BREEDING ADMIXTURE OF CATTLE POPULATIONS KEPT IN THE THABO MOFUTSANYANE DISTRICT

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BREEDING ADMIXTURE OF CATTLE POPULATIONS KEPT IN THE THABO MOFUTSANYANE DISTRICT

by

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August

DECLARATION

I, Nompilo Lucia Hlongwane, student number----------, declare that the dissertation: *Breeding admixture of cattle populations kept in the Thabo Mofutsanyane District*, submitted to the Central University of Technology, Free State for the Magister Technologies: AGRICULTURE is my own independent work and that all the sources used and quoted have been acknowledged by means of complete references; and complies with the code of academic integrity, as well as other relevant policies, procedures, rules and regulation of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person in fulfillment (or partial fulfillment) of the requirements for the attainment of any qualification. I also disclaim the *copyright* of this dissertation in favour of the Central University of Technology, Free State.

Nompilo L Hlongwane DATE

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LIST OF ACRONYMS AND ABBREVIATIONS

N	Alleles
AFLP	Amplified Fragment Length Polymorphism
AD	Anno Domini
BC	Before Christ
AnGR	Animal Genetic Resources
AMOVA	Analysis of Molecular Variance
ARC	Agricultural Research Council
Bp	Base pair
°C	Degree Celsius
DAFF	Department of Agriculture, Forestry and Fisheries
dNTP	Deoxynucleotide
DNA	Deoxyribonucleic acid
ΔK	Delta
H_e	Expected heterozygosity
FAnGR	Farm Animal Genetic Resources
FAO	Food and Agriculture Organization
G	Gauge
F_{ST}	Genetic differentiation
F_{IT}	Global inbreeding coefficient
HWE	Hardy Weinberg Equilibrium
ISAG	International Society for Animal Genetics
Kg	Kilogram

F	Inbreeding coefficient
LFA	Least Frequent Allele
$MgCl_2$	Magnesium chloride
MCMC	Markov Chain Monte Carlo
MNA	Mean Number of Alleles
µL	Microlitre
mM	Millimolar
Min	Minutes
MtDNA	Mitochondrial DNA
MFA	Most frequent allele
D_A	Nei's genetic distance
K	Number of clusters
H_O	Observed heterozygosity
%	Percentage
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
F_{IS}	Population inbreeding coefficient
KCl	Potassium chloride
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
Sec	Seconds
SSR	Simple Sequence Repeats
SNP	Single Nucleotide Polymorphism

SA	South Africa
Tris-HCl	Tris Hydrochloride
U	Units
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

ABSTRACT

The objective of this study was to determine the population structure of the cattle breeds in Thabo Mofutsanyane district using microsatellite markers. Blood samples were collected from three population of Thabo Mofutsanyane district namely: Dihlabeng (n = 32), Phumelela (n = 34) and Maluti-a-Phofung (n = 34). A total of 323 cattle (Nguni, Holstein, Brahman, Hereford, Bonsmara, Afrikaner, Limousin, Jersey, Angus, Drakensberger, Simmentaler and Charolais) were used as reference populations to acquire the population structure. The samples were evaluated for genetic variation, population assignment and genetic distance using 16 different polymorphic microsatellite DNA loci. A high level of genetic variation was observed with a total 139 distinctive alleles detected across the studied populations, with a number of observed alleles ranging from 5 - 17. The average genetic differentiation (F_{ST}) was 0.010, indicating that 0.01 % of genetic diversity can be explained by the genetic differentiation among the populations whereas 99 % can be explained by differences among individual. STRUCTURE analysis revealed no genetic structure between the three populations of Thabo Mofutsanyane. Cattle breeds showed a high admixture population in Dihlabeng with breeds such as Bonsmara (18 %), Simmentaler (14 %), Brahman (12 %) and Drakensberger (11 %) while for Phumelela it was breeds such as Bonsmara (24 %), Charolais (11 %), Brahman, Drakensberger and Simmentaler at 10 %. For Maluti-a-Phofung, breeds such as Charolais (15 %), Bonsmara (14 %), Limousin (13 %) and Drakensberger (11 %) contributed on the observed population admixture. The results presented in this study showed a high level of admixture among the cattle breeds in and can be used as a base line for the development of breeding program in Thabo Mofutsanyane.

CHAPTER 1: BACKGROUND OF THE STUDY

1.1 General Introduction

South Africa has its own unique cattle genetic resources of indigenous and locally developed breeds such as Afrikaner, Bonsmara, Drakensberger and Nguni (Scholtz, 2010). These breeds are highly adaptable to poor quality grazing forages, excessive heat and humidity conditions, resistance to tick-borne diseases (Muchenje *et al*., 2008; Marafu *et al*., 2009). Local cattle breeds have shown to have a huge prospective to produce high quality beef without widespread use of chemicals, acaricides, growth promotants and synthetic feeds (Wollny, 2003; Mapiye *et al*., 2009). Nerpo (2000) hinted that indigenous cattle to be an untapped resource with a potential to increase local and export beef and hides supply. There is a perception that indigenous breeds are inferior because of their small frame as compared to the exotic breeds (Bester *et al*., 2003). However, results from studies conducted by Strydom *et al*. (2000) showed that meat quality produced by indigenous breeds is comparable with that of imported breeds.

In communal areas, cattle are used as the source of food security and a means of storing savings as well as a reliable source of farm incomes. Thus these breeds provide suitable genetic resources to communal farming due to low levels of management as well as high potential to increase sustainability. These have the ability to grow and reproduce under low input system (Scholtz, 1988; Schoeman, 1989). Notably, local livestock remain valued reservoirs of genetic material for adaptive and monetary qualities given the expanded genetic pool that can come as an aid for upcoming tasks (FAO, 2007). Thus indigenous provide a great potential to increase sustainability and improve livelihoods of rural farmers (Anderson, 2003).

At least 1000 breeds have been exposed to the risk of extinction through the introduction of exotic breeds (FAO, 2007). This is because exotic breeds are larger than indigenous cattle breeds and present grading system is biased towards small-framed animals (FAO, 2007). About 16% of livestock are already extinct and it is highly estimated that they were not characterised (FAO, 2007). In communal areas of Southern Africa, indigenous cattle diversity is shrinking with rapid and uncontrolled loss of unique genetic resource due to indiscriminate crossbreeding. Thus this calls for genetic characterization of communal animal as the first step toward understanding their genetic constitute. The use of new biotechnologies may attempt to improve indigenous

breeds as loss of diversity is forever and it is not possible to replace lost diversity. Biotechnology offers the opportunity to better characterize, utilize and access animal genetic resources for food production.

Food and Agriculture Organization (FAO) proposed a program aimed at managing livestock by means of molecular approach for breed characterization (Bjornstad *et al*., 2000). They recommended an international plan intended for administering of genetic resources by means of molecular approach for breed characterisation (Bjornstad & Roed, 2001). Microsatellite markers has been used to trace ancestries so that animals are identified properly, predicting population history as well as calculating inbreeding coefficients among other things. For example Swart *et al*., 2010; Mtileni *et al*., 2011; Soma *et al*., 2012; Qwabe *et al*., 2013; Pienaar *et al*., 2014 have used microsatellite markers in South Africa to study the genetic structure of pigs, chickens, sheep and cattle respectively.

The use of molecular characterization to determine cattle breeds or population's structure in Thabo Mofutsanyane district communal areas will contribute information towards the level of genetic diversity and inbreeding within and between cattle breeds. Characterizing these breeds will assist with conservation and utilisation of indigenous

livestock. Such characterization would provide database information of unknown cattle breeds (FAO, 2000).

1.2 Motivation of the study

Non-descript breed types are caused by the widespread of uncontrolled breeding structures that takes place in communal areas. Breeding programs are a major challenge for small farmers who keep relatively small numbers of local breeds in developing countries. The determination to study herd structure in livestock species from the villages will led to awareness to offer substance meant for maintaining these potential valuable germplasms. The prolong introduction of exotic breeds from industrialised countries is a substantial risk to indigenous breeds in the developing nations. Conservation of these treasured germplasms has been suggested as a way of reducing the loss in livestock diversity over extinction. Sustainable use as well as conservation of genetic diversity is the pillar and strength of viable food preservation. Indigenous cattle breeds need to be conserved as they have an impact on the livelihood of rural people, currently and in future. However it is essential that the population in question be characterized in order to make informed decision with regard to their conservation.

Based on the national survey of an unpublished study conducted by DAFF (unpublished) in Thabo Mofutsanyane district in 2011, it was witnessed that most cattle farmers did not know the breed type of their animals. This was also confirmed by the survey conducted by Motiang and Webb (2014) where they found 35 % of beef cattle being identified as non-descript breeds. Therefore this aimed at genetic characterisation of communal cattle in Thabo Mofutsanyane district.

1.3 Problem statement

There are a significant number (about 30%) of unknown cattle breeds (FAO, 2007b), which are mostly found in communal areas of sub-Saharan Africa. This limit the ability to implement genetic improvement programs in communal areas. The difficulties of accurate recording of cattle breeds are due to the loss of the unique modified stock from the effect of random crossbreeding with unsuitable high upkeep breeds. Exotic breeds have a tendency to not being accustomed to survive and produce in harsh surroundings of the communal set up for underprivileged farmers. These characters consist of tolerance of stressors for instance ticks plus tick-borne illnesses, high temperature, famine and reduced foraging. Moreover, monetary constraints have enforced farmers to purchase cattle as slaughter animals as they no longer have any worth as breeding stock.

Widespread use of cross breeding, destruction of traditional systems and a general thrust towards management systems on greater inputs place certain important gene pool under threat. The introduction of composite cattle breeds into undeveloped structures remains as a severe deprivation of the genetic reserves. Increase threat of indigenous cattle breeds due to gene dilution and indiscriminate cross breeding in communal areas requires a major step so that potential value of local cattle breeds are analysed and conserved. This study thus, emphases the need for genetic characterization on South African cattle population as the first step towards their proper management. Information generated from this study could assist with implementing genetic improvement and conservation programs for the South African livestock breeds.

1.4 Main objective of the study:

The main objective of the study is to determine the breeding structure of cattle breeds in Thabo Mofutsanyane using microsatellite markers.

