identity between the two breeds remains unknown.

The elucidation of genetic variability and genetic relationships among breeds has direct relevance with issues of sustainable use of domestic animal genetic resources (Rehman and Khan, 2009). Molecular biology and

genomics tools have made it possible to study genetic characteristics and variability of livestock. Microsatellite markers and single nucleotide polymorphisms (SNP) are the most powerful and have the potential to estimate genetic diversity and genetic relationships among cattle breeds (Rehman and Khan, 2009). These tools have been widely used to quantify cattle breed relationships across the world, such as the British cattle (Wiener et al., 2004), European cattle (Kantanen et al., 2000), west/central African cattle (Ibeagha-Awemu et al., 2004), Indian cattle (Sharma et al., 2015), Zimbabwean cattle (Gororo et al., 2018) and South African Nguni cattle (Madilindi et al., 2019). A comprehensive genetic characterization study including within-and between-breed genetic diversity and genetic relationships between Tswana and Tuli breeds is required to facilitate effective management and design of sustainable breeding programmes. The current study was undertaken to assess the genetic diversity, the levels of genetic differentiation and genetic identity between the Tswana and Tuli cattle breeds found in Botswana using 12 microsatellite markers.

## Materials And Methods

### Experimental Animals

Twenty-six (26) unrelated Tswana cattle and twenty-five (25) unrelated Tuli cattle kept at Botswana University of Agriculture and Natural Resources (BUAN) farm participated in the study. The BUAN Tswana cattle was assembled in 1989 from animals coming from various parts of the country to safeguard its genetic purity from indiscriminate crossbreeding with exotic cattle breeds and remained a closed population ever since. In 1990 the Department of Agriculture and Research donated Tuli cattle to BUAN for research purposes. The herd has been kept closed since 1990. The cattle are kept under extensive management with very little supplementation.

### Sample Collection and DNA Extraction

Blood samples of 3-5ml were collected from 51 herd of cattle: Tswana (n=26) and Tuli (n=25) from the jugular vein into vacutainer tubes containing EDTA as anticoagulant. Deoxyribonucleic acid (DNA) was extracted from whole blood samples using Quick gDNA blood kit (Zymo, USA) following the manufacturer's protocol. The concentration of gDNA was measured using a spectrophotometer (Nanodrop 2000) and the purity of the gDNA was calculated by the 260/280 absorbance ratio (Thermo Fisher Scientific Inc., Waltham, MA, USA).

### Microsatellite Markers Amplification

A panel of 12 bovine microsatellite markers recommended for estimating genetic diversity in cattle by the International Society for Animal Genetics (ISAG) and Food and Agricultural Organization (FAO) advisory board (FAO, 2011) were used to amplify specific regions of gDNA of Tswana and Tuli cattle breeds (Table 1). All the 12 markers were amplified in a single multiplex polymerase chain reaction using fluorescence-labelled primers at Agricultural Research Council (ARC) molecular genetics laboratory (Irene, Pretoria, South Africa). A 15 µl reaction was prepared with deionized water, 10x PCR buffer optimized with 0.1 µl 50 mM MgCl<sub>2</sub> and 0.1 µl 100 mM deoxynucleotides triphosphates, 0.1 µl 5U DNA taq polymerase (Bioline USA, Inc.), 0.3 µl of 10 mol/µl primers (Applied Biosystems, Foster city, CA, USA) and 5 µl of 50 ng of gDNA. DNA amplification of the 12 marker loci were achieved using GeneAmp PCR System® 9700 gold thermal cycler (Applied Biosystems,

Foster city, CA, USA). A positive control comprising of known DNA profile was included in the study. The negative control comprised of the master mix contents without any DNA template.

