GENETIC VARIATION BETWEEN AND WITHIN SIX SELECTED SOUTH AFRICAN SHEEP BREEDS USING RANDOM AMPLIFIED POLYMORPHIC DNA AND PROTEIN MARKERS

By

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Declaration

I hereby declare that the entire dissertation is my own original work, except where specifically indicated and has not been presented for a degree in any other university and that all sources used in this work have been acknowledged.

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S.R. HLOPHE

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Dedication

This dissertation is dedicated to my lovely parents: Mr. W. Malusi and Mrs.E. Lungiswa Hlophe. Thanks to my two brothers Mazwi and Simiselo and to my three sisters Zethu, Nompilo and Zanele for being who I am and their encouragement during my studies. I would also like to thank my extended family and my friends for all their contributions. I would love to thank God for giving me the potency and being with me.

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Abstract

Six South Africa sheep breeds comprised of indigenous, imported and locally developed sheep were sampled. These breeds include Nguni sheep (Zulu, Swazi and Pedi), South African Merino, Damara and Dorper. Nguni goats were used as an out group. The first objective of this study was to determine the intra and inter-population genetic relationships among six South African (SA) sheep breeds using random amplified polymorphic DNA (RAPD) and protein markers. The second objective was to compare the efficiency between RAPD and protein markers in assessing the genetic variation.

The Nei's genetic distance produced by the RAPD analysis ranged from 0.0430 – 0.1193 for the six selected sheep breeds. The results indicated that Zulu and Swazi breeds were the most genetically identical with a genetic distance of 0.0430. The second closest sheep breeds were the SA Merino and Damara with a genetic distance of 0.0595. The Pedi was related to the Dorper sheep breed with a genetic distance of 0.0814. The greatest genetic distance was between the Pedi and the SA Merino (0.1193) indicating that these two breeds are relatively distant from one another. Genetic identity was the largest (0.9579) between Swazi and Zulu breeds, followed by 0.9423 between SA Merino and Damara breeds. On the UPGMA dendogram the Zulu sheep formed a cluster with the Swazi sheep and the Damara clustered with the SA Merino. The Nguni goats did not cluster with the sheep breeds.

The degree of genetic similarity within the sheep breeds ranged from 66.48 - 88.48 percent. The relationship within the Zulu sheep breed was 72.77%, Pedi 70.76%, Dorper 75.38% and Damara 83.24%. The Swazi sheep breed demonstrated the lowest percentage (66.48%) of genetic similarities between the individuals. On the other hand, the SA Merino breed with 88.48% showed the highest percentage of genetic similarity.

When using the iso-enzymes of transferrin, the Damara and SA Merino formed a cluster with a genetic similarity of 97.13%. Just as in the RAPD results, the Pedi did not form a cluster with other Nguni sheep. The transferrin profile was able to characterise the Nguni goats as an out group species. The dendogram constructed

using the data of the protein profile could not differentiate clearly between the sheep breeds and the Nguni goats.

It can be concluded that although genetic relationships exist, the genetic distances vary among the six sheep breeds. The RAPD analysis demonstrated that these breeds can be treated differently. The genetic variation gave an indication that some breeds such as South African Merino are more uniform. The study showed that the RAPD markers can be used as a tool to estimate the genetic diversity and phylogenetic relationships among breeds of sheep. However, the protein markers must be used with caution. It is recommended that more research should be done to determine the genetic origin of the three Nguni sheep breeds (Zulu, Swazi and Pedi).

CHAPTER ONE

Introduction

Small ruminants like sheep, play an important role in the socio-cultural, subsistence and socio-economic livelihoods of rural and peri-urban communities (Dovie *et al.*, 2006; Kunene and Fossey, 2006; Kosgey and Okeyo, 2007). They are the main source of wealth, and income is derived from the sale of these animals and their products. Sheep provide more products (meat, fat and milk for food, bone and horn for implements, skins and wool for clothing and gut for containers) than any other livestock. Indigenous sheep breeds are an important storehouse of genetic materials due to acclimatization to local, sometimes harsh environmental conditions, nutritional fluctuations and resistance to diseases and parasites (Nsoso *et al.*, 2004; Kunene and Fossey, 2006; Kosgey and Okeyo, 2007; Galal *et al.*, 2008).

In addition to loss of diversity, 20% of domestic animal breeds in the world is believed to be in danger of extinction (FAO, 2000a; FAO, 2000b; Cardellino, 2004; FAO, 2007). According to the Food and Agriculture Organisation (FAO), once the animal genetic diversity is lost it cannot be replaced (FAO, 2000a). Globally it is estimated that 14% of sheep breeds are extinct (Cardellino, 2004). Already 180 sheep breeds are extinct world-wide and 5 of these are from the African continent (FAO, 2007). The grounds for indigenous sheep breeds becoming extinct could be ascribed to economic or socio-political reasons (Taberlet *et al.*, 2008).

Conservation of indigenous animal resources has been proposed as a method for slowing down the loss in diversity in livestock breeds through extinction. Apart from preventing extinction, conservation of indigenous breeds is also important for the future health of the animal industry globally as they could be a resource for novel genes that can permit sustained genetic improvement as well as enabling adaptation to changing breeding objectives and environments (Notter, 1999).

In order to ensure proper conservation and utilization of indigenous breeds, it is necessary to evaluate genetic variations that exist within and among breeds (Kunene *et al.*, 2009). A large proportion of indigenous livestock populations in the developing world have yet to be characterized or evaluated at phenotypic and genetic levels (Hanotte and Jianlin, 2005; Kunene and Fossey, 2006). The FAO has proposed a global programme for the management of genetic resources using molecular methodology for breed characterizations (Bjornstad and Roed, 2001).

The genetic characterization of domestic animals is the first step in considering the sustainable management or conservation of a particular population. It is important to know how unique or how different it is from other populations (FAO, 2000b). It is also valuable to establish DNA archives that can act as benchmark material against which the success of management programmes aimed at the conservation of genetic variability can be monitored (FAO, 2007).

The genetic characterization of domestic animals is part of the FAO Global Strategy for the Management of Farm Animal Genetic Resources. This strategy places a strong emphasis on the use of molecular methods to assist in the conservation of endangered breeds and to determine the genetic status of breeds (FAO, 2000b). Throughout the world researchers utilize molecular technology as a standard approach to establish the genetic distances among breeds. Recently, the loss of genetic diversity between breeds of sheep has been a major concern (Kumar *et al.*, 2008). It is has been suggested by Ali (2003) and FAO (2007) that the highest level of genetic diversity in these populations of livestock is found in the developing world, where record keeping is poor but the risk of extinction is high and is increasing.

The investigation of genetic variation is very important for future monitoring of gene flow in populations, conservation of species, determination of the level of inbreeding and crossbreeding within and between breeds (Hetzel and Drinkwater, 1992; Kunene *et al.*, 2007).

Polymorphism in blood proteins first offered the possibility to study genetic variations in livestock including domestic sheep breeds before the advent of molecular markers (Stasio, 1997). Protein markers have also been used in studying biochemical and molecular characterization of some sheep breeds in Egypt

(Mwacharo *et al.,* 2002). However some authors do not support the variability of protein markers **(**Arranz *et al.,* 1996), on the grounds that they produce low polymorphism and reveal a small proportion of DNA variations (Arranz *et al.,* 1996; Barker *et al*., 1997; 2001; Kantanen *et al*., 2000).

Molecular markers have been identified in sheep populations using microsatellites (Peters *et al.,* 2010), mitochondrial DNA (Hiendleder *et al.,* 1998; 2002) and random amplified polymorphic DNA (RAPD) analysis (Peters *et al.,* 2010). The RAPD markers have been described as a simple and easy method to use for estimation of genetic variability among breeds or species (Kumar *et al.,* 2008). However, because of its low reproducibility, RAPD requires the optimization of protocols (Hassen *et al.,* 2007). They have been used by Duranton *et al.* (2001); Ali (2003) and Zulu (2008) to identify variation in goats, sheep and cattle, respectively. In addition RAPD markers were used by Kunene *et al.* (2008; 2009) in assessing variations between Zulu sheep populations.

The widely used breeds in the sheep production industry in South Africa include the SA Merino, Dorper, Damara and Pedi. The Dorper is a locally developed breed whereas the Merino and Damara are imported breeds. The Zulu and Pedi are indigenous sheep breeds of South Africa collectively known as the Nguni sheep. The Zulu sheep is mainly utilised by the traditional farmers in KwaZulu Natal (Kunene and Fossey, 2006; Kunene *et al.,* 2007; 2008). The Swazi sheep is also classified as Nguni (Ramsay *et al.,* 2000). These six sheep breeds are kept for mutton production, but the SA Merino is also used for its wool (ARC-AII, 2001; Buduram, 2004; Kunene and Fossey, 2006). These sheep breeds have developed unique combinations of adaptive traits to best respond to pressures of the local environment and have settled in a variety of biomes in South Africa (Hammond, 2000; ARC-AII, 2001).

A variety of breeds is essential to provide genetic diversity. Different breeds repond differently to different challenges caused by environmental conditions and diseases vectors. Genetic variation is necessary for selection, adaptation, diseases resistance and production traits. Maintaining genetic variation within breeds reduces chances of inbreeding depression and ensures survival of the breed. The primary aim of the study was to assess the genetic diversity between and within the six selected South African sheep breeds using RAPD and protein markers. Although the FAO has recommended

the use of microsatellite markers for genetic characterization (FAO, 2000), in this study RAPD and protein markers were used because of the simplicity of differentiating genetic variation between and within the sheep breeds. Nguni goats were used as an out-group as means of assessing the efficiency of the markers. The objectives were:

i. To investigate genetic variation within and between the six selected sheep breeds using the random amplified polymorphic DNA (RAPD) markers and protein markers.
ii. To compare the efficiency of the two techniques used in this study (protein markers and random amplified polymorphic DNA) in assessing genetic variation in sheep.

The hypothesis in this study is that there is still some genetic variation between and within the six selected sheep breeds of South Africa. The null hypothesis is that, there is no genetic variation between and within the six selected sheep breeds of South Africa.

CHAPTER TWO

Literature review

2.1 Importance of genetic variation

The loss of genetic variation within and between breeds is detrimental not only from the perspectives of culture, conservation and investigation but also for utility since lost genes may be of future economic interest (Hetzel and Drinkwater, 1992). Within breeds, high rates of loss of genetic variation lead to reduced chances of breed survival due to decreased fitness through inbreeding depression. These breeds become subject to faster changes in gene frequencies, greater rate of loss of genes and genetic constitutions (haplotypes). These are all due to small population sizes or high rates of inbreeding (Meuwissen, 1991). Regrettably, once animal genetic diversity has been lost it cannot be replaced (FAO, 2000b).

Inbreeding can lead to loss of important characteristics of a breed, and can result in the misinterpretation and incorrect classification and identification of breeds (Hetzel and Drinkwater, 1992). Before the advent of genetic studies, the classification of breeds was based on historical and anthropological evidence and morphological characteristics that were and are still not satisfactory or significant for the purpose and objective of conservation, parentage and future monitoring of breeds (Mwacharo *et al.*, 2006).

In order to ensure proper conservation and utilization of indigenous breeds it is important to evaluate genetic variations that exist within and among breeds (Kunene *et al.,* 2008, 2009). Most of the indigenous livestock populations in developing countries have not yet been characterised and evaluated at phenotypic and genetic levels (Hannotte and Jianlin, 2005). Genetic classification of African livestock is essential. Breed classification requires knowledge of genetics that can be effectively measured within and between populations (Nei *et al.,* 1983).

The importance of maintaining domestic animal diversity has been emphasised in several reports (Kantanen *et al*., 1995; FAO, 2007; Hassen *et al.,* 2007). Domestic animal diversity is not only important for food security but also to meet unpredictable future requirements.

There are international organisations currently involved in studying sheep diversity: European (EU-project); International Livestock Research Institute (ILRI); Northern Eurasian (Nordic Genebank for Farm animals (NGH); The Academy of Finland; Ministry of Agriculture and Forestry in Finland **(**ILRI, 2010). Together these organisations aim to provide a global knowledge of genetic diversity of domestic sheep.

2.2 Characteristics of the six selected sheep breeds

2.2.1 Damara Sheep Breed

Although there are claims that the Damara breed of sheep originated in Egypt as long ago as 3000 BC, its recognition as a named breed is more realistically dated to the early twentieth century (New Zealand Rare Breeds, 2007). Others reported that this fat tailed sheep breed arrived in South Africa between 200 and 400 AD (Ramsay *et al.,* 2000). The largest numbers of these animals were found in Namibia. In 1904, this long-legged sheep breed was seen by German explorers in the northern region of Namibia, which was then called Gross Damaland, hence it became known as the Damara Sheep (Damara Breeders' Society, 1993). In 1954, many Damara Sheep were confiscated from commercial farmers who were smuggling sheep through the "Veterinary Cordon Fence", which was erected to separate disease-free areas of southern Namibia from those of the north (Damara Breeder's Society, 1993). The confiscated Damara Sheep were resettled at the Omatjenne Research Station, near Otjiwarongo, Namibia. A Breeders' Association was established at the Omatjenne Research Station in 1986, and many animals were subsequently exported to South Africa, which set up its own Damara Sheep Breeders' Society in 1992 (Damara Breeder's Society, 1993).

Rams are usually horned and heavy whereas ewes are polled and light. This breed contains the following qualities: long legs and short gait for walking long distances;

strong flocking instincts; long productive life; strong mothering ability; high fertility; short lambing intervals; tender and tasty meat with a thin layer of fat; ability to survive on a limited water supply; adaptability to most veld types and temperature tolerance (Ramsay *et al.*, 2000). Damara are almost resistant to most sheep diseases as well as most internal and external parasites (SA Livestock Breeding, 1998; Ramsay *et al.*, 2000). The unique genetic traits of the Damara ensure that it is a most prolific mutton-producing breed. Its ability to survive in a harsh environment and under poor nutritional conditions makes it suitable for the communal areas of Namibia and Southern Africa where extreme conditions are unfavourable. Damaras are smooth haired, predominantly brown sheep but a number of colour varieties occur (Damara Breeders Society, 1993; SA Livestock Breeding, 1998).