1.4.1 Specific objectives are:

- To assess the level of genetic variation and inbreeding of Thabo Mofutsanyane cattle population using autosomal microsatellite markers
- To determine population structure in Thabo Mofutsanyane cattle population using reference data set consisting of other South African purebred commercial lines

1.5 Hypothesis

- Molecular characterization will establish the diversity among and within the indigenous cattle populations.
- Determining the breeding status of the South African cattle will serve as an indicator of any treat to their becoming extinct livestock species.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Extensive cattle management is mainly practised in the communal areas. Communal cattle farming system is different from the commercial system where herds are relatively well characterised (Scholtz *et al*., 1999); valid comparative data is available (Corbet *et al*., 2000) and have links to processors and export market (Delgado, 1999). A broad base of cattle breeds exist in South Africa, which consist of, adapted local breeds and exotic breeds introduced by immigrant's centuries ago. These breeds have evolved through human and natural selection over the years. They have been chosen to fit because of their extensive variety to be able to adapt in severe environmental conditions.

In terms of cultural, social and economic development, these indigenous cattle genotypes play a major role. This includes livestock for their household's milk, meat, hides, horn and income (Chimnyo *et al*., 1999; Simela *et al*., 2006). An introduction of exotic breeds that threatened the status of indigenous breeds has seen the gradual vanishing of native breeds that are accomplished of enduring in extreme surroundings. This destabilizes food and source of revenue safety for the underprivileged while

diminishing the ability of societies to be able to live in rural regions. Haphazard crossbreeding amongst exotic and indigenous breeds have also been attributed to the losses and threat of the indigenous breeds. This leads to the loss of adaptable genetic biodiversity. Clemens (1995) pointed out that absence of proper selection structure lead to breeding strategies with exotic breeds causing indigenous breed replacement. Indigenous breeds possess significance qualities for many reasons but mainly they have acquired distinctive mixture of adaptive features to survive in unforgiving situations (Buduram, 2004).

Efforts to improve livestock production in the communal areas have been based on the policies and development on the use of fast growing imported breeds (Bester *et al*., 2005; Muchenje *et al*., 2008). Since exotic breeds are of large body frame, they are perceived to be superior to local breeds (Bester *et al*., 2005). Exotic breeds are failing to adapt to the harsh environmental and economic circumstances widespread in the communal areas where among other constraints, feed is scarce, level of management is low and disease is extensive (Scholtz, 1988; Collins-Luswet, 2000). These animals need special and often expensive feedstuff, wait to be fed rather than grazing on their own. As a result farmers keeping these imported breeds are likely to acquire additional production expenses. In communal areas, indigenous breeds form the backbone of

relevant and sustainable livestock production compared to their exotic counterparts as genetic characteristics allows them to survive in harsh environments. Therefore, maintenance of genetic diversity is a prerequisite for genetic improvement and conservation of indigenous cattle breeds. The objective of this chapter is to provide an overview on important characteristics of the South Africa cattle breeds, Farm Animal Genetic Resources (FAnGR) conservation and application of genetic markers as well as the importance of performing genetic characterisation.

2.2 South African indigenous cattle breeds

The South African cattle population arise from three types of population; *Bos taurus* (taurine) and *Bos indicus* (Zebu) that are morphologically distinguished by the presence (*Bos indicus*) or absence (*Bos taurus*) of a hump and the Sanga type which are characteristically crescent-shaped horned cervico-thoracic-humped cattle (Mason & Maule, 1960) (Table 2.1). The Sanga is possibly developed from an original cross between the Zebu and humpless cattle (*Bos taurus*).

Table 2.1 Classification of beef breeds in South Africa

Type	Breeds
Bos *taurus* (British)	Angus, Dexter, Hereford, Red Poll, Beef Shorthorn, South Devon, Sussex
Bos *taurus* (European)	Charolais, Braunvieh, Gelbvieh, Limousin, Pinzgauer, Romagnola, Simmentaler
Bos *taurus* (Sanga)	Afrikaner, Ankole, Drakensberger, Nguni, Tuli
Bos *indicus* (Zebu)	Brahman (Grey and Red) Gyr, Guzerat, Nelore and others
Synthetic breeds	Afrigus (Afrikaner x Angus), Afrisim (Afrikaner x Simmentaler), Beefmaster (Brahman x British/European), Bonsmara (Afrikaner x Hereford x Shorthorn), Braford (Brahman x Hereford), Brangus (Brahman x Angus), Hugenoot (Afrikane x Charolais), PinZ2yl (Pinzgauer x Nguni), Sanganer (Nguni x Afrikaner), Santa Getrudis (Brahman x Shorthorn and Hereford), Senepol (N'dama x Red Poll), and Simbra (Simmentaler x Brahman)
Others	Boran (Bos *taurus* and Bos *indicus*, Eastern Africa), Wagu (Bos *taurus*, Japan)

Source: (Bergh, 2013)

The hostile surroundings and ecological constraints, such as Tsetse fly and East coast fever gave warranted the animals to develop well-defined anatomical and physiological adaptation to the harsh environment (Strydom, 2008). Specific development 6000 years BC and association with man, expansion and migration is believed to have established indigenous cattle breeds that are currently found in the continent of Africa (Bachmann, 1983). The Europeans introduced Sanga cattle in this area in the period of the 15th century (Bachman, 1983). Through, the 1970's, the indigenous breeds were destroyed,

because of ruling from the government who considered these breeds to be substandard (Bester *et al*., 2001). Furthermore, the misguided opinion of the indigenous breeds being low-grade by communal communities has led to erosion of their genetic pool because of being replaced and crossbred with exotic types (Strydom, 2008).

Indigenous breeds are established in a natural selection method in a highly contesting surroundings that has the genetic prospective to be accomplished in ideal production settings (Bester *et al*., 2001). Evidence shows that by improving the efficiency on non-commercial farmers, levels of poverty can be reduced (Hazell *et al*., 2007). Indigenous livestock is the pillar and strength of communal areas in South Africa as it provides sustainable livestock under harsh and unfavourable conditions. Communal farmers depend on their livestock for their household's milk, meat, hides, horn and income (Chimonyo *et al*., 1999; Simela *et al*., 2006). Cattle are a good source of dung for manure, fuel and floor polish, and they assist for crops cultivation (Shackleton *et al*., 1999). Livestock serves as a free form of banking. Cattle can also be sold to assist the family's financial needs such as school fees, medical bills and household expenses (Dovie *et al*., 2006; Simela *et al*., 2006). Farming with cattle also benefits the community with employment and some farmers may keep their animals for status and reputation (Shackleton *et al*., 1999).

A variety of indigenous cattle breeds exist in South Africa. The Afrikaner cattle are a typical *Bos indicus* purported as one of the most important indigenous breeds. Jan van Riebeeck (1652) first encountered this breed shortly after he had arrived in the Cape in (Campher *et al*., 1998; Scholtz *et al*., 1999). Little is known about the breed even though the theory is that the Afrikaner originated from Asia cattle of that time and had a Zebu descended. The Afrikaner is best adapted to arid conditions, extreme heat, and tropical diseases in addition to internal and external parasites (De Kock, 2004). It was around 1912 that South African farmers began to understand this small-to-medium frame sized breed with its longer legs, muscular back, loin, rump and thigh.

Early records of the breeds also mentioned draught power, stamina, speed and hardiness (De Kock, 2004). It is furthermore well adapted to the South African climatic conditions that come across under extensive farming being yellow to red in colour with lateral horns. A mature bull weighs 820 to 1 090 kg while a cow weighs 550 to 730 kg. The expected heifer and bull calves weight is 195 kg and 210 kg, respectively. The meat is of high quality as it is tender, tasty and succulent. It is one of the few breeds that can be finished for marketing in a very short time with the most desirable age and carcass mass ranges. Excellent tough leather is also produced from its thick hide.

The Bonsmara breed came about through research and improvement trials at Mara and Messina Research stations in Limpopo Province. The breed was named after Professor J.C. Bonsma and the Mara Research Station. The breed was developed for the subtropical and humid areas of South Africa to be able to survive and produce efficiently in the subtropical savannah regions of the former Transvaal and Natal (Campher *et al*., 1998). At that time, the Afrikaner struggled to reach the desired growth potential and the cows did not calve regularly. Various crosses between indigenous and exotic breeds were experimented with until the breeding admixture of 5/8 Afrikaner and 3/8 Shorthorn/Hereford was decided. Through strict selection over 20 years, a superior cattle breed in the Northern Province was established and it performed better than other breed in the bushveld. It is the only breed in the world that boasts genealogy of the first seven generations (Campher *et al*., 1998). Over the years this medium-framed, smooth-coated, heat and tick tolerant breed has distinguished itself as an 'easy care' breed (De Kock, 1998). Its colour is uniform red to brown with a slightly sloping rump and slight cervico-thoracic hump in the bull ensuring a good beef conformation. Live weight of adult male and female indigenous cattle ranges from 544 to 95 kg and 3000 to 700 kg, respectively (Scholtz, 2010). They have excellent meat qualities such as being tender, tasty and succulent. The Bonsmara breed has gained so much positive reputation

throughout South Africa and in some other parts of the world with its gene pool being recorded as one of the largest in the world (De Kock, 1998).

The Nguni cattle breed known for its ability to perfectly adapt to South Africa's natural environment. It is named after the Nguni people who migrated from the North, Central and East Africa with their Sanga cattle between 590 and 700 AD (Campher *et al*., 1998). Qualities such as fertility, low maintenance inputs, ease of calving, adaptability, resistance to internal and external parasites, resistance to tick-borne diseases, potential as a dam line, sustainable economic profitability, good temperament, longevity, browsing, good walking abilities, cost effective beef production and survival under harsh conditions with limited food and water resources are associated with the Nguni cattle. Adult males and females weigh between 500 to 600 kg and 300 to 500 kg respectively and are known as small to medium framed. Their noses are always black-tipped with variety of horn shapes and can be identified by their multi-coloured skin presenting different patterns (white, brown, golden yellow, black, dappled or spotty). About 14 ecotypes of Nguni have been recorded by Bothma (1993). For the emerging farmers who require a reasonably low upkeep as well as high productivity in an animal, the Nguni is well suitable with their managing style and is appreciated for it genetic material (Bester *et al*., 2001). Internationally, the Nguni is drawing interest because of its

features developed through years of natural selection, which include tick resistance, ease of calving and low calf mortality. Nowadays the Nguni breed is popular for beef production as it was originally used as both beef and dairy production. In terms of meat tenderness when compared to Bonsmara, Afrikaner and Brown Swiss breeds, no differences were detected (Strydom, 2008).

