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GENETIC CHARACTERIZATION OF TSWANA AND TULI CATTLE KEPT AT BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES USING MICROSATELLITE MARKERS.

MASTER OF SCIENCE IN ANIMAL SCIENCE

BY

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OCTOBER 2020

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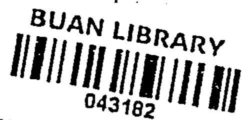

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(ANIMAL BREEDING AND REPRODUCTION)

BY

TIRELO BAKAE

OCTOBER 2020

GENETIC CHARACTERIZATION OF TSWANA AND TULI CATTLE KEPT AT BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES.

A Dissertation submitted in partial fulfilment of the requirement for degree of Master of Science in Animal Science (Animal Breeding and Reproduction)

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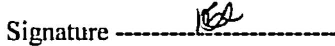
October 2020

DECLARATION

I declare that the thesis hereby submitted by me for the Master of Science Degree (Animal Science) at Botswana University of Agriculture and Natural Resources, is my own independent work and has not been previously submitted by me to another University or Faculty for the award of any other degree or diploma. All assistance towards the production of this work and references contained herein has been duly accredited.

Signature  Date 28/10/2020

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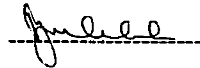
P.M. Kgwatalala	28-10-2020	
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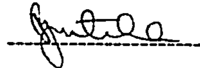
P.M. Kgwatalala	28-10-2020	

GENERAL ABSTRACT

ABSTRACT

Twelve FAO-ISAG-recommended microsatellite markers were used to analyse genetic variation in Tswana and Tuli cattle breeds. All loci were polymorphic and a total of 75 and 77 alleles were genotyped in the Tswana and Tuli breeds, respectively, across all loci. The number of alleles per locus ranged from 2 (BM1818) to 10 (TGLA227) in Tswana cattle and from 3 (BM1818 and ILST006) to 10 (TGLA227) in Tuli cattle with mean number of alleles (MNA) per locus of 6.25 and 6.43, in the two breeds, respectively. A total of 103 unique alleles were genotyped across the two breeds with a total of 49 shared alleles between the two breeds. 26 alleles and 28 alleles were unique to the Tswana and Tuli breeds, respectively. The mean number of shared alleles per locus across the 12 loci was 4.08. The observed heterozygosity ranged from 0.200 (CSSM66) to 1(BM1818) and from 0.00 (CSSM66) to 1 (BM1818) in Tswana and Tuli cattle, respectively, with higher mean observed heterozygosity in Tswana than Tuli cattle (0.6311 vs. 0.555). The PIC values ranged from 0.375 (BM1818) to 0.844 (ETH225) in Tswana cattle and from 0.535 (INTRA23) to 0.833 (TGLA227) in Tuli cattle with mean PIC values of 0.6355 and 0.7156 in Tswana and Tuli cattle, respectively. The within-population inbreeding estimate (Fis) of both Tswana and Tuli cattle were significantly positive. The Fis estimates ranged between -0.054 (TGLA227) and 0.769 (CSSM66) in Tswana cattle and between -0.2 (BM1818) and 1.00 (CSSM66) in Tuli cattle. The within population inbreeding coefficient were 0.20 and 0.332 in Tswana and Tuli cattle, respectively. Tswana cattle were not in Hardy-Weinberg equilibrium at CSSRM60 and CSSM66 marker loci while Tuli cattle were not in Hardy-Weinberg equilibrium at ETH10, ETH225 and CSSM66 marker loci and the rest of the markers were in Hardy-Weinberg equilibrium in the two breeds. Considerable allelic diversity and genetic diversity exist in both

Tswana and Tuli cattle breeds and there was no significant difference in the level of genetic diversity between the two breeds. The genetic identity between Tswana and Tuli cattle breeds was 0.564 indicating that there is 56% genetic similarity between the two breeds. Pairwise genetic differentiation (Fst) value between Tswana and Tuli cattle was 0.0676 indicating that approximately 6.8% of the total genetic variation corresponded to differences between the two breeds, while the remaining 93.2% corresponded to differences among individuals. Tswana and Tuli cattle breeds are thus highly genetically related and therefore crossbreeding between the two breeds is unlikely to benefit from heterosis.

Keywords: **Botswana, Genetic differentiation, Genetic identity, Genetic variability, Inbreeding**

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ACRONYMS AND ABBREVIATIONS

DNA deoxyribonucleic acid

DRUSSA Development Research Uptake in Sub-Saharan Africa

FAO Food and Agricultural Organization

F_{IS} inbreeding coefficient within a subpopulation

GDP gross domestic product

$H_{(e)}$ expected heterozygocity

$H_{(o)}$ observed heterozygocity

HWE Hardy-WeinbergbEquilibrium

ISAG international Society for Animal Genetics

MtDNA mitochondrial deoxyribonucleic acid

Ng nanogram

PIC polymorphic information content

QTL quantitative trait loci

Sd standard deviation

SNP single nucleotide polymorphism

µl Microlitre

°C Degrees Celsius

% Percentage

CHAPTER 1

GENERAL INTRODUCTION

The two beef production sectors in Botswana are the traditional and commercial sectors. The total cattle population in Botswana is about 2.7 million and 82% of the cattle are found in the traditional sector (Masokwane, 1995). The traditional sector keeps mostly the indigenous Tswana cattle breed for both pure-breeding and crossbreeding under the communal free-range system. Tswana cattle are primarily used for beef production and their other uses include milk production and draught power.

Commercial farmers keep mostly exotic cattle breeds and crosses between exotic breeds and crosses between exotic and indigenous cattle breeds of Southern Africa including crosses of exotic and Tswana cattle breeds mostly under fenced ranches. Although the commercial sector accounts for only 18 percent of the total cattle population, the livestock offtake rates are higher at 17 % compared to 8% in the traditional sector (Masokwane, 1995).

The Tswana cattle breed is indigenous to Botswana and is a Sanga type, similar to Barotse and Tuli cattle breeds (Scholtz, 2010). Tswana cattle are predominantly either plain black or multi coloured: usually red pied and rarely black pied (Figure 1.1). They are also found in Southwestern Zimbabwe and the Transvaal region of South Africa (Genus, 1985). Tswana cattle are well adapted to hot and dry environments and have a high level of tick and heat tolerance (Masokwane, 1995).

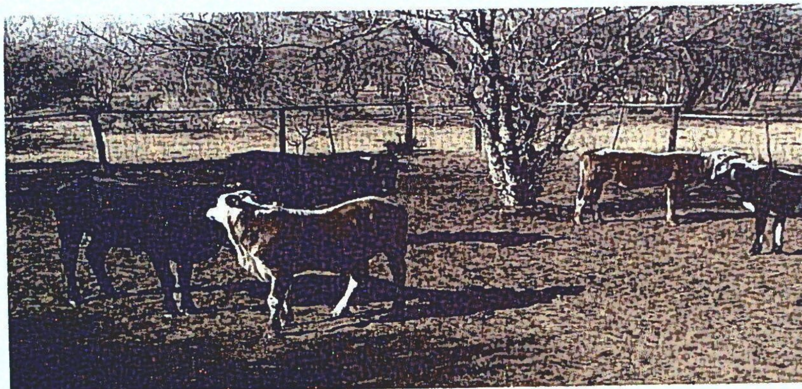

Figure 1.1 Multi-coloured Tswana cattle kept at BUAN.

The Tuli breed was developed in Zimbabwe from the Tswana-type cattle at Tuli research station in Gwanda district of Zimbabwe by Len Harvey about 90 years ago (Scholtz, 2010). The Tuli cattle breed is phenotypically similar to the Tswana cattle breed and share qualities such as hardiness, adaptability, heat resistance/tolerance and tick resistance/tolerance (Masokwane, 1995). Tuli cattle were selected for milk production, carcass, and meat quality traits (Tuli Breeders Society of South Africa, 2016) during development. According to the Tuli breeders Society of South Africa (2016), the development of the Tuli breed in isolation and its unique genetic makeup makes it very successful in crossbreeding programmes and results in a high degree of heterosis and mostly polled progeny.

Some farmers in Botswana practice crossbreeding between Tswana and Tuli cattle breeds but given the common origin of the two breeds and their phenotypic similarities, it is doubtful if the resulting progeny benefit from any significant heterosis. The aim of this study was therefore to assess the genetic diversity of Tswana and Tuli cattle breeds and estimate the degree of genetic differentiation and genetic identity between the two breeds.

Figure 1.2 Tuli cattle breed kept at BUAN

1.1 Justification

Tswana cattle are native to Botswana and are well adapted to the country's hot and dry climatic conditions. Awareness of the value of livestock genetic resources has stimulated the study of the genetic diversity of native breeds (FAO, 2011). Most genetic diversity studies have been carried out on European cattle breeds (Ginjah et al., 2013) and very little information is available on the genetic diversity of cattle breeds indigenous to Botswana and/or Southern Africa in general. Information on the genetic diversity of Tswana cattle remain unknown. Both genetic and phenotypic characterization studies are important basis for conservation programmes (Podisi, 2000). Monitoring and recording of genetic trends over time have become essential for effective protection of livestock breeds.