2.2.2 South African Merino

Merino sheep were introduced into South Africa in 1789 with imports of Spanish Merinos (DAD-IS, 2009). By the mid-1800s the breed was spread over many parts of South Africa. However, it has been reported by Mason (1996) that the South African Merino is a composite of Spanish, Saxony, Rambouillet, American and Australian Merinos. These sheep are found mainly in South Africa and Zimbabwe where the climate is semi-arid to sub-humid and in Kenya and Lesotho at medium to high altitudes under ranching and agro-pastoral management systems (DAGRIS, 2009).

South African Merino sheep breeds are uni-coloured with a white coat and fine-wool. The breed is adapted to high rainfall grassland regions (Rege *et al*., 1996). Animals of this breed are reported to be less susceptible to fly strike because of their smooth body in comparison to sheep with skin folds (Rege *et al*., 1996; SA Livestock Breeding, 1998). Based on the wool type and management level, different types of Merino sheep were developed for different regions in South Africa. Wool types are differentiated from strong wool (25 micron) to the finest wool (16 micron) (DAD-IS, 2005). Sheep varieties vary from plain-bodied, which are meant for the drier Karoo regions to medium bodied, which are meant for the Lucerne lands of the Western Cape, Northern Free State and irrigated areas. The South African Merino is mainly used for its wool. The SA Merino is the only sheep in the world that can produce 10-15% of its own live mass in clean wool **(**SA Livestock Breeding, 1998).

2.2.3 Dorper Sheep Breed

The South African Department of Agriculture and a group of farmers decided to develop a new sheep breed able to produce a maximum number of lambs with good mutton qualities which could be marketed off arid and extensive grazing conditions (Dorper Breeders' of South Africa, 2000). Thus Dorpers are synthetic artificial mutton breeds that were developed in South Africa in the 1930's after crossing Dorset Horn and the Blackhead Persian (Campbell, 1989). Most of the lambs came out black and white and the Dorper breed was selected from those that had the black heads and white bodies and a more muscular body type (Dorper Breeder's of South Africa, 2000).

A few of the lambs came out as all white. They formed the basis of the White Dorper breed. From that group of white lambs there was then an infusion of Van Rooy genetics, which is a fat-tailed breed, very similar to the Black Head Persian except for the lack of a black head. In South Africa, about 85% of all Dorpers are black-headed (Dorper Breeder's of South Africa, 2000).

The Dorper breed was developed for the arid regions of South Africa but has proven that it is adaptable to many conditions throughout the world (Marais and Schoeman, 1990). The rams are known to be early maturing and can breed as early as 100 days. Dorper is a quality single-purpose breed characterised by extended breeding season (3 lamb crops/2 years), excellent mothers, parasite tolerance, fast growing, superb carcass qualities and superior meat (extremely tender and mild). Today Dorpers are the second most popular breed of sheep in South Africa (Dorper Breeder's of South Africa, 2000).

2.2.4 Nguni Sheep Breed

The Nguni sheep are divided into three groups – Pedi, Swazi and the Zulu sheep (ARC-AII, 2001; Kunene and Fossey, 2006; Kunene *et al.,* 2007). The Nguni sheep of Zululand and Swaziland migrated to South Africa with the Nguni people between 200 and 400 AD down the eastern coast to the areas where they are found today (Ramsey *et al.,* 2000). Several routes were taken by the Iron Age people in their

migration to the south. One group came down the east coast into Natal and then dispersed further south. This dispersal was limited by the growing conditions of their crops, such as millet, which could not be grown on the cold plateau of the Highveld (ARC-AII, 2001). These people brought sheep and cattle with them and it is these which are thought to be ancestors of the present Nguni breeds. The fat-tailed Pedi sheep arrived in South Africa between 200 and 400 AD with the Bapedi people who migrated southwards into the Northern Province of South Africa and settled in the area south of Southpansberg (Ramsay *et al.,* 2000).

2.2.4.1 The Pedi breed

The Pedi breed is normally found in the Northern Province of South Africa. A congregate of Pedi sheep was established and maintained at the Stellenbosch breeding station in Sekukuneland in the Northern Province (ARC-AII, 2001) and a second assembly was established for recording and evaluation purposes on Delftzyl farm near Roedtan in the Northern Province in the mid-1980's (Ramsay *et al.,* 2000).

Pedi sheep are predominantly grazers although, in common with other indigenous African animal breeds, they are equally happy browsing. Their extremely hardy nature makes them adaptable to all the varied climatic regions of South Africa including the harsh Karoo and the tropical Natal and Eastern Mpumalanga Provinces (Ramsay *et al.,* 2000). They are small framed and naturally polled. Fat-tailed sheep with a flat shallow body with long legs. The fat tail is usually long and straight, although variations in tail shape do occur. Coat colour varies from uniform brown through white with a red to brown head, to a variety of black and white patterns (Kruger, 2009). The most common colour is white with a red-brown head, which resulted from selection for this trait in the foundation flock at Stellenbosch (Ramsay *et al.,* 2000). They have a natural tolerance of ticks and diseases (Kruger, 2009).

2.2.4.2 The Swazi breed

Swazi sheep are limited to Swaziland where the climate is sub-tropical and sub-humid at low altitudes. The breed consists of coat colour that is usually black; brown or reddish in plain pattern and sometimes with broken colours. The coat is hairy and

long along the back and on the rib cage (DAGRIS, 2009). The body of a Swazi sheep is considered as small; horns are absent in females but usually present in males. Ears are short, almost non-existent in many sheep. Legs are long in proportion to overall size (Ramsay *et al.,* 2000).

2.2.4.3 The Zulu breed

The Zulu sheep have a mixed appearance in that their tails can be either thin or fat, they have different colours and sizes and they can have a coat of either wool or hair (Kunene *et al.,* 2007). This variation seems to point to recently crossbred sheep but in fact is due to a broad ancestral gene pool. This broad gene pool can give rise to a varied physical appearance but also supplies the genes necessary for the animals' adaptation to different and sometimes challenging conditions (Ramsay *et al.,* 2000; Kruger, 2009). This breed can, in fact, survive and flourish where other sheep breeds die. The dominating colours of this breed are brown and white or black and brown (Kunene and Fossey, 2006; Kunene *et al*., 2007). The tail is fat, long and carrot shaped (Ramsay *et al.,* 2000). However, sheep do occur which have long thin tails which contain very little fat. The ears are short and narrow, but sometimes this breed has very small mouse ears (Kunene *et al*., 2007). They tend to be more 'woolly' than breeds such as the Pedi and Damara. Studies report that the number of sheep of this breed are declining very fast (ARC-AII, 2001) because of replacement by imported breeds and also due to the stock theft which is prevalent in KwaZulu-Natal (Nsoso *et al.,* 2004; Kunene and Fossey, 2006; Kosgey and Okeyo, 2007; Galal *et al.,* 2008).

2.3 Genetic conservation

The two methods of conservation are *ex-situ* and *in-situ* conservations (FAO, 1992). *Ex-situ* means literally, "off-site conservation". It is the process of protecting an endangered species of plant or animal outside of its natural habitat. It includes the preservation of DNA segments in frozen blood or other tissues. *In-situ* conservation means "on-site conservation". It is the process of protecting an endangered species of plant or animal in its natural habitat, either by protecting or cleaning up the habitat itself, or by defending the species from predators. *In-situ* conservation of live populations requires no advanced technology. The basic needs of an *in-situ*

programme are already available and affordable throughout the world. *Ex-situ* and *in-situ* conservation are not mutually exclusive (FAO, 1992). Frozen animal genetic resources or captive live zoo populations can play an important role in the support of *in-situ* programmes (FAO, 1992).

The loss of genetic variation between and within animal breeds has resulted in the necessity of conserving certain breeds (Ramsay *et al.*, 2000). It is not only the establishment or existence of meat and wool production industries that has led to crossbreeding and inbreeding processes of the indigenous breeds, but also because of lack of education and awareness on the part of farmers. The conservation of indigenous animal resources has been proposed as a method for slowing down the loss in diversity in livestock breeds through extinction (Notter *et al.*, 2007). Apart from prevention of extinction, conservation of livestock breeds is also important for the future health of the animal industry globally. Conserved animals can be a resource for genes that can permit sustained genetic improvement as well as enable adaptation to changing breeding objectives and environments (Notter, 1999).

2.4 The impact of Molecular Biology

In the past few years, molecular biology has given access to the entire genome by using the DNA techniques (Ali, 2003). It has been reported that the studies of protein and DNA markers are providing useful and practical information at different levels: populations' structure; levels of gene flow; phylogenetic relationships; patterns of historical biogeography; the analysis of parentage and relatedness (Feral, 2002).

The study of the structure and function of genes at the molecular level in a breeding population can help to determine the similarities of the genetic material carried by populations and the genetic variation they possess (Buduram, 2004). Several techniques have been developed to estimate the genetic variation or polymorphism in populations and hence the genetic relationship amongst populations. Such techniques include DNA markers: [DNA hybridization, restriction fragments length polymorphism (RFLP), mitochondrial DNA (mtDNA), microsatellites and random amplified polymorphism DNA (RAPD)] and protein markers: [starch gel

electrophoresis (STAGE) and polyacrylamide gel electrophoresis (PAGE)] (Buduram, 2004).

2.4.1 Random amplification of polymorphic DNA (RAPD)

Random amplification of polymorphic DNA is another technique used in pedigree determination. It is a polymerase chain reaction (PCR) based technique that has been used in the studies of genetic characterization (Williams *et al*., 1990). It uses one short oligonucleotide (±10–12 bp long) to amplify random segments of DNA (Buduram, 2004). This technique has been used successfully in the study of plants (Kantanen *et al*., 1995) and animals (Hassen *et al.*, 2007; Mohammad *et al.*, 2009). The shortcomings of this technique include the fact that RAPDs are dominant markers hence the heterozygotes cannot be detected and they are sensitive to any type of technical changes (Gwakisa *et al*., 1994). The latter can be avoided by considering the prescribed precautions. To achieve reproducible results, it is important to maintain a strictly constant PCR reaction condition and use of good quality DNA (Gwakisa *et al*., 1994). This technique is simple to use, costs less, requires small quantities of template DNA, generates multiple bands per reaction and does not require the use of radioactive isotopes or sequence data for primer construction. Because of the above reasons, the RAPD technique was found useful for the objectives and aims of this study (Gwakisa *et al*., 1994).

Mohammad *et al.* (2009) investigated the genetic diversity of Red Chittagong cattle (RCC) from the Bangladesh livestock research institute (BLRI) farm and from four areas (Chandonish, Anwara, Potia and Satkania) of Chittagong district using RAPD markers. Only two clusters were formed: the Red Chittagong cattle from the Bangladesh Livestock Research Institute (RCC BLRI) and from Anwara. The second cluster was formed by the Red Chittagong cattle from Satkania, Chandonish and Potia. The highest and the lowest diversity were found in cattle from Anwara (0.2925) and Chandonish (0.2147), respectively (Mohammad *et al.*, 2009). Genetic variability of five indigenous Ethiopian cattle (Herro, Sheko, Arsi, Abigar, and Guraghe Highland) was investigated by Hassen *et al.* (2007) using RAPD markers. The gene diversity obtained was found to be moderate and analysis of molecular variance revealed that within-breed genetic variation was much higher than that

between breeds. The smallest genetic divergence was between Guraghe Highland and Abigar followed by Guraghe Highland and Arsi (Hassen *et al.,* 2007). Genetic diversity and phylogenetic relationship of 108 individual sheep from three Turkish breeds (Kivircik, Gokceada and Sakiz) were studied using RAPD analysis by Elmaci (2007). The percentage of polymorphic loci was found to be 80.49%, 78.05% and 73.17% for Kivircik, Gokceada and Sakiz sheep breeds, respectively. The total genetic diversity was 0.2265 and the average coefficient of genetic differentiation was 0.1181. The Gokceada breed was more closely related to the Sakiz breed than to the Kivircik breed (Elmaci, 2007). Fourteen arbitrary primers were used by Appa (1996) to amplify DNA fragments in four species: cattle, buffalo, sheep and goats. Clear and distinct RAPD patterns with a higher level of polymorphism were detected between the species, while fewer polymorphisms were found within the species (Appa, 1996).

2.4.2 Protein Markers

There are two types of methods used in protein analysis: Starch gel electrophoresis (STAGE) and polyacrylamide gel electrophoresis (PAGE). Starch gel electrophoresis is based on horizontal zone electrophoresis, heating and cooling of hydrolyzed starch in an appropriate buffer solution (Deza *et al.,* 2000, Mwacharo *et al.,* 2002). For PAGE, the protein to be analyzed is first mixed with sodium dodecyl sulphate (SDS), an anionic detergent chosen because it binds to and unfolds the protein, giving a near uniform negative charge along the length which denatures secondary and non–disulfide–linked tertiary structures (Laemmli, 1970). Tracking dyes are added to the protein solution to track the progress of the protein solution through the gel during the electrophoretic run (Jurecka *et al.,* 2009). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique that is used in genetics, molecular biology and biochemistry to separate proteins according to their electrophoretic mobility. PAGE is simple, rapid and cheap; however it uses toxic substances like 2-mercaptoethanol that causes burns and is highly toxic if absorbed through skin (Laemmli, 1970). This drawback can be avoided by observing precautions.