The Drakensberger breed are bred and developed in South Africa and known for its remarkable reproductive drive, natural adaptability to a wide range of environmental conditions and tough resilience. For efficient beef production and high financial earnings, it is an ideal mother line breed. Vasco da Gama first recorded in his diary a purchase of a fat black ox from the indigenous people in the Bredasdorp area on the 2nd of December 1497 in exchange of three bracelets (De Kock, 2004). This black in colour cattle breed has a smooth-coated, strikingly long and deep-bodied. The weight of the mature bulls and cows is between 820 to 1 100 kg and 550 to 720 kg with cows remaining in production for as long as 20 years. The Drakensberger enjoys benefits such as adaptability, hardiness and natural resistance against tick-borne diseases compared to other breeds. Its loose skin and short and shiny blue-black hair colour is shown to be resistance to heat (Scholtz, 2010).

2.3 Importance of genetic characterization

Approximately 35 % of mammalian strains are in danger of being extinct and that approximately one breed per week is lost and most of these breeds remain expected to be found in emerging nations (FAO, 2004). According to FAO (2007), 16 % of cattle breeds are considered at risk, 16 % are already extinct and 30 % are unknown. A national survey conducted by Scholtz *et al*. (2008) revealed that 35 % of bulls used in communal /emerging sector are classified as non-descript (Table 2.2). It is thus important to perform genetic characterization as intervention is required to enhance demand by 2025 or else massive importation of livestock products will be needed.

Table 2.2 Dominant beef breeds in the communal and emerging sectors (listed according to the bulls used)

Position	Breed Type	Bulls used (% of total)	% of Herds	% of herds within breeds inherited
1	Non-descript/crossbred	35.0	66.4	--
2	Nguni	22.5	14.2	66.5
3	Brahman	18.2	5.2	72.7
4	Afrikaner	9.9	6.5	42.9
5	Bonsmara	5.1	2.2	57.8
6	Drakensberger	2.8	2.2	59.5
7	Simmentaler	2.1	0.7	18.1
8	Hereford	0.8	0.4	14.3
9	Beefmaster	0.6		33.3
10	Angus	0.6		28.9
	Other Zebu derived types	0.8		
	Other European breed types	0.9		
	Other British breed types	0.4		
	Other Sanga types	0.3		

Source: (Scholtz *et al*., 2008)

Various authors (Kellar & Waller, 2002; FAO, 2007b; Taberlet *et al*., 2008) have reported that loss of sustainability, productiveness, disease resistance as well as the regular incidence of genetic disease ensures undesirable values of genetic erosion and

inbreeding depression. The genetic erosion of a breed that have effects of high degree of inbreeding, genetic distance, introgression and other impacts on the gene pool is also an important indicator of endangerment (Alderson, 2009). It is thus important that comprehensive knowledge of the breed's characteristics is documented in order to effectively manage farm animal genetic resources (Groenveld *et al*., 2010). Performing genetic characterization also ensures that resolutions made on conservation will be knowledgeable and lead to improvement of germplasm management. These include providing reliable information on the geographic distribution, population size, structure, and production environments and within and between breeds genetic diversity.

The unknown livestock population size in addition to extensive uncontrolled breeding practices leading to variable genetics. The damaging effect on genetic development is because of inadequate or no history of record keeping on breeding and other linked activities. Breeding programs are a major challenge for small farmers who keep relatively small numbers of local breeds in developing countries. The effort to study herd structure in livestock species has led to awareness to offer substance meant for maintaining these potential valuable germplasms.

By using livestock technology, this ensures an increase in growth of emerging farmers in the near future. Molecular markers can offer valued evidence on diverse levels as a tool used in evaluating genetic variation and purposes such as structure of animal's populations. The practice of using markers towards promoting the conservation and evaluation of threatened species is strongly emphasized strategy, to determine the genetic status of livestock breeds. Governing and preservation of livestock resources involve consideration of genetic mixture. This is because of complexity of proposing suitable breeding package designed for breeds that have not been characterized genetically. The lack of information on the molecular characterization of cattle breeds of the poor resource cattle farmers hampers the expansion of programs meant for progression of these livestock breeds.

In the past years, development of tools has improved our abilities immensely to be able to characterise breeds. Documenting of phenotypic composition in various strains adapted to diverse surroundings and managing techniques is a simple methodology for characterising animal genetic resources (Taberlet *et al.*, 2011). Genetic markers have performed the most important part in progression of biology. Furthermore in conservation, markers have come to be economical, quicker and contain less invasive sample making it quicker to make decisions (Waples, 1991; Crandall *et al.*, 2000).

Population genetic structures can be assessed using genetic markers for most indigenous breeds since they don't have complete genealogy information. Various DNA markers are appropriate to be used for identification and relationship studies in livestock as described by Teneva *et al*., 2005; Bessa *et al*., 2009; Mukesh *et al*., 2009; Martin-Burriel *et al*., 2011.

In South Africa, Swart *et al*. (2010), Mtileni *et al*. (2011), Soma *et al*. (2012), Qwabe *et al*. (2013) and Pienaar *et al*. (2014) performed genetic characterization on different livestock species using microsatellite markers. Swart *et al*. (2010) found a little differentiation among pig populations of Southern Africa while Mtileni *et al*. (2011) noticed a high within population genetic variation when he evaluated genetic variation within and between the village chicken populations. Soma *et al*. (2012) conducted a genetic analysis of 20 different sheep breeds found in South Africa, population structure results showed a distinction between the fat-tailed indigenous breeds and both the North African/Middle Eastern breeds and the breeds of European origin. Qwabe *et al*. (2013) reported a moderate level of genetic variation when the study was conducted to characterize the Namaqua Afrikaner as a rare breed for conservation as well as a significant differentiation between the three populations was suggested by the population structure results. In both the seed stock and commercial herd of the

Afrikaner cattle breed, genetic variability levels were higher than expected in a study conducted by Pienaar *et al*. (2014).

2.4 Conservation of animal genetic resources

For conservation and utilisation of cattle, it is important to identify the breeds and their population sizes, level of genetic variation and their genetic structure. The FAO refers to conservation as ”human activities including strategies, plans, policies and actions undertaken to ensure that the diversity of farm animal genetic resources is being maintained to contribute to food and agriculture production and productivity, now and in the future” (FAO, 2004). A “Strategic Priority Action Report” initiated by FAO (2002) encourages immediate action for conservation at the country level. Effective conservation of FAnGR can be applied via *in-situ* or *ex-situ* conservation. *In-situ* conservation is the maintenance of live populations of animals in their adaptive environment or as close as possible while *ex-situ* conservation involves the collection and freezing of animal genetic resource in the form of living ova, semen or embryos. Segment of frozen blood or tissues and cryopreservation of germplasm may also be preserved via *ex-situ* (FAO, 2002).

A program for the conservation and development of indigenous livestock has been established by the Department of Agriculture, Forestry and Fisheries together with the Agricultural Research Council (ARC) (Nedambale *et al*., 2010). Research stations and the South African Development Centres are used for the *in-situ* conservation for the maintenance and adaptive management of AnGR (Nedambale *et al*., 2010). The major reasons for conservation of animal genetic resources are financial, scientific, development, sustainability, culture and social are the main motives for conservation of farm animal genetic resources. Conservation aims to sustain utilization, upkeep as well as improvement of genetic resources. The need for conservation comes from the potential rate of decrease of genetic variation (Meuwissen, 1991). Meuwissen (1991) noted that the loss of genetic variation is completely caused by small effective population size and enormous rates of inbreeding.

A relatively narrow genetic base is used to breed commercial livestock over the world due to emphasis being placed on maximum production. Mendelsohn (2003) indicated that for conservation to be effective; the program should prioritise conserving species that genetic basis of the breed. Keeping conservation population of certain breeds may become necessary in the contribution to the possible discovery of previously unknown or unrecognised genes, which could enhance the productivity of existing breeds

(Pieters, 2007). A technique of conserving cattle has been suggested as a way of reducing the loss in livestock diversity over extinction. The conservation of these treasured germplasms has to be considered by means of it being compulsory for upcoming generations. Sustainable use as well as conservation of genetic diversity is the pillar and strength of viable food preservation. This is an expected and justifiable source for production improvement. Cattle breeds in the villages need to be conserved as they have an impact on the livelihood of people, currently and in future. Strategies for conservation and improvements of breeds from the villages includes rapid and dramatic genetic change can be achieved through selection between breeds when there are large differences between breeds to improve traits of economic importance (Simm *et al.*, 1996) but this is not feasible as it will be costly to replace the animals. Breed substitution of exotics for the indigenous breeds and crossbreeding with breeds from temperate regions have also been widely used but have been unsustainable in the long term due to incompatibility of the genotypes (Kosgey *et al.*, 2006). In order for conservation programmes to be successful in the villages it is advisable to involve farmers to back the breeding programme with an effective extension service for maximum effect.

2.5 The history and use of genetic markers

2.5.1 Allozyme markers

Since the 1960s, the most commonly used markers for genetic molecular experiments are allozymes (May *et al.*, 1980; Ryman & Utter, 1987). These markers have been used to measure inbreeding, identifying stock and ancestry studies. Relationships have occurred amongst certain allozyme markers as well as performance characteristics in limited cases (Hallerman *et al.*, 1986; McGoldrick & Hedgecock, 1997). The disadvantages of allozyme include the superiority of tissue sampling necessary and deficiencies in heterozygosity due to null alleles. Moreover, the sequencing of DNA is disguised at the protein level leading to slash visible deviation.