Amplification of the markers was achieved using the following polymerase chain reaction (PCR) conditions: initial denaturation at 98°C for 60 seconds, followed by 30 cycles of 98 for 20s, annealing temperature of 60 for 75s and DNA extension at 72 for 30s, followed by final extension step at 72 for 5 mins. 1.5µl of PCR products were mixed with 11µl of deionised formamide and 0.3µl of GeneScan 500 LIZ size standard and denatured by heating at for 3mins followed by rapid cooling on ice. The PCR products were then separated using capillary electrophoresis ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster city, CA, USA). Data on fragment size were analysed automatically using Genescan Analysis Software v.3.1, which provided information on allele size, and Genotyper 2.5 software/program identified different alleles for each marker.

**Table 1:** Microsatellite markers employed in the genetic analysis of two Sanga type cattle of Botswana.

Chr= chromosomal location

### Statistical Analysis of Data

The MS toolkit software was used to determine the number of alleles per locus, allele frequencies, mean number of alleles per locus, observed and expected heterozygosities and the polymorphic information content (PIC) for each locus in Tswana and Tuli cattle. The inbreeding coefficient ( $F_{is}$ ) for each locus was computed using the program FSTAT (Goudet, 2001). The probability test approach (Guo and Thomson, 1992) implemented in the GENEPOP software (Raymond and Rousset, 1995) was used to test each locus for Hardy-Weinberg equilibrium.

Data on the various population diversity measures were further analysed using General linear Models Procedures of statistical analysis system (SAS, 2009). Means separations were by paired t-test and means were declared significantly different between the two breeds at  $P \leq 0.05$ .

## Results And Discussion

### Alleles in Tswana and Tuli cattle

Twelve markers were successfully amplified in Tswana and Tuli cattle breeds. All amplified loci were polymorphic and yielded a total of 75 and 77 alleles in Tswana and Tuli cattle breeds, respectively (Table 2). The total number of alleles per locus ranged between 2 (BM1818) and 10 (TGLA227) and loci ETH225, ETH10, BM2113, CSRM60 and BM1824 were highly polymorphic registering 6-9 alleles per marker with mean values (MNA) of 6.25 and 6.43 in Tswana and Tuli cattle populations, respectively. The observed allelic diversity in this study was comparable to South African Nguni cattle (6.47), Mozambique indigenous cattle (5.9-6.4) and Southwestern European cattle (6.5) (Beja-Pereira *et. al.*, 2003; Bessa *et al.*, 2009; Sanarana, 2016). The existing allelic diversity of Tswana and Tuli cattle dictates that appropriate management practises be implemented to ensure conservation of the existing diversity for future sustainable breeding programmes.

Unique alleles are important for breed identification and genetic distinctiveness (Senarana *et al.*, 2016). A total of one hundred and three unique alleles were genotyped across the two breeds with a total of 49-shared

Locus	Primer sequences	Chr	Allele range	Dye label	Reference
TGLA227	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	18	79-99	6 FAM	Georges and Massey (1992)
BM2113	GCTGCCTTCTACCAAATACCC CTTAGACAACAGGGGTTTGG	2	120- 144	PET	Sunden et al. (1993)
ETH10	G TTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	5	207- 223	6 FAM	Toldo et al. (1993)
TGLA122	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	21	135- 163	6 FAM	Georges and Massey (1992)
INRA023	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTCA	3	183- 217	NED	Varinman et. al. (1994)
BM1818	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	23	255- 269	NED	Bishop et al (1994)
ETH03	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	19	113- 125	PET	Toldo et al. (1993)
ETH225	GATCACCTTGCCACTATTTCT ACATGACAGCCAGCTGCTACT	9	137- 159	VIC	Steffen et al. (1993)
BM1824	GAGCAAGGTGTTTTTCCAATC CATTCTCCAAGTCTTCCTTG	1	182- 196	PET	Barendse et al. (1994)
CSRM60	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	10	92- 120	PET	Baylor College of Medicine Human Genomics Sequencing
CSSM66	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	14	179- 199	PET	Barendse et al. (1994)
ILST006	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	7	282- 302	VIC	Brezinsky et al. (1993)

alleles between the two breeds, and 26 and 28 alleles being peculiar to the Tswana and Tuli populations, respectively (Table 2). The mean number of shared alleles per locus between Tswana and Tuli cattle breeds is comparable to Sudanese Zebu cattle breeds; between Fuga and Butana (4.4) and between Butana and Kenana (4.0) (Hussain et. al., 2016). The shared alleles indicate an admixture of the two populations or similar ancestral origins of the two breeds. The Tuli population had higher number of alleles than Tswana population and this has also been noted among the Zimbabwean indigenous cattle populations (Gororo et al., 2018).