Blood protein polymorphisms of sheep and goats were used during the 1960's, and revealed a limited number of loci and alleles at a locus (Ndamukong, 1995; Missohou

et al., 1999; Deza *et al.,* 2000). This technique is rapid, affordable and reliable, but requires fresh blood samples. Even though allozyme loci may be found in many different tissues, protein markers are usually best expressed in a specific tissue which is run with a specific buffer (Mwacharo *et al.,* 2002). Blood is mostly used for this analysis, because it is a highly specialized tissue comprising many different kinds of components, the most important of which are red cells, white cells, platelets, and plasma (O'Neil, 2010).

Electrophoresis involves the use of matrices which include agarose and polyacrylamide (Gyllensten *et al.,* 1985; Ordas and Primitivo, 1986; Avise and Vrijenhoek, 1987). They provide a means of separating molecules by size, in that they are porous gels. A porous gel hinders the movement of large macromolecules while allowing smaller molecules to migrate freely. Agarose is used to separate larger macromolecules such as nucleic acids, large proteins and protein complexes while polyacrylamide is used to separate most proteins and small oligonucleotides that require a small gel pore size. These matrices can be stained, scanned and photographed for analysis (Appa, 1996).

Genetic variation was investigated between populations of sheep in Kenya using five blood proteins (Mwacharo *et al.,* 2002). Transferrin, esterase-A and esterase-C were polymorphic in all the populations investigated, while albumin was monomorphic for the S allele in the fat-tailed sheep and haemoglobin was fixed for the B allele in the other populations. Phylogenies derived from the pair-wise genetic distance estimates showed a clear separation between the indigenous sheep populations and Merino. By means of starch gel electrophoresis, genetic variations of horse albumin were reported. Three different albumin phenotypes A, AB and B were described as being controlled by a pair of co-dominant autosomal alleles. Stormont and Brand (1964) demonstrated 16 different transferrin phenotypes of the horse. Family data supported the theory that the observed differences were attributed to the action of six co-dominant autosomal alleles. Ashton (1958) observed at least three pre-albumin types in horses. By using starch electrophoresis, several multiple forms of esterase have been reported to occur in horses. Protein polymorphisms in native goats from Central Argentina were studied (Deza *et al.,* 2000). From a total of 14 loci analyzed, eight were polymorphic in at least one flock. The proportion of polymorphic loci varied

between 21.4% and 42.9% and mean heterozygosity between 0.061 and 0.117. Alleles at loci Catalase, Malic enzyme and Esterase -2 showed significantly different frequencies according to the sample origin (Deza *et al.,* 2000).

CHAPTER THREE

Methods and Materials

3.1 Introduction

Most of the animals included in this study were sampled from regions where each breed is predominantly found. Animal blood samples were collected from different localities to ensure that each population was representative of the breed. All the farmers were interviewed about the pedigree of animals before sampling was done. All the animals sampled were under extensive farming; however the management system was not the same. The Merino, Damara, Dorper and the Pedi samples were collected from breed-registered commercial farmers. Swazi sheep and Nguni goats and some of the Zulu sheep samples were obtained from rural farmers. The other Zulu sheep samples were obtained from research stations.

3.2 Locations for animals sampled

A total of 180 of the selected indigenous sheep and 30 Nguni goats of both sexes were sampled from the different areas presented in Figure 3.1. A total of 30 samples per population of Nguni sheep were collected as follows: Zulu breed (10 Zulu blood samples were collected at University of Zululand (UNIZULU) farm; 10 at Kwa-Mthethwa community; and 10 at Makhathini Research Station). A total of 20 Pedi sheep blood samples were collected from three different farms at Makhado and 10 at Mara Research Station in Limpopo Province. The 30 Swazi sheep samples were collected at Lavumisa, Mafutseni and Dlangeni in Swaziland. The 30 Damara blood samples were collected from 2 localities at Hammarsdale and at Empangeni. The 10 Dorper sheep breeds were collected at Mooi River and 20 blood samples in 2 farms at Empangeni. The 15 SA Merino blood samples were collected at Melmoth and the other 15 SA Merino blood samples at Mooi River. The 30 blood samples of Nguni goats were collected at Kwa-Mthethwa community near in KwaZulu Natal Province.

Figure 3. 1 Geographical locations of the sampled sheep breeds and Zulu goat populations in the eight areas of South Africa and three areas of Swaziland

3.3 Sample collections

Five millilitres of whole blood were collected from the jugular vein of the animals using syringes and needles. The blood was stored in two types of vacuntainer tubes, one containing ethylenediaminetetra-acetic acid (EDTA) as the anticoagulant and the other without anticoagulant (Mwacharo, 2002; Ali, 2003). Before sampling, each farmer was interviewed about the nature of the breeding schemes in order to determine the purity of the animals. The whole blood samples in the EDTA tubes were used for RAPD analysis while the blood samples in the tubes without EDTA were used for protein studies. Because of technical constraints it was impossible to obtain the blood serum of the Swazi sheep breed, hence this was excluded from the protein analysis.

3.4 Random amplified polymorphic DNA (RAPD)

3.4.1 Extraction of DNA

The DNA was extracted from the whole blood with the E.Z.N.A ® Blood DNA Kit (Peqlab Biotechnologie GmbH) as follows. A total of 100 µl of each whole blood sample, 150 µl of elution buffer, 25µl protease and 250 µl of BL buffer were added to sterilized microfuge tubes. The samples were vortexed for 10 seconds and then incubated at 70 ^{0}C for 10 minutes. The samples were vortexed once during incubation after 5 µl of RNase solution was added. Then 250 µl of isopropanol was added to each sample. Each sample was transferred into 2 ml collective tubes and centrifuged for 1 minute at 8,000× g and then transferred into columns. The collection tubes were discarded. Columns were placed in new collection tubes and 500 µl of HB buffer was added to each sample. The samples were centrifuged for 30 seconds at 8,000× g. The liquid phase was discarded.

A total of 600 µl of DNA wash buffer was added to the samples and then centrifuged at 8,000 x g for 60 seconds. This step was repeated twice. Before the DNA wash buffer was added, it was diluted with 100% ethanol in the ratio of 3:2 (DNA wash buffer: ethanol). The columns were dried and 200 µl of preheated elution buffer (10

mM Tris-HCl, pH 8.5) was added to each column. The tube was allowed to set for 2 minutes and centrifuged at 8,000×g for 1 minute. The collected DNA sample was analysed for quality and quantity using a spectrophotometer (NanoDrop, USA). The DNA was diluted with deionized water to a final concentration of 70ng/µl.

3.4.2 PCR buffer protocol and DNA amplification

The DNA Random amplification of polymorphic polymerase chain reaction (RAPD-PCR) protocol was developed. Primers were obtained from Operon technologies (Cologne, Germany) and the PCR reagents by Fermentas (US) were used to initiate RAPD-PCR amplifications. A total of 40 random primers (P01 - P12; OPA01 - OPA16; OPB01, OPB02, OPB04, OPB05, OPB07, OPB10, OPB17, OPB18 and OPC02 - OPC05) were screened using representative samples of the breed. Finally 8 primers (P05, P08, P09, P10, OPA01, OPB01, OPB08 and OPC04) were selected and used for DNA amplification.

The RAPD amplifications were performed in 25 µl reaction mixtures containing 12.5 µl of 2 X PCR Master Mix, 1 µl $MgCl_{2,}$ 0.2 µl Taq DNA polymerase (Fermentas, US), and 1 µl primer (Operon technologies, Cologne, Germany). The appropriate amount of Rnase water (Fermentus, US) was determined by the amount of DNA concentration. This reaction mixture was added to a PCR tube. The amplifications were performed following the PCR conditions in Table (3.1).

Table 3. 1 PCR profile depicting temperature, time and number of cycles

	Temperature (°C)	**Time**	**No. of cycles**
Initial denaturation	95	5 min	**1**
Denaturation	95	30 sec	{ 40 }
Annealing	36	30 sec	
Extension	72	1 min	
Final extension	72	4 min	**1**
Hold temperature	25		42

3.4.3 Loading and running conditions

After amplification, the PCR tubes were removed from the thermal cycler**.** The 4 µl of 6 X Orange DNA loading dye (Fermentas, US) was added to each PCR tube containing DNA samples. The 10 µl of DNA containing mixture was loaded in the agarose gel. Amplification products were resolved in 1.0 % agarose gels which were subsequently stained with 10 mg/mL ethidium bromide in a Tris-acetate-EDTA (TAE) buffer. A voltage of 70(V) was applied in electrophoresis for 1 hour 30 minutes. Gels were photographed under Ultra Violent light using Gene Genius bio-Image and Gene Snap software (SynGene, USA). A 100 base pair (bp) DNA ladder **(**O'Range Ruler 100bp, Fermentas US) was used as a molecular size marker.

3.5 Statistical analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. Presence, absence and intensity of band data obtained were analysed by Gene tool version 1.31 software (SynGene, USA). Data were first exported to Microsoft Excel (2003) to generate a data matrix. All calculations were carried out using the population genetic analysis software, PopGene version 1.31 (Yeh *et al.,* 1999). The similarity matrix data was analyzed using the complete linkage algorithms of Minitab software (1998). The UPGMA dendogram was constructed based on Nei's (1972) genetic distances that were modified from the NEIGHBOR procedure of PHYLIP Version 3.5 (2004).

3.6 Polyacrylamide gel electrophoresis (PAGE)

3.6.1 Analytical Electrophoresis

The blood samples were immediately centrifuged for 20 minutes at 3000 rpm. Red blood cells, plasma and serum were placed in separate test tubes and stored at -20˚C for analysis as recommended by Mwacharo (2002) and Ibeagha *et al.* (2004). Protein and transferring profiles were resolved using vertical discontinuous polyacrylamide gels.

3.6.2 Identification of protein profile

Protein profile identification was done by the method prescribed by Laemmli (1970). The 5 µl of blood serum of the five selected sheep breeds was diluted in a 20 µl sample buffer (0.5M Tris-HCl, pH 6.8, glycerol, 10% sodium dodecylsulfate (SDS), 2-mercaptoethanol and 1% bromophenol blue). A suspension of 5µl was applied to 12% separating gel (0.375M tris (hydroxymethyl) aminomethane pH 8.8) and 4% stacking gel (0.125M tris pH 6.8) (Table 3.2). The samples were run at 90 (V) constant current until they had entered the gel and then at 70 (V) constant current for 1 hour 30 minutes in a 5X electrode buffer (15g/l Tris base , 72g/l glycine and 5g/l SDS) pH 8.3.

Table 3.2 Separating and stacking gels preparation for protein profile

	Separating gel (12%)	Stacking gel (4%)
Distilled H_2O	3.4 ml	6.1 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml	
0.5 M Tris-HCl, pH 6.8		2.5 ml
10% (w/v) SDS	100 µl	100 µl
Acrylamide/Bis-acrylamide	4.0 ml	1.33 ml
(30% stock)		
10% (w/v) ammonium	50 µl	50 µl
persulfate (APS)		
TEMED	5 µl	10 µl

The gel was stained with 0.2% Coomassie Brilliant Blue in 45%: 45%: 10 % methanol, water and acetic acid and destained with methanol: water: acetic acid (45: 45: 10). Gels were photographed using Gene Genius bio-Image and Gene Snap software (SynGene, USA). A molecular weight range of 10 - 250 k daltons was used as a molecular size marker (Precision Plus Protein™ Standards, Bio-Rad, #161-0373, USA).

3.6.3 Identification of transferrin alleles

The serum proteins were separated by non-reducing polyacrylamide gel electrophoresis using the method prescribed by Jurecka *et al.* (2009). The blood serum (5 µl) was diluted in a 15 µl loading buffer (40% sucrose, 1.5% Bromophenol Blue) (Sigma–Aldrich, St. Louis, MO, USA) and 2 µl of each suspension was applied to a 6% stacking and 15% polyacrylamide running gel (Table 3.3). Electrophoresis was carried out in running buffer (72 mMTris, 26mM Boric acid) at 70 (V) constant current for 1 hour.

Bands were stained for 1 hour with 0.04% Coomassie Brilliant Blue dissolved in 3.5% perchloric acid (Jurecka *et al.,* 2009). The gel was photographed using Gene Genius bio-Image and Gene Snap software (SYNGENE, USA). Similarly a molecular weight range of 10 - 250 k daltons was used as a molecular size marker (Precision Plus Protein™ Standards, Bio-Rad, #161-0373, USA).

3.7 Statistical analysis

Present, absence and intensity of bands data were obtained, exported to Microsoft excel (2003) and used to generate a data matrix. The data was analysed using the complete linkage analysis of Minitab (1998). Dendograms of transferrin and protein profile were also constructed using the complete linkage analysis of Minitab (1998).

Table 3.3 Separating and stacking gels preparation for a transferrin

	Separating gel (15%)	Stacking gel (6%)
Distilled H_2O	2.4 ml	5.4 ml
1.5 M Tris-HCl, pH 8.8	2.5 ml	
0.5 M Tris-HCl, pH 6.8		2.5 ml
10% (w/v) SDS	100 µl	100 µl
Acrylamide/Bis-acrylamide (30% stock)	5.0 ml	2 ml
10% (w/v) ammonium persulfate (APS)	50 µl	50 µl
TEMED	5 µl	5 µl

CHAPTER FOUR

Results

4.1 RAPD analysis

4.1.1 Band profile analysis

Eight primers (P05, P08, P09, P10, OPA01, OPB01, OPB08 and OPC04) shown in Table 4.1 gave clear polymorphic bands that were reproducible under similar conditions. These primers were used for the analysis of genetic variation. The guanine-cytosine (G-C) content of the primers ranged from 60 to 80%. The number of bands amplified ranged from 10 to 25. Sizes ranged from 300 to 3000 base pair. The total number of scored bands was 126 and the percentage of polymorphic bands was 80.77.