2.5.2 Mitochondrial Deoxyribonucleic Acid

Mitochondrial DNA (MtDNA) is a tiny round particle, compromising of roughly 37 DNA segments. It seems to be mainly received through the mother's ancestry. Great quantities of alleles for each locus can be revealed through mtDNA. Although the Polymorphic Information Content (PIC) amounts are greater for mtDNA molecule points than for allozymes; the limited number of markers available is lesser than highly variable markers such as microsatellites. For genetic studies, mtDNA needs to be measured as a single locus because of its non-Mendelian style of (Avise, 1994). Challenges that occur in other DNA established markers also arise for MtDNA. These challenges are

back mutation, equivalent substitution and the variation at which locations experience change while being compared to other locations in the similar area.

2.5.3 Restriction Fragment Length Polymorphisms

Restriction Fragment Length Polymorphisms (RFLP) use duplicated DNA components as analyses to hybridise DNA and were first developed in 1980 (Bostein *et al.*, 1980; Schimenti, 1998). They were established to visualise the differences of the gene structure centred on usage of bacteriological constraint enzymes that censored chromosome at locations through particular nucleotide structures (Mburu & Hanotte, 2005). Gel electrophoresis is used to isolate the DNA fragments of varied amounts to enable visualisation of the exact DNA sequence and to subsequently identify RFLPs (Drinkwater & Hetzel, 1991; Smith & Smith, 1993; Albert *et al.*, 1994; Bishop *et al.*, 1995). However, the use of RFLPs takes time coupled with its high cost, notwithstanding it being the first marker to be widely used. Interestingly, simple markers have been developed to obviate the limitations of the aforementioned markers.

2.5.4 Random Amplified Polymorphic Deoxyribonucleic Acid

Random Amplified Polymorphic DNA (RAPD) also known as DNA amplification fingerprint was initially defined in 1990 by Williams *et al.* (1990). This technique is based on the use of polymerase chain reaction (PCR) to produce many duplicates of a

targeted genetic structure. Investigation with RAPD markers is fast and not difficult, even though results remain sensitive to the laboratory environments.

2.5.5 Amplified Fragment Length Polymorphism

The Amplified Fragment Length Polymorphism (AFLP) marker came into existence in the mid-1990 and is perceived as an alternative PCR based technique of producing molecular markers. The AFLP marker is a mixture of RFLP and PCR method (Zabeau & Vos, 1993; Vos *et al*., 1995). It is related to RAPD assay in that not any previous information of the arrangement is necessary and it identifies a bigger amount of loci that is RAPD. Garcia-Mas *et al*. (2000) revealed that AFLPs had greater competence in identifying polymorphism than both RAPD and RFLP markers.

2.5.6 Single Nucleotide Polymorphism

Single Nucleotide Polymorphisms (SNPs) are modifications in distinct base pair which possible can lead to change in phenotype. These markers are gaining popularity particularly when animals are when genotyping aimed at thousands of animals (MacEachern *et al*., 2009). It is beneficial to identify the correct population structure for the conservation of animal genetic resources besides minimisation of inbreeding. Breeders can also precisely trace relations among animals making it valuable to

determine which animals to conserve or mate when trying to retain the utmost genetic variation (Oliehoek *et al*., 2006).

SNPs are fundamental unit because they are numerous in the genome, genetically constant and responsive to high-throughput automated analysis (Nielson, 2000; Heaton *et al*., 2001b; Vignal *et al*., 2002). They can be found in both coding and non-coding areas of the genome in addition being present one SNP in each thousand base pair (Stoneking, 2001; Vignal *et al*., 2002). There are over two million of SNPs identified in cattle to date as more remain to be discovered (Simianer, 2007).

2.5.7 Simple Sequence Repeats

The Simple Sequence Repeats (SSR) used for population genomic studies, similarly branded, as microsatellite remain an outstanding source of genetic markers (Zane *et al*., 2002). Most often microsatellites are used (Beja-Pereira *et al*., 2003; Buduram, 2004) since they are highly polymorphic and are commonly thought-out to be unbiased. Microsatellites are made up of elastic DNA, involving a small number of nucleotides which are 2 to 6 base pairs (bp) recurring numerous times in tandem (Litt & Lutty, 1989). These markers are simply enlarged by means of PCR from DNA removed from blood, hair, skin and faeces. Polymorphism can be visualised on a sequence gel and

the ease of use of automated genetic sequencers tolerates high quantity analysis of huge amount of samples (Goldstein *et al*., 1995; Jarne & Lagoda, 1996).

For multiple purpose microsatellites have been demonstrated to be valuable markers and they are used for identification of animals, evaluation of genetic resources, defining pedigrees, disease studies, determining genetic difference within and among breeds, determining population substructure, reconstruction of phylogenetic relationships among populations and historical studies of domestication and migration of breeds because their high abundance in the genome *(*Albert *et al*., 1994; Bishop *et al*., 1994; MacHugh *et al*., 1994; Stein *et al*., 1996*)*. Goldstein and Pollock (1997) noted that SSR markers were effortlessly suitable out of several of other genetic markers. The reason is because of great variation, modification degree and big quantity, scattered all over the genome and impartiality with selection (Boyce *et al*., 1996).

CHAPTER 3: METHODOLOGY

3.1 Study site

The study was conducted in Thabo Mofutsanyane district (Figure 3.1.), located 28.5333° S, 28.8167° E. Thabo Mofutsanyana District Municipality is situated in the north-eastern Free State between the Orange River in the south and the Vaal River in the north. It nestles in the shade of the Maluti Mountains and for the most part borders on Lesotho and KwaZulu-Natal. The district includes the five local municipalities of Dihlabeng, Maluti-a-Phofung, Nketoana, Phumelela, Setsoto as well as the Thabo Mofutsanyana DMA (Golden Gate Highlands National Park). In terms of geographical distribution, the Free State province is centrally located and represents 10.6 % of the land area of the country. Thabo Mofutsanyane district is one of the districts that have high number of cattle of unknown breeds in the communal areas. Three local municipalities of Thabo Mofutsanyane district were included in this study *viz*; Dihlabeng (DIH), Phumelela (PHU) and Maluti-a-Phofung (MAL).

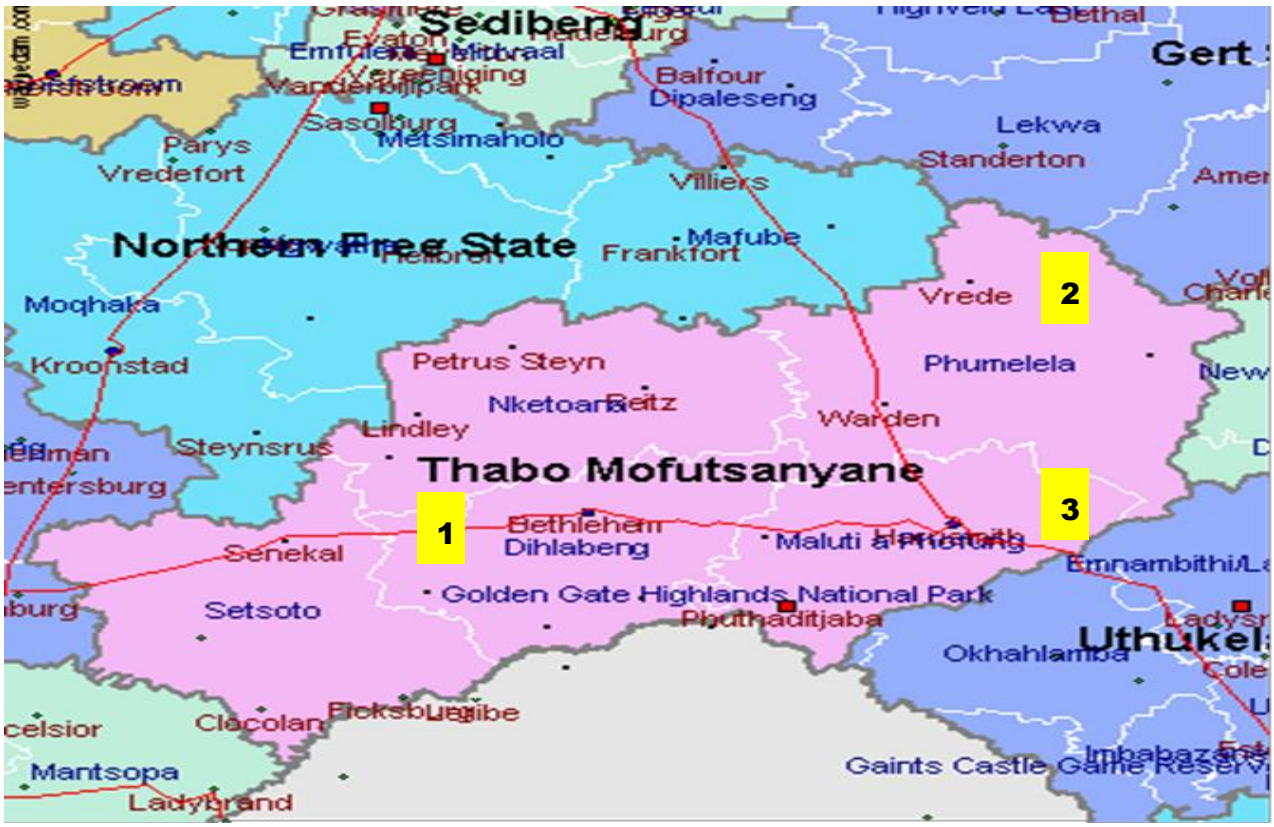

Figure 3.1 Map of Thabo Mofutsanyane district showing the geographical location of three selected municipalities.

3.2 Sampling

As a way of increasing the representativeness and validity of the research findings, 59 farmers were randomly selected, from a total of 419 cattle farmers that were initially interviewed in 2011 at Thabo Mofutsanyane district for animal breed survey. Small-scale and communal farmers were targeted to achieve the set objectives. The samples were representative of different breeds from presumably unrelated individuals of cattle breeds. A total of 100 cattle from three participating populations were sampled (Table

3.1.). Animals were randomly selected from the farmers who participated in the national breed survey. Hundred blood samples from cattle in Thabo Mofutsanyane district of the Free State province were collected from the tail vein using the 16G vacutainer needle. Two cattle per farmer was sampled, a cow and bull. The sampled villages were about 20 – 50 km apart. No full pedigree information was available for any sampled populations; therefore animals with little relations as possible were sampled for further analysis. The sampling process included verbal communication with the different farmers to confirm the purity as well as the representation of the samples within the population.

