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Genetic characterization of South African Nguni cattle ecotypes using microsatellite markers

By

Yandisiwe Patience Sanarana

Submitted in partial fulfilment of the requirements for the degree

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Declaration

I, Yandisiwe Patience Sanarana declare that the thesis/dissertation, which I hereby submit for the degree MSc. Animal Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other tertiary institute.

Signature……..

Date…….

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Yandisiwe Patience Sanarana

Promoter: Prof E. van Marle-Köster

Co-promoter: Prof A. Maiwashe

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Abstract

Genetic characterization is an important step to assess the genetic status of indigenous breeds for informed decision making with regard to genetic improvement and conservation. The Nguni cattle breed is an important indigenous animal genetic resource that is well-adapted to different ecological regions in South Africa. Nguni cattle differ phenotypically in terms of body frame, size of ears, coat colour, horn and head shape and these differences have resulted in the recognition of five major ecotypes within the breed. The aim of this study was to perform a molecular characterization of Makhathini, Pedi, Shangaan and Venda Nguni cattle ecotypes using 22 microsatellite markers. The data was generated from 189 unrelated Nguni cattle individuals sampled from stud herds and research stations. Genetic diversity among Nguni cattle ecotypes was high with heterozygosity values varying from 68% University of Fort Hare (UFH), 69% Shangaan (SHA), 70% Makhathini (MAK), 70% Venda (VEN), 71% Loskop (LOS) to 72% Pedi (PED) with a mean number of alleles that ranged from 6.0 and 6.9. The overall inbreeding value indicated limited inbreeding between the populations (F_{IS}=0.01). The population differentiation (F_{ST}) and AMOVA analyses indicated that 4.8 % of the total variation was due to differences between populations and 95.2% accounted for differences within individuals in the population. The genetic distances revealed shortest relationship between MAK, PEDI and SHA ecotypes. The VEN ecotype differentiated from MAK and PED and was closer to SHA ecotype. In addition, structure analysis depicted the predominance of MAK ecotype into other ecotypes. The results of this study can be applied for the genetic conservation of Nguni bovine ecotypes.

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Abbreviations

AMOVA	Analysis of Molecular Variance
DNA	Deoxyribonucleic acid
DRUSSA	Development Research Uptake in Sub-Saharan Africa
FAO	Food and Agricultural Organization
F_{IS}	Inbreeding coeffient of individuals within a subpopulation
F_{IT}	Inbreeding coeffient of individuals within the total population
F_{ST}	The amount of genetic differentiation within the total population
g	Gram
ISAG	International Society for Animal Genetics
K	Number of assumed populations
Kg	Kilograms
Ln Pr (X|K)	Ln probability of the data
m	Meters
MAK	Makhathini
mm	Millimetres
$MgCl_2$	Magnesium Chloride
mtDNA	Mitochondrial Deoxyribonucleic acid
ng	Nanogram
nm	Nanometer
PED	Pedi
QTL	Quantitative trait loci
R_{ST}	Coefficient index under the step-wise mutation model
SD	Standard deviation
SHA	Shangaan
SNPs	Single Nucleotide polymorphisms
VEN	Venda

CHAPTER 1

INTRODUCTION

Domestication of livestock species was a vital step in human demographic and cultural development that took place years ago (Clutton-Brock, 1992). It was a complex and gradual process that changed lifestyles of humans dramatically, enabled people to consolidate food resources and became more independent from environmental fluctuations (MacHugh, 1996; Strydom, 2008). It is primary evolutional forces such as migration, selection, mutation and genetic drift that shaped ancestral animals into cultured domesticated species (Vila *et al.*, 2005). This led to the formation of vast array of differences in morphology, physiology and production traits (Clutton-Brock, 1994). To single out among evolutional processes, migration was the main force that allowed animals to spread into different continents (Diamond, 2002). Later, this resulted in a number of livestock species followed by the development of breeds that are well adapted to different environments. Since the first breed development and selection, there has been a trend towards changes in genetic variation and in some cases a loss in valuable resources (Meuwissen, 2002; Zulu, 2008).

Cattle were the second species to be domesticated after sheep and goats (Clutter-Brock, 1999). They belong to mammalia group of *Bovidae* family (Bradley *et al*., 1996) and diverged from a common ancestor of the Auroch (*Bos primigenius*) about 250,000 years ago. This ancestra of wild cattle became extinct approximately 2,000 years ago (Burt, 2009). Cattle domestication took place about 10,000 years ago Near East of Asia in Fertile Crescent (Edwards *et al.*, 2007; Decker *et al.*, 2014) and later in the African continent also known as the centre of domestication (Hanotte *et al*., 1998). The origin of domestication in Africa can be traced to the ancient East with Semitic tribes of southern Arabia who first introduced *Zebu* and *Bos taurus* to Northern, Eastern and Southern Africa (Figure 1). This was later followed by hybrid *Bos taurus indicus* cattle that developed from the hybridization of *Bos indicus* and *Bos taurus* (MacHugh, 1996).

Presently, three main types of cattle breeds found in Africa continent are classified as unhumped *Bos taurus* (exotic), humped *Bos indicus* (Zebu) and *Bos taurus Africanus* (Sanga) (Schoeman, 1989). The Sanga is a variation that is mainly found in East and Southern of

Africa (Bradley *et al*., 1996); an admixture that descended from both *indicine* and *taurine* breeds. The Sanga made its way in the African continent 8000 years ago with nomadic people (MacHugh, 1996; Hanotte *et al*., 1998). Under Sanga group there is a large variety of indigenous cattle breeds representing rich genetic variation compared to the rest of the world. This is due to the fact that most African breeds have never been or under intense selection unlike in the developed countries where breeds have been subjected to intensive artificial selection that contributed to reduced gene diversity (Giovambattista, 2001).

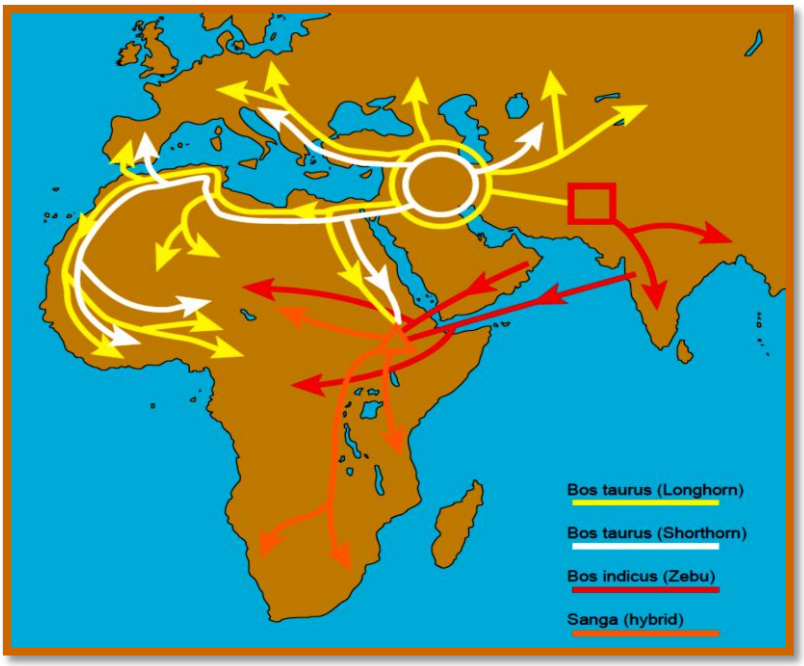

Figure 1.1. Domestication origin, routes and scattering of cattle in Africa (MacHugh, 1996)

In Africa, indigenous cattle breeds are an important animal genetic resource that supports range of diverse human communities with reasonable products and by products such as food, wealth and economic security (Adhiambo, 2002). The products that cattle produce include meat, fat, and milk, hides and production of manure for plants in mixed farming systems. Additionally, they play an important role in socio-cultural (traditional and special ceremonies), subsistence and socio-economic activities (draught animal power, and foreign exchanges). Despite the fact that they are an important protein source and wealth there are still some African countries that regards indigenous cattle breeds inferior due to low production outputs (Charoensook *et al*., 2013). In South Africa indigenous types are important genetic resources due to their adaptability and are seen as reservoir of genes that could be assets for future use and long-term sustainable production (Zulu, 2008). The country

has a pool of indigenous and locally developed livestock breeds representing a valuable rich biodiversity and has access to pure nucleus herds (Ramsay *et al*., 2000). The indigenous cattle breeds in South Africa include Afrikaner, Drakensberger and Nguni and regarded as important genetic resources possessing valuable traits that make them able to survive and reproduce under local harsh environmental conditions (Scholtz & Ramsay 2007). They have a higher tolerance to difficult conditions, parasites and diseases compared to exotic breeds (Marufu *et al*., 2011).

The Food and Agricultural Organisation (FAO) reported that indigenous breeds are at risk of disappearing without ever being documented or characterized (FAO, 2007a). This is due to breed substitutions through cross breeding with exotic breeds and also from the absence of cross- breeding programmes (Scherf, 2000). According to Groeneveld *et al*. (2010), the disappearing animal diversity is highly enhanced by dilution of the indigenous cattle breeds nucleus with exotic cattle breeds for the improvement of production performance and economically important traits. Reports further estimated that 35% mammalian breeds and 63% avian breeds are at risk of extinction (FAO, 2012a). This has raised concerns about the erosion of Animal Genetic Resources (AnGR) and the loss of animal genetic diversity among breeds with regard to the potential negative effect on the sustainability of animal production (FAO, 2007a). Due to this, the Food and Agriculture Organization-United Nations (FAO-UN) proposed molecular characterization of Animal Genetic Resources to determine the genetic status within and between indigenous populations (AnGRs) (FAO, 2011). The genetic diversity of cattle breeds is essential to meet production needs for current and future environments and enable adaptation with changes in breeding objectives (Notter, 1999).

The genetic characterization involves describing and classifying of species at molecular level using DNA analysis (Boettcher *et al.*, 2010). The DNA conveys knowledge of genetic variation which can be effectively measured within and between populations (van Marle-Köster & Nel, 2003). Microsatellite markers have been acknowledged as the choice of markers to assess genetic diversity among populations by the FAO-UN in agreement with the International Society for Animal Genetics (ISAG) (FAO, 2011).

1.2. Aim of the study

South African Nguni cattle ecotypes have not been characterized at genetic level and their population structure is unknown. Their main classification is only based on phenotypic data and type description (Nguni Cattle Breeders Society, 2008). Population differentiation among Nguni cattle ecotypes is expected due to geographical isolation. The Pedi and Zulu Nguni cattle ecotypes are listed by FAO (http://agtr.ilri.cgiar.org/library/docs/worldwatchlist.pdf) as endangered and there is no genetic information documented for these populations. Therefore, genetic characterization of Nguni cattle ecotypes based on DNA markers is essential to obtain reliable genotypic information.

The aim of this study was to perform genetic characterization of four South African Nguni cattle ecotypes using 22 microsatellite markers recommended for genetic diversity studies by FAO-ISAG advisory panel (FAO, 2011).

1.3. Objectives:

i. To evaluate the genetic diversity between and within South African Nguni cattle ecotypes using microsatellite markers.
ii. To estimate the genetic relationship among Nguni ecotypes
iii. To determine the population structure of the Nguni cattle ecotypes.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Characterization of farm animal genetic resources (FAnGRs) has received more attention since the first establishment of Domestic Animal Diversity Information System (DAD-IS) maintained by Food and Agriculture Organization of the United Nations (FAO-UN) (FAO, 2012b). Evaluating the genetic diversity of landrace breeds is a global implemented strategy to save landrace breeds from becoming extinct without ever documented. Presently, the genetic diversity documentation among farm animal genetic resources species vary largely between countries (Tixier-Boichard, 2014). In a number of countries especially in Africa there is a need for breed documentation and characterization for appropriate management and conservation (Bett *et al*., 2013). The aim of this chapter was to review approaches related to genetic diversity between and within populations with special reference to genetic diversity in South African landrace cattle breeds. The history of the South African Nguni cattle is also covered focusing on aspects that relate to breed development.

2.2. Nguni cattle in South Africa

The Nguni is one of South Africa most popular indigenous cattle breeds (Nguni Cattle Breed Society, 2008). The name Nguni was derived from black African people collectively known as Nguni speaking people (Schoeman, 1989). It is considered as one of sub – types of Sanga that originated from the imported Arabian Peninsula bulls (Hanotte *et al*., 1998). The breed was brought along to the eastern and southern areas of Africa by nomadic people who migrated from North, Central and West Africa escaping from environmental pressures of war and trade (Bester *et al.*, 2003). The mitochondrial based analysis evidently pointed out Nguni breed as an admixture of humped *Bos indicus* and humpless *Bos taurus* cattle (Parfitt & Huisman, 1998).

To date, the genotypes of Nguni cattle are traced in Sub-Sahara African countries that include South Africa, Swaziland, Namibia, Mozambique, Zimbabwe, Malawi and Zambia resultant from three migration routes through Southern Africa (Hanotte *et al.*, 1998; Maciel *et al*., 2013). In South Africa, the Nguni breed is believed to have been arrived approximately 2000

years ago via the banks of Limpopo River (Ramsay *et al*., 2000), re - evolved and settled with different tribes. Some were traced back to Limpopo and Northern Kwa – Zulu Natal. These tribes selected their Nguni cattle based on phenotypes such as horn shape and size; body conformation and coat colour patterns (Oosthuizen, 1996). Accordingly, as tribes were splitting up to settle in different geographic regions of South Africa, distinctive Nguni cattle ecotypes developed and adapted into different environmental regions (Bester *et al*., 2003).

Decades ago, Nguni cattle were perceived as inferior by the South African industrial beef sector due to low production outputs (Bester *et al.*, 2003). During colonization there was a lack of performance recording schemes thus no attention was paid to the potential of indigenous cattle breeds in South Africa, except for the Afrikaner cattle breed. The Afrikaner cattle Breed Society was established in 1912 due to the breed's outstanding qualities that were identified by Potchefstroom College of Agriculture former director, Alex Holm (Scholtz & Ramsay, 2007). Apart from that, some commercial farmers valued the Nguni breed's adaptive traits and used it in uncontrolled crossbreeding programs (Matjuda, 2012). A number of events occurred during the early 1900s including failure of exotic breeds. This resulted in crossbreeding initiative to develop breeds that could adapt and perform well under local environmental conditions. The late Professor HH Curzon identified this problem and a committee was appointed by the Department of Agriculture to report on the performance of landrace livestock for conservation and preservation (Bonsma *et al*., 1950). The committee aimed to end the decline of landrace cattle and established a 500 Nguni breeding herd to investigate the growth, production and reproduction potential and consequently serve as the pure herd nucleus for stud breeding in KwaZulu Natal province (Kars, 1993).

The Bartlow Combine Station was established in 1954 from Nguni cattle stud dating back to 1931 (Kars *et al*., 1994). This consisted of one cow, four heifers and one bull purchased from Chief Mtubatuba at Mhlabisa district along with one mature bull, three cows and three heifers purchased from Nongoma district (Kars, 1993). The national recording schemes of all beef cattle were established in 1959 (Hofmeyr, 1994) while Nguni cattle breeders' society was established in 1986 (Scholtz & Ramsay, 2007). A Department of Agriculture research facility in Loskop South (location) currently known as Agricultural Research Council Loskop South farm was later established for Nguni cattle research (Matjuda, 2012). Since then, Nguni cattle numbers in commercial sector have grown, well established and recorded in Livestock Improvement Act 25 of 1977.

To date, the importance of Nguni as a "universal breed" has been embraced as demonstrated by large number of stud Nguni herds in the ownership of research institutions, farmers and Universities where some Nguni populations are kept and conserved (Figure 2.1). This adds to an initiative supported by the National Department of Agriculture, Forestry and Fisheries (DAFF) in collaboration with Industrial Development Corporation (IDC) working with emerging farmers to grow Nguni herds in rural communities. The IDC Nguni project is implemented in all provinces of South Africa promoting the farming of Nguni by providing farmers with Nguni cattle (De Waal, 2014). This includes a number of pregnant heifers and one or two bulls on loan. Over a period of five years, stock given to the community is returned back to the owners as the same number of heifers and bulls given away (www.drussa.net/index).

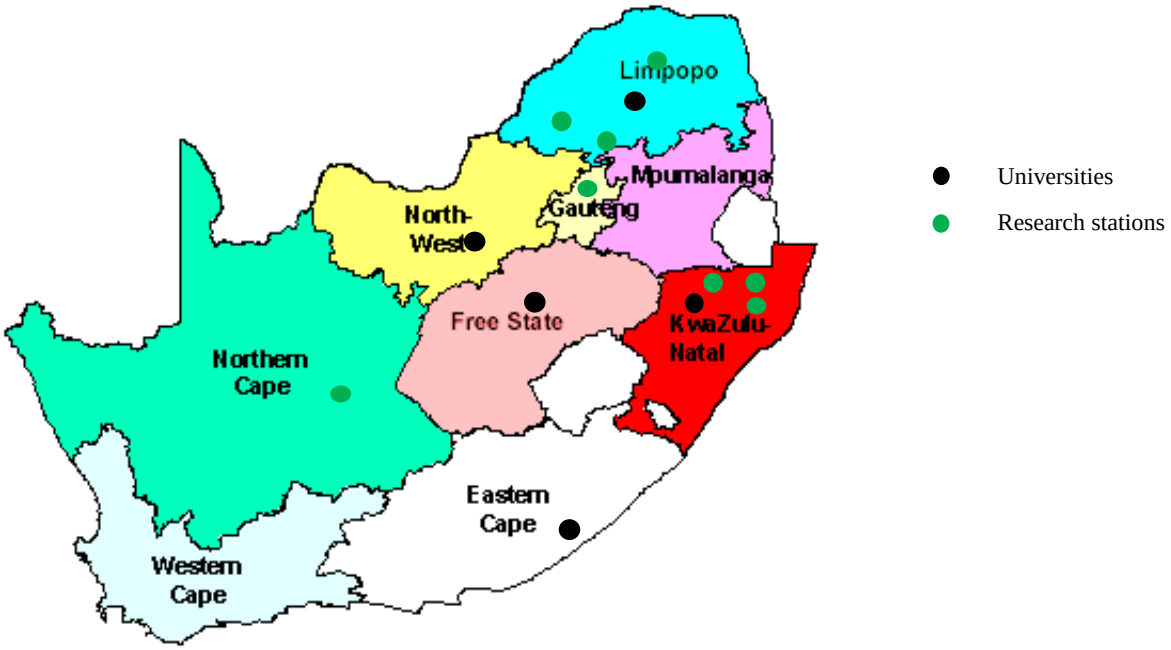

Figure 2.1. Universities and research stations keeping Nguni cattle populations in South Africa

The recent statistics released in South African Stud Book Annual Logix Beef report (2014) indicated Nguni as a second most popular breed that is being recorded after Bonsmara (Table 2.1). This includes the number of herds (407) registered, individual females (54,748) and males (20,407). However, these figures exclude the large number of Nguni cattle in rural areas. Since the number of active Nguni females is greater than 10,000 (normal >10, 000) it could be concluded that the Nguni breed is not in danger of extinction (Tada *et al*., 2013).