The results illustrated that the total number of loci amplified with the eight primers was 247 in all the sheep breeds and the goats (Table 4.2). The Swazi and Merino breeds both had 36 loci, of which 23.08 % were polymorphic. The Zulu breed had a total number of 37 loci, of which 23.72% were polymorphic. The Dorper breed had the highest number of loci (49) with the highest level of RAPD polymorphism of 31.41%. Pedi sheep showed the lowest number of loci (30), with only 19.23% being polymorphic. The Zulu goat had 15 loci and had the lowest level of polymorphism.

Table 4. 1 Degree of polymorphism and information content for the eight primers applied to the six sheep breeds and Zulu goats

Primer Name	Primer sequence (5'-3')	Percentage of G+C	Number of scored bands	Bands size range (bp)
P05	GCATGCGATC	60%	14	500-3000
P08	GACCGCTTGT	60%	16	500-2500
P09	AACGCGTCGG	70%	14	400-2000
P10	GCGCGCACTC	80%	23	400-3100
OPA-01	CAGGCCCTTC	70%	13	400-1400
OPB-01	GTTTCGCTCC	60%	25	400-1300
OPB-08	GTCCACACGG	70%	11	300-1300
OPC-04	CCGCATCTAC	60%	10	300-1300
Total			**126**	

Table 4. 2 Primer sequence and degree of polymorphism

Breeds/ Populations	Number of loci	Percentage of Polymorphic loci
Zulu	37	23.72
Swazi	36	23.08
Pedi	30	19.23
Dorper	49	31.41
Damara	44	28.21
Merino	36	23.08
Goats	15	9.62
Total	**247**	
Mean	**35.3**	**22.6**

The RAPD fingerprints of the Zulu, Swazi, Pedi, Dorper, Damara Merino sheep and Zulu goats that were obtained using the primers OPA-01, OPB-01 and P-10 are presented in Figures 4.1, 4.2 and 4.3, respectively. Figure 4.1 indicates a band (B) common in both sheep and Nguni goats, and band (A) which demonstrates that the goats were different from the sheep profiles. Figure 4.2 shows the difference between Nguni sheep (Zulu, Swazi and Pedi) and other three sheep breeds (Dorper, Damara and Merino). In this diagram the band D highlighted the difference between Nguni goats and the 6 sheep breeds. Figure 4.3 demonstrates two common bands within the Zulu sheep breed and the one band that was observed in some Zulu sheep individuals.

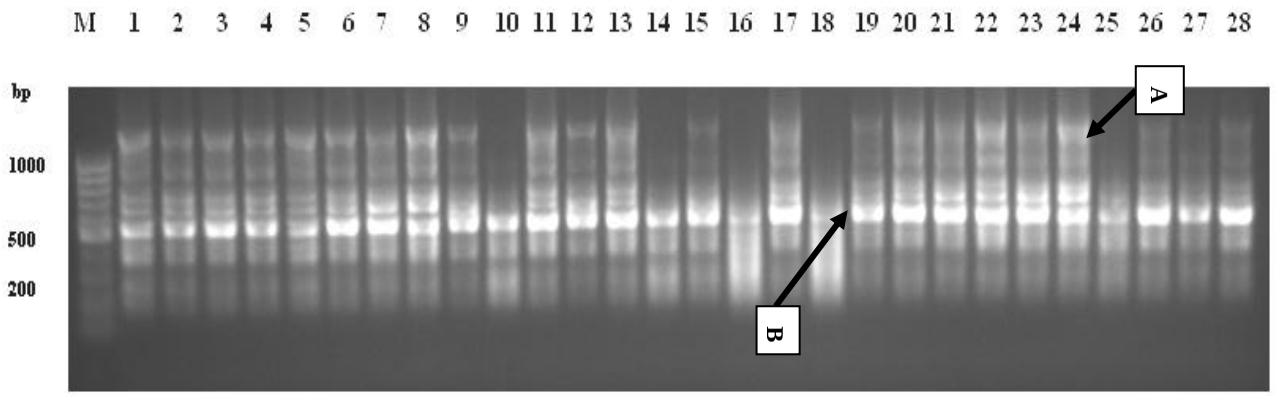

Figure 4.1 RAPD profile generated by using OPA-01 primer. Lanes 1-4 represent Zulu, lanes 5-8 Swazi, lanes 9-12 Pedi, lanes 13-16 Dorper, lanes 17-20 Damara, lanes 21-24 Merino and lanes 25-28 Zulu goats. The first lane represents the molecular size marker (O'RangeRuler, 100 bp Fermentas, US). Arrow A indicates the band that was common to all the sheep breeds but not in Nguni goats and arrow B indicates the universal band that was found in all sheep breeds and Nguni goats.

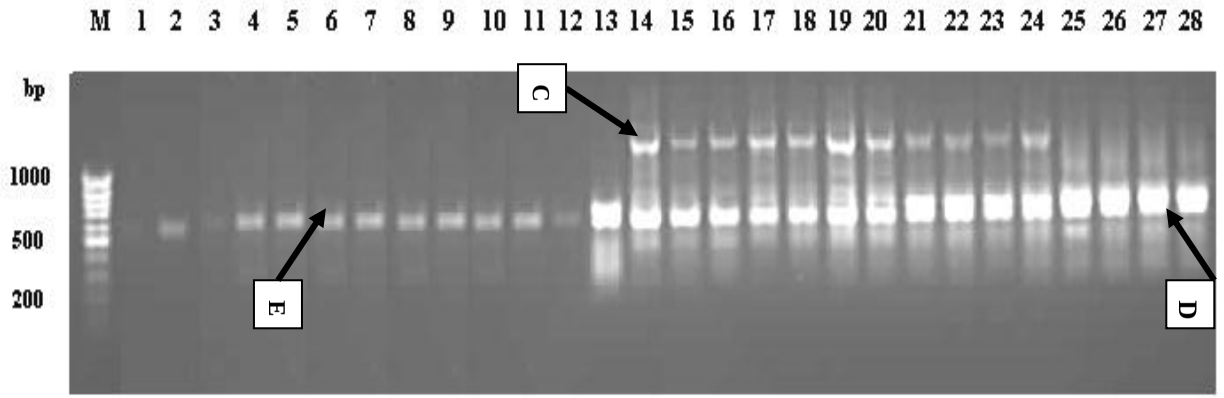

Figure 4. 2 RAPD profile generated by using OPB-01 primer. Lanes 1-12 represent Nguni sheep, lanes 13-16 Dorper, lanes 17-20 Damara, lanes 21-24 Merino and lanes 25-28 Zulu goats. The first lane represents the molecular size marker (O'RangeRuler, 100 bp Fermentas, US). Arrow C indicates band that was only common among the Dorper, Damara and Merino sheep breeds. The intensity of band D varies between species and between breeds. Arrow E indicates band common in Nguni sheep breeds.

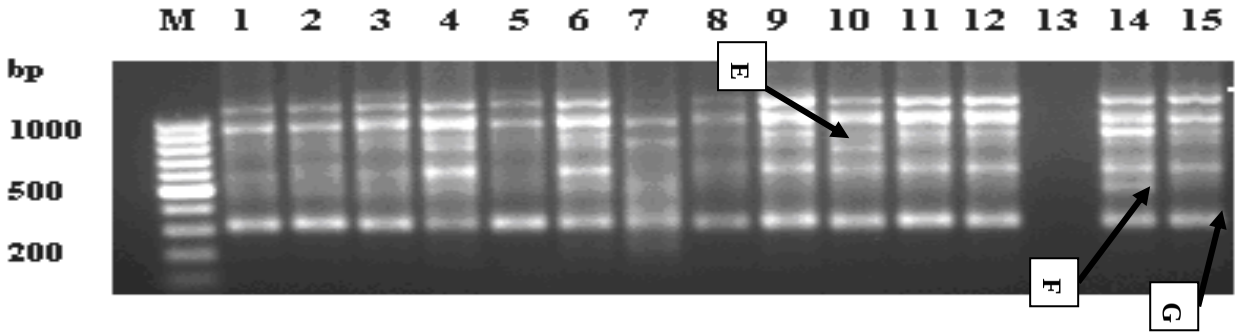

Figure 4. 3 RAPD fingerprints generated by using primer P-10 within the Zulu sheep breed. Lanes 1-15 represents individuals of Zulu sheep. The first lane in each of the profile represents the molecular size marker (O'RangeRuler, 100 bp Fermentas, US). E is a band found in some Zulu sheep individuals. The arrows F and G indicate the bands that are common within the individuals of the Zulu sheep breed.

4.1.2 Genetic diversity and relationships among the investigated sheep breeds

The genetic variations in the six selected South African sheep breeds is shown in Table 4.3 as Nei's gene diversity (h) and Shannon's information (I). The Nei's gene diversity was more or less similar for some populations and ranged from 0.0500 - 0.0962 (Table 4.3). The highest level of heterozygosity (h = 0.0962) in sheep was observed in the Dorper. Lowest level of genetic diversity was observed in Pedi with h = 0.0500. A mean genetic diversity of 0.0713 was obtained among the six selected sheep breeds (Table 4.3). The Nguni goats were also shown to be very closely related (h = 0.0333). The Shannon's Information index ranged from 0.0787 (Pedi) to 0.1473 (Dorper) as shown in Table 4.3.

The Nei's genetic identity and genetic distance are presented in Table 4.4. These were calculated using Nei's (1972) equations through POPGENE software version 1.31. The Nei's genetic distance obtained ranged from 0.0430 – 0.1193 between the six selected sheep breeds. Results indicated that the Zulu and Swazi sheep breeds were the most closely related with a genetic distance of 0.0430. A genetic distance of 0.0595 was observed between Damara and Merino breeds. Pedi sheep were closer to Dorper (0.0814) than to the other sheep breeds and were slightly closer to the Swazi (0.0781) than to the Zulu sheep (0.0822). The highest genetic distance in the six selected sheep breeds was observed between Pedi and Merino with 0.1193. Similarly the goat species showed a large genetic distance (0.1094-0.1316) with the sheep breeds (Table 4.4). The highest genetic identity was observed between Swazi and Zulu breeds (0.9579) as well as between Damara and Merino breeds (0.9423). The lowest genetic identity (0.8714) was between Damara sheep and Nguni goats, which was almost similar to that of Nguni goats and Merino sheep (0.8767).

Table 4. 3 Estimation of genetic variation among the six selected sheep breeds and Nguni goats

Population	Locus	n*	h*	I*
Zulu	Mean	25	0.0663	0.1022
Swazi	Mean	25	0.0556	0.0894
Pedi	Mean	26	0.0500	0.0787
Dorper	Mean	26	0.0962	0.1473
Damara	Mean	26	0.0902	0.1369
Merino	Mean	26	0.0692	0.1057
Goats	Mean	26	0.0333	0.0496
	Mean		**0.0713**	

* n = Sample size

* h = Nei's gene diversity = heterozygosity (Nei, 1973)

* I = Shannon's Information index [Lewontin (1972)]

Table 4. 4 Genetic distance and identity between the six sheep breeds and the Nguni goats

Breeds	Zulu	Swazi	Pedi	Dorper	Damara	Merino	Goat
Zulu	****	0.9579	0.9211	0.9279	0.9055	0.9063	0.8964
Swazi	0.0430	****	0.9249	0.9280	0.9087	0.9118	0.9016
Pedi	0.0822	0.0781	****	0.9218	0.8934	0.8876	0.8861
Dorper	0.0748	0.0747	0.0814	****	0.9193	0.9149	0.8930
Damara	0.0993	0.0957	0.1128	0.0841	****	0.9423	0.8714
Merino	0.0984	0.0957	0.1193	0.0889	0.0595	****	0.8767
Goat	0.1094	0.1036	0.1209	0.1132	0.1132	0.1316	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

The dendogram in Figure 4.4 was created using a POPGENE software package version 1.31 (Yeh *et al.*, 1999). It is based on genetic distance using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis. It depicts the relationship among the sheep as well as the goats. The first cluster branch consisted of the Nguni goats. The second cluster was made of the Damara and the SA Merino. Another cluster was formed by the Swazi and the Zulu sheep breeds as seen in Figure 4.4. The results indicated that the Pedi were closer to the Dorper than to the Zulu and Swazi sheep, which are also classified as Nguni.

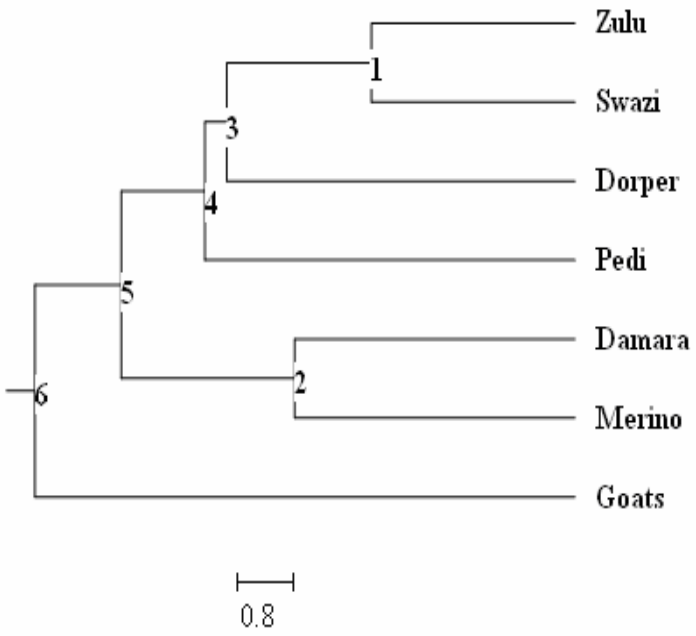

Figure 4. 4 UPGMA dendogram showing differentiation between the six selected sheep breeds and the Nguni goats, based on Nei's (1972) genetic distance using POPGENE software package version 1.31.