Table 3.1 Selected areas sampled in Thabo Mofutsanyane district

Municipality	Areas	Number of Blood samples
Dihlabeng	Paul Roux	10
	Rosendal	10
	Clarens	12
Phumelela	Memel	10
	Vrede	12
	Warden	12
Maluti-a-Phofung	Kestelll	10
	Harrismith	12
	Phuthaditjhaba	12

3.3 DNA Isolation and quantification

The DNA sample was extracted from the whole blood using Roche commercial extraction and purification kit. The following DNA extraction procedure used: 20 ml of absolute ethanol was added to inhibitor removal buffer. 80 ml of absolute ethanol was added to washing buffer. 4.5 ml of sterile water was added to Proteinase K. Took 300 µl of whole blood. Added 200 µl of binding buffer and 40 µl of Proteinase K, mixed immediately and incubated for 10 minutes at 70 ° C. Added 100 µl isopropanol, mixed well and applied mixture to a High Pure filter tube, centrifuged for 1 minute at 8,000 x g. Added 500 µl inhibitor removal buffer and centrifuged for 1 minute at 8,000 x g then discarded flow through and collection tube. Added 500 µl wash buffer and centrifuged for 1 minute at 8,000 x g then discarded flow through and collection tube (Repeat this step). Centrifuged for 10 seconds at 13,000 x g then discarded flow through a collection tube. A 50 µl of elution buffer was added into a new tube that was maintained at 70º C. Centrifuged for 1 minute at 8,000 x g. Extracted DNA was stored at -20º C until analysis in the polymerase chain reaction (PCR). The DNA was quantified and qualified through spectrophotometer using NanoDrop® ND-100 (NanoDrop Technologies Inc., Washington, USA). The concentration was measured in ng/ µl. Samples of 20 ng/ µl were used as they are known to give good profile.

3.4 Amplification and Genotyping

The DNA amplification was performed as follows for each sample: 7.5 µL of the total reaction volume containing 6.5 µL of primer cocktail mix which was made of 1.00 µl primer mix (16 microsatellites), 0.40 µL Supertherm Gold Taq DNA polymerase (5 U/ µL), 1.50 µL Supertherm Gold reaction buffer (20 mM Tris-HCl pH 8.3, 15 mM $MgCl_2$, 50 mM KCl), 0.75 µL dNTP's, 0.18 µL Tween and 3.67 µL deionised water together 1 µL of DNA using Perkin Elmer Gene Amp PCR System 9700 thermocycler (Figure 3.3). PCR cycling conditions employed were: Initialization for 10 min at 95 ºC followed by 33 cycles of denaturation for 45 sec at 94 ºC, annealing for 90 sec at 61 ºC, extended for 60 sec at 72 ºC and final extension for 60 min at 72 ºC and cooled to 4 ºC. Compatible multiplexes were pooled and mixed with formamide and Liz^{TM} internal size standard before being denatured for 5 min at 95 ºC. An internal bovine control DNA sample was included in each PCR. The bovine control DNA serves to indicate a problem with the PCR, or within the sample DNA. Thus allowing for the monitoring of the sizing accuracy since it sizing and labelling is known. Genotyping was carried out on an ABI 3130xl Genetic Analyser and allele scoring was performed using Genemapper software version 4.0 (Applied Biosystems, Foster City, CA).

A set of 16 autosomal microsatellite markers recommended by the Food and Agricultural Organization (FAO) and International Society for Animal Genetics (ISAG) was used for genotyping (Table 3.2). The markers were chosen considering the polymorphism, compatibility in the variety of allele size as well as their capability to enlarging in complex PCR reactions.

Table 3.2 Microsatellite marker information applied in this study

Primer	Sequences	Chromosome Number	Size range (bp)	Annealing Temp (° C)	Original reference
CSSM66	F: ACACAAATCCTTTCTGCCAGCTGA	14	55-65	171-209	Barendse *et al.* (1994)
	R: AATTTAATGCACTGAGGAGCTTGG				
BM1824	F: GAGCAAGGTGTTTTTCCAATC	1	176-197	55-60	Barendse *et al.* (1994)
	R: CATTCTCCAACTGCTTCCTTG				
HAUT27	F: TTTTATGTTCATTTTTTGACTGG	26	120-158	57	Thiven *et al.* (1997)
	R: AACTGCTGAAATCTCCATCTTA				
TGLA227	F: CGAATTCCAAATCTGTTAATTTGCT	18	75-105	55-56	Georges & Massey (1992)
	R: ACAGACAGAAACTCAATGAAAGCA				
BM1818	F: AGCTGGGAATATAACCAAAGG	23	248-278	56-60	Bishop *et al.* (1994)
	R: R: AGTGCTTTCAAGGTCCATGC				
TGLA53	F: CCCTCCTCCAGGTAAATCAGC	16	143-191	55	Georges & Massey (1992)
	R: ATCTTCACATGATATTACAGCAGA				
INRA023	F: GAGTAGAGCTACAAGATAAACTTC	3	195-225	55	Vaiman *et al.* (1994)
	R: TAACTACAGGGTGTTAGATGAACTC				
ETH10	F: GAGTAGAGCTACAAGATAAACTTC	5	207-231	55-65	Solinas-Toldo *et al.* (1993)
	R: CCTCCAGCCCACTTTCTCTTCTC				
TGLA122	F: CCCTCCTCCAGGTAAATCAGC	21	136-184	55-58	Georges & Massey (1992)
	R: AATCACATGGCAAATAAGTACATA				
ETH225	F: GATCACCTTGCCACTATTTCCT	9	131-159	55-65	Steffen *et al.* (1993)
	R: ACATGACAGCCAGCTGCTACT				
BM2113	F: GCTGCCTTCTACCAAATACCC	2	122-156	55-60	Sunden *et al.* (1993)
	R: CTTCCTGAGAGAAGCAACACC				
SPS115	F: AAAGTGACACAACAGCTTCTCCAG	15	234-258	55-60	**BCM (2006)
	R: AACGAGTGTCCTAGTTTGGCTGTG				
TGLA126	F: CTAATTTAGAATGAGAGAGGCTTCT	20	115-131	55-65	Georges & Massey (1992)
	R: TTGGTCTCTATTCTCTGAATATTCC				
ETH3	F: GAACCTGCCTCTCCTGCATTGG	19	103-133	55-65	Solinas-Toldo *et al.* (1993)
	R: ACTCTGCCTGTGGCCAAGTAGG				
CSRM60	F: AAGATGTGATCCAAGAGAGAGGCA	10	79-115	58-67	**BCM (2006)
	R: AGGACCAGATCGTGAAAGGCATAG				
ILST006	F: TGTCTGTATTTCTGCTGTGG	7	277-309	55	Brezinsky *et al.* (1993)
	R: ACACGGAAGCGATCTAAACG				

**BCM - Baylor College of Medicine Human Genome Sequencing Center

3.5 Statistical Analysis

3.5.1 Genetic diversity

Allele frequencies, heterozygosity, Polymorphic Information Content (PIC) and genetic variation estimates were calculated using MSToolkit (Park, 2001). For diversity measures, evaluations of mean number of alleles (MNA), expected (H_e) and observed (H_o) heterozyosities are shown to be good pointers of within breed genetic variability in a population. Heterozygosity values were calculated to determine the level of genetic variation within all populations. The H_o and H_e were computed according to Nei (1987). To test deviation from Hardy Weinberg Equilibrium at each locus overall population, GENEPOP version 4.3 software was used (Raymond & Rousset, 1995).

To compute Wright's *F*-statistics for each locus, FSTAT version 2.9.3.2 (Goudet, 1995) program was used. This includes F, Θ and f, which are analogous to Wright's (1978) F_{IT}, F_{ST}, and F_{IS}. Jack-knifing procedure was applied over the loci in deriving their significance levels using the Weir and Cockerham (1984) estimation of F_{IT}, F_{ST}, and F_{IS} and performed for every locus among populations. F_{IS} was also calculated between he populations using FSTAT version 2.9.3.2 (Goudet, 1995). Arlequin version 3.1 (Excoffier *et al.*, 2005) was performed to indicate the differentiation between populations. The analysis of molecular variance (AMOVA) was used to illustrate the

partitioning of gene diversity (Excoffier *et al.*, 2005). The total gene diversity is partitioned into a component within breeds or populations and between breeds or populations in a structured population (Toro *et al.*, 2009).

3.5.2 Population assignment

To describe the genetic structure of the cattle population, STRUCTURE software (Pritchard *et al.*, 2000) was used to implement a Bayesian assignment approach for a multi-locus genotype analysis. Individuals were assigned to clusters (K = 12) and admixture proportions of individuals were estimated. All runs consisted of burn-in period of 100 000 steps that were followed by 300 000 Markov Chain Monte Carlo (MCMC) iterations, for values of 2 = K = 20, with twelve replications of each K-value. Structure Harvester v0.6.93 (Earl & vonHodlt, 2011) was used to determine DeltaK (ΔK) (Evanno *et al.*, 2005) from –Ln probability values to determine the correct number of clusters identified with STRUCTURE software. Monte Carlo Markov Chain method is used to estimates the natural logarithm of the probability (Pr) of the observed genotypic array (*X*), given a pre-defined number of clusters (K-values) in the data set (Pritchard *et al.*, 2000).

3.5.3 Genetic distance

Genetic variation between populations was measured using Nei *et al*. (1983) angular genetic distance (D_A). GENEPOP package, version 4.2 was used to estimate D_A genetic distance between the studied populations. Due to its superior performance in phylogeny reconstruction, the D_A measure of genetic distance was computed. For dendrogram construction, with the neighbour-joining (NJ) algorithm was used to determine the genetic distance between populations (Saitou & Nei, 1987). Using the algorithm implemented in MEGA version 6 (Tamura *et al*., 2013), the phylogenetic tree of populations' relationships was constructed. A 1000 bootstrap resampling was performed to test the robustness of the dendrogram topology.