Although the South African Nguni cattle breed is regarded as popular with large census population size, genetic variation within the breed and population structure is unknown.

Table 2.1. Numbers of stud animals for four South African beef cattle breeds (Studbook, 2014)

Breed	Males		Females		
	Registered	Logix	Registered	Logix	Herds
Afrikaner	3026	2948	8719	8383	54
Bonsmara	41215	40958	109005	108532	411
Drankensburger	5325	5321	12757	12728	81
Nguni	20407	7856	54748	19136	407

2.3. Traits of economic importance in Nguni cattle

Nguni cattle are important indigenous genetic resource in South Africa possessing valuable traits resulting in a breed that can survive and reproduce efficiently in marginal production areas (Mapiye *et al.*, 2007). It is a multi-purpose breed and play an important role in a variety of communal farming systems in South Africa (Scholtz, 2010). The Nguni cattle breed is described by the breed society as having specific breed standards (Figure 2.2).

Figure 2.2. Typical Nguni male

Nguni cattle are small to medium in frame size. The body conformation ensures its adaptation; with long legs strong hooves that enable the breed to walk long distances and search for grazing (Nguni Cattle Breeding Society, 2008). The bulls are well developed with a rounded cervio – thoracic humps and muscular rather than fatty, weighing 500 – 700kg

while cows are small with almost non-existant humps weighing in between 320 – 440kg. The breed is heat tolerant with thick pigmented skin covered with fine short hair of different attractive colours (black, white, brown, cream and red) and a sleek-glossy skin that prevents the attachment of ticks (Muchenje *et al*., 2008b).

According to Marufu *et al*. (2011; 2014) Nguni cattle are not only able to tolerate difficult conditions where there is scarcity of forage but showed a high tick tolerance, natural immunity to tick – borne diseases and parasites that limit livestock production. The preliminary results obtained from a genome wide association study (GWAS) revealed genomic regions underlying tick resistance in Nguni cattle (Mapholi *et al*., 2014). In addition, that tick load burden occurrences in Nguni cattle adapted whether in hotter or colder regions of the country might not lead to tick-borne diseases susceptibility.

Nguni cattle have long productive lives as cows can produce 10 or more calves. Nguni heifers mature early; are highly fertile and have low calf mortalities (Matjuda, 2012). In addition, they have good temperament and mothering ability and this is linked to the historical development of the breed (Nguni Cattle Breeders Society, 2008). Nguni cows show great efficiency and often wean calves that weigh 45 – 50 % (153 kg) of their body mass (Table 2.2). They are less prone to dystocia and this is ascribed to their sloping, small uterus and low birth mass (Maciel *et al*., 2013).

Nguni cattle are excellent foragers with medium stomach capacity. They graze in natural pastures, browse in thick bushveld, extract the required quantity of nutrients (Muchenje *et al*., 2008a) and produce a carcass of good quality, approximately 180 – 298 kg with good marbling and a thin covering of fat (Muchenje, 2007; Strydom, 2008). Tables 2.2 a and b present comparison of performance of the Nguni cattle with other breeds.

Table 2.2a. Traits performance data of Nguni cattle females in comparison to Afrikaner, Bonsmara and Drakensberger breeds (Studbook, 2014).

SA Breeds	Traits								
Females	Birth Weight (kg)	Pre-wean (kg)	Wean weight (kg)	Heifers at 12 months	Heifers at 18 months	Age at first calving	Inter-calving period	Cow weights	
								At calving	At weaning
Afrikaner	30.0	116	192	235	302	32.8	452	435	468
Bonsmara	34.1	125	219	258	336	30.2	410	496	501
Drankensburger	33.5	126	214	228	313	32.8	419	466	497
Nguni	24.8	85	153	173	227	29.7	400	342	359

Table 2.2b. On-farm testing traits performance data (Phase B and D) in comparison to Afrikaner, Bonsmara and Drakensberger bulls (Studbook, 2014).

SA Breeds	Traits										
Males	Final weight (kg)	ADG (g)	FCR (kg)	Height (mm)	Length (mm)	Skin thickness (mm)	Scrotal circum. (mm)	Rib fat (mm)	Rump fat (mm)	Eye muscle (cm^2)	Marbling (%)
Afrikaner	339	1245	7.96	1193	1329	15	333	-	-	-	-
Bonsmara	403	1673	5.74	1194	1413	15	337	2.9	4.8	67	2.6
Drankensburger	375	1526	5.95	1169	1386	15	320	2.2	3.1	57	2.4
Nguni	324	1299	6.62	1105	1337	14	287	2.7	4.1	58	2.4

2.4. Nguni cattle ecotypes

In South Africa there are five different Nguni ecotypes that differ in size, coat colour, ears, teat, head and horn shape but retaining the actual adaptation traits of the original Sanga breed (Schoeman, 1989). The frame size among Nguni cattle ecotypes are attributed to different environmental factors such as veld type, mineral status of the soil, temperatures, humidity and rainfall (Ramsay, 1988). Phenotypic description of the four Nguni ecotypes is presented in Table 2.3.

Table 2.3. South African Nguni cattle ecotypes descriptions (Nguni Cattle Breeders Society, 2008; http://dad.fao.org/)

	Venda	**Pedi**	**Shangaan**	**Makhathini**
Frame	Medium	Large	Large	Small
Bulls	480-650kg	550-700kg	550-800kg	400-550kg
Cows	330-370	380-420	360-500	280-360
Head	Short	Long	Long	Short, narrow and "v" shaped between horns.
Horn shape	Lyre	Medium lyre	lyre	Open lyre
Ears	Medium	Large	Large	Small
Coat	Smooth	Coarser	Smooth	Smooth
Coat patterns	Black & White	Black &White	Black & White	Black &White
	Red &White	Grey to black	Red & White	Red & White
	Black	Grey to white	Grey to white	Black
		Grey	Grey	Grey
		White	Red	Red
Tail	Thin	Thicker	Thicker	Thin
Teat	Thin	Thicker	Thicker	Thin

The Nguni cattle ecotypes in South Africa include Venda, Pedi, Shangaan, Bartlow and Makhathini (Bester *et al*., 2003) and these names are linked to geographic areas where they were distributed, related to tribal segregation and traditional historic existence (Matjuda, 2012). These populations are preserved and managed by stud farmers and governmental research stations in different South African provinces. The ecotypes are shown from Figure 2.3 – 2.6 presenting different phenotypes variation recognised by South African Nguni Society farmers.

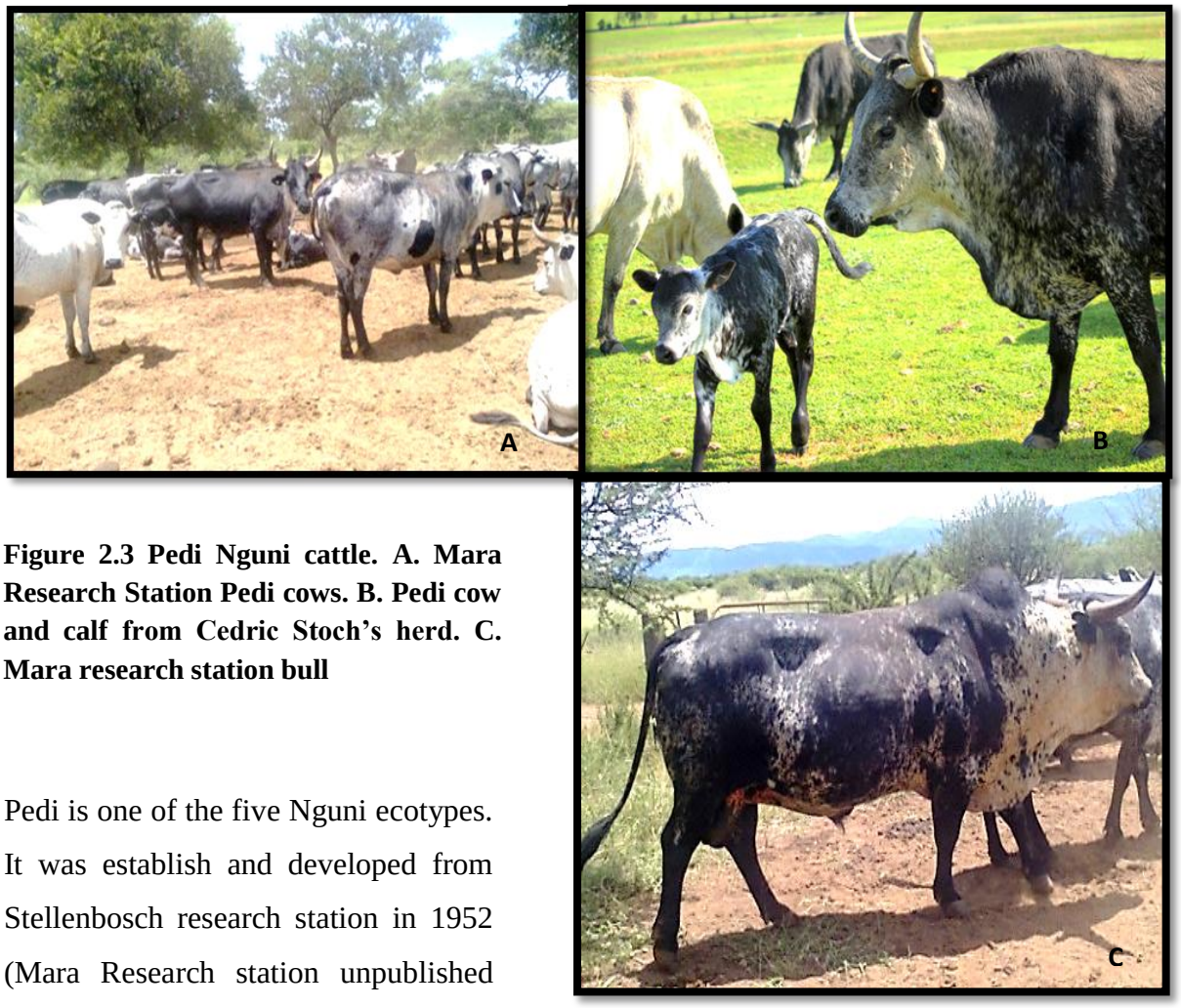

Figure 2.3 Pedi Nguni cattle. A. Mara Research Station Pedi cows. B. Pedi cow and calf from Cedric Stoch's herd. C. Mara research station bull

Pedi is one of the five Nguni ecotypes. It was establish and developed from Stellenbosch research station in 1952 (Mara Research station unpublished report). The Pedi type is the largest ecotype population group among five South African Nguni cattle ecotypes (http://www.tafelsigngunis.co.za/). These groups are adopted and conserved by few numbers of stud breeders in located in three provinces of the country including include Limpopo Department of Agriculture at Mara Research Station in Makhado, Venda; Mr De Beers in Balfour, Mpumalanga and Cedric Stoch in Malmesbury, Western Cape (Nguni Cattle Breeders Society, 2008). Pedi Nguni type is characterized with mainly three coat colour patterns that is grey, grey white and black. It has medium sized lyre shaped horns and large body frame (DAD-IS) (http://dad.fao.org/).

Shangaan Nguni cattle ecotype also known as Gazankulu type was established 38 years ago in Hartbees farm in Giyani in Limpopo province. The foundation herd included five herds from different rural communities (Mara Research Station, unpublished report). They were selected based on coat colour variation including white and black, red and white, white which appears with some form of poor pigmentation. The Shangaan ecotype is known for being highly fertile and females that ease in calving due to the large body frame than other ecotypes (http://dad.fao.org/).

Figure 2.4. A. Shangaan cows. B. Shangaan Bull of Mara Research station

Figure 2.5. A. Venda cow. B. Venda bull at Mara Research station

The Venda ecotype originated 30 years ago in Doppie farm used to be the government property but ownership was taken over by the community through land claim (Mara Research Station, unpublished reports). This population was established around Nguni cattle that were bought from the former Venda homelands communities and were selected on basis of coat colour patterns. The Venda ecotype is distributed mainly in Sibasa in the northern region of Limpopo Province (Mara Research Station, unpublished report). It differs from other ecotypes and mainly characterized by the medium body frame, short head and coat colour patterns varying from black; black and white to red and white (Nguni Cattle Breeders Society, 2008).

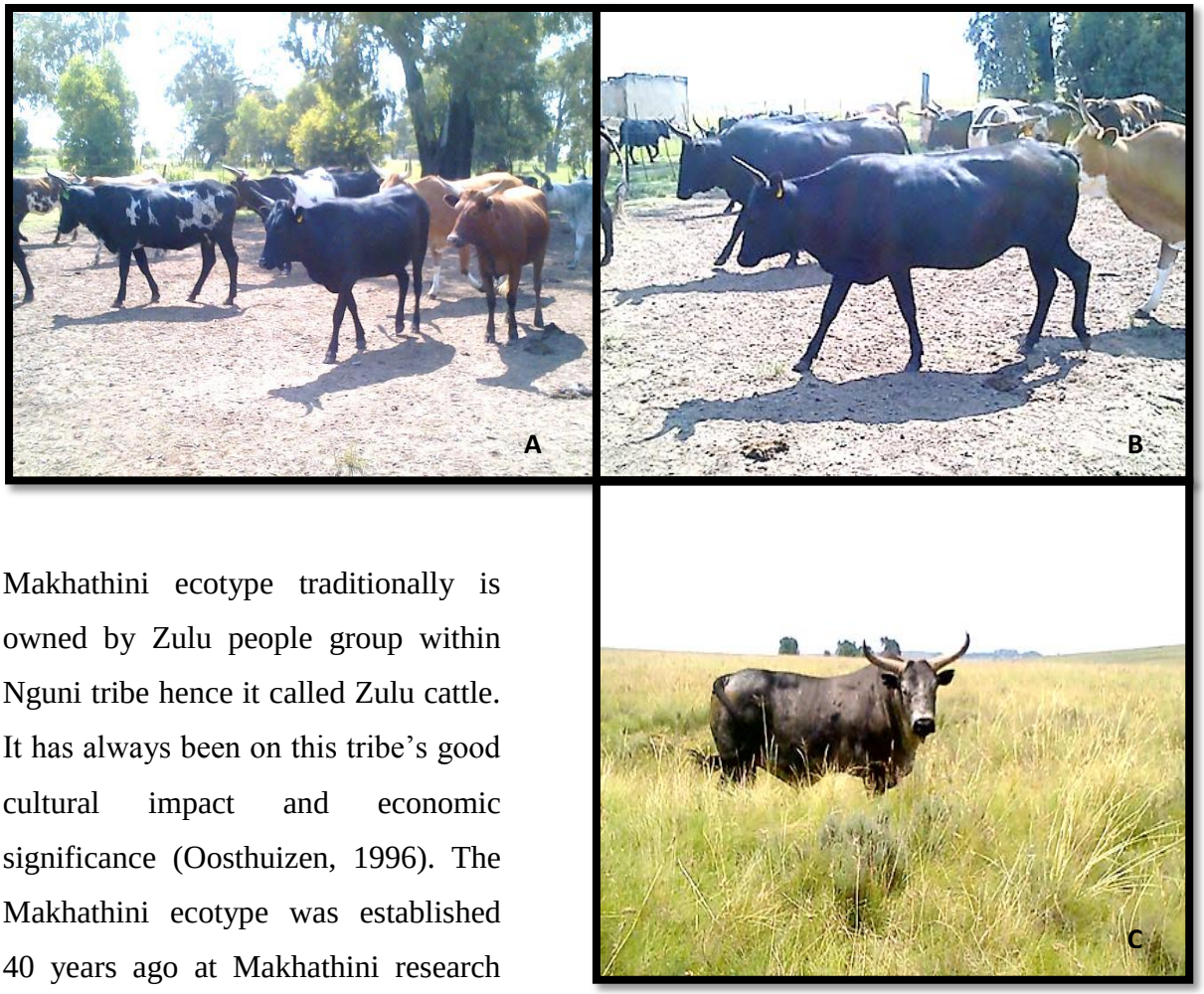

Figure 2.6. A-C. Zulu cattle at KwaMakhathini farm

Makhathini ecotype traditionally is owned by Zulu people group within Nguni tribe hence it called Zulu cattle. It has always been on this tribe's good cultural impact and economic significance (Oosthuizen, 1996). The Makhathini ecotype was established 40 years ago at Makhathini research station in north-east of KwaZulu Natal province. The Makhathini ecotype resemble all other Nguni cattle ecotypes coat colour variations. It is only distinct from other ecotypes by a small body size, "V" shaped short head and open lyre horns (Nguni Cattle Breeders Society, 2008).