4.1.3 Phylogenetic relationships among the six sheep breeds and Nguni goats

To show the genetic similarities between and within the breeds, Figures 4.5 and 4.6 were constructed using the complete linkage analysis of Minitab (1998). Figure 4.5 depicts that the Nguni goats formed an out group to the sheep breeds. The genetic similarity between the six selected South African sheep breeds ranged from 53.02% to 80.60 %. The highest degree of similarity was observed between the Damara and SA Merino (80.6%). The Zulu and the Swazi sheep formed a cluster with a genetic similarity of 53.03%. A genetic similarity of 70.38% between Dorper, Damara, and SA Merino breeds was also observed. The six sheep breeds investigated had a genetic relationship of 50.98% with the Nguni goats.

Figure 4.6 demonstrates the genetic similarity within the six selected sheep breeds without the inclusion of the Nguni goat species. The degree of intra-breed genetic similarity ranged from 66.48 to 88.48 percent. Within-breeds similarities were found to be: 72.77% for Zulu; Swazi 66.48%; Pedi 70.76%; Dorper 75.38%; Damara 83.24% and SA Merino with 88.48%. The Swazi sheep, with 66.48 %, demonstrated the lowest percentage of genetic similarity between the individuals whereas the SA Merino sheep, with 88.48 %, had shown highest percentage of genetic similarities. Thus the lowest (11.52%) genetic diversity on the latter compared to the other sheep breeds. The Pedi and Zulu sheep had relatively high genetic variation of 29.24 % and 27.23% respectively.

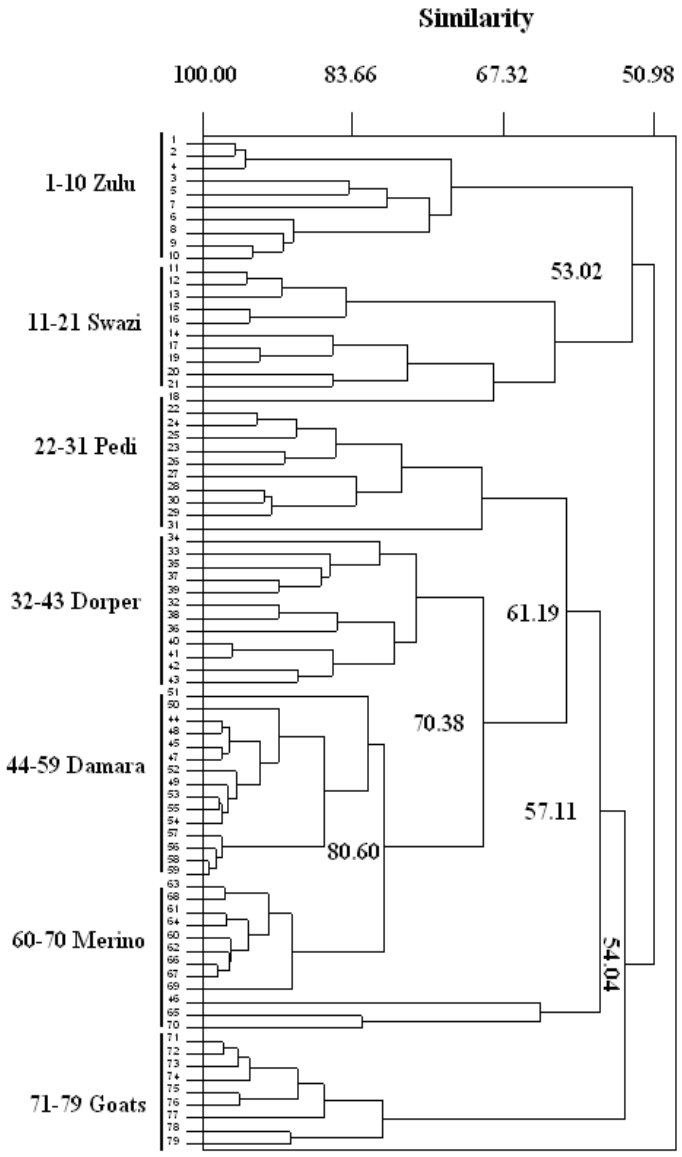

Figure 4. 5 The relationship between the six selected South African sheep breeds and the Nguni goats according to RAPD analysis using the complete linkage analysis of Minitab (1998).

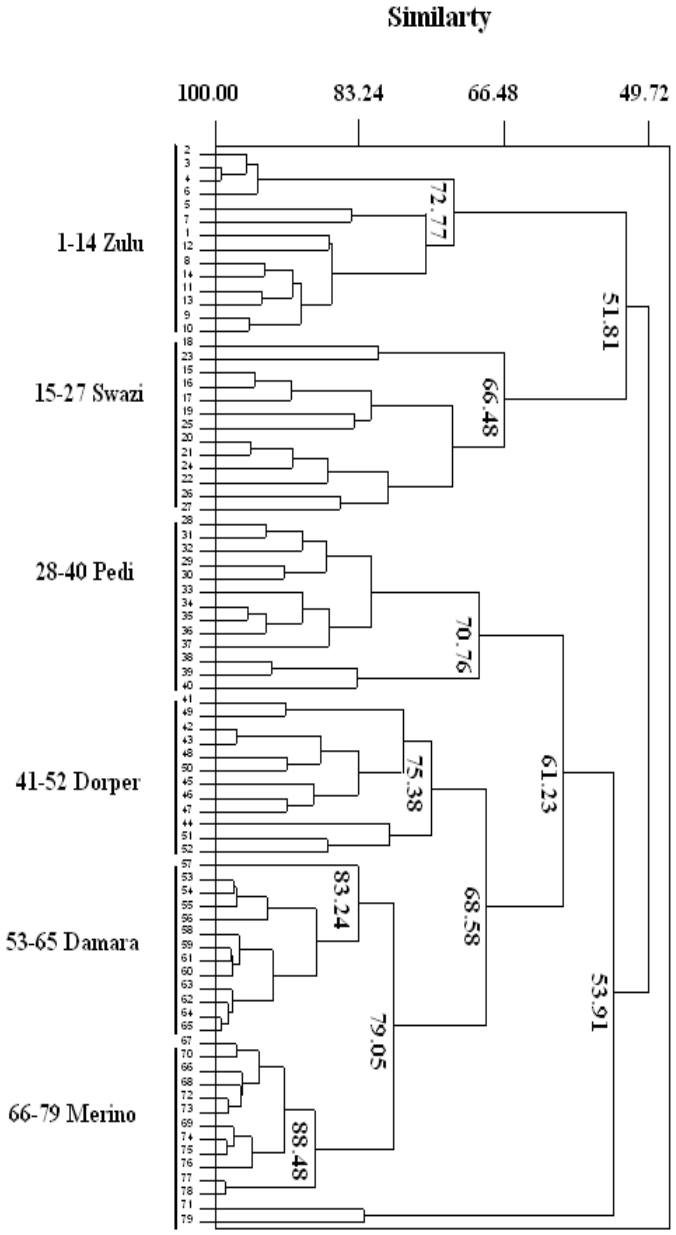

Figure 4. 6 Dendogram showing the genetic relationship within the six selected sheep breeds based on RAPD data analysed using the complete linkage analysis of Minitab (1998).

4.2 PAGE analysis

4.2.1 Genetic variations among populations obtained from the transferrin enzyme profiles

Figure 4.7 demonstrates the profiles of the iso-enzymes of transferrin of the Zulu and Damara sheep breeds. In this illustration, the polymorphism shown by the iso-enzymes of transferrin was able to differentiate between the Damara and Zulu sheep breeds.

The locus transferrin (Tf) exhibited six alleles A, B, C, D, E and F. The polymorphic locus exhibited five alleles in the Dorper and Damara sheep breeds, followed by four alleles in the SA Merino and Nguni goats and three alleles in the Zulu and Pedi breeds (Table 4.5). TfE and TfF alleles were present in both species, with TfE occurring at the highest frequencies. The TfC allele was observed only in Damara and in SA Merino (Table 4.5).

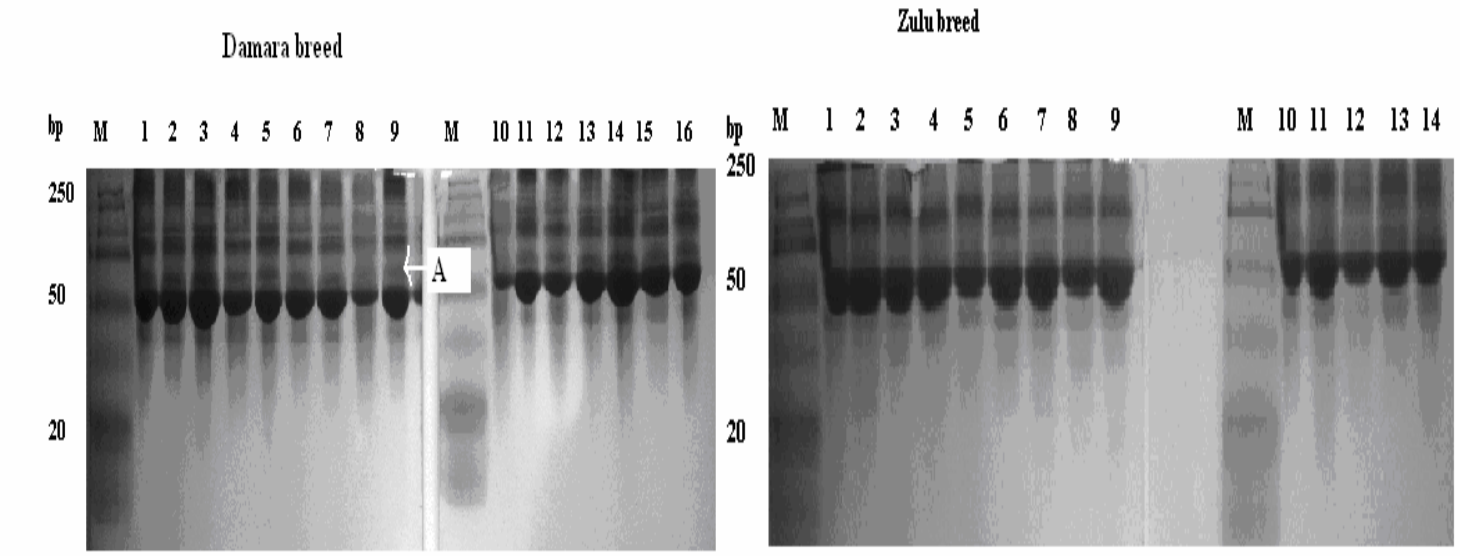

Figure 4. 7 Iso-enzymes of transferrin fingerprints generated from selected indigenous sheep using PAGE. Only the two sheep breeds' representatives are shown. Arrow A in the Damara breed fingerprint indicates that the D allele is present in Damara sheep but not in the Zulu sheep. The M lane in each of the profiles represents the molecular size marker (Precision Plus Protein, BIO-RAD). Lanes 1 to 16 and lanes 1-14 are Damara and Zulu sheep, respectively.

Table 4. 5 Gene frequency of transferrin alleles among the sheep and goat populations

Locus	Allele	Zulu	Pedi	Dorper	Damara	SA Merino	Nguni goat
Tf	A	-	-	1	1	1	1
	B	1	1	1	-	-	-
	C	-	-	-	1	1	-
	D	-	0.938	0.938	0.563	-	0.063
	E	1	1	0.938	1	1	1
	F	0.250	0.938	0.938	0.875	1	0.938

4.2.2 Relationship between sheep breeds shown by dendograms constructed from the transferrin enzyme profile.

The dendogram showing the relationship between sheep breeds using iso-enzymes of transferrin was constructed using the complete linkage analysis of Minitab, 1998. Figure 4.8 showed that the Nguni goats were an out group to the sheep breeds. Genetic similarity between the five sheep breeds ranged from 87.85% to 97.95 %. The degree of similarity between the Damara and SA Merino was the highest (97.13%). The five sheep breeds investigated had a similarity of 87.85% with the Nguni goats.

Figure 4.9 shows a dendogram of the relationship between the sheep breeds only. The percentage of genetic similarity between the five sheep breeds investigated was 90.15 (Figure 4.9). The genetic similarity within the Damara and Pedi breeds was 98.98 % and 97.74% respectively. Some individuals of the Pedi and Dorper sheep breeds overlapped, as did some individuals of the Damara and SA Merino groups (Figure 4.8 and 4.9).