Tswana cattle are indigenous to Botswana and kept by a majority of the households throughout the country. Cattle farmers in Botswana cross Tswana cattle with exotic breeds such as Brahman, Simmental and Charolois, and with other indigenous cattle breeds of Southern Africa such as the Tuli to improve traits of economic importance. The Tuli breed was developed in Zimbabwe from the Tswana-type cattle in Gwanda district of Zimbabwe (Buck *et al.*, 1982).

The Tswana and Tuli cattle breeds share many phenotypic similarities and common attributes of the two breeds include hardiness, adaptability to hot and dry environments, heat tolerance and tick resistance/tolerance. Although some local farmers practice crossbreeding between Tswana and Tuli breeds, it is doubtful if the resulting crosses benefit from any significant heterosis due to the phenotypic similarities between the breeds. No studies to date have been carried out to compare molecular genetic profiles of Tswana and Tuli cattle breeds and their degree of genetic differentiation and genetic identity. Hence the importance of the current study.

1.2 Aim

To characterize Tswana and Tuli cattle breeds using a panel of twelve Microsatellite markers.

1.3 Objectives

-To evaluate the genetic diversity of Tswana and Tuli cattle using a panel of twelve (12) microsatellite markers recommended by FAO-ISAG Advisory Panel (2011).

-To compare the genetic diversity between Tswana and Tuli cattle breeds

- To estimate the genetic relationship (genetic identity and genetic differentiation) between Tswana and Tuli cattle breeds.

1.4 Hypothesis

- H_0: There is lack of genetic diversity in the Tswana cattle population kept at Botswana University of Agriculture and Natural Resources farm.
- H_A: There is genetic diversity in the Tswana cattle population kept at Botswana University of Agriculture and Natural Resources farm.
- H_0: There is no significant difference in genetic diversity between Tswana and Tuli cattle breeds kept at Botswana University of Agriculture and Natural Resources farm.
- H_A: There is a significant difference in the genetic diversity of Tswana and Tuli cattle breeds kept at Botswana University of Agriculture and Natural Resources farm.
- H_0: There is no significant genetic differentiation between Tswana and Tuli cattle breeds
- H_A; There is significant genetic differentiation between Tswana and Tuli cattle breeds

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CHAPTER 2

LITERATURE REVIEW

2.1 Tswana Cattle Breed

2.1.1 History of Tswana cattle

The Tswana cattle belong to the group of cattle called Humped cattle and the two types of humped cattle are the Sanga and the Zebu type. Tswana cattle are an indigenous Sanga beef cattle breed found in most parts of Botswana (APRU, 1989). Tswana cattle are mostly red, black, red and white or black and white in colour. The most typical Tswana cattle currently occur mainly in the North and North-west of the country, in Ngamiland and along Boteti river (Buck, 1982). Tswana cattle are closely associated with the Damara cattle in Namibia since they came through the same route of migration from Ethiopia and Somalia (APRU, 1989). They are well adapted to hot and dry conditions of the local environment. This environmental adaptation is particularly important in respect of resistance to local endemic diseases and ability to withstand nutritional stress during the dry season (Mpofu, 1996). Research has shown that they are heat and tick resistant (Mpofu, 1996).

2.1.2 Importance of Tswana cattle

In Botswana, beef production is a major agricultural activity and beef production is mainly based on extensive grazing in natural rangelands. To support beef production, the country has set aside 581 000 km^2 of land for extensive grazing of ruminant livestock (Buck, 1982). Tswana cattle breed was the main source of beef nationwide in the 1980s (ARPU, 1989). Botswana Meat Commission (BMC) that started its operations in 1965 existed to promote the development of the country's beef and related products globally and during inception relied

heavily on the Tswana cattle breed. The BMC has had a significant contribution to the development of the country over the years by exporting beef to Europe, South Africa and Angola and other importing areas. Other uses of Tswana cattle include milk production, draught power and production of dung used as fuel and manure for horticultural production.

Tswana cattle also have Socio-cultural function and the relative importance of each of the cattle functions vary with farmers' objectives, production system, rangeland type, region and socio-economic factors such as gender, marital status, age, education and religion of the keepers (Chimonyo et al., 2000, Simela *et al.*, 2006). Cattle are reserved for special ceremonial gatherings such as marriage feasts, weddings, funerals, circumcision and ancestral communion (FAO, 2007, Bayer, *et al.*, 2004). Cattle are given as gifts to visitors and relatives, and as starting capital for youth and newly married couples (Musemwa *et al.*, 2008). They are used to strengthen relationships with in-laws and to maintain family contacts by entrusting them to other family members (Dovie *et al.*, 2006). Cattle play an important role in installation of spirit mediums, exorcism of evil spirits and are given as sacrificial offerings to appease avenging spirits (Bayer *et al.*, 2004, 2009)). Some farmers keep cattle for prestige and pleasure.

2.1.3 Attributes of Tswana cattle

The Ministry of Agriculture Development and Food security through the Department of Agricultural Research (DAR) conducted a series of studies on Tswana cattle and concluded that Tswana cattle have good beef, high fertility, average milk yield and good maternal ability (APRU, 1989). In terms of reproductive performance, Tswana cattle have easy calving due to large birth canal and small birth weight (Buck, *et al.*, 1982). Tswana cattle also mature early, are of medium body size (Adult size 400kg) and have low feed requirements. Tswana cattle

also have a unique ruminal digestion that confer on them the ability to utilize low quality standing grass and crop residues better that temperate breeds (Mpofu, 1996).

2.1.4 Crossbreeding of Tswana cattle with exotic breeds

In the early 19th century, Tswana cattle were the main source of beef to the country and European settlers in Botswana brought in Friesian, Afrikaner, Sussex and other exotic breeds and crossbred them with Tswana cattle (Mpofu, 1996). Crossbreeding between indigenous Tswana cattle and exotic breeds is aimed at improving growth performance and meat quality of Tswana cattle and to provide a more profitable market product for BMC (APRU, 1989). Then the government embraced the concept of crossbreeding and through the Ministry of Agriculture established Livestock Improvement Centres (LIC) at each Tribal Reserve (TR) across the country to produce crossbred bulls and heifers to improve Tswana cattle (Lethola *et. al.*, 1983). The introduction of crossbreeding between exotic breeds and indigenous Tswana cattle has led to genetic erosion of the Tswana cattle breed and poses a threat to the genetic diversity of the well-adapted Tswana cattle.

2.1.5 Conservation of Tswana Cattle

The implementation of Tswana cattle conservation project by DAR started after realizing that the genetic uniqueness of Tswana cattle was threatened by crossbreeding with other exotic breeds (Podisi, 2000). DAR established a foundation herd of purebred Tswana cattle herd for conservation purposes in 1989. The foundation herd comprised purebred Tswana cattle from communal farmers across the country and included Tswana cattle coming from Kweneng, Kgalagadi, South and Central regions of Botswana (APRRU, 1990) in order to establish as broad a genetic base as possible. The foundation herd was divided into three groups comprising 250-300 Tswana cattle each, and one of the groups served as the unselected control line, the other group was selected for pre-weaning growth performance or maternal performance and

the last group was selected for post-weaning growth performance. The three groups or lines have remained closed since 1989 in government ranches at Musi, Dikgathong and Morapedi, respectively. Botswana University of Agriculture and Natural Resources (BUAN) received cattle from the unselected control line in 1989 and the herd has since remained closed with no intentional selection for any trait of economic importance.

2.2 Tuli Cattle Breed

2.2.1 History of Tuli cattle

According to Scholtz (2010), Tuli cattle breed was developed from Sanga-type cattle by Mr Len Harvey at a Government station in Gwanda (Zimbabwe) (Buck *et al.*, 1982). The Tuli is of Tswana descent (From Botswana) and is closely related to the Tswana cattle breed of Botswana. The Tswana breed from which the Tuli was developed, underwent natural selection for over 5000 years and became ideally suited to a wide range of conditions across Zimbabwe while retaining a high level of fertility, early maturity and good meat carcass (Zimbabwe Tuli Society, 2015). From Zimbabwe, the Tuli spread to other Southern African countries especially to Botswana and South Africa and is also currently found in Australia, USA, Canada, Mexico, Argentina and others (Tuli cattle, 2015).

2.2.2 Physical Breed Characteristics

The Tuli is a Sanga type cattle characterized by an erect small cervico-thoracic hump. Tuli cattle are one-coloured and the colour variations range from yellow, golden-brown, red, white, brownish-grey, beige and tan (Tuli cattle, 2015). The Tuli cattle breed is predominantly yellow in colour with a uniform coat pattern and straight short hair. The face profile of the Tuli is flat with a pigmented or non-pigmented muzzle. The Tuli breed can be horned but mostly polled

with ears that are round-shaped with lateral orientation and pigmented at the ends. The body frame is medium-sized and the udder is small with medium-sized teats (Tuli cattle, 2015).