CHAPTER 4: RESULTS

4.1 Marker polymorphism, within and between population variations

A total of 139 different alleles were detected and the number of alleles observed across microsatellite markers ranged from five to seventeen at loci BM1824 and TGLA53 over three populations respectively. The average number of alleles observed across all the populations was 9.0. Overall, the average values for PIC in the tested markers had high levels of polymorphism. The PIC values ranged between 0.456 (SPS115 - PHU) and 0.867 (TGLA53- MAL) (Table 4.1).

Levels of genetic variability were estimated using allelic diversity, involving the observed (H_o) versus unbiased expected (H_e) heterozygosities. The mean H_o and H_e observed across the three population was 0.733 (73 %) and 0.780 (78 %) for DIH; 0.779 (78 %) and 0.806 (81 %) for PHU; 0.760 (76 %) and 0.798 (80 %) for MAL (Table 4.1). The lowest H_o and H_e were observed in DIH and PHU at 0.182 (HAUT27) and 0.488 (SPS115) while the highest H_o and H_e were observed in both DIH and PHU at 1.000 (TGLA53) and 0.916 (TGLA53) respectively.

Markers HAUT27 and ILST006 were not in HWE in all the three populations (Table 4.1). A total of five markers were not in HWE for DIH and MAL populations while a total of

three markers were not in HWE for PHU population. The average HWE for all populations were 0.4103, 0.3853 and 0.3932 for DIH, PHU and MAL respectively. Eleven markers were in HWE ($P \leq 0.05$) for both DIH and MAL populations and thirteen markers for PHU population respectively therefore few loci deviated significantly from the expected HWE.

Table 4.1 Descriptive statistics of the 16 microsatellite marker loci. Statistics reported for each population separately and overall, consolidating all cattle: alleles (N), most frequent alleles (MFA), least frequent alleles (LFA), observed heterozygosity (Ho), expected heterozygosity (H_e), polymorphic information content (PIC) and Hardy Weinberg Equilibrium (HWE)

	DIH							PHU							MAL						
Locus	**N**	**MFA**	**LFA**	**H_o**	**H_e**	**PIC**	**HWE**	**N**	**MFA**	**LFA**	**H_o**	**H_e**	**PIC**	**HWE**	**N**	**MFA**	**LFA**	**H_o**	**H_e**	**PIC**	**HWE**
BM2113	10	139	125	0.827	0.884	0.858	**0.0342**	10	133	135	0.931	0.874	0.843	0.3327	10	133	141	0.778	0.877	0.850	0.2555
ETH10	8	217	215	0.857	0.822	0.791	0.9656	7	217	215	0.897	0.808	0.765	0.7680	9	221	215	0.778	0.821	0.786	**0.0845**
SPS115	6	248	252	0.556	0.519	0.488	0.4822	7	248	246	0.462	**0.484**	**0.456**	0.1341	8	248	246	0.706	0.697	0.659	0.1969
TGLA227	12	77	103	0.800	0.856	0.826	**0.0577**	11	77	103	0.828	0.888	0.859	0.1301	10	77	83	0.889	0.838	0.804	0.7429
TGLA53	17	160	174	0.971	**0.916**	0.895	0.8929	15	160	174	**1.000**	**0.916**	0.892	0.9004	15	160	158	0.917	0.890	**0.867**	0.9468
INRA23	11	214	210	0.971	0.857	0.828	0.7085	10	214	216	0.793	0.839	0.802	0.6146	12	214	194	0.778	0.815	0.785	0.4597
TGLA122	10	151	183	0.829	0.800	0.761	0.4819	11	151	183	0.793	0.834	0.797	0.5987	11	143	183	0.861	0.800	0.762	0.9219
TGLA126	7	115	119	0.771	0.776	0.730	0.6918	7	115	113	0.828	0.785	0.740	0.5693	7	115	113	0.556	0.615	0.580	0.3491
BM1824	6	180	190	0.600	0.690	0.624	0.1785	5	180	192	0.655	0.732	0.679	0.2018	6	280	190	0.778	0.748	0.694	0.8755
ETH225	9	150	160	0.800	0.830	0.795	0.8458	8	144	146	0.793	0.852	0.816	0.3623	8	150	152	0.917	0.828	0.791	**0.0613**
ETH3	7	117	103	0.743	0.707	0.668	0.1623	9	117	103	0.690	0.746	0.709	0.5157	8	117	123	0.556	0.680	0.640	**0.0042**
BM1818	7	266	258	0.771	0.758	0.715	0.7890	8	262	260	0.897	0.804	0.760	**0.0110**	9	266	268	0.806	0.781	0.740	0.1751
CSRM60	10	102	94	0.571	0.779	0.736	**0.0080**	9	102	116	0.823	0.803	0.763	0.8172	9	94	92	0.778	0.806	0.768	0.6400
HAUT27	7	146	142	**0.182**	0.577	0.538	**0.0000**	7	150	156	0.214	0.839	0.782	**0.0000**	8	136	144	0.278	0.844	0.798	**0.0000**
CSSM66	11	185	191	0.829	0.875	0.849	0.2617	8	185	195	0.897	0.858	0.825	0.1817	10	185	195	0.889	0.866	0.837	0.5395
ILST006	11	294	288	0.657	0.835	0.801	**0.0050**	11	294	288	0.966	0.837	0.780	**0.0267**	11	296	288	0.889	0.862	0.833	**0.0383**
Average	**9**	**173**	**175**	**0.733**	**0.780**	**0.744**	**0.4103**	**9**	**172**	**178**	**0.779**	**0.806**	**0.767**	**0.3853**	**9**	**177**	**174**	**0.760**	**0.798**	**0.762**	**0.3932**

Results of the *F*-Statistics for each of the 16 analysed loci are shown in Table 4.1. Fixation indices (F_{IT}, F_{ST} and F_{IS}) were used to evaluate population differentiation for each of the sixteen markers across the three population of Thabo Mofutsanyane district. The mean estimates of *F*-statistics obtained by jack-knifing over loci were: F_{IT} = 0.057, F_{ST} = 0.010, F_{IS} = 0.048. Of the sixteen markers, 7 markers showed negative F_{IT}, 7 markers showed positive F_{ST} and 8 markers showed negative F_{IS} from the total of 16 markers that were used. The total inbreeding coefficient was 5.7 %. A significant deficit was observed at loci HAUT27 at 0.729. The average genetic differentiation was 0.010, indicating that 0.01 % of genetic diversity can be explained by the genetic differentiation among the populations whereas 99 % can be explained by differences among individual. The average F_{IS} of 0.048 was low positive, indicating limited inbreeding. The F_{IS} negative values witnessed in some of the markers may be explained by the Wahlund effect. The F_{IS} per population varied from 0.035 (PHU), 0.050 (MAL) and 0.061 (DIH) and was lower than that of purebred lines.

Table 4.2 Wright's *F*-statistical for 16 microsatellite loci (F_{IT}, F_{ST}, F_{IS}) for each locus

Locus	F_{IT} (F^1)	F_{ST} (Θ^2)	F_{IS} (f^3)
BM2113	0.046*	0.001	0.044*
ETH10	-0.008	0.020	-0.028
SPS115	0.000	0.014	-0.014
TGLA227	0.029	0.007*	0.022
TGLA53	-0.064	-0.003	-0.060
INRA23	-0.010	0.007*	-0.017
TGLA122	-0.032	-0.007	-0.025
TGLA126	0.049*	0.035	0.015
BM1824	0.056*	-0.005	0.061
ETH225	-0.003	0.002	-0.005
ETH3	0.059*	-0.010	0.069
BM1818	-0.031	0.021	-0.053
CSRM60	0.087	-0.011	-0.020
HAUT27	0.729**	0.088*	0.703**
CSSM66	-0.006	-0.002	-0.004
ILST006	0.017	-0.001	0.019
Overall	**0.057***	**0.010***	**0.048***

[1]Total inbreeding estimate; [2]Measure of population differentiation; [3]Within-population inbreeding estimate. Statistical significance from permutation tests: * P < 0.001, ** P < 0.05

In the AMOVA analysis (Table 4.3), the partitioning of genetic variation was further explained and virtually no variation was detected between populations, with only 0.01 % of variation attributed to differences among populations. The remaining 99 % of variation was observed within populations.

Table 4.3 AMOVA analyses for the three populations of Thabo Mofutsanyane

Source of variation	Sum of squares	Variance Components	Percentage Variation
Among Populations	13.637	0.01737	0.01
Within Populations	1116.103	5.66550	99.99
Total	1129.740	5.68287	

4.2 Cluster analysis

The population structure and its variant over time were studied using the Bayesian approach and implemented in the software STRUCTURE version 2.3.4. To identify the optimal K value, the methodology described by Evanno *et al*. (2005) was applied. Evanno's transformation is useful for assessing K and it is based on the second order derivation on the variance of the maximum likelihood estimation of your model given a specific K. The cluster analysis was performed without prior information on breed groups evidenced by a meaningful pattern of mean Ln Pr (X | K) values from K = 2 to K = 20 (Figure 4.1). The mean value of Ln Pr (X | K) increased noticeably up to K = 12 and dropped afterwards, with a large increase in its variance. The most probable clustering was found at K = 12.

