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Genetic and phenotypic characterisation of the South African Namaqua Afrikaner sheep breed

By

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Submitted in partial fulfilment of the requirements for the degree
MSc (Agric) Animal Science

Faculty of Natural and Agricultural Sciences
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Declaration

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

Signature……..

Date……..

Acknowledgement

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Abstract

Genetic and phenotypic characterisation is essential for the conservation and utilisation of farm animal genetic resources, especially indigenous types that are often disregarded due to lower production potential compared to commercial breeds. In this study a genetic characterisation was performed on 144 Namaqua Afrikaner sheep kept at the Karakul Experimental Station (KES) and Carnarvon Experimental Station (CES) and a private farm Welgeluk (WGK) using 22 ISAG recommended microsatellite markers. Results of this study showed that the mean number of alleles were low (3.6 for KES to 4.2 for WGK) for the loci tested. Heterozygosity values across loci ranged between 46% for WGK, 48% for KES and 55% for CES, indicating low to moderate genetic variation within the different populations. The AMOVA analyses revealed that 89.5% of the genetic variation in the breed was due to the differences within populations and 10.5% due to differences between populations. The genetic distance estimates revealed a close relationship between the CES and WGK populations. The population structure confirmed the differentiation of three clusters with relationships between the CES and WGK populations. Phenotypic characterisation of the breed was limited to the Carnarvon flock, where production and morphological data were recorded. Morphological measurements indicated an average body length of 71.2 cm and 68.7 cm for rams and ewes respectively. Over 60% of the sheep had their tail twisted to the left. The molecular data provided by this study will serve as a reference for genetic management and breeding strategies of the indigenous Namaqua Afrikaner sheep.

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Abbreviations

AFLP	Amplified Fragment Polymorphic DNA
AD	Anno Domini – number of years since the time of Jesus Christ
AMOVA	Analysis of molecular variance
ARC	Agricultural Research Council
CES	Carnarvon Experimental Station
DNA	Deoxyribonucleic acid
DAD-is	Domestic Animals Diversity Information System
DAFF	Department of Agriculture, Forestry and Fisheries
FAO	Food and Agricultural Organisation of the United Nations
FanGR	Farm animal genetic resource
F_{it}	Inbreeding coefficient of an individual (i) relative to the total population (t)
F_{is}	Inbreeding coefficient of an individual relative to the subpopulation (s) compare to the total population (t)
Fs_t	The amount of differentiation among subpopulations relative to the limiting amount under complete fixation
GADI	Grootfontein Agricultural Development Institute
He	Expected heterozygosity
Hobs	Observed heterozygosity
HWE	Hardy Weinberg Equilibrium
ISAG	International Society of Animal Genetics
WGK	Welgeluk farm
K	Number of assumed populations
KES	Karakul Experimental Station
Ln Pr (X|K)	Ln probability of the data
N	Number of individuals
NCDALRRD	Northern Cape Department of Agriculture, Land Reform and Rural Development
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
Q	The estimated membership coefficient of each individual in each cluster
RAPD	Random Amplified Polymorphic DNA Fragment
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
UN	United Nation
UPGMA	Unweighted pair group method with arithmetic mean
VNTR	Variable Tandem Repeat

Chapter 1

1.1 Introduction

Indigenous breeds have the ability to adapt and survive in often challenging environments. They adapt to a variety of ecological areas and thus represent an important genetic resource for livelihood of rural inhabitants (Anderson, 2003). In developing countries indigenous breeds play an important role in the livelihoods of herders and smallholders and in the utilisation of marginal ecological areas. Their role often includes the provision of traction and manure, a source of savings, insurance, and serving a socio-cultural purpose (e.g. dowry payment and / or slaughter during special ceremonies) (Kunene *et al.*, 2009). A number of factors have contributed to the severe erosion of indigenous genetic resources and even extinction of indigenous breeds. These include the use of exotic breeds, changes in breeder's preferences due to short-term socio-economic influences, degradation of the ecosystem in which the breeds were developed as well as natural disasters such as drought and diseases (FAO, 1998; FAO, 2000). Therefore, there is a need to characterise indigenous breeds in order to understand the existing diversity to facilitate the development of rational utilisation and conservation strategies for these breeds (Hanotte & Jianlin, 2005).