2.2.3 Production traits:

The Tuli is a medium-sized cattle breed with excellent maternal traits such as high fertility, calf survival and weaning weight and is renowned for its hardiness and adaptability to hot and dry conditions (Tuli cattle, 2015). The Tuli breed is characterized by early maturity, high docility, excellent mothering ability and good milking qualities. The Tuli breed is also renowned all over the world for its exceptional beef qualities characterized by tender and juicy meat with relatively low levels of fat but sufficient enough to give excellent marbling. Some adaptive traits of the Tuli include; tolerance to high environmental temperature, bulls are ready to serve at 2 years of age and heifers ready for bulling at 20 to 24 months of age, low incidence of dystocia, natural resistance/tolerance to ticks, flies and internal parasites and natural ability to survive and reproduce under harsh environments (Zimbabwe Tuli Society). 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.

It has been noted that due to its unique genotype, the Tuli breed offers high hybrid vigour and therefore, widely suitable for crossbreeding programmes. Small-scale farmers in communal areas in pursuit of the benefits of heterosis and complementarity in the resulting crosses often practise crossbreeding between Tuli and Tswana cattle breeds. It is however, doubtful if such crossbreeding yield the pursued benefits given the common origins and phenotypic similarities between the two breeds. According to Buck *et al.* (1982) there were no significant differences in weights between pure Tswana and Tuli x Tswana crossbreds under communal and ranch management systems.

2.3 Application of genomics in conservation studies

Since the 1980s, developments in Molecular Biology and genomics have made it possible to study some genetic parameters in livestock populations including cattle. Variations in genomic sequences provide novel tools to analyse molecular data and provide answers instrumental in conservation studies. These tools have evolved from Variable Number Tandem Repeats (VNTRs) then Short Tandem Repeats, Single Nucleotide Polymorphisms and Sequencing (Pokhriyal *et al.*, 2012).

2.3.1 Variable Number Tandem Repeats (VNTR)

Variable Number Tandem Repeats are sequences found in the genome as repeating non-coding sequences. The sequences vary in length among different genomes. VNTR were used to show variations in alleles within a population and their major drawback was the requirement of large amounts of Biological material to analyse. VNTR comprised tandemly repeated bases comprising 10-60 bps in length (Goodwill *et al.*, 2007).

2.3.2 Single Nucleotide Polymorphism (SNP)

SNPs represent a single base change between two individuals at a defined location in the genetic material or DNA. The three different categories of SNPs include transitions (C/T or G/A), transversions (C/G, A/T, C/A or T/G) and small deletions/ insertions (Duran *et al.*, 2009). SNPs are estimated to occur about once in every 1000 base pairs throughout the entire genome in coding promoters, exons and introns (Vignal *et al.*, 2002). SNPs have become markers of choice due to their abundance in genomes. They are extremely useful for creating high-density genetic maps. Other genetic marker classes cannot achieve this high-density property of SNP. Due to their abundance in mammalian genomes, SNPs have the potential to provide basis of a superior and highly informative genotyping assay.

SNPs are largely bi-allelic in nature and are less mutable compared to other types of markers, particularly microsatellites. The low rates of recurrent mutations make them evolutionarily stable. They are excellent markers for studying complex genetic traits and for understanding genomic evolution. To date, genomic wide analysis brought insights on patterns of ancestry, divergence and admixture (Hanette, *et al*., 2005). Several cattle studies have used SNPs to characterize genomic wide genetic diversity in cattle including South African cattle breeds (Makina *et al*., 2014), West African cattle population (Gauter *et al*., 2009) and the Eastern African Short Horn Zebu cattle population (Mbole-Kariuki *et al*., 2014). Illumina 50K SNP bead chip is commonly used in cattle studies and to date the SNP chip has brought insights on patterns of ancestry, divergence and admixture (Hanette, *et al*., 2005).

2.3.3 Next Generation Sequencing (NGS)

NGS is a massively parallel DNA sequencing technology that can sequence the whole genome in one day. NGS platform perform sequencing of millions of small fragments of DNA in parallel. Bioinformatics analyses is then used to put these fragments together by mapping the individual reads to the reference sample (Behjati and Tarpey, 2013). The method is robust and efficient in genomic analysis. It differs from the Sanger Sequencing technology with the volume that can be analysed at a time. Less amount of template DNA is required to do the analysis. It is cheaper and more accurate for operations such as Forensic Science and Genetic Characterization (Multz *et al*., 2012). NGS is completely unselective and used to interrogate full genome or exons to discover entirely novel mutations and disease causing genes (Sam Behjati *et al*., 2013). The genome-wide sequencing of indigenous cattle will allow for the development of African cattle specific genetic tools such as genetic markers and SNP chips.

The NGS platform is credited with establishing genetic differences between the *Bos Taurus* and *Bos indicus* ancestry in cattle (Ajmone-Marsan *et al.*, 2010).

2.3.4 Microsatellite Markers

Microsatellite markers are used in Marker Assisted Selection (MAS) and for QTLs detection. Microsatellite markers have been used for mapping functional genes and production genes in domesticated animals (Vignal *et al.*, 2002). Microsatellite markers are more powerful for parentage determination because they are highly polymorphic. Microsatellite markers are also currently used as tools for sex determination and animal products screening. In population genetics studies, microsatellite markers elucidate the relationships between individuals and populations and for estimating levels of heterozygosity and inbreeding levels. Microsatellite markers are also used for estimating the degree of introgression from other species and the degree of genetic differentiation and admixture among breeds.

2.4 Measurements of Genetic Diversity from Molecular Data

2.4.1 Allelic diversity

The number of alleles per marker provides an overview of the genetic variability of a microsatellite marker. It is useful in determining the resolution power of a marker being used in genetic diversity studies. Markers with a high number of alleles are preferred over markers with low number of alleles. According to the selective standard of FAO (2004), the microsatellite markers used in genetic diversity studies should have a minimum of five (5) distinct alleles (Chaudhari *et al.*, 2009). The Mean Number of Alleles (MNA) for a given breed indicates the level of allelic variability. In Africa, The South African Nguni had MNA of 6.5 (Saranana, 2015) and in Mozambique MNA of 7.7 was recorded on indigenous breeds (Bessa *et al.*, 2009). South-western Europe cattle breeds had MNA of 6.47 (Beja-Pereira *et al.*, 2003).

Bulgarian cattle had MNA of 7.6 (Teneva *et al.*, 2009) and Pakistani cattle breeds had MNA of 22.6 (Hussain *et al.*, 2016).

Private alleles are used to differentiate breeds (Chaudhari *et al.*, 2009) and are common in diversity studies of indigenous livestock. In principle, private alleles define the genetic uniqueness of a particular breed among a collection of breeds (Ngono *et al.*. 2014). Eight (8) private alleles have been found in Nguni cattle using a panel of 21 markers (Sanarana, 2015). The detection of some private alleles in a population or populations might be reflective of the origin of the breed and /or gene flow within the breed. The detection of an allele *BM21143-113* is usually associated with West African Taurine cattle (McHugh *et al.*, 1996) and the presence of *BM2113-143* allele and *ETH152-193* allele is usually associated with Zebu Sudanese cattle (McHugh, *et al.*, 1996).

2.4.2 Observed and Expected Heterozygosity

Heterozygosity provides a measure of the presence of heterozygotes in a population. The more the heterozygotes the more the gene pool of that particular population. Deficiency of heterozygotes indicate high levels of inbreeding. Under random mating, alleles at a locus are randomly inherited and heterozygosity is used to measure the level of variability at each locus (Nei *et al.*, 1983). It also measures the availability of heterozygotes as opposed to homozygotes in a population. The measures of heterozygosity range between 0 and 1 (Koundante *et al.*, 2009). High levels of expected heterozygosity (He) indicates adaptation to an environment with more breeds or strains in the population. Low levels of heterozygosity indicate isolation and genetic drift resulting in genetic diversity loss over the years (Saranana, 2015). When observed heterozygosity is less than expected heterozygosity (Ho < He) it indicates that there might be forces such as inbreeding which leads to deficiency of heterozygotes. Ho > He may indicate

crossing of breeds which were once isolated while Ho=He indicates that that the population is likely to be random mating (Khanyile *et al.*, 2015).

The mean expected heterozygosity of South African Nguni was 0.701 (Sanarana, 2015) with mean observed heterozygosity of 0.694. Thus Ho < He indicating the deficiency of heterozygotes in the general population. Sudanese Zebu cattle had mean expected heterozygosity of 0.609 (Upreti *et al.*, 2012) and mean observed heterozygosity of 0.73. Thus Ho > He indicating the presence of more heterozygotes in Sudanese Zebu cattle. In Pakistani cattle (Hussain, 2016) the mean expected heterozygosity was 0.8164 and the mean observed heterozygosity was 0.4943. In Portugenese native cattle, mean expected Heterozygosity was 0.689 while the mean observed heterozygosity was 0.667.

2.4.3 Polymorphic Information Content (PIC)

PIC is a parameter that indicates genetic variation and measures the informativeness of the given microsatellite marker (Cho *et al.*, 2014). PIC values range between 0 and 1. PIC values closer to 1 indicate that the microsatellite markers are highly informative, those between 0.25 and 0.5 indicate that the microsatellite markers are moderately informative while markers with PIC values less than 0.25 are considered less informative. Microsatellite markers with high PIC values are more useful in population studies, parentage verification and other molecular genetics applications such as Forensics.