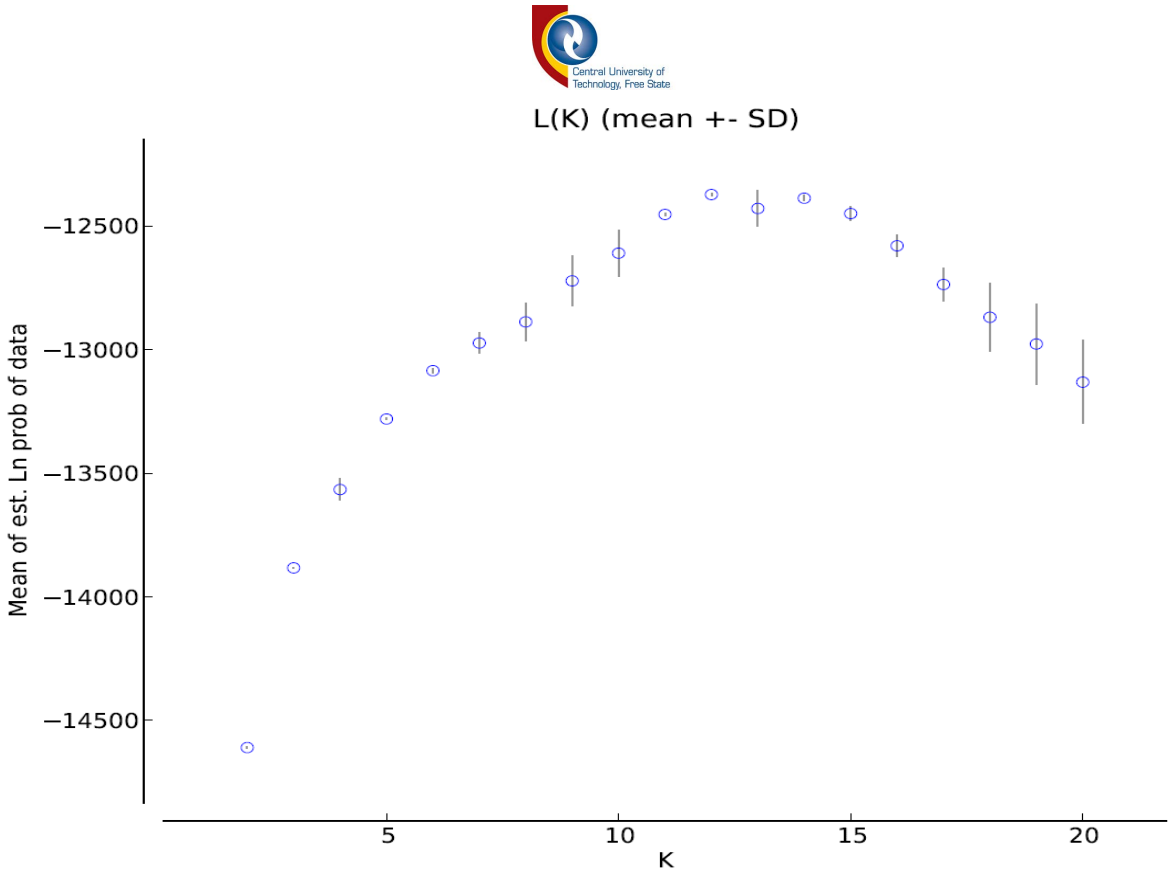

Figure 4.1 Plot of estimated posterior probabilities of the data [Ln Pr (X|K)] for different number of inferred clusters (K=2 to K=20). (a) [Ln Pr (X|K)] values are presented as a function of the number of clusters (Pritchard *et al.*, 2000). Mean [Ln Pr (X|K)] values within each K are presented by solid circles

To further investigate variation of cluster membership in the studied population and variation in proportion of memberships or admixture between DIH, PHU and MAL individuals, STRUCTURE analysis was performed. For K = 12 most of the reference population could be recognizably assigned to the twelve individual clusters. Table 4.4 demonstrates the proportion of membership of the twelve reference breeds in each of the twelve clusters. The breeds were clustered as follows: Cluster 1 = Nguni, Cluster 2 = Holstein, Cluster 3 = Brahman, Cluster 4 = Hereford, Cluster 5 = Bonsmara, Cluster 6 = Afrikaner, Cluster 7 = Limousin, Cluster 8 = Jersey, Cluster 9 = Angus, Cluster 10 = Drakensberger, Cluster 11 = Simmentaler and Cluster 12 =

Charolais. Cattle breeds showed a high admixture population in DIH with breeds such as Bonsmara (18 %), Simmentaler (14 %), Brahman (12 %) and Drakensberger (11 %), while cattle population in PHU include breeds such as Bonsmara (24 %), Charolais (11 %), Brahman, Drakensberger and Simmentaler at 10 % and for MAL it was breeds such as Charolais (15 %), Bonsmara (14 %), Limousin (13 %) and Drakensberger (11 %). The results presented in Figure 4.2 demonstrated that the population of Thabo Mofutsanyane have high levels of admixture.

Table 4.4 Proportion of membership of the analysed cattle populations in each of the twelve clusters inferred in the structure program

Given Pop	Inferred Clusters												n
	1	2	3	4	5	6	7	8	9	10	11	12	
DIH	0.077	0.098	**0.119**	0.052	**0.184**	0.003	0.075	0.043	0.052	**0.105**	**0.137**	0.055	32
PHU	0.088	0.055	**0.104**	0.042	**0.236**	0.003	0.098	0.023	0.037	**0.104**	**0.101**	**0.109**	34
MAL	0.091	0.086	0.087	0.044	**0.144**	0.003	**0.134**	0.052	0.058	**0.111**	0.041	**0.149**	34
ANG	0.006	0.001	0.004	0.013	0.008	0.002	0.001	0.002	**0.970**	0.010	0.002	0.002	28
BRA	0.011	0.006	**0.947**	0.005	0.010	0.002	0.006	0.007	0.006	0.007	0.006	0.020	28
NGU	**0.953**	0.007	0.008	0.009	0.011	0.002	0.001	0.008	0.004	0.006	0.006	0.006	26
BON	0.023	0.017	0.009	0.007	**0.937**	0.003	0.001	0.007	0.014	0.002	0.010	0.018	30
DRA	0.018	0.030	0.002	0.004	0.028	0.003	0.002	0.010	0.010	**0.960**	0.001	0.003	30
AFR	0.002	0.003	0.002	0.002	0.003	**0.973**	0.003	0.002	0.003	0.003	0.002	0.003	30
HER	0.007	0.011	0.004	**0.929**	0.007	0.002	0.001	0.016	0.013	0.002	0.016	0.041	24
SIM	0.010	0.016	0.004	0.012	0.008	0.002	0.004	0.002	0.019	0.002	**0.914**	0.015	22
HOL	0.008	**0.980**	0.006	0.006	0.009	0.002	0.013	0.008	0.009	0.003	0.011	0.005	20
JER	0.005	0.008	0.005	0.011	0.005	0.002	0.008	**0.922**	0.011	0.008	0.008	0.007	25
CHA	0.008	0.014	0.005	0.005	0.014	0.002	0.016	0.013	0.078	0.002	0.005	**0.902**	30
LIM	0.001	0.017	0.008	0.021	0.007	0.002	**0.938**	0.004	0.002	0.016	0.036	0.045	30

n = number of individuals

Assuming K = 12, the proportional contribution of the assumed populations to each of the current breeds was computed, the corresponding results is shown in Figure 4.2. Table 4.4 illustrates the three experimental breeds and the twelve reference breeds that were used in this study. A thin vertical line consisting of only one colour represents populations without admixture (Pritchard *et al*., 2000). The different colours occuring in one population represents the admixture proportions. There were no clear differences between the three types of populations of Thabo Mofutsanyane from the observed genetic structure. This illustrates that these populations had no genetic structure for all observed clusters for the studied population.

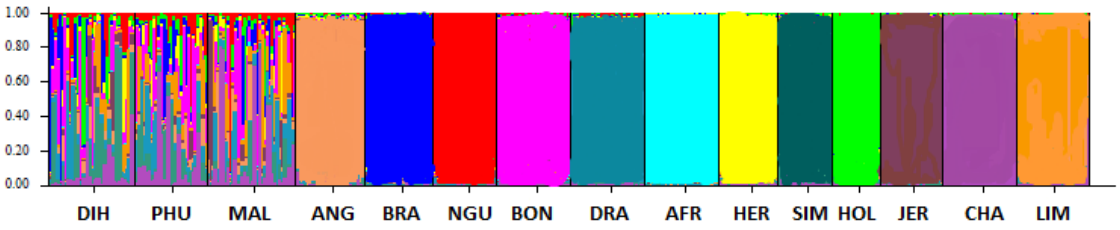

Figure 4.2 A summary plot of the estimate of Q, with each individual represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the fourteen inferred clusters

4.3 Genetic distance

To evaluate the genetic relationships between the three populations, genetic distance was performed (Table 4.5). The genetic distance estimate ranged from

0.0053 between DIH and PHU to 0.0091 between DIH and MAL. For PHU and MAL, the genetic distance was 0.0062, indicating a close relationship between DIH and PHU populations.

Table 4.5 D_A genetic distance between Thabo Mofutsanyane genotypes

Pop ID	DIH	PHU	MAL
DIH	****		
PHU	0.0053	****	
MAL	0.0091	0.0062	****

The D_A distances were calculated to construct a neighbour-joining (NJ) topology tree relating for the three experimental populations as presented in Table 4.5. The dendrogram representing distances among breeds enables the visualization of genetic relationships between two populations.

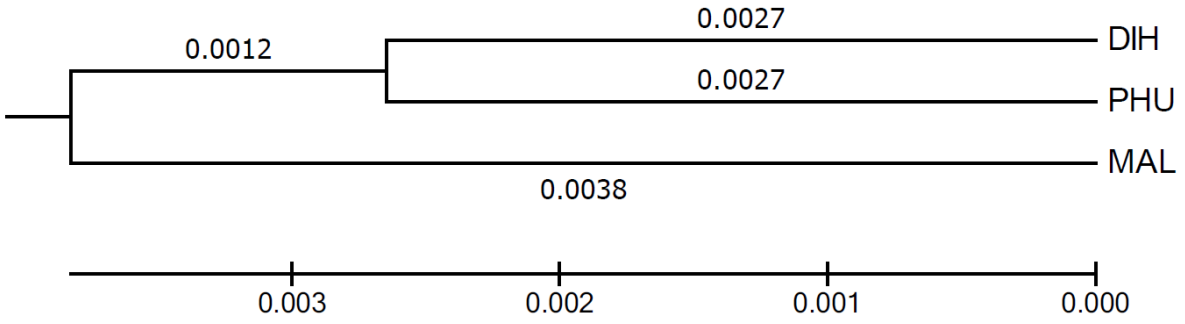

Figure 4.3 Phylogenetic tree of genetic relationship among the three populations of the experimental cattle located at the Thabo Mofutsanyane district, which was based on D_A genetic distances (Nei, 1983) estimated with 16 microsatellites. Number on the nodes in the tree are bootstrap values of 10,000 replication

For instance, two populations of DIH and PHU were clustered closer together with MAL forming a well-separated cluster. The phylogenetic tree in Figure 4.3 also confirmed the genetic relationships of the populations.