Given the abovementioned phenotypic variation among Nguni cattle ecotypes it is essential that this variation is validated using the genotypic data in order understand the current Nguni ecotypes population structure in order to meet Domestic Animal Diversity Information System (DAD-IS) breeds' documentation strategies and regulations (FAO, 2012a; Bett *et al.*, 2013). The DAD-IS is the global communication and information system network that aims at implementing conservation strategies for management of animal genetic resources used for the production of food and agriculture (http://dad.fao.org/).

2.5. Maintenance of genetic diversity in livestock breeds

Genetic diversity is defined as the variety of alleles and genotypes present in a population. It is normally reflected by morphology, physiology and behavioural differences between individuals in a population (Meuwissen, 2009). It provides information on how livestock should be raised in a wide range of production environments and the basis of livestock populations adapting to future environmental changes (Taberlet *et al.*, 2008). The animal genetic resources diversity is an important component of the biological basis of agriculture and food production.

Livestock contributes about 30 percent of agricultural gross domestic product in developing countries by providing a wide range of products and services such as food, fibres, manure and draught power to serve millions of people. Although livestock has a major role in food safety and plays a role in sustainable production systems, the genetic diversity among livestock breeds is threatened by numerous factors resulting in declining numbers of livestock breeds (Scherf, 2000). These include the programs such as the new reproduction technologies i.e. artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) (Bett *et al.*, 2013); crossbreeding with exotic breeds and lack of breeding systems. The decline in diversity of AnGR is a major threat in food security hence maintaining the genetic diversity is essential (Taberlet *et al.*, 2008; FAO, 2011). Strategic measures to promote the sustainable use, maintenance and conservation of animal genetic resources require adequate details on diversity of Animal Genetic Resources populations (Scherf, 2013).

The maintenance of genetic diversity is important to prevent low performance that could be the result of inbreeding (Meuwissen, 2002). According to Engelsma (2012) the genetic diversity is important for two main reasons. Firstly, to develop within breed long-term genetic improvement for new traits. Secondly, to fulfil specific requirements that might be needed in future to support maintenance of genetic variation. The importance of genetic

diversity and maintenance for conservation has been emphasised in several studies (Hanotte & Jianlin, 2005; Taberlet *et al*., 2008; Groeneveld *et al*., 2010; Bett *et al*., 2013).

The FAO implemented two important strategies to facilitate the monitoring of AnGR at all levels. These have come with good outputs that indicate livestock trends globally. Firstly, the Convention on Biological Diversity (CBD) program which was initiated in 1992 to implement Global Strategy for the Management of farm animal genetic resources (FAGRs) (Scherf, 2000). This program was successful as it obligated countries to conserve their landrace breeds. Secondly, is the information system called the Domestic Animal Diversity Information System (DAD-IS) that aims at assisting countries by providing extensive searchable databases and guidelines for better characterization, utilization and conservation of animal genetic resources (Gibson *et al*., 2006)

Since FAO strategic plans, the current trends in the past twelve years communicated in third edition of World Watch document indicated that there are about 6 379 breed populations comprising of thirty mammalian and avian species in Global Databank for Farm Animal Genetic Resources (Figure 2.11) (FAO, 2012b; DAD-IS). The DAD-IS figures further indicated about 10 507 breeds of livestock globally, belonging to 37 domesticated species in 182 countries that have been documented. Out of that, there were 8054 breeds reported, 631 breeds classified as extinct and 7 transboundary breed (FAO, 2007b) in Africa and it has been identified that out of a total of 684 livestock breeds, 388 are non-descript breeds. Due to these reports, the documentation status of breeds is known and is applied for conservation strategies (Figure 2.7).

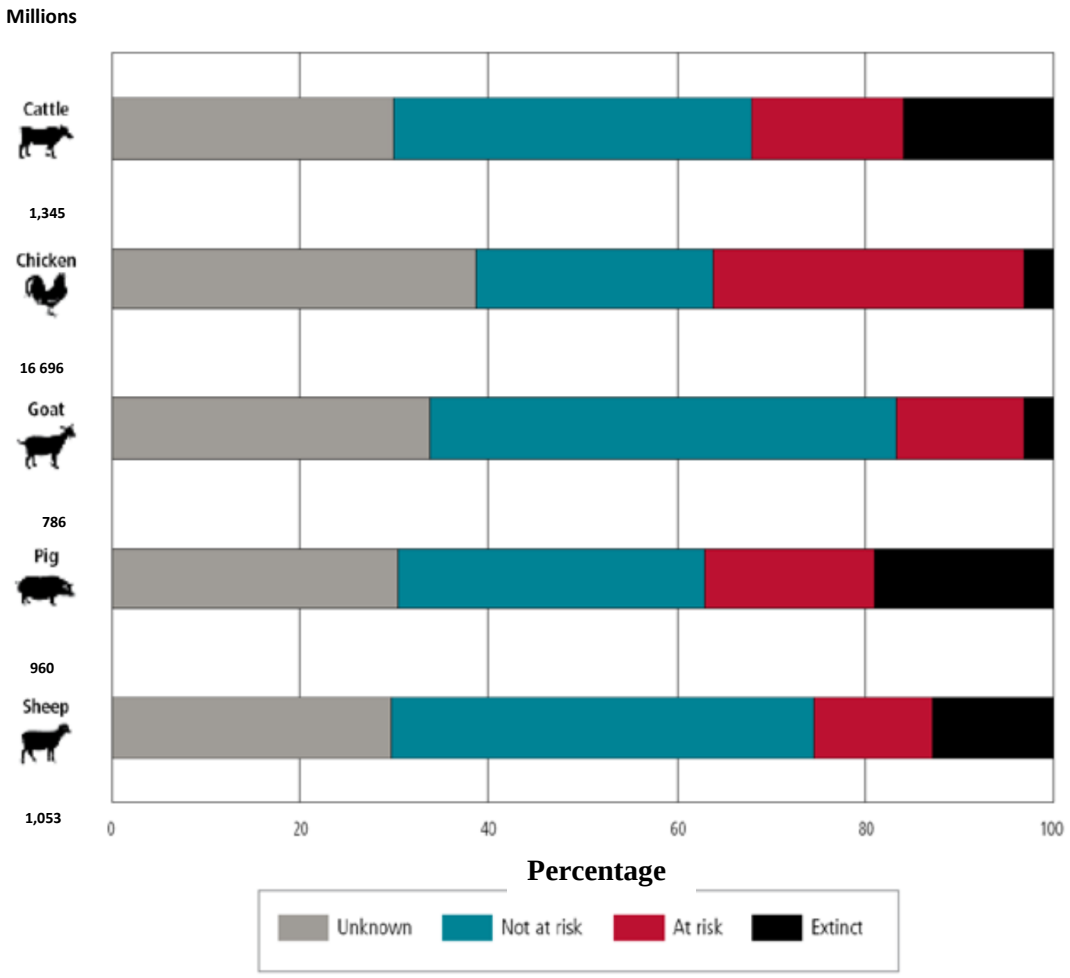

Figure 2.7. Global breed risk status of major livestock species (FAO, 2012a)

It is important to initially accumulate comprehensive breed's information in order to succeed in the management and conservation of Farm Animal Genetic Resources (FAO, 2012a). This includes population size, structure, ecological distribution and production management data (Groeneveld *et al*., 2010). This information is useful to determine the risk status of a specific breed or population as categorized in Table 2.4. This is because more domestic animal breeds are in danger of becoming extinct without being documented. Documenting the current status and possible future dynamics of livestock breeds is an important step in the management of African indigenous Animal Genetic Resources and to meet DAD-IS standards.

Table 2.4. Global FAO World watch categories for breeds risk status (Bodo, 1989; Scherf, 2000).

Risk status	Number of breeding ♀	Number of breeding ♂	Population size	Description
Extinct	0	0	0	No traces of the breed population found.
Critical	<100	<5	120	Reduced gene diversity, close to extinction and population increase is needed.
Endangered	100 - 1000	20	1200	In danger of extinction due to smaller population size.
Insecure	1-5000	-	-	Decline in population numbers.
Vulnerable	5-10 000	-	-	Rapid decline in population numbers.
Normal	10 000	-	-	No visible changes in population size.
Critical/Endangered -maintained	-	-	-	Populations conserved and maintained by stud breeders or in research institutes.

Conservation of endangered breed's genetic diversity is an important strategy to prevent erosion of Farm Animal Genetic Resources and is prioritized (Engelsma, 2012). FAnGRs conservation is globally emphasized to ensure proper utilization of indigenous breeds to keep the unique alleles, while simultaneously minimizing the loss of genetic diversity (Oliehoek *et al*., 2006). There is a vital need to investigate their genetic variation since most productive and adapted animals for specific environment must be selected for breeding purposes in that certain environment (Philipsson, 2000). There are different methods to conserve livestock, namely - *ex situ in vivo; ex in vitro* and *in situ*. These are all relevant when the breed is rare or near extinction (FAO, 2013).

In situ conservation is the preservation of livestock in their own habitat using specific breeding programmes. It is the preferred method of conservation where maintenance and management of the FAnGRs is the best available livelihood option for the societies involved

with less expensive costs (Gibson *et al.*, 2006). Keeping the breed in its adapted habitat justifies its cultural and socio-economic role, adaption in changing conditions and continues evolving (Engelsma, 2012). Therefore, *in situ* conservation should be established as a control to protect against loss of the FAnGRs. E*x in vivo* is defined as the management of animals out of their adapted habitat (off-farm or zoo); it is an alternative when an *in situ* is not possible in a small population faced by extinction. However, it does not guarantee greater maintenance of genetic diversity (Bett *et al*., 2013). This method of conservation is practised in Swaziland for the re-establishment of pure Swazi Nguni cattle ecotype that was eroded to a remnant of its size (Farmer's weekly, 2013). E*x situ in vitro* is the preservation of animal genetic information in the form of cryopreservation (semen or embryo) as a source of germplasm to preserve genetic diversity of breeds for future purposes (Engelsma, 2012).

The importance of genetic diversity maintenance is the main reason for FAO-UN proposed the programme for management of farm Animal Genetic Resources engaging prominence in use of molecular markers (Gibson *et al.*, 2006). The plan is to determine the genetic status, to assist for proper management and conservation of indigenous germplasm, endangered or critical status breeds (Bjornstad & Roed, 2001; Singh *et al.*, 2014).

2.6. Genetic characterization of livestock using molecular markers

The improvement of livestock was traditionally based on phenotypic data selection (Teneva, 2009). The first work on detection of genomic variation was done based on morphological, chromosomal and biochemical markers (Walsh, 2000). Currently, selection using genotype has become an important tool in the breeding of livestock. The use of molecular data in population genetic diversity studies is increasingly becoming common (Vignal *et al*., 2002). Molecular markers have a number of valuable characteristics compared with phenotypic and quantitative traits as these are not influenced by environmental effects, heterogeneity, pleiotropy and complex gene interactions (Ajmone-Marsan *et al*., 2001).

Over time, a wide of range molecular markers have been mapped and classified. There are three categories of molecular markers (Lenstra *et al.*, 2012). These include mitochondrial DNA sequences (mtDNA) maternal lineage (White *et al.*, 2008); Y-chromosomal paternal lineage (Boettcher *et al*., 2010) and autosomal Mendelian (biparental) markers (Mburu & Hanotte, 2005). The different autosomal markers have been developed, classified and utilized (Lenstra *et al*., 2012). They are classified as single and multi-locus markers (Toro *et al.*, 2009). Multilocus markers include Amplified Length Polymorphism (AFLPs), Restriction

Fragment Length Polymorphism (RFLPs) and Variable Number Tandem Repeats (VNTPs) known as minisatellite whereas single locus markers include microsatellites also known Simple Sequence Repeats (SSRs), Random Amplified Length Polymorphic DNAs (RAPDs) and Single Nucleotides Polymorphisms (SNPs) (Erhardt & Weimann, 2007). Currently, microsatellite markers and SNP's are the most commonly used markers; these differ in genetic information, interpretation and standardization (Vignal *et al*., 2002).

Single Nucleotide Polymorphism (SNP) is the variation in the DNA sequence that occurs when a single nucleotide (A, T, C or G) in the genome is altered and changes the DNA sequence, that is, in a stretch of DNA (A**A**GGCTAA to A**T**GGCTAA), the single base pair substituted creates a SNP (Brookes, 1999). The SNP markers are captured in a DNA arrays or DNA based "chips" (Lenstra *et al*., 2012). After the identification of thousands and millions of SNPs these markers have superseded other markers for many applications. Currently, commercial panels of 50 000K and higher (770K) are available (Bovine HapMap Consortium *et al.*, 2009; Mutumakali *et al*., 2009; Lenstra *et al*., 2012). It is estimated that three to eight bi-allelic SNPs are as informative as one microsatellite locus (Lenstra, *et al*., 2012). This has resulted in new opportunities to estimate genetic diversity in detailed way to contribute to available methods of studying genetic diversity (Engelsma, 2012). The analysis of this marker type is based on a number of modern highly effective approaches, in particular, application of DNA microarrays. SNP markers has been currently used in studies ranging from gene expression for the identification of single nucleotide polymorphisms (SNPs) or differences in DNA sequences amongst genotypes and Genome Wide Association studies (Wang *et al*., 1998; Hayes *et al*., 2009). Compared to other types of DNA markers, the use of SNPs allows automated analysis and enhances the efficiency of genotype analysis by an order of magnitude (Khlestkina & Salina, 2006). SNP markers have less alleles per marker that results in less information and therefore thousands of SNPs need to be genotyped in order to provide adequate information (Lenstra *et al*., 2012). Compared to microsatellite markers, SNP markers use more DNA. In addition, commercial SNP chips were developed in mapping information from exotic breeds and indigenous stock was not included, resulting in less information about indigenous breeds (Wollstein *et al*., 2010).

Microsatellites markers are DNA sequences consisting of short tandem repeats (STR) of 1–6 base pairs of nucleotides, occurring throughout the genome but likely in noncoding regions (Zane *et al*., 2002). The repeated units can be mono, di, tri, tetra, penta or hexa nucleotides

and di is the most repeated hence known as Short Sequence Repeats (SSR) (Tautz & Schlotterer, 1994). Microsatellite markers are among the most versatile and popular genetic markers, being used in livestock genetic diversity studies (Dorji & Daugjinda, 2014). This is due their genetic codominance, abundance, dispersal throughout the genome, multi-allelic variation, high reproducibility and high level of polymorphism that is easy to interpret (Mburu & Hanotte, 2005). This high level of polymorphism is due to mutation affecting the number of repeat units. It is these characteristics that make microsatellites become popular markers in association studies, population genetics, and forensics (Erhardt & Weimann, 2007).

Microsatellites have several advantages. A small amount of template DNA is used; they are easily amplified by PCR and can be used in wide range of sample material such as blood, hair, meat and skin. Furthermore, their genetic systems are easily automated enabling the analysis of a large number of samples (Erhardt & Weimann, 2007). Allele sizes can be determined with high accuracy; comparison across different gels possible using size standard and capable of detecting genetic variation and revealing polymorphism existing among individuals in the population (Beaumont & Bruford, 1999). They can prove some degree of linkage of the QTL affecting the trait and the marker (Vignal *et al*., 2002). However, disadvantages about microsatellite markers is that, heterozygotes may be misclassified as homozygotes when null-alleles occur due to mutation in the primer annealing sites and stutter bands may complicate accurate scoring of polymorphisms (Lenstra *et al*., 2012). Microsatellite markers provide useful molecular data (MacHugh *et al*., 1997). The analysis provides information with regard to allele frequency differences among populations and cladistics relationship between alleles and group of alleles by comparing the differences in allelic repeat length (Uggla, 2008). Due to this, they have been used in a wide range of genetic diversity studies with recommendation received from the ISAG and FAO (FAO, 2007a). The ISAG/FAO society endorsed a list of 30 standardized bovine microsatellite markers available at (http://dad.fao.org/) (FAO, 2011). To date, there is a wide range of genetic diversity studies that have been piloted globally. Table 2.5 provides a summary of genetic characterization studies performed based on microsatellite markers in number of indigenous cattle breeds globally including in the Sub Saharan region.

Table 2.5. Global genetic characterisation studies of the cattle breeds.

Cattle breeds	Title of study	References
East African cattle breeds	Characterization of genetic diversity of East African cattle breeds using microsatellite DNA markers.	Adhiambo., 2002
Aberdeen Angus cattle breeds (Brazil).	Genetic characterization of Aberdeen Angus cattle using molecular markers.	Vasconcellos *et al*., 2003
China Native cattle breeds (China).	Analysis of Microsatellite DNA Polymorphisms in Five China Native Cattle.	Hai-Guo *et al*., 2005
Kherigarh cattle breed (India).	Genetic diversity studies of Kherigarh cattle based on microsatellite markers.	Pandey *et al*., 2006
Burlina breed (Italy).	Genetic characterization of the Burlina breeds using microsatellite markers.	Dalvit *et al*., 2008
Ankole Longhorn cattle (Kenya).	Genetic and morphological characterization of the Ankole Longhorn cattle in the African Great Lakes region.	Ndumu *et al.*, 2008
Angone, Landim and Bovino de Tete (Mozambique).	Genetic diversity and relationship among indigenous Mozambican cattle breeds.	Bessa *et al*., 2009
Portuguese and Spain breeds (Spain).	Genetic diversity, structure and breed relationships in Iberian cattle.	Martin-Burriel *et al*., 2011
Ankole cattle (Uganda)	Genetic diversity and differentiation of Ankole cattle populations inferred from microsatellite data.	Kugonza *et al*., 2011
Gir and Kankrej breeds (India).	Genetic diversity of indigenous cattle population of Rajasthan using microsatellite markers.	Upreti *et al*., 2012
Cuban Creole (Cuba)	Genetic characterization and differentiation of five Cuban cattle using 30 microsatellite markers.	Acosta *et al*., 2013
Iberoamerican cattle (Portugal)	Analysis of conservation priorities of Iberoamerican cattle based on autosomal microsatellite markers.	Ginja *et al*., 2013
Cameroonian indigenous cattle (Cameroon)	Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers.	Ngono Ema *et al*., 2014
Afrikaner (South Africa)	Genetic diversity in the Afrikaner cattle breed	Pienaar, 2014

These aforementioned studies have evidently proved microsatellite markers as the useful tool to study the genetic diversity within and between cattle population breeds globally. Hence, they have been chosen to generate genotypic data in evaluating the genetic diversity of South African Nguni cattle ecotypes in order to make a decision with regard to ecotypes conservation.