In Gaolao cattle six markers (BM1824, ETH3, ETH152, HEL51, ILST005, and ILST006) out of a panel of 25 markers had low PIC values (less than 0.25) while in Kenkhara cattle five microsatellite markers (BM1824, CSRM60, ETH152, ILST005 and ILST006) out of a panel of 25 markers were less informative (Chaudhari *et al.*, 2009). The South African Nguni cattle had the mean PIC value of 0.655 (Sanarana, 2015) from a panel of 28 microsatellite markers

and Pakistani cattle had mean PIC value of 0.81 from a panel of 21 microsatellite markers (Hussain, 2016).

2.4.4 Test for Hardy-Weinberg Equilibrium

Hardy-Weinberg Equilibrium (HWE) is a measure of the Genetic stability of the population (Ginja *et al.*, 2009). Deviations from HWE may indicate genetic influences that could be due to some form of Selection that may have occurred in that population (Podisi, 2000). When the HWE P-value of a microsatellite marker is greater than 0.05 then the marker is in Hardy-Weinberg Equilibrium. Microsatellite markers with HWE P-values less than 0.05 indicate that the microsatellite markers are not in Hardy-Weinberg Equilibrium.

Out of the twenty-eight microsatellite markers used in the genetic characterization of South African Nguni cattle, 3 markers (INRA23, ETH225 and INRA37) were not in Hardy-Weinberg Equilibrium (Sanarana, 2015). The adherence of most of the markers to HWE indicated that the allelic frequencies of most markers in Nguni cattle populations remained constant from generation to generation (Dorji and Daugjinda, 2014). In Latin-American Creole cattle, 3 out of 19 microsatellite markers assessed were not in Hardy-Weinberg Equilibrium. In Portuguese native cattle, 5 out of 39 microsatellite markers employed in the study were not in Hardy-Weinberg Equilibrium (Ginja, *et al.*, 2009) in the 16 cattle breeds under investigation. When a population is in a Hardy-Weinberg Equilibrium (HWE) for a locus it means there is random mating, no selection pressure, no mutation, no gene flow and that the population is large enough to avoid effects of genetic drift (Jon-Barker's curriculum unit). Deviation of a marker from HWE imply the presence of a force or forces either acting singly or in combination capable of changing allele frequencies in the population such as selection, mutation, migration or chance changes in allele frequencies common in small populations.

2.4.5 Inbreeding Coefficient (Fis)

The F_{IS} values range from a minimum of -1 (outbreeding) to a maximum of +1 (inbreeding). In South African Nguni, F_{is} was 0.010 indicating very low levels of inbreeding within the South African Nguni cattle probably due to outbreeding and avoidance of mating between closely related animals. In Portuguese cattle breeds, F_{is} was significantly higher than zero indicating high levels of inbreeding. This was due to mating between closely related animals and the *wahblund* effect in Brava de Lide and Mertolenga independent lineages in these breeds and evidence of genetic erosion due to low effective number of males in Alentejena (Carolino and Gama 2008). In Pakistan cattle, the inbreeding coeficient (Fis) was 0.2819 and the mild directional selective pressure, non-random mating due to intense reproductive management and the use of small number of bulls as semen donars in assisted reproduction practices could be the causes of the observed inbreeding levels in Pakistani cattle (Hussain, 2016).

2.4.6 Genetic Differentiation (F_{ST})

Genetic differentiation (F_{ST}) is used to determine the existence of gene flow between populations and to detect the effect of genetic drift due to evolutionary forces (Kalinowski, 2002). Fst is the coefficient of the subpopulation within the total population. It is widely used to indicate genetic differentiation between sub populations based on allelic frequencies. According to Hussain (2016) it is generally accepted that Fst values ranging from 0 to 0.05 indicate low genetic differentiation; a value ranging between 0.05 and 0.15, medium differentiation; a value ranging between 0.15 and 0.25, significant differentiation; and a value above 0.25, highly significant genetic differentiation. Medium genetic differentiation has been reported among 12 African *Bos indicus* and *Bos taurus* cattle breeds, among Sudanese Zebu cattle breeds, among Latin-American Creole cattle breeds and among European cattle breeds (Ibeagha-Awemu and Erhardt, 2005; Hussein *et al.*, 2014; Delgado *et al.*, 2011). In South

African Nguni ecotypes mean F_{st} was 0.007 and this figure depicts more genetic variability within the population and less variability between populations (Sanarana, 2015). In Parkistani cattle mean F_{st} was 0.1456 indicating that 14% of the variation was due to differences among populations and 86% was due to within population differences (Hussain, 2016).

2.4.7 Genetic identity

Genetic identity is a measure of the proportion of genes that are identical in two populations. This is common in populations with similar origin. The geographical isolation between related breeds ultimately results in genetic loss over time and new mutations causing significant deviations between geographically isolated but related populations. Genetic identity gives information about the ancestral history of populations. Common ancestry has been reported between the Ayrshire and Friesian breeds (Zenger *et al.*, 2007). On the other hand, little genetic identity was reported between Danish Jersey and Friesian breeds which do not share a common ancestral history (Kantanen *et al.*, 2000).

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CHAPTER 3:

ASSESSMENT OF GENETIC DIVERSITY IN TSWANA CATTLE KEPT AT BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES USING DNA MICROSATELLITE MARKERS

Abstract

The aim of this study was to assess the genetic diversity in Tswana cattle at Botswana University of Agriculture and Natural Resources farm. 12 FAO recommended microsatellite markers (TGLA227, TGLA122, ETH3, ETH10, ETH225, BM181 81, BM1824, BM2113, CSSM60, CSSM66, ILST006 and INRA023 TGLA153, TGLA126, HAUT27 and SPS115) were used for the assessment of genetic diversity in 26 Tswana cattle. Seventy-five alleles were found across all loci with the mean number of alleles per marker of 6.25±2.60. The mean expected and observed heterozygosity were 0.7895±0.0406 and 0.6311±0.0414, respectively.

The PIC values which are used for the assessment of the informativeness of the individual markers, ranged between 0.375 (BM1818) and 0.8439 (ETH225) with overall mean PIC of 0.6692. TGLA227, BM2113, ETH10, TGLA122, ETH3, ETH225, CMSS60, CMSS66, and ILST006 were highly informative in the genetic analysis of Tswana cattle. Ten markers were in HWE Equilibrium while two markers (CMSS60 with P=0.0437±0.0002) and (CMSS66 with P=0.0030±0.0001) were not in Hardy-Weinberg equilibrium. The inbreeding coefficient of BUAN Tswana cattle was 20%. The Tswana cattle at BUAN farm have high levels of allelic and genetic diversity. The level of inbreeding in the herd is also high which calls for the introduction of new bulls for breeding to mitigate the negative effects of inbreeding on fitness traits and on performance in traits of economic importance.

Key words: genetic diversity, inbreeding coefficient, indigenous Cattle

3.1 INTRODUCTION

The Tswana cattle breed is indigenous to Botswana and is well adapted to the country's harsh environmental conditions. Notable attributes of the Tswana cattle include disease tolerance/resistance, high fertility, heat tolerance, good maternal qualities, longevity and ability to survive and reproduce under stressful environmental conditions and poor quality feeds (Buck *et al*, 1982). The indigenous Tswana cattle breed is at risk of extinction due to indiscriminate crossbreeding with exotic cattle breeds in order to improve their productivity, persistent droughts and outbreak of cattle diseases (Senyatso and Masilo, 1996; Podisi, 2000). Although indigenous Tswana cattle may appear less productive than their highly selected and highly specialized exotic counterparts, they are highly productive in their use of local resources and under prevailing local production environment and may be more sustainable in the long term. Besides, they carry useful genes that might prove highly beneficial in the future to mitigate the effects of global warming and climate change on livestock production and productivity.

In an attempt to conserve the indigenous Tswana cattle, the Department of Agriculture Research under the Ministry of Agricultural Development and Food Security implemented Tswana Cattle Conservation Project in 1989. The base population of the Tswana Cattle Conservation Project was assembled using different strains of Tswana cattle from Kgalagadi, Kweneng, Southern and Central regions of Botswana (APRU, 1989). From the base population, some cattle remained as the unselected control line and the remainder were selected for reproductive performance and post-weaning growth performance have since remained closed populations in various government ranches. BUAN received cattle from the unselected control line in 1989 and the herd has since remained closed with no intentional selection for any trait of economic importance. The BUAN Tswana cattle herd has remained purebred since 1989 through the rotation of Tswana bulls supplied by the Department of Agricultural Research in order to minimize inbreeding. Because of crossbreeding that is very common in communal

grazing areas, it is very difficult to ascertain the genetic purity of the remaining Tswana cattle in communal areas. The closed populations of purebred Tswana cattle in government ranches and at BUAN are therefore representative of the indigenous Tswana cattle breed in terms of both genetic purity and composition (sampled from various parts of the country).