CHAPTER 5: DISCUSSION

The least number for alleles detected for each microsatellite locus was five. This was in line with the number proposed for proficient judgement of a minimum of four distinct alleles per locus of genetic differentiation between breeds standards set by the National Farm Animal Genetic Resources, using reference Microsatellite as reported by FAO (2004). All markers in all three populations had PIC value of greater than 0.5 except for SPS115 in DIH and PHU, respectively. Buchanan *et al*. (1994) observed that PIC value to be reasonably small when one or two alleles dominate.This indicated the usefulness of the studied markers for genetic diversity studies and the mean PIC values for DIH (0.78), PHU (0.78) and MAL (0.76) indicated high levels of information from the markers that were used for this study.

The most widely used parameter used to measure genetic diversity is unbiased heterozygosity (Toro *et al*., 2009). Results of calculated average heterozygosity for all three populations in this study exceed 0.7. Teneva *et al*. (2007) also reported the high level of genetic variation with a mean value of 0.7858 for H_e and 0.7513 for H_o. The lowest levels of heterozygosity were noticed at loci HAUT27 in all the populations at 0.182 for DIH, 0.2124 for PHU and 0.278 for MAL. This differs with Mukesh *et al*. (2009), who reported high levels of heterozygosity for loci HAUT27.

The reason for higher variability observed in the three populations may be that these cattle were not subjected to strict selection. The excess heterozygosity observed in the populations can almost certainly be caused by the high rate of crossbreeding and genetic erosion (Ginja *et al*., 2010b). Heterozygosity values in this study are comparable with values reported in previous studies (Teneva *et al*., 2007) but higher than the average reported by MartÍn-Burriel *et al*. (1998). There is a large level of genetic variation in TM cattle breeds despite the high level of admixture that exist there. Highly selected commercial breeds are much less diverse and more inbred than local breeds (Hansen *et al*., 2002; Maudet *et al*., 2002), emphasizing the importance of local breeds for genetic diversity as they have high potential to be preserved as genetic resources. High levels of genetic diversity were observed from cattle developed from the admixture of many breeds (Kantanen *et al*., 2000). Indiscriminate mating seems to have contributed to maintain a high level of genetic diversity, since there are no proper breeding strategies in the communal areas.

Hardy Weinberg Equilibrium test was used to predict whether the population is stable or not. Deficit of heterozygous individuals may have resulted in the deviation from HWE on the five markers due to the presence of null alleles, Wahlud effect, inbreeding or selection towards homozygosities (Maudet *et al*., 2002). Genetic drift and small sample size may also cause the deviation from equilibrium. Population size could be the reason for differences in the levels of genetic diversity. Farmers in

the communal areas of the Free State province have numerically small herds of about 14 cattle per herd (Scholtz *et al.*, 2008).

A significant amount of genetic variation is maintained in the cattle population as shown by the genotype data analysed in the current study. The low values from observed heterozygosity versus majority of loci, which had higher expected heterozygosity, reflected the existence of variation in the studied populations. Moioli *et al*. (2001) indicated that even in populations of small sizes, a higher amount of genetic variability could be achieved. The results of the current study are within the acceptable range of 0.3 – 0.8 to be a useful marker for measuring genetic variation (Takezaki & Nei, 1996).

Results of the 5.7 % for inbreeding levels came as a surprise as there are no formal breeding plans in place as the experimental cattle breeds mate randomly. MartÍn-Burriel *et al*. (2007) also reported lower values of inbreeding co-efficient. Factors such as assortative mating, linkage with loci under selection or population heterogeneity may attribute to lower heterozygotes and excess of homozygotes. (F_{IS} > 0). Makina *et al*. (2014) noted that allele frequencies may be poor in indicating the real status of inbreeding within cattle breeds and thus assessment of the inbreeding levels should be perfomed every 5 years to determine any unfavourable change and take appropriate steps to prevent increases in inbreeding. The positive F_{is} values

ranging from 0.035 to 0.061 per population indicated a reduction of the observed heterozygosity compared to what is expected under random mating and serves as an indication of inbreeding within the population (Hartl, 1998). This might be due to sub-structure within the population avoiding non-random mating.

The genetic differentiation levels of 1.0 % observed in this study was much lower than that observed among other studies. For instance, moderate differentiation levels (F_{ST}) of 6.0 % was observed by Ibeagha-Awemu *et al*. (2005) while much higher values (11 %) were reported in a study conducted in western India cattle breeds by Sodhi *et al*. (2005). This might be caused by the farmers' traditional and cultural practices, as well as genetic variation to be presented within sub-population. The easy accessibility of the Thabo Mofutsanyane district may have a role in the easy movement of cattle populations from the neighbouring districts.

Genetic structure, breed assignments and the degree of admixture were investigated using the Bayesian clustering implemented STRUCTURE version 2.3.4 (Pritchard *et al*., 2000). The STRUCTURE analysis reveals a clear evidence of admixture, with various reference cattle populations contributing differently to the studied populations. A cluster analysis clearly illustrates that there was no genetic structure (K = 1) among the population of Thabo Mofutsanyane district. This is in agreement with a study conducted by Scholtz *et al*. (2008) who reported that 66.4 % of herds in

the communal and emerging sector to be crossbred. Very limited number of bulls available for communal farmers also plays a role. Conservation actions including exchange of bulls amongst the farmers as well as germplasm sampling and cryo-conservation may assist in conservation programmes. Population structure information is thus important for communal cattle breeds for genetic improvement.

Cattle breeds in Thabo Mofutsanyane district were shown to be highly mixed with breeds such as Bonsmara, Brahman, Limousin, Drakensberger, Simmentaler and Charolais, while other breeds contributed less than 10 % of the entire cattle population studied. The results showed small genetic contributions from Nguni, Afrikaner, Hereford, Holstein and Jersey breeds. This high level of admixture reveals high levels of cross-breeding. Bonsmara and Drakensberger breeds seem to be well represented in all the populations of TM and this indicate their popularity among the communal cattle farmers. Brahman and Simmentaler breeds were a common denominator in DIH and PHU while Charolais breed was common in PHU and MAL. Ndumu *et al*. (2008) noticed that as a result of trade and cultural exchange taking place in rural areas, gene flow usually occcurs. Past and ongoing cattle trade accounts for gene flow observed in these populations. Directional matings from the exotic breeds into the local genomes is noticeable from this study.

A study conducted by Mapiye *et al*. (2009) found the crossbreds to be commonly distributed, consisting of exotic and Nguni breeds while breeds such as Bonsmara, Hereford, Brahman and Drakensberger were found to be popular at 20 %. This report differs from our study as the Nguni breed only contributed less than 10 % of the genetic material in the studied population. The varied reportscould be attributed to differences in the geographical locations, as the study by Mapiye *et al*. (2009) was conducted in the Eastern Cape. Interestingly, the national survey results by Scholtz *et al*. (2008) revealed that 22.5 % of bulls used in communal areas are predominantly Nguni breed. However, consideration is taken that this is the first genetic characterization study to be conducted for cattle in communal areas using microsatellite markers.

Several recent studies have considered the use of microsatellite markers in cattle to analyse the intensity of breed admixture in cattle (Freeman *et al*., 2005; MartÍn-Burriel *et al*., 2011; Pienaar *et al*., 2014). MartÍn-Burriel *et al*. (2011) justified the reasons for admixture due to the traditional extensive management system and the late arrival of the modern genetic management such as parentage testing. Overall, the breeds displayed a greater degree of admixture reflecting crossbreeding between the breeds. Genetically purebred herds seem to not exist or if they do, they are rare as there is an influx of exotic genetic material in the communal sector.

The genetic characterization of cattle breeds from the communal sector for determining genetic relationships will assist in prioritising breeding programs on the basis of their genetic make-up. Relationships between the populations were analysed by Raymond and Rousset's genetic distance. The DIH and PHU populations distanced themselves from the MAL. This was consistent with the geographically proximity as PHU and MAL are geographically closer together but was suprising as well as all these three populations are within the boundaries of each other. The geneflow between the populations did not have any significant isolation by distance.

The genetic distance shows that MAL population was the most divergent from the others. The relationships in the dendrogram are not too robust as this is shown by the low bootstrap values. This could be attributed to the genetic drift as a main factor in differentiation among closely related populations (Takezaki & Nei, 1996; Weir, 1996). However, the high levels of indiscriminate crossbreeding appear to encourage a high level of gene flow and thereby supporting a low level of differentiation. The results of the analysis with genetic distance were mostly in agreement with the UPGMA phylogenetic tree. Proper management programs must be employed to make sure that the genetic pool represented by these breeds is not lost due to uncontrolled crossbreeding.

CHAPTER 6: CONCLUSSION AND RECOMMENDATIONS

Results for this study revealed a significant decline in the numbers of purebred cattle in the communal areas due to a greater degree of unplanned crossbreeding that is practiced by communal farmers. The inbreeding coefficients revealed low levels of inbreeding. To make sure that inbreeding is maintained at the low level, evaluation of the inbreeding level should be done every five years so as to determine any unfavourable breeding program that needs adjustment. A lack of genetic differentiation as shown by the STRUCTURE analysis was expected, mostly due to the fact that there are no proper breeding management of the cattle herds in the communal cattle production sector. To accurately evaluate the availability of genetic resources, as the first step to an effective breeding program, it is important to consider the level of admixture when trying to estimate the future breeding potential of a given livestock. The level of highly cross-bred cattle population observed at the Thabo Mofutsanyane district should be noted and steps toward assisting these communal farmers to develop appropriate breeding strategies should be prioritised.

Further analysis could be helpful in providing the basis for a rational exploitation of livestock. Future population genetics studies of cattle from the communal farms should consider the use of SNP markers as long as relevant partnerships and funding are available to be able to provide more accurate information on breed

relationships and population genetic structure. Phenotypic data and genetic studies should be wisely combined with greater numbers of representatives as well as greater numbers of population in order to be able to make comprehensive decisions for the future breeding programmes and utilization of cattle breeds in the communal areas. This is vital for the future production of food and thus a being able to alleviate poverty and act as an important economic resource for rural development. In conclusion, results presented have shown the importance of genetic characterization in knowing the genetic structure of the population thus providing information on the status of the high level of admixture of cattle breeds in Thabo Mofutsanyane.

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