**Table 2:** Number of alleles based on 12 microsatellite analysis of Tswana and Tuli cattle

<b>Locus</b>	<b>Breed</b>	<b>Observed alleles</b>	<b>TNA</b>	<b>Shared Alleles</b>
TGLA227	Tswana	77, 79, 81, 83, 87, 89, 97, 99, 101, 103	10	8
	Tuli	77, 79, 81, 83, 87, 89, 91, 95, 97, 101	10	
BM2113	Tswana	121, 125, 127, 133, 135, 137, 139, 141, 143	9	8
	Tuli	121, 125, 127, 133, 135, 137, 139, 141	8	
ETH10	Tswana	206, 213, 214, 215, 217, 218, 219, 221, 225	9	3
	Tuli	207, 211, 216, 217, 218, 219, 223	7	
TGLA122	Tswana	137, 143, 151, 161, 179, 181, 183	7	4
	Tuli	137, 143, 151, 177, 179	5	
INRA23	Tswana	196, 198, 208, 214	4	3
	Tuli	198, 208, 210, 214	4	
BM1818	Tswana	262, 264	2	1
	Tuli	261, 262, 266	3	
ETH3	Tswana	115, 117, 125, 127, 129	5	5
	Tuli	107, 108, 115, 117, 119, 121, 125, 127, 129	9	
ETH225	Tswana	137, 140, 144, 146, 150, 154, 159, 176, 180	9	5
	Tuli	140, 144, 148, 150, 152, 154, 159	7	
BM1824	Tswana	146, 176, 178, 180, 182, 195	6	3
	Tuli	140, 154, 159, 178, 180, 182, 188, 192	8	
CSRM60	Tswana	92, 96, 100, 102, 110, 114	6	5
	Tuli	92, 94, 96, 98, 100, 102, 110	7	
CSSM66	Tswana	179, 181, 183, 187, 195	5	1
	Tuli	179, 185, 193	3	
ILST006	Tswana	286, 294, 296	3	3
	Tuli	286, 290, 294, 296, 298, 300	6	
Total	Tswana		<b>75</b>	<b>49</b>
	Tuli		<b>77</b>	
<b>Mean (MN)</b>			<b>6.25</b>	<b>4.08</b>
			<b>6.43</b>	

TNA= Total Number of Alleles

### Genetic Diversity of Tswana and Tuli cattle

The polymorphic information content (PIC) of the 12 microsatellite markers were significantly high (PIC >0.5) except for BM1818 (0.375) and INTRA23 (0.393) that were moderately informative in Tswana cattle population. The mean PIC values were 0.636 and 0.7156 in Tswana and Tuli cattle, respectively (Table 3) and there was no significant difference in mean PIC values of Tswana and Tuli cattle (P=0.367). Similar mean PIC values have been reported in Sudanese Zebu breeds (0.647), South African Nguni cattle breed (0.655) and Zimbabwean Sanga cattle type (0.664) (Hussain et al., 2016, Sanarana et al., 2016; Gororo et al., 2018). This indicates that all the microsatellite markers used in this study were highly informative and useful for assessment of genetic diversity in the studied cattle populations.