According to Teveva *et al.* (2005), the first step in assessing genetic conservation needs is the development of baseline information for genetic diversity. The BUAN Tswana cattle herd has never been genetically characterized and therefore no baseline data exists for future monitoring of genetic trends. The purpose of this study was therefore to assess the genetic diversity of Tswana cattle population (unselected control line from the initial base population of 1989) at BUAN using a panel of 12 microsatellite markers. This will establish baseline data for future monitoring of genetic trends and inform utilization and conservation programmes.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Animals

The BUAN Tswana cattle herd was assembled in 1989 from animals coming from various parts of the country in an effort to conserve the breed and safeguard its genetic purity. The BUAN Tswana herd has remained a closed population since 1989.

3.2.2 Sample Collection and DNA Extraction

Blood samples were collected from 26 randomly selected and unrelated purebred Tswana cattle kept at BUAN farm in EDTA tubes. Pedigree records ascertained that sampled animals were unrelated. The age of the sampled animals ranged between 2 years and 8 years of age. DNA was extracted from whole blood samples using Quick gDNA blood kit (Zymo research, USA) following the manufacturer's protocol. The concentration of gDNA was measured using a

spectrophotometer (Nanodrop 2000) and the purity of the extracted gDNA was calculated by the 260/280 absorbance ratio (Thermo Fisher Scientific Inc., Waltham, MA, USA).

3.2.3 Polymerase Chain Reaction (PCR)

A panel of 12 bovine microsatellite markers endorsed for estimating genetic diversity by the ISAG and FAO advisory board (FAO, 2011) was used to amplify specific regions of gDNA. The microsatellite markers, their chromosomal positions, size range and primers used for their amplification are shown in Table 3.2.1

Table 3.2.1 Microsatellite markers employed in the characterization of Tswana cattle

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

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, 10 x PCR buffer optimized with 50 mM $Mgcl_2$ and 100 mM deoxynucleotides triphosphates, 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 was achieved using GeneAmp PCR System® 9700 gold thermal cycler (Applied Biosystems, Foster city, CA, USA). Amplification of the markers was achieved using the following PCR conditions- initial denaturation at 98°C for 60 seconds, followed by 30 cycles of 98°C for 20s, annealing temperature of 60°C for 75s and DNA extension at 72°C for 30s, followed by final extension step at 72°C for 5mins.

3.2.4 Genotyping

1.5µl of PCR products were then mixed with 11µl of deionised formamide and 0.3µl of GeneScan 500 LIZ size standard and denatured by heating at 95°C for 3mins followed by cooling on ice. The PCR products were then separated using capillary electrophoresis ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster city, CA, USA).

3.2.5 Fragment Analysis

Data on fragment size were analysed automatically using Genescan Analysis Software v.3.1 which provided information on allele size.

3.2.6 Statistical Analysis of Data

The MS toolkit software (Kim, 2002) was used to determine number of alleles per locus, allele frequencies, mean number of alleles per locus, observed and expected heterozygosity and the

polymorphic information content (PIC) for each locus. 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.

3.3 RESULTS AND DISCUSSION

Alleles at the 12-microsatellite marker loci used for genetic characterization of Tswana cattle are shown in Table 3.3.1. All the 12 microsatellite markers were polymorphic and yielded 75 alleles in Tswana cattle. The number of alleles per marker in Tswana cattle ranged between 2 for BM1818 marker and 10 for TGLA227 marker with mean number of alleles per marker of 6.25. Microsatellite markers ETH225, ETH10 and BM2113 were also highly polymorphic each with nine alleles per marker. Teneva *et al.* (2005) also reported high allelic diversity in markers TGLA227 (10 alleles), BM2113 (7 alleles) and ETH225 (6 alleles) with mean number of alleles per marker of 7.6 in Bulgarian grey cattle. A much higher allelic diversity than that found in Tswana cattle was reported in Latin-America Creole cattle with a range of 7 to 21 alleles per marker and mean number of alleles per marker of 13.65 (Delgado *et. al.*, 2011). Higher allelic diversity was also reported in native Turkish cattle (7 to 28 alleles per marker and average of 13.45 alleles per marker) and Pakistani cattle breeds (10 to 43 alleles per marker and average of 22.67 alleles per marker) (Hussain *et. al.*, 2016).

Table 3.3.1: Alleles of 12 microsatellite markers found in Tswana Cattle at BUAN farm.

Locus	Observed No. of allele	Alleles sizes(bps) and their frequencies									
TGLA227	10	77	79	81	83	87	89	97	99	101	103
BM2113	9	121	125	127	133	135	137	139	141	143	
ETH10	9	206	213	214	215	217	218	219	221	225	
TGLA122	7	137	143	151	161	179	181	183			
INRA23	4	196	198	208	214						
BM1818	2	262	264								
ETH3	5	115	117	125	127	129					
ETH225	9	137	140	144	146	150	154	159	176	180	
BM1824	6	146	176	178	180	182	195				
CSRM60	6	92	96	100	102	110	114				
CSSM66	5	179	181	183	187	195					
ILST006	3	286	294	296							
Total	75										
Mean (MNA)	6.25±2.6										

Higher allelic diversity was also reported in Mozambique indigenous cattle with mean number of alleles per marker of 7.7 (Bessa *et al.*, 2009). The allelic diversity of Tswana cattle was comparable to that of South African Nguni cattle (MNA=6.47) and South-Western European bovine breeds (MNA=6.5) (Saranana, 2015; Beja-Pereira *et al.*, 2003). Tswana cattle had higher allelic diversity than five native Indonesian cattle breeds with MNA per marker of 4.2 (Sutarno *et. al.*, 2015) and Ongole and Deoni Indian cattle breeds with MNA per marker of 4.5 and 4.1, respectively (Metta *et. al.*, 2004). According to Pandey *et al.* (2006), FAO specified a

minimum of four alleles per marker for effective screening of genetic differences between breeds. All the markers used in the current study with the exception of BM1818 and ILST0076 therefore exhibited sufficient allelic polymorphism for Tswana cattle breed characterization and estimation of genetic differences within and between breeds. Allele frequencies varied per marker and ranged between 0.001 for allele 100 of CSSM60 marker and 0.750 for allele 198 of INTRA23 marker. The BUAN Tswana cattle conservation herd therefore still exhibit high levels of allelic diversity despite remaining a closed population since 1989. The high allelic diversity could be because of lack of any selection program to improve traits of economic importance and limited inbreeding resulting from bull rotations every breeding season. The high allelic diversity also attests to the broad genetic base resulting from the admixture of Tswana cattle strains from different regions of Botswana during assembly of the base population in 1989.

A total of 6 private alleles were detected in Tswana cattle. According to Ngono Ema *et al.* (2014) private alleles are common in diversity studies on indigenous livestock. Private alleles define the genetic uniqueness of a particular breed or population among a collection of breeds (Erhardt and Weinmann, 2007). Private alleles are also be used as a tool for the measurement of the genetic distinctiveness of a population (Szpiech and Rosenburg, 2011).

Apart from allelic diversity, other measures of genetic diversity include observed heterozygosity (Ho), expected heterozygosity (He) and polymorphic information content (PIC) (Table 3.3.2). Measures of genetic diversity (Ho, He and PIC) in Tswana cattle indicated that most of the microsatellite markers were highly polymorphic.

Ho and He ranged between 0.20 (CSSM66) and 1(BM1818) and between 0.45(INTRA23) and 1 (BM1818), respectively, with mean Ho and He across all loci of 0.63 and 0.79, respectively. The average Ho of 0.63 and average He of 0.79 indicate high levels of genetic variability or

genetic diversity in BUAN Tswana cattle conservation herd since it is interpreted as such when the heterozygosity values exceed 0.5 (Melendez *et. al.*, 2014). The mean observed heterozygosity of Tswana cattle (0.63) is comparable to that of the South African Nguni cattle (0.69) and Kenana Sudanese Zebu breed (0.692) (Sanarana, 2015; Hussein *et. al.*, 2015). It is however higher than that of the Pakistani cattle breeds (0.4943) and native Gir breed of Rajasthan (0.4236) but lower than that of the Hallikar breed of India (0.7515) and Bulgarian grey cattle (0.78) (Upreti *et. al.*, 2012; Kumar *et. al.*, 2005; Teneva *et. al.*, 2005).

The average He (considered the unbiased estimate of gene diversity) of Tswana cattle (0.79) is comparable to that of the Hallikar breed of India (0.785) and Pakistani cattle breeds (0.8164) (Kumar *et. al.*, 2006; Hussain *et. al.*, 2016). It is however higher than that of the Gir and Kankrej breeds of Rajasthan (0.4520 and 0.5403, respectively) and Sudanese Zebu breeds (0.690) but lower than that of Bulgarian grey cattle (0.86) (Upreti *et. al.*, 2012; Hussain *et. al.*, 2016; Teneva *et. al.*, 2005).

Tekezaki and Nei (1987) indicated that for markers to be useful in measuring genetic variation they must have average heterozygosity between 0.3 and 0.8, and the mean Ho and He in Tswana cattle fall within range, clearly indicating the suitability of the 12 markers for assessing genetic diversity in Tswana cattle. Higher He than Ho in Tswana cattle (0.79 vs. 0.63) indicates deficiency of heterozygotes in the study population (Khanyile *et. al.*, 2015). BUAN Tswana cattle herd has been closed since 1989 which tends to encourage homozygosity at the expense of heterozygosity. The high genetic diversity in BUAN Tswana cattle herd (0.79) could be attributed to lack of any artificial selection program to improve traits of economic importance and the admixture of different Tswana cattle subpopulations during the assembly of the base population in 1989 (Ojango *et al.*, 2011).