2.7. Statistics for molecular characterization

The recent developments in molecular tools and computerized techniques have made it easy to investigate the genetic diversity among and between populations in order to understand the influence of evolution, mating systems and breeding techniques. Genetic variation is measured by estimating the basic population genetic descriptive statistics for each marker and population. This includes estimating the mean number of alleles, gene frequency, heterozygosities (observed and expected) (Park, 2001), the exact test of Hardy – Weinberg Equilibrium (Toro & Caballero 2005), genetic distances, viewing of population structure in allele frequencies (Nei, 1987), Wright's fixation indices (Wright, 1969) and Bayesian structure cluster analysis (Pritchard *et al.*, 2000).

Heterozygosity is the main parameter used to describe or understand genetic variation at single locus or at number of loci and assuming that alleles were randomly chosen in different populations (Nei *et al.*, 1983). Additionally, it is a good predicator of chances for long-term survival of a population and also reflects the number of genetic options available within a population (Hedrick, 2005). The heterozygosity is also called as the gene diversity and the measure ranges from 0 – 1 (Mburu & Hanotte, 2005). High expected heterozygosity (H_E) value is an indicator of long-term natural population adapted in an environment with mixed nature of the breeds or mixing of strains of different populations whereas low level of expected heterozygosity value may be due to isolation and genetic drift resulting in loss of genetic diversity (Ojango *et al.*, 2011).

The observed heterozygosity (H_O) is the percentage of loci heterozygous per individual of individuals heterozygous per locus. When observed heterozygosity is lower than expected ($H_O<H_E$) this might be due to forces such as inbreeding resulting in deficit of heterozygotes; when the observed is higher than expected ($H_O>H_E$) then that might be due to the mixing of two previously isolated populations and when $H_E=H_O$ the population is likely to be in random mating (Mburu & Hanotte, 2005).

Mean number of alleles (MNA) normally corresponds with heterozygosity parameter when the genetic variation is examined and well used as determinant of allelic richness. Similarly, high MNA represent great allelic genetic diversity whereas low MNA implies low genetic variation (Dorji & Daugjinda, 2014). Therefore, heterozygosity and MNA are called the genetic diversity parameters that in parallel and also heterozygosity is estimated in a large number of alleles. These aforementioned parameters can be calculated using a variety of computer programs such as FSTAT (Goudet, 1995); Genetix (Belkhir *et al*., 1996-2004); Microsatellite Tool kit (Park, 2001); R-package Microsatellite Analyzer (Dieringer & Schlstterer, 2003) and also GenAlex (Peakall & Smouse, 2006). Allele frequencies and private alleles can be calculated manually by direct counting from total alleles (Ojango *et al*., 2011).

However, heterozygosity is always compared to what is expected under Hardy-Weinberg equilibrium (HWE). The law of HWE states that gene and genotype frequencies remain constant from generation to generation with underlying conditions that the population is not under any genetic force as it may result in an increase or decrease in heterozygotes of the population (Dorji & Daugjinda, 2014). Accordingly, deviation of population from HWE is an indication of possible inbreeding and problems with genotyping. The test of population from HWE deviation can be performed using any of the following three methods. These include chi square (Deka *et al*., 1995; Rousset & Raymond, 1997), likelihood ratio test criterion (G statistics) (Deka *et al*., 1995) and Fisher's exact test (Weir, 1996). Computer programmes like GenAlex, Genepop, FSTAT and Arlequin can be used to estimate HWE exact test of deviation (Ojango *et al*., 2011).

Genetic differentiation within and between sub-divided populations can be quantified using two approaches namely – Wright's F-statistics coefficient (Three Fixation indices) and AMOVA (Analysis of Molecular Variance). AMOVA is used to describe the partitioning of genetic differentiation between and within breeds or populations and to test user defined grouping populations (Toro *et al*., 2009). It differs from Analysis of variance (ANOVA) because of hierarchically arranged data; mean squares are computed for populations at hierarchy levels. Wright's F-statistics is an important tool that provides insight on the evolutionary forces that influenced the structure of genetic variation within and among populations (Wright, 1942). The most used metrics to detect genetic differentiation are F-statistics developed in a conceptual and mathematical framework to describe the distribution of genetic variation within populations using the series of inbreeding fixation indices (F_{IS}, F_{IT}

and F_{ST}). Fixation indices are parameters to determine breeds diversity; to analyse the degree of subpopulation division and breeding. The fixation indices are calculated as summarized in Table 2.6. These fixations are interpreted based on allele frequencies distribution among the populations. The F_{IS} coefficient of an individual within a subpopulation and is also known as genetic inbreeding coefficient (Toro *et al*., 2009). It indicates whether individuals of the subpopulation are under non-random mating system or not and therefore, denotes the degree of HWE departure in subpopulations due to genetic inbreeding (Dorji & Daugjinda, 2014). The F_{IS} values range from −1 to 0 outbreeding to a maximum of 1 inbreeding (Norberg & Sørensen, 2007; Dorji & Daugjinda, 2014). The F_{IT} coefficient is an overall inbreeding index of an individual within the total population. It is rarely being used under HWE deviation in total population and thus combines contributions from non-random mating with populations and effects of random drift among populations. Similarly, it also ranges from −1 to 0 outbreeding to a maximum of 1 inbreeding (Dorji & Daugjinda, 2014). The F_{ST} is the coefficient of a subpopulation within the total population (Hanotte & Julian, 2005). It is a widely used index to evaluate the degree of genetic differentiation between subpopulations based on allele frequency in determining the existence of gene flow and more to detect the effect of genetic drift relative to other evolutionary forces (Kalinowski, 2002). The F_{ST} coefficient ranges from 0 to 1 (Norberg & Sørensen, 2007). The F_{ST} value ranging between 0-0.05 indicates little genetic variation; 0.05-0.15 moderate genetic variation; 0.15-0.25 great variation and a value above 0.25 indicates a high genetic variation (Wright, 1978).

Table 2.6. Fixation indices formulas (Dorji & Daugjinda, 2014).

Fixation index	Formula
$\boldsymbol{F_{IS}}$	$\frac{(H_s - H_I)}{H_s}$
$\boldsymbol{F_{IT}}$	$\frac{(H_T - H_I)}{H_T}$
$\boldsymbol{F_{ST}}$	$\frac{(H_T - H_S)}{H_T}$

H_s = mean expected heterozygosity within random mating subpopulations.

H_T = expected heterozygosity in random mating total population.

H_I = mean observed heterozygosity per individual within subpopulations.

Genetic population structure and cluster analysis can be assessed by using developed and implemented computer program STRUCTURE (Pritchard *et al*., 2000). This program is model-based cluster analysis hence is called Bayesian clustering analysis. It is used to infer subpopulations, to reveal the underlying evolutionary history and admixture among populations using multilocus genotypic data (Ojango *et al.*, 2011). The STRUCTURE is implemented in a way that assumes a model has *K* populations that are unknown and each is characterized by a set of allele frequencies at each locus (Evanno *et al.*, 2005). The clustering analysis includes the calculation of pairwise distance matrices, whose entries give an estimate of the distance between populations indicating the degree of closeness in gene frequency (Nei & Rhochoudhury, 1974). When the allele frequencies are similar the genetic distance approaches zero (Frankham *et al.*, 2004). Genetic distance estimation based methods have been widely used as a measure of genetic differentiation among populations but Nei's genetic distance has been the ideal approach (Nei, 1972).

2.8. Conclusion

Genetic characterization of indigenous livestock breeds is an acknowledged component of biodiversity. It is the first step prior to conservation to save unique genetics and to ensure proper utilization of the indigenous breeds. The description of Nguni cattle ecotypes was principally based on remarkable phenotypic measurements. Therefore, it was vital to validate Nguni cattle ecotypes phenotypic measurements with genotypic data in order to establish an appropriate management and conservation strategy.

CHAPTER 3

MATERIALS AND METHODS

3.1. Introduction

In South Africa there are five Nguni cattle ecotypes. Due to resource constraints only four South African Nguni cattle ecotypes were studied. Four Nguni cattle ecotypes and two Nguni cattle populations treated as genetically unknown entities were molecularly characterized using 22 microsatellite markers. These markers are recommended for genetic diversity studies by Food and Agricultural Organization of the United Nations and the International Society for Animal Genetics Advisory Group (FAO, 2011). The South African Nguni ecotypes characterized include Makhathini (MAK), Pedi (PED), Shangaan (SHA) and Venda (VEN) ecotypes whereas the unknown populations considered were the Nguni purebred herds located at Agricultural Research Council-Animal Production Institute in Loskop South farm, Limpopo and at the University of Fort Hare, Eastern Cape. The study was performed according and with approval of the Animal Ethic Committee (AEC) under the University of Pretoria (EC111-13).

3.2. History of the populations used for the study

Limpopo Department of Agriculture in Mara Research Station is a "home" to three South African Nguni cattle ecotypes. These include Pedi, Shangaan and Venda and have been preserved in Mara for more than about 20 years and kept separately to prevent uncontrolled mating or gene flow. These ecotypes' history could be traced to different farms in Limpopo province with each ecotype having been selected based on coat colour. Pedi is the most numerous of the three ecotypes in Mara Research Station.

The Pedi ecotype originated from Sekhukhune district situated in the Southern region of Limpopo province. The first group dates back to 1952 in Stellenbosch Research Station with 108 cows and eight bulls from different farms. These were 50 cows from Blouberg, Gopeng and Moloto; 25 from Potgietersrus, and 25 from Blouberg and eight bulls with mainly grey and white and black coat colour. In 1961 the herd had grown to 555 cattle with 189 breeding cows. The first group of Pedi animals consisting of 10 cows and one bull arrived in Mara

Research Station about 20 years ago and the second group of 20 females and two bulls arrived in 2006. Currently, the population has grown to 99 breeding females and 6 bulls.

The breeding of Shangaan ecotype started in 1976 at Hartebees farm owned by then Gazankulu government of Giyani. The population had 10 breeding lines that originated from Mashawa and Magwena farms, comprised of 9 lines and 1 poor pigmentation line. Shangaan ecotype was brought to Mara Research Station approximately 18 years ago with 10 cows and two bulls and the population currently consists of about 52 breeding females and two bulls.

Venda ecotypes originated from Doppie farm back then owned by the former Venda government under the cabinet memo 40/84 in 1984. In 1985 major farm development was done and Venda Nguni cattle were bought from farms within Venda districts. Five breeding lines were identified according to area of origin (i.e. Mulenzhe, Mangaya, Tshimbupfe, Vyeboom and Mutale areas). They were brought to Mara Research Station approximately 20 years ago in a group of 10 cows and three bulls. Currently, the population has grown to 50 breeding females and two bulls.

Another Pedi population included in the study was taken from Mr De Beer's farm in Balfour. His herd comprises of 300 breeding females and 17 males. Some of Mr De Beer's Pedi Nguni's originated from Stellenbosch Research Station and Mr Cedric Stoch in the Western Cape (a prominent Nguni stud breeder) and his herd was herd was originally sourced from five herds across Swaziland (Horsburg *et al*., 2013).

Makhathini ecotype is kept by Mr Barry and Naledi Roberts at Kwa Makhathini Farm in Reitz, North-East of Free State. This herd comprises of 400 breeding females and 20 bulls. They were bought from private communal farmers of Northern KwaZulu Natal Province from the districts of Jozini, Hluhluwe and Nongoma and other farms around South Africa. Some Makhathini types are found in Amajuba Stud in Newcastle maintained and managed by Mr Mohammed. In Mpumalanga at Ermelo there is another Makhathini population conserved by Mr Mike Greyling with MAK brand mark standing for Makhathini Nguni's. There is a relationship between these three aforementioned populations hence samples from Roberts and Amajuba herds were used for this study.

The University of Fort Hare Nguni stud population dates back to 1968 from E.A Reilly's herd from Swaziland. Since the 80's the herd has been using bloodlines from different farms mostly from KwaZulu Natal (KZN) and Eastern Cape provinces. These farms include LBC

Biggs Trust and Mr and Mrs Roberts MAK population in KZN as well as some farmers around Eastern Cape whom their herd's origin some are traced to KwaZulu namely - Hobbs PM, Lovemore GA and Son, Peet Steenkamp Family Trust, Slaagboom Nguni's and Peet Steenkamp Family Trust. The University of Fort Hare Nguni population played a prominent role in the Eastern Cape rural areas Nguni cattle conservation programme funded by Industrial Development Corporation (IDC) in agreement with Eastern Cape Department of Rural Development and Agrarian Reform (ECDRDAR) (Somoro, 2009). The project started in 1990 with two community villages in Amathole regions through funding provisioned by the Norwegian government and it was facilitated by Prof Jan Raats. It was aimed at empowering communal and small-scale enterprises in the rural areas to practise *in situ* conservation. Communities were provided with ten pregnant female and two bulls and after five years have to return back the original stock. To date, the project has established 72 nucleus herds managed at communal and small-scale enterprises (Tada *et al*., 2013).

The Loskop Nguni cattle population is a conservation herd belonging to the Agricultural Research Council – Animal Production Institute, Limpopo Province. The population could be traced to few cows and bulls that were bought from Bartlow Combine Research Station in KwaZulu Natal approximately 25 years ago. Presently, the population consists of 45 bulls and approximately 1200 breeding females.

All these aforementioned populations participate in animal recording programme. They are registered with the South African Nguni Cattle Breeders Society. The areas where the samples were collected are depicted in Figure 3.1.

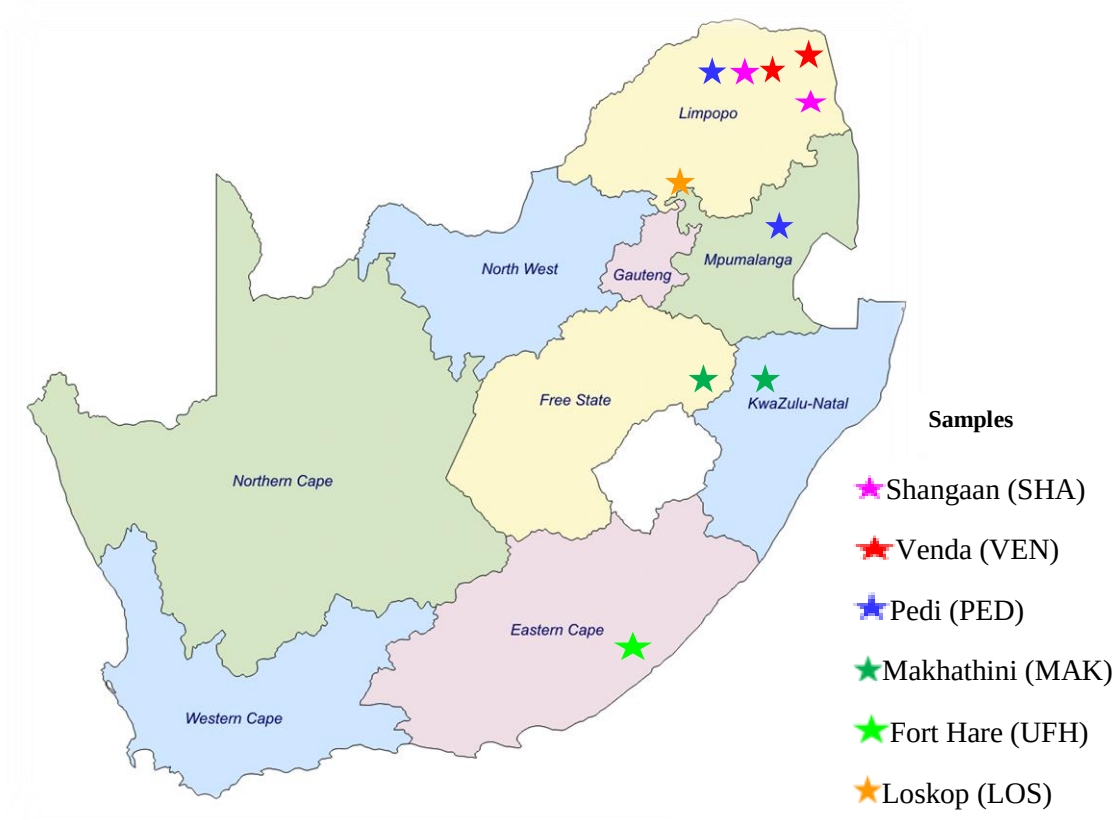

Figure 3.1. South African map showing the geographic distribution of Nguni cattle genotypes sampled for the study

3.3. Study cohorts and geographic descriptions

All farmers were interviewed regarding the origin of their Nguni cattle herd populations. Sample collection was piloted in five South African provinces, stationed as indicated in Table 3.1.

Table 3.1. Districts where Nguni ecotypes were sampled

District	Site	Ecotype	N	Owner
Vhembe	Mara Research Station	Pedi (PED)	17	Limpopo Department of Agriculture.
		Shangaan (SHA)	20	
		Venda (VEN)	19	
	Makhado	Venda (VEN)	12	Private farmers.
Mopani	Giyani	Shangaan (SHA)	10	Private farmers.
Gert Sibande	Balfour	Pedi (PED)	15	Mr De Beers.
Thabo Motsunyana	Rietz	Makhathini (MAK)	30	Mr & Mrs Roberts.
Amajuba	Newcastle	Makhathini (MAK)	2	Mr Mohammed.
Sekhukhune	Loskop	Unknown (LOS)	32	ARC-API (Outstation)
Amathole	Alice	Unknown (UFH)	32	University of Fort Hare.