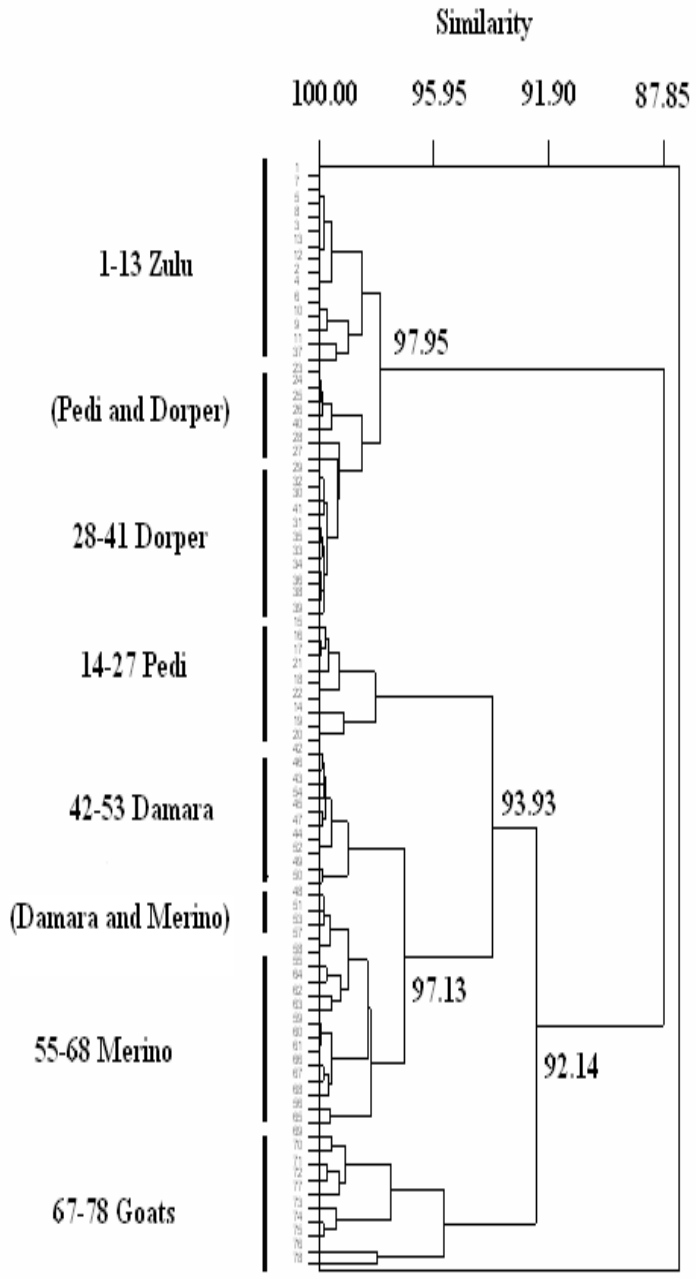

Figure 4. 8 Dendogram showing the genetic relationship between the selected sheep breeds and the Nguni goats using the enzyme transferrin profile.

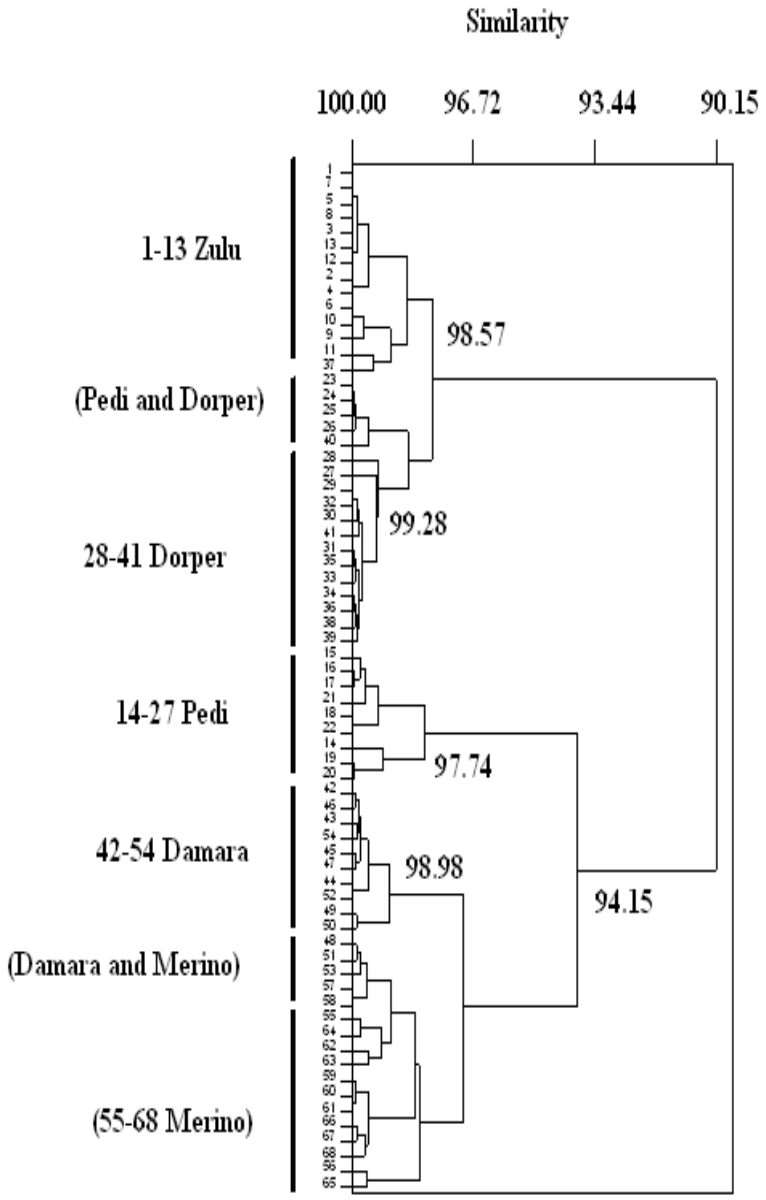

Figure 4. 9 Dendogram showing clusters based on transferrin profile analysis within the selected sheep breeds using the complete linkage analysis of Minitab (1998).

4.2.3 Genetic relationship using the total protein profile.

The total protein profile generated through PAGE is seen in Figure 4.10 A and B. The degree of polymorphism was very low and this is depicted clearly on the dendogram constructed (Figure 4.11) from protein profile.

The Dendogram in Figure 4.11 exhibits the genetic relationship developed from the protein profiles and was assembled using the complete linkage analysis of Minitab, 1998. The overall genetic similarity revealed between the sheep and Nguni goats was 47.98 %. The protein profile dendogram indicated several numbers of overlaps between the breeds. Overlaps include: cluster A which contains two breeds (Zulu and Pedi); cluster B, three breeds (Pedi, Damara and SA Merino); cluster C, three breeds (Pedi, Dorper and Damara); cluster D, three breeds (Zulu, Damara and SA Merino); cluster E, two breeds (SA Merino and Pedi); cluster F, three breeds (Zulu, Pedi and SA Merino); cluster G, three breeds (Dorper, Damara and SA Merino); cluster H, two breeds (Pedi and SA Merino); clusters I and J, two breeds (Zulu sheep and Nguni goats).

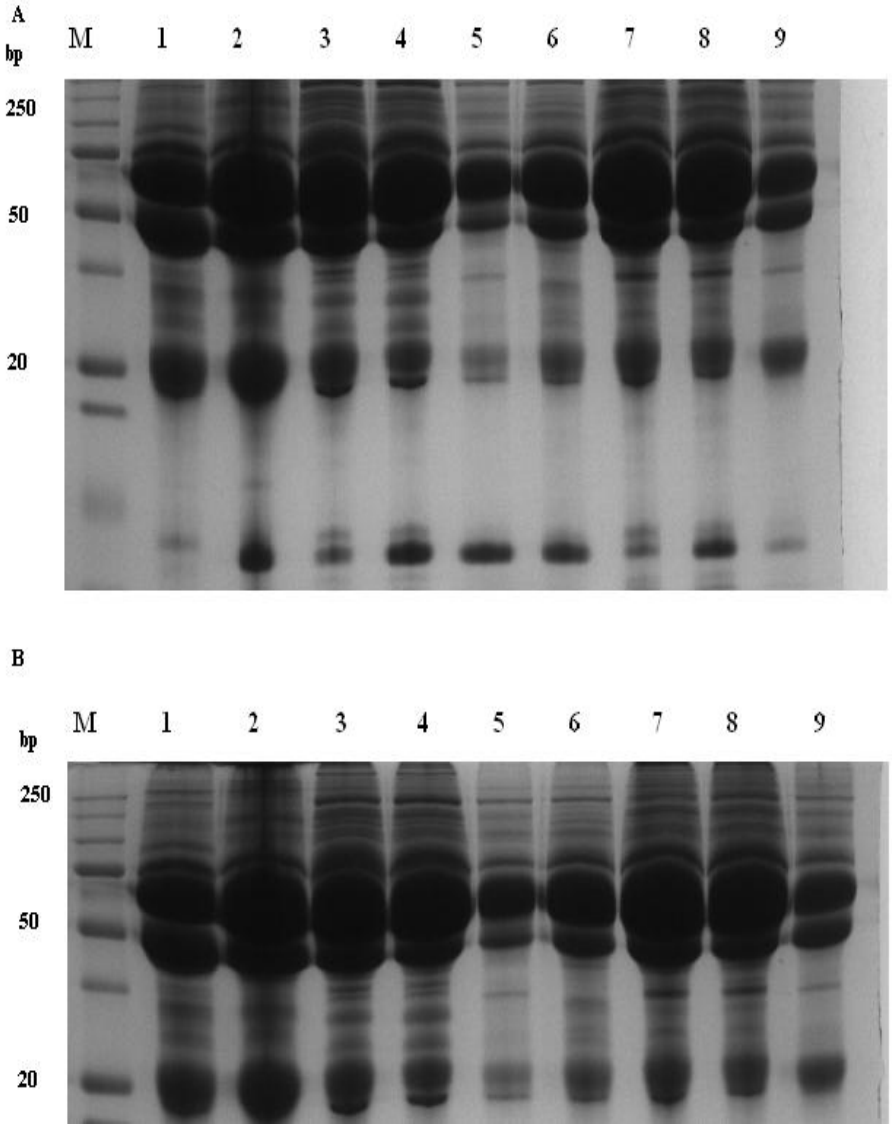

Figure 4. 10 Protein profile fingerprints generated from selected indigenous sheep using PAGE. The first lane in each of the profiles represents the molecular size marker (Precision Plus Protein, BIO-RAD). Lanes 1 – 2 Zulu; lanes 3 - 4 Swazi; lanes 5-6 Dorper; lane 7 Damara; lane 8 SA Merino and lane 9 Nguni goat.

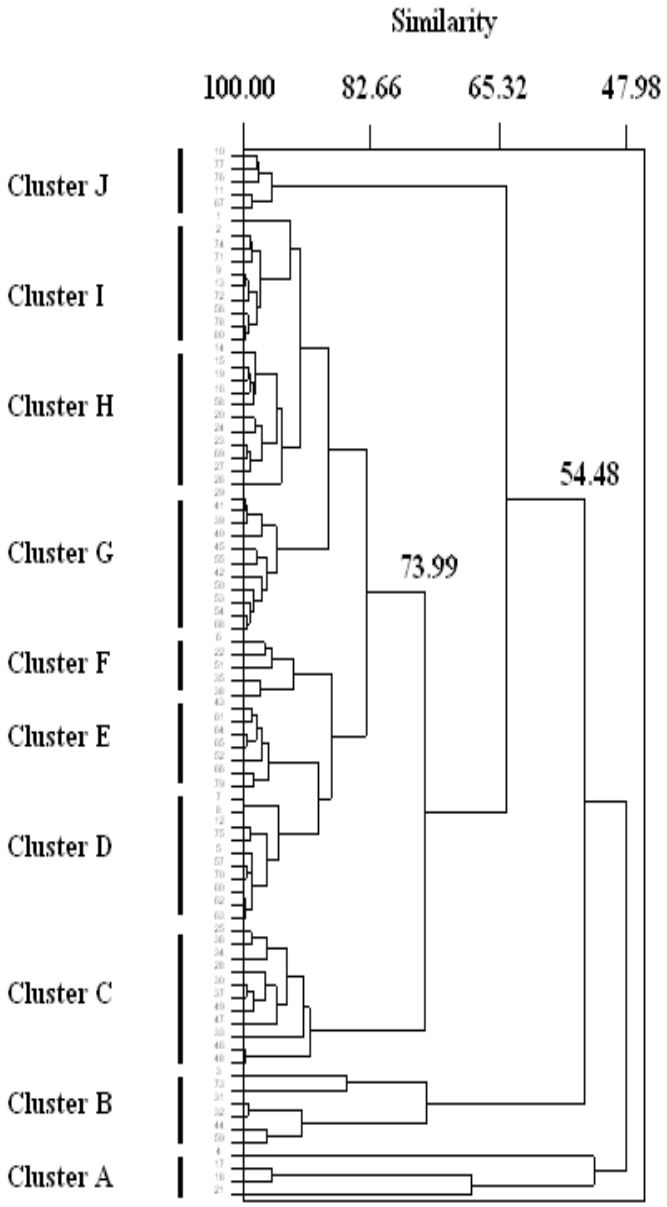

Figure 4. 11 Dendogram showing clusters based on protein profile analysis between and within sheep breeds. Cluster A contains (Zulu and Pedi), cluster B (Pedi, Damara and SA Merino), cluster C (Pedi, Dorper and Damara), cluster D (Zulu, Damara and SA Merino), cluster E (SA Merino and Pedi), cluster F (Zulu, Pedi and SA Merino), cluster G (Dorper, Damara and SA Merino), cluster H (Pedi and SA Merino), clusters I and J (Zulu sheep and Nguni goats).

CHAPTER FIVE

Discussion

5.1 RAPD

There was no relationship between primer GC content and the number of bands it amplified. This corresponds to the results obtained by Devrim *et al.* (2007) when working with genomic polymorphism and diversity in sheep breeds in north eastern Anatolia. In addition, when Kumar (2008) used the same DNA makers to assess the genetic relationship among four Indian sheep breeds, the results revealed no relationship between the GC content and the number of bands it amplified. Similar trends of results were reported by Zulu (2008) on genetic characterization of Zambian indigenous cattle breeds using RAPD markers.

The total number of amplified loci shown by POPGENE software was 126 and the percentage of polymorphic bands was 80.77 %. This percentage of polymorphic band was lower when compared to the percentages of polymorphism (95%) obtained by Buduram (2004) when working with the various sheep breeds of South Africa. The locally developed Dorper produced the highest number (49) of loci and showed the highest percentage (31.41%) of polymorphism among all the selected sheep breeds (Table 4.2).

Nei's genetic distance ranged from 0.0430 – 0.1193 between the six selected sheep breeds. The results indicated that the Zulu and Swazi breeds were the closest sheep breeds with a genetic distance of 0.0430. The close genetic relationship observed between these breeds is possibly due to their common ancestors (Nguni) and geographical locations. When using microsatellite markers, Buduram (2004) found the Zulu and the Swazi sheep to be genetically closer to each other than to any other SA sheep breeds. A genetic distance of 0.182 between the two breeds was reported.