Table 3.3.2 Heterozygocity (He), Polymophic information content (PIC), Inbreeding coefficient (F_{is})and Hardy-Weinberg Equilibium (HWE) for each locus in Tswana cattle

Microsatellite locus	Genetic Parameters			
	$H_{(E)}$	PIC	F_{IS}	HWE P-value
TGLA227	0.873	0.833	-0.018	0.6613±0.0004
BM2113	0.837	0.787	-0.035	0.2666±0.0003
ETH10	0.901	0.819	0.179	0.0642±0.0002
TGLA122	0.756	0.697	0.207	0.1298±0.0005
INRA23	0.455	0.393	0.267	0.1333±0.0004
BM1818	1.00	0.375	0.00	1.0000±0.0000
ETH3	0.696	0.615	0.281	0.3329±0.0006
ETH225	0.887	0.844	0.225	0.0589±0.0003
BM1324	0.679	0.623	0.172	0.1433±0.0003
CSSM60	0.774	0.709	0.384	0.0454±0.0002
CSSM66	0.867	0.745	0.769	0.0030±0.0001
ILST006	0.750	0.582	0.00	0.4676±0.0005
Mean	0.790±0.041	0.669±0.020	0.203±0.023	0.6613±0.0004

Polymorphic information Content (PIC) is a parameter that indicates genetic variation and measures the marker's usefulness in population studies (Guo and Thomson, 1992). PIC values range between 0 (no genetic variation and less usefulness of the marker in population studies) and 1 (high genetic variation and high usefulness of the marker in population studies. The PIC in Tswana cattle ranged between 0.375 for marker BM1818 and 0.8439 for marker ETH225 with mean PIC value across all the 12 loci of 0.6692. The mean PIC value in Tswana cattle is comparable to that of South African Nguni cattle (0.655) and, Sudanese Zebu cattle breeds (0.63) (Sanarana *et. al.*, 2015; Hussein *et. al.*, 2016). It is however higher than that of five Indonesian native cattle breeds (0.55) but lower than that of Bulgarian grey cattle (0.72) and Pakistani cattle breeds (0.81) (Sutarno *et. al.*, 2015; Teneva *et. al.*, 2005; Hussain *et. al.*, 2016).

Markers with PIC values less than 0.25 are considered less informative, those with PIC values between 0.25 and 0.5 moderately informative and those with PIC values of 0.5 or greater highly informative or useful in population molecular genetics studies (Montenegro *et. al.*, 2015). Markers INTRA23 and BM1818 are therefore moderately informative while the rest of the markers are highly informative making them suitable for assessment of genetic variation or diversity in Tswana cattle population and for other molecular applications such as forensics (individual identification), parentage verification or segregation analysis in Tswana cattle.

The inbreeding coefficient (F_{IS}) describe how genetic diversity is partitioned in a population. F_{is} values determine whether populations or subpopulations have fewer or more heterozygous individuals than expected. If the markers have a positive sign it indicates an excess of homozygotes while a negative sign indicates excess of heterozygotes (Montenegro *et. al.*, 2015). In the current study F_{is} values per locus ranged between -0.035 (BM2113) and 0.769 (CSSM66) with average inbreeding coefficient across all loci of 0.203, indicating moderate

levels of inbreeding in BUAN Tswana cattle herd. There is therefore a 20% shortfall of heterozygotes in BUAN Tswana cattle population most probably because of inbreeding which accumulates rapidly in small and closed populations. All the microsatellite markers with the exception of TGLA227, BM2113, BM1818 and ILST006 contributed to the observed 20% heterozygote deficit. According to Nei (1987), inbreeding, genetic hitchhiking, null alleles and the occurrence of population substructure are some of the established reasons for heterozygote deficiencies. The contribution of most markers to the inbreeding coefficient of the whole population suggests inbreeding as the likely cause of the deficiency of heterozygotes in BUAN Tswana cattle population. In the absence of any selection program in Tswana cattle, the confinement of Tswana cattle to a restricted geographical area and the use of limited number of bulls for breeding, as is the case at BUAN, may all result in an excess of homozygotes because of inbreeding.

Ten out of 12 microsatellite markers used in the current study were in Hardy-Weinberg Equilibrium (HWE) and only two markers (CSSM60 and CSSM66) deviated from HWE. The conformation of 10 out of 12 markers to HWE indicates the high genetic stability of BUAN Tswana cattle population. The high genetic stability of BUAN Tswana cattle population suggests that the population might be random mating (deliberate efforts made to minimize inbreeding), is not undergoing any artificial selection and not subjected to other evolutionary forces such as mutation and migration capable of altering gene and genotype frequencies and causing significant deviations from HWE. Significant deviation from HWE for markers CSRM60 and CSSM66 could be due to non-random mating with respect to the two markers or due to natural selection with respect to the two markers.

Quantitative trait loci (QTL) for milk production, milk composition and growth traits (post weaning average daily weight and back-fat thickness) and functional genes underlying those QTL have been mapped to BTA14 (Wibowo *et. al.*, 2008) where microsatellite marker CSSM66 is also found. Natural selection for low milk production and slow growth rate in Tswana cattle over centuries might have fixed alleles for those traits, which might be in linkage disequilibrium with alleles at CSSM66 locus resulting in significant departure of CSSM66 marker from HWE. A QTL for tick resistance/susceptibility has been mapped to BTA10 (Machado *et. al.*, 2010) where the microsatellite marker CSRM60 is also mapped. Natural selection for tick resistance in Tswana cattle over centuries might have fixed the tick resistance alleles which might be in linkage disequilibrium with alleles at CSRM60 locus (tick resistance alleles and marker alleles always inherited together) resulting significant deviation of CSRM60 marker from HWE.

3.4 CONCLUSIONS

The BUAN Tswana cattle herd exhibits high level of genetic diversity, which can be exploited in the future for improving performance in various traits of economic importance. However, inbreeding poses a threat to the existing genetic diversity of BUAN Tswana cattle conservation herd.

3.5 RECOMMENDATIONS

The genetic diversity found in the BUAN Tswana cattle calls for continued preservation of the herd/breed for future exploitation in breeding programmes. Outbreeding of the BUAN Tswana cattle herd is recommended to reverse the negative effects of inbreeding on genetic diversity

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CHAPTER 4

MICROSATELLITE MARKERS BASED GENETIC ASSESSMENT OF TSWANA AND TULI CATTLE BREEDS KEPT FOR CONSERVATION PURPOSES AT BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES FARM

Abstract

Twelve FAO-ISAG-recommended microsatellite markers were used to analyse genetic variation in Tswana and Tuli cattle breeds. All amplified loci were polymorphic and 75 and 77 alleles were genotyped in the Tswana and Tuli breeds, respectively. The number of alleles per locus ranged between 2 (BM1818) and 10 (TGLA227) in Tswana cattle and between 3 (BM1818 and ILST006) and 10 (TGLA227) in Tuli cattle with mean number of alleles (MNA) per locus of 6.25 and 6.4 in the two breeds, respectively. One Hundred and three unique alleles were genotyped across the two breeds with 49-shared alleles between the two breeds. Twenty-six alleles and 28 alleles were unique to the Tswana and Tuli breeds, respectively. The observed heterozygosity ranged between 0.200 (CSSM66) and 1(BM1818) and between 0.00 (CSSM66) and 1 (BM1818) in Tswana and Tuli cattle, respectively and there was no significant difference in mean observed heterozygosity between Tswana than Tuli cattle (0.6311 vs. 0.555). The PIC values ranged from 0.375 (BM1818) to 0.844 (ETH225) in Tswana cattle and from 0.535 (INRA23) to 0.833 (TGLA227) in Tuli cattle with mean PIC values of 0.6355 and 0.7156 in Tswana and Tuli cattle, respectively. The within population inbreeding coefficient were 0.20 and 0.332 in Tswana and Tuli cattle, respectively. The Tswana cattle population was not in Hardy-Weinberg equilibrium at CSSRM60 and CSSM66 marker loci while the Tuli cattle population was not in Hardy-Weinberg equilibrium at ETH10, ETH225 and CSSM66 marker loci while the rest of the markers were in Hardy-Weinberg equilibrium in the two breeds. The degree of genetic differentiation (Fst) value between Tswana and Tuli cattle was 0.0676

indicating that approximately 6.8% of the total genetic variation corresponded to differences between the two breeds, while the remaining 93.2% corresponded to differences among individuals. The genetic identity between the two breeds was 56% and there were no significant differences in allelic diversity and in genetic diversity between the Tswana and Tuli cattle breeds. The use of Tswana and Tuli breeds in a crossbreeding program is likely to result in minimal heterosis and therefore not recommended.