N=Number of samples

The geographical description of the various regions where samples were collected is presented in Table 3.2. The table shows the location, gps coordinates, altitude, annually temperature variation, biomes (Bushveld, Grassland and False Thornveld) and mean annual rainfall (www.saexplorer.co.za/south-africa/climate).

Table 3.2. Geographic descriptions of sampled locations for Nguni cattle genotypes (www.saexplorer.co.za/south-africa/climate)

Province	Location	Coordinates	Vegetation type (Acocks,1975; 1988)	Altitude (m)	Temperature	Mean rainfall (mm)
Limpopo	Mara	23°.05' South and 29°.25' East.	Bushveld	850-1200	20.2°C in Jun to 30.0°C in Jan.	495
Limpopo	Giyani	23°.31' South and 30°.70' East.	Bushveld	300-600	23.9°C in Jun to 31.0°C in Jan.	421
Limpopo	Loskop South	25°.18' South and 29°.20' East.	Bushveld	950-1300	20.9°C in Jun to 28.9°C in Jan.	506
Mpumalanga	Balfour	26°.65' South and 28°.58' East.	Grassland	1580-1860	16.2°C in Jun to 26.5°C in Jan.	568
Free State	Reitz	27°.80' South and 28°.43' East.	Grassland	1460-1800	15.5°C in Jun to 26.1°C in Jan.	565
KwaZulu Natal	Newcastle	27°.70' South and 29°.09' East.	Grassland	1100-1540	19.5°C in Jun to 27.6°C in Jan	687
Eastern Cape	Alice	32º.46' South and 26º.50' East.	False Thornveld	480-535	19.0°C in Jun to 27.6°C in Jan	535

3.4. Sample collection and preparation

A total of 189 unrelated adult cattle selected from pedigree records were sampled for hair and blood samples. During sampling, the animals were clamped gently in hospital pens. Hair samples were collected from the tail by pulling towards the animal to obtain good quality root hairs and kept in different envelopes to prevent contamination. Whole blood samples were collected by puncturing jugular vein from the neck using 6ml vacutainer tubes containing the anticoagulant ethylenediaminetetra-acetic acid (EDTA) combined with veterinary collection needles. Precautions were taken during blood collection; tubes were kept at 4°C and after collection, were transported to the laboratory. When the samples reach the laboratory, twenty root hairs were cut into 1.5 ml eppendorf tubes for Phenol chloroform extraction whereas blood samples were aliquated into 2 ml cryo tubes, stored at -22°C DNA isolation. Hair samples were primarily used in the study while blood samples were used as reserve in case insufficient DNA was obtained from hair samples.

3.5. DNA extraction

Genomic DNA (gDNA) was isolated from 20 root hairs per sample using modified Phenol chloroform protocol (ARC - Animal Genetics laboratory) (Sambrook *et al*., 1989) while DNA from 200 µl blood was extracted using Roche High Pure PCR Template Preparation kit (Roche, IN, USA). The concentration of the genomic DNA was measured using spectrophotometer (Nanodrop 2000) and the purity was verified by the 260/280 absorbance ratio (Thermo Fisher Scientific Inc., Waltham, MA, USA). The quality of the gDNA was inspected using 1% agarose gel electrophoresis in Animal breeding and genetics laboratory at University of Pretoria. Three microliter of DNA and 2 µl of loading dye were mixed, loaded in each well of the gel and subjected to the electrophoresis stained with ethidium bromide. DNA bands were visualised under Ultraviolet (UV) trans – illuminator and photos were taken. The samples were thereafter stored at -22°C until further step of Polymerase Chain Reaction (PCR).

3.6. Marker selection

A bovine microsatellite marker panel of 30 markers recommended by the ISAG and Food and Agricultural Organisation of the United Nations advisory board (FAO, 2011) were used to select 22 microsatellite markers (Table 3.3). These markers were selected, optimized and designed into two plexes with consideration for annealing temperature, fragment size and four dyes fluorescent labels including FAM (Blue), VIC (Green), NED (Yellow) and PET (Red) provided by Life Technology (Applied Biosystems, CA, USA).

3.7. Polymerase Chain Reaction and genotyping

PCR and genotyping were performed in Animal breeding and genetics laboratory at the University of Pretoria. The PCR was carried out in volume of 15 µl per sample. The reaction was prepared with 6.1 µl molecular water; 3 µl buffer optimized with MgCl2 and deoxynucleotides triphosphates (dATP, dCTP, dGTP and dTTP) (Bioline, USA, Inc.); 0.6 µl primers (reverse and forward) and 0.3 µl Bioline MyTaq DNA polymerase® (Bioline, USA, Inc.). The amplification of the DNA samples was performed using Perkin Elmer Gene Amp PCR System® 9700 Thermo cycler (Applied Biosystems, Foster city, CA, USA). It was programed to run bovine amplification. This was 94° C for 10 minutes; 3 steps at 33 cycles per loci (denaturation at 94° C for 45 seconds, specific marker annealing temperature for 1.5 minutes and replication at 72 ° C for 60 seconds) and final extension with time depended on marker and holds at 4 ° C. After amplification, the PCR products were quantified using 3% agarose gel stained with ethidium bromide and electrophoresed for 15 min in tank buffer; visualised under UV trans – illuminator and photo was taken.

Genotyping was performed by diluting the PCR products with de-ionized water. Formamide was mixed with GeneScan™ – 500 Liz® size standard (Life technology and Applied Biosystems, Foster city, CA, USA). The mix of 9 µl and 1 µl of fragmented DNA were loaded in 96 well plate. The samples were denatured at 95°C for 3 minutes and immediately chilled at 4°C using Perkin Elmer Gene Amp PCR System 9700 Thermo cycler (Applied Biosystems, Foster City, CA, USA). The amplicons were separated and determined by capillary electrophoresis using ABI PRISM 3130 automatic sequencer (Applied Biosystems, Foster city, CA, USA) at the Forestry and Agricultural Biotechnology Institute (FABI). The data was captured using GeneScan 3.1™ software (Applied Biosystems, CA, USA) and imported to GeneMarker 1.95™ software (Applied Biosystems, CA, USA) for analysis. The allelic table was created from the analyzed data and exported to Microsatellite Tool Kit (Park, 2001).

3.3. Characteristics of the microsatellite markers selected for the study

Primer name	Allelic Range	Primer sequences	T_A	Chromosome number	Dye	References
HEL1	98-112	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	55	15	6-FAM	Bishop *et al*. (1994)
INRA63	162-184	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	55	18	6-FAM	Vaiman *et al*. (1994)
ETH185	220-238	TGCATGGACAGAGCAGCCTGGC GCACCCCAACGAAAGCTCCCAG	55	17	6-FAM	Steffen *et al*. *(*1993)
TGLA126	116-128	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	60	20	VIC	Georges & Massey (1992)
HEL5	150-168	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	55	21	VIC	Bishop *et al*. (1994)
ILSTS006	282-302	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	55	7	VIC	Brezinsky *et al*. (1993)
INRA37	122-130	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	55	10	NED	Vaiman *et al*. (1994)
ETH152	160-170	AGGGAGGGTCACCTCTGC CTTGTACTCGTAGGGCAGGC	55	5	NED	Steffen *et al*. *(*1993)
INRA23	183-217	GAGTAGAGCTACAAGATAAAC TAACTACAGGGTGTTAGATGAACTCA	55	3	NED	Vaiman *et al*. (1994)
CSRM60	92-120	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	50	10	PET	Baylor Collage of Medicine Human Genome Sequencing Center (2006)
CSSM66	179-199	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	55	14	PET	Barendse *et al*. (1994)

TGLA227	79-99	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	50	18	6-FAM	Georges &Massey (1992)
TGLA122	135-163	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	60	21	6-FAM	Georges & Massey (1992)
ETH10	207-223	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	60	5	6-FAM	Solinas *et al*. (1993)
INRA35	100-104	ATCCTTTGCAGCCTCCACATTG TTGTGCTTTATGACACTATCCG	55	16	VIC	deGortari *et al*. (1998)
ETH225	137-159	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	60	9	VIC	Steffen *et al*. *(*1993)
ETH3	113-125	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	60	19	NED	Solinas *et al*. (1993)
TGLA53	152-188	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	50	16	NED	George & Massey (1992)
BM1818	255-269	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	60	23	NED	Bishop *et al*. (1994)
BM2113	120-144	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	60	2	PET	Sunden *et al*. (1993)
BM1824	182-196	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	50	1	PET	Barendse *et al*. (1994)
SPS115	244-262	AAAGTGACACAACAGCTTCTCCAG AACGCGTGTCCTAGTTTGGCTGTG	60	15	PET	Baylor Collage of Medicine Human Genome Sequencing Center (2006)

3.8. Computation and statistical analysis

Microsatellite toolkit was used to calculate genetic descriptive statistics per locus and population (Park, 2001). These were heterozygosities (H_O) and (H_E) values, allele frequencies, total number of alleles, mean number of alleles (MNA), private alleles and polymorphic information content (PIC) values. Convert version 1.31 computer program was used to translate diploid genotypic data from Microsatellite toolkit to different input format files for other population genetic software packages (Glaubitz, 2004). The exact test of deviation from HWE per locus for all populations was conducted using GenePOP version 4.0 (Raymond & Rousset, 1995). Wright's statistics (F_{IS}, F_{ST}, F_{IT}) and breed differentiation detected by locus under the step-wise mutation model (R_{ST}) were calculated for each locus and across the genome using FSTAT version 2.9.3.2 (Goudet, 2002). Weir and Cockerham (1984) estimation of F_{IS} (f), F_{ST} (θ) and F_{IT} (F) were performed by Jack-knifing procedure in order to generate significance values. Arlequin version 3.1 was used to perform locus by locus analysis of molecular variance (AMOVA) to determine the differentiation within and between the populations (Excoffier *et al*., 2005). Genetic relationship among Nguni cattle populations was determined according to Nei's standards (Nei, 1987) using POPGene.

The genetic population structure analysis of Nguni cattle ecotypes was assessed using Bayesian admixture procedure implemented in STRUCTURE 2.3.4 (Pritchard *et al*., 2000) to infer the most likely number of clusters. The software was programmed to run using the admixture model and correlated allele frequency. The number of assumed populations (K) was estimated for K ranging from 2-12. Five repetition were routed per each K with a burn-in period of 100 000 following with 500 000 Markov Chain Monte Carlo (MCMC) iterations to obtain the corresponding Ln Pr (X|K). The most probable number of populations was determined following the recommendation of Evanno *et al*. (2005). Different values of the number (K) of a priori defined clusters were compared and used to calculate the Ln Pr (X|K). Genetic structure for genetically unknown Nguni populations from Loskop and University of Fort Hare was also evaluated in comparison to known genetically Nguni cattle ecotype. Similar sets of runs for genetic structure were routed and K value was estimated. To validate structure results multivariate analysis of microsatellite allele frequencies (Principal Component Analysis, PCA) was performed to reveal the underlying evolutionary history; admixture among populations using GenAlex 6.41 (Peakall & Smouse, 2006).

CHAPTER 4

RESULTS

4.1. Introduction

Twenty two microsatellite markers were used to perform the genetic characterization of six South African Nguni cattle populations. These were four known SA Nguni cattle ecotypes that included Pedi (PED), Shangaan (SHA), Venda (VEN) and Makhathini (MAK) and two genetically unknown populations (UFH and LOS). All markers successfully amplified and were divided into two sets in consideration of product size and fluorescent dye label. No monomorphic alleles were observed. Only INRA35 marker showed low polymorphic information content (PIC). Other markers indicated medium to high polymorphism for evaluating genetic variability within and exploring genetic differences between Nguni cattle populations.

The genetic descriptive statistics of this study is attached as APPENDIX A. A total of 199 alleles were detected across 22 microsatellite loci in six populations studied with 9.0 loci overall mean (Table 4.1). The lowest number of alleles were found in locus INRA35 (three alleles) while the highest number of alleles was observed in locus TGLA53 depicting the amount of allele richness (Table 4.1).

Table 4.1. Number of alleles observed for the 22 microsatellite markers

Locus	Observed alleles	N
HEL1	98, 100, 102, 104, 106, 108, 110, 112	8
INRA63	162, 166, 168, 174, 176, 178, 180, 182, 184	9
ETH185	220, 222, 228, 230, 232, 234, 236, 238	8
TGLA126	116, 118, 120, 122, 124, 126, 128	7
HEL5	150, 152, 154, 160, 162, 164, 166, 168	8
ILSTS006	282, 284, 288, 290, 292, 294, 296, 298, 300, 302	10
INRA37	122, 124, 126, 128, 130	5
ETH152	160, 164, 166, 168, 170	5
INRA23	183, 185, 189, 193, 197, 199, 203, 207, 209, 213, 215, 217	12
CSRM60	92, 94, 96, 98, 100, 102, 104, 106, 112, 114, 116, 118, 120	13
CSSM66	179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201	12
TGLA227	79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99	11
TGLA122	135, 137, 139, 141, 143, 145, 147, 149, 151, 161, 163	11
ETH10	207, 209, 211, 215, 217, 219, 221, 223	8
INRA35	100, 102, 104	3
ETH225	137, 139, 141, 143, 145, 147, 149, 151, 153, 157, 159	11
ETH3	113, 115, 117, 119, 121, 123, 125	7
TGLA53	152, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188	18
BM1818	255, 257, 259, 261, 263, 265, 267, 269	8
BM2113	120, 122, 126, 128, 130, 134, 136, 138, 140, 142, 144	11
BM1824	182, 184, 186, 190, 192, 194, 196	7
SPS115	244, 246, 248, 250, 252, 254, 262	7
Total		**199**
Mean		**9**

N- number of alleles detected.

Eleven microsatellite markers showed a total of eighteen distinct (9%) private alleles ranging between one and three per locus (Table 4.2). The private alleles were confirmed by checking genotyping errors. Seven (INRA63, ILSTS006, ETH152, TGLA122, ETH225, BM2113, SPS115) from the eleven loci with private alleles had allele frequency higher than 9% and were considered as magnitude for genetic uniqueness in Nguni cattle ecotypes (Table 4.2). The allelic frequencies table is attached as APPENDIX B.

Table 4.2. Private alleles and frequencies of the 22 microsatellite markers

Locus	Private alleles	Allele Frequency
HEL1	0	0.125
INRA63	2	**0.111**
ETH185	0	0.125
TGLA126	0	0.143
HEL5	0	0.125
ILSTS006	2	**0.100**
INRA37	0	0.140
ETH152	1	**0.200**
INRA23	2	0.083
CSRM60	1	0.077
CSSM66	2	0.083
TGLA227	0	0.091
TGLA122	3	**0.091**
ETH10	0	0.125
INRA35	0	0.033
ETH225	1	**0.110**
ETH3	0	0.143
TGLA53	1	0.055
BM1818	0	0.125
BM2113	1	**0.910**
BM1824	0	0.143
SPS115	2	**0.143**
Total	**18**	

Bold – unique alleles with high frequencies

4.2. Polymorphism of microsatellite markers

The heterozygosity and polymorphism information content (PIC) and Hardy-Weinberg Equilibrium for the twenty two microsatellite markers are summarized in Table 4.3. Most markers (90.9%) showed moderate to high levels of polymorphism and heterozygosity. The polymorphic information content (PIC) in all loci varied between 0.23 (INRA35) and 0.78 (ILSTS006) with a high overall mean of 0.65. The expected heterozygosity (H_E) values across the loci ranged from 0.27 (INRA35) to 0.82 (ILSTS006) with an overall mean of 0.70 while the observed heterozygosity (H_O) across loci ranged from 0.23 (INRA35) to 0.87 (BM2113) with an overall mean of 0.69 (Table 4.2). Out of twenty two, nineteen loci were in Hardy Weinberg Equilibrium (P>0.05) and only three loci (INRA23, INRA35 and SPS115) deviated from HWE (Table 4.3).

Table 4.3. Expected heterozygosity (H_E), Observed heterozygosity (H_O), polymorphic information content (PIC) values and HWE test of deviation for 22 loci

Locus	H_E	H_O	PIC	P-value HWE
HEL1	0.718	0.765	0.658	0.853±0.010
INRA63	0.677	0.708	0.619	0.508±0.015
ETH185	0.699	0.705	0.653	0.498±0.016
TGLA126	0.810	0.833	0.768	0.833±0.009
HEL5	0.750	0.726	0.705	0.389±0.018
ILSTS006	0.817	0.830	0.777	0.393±0.017
INRA37	0.735	0.685	0.680	0.052±0.004
ETH152	0.619	0.653	0.538	0.688±0.007
INRA23	0.690	0.594	0.640	**0.000±0.000**
CSRM60	0.745	0.720	0.692	0.141±0.011
CSSM66	0.756	0.737	0.705	0.446±0.018
TGLA227	0.792	0.734	0.749	0.098±0.010
TGLA122	0.758	0.743	0.710	0.095±0.007
ETH10	0.779	0.832	0.735	0.979±0.003
INRA35	0.266	0.207	0.232	**0.000±0.000**
ETH225	0.766	0.725	0.717	**0.007±0.002**
ETH3	0.615	0.617	0.556	0.542±0.013
TGLA53	0.777	0.790	0.742	0.145±0.018
BM1818	0.752	0.728	0.702	0.320±0.015
BM2113	0.805	0.856	0.765	0.647±0.015
BM1824	0.599	0.599	0.537	0.641±0.011
SPS115	0.487	0.494	0.443	0.766±0.010
Mean	**0.700**	**0.694**	**0.651**	

HWE (P<0.05) P-values in bold did not adhere to HWE

Population differentiation across all the markers was evaluated using Wright's fixation indices (F_{IS}, F_{ST} and F_{IT}) in Table 4.4. The overall loci estimates of inbreeding indicated two markers with reduced heterozygosity (INRA23 and INRA35) at a significance level of $p<0.05$ while the other markers showed moderate and negative F_{IS} values with a mean of 0.01 indicating limited inbreeding. All F_{ST} values were significant across the loci ($p<0.01$) with TGLA122 marker showing high value of 0.128 and the average number 0.048. The population differentiation was estimated under F_{ST} and step-wise mutation R_{ST} models. The mean value for R_{ST} was higher than F_{ST} indicating that 4.8% of the total genetic variation corresponded to differences among populations whereas 95.2% depicts differences among individuals within the populations.