The observed heterozygosity ( $H_o$ ) varied from 0.00 (CSSM66) to 1.00 (BM1818) with mean values of  $0.6311 \pm 0.091$  in Tswana and  $0.556 \pm 0.021$  in Tuli cattle and the expected heterozygosity ( $H_e$ ) varied from 0.455 (INRA23) to 1.00 (BM1818) with mean values of  $0.789 \pm 0.033$  in Tswana and  $0.812 \pm 0.033$  in Tuli cattle populations (Table 3). There were no significant differences in both mean observed heterozygosity (P=0.451)- and mean expected heterozygosity (P=0.617) between Tswana and Tuli cattle breeds. Similar mean  $H_e$  values have been noted in different indigenous cattle in the southern African region; the Nguni cattle breed of South Africa (Sanarana et al., 2016) and the Zimbabwean Sanga cattle type (Gororo et al., 2018). The mean  $H_o$  and  $H_e$  values of the two breeds indicate that considerable genetic variation still exists in Tswana and Tuli cattle breeds which is relevant for adaptation and could be exploited in selection programmes to bring improvements in traits of economic importance. However, the higher average expected heterozygosity value over the observed heterozygosity is of concern and could be attributed to; null alleles, Wahlund effect, assortative mating and inbreeding (Cervini et al., 2006). Heterozygote deficiency ( $H_e > H_o$ ) has also been reported in different African countries for different indigenous cattle like Mozambique (Bessa et al., 2009) South Africa (Sanarana et al., 2016), Senegal (Ndiaye et al., 2015) and Cameroon (Ema et al., 2014).

**Table 3:** Measures of Genetic Variability of the loci analysed.

Locus	Ho		He		PIC	
	Tswana	Tuli	Tswana	Tuli	Tswana	Tuli
TGLA227	0.889	0.833	0.873	0.875	0.833	0.833
BM2113	0.867	0.625	0.837	0.865	0.787	0.818
ETH10	0.714	0.375	0.901	0.892	0.819	0.814
TGLA122	0.600	0.474	0.756	0.724	0.695	0.649
INRA23	0.333	0.600	0.455	0.644	0.393	0.535
BM1818	1.00	1.00	1.00	0.833	0.375	0.555
ETH3	0.500	0.667	0.696	0.794	0.615	0.745
ETH225	0.688	0.500	0.887	0.839	0.844	0.787
BM1324	0.563	0.500	0.679	0.845	0.623	0.797
CSRM60	0.471	0.688	0.774	0.835	0.709	0.784
CSSM66	0.200	0.00	0.867	0.714	0.745	0.555
ILST006	0.750	0.400	0.750	0.899	0.582	0.772
<b>Mean</b>	<b>0.631±0.091</b>	<b>0.556±0.021</b>	<b>0.790±0.033</b>	<b>0.812±0.033</b>	<b>0.636±0.013</b>	<b>0.716±0.005</b>

### Inbreeding and test for Hardy-Weinberg Equilibrium in Tswana and Tuli cattle

The Tswana cattle population had an inbreeding estimate ( $F_{is}$ ) ranging between -0.054 (TGLA227) and 0.769 (CSSM66) while the Tuli cattle population ranged between -0.2 (BM1818) and 1.00 (CSSM66) (Table 4). The overall mean inbreeding coefficient for Tswana population was  $0.200 \pm 0.002$  and  $0.332 \pm 0.0001$  for Tuli population, indicating 20% and 33% shortfall of heterozygotes in the two breeds, respectively. Similar values of inbreeding coefficient have also been obtained in Indian Bachaur cattle breed (0.22) (Sharma et al., 2015) and Indian Ongole cattle breed (0.36) (Metta et al., 2004). Contrary to our current findings, Gororo et al. (2018) reported lower inbreeding coefficient values for conserved Zimbabwean cattle; Tuli (-0.047), Mashona (0.003) and Nkone (-0.052). The difference in the studies could be the sample sizes and limited number of breeding bulls at the BUAN conservation farm as well as other factors such as linkage with loci under selection and the presence of null alleles (Rehman and Khan, 2009). The observed higher inbreeding coefficient values in Tuli than Tswana cattle (0.332 vs 0.20) is probably due to intensive selection practised during the development of the Tuli breed in Zimbabwe while no selection pressure has been applied to Tswana cattle at BUAN farm. According to Mpofu (2002) the Tuli breed was distributed from a founding herd in Zimbabwe and most of the bulls used for mating are technically from the same genetic pool.