The Namaqua Afrikaner is one of the oldest sheep breeds in South Africa. The original Namaqua Afrikaner sheep migrated south with the Khoisan people and entered South Africa between 200 and 400 AD (Cloete, 1978; Ramsay *et al.*, 2001). The Nama people kept the original Namaqua Afrikaner in the harsh dry areas of the north-west Cape and southern Namibia. This breed was facing extinction when the Department of Agriculture bought one of the last purebred flocks from Mr. P. J. Maas from Namies, Springbok in 1966 and since then this flock has been kept at the Carnarvon Experimental Station in the Northen Cape. In March 1985, 30 ewes and five rams from the Carnarvon flock were transferred to the Tarka Conservation area near Hofmeyer in the Eastern Cape Province. Their numbers were allowed to increase to approximately 100 breeding ewes. In 1991 this flock was transferred to the Grootfontein Agricultural Development Institute (GADI) and since August 1995, the flock has been kept at the Karakul Experimental Station near Upington. (Snyman et al., 2005c) Two Namaqua Afrikaner herds of approximately 120 ewes each are currently maintained by the Northern Cape Department of Agriculture, one at the Carnarvon Experimental Station and the other at the Karakul Experimental Station. The aim of maintaining these flocks is to preserve this unique genetic pool and also to collect production and reproduction data on this endangered breed.

The Namaqua Afrikaner is a fat tailed meat sheep (Figure 1.1) with a soft, shiny, covering of wool and hair. The undercoat is white fluffy wool with a silky feel, while the outer coat consists of long hair. The body covering is shed during summer after forming a mat (Ramsay *et al*., 2001). The Namaqua sheep mostly have black or red/brown heads with white bodies (Figure 1.2). Sheep with black heads have black hooves and horns and their skins also have black pigmentation. Sheep with red heads have amber coloured hooves and horns and brown pigmentation (Cloete, 1978; Ramsay *et al*., 2001).

Figure 1.1 Namaqua Afrikaner sheep showing a fat tail with more than one twist to the right and no left twist

The tail extends as far as the hocks where it makes either a twist to left or right “draaistert” or stands out at right angles to the first portion of the tail “wipstert” (Figure 1.1). As more fat is deposited in the tail the twist becomes more pronounced, resulting in more than one full turn (Cloete, 1978). The skin of the Namaqua Afrikaner is well suited to be processed into leather goods (Snyman & Jackson-Moss, 2005).

Figure 1.2 Namaqua Afrikaner sheep at the Carnarvon Experimental Station showing black and red/brown heads with white bodies

Figure 1.3 Namaqua Afrikaner ram at the Carnarvon Experimental Station displaying long legged phenotype

The Namaqua is a virile sheep breed that has a long life span and their reproduction performance recorded under extreme conditions compare favourably with other South African breeds such as Afrino and Dorper sheep (Snyman *et al*., 1993; Snyman *et al*., 2005b; Snyman *et al*., 2005d). The ewes lamb at an early age and throughout the year. Snyman *et al*. (1993) reported the average age at first lambing to be 16.5 months at an average weight of 45.5 kg at the Tarka Conservation Area. A conception rate of 86.0 ± 2.0% and fecundity rate of 156.4 ± 1.5 were reported by Snyman *et al*. (1993). By utilising their fat reserves, the Namaqua ewes are able to wean heavy lambs even under severe drought conditions. Their mothering ability is excellent, and they will fiercely protect their lambs against predators. Namaqua sheep mature early and have an average lambing cycle of nine months when run under a free-mating system.

The Namaqua sheep has long legs and can walk long distances in search for food and water (Figure 1.3). The breed can tolerate extreme temperatures and also have a remarkable resistance to most African sheep diseases and internal and external parasites. However, comprehensive research data on the genetic potential regarding disease resistance and adaptation mechanisms of indigenous livestock are limited and this has also emphasised the need for a genetic and phenotypic characterisation of the Namaqua Afrikaner as a genetic resource. Indigenous breeds such as the Namaqua Afrikaner are particularly vulnerable as selection for improvement in production and uncontrolled mating strategies may lead to genetic dilution and loss of genetic variation within these breeds, leading to their eventual extinction (Shresta, 2005; Scherf *et al*., 2006). Currently the Namaqua Afrikaner is one of the few indigenous breeds in South Africa for which at least scientific production and reproduction norms are available (Snyman, 2007). It is crucial that endangered resources such as Namaqua Afrikaner sheep will not only be conserved, but also utilised. There is therefore a need for baseline information of the breed for effective conservation and utilisation.