Table 4.4. Wright's F-statistics (F_{IT}, F_{ST} and F_{IS}) and population differentiation under the step-wise mutation model (R_{ST}) for each locus.

Locus	$F_{IS}(\theta)$	$F_{IT}(F)$	$F_{ST}(f)$	R_{ST}
HEL1	-0.067	-0.036	0.029*	0.004
INRA63	-0.051	-0.024	0.027*	0.030
ETH185	-0.010	0.055	0.065*	0.064
TGLA126	-0.030	0.006	0.035*	0.010
HEL5	0.032	0.088	0.061*	0.044
ILSTS006	-0.010	0.019	0.028*	0.011
INRA37	0.067	0.096	0.031*	0.019
ETH152	-0.055	-0.034	0.020*	0.035
INRA23	0.140	0.238	0.113*	0.171
CSRM60	0.041	0.080	0.042*	0.040
CSSM66	0.023	0.047	0.025*	-0.006
TGLA227	0.071	0.085	0.015*	0.012
TGLA122	0.019	0.144	0.128*	0.116
ETH10	-0.070	-0.036	0.031*	-0.005
INRA35	0.239	0.249	0.012*	0.028
ETH225	0.053	0.093	0.042*	0.077
ETH3	-0.004	0.060	0.064*	0.098
TGLA53	-0.022	0.024	0.045*	0.038
BM1818	0.033	0.109	0.078*	0.055
BM2113	-0.064	-0.025	0.038*	0.069
BM1824	0.000	0.040	0.040*	0.052
SPS115	-0.022	0.016	0.037*	0.033
Mean	**0.010**	**0.057**	**0.048**	**0.061**
SD	**0.006**	**0.016**	**0.007**	**0.020**

P<0.05;**P*<0.01

4.3. Genetic diversity of the populations

The descriptive statistics for the genetic diversity found within and between South African Nguni cattle populations are presented in Table 4.5. High genetic diversity was found across the six populations with an average of 70% heterozygosity. The observed heterozygosity (H_O) of UFH and LOS populations were higher than the expected heterozygosity (H_E). The overall mean number of alleles for the populations was 6.47. The PED population was found to be the most diverse population with highest expected heterozygosity (H_E=72% and H_O=69%) and MNA (6.82) which was slightly higher than the overall (6.47). There were 18 distinct private alleles found in MAK (4), UFH (4) as well as in PED (3), VEN (3) and LOS (4). No private alleles were found in SHA population. The overall estimate of inbreeding showed limited inbreeding in the six Nguni cattle populations (0.01) consistent with the

results obtained in Table 4.4. Low amount of inbreeding was observed in UFH (-0.01) and LOS (-0.01) populations.

Table 4.5. Descriptive statistics for six Nguni cattle populations

Population	Sample size	Loci typed	Expected Hz ± SD	Observed Hz ± SD	Number Alleles ± SD	F_{IS}
PED	32	22	0.717±0.030	0.689±0.010	6.82±2.20	0.04
SHA	30	22	0.688±0.030	0.642±0.010	6.27±1.70	0.07
VEN	31	22	0.701±0.020	0.682±0.010	6.32±1.90	0.03
MAK	32	22	0.702±0.030	0.667±0.010	6.70±2.40	0.05
UFH	32	22	0.687±0.030	0.696±0.010	6.68±2.40	-0.01
LOS	32	22	0.708±0.020	0.789±0.010	6.00±1.80	-0.10
Mean			**0.701**	**0.694**	**6.47**	**0.01**

P<0.05

4.4. Population differentiation

The analysis of Molecular Variance (AMOVA) in Table 4.6 revealed 4.8 % of the total variation was due to differences among populations and 95.2% was accounted for differences within individuals in the populations similar to results obtained by F_{ST} in Table 4.4.

Table 4.6. AMOVA analyses for six Nguni cattle populations

Source of variation	Sum of squares	Variance components	Percentage of variation	P-value
Between populations	155.678	0.39204	4.81702	0.001
Within populations	2786.648	7.74652	95.18297	0.001
Total	2942.326	8.13856		

4.5. The genetics distances of the six Nguni cattle populations

Pairwise genetic differentiation quantified by F_{ST} estimate in Table 4.7 identified PED to be closer with MAK (0.034) and SHA (0.035) ecotypes. The VEN (0.048) was found distant from the two (MAK and PED) ecotypes and mostly closer to SHA while LOS differentiated from all the populations (0.060). These results were similar to the results obtained using Nei's genetic distance matrix in Table 4.8.

Table 4.7. Pair-wise population matrix of F_{ST} values between six Nguni cattle populations analyzed

	PED	SHA	VEN	MAK	UFH	LOS
PED	0					
SHA	0.034	0				
VEN	0.048	0.035	0			
MAK	0.035	0.049	0.051	0		
UFH	0.038	0.047	0.053	0.028	0	
LOS	0.060	0.058	0.062	0.043	0.040	0

Nei's genetic distances among populations are illustrated in Table 4.8. Shorter distances were observed between PED, MAK and SHA and also in SHA and VEN ecotypes. It was observed that MAK ecotype had a shorter distance with UFH. LOS was distant from all other Nguni cattle populations with a higher genetic distance (0.223).

Table 4.8. Pairwise matrix of Nei's Genetic Distance (D_A) for six Nguni cattle populations analyzed

	PED	SHA	VEN	MAK	UFH	LOS
PED	*****					
SHA	0.132	*****				
VEN	0.171	0.127	*****			
MAK	0.126	0.148	0.163	*****		
UFH	0.129	0.150	0.150	0.112	*****	
LOS	0.210	0.223	0.209	0.203	0.169	*****

4.6. Population structure

The proportion of genetically known entities of South African Nguni cattle ecotypes belonging to each of the four clusters is illustrated in Table 4.9.

Table 4.9. Proportion of analyzed four Nguni cattle ecotypes in each of the four clusters (*K*=4)

Populations	Inferred clusters			
	1	2	3	4
PED	**0.668**	0.080	0.038	0.214
SHA	0.137	**0.553**	0.188	0.122
VEN	0.023	0.133	**0.751**	0.093
MAK	0.028	0.084	0.043	**0.850**

The PED ecotype was assigned in cluster 1 (67%), separated by cluster 4 (21%), followed by cluster 2 (8%) and a small proportion of cluster 3 (4%). Cluster 2 was dispersed with SHA (55%), PED (19%), MAK (14%) and VEN (12%). Cluster 3 was separated by VEN (75%) and SHA (13%) while cluster 4 was assigned for the MAK ecotype (85%).

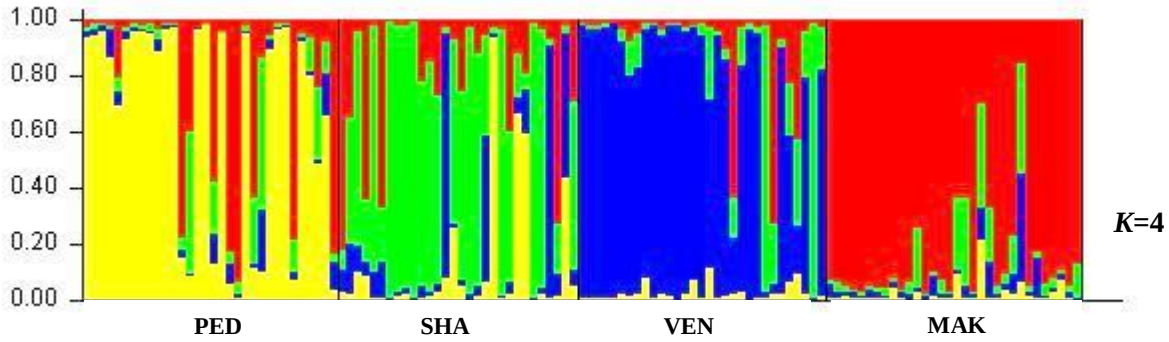

Figure 4.1. Clustering assignment of 125 animals representing four South African Nguni cattle ecotypes at *K*=4

Table 4.10 indicates the membership of four genetically known Nguni cattle ecotypes and two unknown genetic entities of the Nguni cattle populations (Loskop and University of Fort Hare). The assumed K ranged from 2 to 12, the peak was reached at K=5 and the LnP (D) decreased at 6.

Table 4.10. Proportion of analyzed six Nguni cattle populations in six clusters at *K*=5.

Populations	Inferred clusters				
	1	**2**	**3**	**4**	**5**
PED	**0.640**	0.104	0.041	0.125	0.089
SHA	0.139	**0.460**	0.254	0.121	0.026
VEN	0.026	0.116	**0.737**	0.099	0.022
MAK	0.065	0.171	0.077	**0.594**	0.092
UFH	0.087	0.081	0.042	**0.670**	0.120
LOS	0.033	0.024	0.043	0.100	**0.800**

The PED was again assigned to cluster 1 (64%), separated by 10% of SHA and 13% MAK of cluster four and five. SHA (46%) was assigned to cluster 2 separated by VEN (25%), PED (14%), and MAK and UFH (12%). VEN (74%) was assigned to cluster 3 and was separated by 12% of SHA. It was observed that MAK (59%) and UFH (67%) clustered together in cluster 4 and separated by LOS (21%). This confirmed the common origin between MAK

and UFH populations. LOS (80%) was assigned in cluster 6 and was separated by MAK (10%).

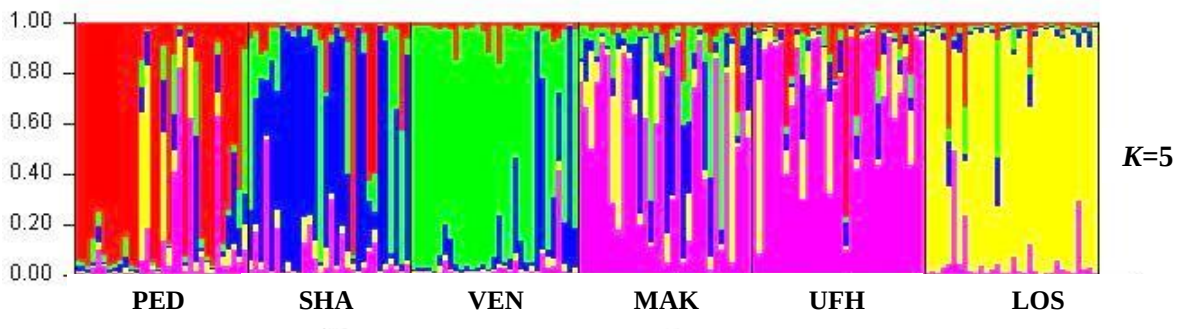

Figure 4.2. Clustering assignment of 189 animals representing six Nguni cattle populations at *K*=5

The Principal Coordinates Analysis (PCA) was performed to further investigate possible genetic relationship among Nguni cattle ecotypes and other Nguni cattle populations (Figure 4.3).

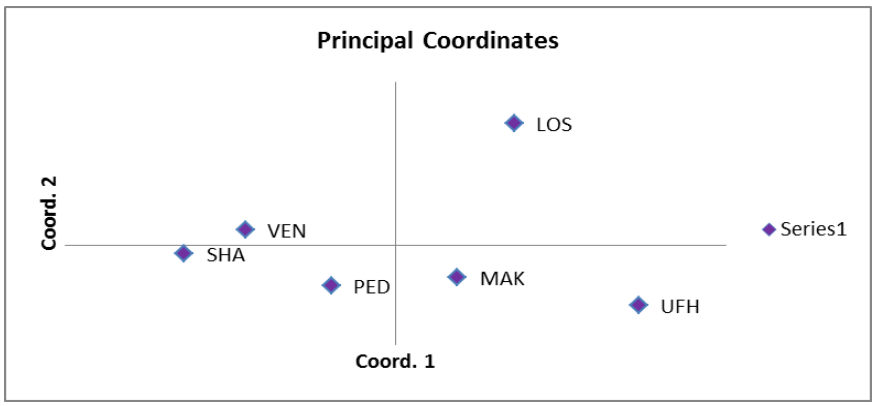

Figure 4.3. Principal Coordinates Analysis (PCA) via Covariance matrix with data standardization

The first three components of the Principal Coordinates Analysis indicated that PC1 (35.45); PC2 (41.48) and PC3 (17.82) accounted for 95% of the total variation. This analysis evidently distinguished VEN and LOS populations from other populations while defined SHA and PED plus MAK and UFH as pairs and closer related ecotypes.

CHAPTER 5

DISCUSSION

Genetic characterization of livestock is essential in designing appropriate management and conservation strategies for animal genetic resources. It is a strategy advocated by Food and Agricultural Organisation to save rare eroding traits that can be useful in future for food security in changing environmental conditions (Tixier-Boichard, 2014). To support FAO strategy, South African Nguni cattle ecotypes were characterized to evaluate their genetic diversity and population structure. This was vital as no studies have reported about genetic diversity in South African Nguni cattle ecotypes using microsatellites.

Twenty two microsatellite markers (FAO-ISAG) were used to perform genetic characterization of the South African Nguni cattle ecotypes. A higher number of microsatellite markers have been used by Dalvit *et al*. (2008) (n=25); Ibeagha-Awewu (2004) (n=28) and Acosta *et al*. (2013) (n=30). In other genetic diversity studies based on Sanga breeds, a lower number of markers were used by Bessa *et al*. (2009) (n=13) and Pienaar (2014) (n=9) compared to the 22 in the current study. The results from this study based on 22 markers indicated sufficient information for characterization of Nguni cattle ecotypes regarding number of alleles, heterozygosity values, and fixation indices.

A total number of 199 alleles were observed across the populations with a mean number of 9.0 alleles and allele frequency proportion ranging from 0.033 to 0.21. This number is higher than the mean number of alleles detected in indigenous cattle breeds of Mozambique cattle (n=7.7) (Bessa *et al.*, 2009). A high mean number of alleles were observed in several African cattle genetic diversity studies in Cameroon cattle (n=10.7) (Ngono-Ema *et al*., 2014); Kenya cattle (11.6) (Adhiambo, 2002); Ankole longhorn cattle in African Great Lakes region (13.8) (Ndumu *et al.*, 2008) and Ankole cattle of Uganda (10.5) (Kugonza *et al.*, 2011). High number of detected alleles, allele frequency and the mean number of alleles is an indication of genetic variation of the loci used that has direct effect on within breed variability (Buchanan *et al.*, 1994; Hanotte, 2000). All markers were polymorphic with at least three alleles detected per locus with an average PIC value of 0.65 observed signifying the informativeness of the markers. It should be noted that the panel of markers used here were approved selected microsatellite markers recommended by Food and Agricultural Organization guidelines (FAO, 2011).

Eight private alleles (9%) were found across eleven markers and each studied population had three to four private alleles except SHA population. This was in MAK (4), PED (3), VEN (3), LOS (4) and UFH (4). In SHA population no private alleles were found pointing out the introgression and gene flow of this population to other populations resulting in absence of unique alleles. Private alleles are important for conservation and are recognized as a tool to measure population genetic distinctiveness since they specify uniqueness of the population among the broader collection of populations (Szpiech & Rosenburg, 2011). The absence of private alleles in SHA ecotype population can be evidenced by this population's high phenotypic resemblance to all other ecotypes especially for PED type such as colour patterns, body size and frame (Nguni Cattle Breeders Society, 2008).

Population differentiation based on twenty two markers was quantified using fixation indices (F_{IS}; F_{ST} and F_{IT}) and step-wise mutation (R_{ST}). Only four markers (INRA37, INRA23, TGLA227 and INRA35) showed reduced heterozygote with an overall inbreeding coefficient showing low positive inbreeding value (0.010) supporting heterozygote deficit obtained in low H_O overall mean of markers across the populations. This is normally caused by inbreeding and substructure in the populations. In addition, high estimation value of R_{ST} than F_{ST} is an indication that differentiation among Nguni populations did not only involved allelic frequencies but also differences in allele sizes caused by microsatellite loci tendencies to mutate (Egito *et al*., 2007; Acosta *et al*., 2013).

All markers considered were in Hardy-Weinberg Equilibrium except for three loci (INRA23, INRA37 and ETH225). The effect of these markers (86%) adhered to HWE is an indication that the allele frequency among Nguni populations studied has remained constant from generation to generation (Dorji & Daugjinda, 2014). The deviation from HWE of the three markers could be ascribed to factors such as existence of null alleles (unamplified alleles), reduced heterozygosity, genetic drift, inbreeding and sub-structuring of the population (Wahlund's principle) resulting in reduced allele frequency of loci in a chromosome (Goddard, 1992; Falconer & Mackay, 1996; Tripathy & Reddy, 2007). In addition, the latter factors were observed in UFH population when locus INRA23 amplified in all other five populations except UFH population showing the presence of null alleles. This was expected due to relevant history of this population sub-structuring and in the fact that null alleles are likely encountered in populations with large effective population size (Chapuis & Estoup, 2006; Dharmarajan *et al*., 2013).