Key words: Inbreeding, Genetic diversity, Microsatellite markers, Tswana cattle, Tuli cattle

4.1 INTRODUCTION

Indigenous cattle are mostly kept under extensive production systems and play a pivotal role in sustainable livestock production of smallholder farmers in support of food security and livelihoods (Podisi, 2000). Indigenous cattle breeds have evolved under local climatic and production environments and are therefore more adapted to the region's harsh environments than their exotic counterparts (FAO, 2018). Indigenous cattle breeds such as the Tswana and Tuli are components of the beef production systems in Southern Africa (FAO, 2018). Tswana cattle are indigenous to Botswana and Tswana-type cattle can be found in South-western Zimbabwe and in the Northern Cape of Transvaal. The Tuli 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 some 80 years ago (Scholtz, 2010).

Phenotypic similarities exist between the Tswana and Tuli cattle breeds and some notable attributes of the two breeds include hardiness and adaptability, heat tolerance, tick resistance/tolerance, high fertility and longevity and excellent mothering ability (Mpofu, 2002). In addition to the above attributes, the Tuli was selected for milk production, carcass, and meat quality traits. The development of Tuli cattle in isolation from the base population of Tswana-type cattle and intentional selection for traits of economic importance has resulted in unique genetic constitution of the Tuli making the breed very successful in crossbreeding program (Tuli Breeders Society of South Africa, 2016) due to a high degree of heterosis. 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. According to Rehman and Khan, the elucidation of genetic variability and genetic relationships among breeds has direct relevance with issues of sustainable use of domestic animal genetic

resources. Microsatellite markers and SNPs have become markers of choice in genetic characterization of cattle breeds and in the estimation of genetic diversity and genetic relationships among cattle breeds (Rehman and Khan, 2009). The current study was undertaken to assess the genetic diversity, the level of genetic differentiation and genetic identity between the Tswana and Tuli cattle breeds of Southern Africa.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Animals

Twenty-six unrelated Tswana cattle and 25 unrelated Tuli cattle kept at BUAN farm participated in the study. The BUAN Tswana cattle was assembled in 1989 from animals coming from various parts of the country in an effort to conserve the breed and safeguard its genetic purity from indiscriminate crossbreeding with exotic cattle breeds. The BUAN Tswana cattle herd has remained a closed population since 1989.

In 1990 DAR donated Tuli cattle to BUAN for research purposes. The herd was been kept closed since 1990. Twenty-five (25) of the above mentioned Tuli herd was sample and used in the study.

4.2.2 Sample Collection and DNA Extraction and quantitation

Blood samples were collected from 26 randomly selected purebred Tswana cattle and 25 purebred Tuli cattle kept at BUAN farm in EDTA tubes. The age of the sampled animals ranged between 2 years and 8 years of age in both breeds. 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).

4.2.3 Microsatellite Markers Amplification and Analysis

A panel of 12 bovine microsatellite markers recommended for estimating genetic diversity in cattle by the ISAG and FAO advisory board (FAO, 2011) were used to amplify specific regions of gDNA of Tswana and Tuli cattle breeds (Table 3.2.3). The microsatellite markers, their chromosomal positions, size range and primers used for their amplification are shown in table 3.2.3. 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 $Mgcl_2$ 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 PCR conditions- initial denaturation at 98°C for 60 seconds, followed by 30 cycles of 98°C for 20s, annealing temperature of 60°C for 75s and DNA extension at 72°C for 30s, followed by final extension step at 72°C 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 95°C 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.

4.2.4 Statistical Analysis of Data

The MS toolkit software (Kim, 2002) 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.

4.3 RESULTS AND DISCUSSION

4.3.1 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 breeds, respectively (Table 4.3.1). The number of alleles per locus ranged between two (BM1818) and 10 (TGLA227) in Tswana cattle and between three (BM1818 and ILST006) and 10 (TGLA227) in Tuli cattle. The mean number of alleles (MNA) per locus were 6.25 and 6.43 for the Tswana and Tuli cattle, respectively. There was no significant difference in allelic diversity between Tswana and Tuli cattle breeds (P=0.678). The MNA per locus found in the two breeds is like those of Portuguese native cattle (MNA=6.7), Hallikar cattle breed of India (6.368), South African Nguni cattle (MNA=6.47) and South-western European cattle breeds (MNA=6.5) (Ginja *et. al.*, 2010; Kumar *et. al.*, 2005; Saranana, 2015; Beja-Pereira *et. al.*, 2003). The MNA per locus in Tswana and Tuli cattle breeds were however lower than those of Pakistani cattle breeds (MNA=22.67) and Bulgarian grey cattle (MNA=7.6) (Hussain *et. al.*, 2016; Teneva *et. al.*, 2005).

Table 4.3.1: Alleles (bps) in the 12 microsatellite markers analysed in Tswana and Tuli cattle breeds at BUAN farm

Locus	Breed	Observed Allele	N	Shared alleles
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	
	Tuli	140, 154, 159, 178, 180, 182, 188, 192	8	3
CSRM60	Tswana	92, 96, 100, 102, 110, 114	6	
	Tuli	92, 94, 96, 98, 100, 102, 110	7	5
CSSM66	Tswana	179, 181, 183, 187, 195	5	
	Tuli	179, 185, 193	3	1
ILST006	Tswana	286, 294, 296	3	
	Tuli	286, 290, 294, 296, 298, 300	6	3
Total	Tswana		75	
	Tuli		77	49
Mean (MNA)	Tswana		6.25	
	Tuli		6.43	4.08

Microsatellite marker loci ETH225, ETH10, BM2113, CSRM60 and BM1824 were highly polymorphic in Tswana and Tuli cattle with a total of 6-9 alleles per marker in both breeds. According to FA0 2018, microsatellite loci must have at least four alleles to be considered useful for evaluation of genetic diversity. One hundred and three unique alleles were genotyped across the two breeds with a total of 49-shared alleles between the two breeds and 26 alleles and 28 alleles were unique to the Tswana and Tuli cattle breeds, respectively. The number of shared alleles between the two breeds ranged between one (BM1818 and CSRM60) and 8 (TGLA227 and BM2113) with mean number of shared alleles across the 12 loci of 4.08. The mean number of shared alleles between Tswana and Tuli cattle breeds (4.08) is similar to the mean number of shared alleles between Fuga and Butana (4.4) and between the Butana and Kenana (4.0) Sudanese Zebu cattle breeds (Hussain *et. al.*, 2016).

4.3.2 Genetic Diversity of Tswana and Tuli cattle

The observed heterozygosity (Ho) ranged between 0.200 (CSSM66) and one (BM1818) and between 0.00 (CSSM66) and one (BM1818) in Tswana and Tuli cattle, respectively (Table 4.3.2). The mean Ho across all the 12 loci were 0.6311 and 0.555 in Tswana and Tuli cattle, respectively. There was no significant difference in mean Ho between Tswana and Tuli cattle breeds (P=0.451). The expected heterozygosity (He) which is considered a more accurate measure of genetic or gene diversity ranged between 0.455 (INRA23) and one (BM1818) in Tswana cattle and between 0.664 (INRA23) and 0.899 (ILST006) in Tuli cattle. There was no significant difference (P=0.617) in mean He between Tswana and Tuli cattle breeds (0.790 vs. 0.813, respectively).

The mean He of both Tswana and Tuli cattle breeds are consistent with those of the Hallikar breed of India (He=0.785), the Nguni cattle breed of South Africa (He=0.701), Pinzgauer (He=0.71), Vosges (He=0.68) and Simmental (He=0.58) (Kumar *et. al.*, 2005: Sanarana, 2015; Edwards, 2000). The mean He of both Tswana and Tuli cattle breeds found in the current study indicate that considerable genetic variation still exist in Tswana and Tuli cattle breeds which could be exploited in selection programmes to bring about improvements in traits of

economic importance. The existing genetic variation in Tswana and Tuli cattle dictates that appropriate management programs be implemented to ensure that the existing gene pool is not lost to inbreeding or uncontrolled crossbreeding (Delgado *et. al.*, 2011). Mean He values were higher than mean Ho values in both breeds indicating a deficiency of heterozygotes in the sampled populations probably due to inbreeding which increases the number of homozygotes at the expense of heterozygotes in the population.

Table 4.3.2: 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
Mean	0.6311±0.091	0.5555±0.021	0.7895±0.033	0.8123±0.033	0.6355±0.013	0.7156±0.005

The PIC values ranged between 0.375 (BM1818) and 0.844 (ETH225) in Tswana cattle and between 0.535 (INTRA23) and 0.833 (TGLA227) in Tuli cattle with mean PIC values of 0.6355 and 0.7156 in Tswana and Tuli cattle, respectively. There was no significant difference in mean PIC values of Tswana and Tuli cattle breeds (P=0.367). The mean PIC values of both Tswana cattle and Tuli cattle breeds are similar to those of Fuga (0.664) and Butana (0.630) Sudanese Zebu breeds, South African Nguni cattle breed (0.655) and Hariana (0.749) and Hissar (0.712) cattle breeds of Pakistan (Hussein *et. al.*, 2016, Sanarana, 2015; Rehman and Khan, 2009). According to Montenegro *et al.* (2015), markers with PIC values greater than 0.5 are highly informative, those with PIC values between 0.25 and 0.5 are moderately informative

and those with PIC values less than 0.25 are less informative. Based on this criterion, all the 12 microsatellite markers were therefore highly informative in Tuli cattle and all the markers with the exception of BM1818 and INTRA23 were highly informative in Tswana cattle and therefore useful for the assessment of genetic diversity in the two breeds. BM1818 and INRA23 markers were moderately informative in Tswana cattle.