The author attributed this to the lack of a geographical barrier between the two sheep breeds.

The range of genetic identities (0.8876 - 0.9579) is larger than with the lowest and largest genetic identity (0.561 - 0.834) obtained by Buduram (2004) on the same sheep breeds. This could still be due to the fact that different DNA markers were used for the two studies. The genetic identity was highest between the Swazi and Zulu sheep breeds (0.9579). The result confirms the closeness of the Zulu and Swazi sheep. The lowest value of genetic identity (0.8876) was found between the SA Merino and the Pedi sheep breeds indicating that these two breeds are genetically distant from each other.

Furthermore, the dendogram in Figure 4.5 also confirmed the close proximity of the Zulu and Swazi breeds. It was expected that these two breeds would form a cluster because they have been classified as a subgroup of the Nguni sheep (Ramsay *et al.*, 2000). Similar trend of results were observed by Buduram (2004) when using microsatellite markers. In addition these sheep breeds share certain characteristics, including small body size, hardiness and colour patterns.

In accordance with Nei's genetic identity, genetic distance and the dendograms presented, the Pedi breed did not cluster with the Zulu or Swazi breeds. This corresponds to the results obtained by Buduram (2004), which showed that the Pedi was found to be genetically distant from the indigenous breeds like Zulu, Swazi and Afrikaner types. The findings of the current study also indicate that the Pedi were even more distant from the Damara breed, with a genetic distance of 0.1128. Using microsatellites, Buduram (2004) also reported a relatively higher genetic distance (0.572) between the two breeds (Pedi and Damara) and concluded that the Pedi and Damara sheep breeds are different.

According to the Nei's dendogram (Figure 4.4), the Pedi was closer to the Dorper. This was not expected because Dorper is not part of the Nguni population. The genetic distance shown by microsatellite markers indicated that Dorper was more closely related to Nguni (Zulu, Swazi and Pedi) breeds (0.325, 0.340 and 0.513 respectively) compared to the Damara (0.871) sheep breed (Buduram, 2004). Phenotypically the Dorper is white and has a characteristic black or white head. On

the other hand, the Pedi sheep colours vary from uniform brown through white with a red to brown head to a variety of black and white patterns (Ramsay *et al*., 2000). However, these two breeds have many characteristics in common. They both have a quality skin (Dorper Breeders' of South Africa, 2000). Both Dorper and Pedi breeds are fertile, excellent mothers, parasite tolerant and more superior meat - extremely tender (Ramsay *et al*., 2000). Both breeds are classified as having more hair than wool.

In the Nei's dendogram (Figure 4.4) and genetic distance (Table 4.4), there was a relatively closer relationship between the two groups; Dorper and Pedi as a group with the Swazi and Zulu sheep as a group than with the Damara and SA Merino sheep breeds. This also corresponds with results obtained by Buduram (2004) and Peter *et al.* (2010) where Damara and Dorper were genetically different. It was expected that the Damara and Dorper would be genetically further apart because of their origin. Damara breed is indigenous to Namibia and it has been speculated that the Khoi-Khoi people migrated with Damara sheep southwards (Buduram, 2004). On the other hand the Dorper is a man-made breed which was developed in South Africa after crossing the mutton producing Dorset Horn and the hardy Blackhead Persian. The Dorset Horn is of British origin, developed from crossing the Merino with the horned sheep, whereas the Blackhead Persian is believed to be an indigenous sheep of South Africa because it was developed from the local breeds (Campbell, 1989; Nel, 1993; Dorper Breeders of South Africa, 2000).

The greatest genetic distance of 0.1193 between the six selected South African sheep breeds was observed between Pedi and SA Merino. The characteristics of the Pedi and SA Merino have been reported to be different (Ramsay *et al.*, 2000; DAD-IS, 2009). The SA Merino is a non-selective grazer, known as a true dual purpose mutton-wool breed and well known for excellent reproductive ability (DAD-IS, 2009). The Pedi sheep is a strong breed, and it is a heat and tick-borne disease-tolerant breed compared to the SA Merino sheep. It has more hair than wool (Ramsay *et al.*, 2000). Pedi sheep are mostly grazers although, in common with other indigenous African animal breeds, they are equally browsers. They are found predominantly in the sub tropical bushveld and semi arid savannah. The Pedi were brought to the north of South Africa by the migration of the Bapedi people (Ramsay

et al., 2000). Initially the ancestral stock of this sheep was seen in Egypt (DAGRIS, 2009). On the other hand, SA Merino was imported into South Africa from Spain (DAD –IS, 2009).

Nei's genetic identity and genetic distance, UPGMA dendogram and Minitab dendograms reflected a close genetic relationship between the Damara and SA Merino breeds. This was an interesting finding because there is no evidence of similarity between these two breeds. Unlike the SA Merino, the Damara is known as a fat tailed hair breed, it has the ability to survive in harsh environments and in poor nutritional conditions, which make it suitable for the communal areas of Namibia and Southern Africa (Du Toit, 2008). The close genetic relationship is difficult to explain because of the different properties and origins. Perhaps a study using mitochondrial DNA and microsatellite markers can provide more accurate estimates on the origin and genetic relationship between these two breeds. This is because mtDNA is transmitted only through the maternal line in most species (Gyllensten *et al*., 1985; Avise and Vrijenhoek, 1987). It shows haplotype diversity within species which makes it a useful tool in establishing phylogenetic relationships at or below the species level (Avise and Vrijenhoek, 1987). It has been used in studies of the origins of sheep (Hiendleder *et al*., 1998; 2002; Tapio *et al.*, 2001). Microsatellite markers can reveal degrees of polymorphism that are easy to interpret. Hypervariability is important in detecting differences in a population and between individuals. Microsatellite markers are also recommended by the Food and Agricultural Organization (FAO) for animal genetic resources studies (FAO, 2000).

In general, the phylogenetic trees constructed separated the exotic Merino from the Nguni and Dorper sheep, perhaps suggesting early prehistoric separation of origins.

Despite the fact that the indigenous Zulu breed was sampled from three diverse areas (UNIZULU, Makhathini Research Station and from the KwaMthethwa community), the genetic similarity within this breed was relatively high (72.77%). The heterozygosity within the Zulu sheep breed was therefore 27.23%. These results are not far from the 28% genetic diversity which was reported by Kunene *et al.* (2008) within this breed. The genetic diversity within the Swazi breed was the highest (33.52%) among all the sheep breeds showing a higher proportion of heterozygotes in the Swazi breed.

The results demonstrated that SA Merino had the lowest genetic diversity (11.52 %). This suggested that Merino had lowest proportion of heterozygote in comparison to all the other sheep breeds investigated. The reason could be due to the fact that there was more probability for selection rather than random mating, thus accounting for low variation within the breed. Selection for economically important traits such as wool characteristics has been widely practiced in this breed (Bidinost *et al.,* 2008).

The genetic variation within the Damara population was relatively low (16.76%) compared to the Dorper (24.62%) and the Pedi (29.24%). The higher the genetic variation in a breed the higher the chances of survival, whereas low genetic variation is often the basis for traits, such as high disease susceptibility and low reproductive success, which result in species vulnerability (O'Brien *et al.,* 1985; Wildt *et al.,* 1987; O'Brien, 1994). However it may be necessary to expand the number of populations for Damara sheep in order to make conclusive statements about the genetic diversity within this breed.

Nguni goats showed the lowest number of loci and percentage of polymorphism compared to the sheep breeds. The highest range (0.1036-0.1316) of genetic distance and the lowest range (0.8714-0.9016) of genetic identity in goats compared with all the sheep breeds are shown in Table 4.4. Such observations were expected because these are different species. In this study a similarity of 51% was observed between the six SA sheep breeds and the Nguni goats. Kunene *et al.* (2009) reported a genetic similarity of 64% between Nguni sheep and goats. The UPGMA Dendograms based on Nei's genetic distance and dendograms based on complete linkage algorithm showed Nguni goats as an out group breed.

5.2 Polyacrylamide gel electrophoresis (PAGE)

The allelic constitution observed was generally comparable in all the populations studied. Six alleles of transferrin were observed, with the TfE and TfF turning out to be the most frequent alleles in all the sheep breeds as well as with the Nguni goats. Four alleles of transferrin were observed in the SA Merino breed. These results were different to those observed by Mwacharo *et al. (*2002) who reported five alleles in

Merino in Kenya. The B, D and E alleles in the transferrin system showed a genetic relationship between Pedi and Dorper.

The results presented by Minitab dendograms (Figure 4.8 and 4.9) show a close genetic relationship between Damara and SA Merino breeds; this was also observed with the RAPD markers. The degree of similarity (97.13%) was very high between these breeds. This relationship was higher than the value observed using the RAPD markers (80.60%) between the same breeds. However on phylogeny tree (Figure 4.8 and 4.9) a few individuals overlapped between these two sheep breeds. In general the enzyme transferrin profile separated the exotic SA Merino from the indigenous Nguni sheep breeds, suggesting either early prehistoric separation of origins. In addition when Mwacharo *et al.* (2002) used protein markers, Merino sheep did not form a cluster with fat tailed sheep.

Although the Dorper was more closely related to the Zulu sheep, few individuals of the Pedi sheep joined with those of the Dorper (Figure 4.8 and 4.9). The majority of Pedi sheep formed their own cluster and were closer to the Damara and Merino then to the Dorper and the Zulu sheep. These results to some extent still show that the Pedi sheep is not so close to the Nguni sheep. However, since the Swazi breed was not included on the protein analysis one may not know if it would have formed a cluster with the Zulu breed as was in the RAPD analysis.

The iso-enzymes of transferrin also revealed that Nguni goats were genetically different from the five selected sheep breeds. Although the use of transferrin enzyme markers had certain difficulties in characterising the genetic diversity within and between some of the populations, this system managed to differentiate Nguni goats from the sheep breeds investigated.

The dendogram based on total protein profile failed to characterise the genetic relationship between the breeds and between the species. More overlaps were identified in the protein profile when genetic relationship was measured between the populations. Perhaps the DNA markers are more accurate to estimate the genetic relationship between these breeds than use of protein profile.

CHAPTER SIX

Conclusion and Recommendations

This study demonstrated the usefulness of the RAPD assay in detecting genetic variation and similarity within and between selected sheep breeds. It showed that RAPD markers can be used to indicate less genetically similar individuals within the breeds or populations. Such results can be used to assist in selecting animals to be used in *ex-situ* conservation so that the germplasm bank is able to hold the maximum genetic variations which exist in populations.

It was very interesting to observe that although the ancestry of Pedi, Zulu and Swazi sheep is said to be Nguni, the Pedi was closer to the Dorper. More research needs to be done on the origin of, and on the genetic relationship between the Pedi and two breeds (Zulu and Swazi sheep). Perhaps the mitochondrial DNA would shed some light on this aspect, because population studies have shown that mtDNA is transmitted only through the maternal line in most species.

Random amplified polymorphic DNA markers managed to identify the genetic diversity between and within the six sheep breeds. There is enough evidence to conclude that the Zulu and the Swazi sheep breeds are genetically related. It was also important that the trend of results of the genetic work that was done by other researcher using microsatellite markers on similar SA sheep breeds was relatively similar to the trend of results realised in this research using RAPD analysis. However more research using other DNA markers needs to be conducted to investigate the relationship between the Damara and the SA Merino.

The protein profile was unable to clearly identify genetic variation between and within sheep breeds, nor even between species. The transferrin enzyme profile managed to some extent to demonstrate the genetic variations between the breeds. In addition it was also able to distinguish between the goats and sheep. It seems that

some protein markers may be used to identify different species as it was realised with the transferrin enzyme profile**.** Therefore, even the second objective of this study, which was to compare the efficiency of protein markers and random amplified polymorphic DNA while assessing the genetic variation between and within sheep breeds, was therefore met.

These data are important for developing a database for sheep breeds and are very important in breed characterisation and conservation studies as well as in selection programmes. As it is possible to estimate the time of domestication of a species using mtDNA therefore it is recommended that more studies should be done using such markers to improve the present genetic database records of the sheep breeds of South Africa.

References

Ali, A.B., 2003. Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers. African Journal of Biotechnology 2 (7), pp.194–197.

Appa, K.B.C., Bhat, K.V., Totey, S.M., 1996. Detection of species-specific genetic markers in farm animals through random amplified polymorphic DNA (RAPD). Genetic Analysis of Biomolecular Engineering 13, pp.135–138.

ARC-AII, 2001. The origin of the indigenous livestock of South Africa: http://www.arc.agric.za/institutes/aii/main/divisions/animalbreedgen/reproduction/genresdep.htm#top.

Arranz, J.J., Bayon,Y., Primitivo, F. S., 1996. Comparison of protein markers and microsatellites in differentiation of cattle populations. Animal Genetic 27, pp. 415–419.

Ashton, C., 1958. Serum protein variations in horses. Nature 182, pp. 1029-1030.

Avise, J.C., Vrijenhoek, R.C., 1987. Mode of inheritance and variation of mitochondrial DNA in hybridogenetic fishes of the genus Poeciliopsis. Molecular Biology and Evolution 4, pp. 514-525.

Barker, J.S.F., Moore, S.S., Hetzel, D.J.S., 1997. Genetic diversity of Asian water buffalo (*Bubalus bubalis*): microsatellite variation and a comparison with protein-coding loci. Animal Genetics, 28, 103–115.

Barker, J.S.F., Tan, S.G., Moore, S.S., 2001. Genetic variation within and relationships among populations of Asian goats (*Capra hircus*). Journal of Animal Breeding and Genetics 118, 213–233.

Bidinost, F., Roldán, D.L., Dodero, A.M., 2008. Wool quantitative tract loci in Merino sheep. Small Ruminant Research, 74: pp. 113-118.