The gene diversity values obtained among studied populations were high and it varied among the ecotypes (72% Pedi, 69% Shangaan, 70% Venda, 70% Makhathini, 69% University of Fort Hare, 71% Loskop populations). Based on these observations the gene diversity in Nguni cattle ecotypes was higher than the genetic diversity reported in Afrikaner cattle populations (57%) by Pienaar (2014). The heterozygosities observed in the current study were comparable to high values reported in Landim cattle (67%) (Nguni cattle breed strain in Mozambique), Angone (69%); Bovino de Tete (67%); Mashona (64%) by Bessa *et al.* (2009). The high H_E values obtained in this study indicated high genetic variation that exists among Nguni cattle ecotypes; a desirable point for population improvement and conservation. High gene diversity levels are normally associated with long-term natural selection for adaptation and the historic mixing of different populations (Ojango *et al.*, 2011). It was detected that the observed heterozygosity in UFH and LOS populations was higher than the expected heterozygosity and also F_{IS} values corresponded with H_O values pointing out lower inbreeding and selection in these two populations (UFH and LOS) and may also be explained by the fact that these two populations have higher census population sizes. High diversity and limited inbreeding shows that there is a room for selection within these ecotypes.

The Analysis of Molecular Variation revealed moderate differentiation between Nguni cattle populations with a variation of 4.8% (F_{ST} = 0.048). Similar figures were obtained from Mozambique cattle breeds (0.047) by Bessa *et al.* (2009). Higher F_{ST} levels were reported in Cameroon landrace breeds F_{ST}=0.061 (Ngono-Ema *et al.*, 2014), Ankole Longhorn cattle (0.090) (Ndumu *et al.*, 2008), North European breeds F_{ST}=0.107 (McHugh *et al.*, 1998) and from seven European cattle breeds F_{ST}=0.112 (Kantanen *et al.*, 2000). More variation was observed within Nguni cattle populations (95.2%). These figures depicted more genetic variation within populations and less genetic variation between populations. This shows that high genetic variation levels are found between European and North African cattle breeds.

In general, both F_{ST} genetic differentiation and unbiased Nei's genetic distance pairwise matrix estimates revealed close genetic relationship among Nguni cattle ecotypes. A shortest distance was found between PED, SHA and MAK Nguni ecotypes, followed by the SHA to VEN. However, VEN was found to be little distant from PED and MAK. These differences in ecotypes could be attributes to tribes selected ecotypes for specific colour variation, horn shape and other traits over many years (Oosthuizen, 1996). Geographical and topography differences for example mountain ranges could also contribute to isolation of populations and resulting in a more uniform population with certain morphological characteristics. Similar,

with results obtained by Van Marle-Köster *et al*. (2008) which indicated Lebowa-Venda chickens to be distant from other chicken lines. Again, this was attributed to its origin as it was geographically isolated from other lines and had unique morphological traits. The closer relationship between VEN and SHA ecotype can be explained with proximate ecological distance that the two ecotypes exhibit. The genetic distances obtained in this study are evidenced with some phenotypes features such as coat colour patterns, horn shape and body frame that are analogous within Nguni cattle ecotypes confirming the originality from the Sanga breed (Nguni Cattle Breeders Society, 2008; DAD-IS). It was observed that UFH population is the MAK ecotype and it was only LOS population that exhibited the most differentiation among the populations. The closer distance between MAK ecotype and UFH population is due to the fact that the two populations have a common origin since most of the UFH animals originated from KwaZulu Natal herds and half of the herd from Eastern Cape farmers that their herds originated in KwaZulu Natal farms. LOS distance from other population can be explained with the fact that this population has been geographically separated conserved in a closed environment at Agricultural Research Council-Loskop South Farm for more than 25 years from its counterparts of Zulu cattle.

The genetic introgression between Nguni cattle ecotypes was observed in structure cluster analysis confirming the results obtained in genetic distance matrix. The genetic clusters of Nguni cattle ecotypes can further be explained with phenotypic variation underlying Nguni cattle ecotypes and adaptation traits from the original Sanga (Matjuda, 2012). The phenotypic variation can visibly be observed between SHA and PED ecotypes populations that share similar qualitative traits such as coat colour patterns and body size frame. These traits are simple inherited through non-additive gene action and are not controlled by environment factors but with one or few gene pairs that have large effect on body size, coat colour patterns and horns (Bourdon, 2000). The predominance of MAK to other ecotypes can be attributed from Sanga origination; MAK inherited and own all other ecotypes coat colour patterns and only distinct from other ecotypes with short “V” shaped head and small body size. The validation of UFH and LOS Nguni cattle populations in comparison with four Nguni cattle ecotypes performed using structure cluster analysis presented UFH and MAK populations clustering together, confirming relatedness and common origin. LOS clustered as a separate population with little signal of MAK and traces of other populations indicating good long term maintained and conserved population in separate closed environment. In addition, LOS

has a large population size and there has been lot of selection in this population (Agricultural Research Council, unpublished reports).

The structure analysis results were confirmed with Principal Component Analysis (PCA). The PCA plot revealed distinctness of VEN and LOS populations similar to results with structure patterns analysis. Likewise, SHA and PED populations grouped together and similarly MAK and UFH indicating the relationship among the populations. It was observed that VEN ecotype share a component alone but closer in borderline to a component of SHA and PED whereas LOS was distinct shares no component. Similarly, MAK shared a component with UFH but closer in borderline with PED and SHA.

Based on the results obtained in this study it can be confirmed that there are genetic relationships among MAK, PED and SHA ecotypes. It has been revealed that the genetic diversity between the Nguni populations is maintained. Despite, the overall genetic diversity it would be important to focus on diversity of ecotypes for long term improvement. Selection of new traits to meet specific requirements is needed for future changing environmental condition. Selection programs should avoid more genetic dilution among Nguni cattle ecotypes to keep the existing ecotypes pure. The private alleles observed in some populations could be important for future plans towards Nguni cattle ecotypes. It is further recommended that the genetic diversity and population structure of South African Nguni ecotypes populations be evaluated periodically because the conservation of Nguni ecotypes is a priority.

Evaluating the genetic diversity and population structure in South African Nguni cattle ecotypes has generated baseline data with regard to within and between genetic diversity in Nguni ecotypes. Therefore, results from this study will contribute in conservation of Nguni cattle ecotypes maintained at research stations, private farms and universities around South Africa for selection programmes. This data will continuously be used as reference in detecting the genetic diversity that exists in South African communal and in comparison with other Nguni cattle genotypes found in South Africa neighbouring countries such as Botswana, Malawi, Mozambique, Swaziland, Namibia, Zambia and Zimbabwe. In addition, it will assist in sampling of animals for *ex situ* conservation that can be used in creation of germplasm for bio banking in order to preserve genetic diversity of Nguni cattle ecotypes for future purposes.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

This is the first study to evaluate and report on the genetic characterization of South African Nguni cattle ecotypes using twenty two microsatellite markers. These markers have shown effectiveness to detect genetic diversity in Nguni cattle ecotypes. The present results will contribute in understanding the existing genetic diversity, relationship and population structure among South African Nguni cattle ecotypes populations. In addition, these results will be used to improve breeding programs and conservation of South African Nguni cattle ecotypes.

Microsatellite markers with high polymorphic information content that have been used in evaluating the genetic diversity of South African Nguni cattle ecotypes should be included in future genetic characterization studies for South African cattle breeds. The genetic diversity parameters showed high values and the inbreeding estimate was negative to low positive. These indicated well maintained genetic diversity and controlled inbreeding within the populations especially UFH and LOS populations as they have large population sizes. The subpopulation differentiation and AMOVA depicted that most genetic differentiation occurred among individuals within populations than among populations. Therefore, this shows no intensive selections of any special production traits that might have been conducted between the populations. Based on the results of this study, genetic relationship and population structure within Nguni cattle ecotypes can be explained by phenotypic variation underlying as the main effect of differentiation. However, it is recommended that the existing Nguni cattle ecotypes population diversity be conserved. Venda should be conserved alone to maintain its distinct characters, while MAK, PED and SHA can be conserved together. Conserving population in a closed herd shown to promote the distinctive character of LOS, thus this population may be conserve in a closed population system.

It would be worthwhile to assess the genetic variation that exists in communal Nguni cattle populations using the data from this study as reference. There is a need to investigate also Bartlow Combine Station population genetic status in comparison with Loskop genetic database available. In addition, origin patterns, trace of ancestry and the gene responsible for phenotypic variation among Nguni ecotypes are all unknown and therefore it would be vital

to trace the genetic history of Nguni cattle ecotypes and specific genes responsible for phenotypic variation using the SNPs markers based technology.

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APPENDICES

APPENDIX A

Heterozygosities and PIC for all populations by locus

Expected heterozygosities

	Populations					
Locus	**PED**	**SHA**	**VEN**	**MAK**	**UFH**	**LOS**
HEL1	0.735	0.699	0.758	0.690	0.768	0.655
INRA63	0.673	0.766	0.564	0.649	0.728	0.682
ETH185	0.738	0.643	0.790	0.703	0.549	0.771
TGLA126	0.803	0.820	0.828	0.800	0.764	0.843
HEL5	0.799	0.721	0.763	0.753	0.769	0.698
ILSTS006	0.811	0.855	0.819	0.808	0.801	0.806
INRA37	0.747	0.750	0.663	0.776	0.719	0.754
ETH152	0.635	0.622	0.580	0.576	0.635	0.666
INRA23	0.855	0.690	0.860	0.713	0.250	0.773
CSRM60	0.790	0.795	0.734	0.766	0.663	0.723
CSSM66	0.793	0.671	0.860	0.814	0.702	0.699
TGLA227	0.743	0.814	0.743	0.791	0.846	0.814
TGLA122	0.776	0.776	0.696	0.789	0.801	0.708
ETH10	0.838	0.809	0.690	0.785	0.766	0.788
INRA35	0.411	0.097	0.337	0.246	0.260	0.246
ETH225	0.704	0.667	0.775	0.771	0.833	0.848
ETH3	0.614	0.695	0.738	0.507	0.543	0.593
TGLA53	0.817	0.655	0.692	0.811	0.856	0.829
BM1818	0.739	0.747	0.711	0.817	0.802	0.694
BM2113	0.810	0.841	0.769	0.842	0.792	0.778
BM1824	0.438	0.678	0.628	0.513	0.625	0.709
SPS115	0.507	0.321	0.428	0.510	0.646	0.509

PIC values

	Populations					
Locus	**PED**	**SHA**	**VEN**	**MAK**	**UFH**	**LOS**
HEL1	0.682	0.631	0.706	0.627	0.716	0.585
INRA63	0.615	0.713	0.514	0.591	0.678	0.605
ETH185	0.686	0.603	0.741	0.655	0.514	0.719
TGLA126	0.760	0.779	0.788	0.755	0.719	0.807
HEL5	0.753	0.680	0.720	0.711	0.727	0.642
ILSTS006	0.774	0.822	0.778	0.765	0.759	0.763
INRA37	0.689	0.697	0.613	0.726	0.657	0.697
ETH152	0.550	0.532	0.516	0.498	0.551	0.581
INRA23	0.820	0.613	0.829	0.660	0.195	0.722
CSRM60	0.750	0.746	0.675	0.718	0.603	0.659
CSSM66	0.749	0.602	0.827	0.775	0.637	0.643

TGLA227	0.693	0.773	0.693	0.751	0.813	0.772
TGLA122	0.727	0.727	0.644	0.743	0.756	0.662
ETH10	0.800	0.766	0.641	0.736	0.720	0.744
INRA35	0.367	0.090	0.277	0.212	0.235	0.212
ETH225	0.646	0.595	0.721	0.731	0.796	0.815
ETH3	0.564	0.627	0.682	0.455	0.508	0.498
TGLA53	0.788	0.622	0.651	0.774	0.823	0.796
BM1818	0.686	0.695	0.655	0.778	0.763	0.633
BM2113	0.766	0.805	0.730	0.807	0.746	0.734
BM1824	0.400	0.621	0.552	0.465	0.536	0.650
SPS115	0.415	0.303	0.390	0.478	0.594	0.477

Observed heterozygosities

	Populations					
Locus	**PED**	**SHA**	**VEN**	**MAK**	**UFH**	**LOS**
HEL1	0.750	0.767	0.677	0.781	0.800	0.813
INRA63	0.719	0.690	0.581	0.656	0.633	0.969
ETH185	0.781	0.643	0.742	0.594	0.563	0.906
TGLA126	0.938	0.786	0.935	0.719	0.774	0.844
HEL5	0.781	0.667	0.742	0.677	0.645	0.844
ILSTS006	0.813	0.800	0.839	0.750	0.871	0.906
INRA37	0.688	0.655	0.516	0.688	0.781	0.781
ETH152	0.613	0.690	0.645	0.594	0.625	0.750
INRA23	0.621	0.526	0.800	0.536	0.250	0.833
CSRM60	0.750	0.704	0.742	0.719	0.719	0.688
CSSM66	0.906	0.571	0.857	0.774	0.594	0.719
TGLA227	0.594	0.600	0.677	0.719	0.938	0.875
TGLA122	0.781	0.724	0.645	0.688	0.839	0.781
ETH10	0.839	0.724	0.833	0.844	0.844	0.906
INRA35	0.188	0.100	0.290	0.156	0.226	0.281
ETH225	0.615	0.636	0.750	0.733	0.710	0.906
ETH3	0.567	0.600	0.613	0.594	0.581	0.750
TGLA53	0.774	0.667	0.677	0.813	0.903	0.906
BM1818	0.742	0.800	0.516	0.781	0.839	0.688
BM2113	0.806	0.800	0.871	0.844	0.844	0.969
BM1824	0.469	0.667	0.581	0.531	0.656	0.688
SPS115	0.419	0.321	0.484	0.500	0.677	0.563

APPENDIX B

Allele frequency comparison over populations
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Key to Population Names:
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Pop1 Pedi (PED)
Pop2 Shangaan (SHA)
Pop3 Venda (Venda)
Pop4 Makhathini (MAK)
Pop5 University of Fort Hare (UFH)
Pop6 Loskop (LOS)

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
HEL1	1	98	0.0781	0.0000	0.0484	0.0000	0.0000	0.0000	0.0214
HEL1	2	100	0.0000	0.0000	0.0323	0.0312	0.1167	0.0000	0.0294
HEL1	3	102	0.4062	0.4000	0.3710	0.4531	0.2167	0.4844	0.3904
HEL1	4	104	0.2969	0.1500	0.1774	0.2969	0.2667	0.0938	0.2139
HEL1	5	106	0.0938	0.3500	0.2742	0.1562	0.3333	0.3281	0.2540
HEL1	6	108	0.0469	0.0167	0.0806	0.0312	0.0167	0.0156	0.0348
HEL1	7	110	0.0000	0.0000	0.0000	0.0000	0.0167	0.0156	0.0053
HEL1	8	112	0.0781	0.0833	0.0161	0.0312	0.0333	0.0625	0.0508
HEL1	# samples:		32	30	31	32	30	32	187

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
INRA63	1	162	0.0000	0.0000	0.0000	0.0000	0.0667	0.0000	0.0108
INRA63	2	166	0.0156	0.0000	0.0000	0.0312	0.0000	0.0000	0.0081
INRA63	3	168	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0027
INRA63	4	174	0.0938	0.0862	0.0161	0.0156	0.1000	0.0000	0.0511
INRA63	5	176	0.1250	0.2586	0.1452	0.2188	0.1833	0.4062	0.2231
INRA63	6	178	0.0000	0.0345	0.0000	0.0000	0.0000	0.0312	0.0108
INRA63	7	180	0.2500	0.2241	0.1613	0.1719	0.1833	0.2344	0.2043
INRA63	8	182	0.5000	0.3448	0.6290	0.5312	0.4500	0.3281	0.4651
INRA63	9	184	0.0156	0.0517	0.0484	0.0156	0.0167	0.0000	0.0242
INRA63	# samples:		32	29	31	32	30	32	186

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
ETH185	1	220	0.0938	0.0000	0.0484	0.0000	0.0156	0.0000	0.0267
ETH185	2	222	0.2188	0.1607	0.2097	0.2031	0.1094	0.1250	0.1711
ETH185	3	228	0.4219	0.5714	0.2742	0.4844	0.6562	0.2656	0.4439
ETH185	4	230	0.0156	0.0357	0.0161	0.0625	0.0156	0.0000	0.0241
ETH185	5	232	0.1875	0.0357	0.0161	0.1562	0.1094	0.3125	0.1390
ETH185	6	234	0.0625	0.0536	0.2742	0.0469	0.0312	0.0312	0.0829
ETH185	7	236	0.0000	0.0536	0.1613	0.0312	0.0625	0.2344	0.0909
ETH185	8	238	0.0000	0.0893	0.0000	0.0156	0.0000	0.0312	0.0214
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ETH185	# samples:	32	28	31	32	32	32	187	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
TGLA126	1	116	0.0156	0.0536	0.0323	0.0000	0.0161	0.1094	0.0376
TGLA126	2	118	0.1094	0.1071	0.1935	0.1406	0.2097	0.1406	0.1505
TGLA126	3	120	0.1562	0.1964	0.2581	0.1250	0.0806	0.0469	0.1425
TGLA126	4	122	0.2656	0.0536	0.0484	0.0156	0.1129	0.2031	0.1183
TGLA126	5	124	0.0469	0.0893	0.0968	0.1875	0.0323	0.2031	0.1102
TGLA126	6	126	0.2969	0.1964	0.1935	0.2812	0.4032	0.0781	0.2419
TGLA126	7	128	0.1094	0.3036	0.1774	0.2500	0.1452	0.2188	0.1989
TGLA126	# samples:	32	28	31	32	31	32	186	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
HEL5	1	150	0.0156	0.0167	0.0645	0.0968	0.0161	0.0000	0.0348
HEL5	2	152	0.0938	0.1333	0.1774	0.0323	0.1452	0.0156	0.0989
HEL5	3	154	0.0156	0.1167	0.1129	0.1613	0.0645	0.4688	0.1578
HEL5	4	160	0.0000	0.0000	0.0161	0.0161	0.0161	0.0469	0.0160
HEL5	5	162	0.2656	0.0833	0.0645	0.0806	0.0645	0.0625	0.1043
HEL5	6	164	0.2188	0.1333	0.1452	0.1613	0.2097	0.2500	0.1872
HEL5	7	166	0.2656	0.4833	0.4194	0.4355	0.4032	0.1562	0.3583
HEL5	8	168	0.1250	0.0333	0.0000	0.0161	0.0806	0.0000	0.0428
HEL5	# samples:	32	30	31	31	31	32	187	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
ILSTS006	1	282	0.0000	0.0000	0.0000	0.0000	0.0000	0.0469	0.0080
ILSTS006	2	284	0.2031	0.1333	0.2581	0.2188	0.1613	0.2656	0.2074
ILSTS006	3	288	0.0156	0.0667	0.0000	0.0156	0.0645	0.0000	0.0266
ILSTS006	4	290	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0027
ILSTS006	5	292	0.3438	0.2167	0.1935	0.0781	0.3226	0.2656	0.2367
ILSTS006	6	294	0.1094	0.0333	0.2581	0.2812	0.2581	0.1406	0.1809
ILSTS006	7	296	0.0469	0.2333	0.0323	0.2344	0.1129	0.1719	0.1383
ILSTS006	8	298	0.0625	0.1167	0.0968	0.0156	0.0161	0.0000	0.0505
ILSTS006	9	300	0.1094	0.1167	0.0645	0.0156	0.0161	0.0312	0.0585
ILSTS006	10	302	0.1094	0.0833	0.0968	0.1250	0.0484	0.0781	0.0904
ILSTS006	# samples:	32	30	31	32	31	32	188	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
INRA37	1	122	0.0469	0.0517	0.0806	0.0781	0.0781	0.2031	0.0904
INRA37	2	124	0.0938	0.2069	0.0645	0.1562	0.2031	0.1406	0.1436
INRA37	3	126	0.2500	0.3966	0.5323	0.2500	0.2812	0.2969	0.3324
INRA37	4	128	0.2500	0.1897	0.1290	0.1875	0.0312	0.0312	0.1356
INRA37	5	130	0.3594	0.1552	0.1935	0.3281	0.4062	0.3281	0.2979
INRA37	# samples:	32	29	31	32	32	32	188	