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

The within-population inbreeding estimate (Fis) of both Tswana and Tuli cattle were significantly positive (Table 4.3.3). The Fis estimates ranged between -0.054 (TGLA227) and 0.769 (CSSM66) in Tswana cattle and between -0.2 (BM1818) and 1.00 (CSSM66) in Tuli cattle. The within population inbreeding coefficient were 0.20 and 0.332 in Tswana and Tuli cattle, respectively, indicating 20% and 33% shortfall of heterozygotes in the two breeds, respectively.

Table 4.3.3: Inbreeding and test for Hardy-Weinberg Equilibrium in Tswana and Tuli cattle breeds at BUAN farm

Locus	Fis		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	-0.2	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
Mean	0.200±0.002	0.332±0.0001		

The within breed inbreeding coefficient of Tswana cattle (Fis=0.200) is similar to the inbreeding coefficient of Indian Bachaur cattle breed (Fis=0.22) while that of the Tuli cattle (Fis=0.332) is similar to that of Ongole cattle breed (Fis=0.36) (Sharma *et. al.*, 2006; Metta, 2004). Contrary to our current findings, Hussein *et al.* (2014) reported negative within population inbreeding coefficients in Butana (Fis=-0.830), Kenana (Fis=-0.195) and Fuga (Fis=-0.091) Sudanese cattle breeds indicating an excess of heterozygotes in the three Sudanese

Zebu breeds because of outbreeding. Inbreeding likely contributed to the heterozygotes deficiency in Tswana and Tuli cattle breeds emanating from the confinement of the two breeds to BUAN farm since 1989 and the use of a limited number of breeding bulls. Other factors that contribute to deficiency of heterozygotes and excess of homozygotes include assortative mating, linkage with loci under selection and the presence of null alleles (Rehman and Khan, 2009). Higher inbreeding coefficient in Tuli than Tswana cattle (0.332 vs 0.20) is likely 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 (2004) the high inbreeding coefficient of the Tuli breed could also be due to the fact that 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 (Mpofu, 2004).

The Hardy-Weinberg equilibrium test is used to assess the genetic stability of the population. The Tswana cattle population was not in Hardy-Weinberg equilibrium at CSSRM60 and CSSM66 marker loci while the Tuli cattle population was not in Hardy-Weinberg equilibrium at ETH10, ETH225 and CSSM66 marker loci while the rest of the markers were in Hardy-Weinberg equilibrium in the two breeds. The deviation of some markers from Hardy-Weinberg equilibrium might be due to several causes including selection, genetic drift and small sample size and problems with genotyping (Kumar *et al.*, 2005). Significant departure from Hardy-Weinberg equilibrium of CSSM66 marker in both Tswana and Tuli cattle could be due to the effects of natural selection with respect to the two markers. QTL and functional genes for milk production, milk composition and growth traits have been mapped to BTA 14 where CSSM66 marker is also located (Wibobo *et. al.*, 2008). Natural selection for low milk production and slow growth rate in Tswana and Tuli cattle over centuries might have eliminated some alleles of respective functional genes and left currently existing alleles which might be in linkage disequilibrium with alleles at CSSM66 locus (always inherited together) resulting in non-

random mating with respect to that marker locus and significant departure from Hardy-Weinberg equilibrium.

Significant departures from Hardy-Weinberg equilibrium of ETH10 and ETH225 markers only in Tuli cattle might be attributed to the effects of selection. QTLs for growth and carcass traits have been mapped to BTA5 where ETH10 marker is also found and QTLs for feed efficiency have been mapped to BTA9 where ETH225 marker is also found (McNeil and Grosz, 2002; Pereira *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 (Linkage disequilibrium). The results observed significantly deviates from hardy-Weinberg equilibrium. Natural selection for tick resistance/tolerance in Tswana cattle could also have resulted in non-random mating with respect to CSRM60 marker (QTL for tick resistance/tolerance and CSRM60 marker found on BTA 10 and possibly in linkage disequilibrium). Thus, resulting in the observed significant departure of CSSRM marker from Hardy-Weinberg equilibrium in Tswana cattle (McNeil and Grosz, 2002; Pereira *et al.*, 2005)

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4.3.5 Genetic relationship between breeds

The genetic identity between Tswana and Tuli cattle breeds was 0.564 indicating that there is 56% genetic similarity between the two breeds. Both the Tswana and Tuli cattle breeds are classified into the Sanga breed group and the high genetic identity between the two breeds likely indicate that the two breeds share 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 (Fst) value between Tswana and Tuli cattle was 0.0676 indicating that approximately 6.8% of the total

genetic variation corresponded to differences between the two breeds, while the remaining 93.2% corresponded to differences among individuals.

According to Hussain *et al.* (2016) it is generally accepted that Fst value ranging from 0 to 0.05 indicates low genetic differentiation; a value ranging between 0.05 and 0.15, medium differentiation; a value ranging between 0.15 and 0.25, significant differentiation; and a value above 0.25, highly significant genetic differentiation. Applying the same classification criteria, there is therefore moderate or medium genetic differentiation between Tswana and Tuli cattle breeds which is in agreement with the high genetic identity between the two breeds and supports the hypothesis of closer evolutionary history or common origin of the two breeds. Medium genetic differentiation has also been reported among 12 African *Bos indicus* and *Bos taurus* cattle breeds, among Sudanese Zebu cattle breeds, among Latin-American Creole cattle breeds and among European cattle breeds (Ibeagha-Awemu and Erhardt, 2005; Hussain *et. al.*, 2016; Delgado *et. al.*, 2011; Loftus *et. al.*, 1994). The moderate genetic differentiation between Tswana and Tuli cattle breeds is higher than the genetic differentiation (Fst=0.016) among Red Bororo and White Fulani cattle breeds of Nigeria and Cameroon (Ibeagha-Awemu and Erhardt, 2006). The moderate genetic differentiation between Tswana and Tuli cattle breeds (Fst=0.0676) may indicate the presence of gene flow between the two breeds and /or the presence of gene flow between the two breeds may be due to their common origin (Hussain et al., 2016, Loftus *et al.*, 1994).

4.4 CONCLUSIONS

Both Tuli and Tswana have high levels of genetic diversity as individual breeds and there is no significant difference in their levels of genetic diversity. There is 56% genetic identity between Tswana and Tuli cattle breeds

4.5 RECOMMENDATION

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.

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CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATIONS

The markers used in the study are highly polymorphic therefore they are recommended for genetic characterization of both the Tswana cattle and Tuli cattle breeds. The genetic parameters that were used to analyse the Tswana Breed in the study are high indicating high levels of genetic diversity even though the inbreeding level was high. Strict breeding mechanisms should be established in order to maintain the high level of genetic diversity and the vast gene pool of Tswana cattle while minimizing the negative effects of inbreeding on fitness and production traits in the BUAN conservation herd. It will also be worthwhile to do a similar study on communal Tswana Herds and use these results as a reference to estimate the extent of admixture of the communal herds with exotic breeds which is rampant under communal grazing system. Further studies should be carried out to unravel the genetic architecture of valuable traits in Tswana cattle such as tick resistance and heat tolerance using DNA sequencing technology and single nucleotides polymorphisms. In addition to phylogenetic distances between-populations, genetic variability and gene losses should be considered when breeds are selected for conservation purposes. Not only purebreds are important but representative populations from whole breed groups should be conserved in order to maximise the amount of genetic diversity in the conserved breed.

The mean values Heterozygocity, Homozygocity, Polymophic Index Content, which are used to measure genetic diversity calculated from the microsatellite data were similar between the Tswana and Tuli cattle breeds. This is consistent with observed phenotypic similarities between the two breeds and the common origins of the two breeds and their geographical distribution along the Botswana-Zimbabwean, hence the genetic similarities. The 6% genetic differentiation between the two breeds also indicate moderate genetic differentiation between

the two breeds resulting from the relative isolation of the Tuli during its development about 70 years ago and artificial selection for carcass and meat quality traits and milk production. The 56% genetic identity between the two breeds also attest to their genetic similarities and their common origins. Thus the modern practice of crossing Tswana and Tuli cattle breeds does not yield any significant benefit in terms heterosis and should therefore be discouraged in favour of pure breeding of the two breeds.

5.1 Conclusions

The BUAN Tswana cattle herd exhibits high level of genetic variability which can be explored in future for improving performance in various traits of economic importance.

Both Tuli and Tswana have high levels of genetic diversity as individual breeds and there is no significant difference in their levels of genetic diversity.

There is 56% genetic identity between the Tswana and Tuli cattle breeds. Therefore crossbreeding the two breeds does not benefit from heterosis. Farmers are advised not to cross the two breeds for purposes of improving genetic performance on economic traits.

5.2 Recommendations

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. The genetic diversity found in the BUAN Tswana cattle calls for continued preservation of the herd/breed for future exploitation in breeding programmes.