The Hardy-Weinberg equilibrium test is used to assess the genetic stability of the population. Considering two populations, many of the markers were in Hardy-Weinberg equilibrium (Table 4). The loci that significantly deviated ( $P < 0.05$ ) from HWE were CSSRM60 and CSSM66 for Tswana population and ETH10, ETH225 and CSSM66 for Tuli population. The deviation of some markers from Hardy-Weinberg equilibrium could be due to genotyping errors, selection, genetic drift and small sample size (Kumar *et al.*, 2005). Selection for growth and carcass traits and feed efficiency during the development of the Tuli breed could have resulted in non-random mating with respect to the two markers ETH10 and ETH225. Natural selection for tick resistance/tolerance in Tswana cattle could also have resulted in non-random mating with respect to CSRM60 marker (McNeil and Grosz, 2002; Pereira *et al.*, 2005).

**Table 4:** Inbreeding and test for Hardy-Weinberg Equilibrium in Tswana and Tuli cattle breeds at BUAN farm

Locus	$F_{IS}$		HWE	
	Tswana	Tuli	Tswana	Tuli
TGLA227	-0.054	0.666	0.66±0.0004	0.8953±0.0003
BM2113	-0.004	0.723	0.27±0.0003	0.0976±0.0003
ETH10	0.841	0.579	0.06±0.0002	0.0049±0.0001
TGLA122	0.207	0.345	0.13±0.0005	0.061090.0002
INRA23	0.267	0.069	0.13±0.0004	0.1902±0.0004
BM1818	0.000	-0.20	1.00±0.0000	1.000±0.0000
ETH3	0.281	0.160	0.33±0.0006	0.0723±0.0022
ETH225	0.225	0.071	0.059±0.0003	0.01243±0.001
BM1324	0.165	0.221	0.14±0.0003	0.6705±0.0005
CSRM60	0.361	0.176	0.05±0.0002	0.4259±0.0001
CSSM66	0.769	1.000	0.00±0.0001	0.0278±0.0001
ILST006	0.332	0.033	0.47±0.0001	0.9078±0.0003
<b>Mean</b>	<b>0.200±0.002</b>	<b>0.332±0.0001</b>		

### Genetic relationship between breeds

The genetic identity between Tswana and Tuli cattle breeds was 0.56 indicating 56% genetic similarity between the two breeds. The high genetic identity indicates that the two breeds share common ancestry or a closer evolutionary history as they occupy adjacent habitats (Tuli developed in Gwanda region of Zimbabwe adjacent to Tswana cattle in Bobirwa region of Botswana just separated by the border). Pairwise genetic differentiation ( $F_{st}$ ) also indicated moderate genetic differentiation of 6.8% while the remaining 93.2%

corresponded to differences within each population. Medium genetic differentiation has been reported in Mozambique indigenous cattle (Bessa et al., 2009), South African indigenous cattle (Sanarana et al., 2016) and indigenous cattle of Zimbabwe (Gororo et al., 2018). The moderate genetic differentiation between Tswana and Tuli cattle breeds may also indicate the presence of gene flow between the two breeds, co-ancestry (Hussain et al., 2016, Loftus et al., 1994), reproductive isolation or moderate selection pressure in the populations kept at BUAN farm (Feliuss et al., 2014)

## Conclusions

The two Sanga type cattle population of Botswana possess high genetic diversity that is relevant for sustainable breeding programmes and incessant conservation to ensure that the existing gene pool is not lost. There are genetic similarities between the Tswana and Tuli cattle breeds and crossbreeding between the two breeds should be discouraged in favour of pure breeding of the two breeds. Crossing the two will likely result in minimal heterosis benefits.

## Declarations

### Statement and Declarations

The authors declare no conflict of interest.

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### Animal Ethics Statement

The handling and sampling of cattle were in conformity with the guidelines of Botswana University of Agriculture and Natural Resources ethical committee.

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