Bjornstad, G., Roed, K.H., 2001. Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers. Animal Genetics 32, pp. 59-65.

Buduram, P., 2004. Genetic Characterisation of South African Sheep Breeds Using DNA markers. M.Sc. thesis, University of Free State, South Africa.

Campbell, Q.P., 1989. Make money with mutton sheep. Cedar printing and silks screening, P.O. Box 2881, North End 6056, South Africa.

Cardellino, R. A., 2004. Conservation of farm animal genetic resources a global view. Farm Animals Genetic Resources 30, pp. 1-14.

DAD-IS (Domestic animal diversity information system), 2005. http://www.dad.fao.org/en/home.htm.

DAD-IS (Domestic animal diversity information system), 2009. http://www.dad.fao.org/en/home.htm.

DAGRIS (Domestic animal genetic resources information system), 2009. October, 2009. http://www.dagris.ilri.cgiar.org/browse.asp?SC=&BN=Merino.

Damara Breeders' society of South Africa, 1993. Damara Sheep Journal, Ministry of Agriculture, Water and Rural Development, Republic of Namibia, Private Bag 13184, Windhoek, 9000 http://www.damarasheep.co.za.

Devrim, A.K., Kaya, N., Guven, A., Kocamis, H., 2007. A study of genomic polymorphism and diversity in sheep breeds in northeastern Anatolia. Small Ruminant Research 73, pp.291–295.

Deza, C., Perez, G.T., Gardenal, C.N., Varela, L., Villar, M., Rubiales, S., Barrioglio, C., 2000. Protein polymorphisms in the native goats from central Argentina, Small Ruminant Research 35 (3), pp. 195-201.

Dorper Breeders' Society of South Africa, 2000. Small Ruminant Research Volume 36, issue 2, May 2000, pp. 99-102. http://www.dorperbreeders/SA.

Dovie, D.B.K., Shackleton, C.M., Witkowski, E.T.F., 2006. Valuation of communal area livestock benefits, rural livelihoods and related policy issues. Land use policy 23, pp. 260-271.

Du Toit, D.J., 2008. The indigenous livestock of Southern Africa. http://www.damarasheep.co.za/files/ParisRoundtable.pdf.

Duranton, C., Dorchies, P., Jourdane, J., Durand, P., 2001. Genetic structure of *Oestrus ovis* populations in sheep and goats. Volume 104, Issue 2, pp. 167-173.

Elmaci, C., Oner, Y., Seniz, O., Ozis, S., Tuncel, E., 2007. RAPD analysis of DNA polymorphism in Turkish sheep breeds. Biochemical Genetics 45, pp. 691–696.

FAO, 1992. Domestic Animal Diversity Information System (DAD-IS): http://www.fao.org/docrep, World Watch List for Domestic Animal Diversity, 3rd edition – FAO, October, 1992.

FAO, 2000. World Watch List for domestic animal diversity FAO, Rome. October, 2000.

FAO, 2000a. Domestic Animal Diversity Information System (DAD-IS 2.0): http://dad.fao.org/dad-is/home.htm.

FAO, 2000b. In: Scherf, B. (ed), World Watch List for Domestic Animal Diversity, 3rd ed., Rome.

FAO, 2007. The state of the world's animal genetic resources for food agriculture. http://www.fao.org/docrep/010/a1250e/a1250e00.htm.

Feral, J. P., 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity. Marine Biology and Ecology 268: 121-145. Genetic variation. Animal Genetics 26, pp. 315–320.

Galal, S., Gürsoy, O., Shaat, I., 2008. Awassi sheep as a genetic resource and efforts for their genetic improvement review. Small Ruminant Research 79, pp. 99–108.

Gwakisa, P.S., Kemp, S.J., Teale, A.J., 1994. Characterization of zebu cattle breed in Tanzania using Random Amplified Polymorphism DNA marker. Animal Genetics 25, pp. 89-94.

Gyllensten, U., Wharton, D., Wilson, A.C., 1985. Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. Journal of Heredity 76, pp. 321-324.

Hammond, K., 2000. A global strategy for the development of animal breeding programmes in lower-input production environments. Animal Genetic Resources, Animal Production and Health Division, FAO, Rome, Italy.

Hannotte, O., Jianlin, H., 2005. Genetic characterization of livestock populations and its use in conservation decision making, pp. 89-96.

Hassen, F., Bekele, E., Ayalew, W., Dessie, T., 2007. Genetic variability of five indigenous Ethiopian cattle breeds using RAPD marker. African Journal Biotechnology 6, pp. 2274-2279.

Hetzel, D.J.S., Drinkwater, R.D., 1992. The use of DNA technologies for the conservation and improvement of animal genetic resources. FAO Expert Consultation on the Management of Global Animal Genetic Resources, Rome, April 1992.

Hiendleder S., Kaupe, B., Wassmuth, R., Janke, A., 2002. Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. Production of the Royal Society Biological Science 269, pp. 893–904.

Hiendleder, S., Mainz, K., Plante, Y., Lewalski, H., 1998. Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources. No evidence for contributions from Urial and Argali sheep. Journal of Heredity 89, pp. 113-120.

Ibeagha, A. E. M., Jann, C. O., Weimann, C., Erhardt, G., 2004. Genetic diversity, introgression and relationships among West/Central African cattle breed. Genetics Selection Evolution 36:673–690. Issues, Land Use Policy 23, pp. 260–271.

ILRI, 2010. International livestock research institute. http://www.ilri.org/

Jurecka, P., Irnazarow, I., Westphal, A. H., Forlenza, M., Arts, A. J., Savelkoul, F.K., Wiegertjes, F.G., 2009. Allelic discrimination, three-dimensional analysis and gene expression of multiple transferrin alleles of common carp (Cyprinus carpio L.), Fish Shellfish Immunol, 26, 4, pp. 1050-4648.

Kantanen, J., Olsaker, I., Holm, L., 2000. Genetic diversity and population structure of 20 North European cattle breeds. Journal of Heredity, 91, pp. 446–457.

Kantanen, J., Vilkki, J., Elo, K., Maki-Tanila, A., 1995. Random amplified polymorphic DNA in cattle and sheep: application for detecting genetic variation. Animal Genetics. 26, pp. 315–320.

Kosgey, I.S., Okeyo, A.M., 2007. Genetic improvements of small ruminants in low-input, smallholder production systems: Technical and infrastructural issues. In: Notter, D.R., Barker, R.L., Cockett, N. (Eds.), The Outlook for Quantitative and Molecular Genetic Applications in Improving Sheep and Goats. Small Ruminant Research 70, pp.76–88.

Kruger, I., 2009. The indigenous sheep of South Africa. http://www.arc.agric.za/home.asp?pid=2699

Kumar, K.G., Kumar, P., Bhattacharya, T.K., Bhushan, B., Patel, A.K., Choudhary, V., Sharma, A., 2008. Genetic relationship among four Indian breeds of sheep using RAPD-PCR. Journal of Applied Agricultural Research 42, pp. 177–183.

Kunene, N.W., Bezuidenhout, C.C., Nsahlaic, I.V., 2009. Genetic and phenotypic diversity in Zulu sheep populations: Implications for exploitation and conservation. Small Ruminant Research 84 (2009), pp. 100–107.

Kunene, N.W., Bezuidenhout, C.C., Nshalai, I.V., 2008. Use of RAPD markers for detecting genetic similarity between and within Zulu (Nguni) sheep populations. World Congress on Animal Production, Cape Town, 23–28 November.

Kunene, N.W., Fossey, A., 2006. A survey on livestock production in some traditional areas of Northern KwaZulu-Natal in South Africa. Livestock Research for Rural Development 18, pp.113.

Kunene, N.W., Nesamvuni, E.A., Fossey, A., 2007. Characterization of Zulu (Nguni) sheep using linear body measurements and some environmental factors affecting these measurements. South African Journal Animal Science. 37 (1), pp. 11–20.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227(259), pp. 680-685.

Marais, P.G., Schoeman, A., 1990. Geographic distribution of Dorper sheep in the republic. Dorper News 46**,** pp. 4–5.

Mason, I.L., 1996. A World Dictionary of Livestock Breeds, Types and Varieties, 4th ed. CAB International, Wallingford, UK.

Meuwissen, T.H., 1991. Reduction of selection differentials in finite populations with a nested full-half sib family structure. Biometrics 47(1), pp.195-203.

Minitab Inc., 1998. Minitab Reference Manual. Minitab Inc., United States of America.

Missohou, A., Nguyen, T.C., Sow, R., Gueye, A., 1999. Blood polymorphism in West African breeds of sheep. Tropical Animal Health Production, 31(3), pp. 175-179

Mohammad, M. R. M., Parvin, M., Kumar, G.D., Nahar, K., Khan, S. H., 2009. Genetic diversity of Red Chittagong cattle using randomly amplified polymorphic DNA markers. American Journal of Animal and Veterinary Sciences 4 (1): pp. 1-5. Animal Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka -1341, Bangladesh

Mwacharo, J. M., Okeyo, A.M., Kamande, K.G., Rege, J. E. O., 2006. The small East Africa shorthorn Zebu cows in Kenya. I: Linear body measurements. Tropical Animal Production 38, pp. 65-74.

Mwacharo, J.M., Otieno, C.J., Okeyo, A.M., 2002. Genetic variations between indigenous fat-tailed sheep populations in Kenya. Small Ruminant Research 44, pp. 173–178.

Ndamukong, K.J.N., 1995. Haemoglobin polymorphism in Grassland Dwarf sheep and goats of the North West Province of Cameroon. Bulletin Animal Health and Production. Africa 43, pp. 53–56.

Nei, M., 1972. Genetic distance between populations. American Naturalist 106, pp. 283–292.

Nei, M., Tajima, F., Tateno, Y., 1983. Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution 19, pp.153–170.

Nel, J.A., 1993. History of the Dorper. The Dorper Breeders'Association of South Africa. PO Box 26, Middelburg CP 5900, South Africa

New Zealand Rare Breeds, 2007. Website: www.rarebreeds.co.nz – Information, 2007-2010

Notter, D. R., 1999. The importance of genetic diversity in livestock populations of the future. Animal Science 77, pp. 61–69.

Notter, D.R., Barker, R.L., Cockett, N., 2007. The Outlook for Quantitative and Molecular Genetic Applications in Improving Sheep and Goats. Small Ruminant Research 70, pp. 76–88.

Nsoso, S.J., Podisi, B., Otsogile, E., Mokhutshwane, B.S., Ahmadu, B., 2004. Phenotypic characterization of indigenous Tswana goats and sheep breeds in Botswana: continuous traits. Tropical Animal Health and Production. 36, pp. 789–800.

O'Brien, S.J., 1994. A role for molecular genetics in biological conservation. Proceeding of the National academy of Science, USA, 91, pp. 5748–5755.

O'Brien, S.J., Roelke, M.E., Marker, L., Newman, A., Winkler, C.A., Meltzer, D., Colly, L., Everman, J.F., Bush, M., Wildt, D.E., 1985. Genetic basis for species vulnerability in the cheetah. Science 227, pp. 1428–1434.

O'Neil, D., 2010. Blood Components. Copyright © 1999-2010. http://anthro.palomar.edu/blood/blood_components.htm. September 21, 2010

Ordas, J.G., Primitivo, S. F., 1986. Genetic variations in blood proteins within and between Spanish dairy sheep breeds. Animal Genetics 17, pp. 255–266.

Peters, F.W., Kotze A., Vander Bank F.H., Soma, P., Grobler J.P., 2010. Genetic profile of the locally developed Meatmaster sheep breed in South Africa based on microsatellite analysis. Volume 90, Issues 1-3, pp. 101-108.

Ramsay, K., Harris, L., Kotze, A., 2000. Landrace Breeds: South Africa's Indigenous and Locally Developed Farm Animals. Farm Animal Conservation Trust, Pretoria.

Rege, J.E.O., Yapi-Gnaore, C.V., Tawah, C.L., 1996. The indigenous domestic ruminant genetic resources of Africa. Africa Conference on Animal Agriculture 1/4/1996, Pretoria, South Africa.

South African Livestock Breeding, 1998. SA Studbook Association of South Africa. http://www.studbook.co.za/

Stasio Di, L., 1997. Biochemical genetics. Genetics of sheep. Piper, L. and Runvisky, A. (Eds), Oxon, CAB International, pp.133-148.

Stormont, C., Brand, M., 1964. Studies on hemoglobin and transferrin types of horses. Nord. Veterinarni Medicina, 16, pp. 31-37.

Taberlet, P., Valentini, A., Rezaei, R., Naderi, S., Pompanon, F., Negrini, R., Ajomone-Marsani, P., 2008. Are cattle, sheep and goats endangered species? Molecular Ecology 17, pp. 275–284.

Tapio, N., Miceikeikiene, L., Vilkki, J., Kantanen, J., 2001. Comparison of microsatellite and blood protein diversity in sheep, inconsistencies in fragmented breeds. Molecular Ecology, 12, pp.2045-2056.

Wildt, D.E., Bush, M., Goodrowe, K.L., Packer, C., Pusey, A.E., Brown J.L., Joslin, P., O'Brien, S.J., 1987. Reproductive and genetic consequence of founding isolated populations. Nature (London) 329, pp. 328–331.

Williams, J.G.K., Kubelik, A.R., Livak, J.K., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Research 18, pp. 6531-6535.

Yeh, F.C., Boyle, T., Rongcai, Y. Ye. Z., Xian, J.M., 1999. POPGENE, Version 1.31. A Microsoft window based free ware for population genetic analysis. University of Alberta, Edmonton. Canada.

Zulu, D.N., 2008. Genetic Characterization of Zambian Native Cattle Breeds by DNA Markers. MSc. Thesis. Blacksburg, Virginia Polytechnic Institute and State University.