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
ETH152	1	160	0.1935	0.1207	0.1452	0.2812	0.1250	0.2500	0.1872
ETH152	2	164	0.4839	0.4655	0.5968	0.5781	0.4531	0.3906	0.4947
ETH152	3	166	0.3226	0.3966	0.2258	0.1406	0.3906	0.3594	0.3048
ETH152	4	168	0.0000	0.0172	0.0323	0.0000	0.0000	0.0000	0.0080
ETH152	5	170	0.0000	0.0000	0.0000	0.0000	0.0312	0.0000	0.0053
ETH152	# samples:		31	29	31	32	32	32	187

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
INRA23	1	183	0.0172	0.0000	0.2500	0.0000	0.0000	0.0000	0.0571
INRA23	2	185	0.1897	0.0000	0.2333	0.0000	0.0000	0.0000	0.0893
INRA23	3	189	0.1897	0.0526	0.0333	0.0000	0.0000	0.0000	0.0536
INRA23	4	193	0.0172	0.0000	0.0833	0.0000	0.0000	0.0000	0.0214
INRA23	5	197	0.1207	0.3947	0.1000	0.1250	0.1250	0.1833	0.1679
INRA23	6	199	0.1552	0.3947	0.0500	0.2321	0.0000	0.2833	0.2036
INRA23	7	203	0.0345	0.0000	0.0833	0.4643	0.8750	0.3333	0.2143
INRA23	8	207	0.0690	0.0526	0.0500	0.0536	0.0000	0.0500	0.0536
INRA23	9	209	0.0000	0.0000	0.0000	0.0179	0.0000	0.0500	0.0143
INRA23	10	213	0.2069	0.1053	0.0167	0.1071	0.0000	0.1000	0.1036
INRA23	11	215	0.0000	0.0000	0.0167	0.0000	0.0000	0.0000	0.0036
INRA23	12	217	0.0000	0.0000	0.0833	0.0000	0.0000	0.0000	0.0179
INRA23	# samples:		29	19	30	28	4	30	140

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
CSRM60	1	92	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0027
CSRM60	2	94	0.3750	0.2321	0.3387	0.2969	0.2969	0.3750	0.3209
CSRM60	3	96	0.0781	0.1071	0.0323	0.0625	0.0625	0.0000	0.0561
CSRM60	4	98	0.1719	0.0357	0.0645	0.0469	0.0312	0.0156	0.0615
CSRM60	5	100	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0027
CSRM60	6	102	0.0781	0.0179	0.0000	0.0000	0.0312	0.2031	0.0561
CSRM60	7	104	0.1875	0.2679	0.1774	0.3594	0.5000	0.3125	0.3021
CSRM60	8	106	0.0000	0.0000	0.0161	0.0156	0.0000	0.0000	0.0053
CSRM60	9	112	0.0469	0.2500	0.3548	0.1406	0.0469	0.0938	0.1524
CSRM60	10	114	0.0000	0.0000	0.0161	0.0000	0.0312	0.0000	0.0080
CSRM60	11	116	0.0469	0.0536	0.0000	0.0469	0.0000	0.0000	0.0241
CSRM60	12	118	0.0000	0.0357	0.0000	0.0000	0.0000	0.0000	0.0053
CSRM60	13	120	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0027
CSRM60	# samples:		32	28	31	32	32	32	187

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
CSSM66	1	179	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0027
CSSM66	2	181	0.0000	0.0000	0.0714	0.0968	0.0312	0.0000	0.0328
CSSM66	3	183	0.1562	0.0179	0.1071	0.1613	0.0156	0.1875	0.1093
CSSM66	4	185	0.0156	0.0357	0.1250	0.0645	0.0312	0.0156	0.0464
CSSM66	5	187	0.2969	0.4643	0.2679	0.2097	0.3906	0.2188	0.3060
CSSM66	6	189	0.0938	0.0893	0.1250	0.0968	0.1562	0.0938	0.1093

CSSM66	7	191	0.2969	0.3393	0.1607	0.3226	0.3594	0.4688	0.3279
CSSM66	8	193	0.0156	0.0000	0.0000	0.0000	0.0000	0.0000	0.0027
CSSM66	9	195	0.0000	0.0179	0.0000	0.0000	0.0156	0.0000	0.0055
CSSM66	10	197	0.0156	0.0000	0.0714	0.0000	0.0000	0.0000	0.0137
CSSM66	11	199	0.0000	0.0179	0.0000	0.0000	0.0000	0.0156	0.0055
CSSM66	12	201	0.0938	0.0179	0.0714	0.0484	0.0000	0.0000	0.0383
CSSM66	# samples:		32	28	28	31	32	32	183

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
TGLA227	1	79	0.4062	0.3000	0.4194	0.2656	0.2500	0.2656	0.3175
TGLA227	2	81	0.0312	0.0500	0.0806	0.0781	0.0781	0.2344	0.0926
TGLA227	3	83	0.0781	0.2000	0.1613	0.0469	0.1094	0.0469	0.1058
TGLA227	4	85	0.0000	0.0667	0.0000	0.0156	0.0469	0.0000	0.0212
TGLA227	5	87	0.0156	0.0000	0.0000	0.0000	0.0156	0.0000	0.0053
TGLA227	6	89	0.2812	0.2333	0.2258	0.3594	0.2500	0.2188	0.2619
TGLA227	7	91	0.1250	0.0000	0.0161	0.0156	0.1250	0.1406	0.0714
TGLA227	8	93	0.0312	0.0333	0.0000	0.0469	0.0000	0.0156	0.0212
TGLA227	9	95	0.0000	0.0500	0.0000	0.0312	0.0156	0.0469	0.0238
TGLA227	10	97	0.0156	0.0000	0.0000	0.0625	0.0469	0.0000	0.0212
TGLA227	11	99	0.0156	0.0667	0.0968	0.0781	0.0625	0.0312	0.0582
TGLA227	# samples:		32	30	31	32	32	32	189

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
TGLA122	1	135	0.2344	0.0862	0.1129	0.3125	0.1774	0.0938	0.1711
TGLA122	2	137	0.0000	0.0000	0.0645	0.0469	0.0000	0.0000	0.0187
TGLA122	3	139	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0027
TGLA122	4	141	0.0312	0.0345	0.0000	0.2812	0.3065	0.4844	0.1925
TGLA122	5	143	0.3438	0.3448	0.2258	0.0781	0.0000	0.1406	0.1872
TGLA122	6	145	0.2031	0.0517	0.0000	0.0000	0.0645	0.0000	0.0535
TGLA122	7	147	0.0312	0.0345	0.0000	0.0938	0.1935	0.1406	0.0829
TGLA122	8	149	0.1406	0.1724	0.1129	0.1719	0.2097	0.1406	0.1578
TGLA122	9	151	0.0156	0.2759	0.4839	0.0000	0.0000	0.0000	0.1257
TGLA122	10	161	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0027
TGLA122	11	163	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0053
TGLA122	# samples:		32	29	31	32	31	32	187

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
ETH10	1	207	0.1452	0.1724	0.2333	0.2188	0.1094	0.0938	0.1613
ETH10	2	209	0.1129	0.0690	0.0833	0.1562	0.2188	0.2344	0.1478
ETH10	3	211	0.1452	0.1379	0.0500	0.0469	0.0312	0.1250	0.0887
ETH10	4	215	0.2581	0.2241	0.0667	0.0000	0.0469	0.0469	0.1048
ETH10	5	217	0.1290	0.0690	0.0500	0.2344	0.1719	0.1562	0.1371
ETH10	6	219	0.1935	0.3103	0.5000	0.3125	0.3906	0.3438	0.3414
ETH10	7	221	0.0161	0.0172	0.0167	0.0156	0.0000	0.0000	0.0108
ETH10	8	223	0.0000	0.0000	0.0000	0.0156	0.0312	0.0000	0.0081
ETH10	# samples:		31	29	30	32	32	32	186

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
INRA35	1	100	0.0938	0.0000	0.0000	0.0000	0.0323	0.0000	0.0213
INRA35	2	102	0.1562	0.0500	0.2097	0.1406	0.1129	0.1406	0.1356
INRA35	3	104	0.7500	0.9500	0.7903	0.8594	0.8548	0.8594	0.8431
INRA35	# samples:		32	30	31	32	31	32	188

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
ETH225	1	137	0.4615	0.5455	0.3542	0.4167	0.2258	0.1875	0.3377
ETH225	2	139	0.0000	0.0000	0.0000	0.0000	0.0000	0.0469	0.0097
ETH225	3	141	0.0000	0.0455	0.0417	0.0500	0.0323	0.0000	0.0260
ETH225	4	143	0.0000	0.0000	0.0000	0.0000	0.0161	0.0938	0.0227
ETH225	5	145	0.0962	0.0455	0.2083	0.1000	0.0484	0.1094	0.1039
ETH225	6	147	0.2692	0.0909	0.1875	0.0167	0.0484	0.1250	0.1201
ETH225	7	149	0.0192	0.0000	0.0000	0.1500	0.1129	0.1406	0.0844
ETH225	8	151	0.0192	0.0000	0.0208	0.0000	0.0323	0.0000	0.0130
ETH225	9	153	0.0000	0.0000	0.0000	0.0667	0.0161	0.0000	0.0162
ETH225	10	157	0.1154	0.2273	0.1875	0.1667	0.2419	0.2656	0.2013
ETH225	11	159	0.0192	0.0455	0.0000	0.0333	0.2258	0.0312	0.0649
ETH225	# samples:		26	11	24	30	31	32	154

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
ETH3	1	113	0.1667	0.0500	0.0968	0.0625	0.0968	0.0312	0.0833
ETH3	2	115	0.5833	0.4167	0.3065	0.6719	0.6613	0.4219	0.5108
ETH3	3	117	0.0167	0.0000	0.0161	0.0000	0.0645	0.0000	0.0161
ETH3	4	119	0.0000	0.0333	0.0645	0.0000	0.0000	0.0469	0.0242
ETH3	5	121	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0027
ETH3	6	123	0.1333	0.3333	0.3871	0.2031	0.1129	0.4844	0.2769
ETH3	7	125	0.1000	0.1667	0.1290	0.0625	0.0484	0.0156	0.0860
ETH3	# samples:		30	30	31	32	31	32	186

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
TGLA53	1	152	0.0000	0.0000	0.0000	0.0000	0.0161	0.0000	0.0027
TGLA53	2	156	0.0000	0.0000	0.0000	0.0000	0.0323	0.0000	0.0053
TGLA53	3	158	0.3871	0.5667	0.5161	0.2344	0.2419	0.3438	0.3797
TGLA53	4	160	0.1129	0.0500	0.0161	0.0000	0.1774	0.0625	0.0695
TGLA53	5	162	0.0000	0.0000	0.0000	0.0312	0.0000	0.0000	0.0053
TGLA53	6	164	0.0323	0.0167	0.0000	0.0156	0.0161	0.0938	0.0294
TGLA53	7	166	0.0323	0.0000	0.0161	0.0000	0.0645	0.0000	0.0187
TGLA53	8	168	0.0161	0.0000	0.0000	0.0312	0.0161	0.0000	0.0107
TGLA53	9	170	0.0323	0.0000	0.0000	0.0156	0.1774	0.0156	0.0401
TGLA53	10	172	0.0161	0.0167	0.0323	0.0312	0.0000	0.0000	0.0160
TGLA53	11	174	0.1129	0.1500	0.0968	0.3438	0.1613	0.1875	0.1765
TGLA53	12	176	0.0645	0.0333	0.0484	0.0312	0.0000	0.0000	0.0294

TGLA53	13	178	0.0968	0.0500	0.0161	0.0469	0.0484	0.1094	0.0615
TGLA53	14	180	0.0323	0.0500	0.1935	0.1406	0.0323	0.0156	0.0775
TGLA53	15	182	0.0484	0.0167	0.0323	0.0469	0.0161	0.1406	0.0508
TGLA53	16	184	0.0161	0.0000	0.0000	0.0156	0.0000	0.0000	0.0053
TGLA53	17	186	0.0000	0.0500	0.0323	0.0156	0.0000	0.0000	0.0160
TGLA53	18	188	0.0000	0.0000	0.0000	0.0000	0.0000	0.0312	0.0053
TGLA53	# samples:		31	30	31	32	31	32	187

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
BM1818	1	255	0.0323	0.0000	0.0000	0.1094	0.0645	0.0312	0.0401
BM1818	2	257	0.0000	0.1167	0.0806	0.0625	0.1129	0.0469	0.0695
BM1818	3	259	0.1613	0.1167	0.4516	0.1406	0.1935	0.1406	0.2005
BM1818	4	261	0.2097	0.2667	0.1935	0.2812	0.1129	0.0156	0.1791
BM1818	5	263	0.4194	0.4000	0.2258	0.2656	0.1290	0.2969	0.2888
BM1818	6	265	0.1613	0.0167	0.0161	0.0938	0.3548	0.4531	0.1845
BM1818	7	267	0.0000	0.0000	0.0000	0.0156	0.0323	0.0156	0.0107
BM1818	8	269	0.0161	0.0833	0.0323	0.0312	0.0000	0.0000	0.0267
BM1818	# samples:		31	30	31	32	31	32	187

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
BM2113	1	120	0.0000	0.0000	0.0000	0.0000	0.0156	0.0000	0.0027
BM2113	2	122	0.1935	0.2500	0.1290	0.0781	0.2812	0.2812	0.2021
BM2113	3	126	0.0000	0.0000	0.0161	0.0000	0.0000	0.0625	0.0133
BM2113	4	128	0.0000	0.0500	0.0000	0.0156	0.0000	0.0156	0.0133
BM2113	5	130	0.0968	0.0500	0.0645	0.0625	0.0312	0.0781	0.0638
BM2113	6	134	0.2581	0.1167	0.1290	0.2031	0.2812	0.3594	0.2261
BM2113	7	136	0.2581	0.1500	0.4194	0.1406	0.0625	0.0938	0.1862
BM2113	8	138	0.0000	0.0333	0.0000	0.0469	0.0156	0.0000	0.0160
BM2113	9	140	0.1452	0.2333	0.1129	0.2500	0.2188	0.0781	0.1729
BM2113	10	142	0.0484	0.1167	0.1290	0.1875	0.0938	0.0312	0.1011
BM2113	11	144	0.0000	0.0000	0.0000	0.0156	0.0000	0.0000	0.0027
BM2113	# samples:		31	30	31	32	32	32	188

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
BM1824	1	182	0.0156	0.1167	0.0323	0.1094	0.0000	0.0156	0.0476
BM1824	2	184	0.7344	0.5000	0.5161	0.6719	0.4688	0.4375	0.5556
BM1824	3	186	0.0625	0.2167	0.3065	0.1719	0.3750	0.1875	0.2196
BM1824	4	190	0.0156	0.0000	0.0000	0.0000	0.0000	0.0469	0.0106
BM1824	5	192	0.0156	0.0000	0.0000	0.0000	0.0000	0.0469	0.0106
BM1824	6	194	0.0000	0.0167	0.0000	0.0000	0.0000	0.0000	0.0026
BM1824	7	196	0.1562	0.1500	0.1452	0.0469	0.1562	0.2656	0.1534
BM1824	# samples:		32	30	31	32	32	32	189

Locus	Allele#	Size	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Overall
SPS115	1	244	0.3226	0.0536	0.0161	0.0938	0.1935	0.0938	0.1297
SPS115	2	246	0.6290	0.8214	0.7419	0.6875	0.5484	0.7031	0.6865
SPS115	3	248	0.0161	0.0179	0.1452	0.0781	0.0323	0.0625	0.0595
SPS115	4	250	0.0000	0.0000	0.0161	0.0000	0.0000	0.0000	0.0027
SPS115	5	252	0.0000	0.0357	0.0000	0.0625	0.1129	0.0000	0.0351
SPS115	6	254	0.0323	0.0714	0.0806	0.0781	0.1129	0.0938	0.0784
SPS115	7	262	0.0000	0.0000	0.0000	0.0000	0.0000	0.0469	0.0081
SPS115	# samples:		31	28	31	32	31	32	185