A breed is considered endangered if the total number of breeding females varies between 100 and 1000 and the total number of breeding males between six and 20. The breed is also considered at risk of becoming endangered when the overall population size is slightly above 1000 but decreasing with the percentage of females being bred pure below 80% (FAO, 2000). In 1995, it was estimated that approximately 2000 Namaqua Afrikaner sheep were left in the country (Campell, 1995) and therefore an attempt was made in 1997 to establish a breeding interest group for both the Namaqua and Ronderib Afrikaner sheep breeds. A request was made in the popular press for owners of Namaqua Afrikaner sheep to contact Grootfontein Agricultural Development Institute (GADI) and take part in planning of the future of the breed. Very few Namaqua sheep breed owners made contact and those who did had only a few sheep. currently the majority of the Namaqua breed is kept by the Northern Cape Department of Agriculture, Land Reform and Rural Development (NCDALRRD) at the Carnarvon and the Karakul Experimental Stations (Snyman, 2007). It was time to set the protection and conservation of the breed as a priority.

1.2 Aim of the study

The development of DNA technology has made it possible to study genetic diversity at a genomic level and several small stock breeds in South Africa have been characterised at molecular level. These include Pedi, Swazi, SA Mutton Merino, Karakul, Black and White Persian sheep (Buduram, 2004); and SA Boer goats and Kalahari Red goats (Visser *et al*., 2004), SA Angora goats (Visser & van Marle-Koster, 2009) and Nguni sheep (Kunene *et al.*, 2009).

To date the experimental population of Namaqua Afrikaner sheep has not been characterised and since these are kept for future conservation and utilization the first step was to collect blood for long term DNA storage in the GADI biobank. Since 2007 blood samples of both the experimental populations have been collected and stored in the GADI biobank. Phenotypic traits were however only recorded for the Carnavon population.

In this study the aim was to perform a genetic and phenotypic characterisation of the indigenous Namaqua Afrikaner sheep using 22 microsatellite markers from the recommended panel for diversity by the International Society of Animal Genetics (ISAG). The available production and morphological records were analysed to set breed standards for the breed.

The objectives included:

- To perform a genetic characterisation using an International Society of Animal Genetics (ISAG) panel of microsatellite markers
- To genetically compare the Namaqua Afrikaner sheep with other South African sheep breeds
- To phenotypically describe the Namaqua Afrikaner sheep breed and set breed standards.

Chapter 2

Literature review

2.1 Introduction

Conservation of farm animal genetic resources refers to all human activities including strategies, plans, policies and actions undertaken to ensure that the diversity of farm animal genetic resources is maintained to contribute to food and agricultural production and productivity, now and in future (Scherf, 2000). The idea of conserving animal genetic resources focuses on two separate but interlinked concepts. The first is the conservation of 'genes' and the second, the conservation of 'breeds' or populations (FAO, 2005). Both conservation of genes and breeds is essential to meet future needs in Africa including Southern Africa. In order to cope with an unpredictable future, genetic reserves that can respond and adapt to a broad spectrum of environments must be conserved. These act as storehouses for genetic diversity, which forms the basis for selection and may be drawn upon in times of biological stress such as famine, drought or disease epidemics (FAO, 2005; FAO, 2010). The aim of this chapter is to review appropriate aspects of farm animal genetic resources conservation and application of molecular markers for studying genetic diversity.

2.2 Conservation

Farm animal genetic resources is defined as all species, breeds and strains of animals, particularly those of economic, scientific and cultural interest to mankind for agriculture either at present or potentially in the future (Alderson, 2010). A breed is defined as a homogenous group of domestic livestock with definable and identifiable external characteristics that allow it to be separated by visual appraisal from other similarly defined groups within the same species Scherf (2000). Breeds may share a large proportion of their genome with other breeds, but each possesses distinctive combination of genes. These may include distinctive traits particularly for adaptation to a specific environment (Alderson, 2010). Breeds are also linked to their origin with regard to tradition and history or a geographical region (Alderson, 2010).

Indigenous breeds in general demonstrate low production figures when compared to commercial stock, however they may hold potential due to years of adaption to the pressures of the specific local environment. Adaptive traits that are usually associated with the indigenous breeds include: tolerance to various diseases, tolerance to extreme temperatures and humidity, tolerance to change in the availability of feed, adaptation to low capacity management and ability to survive, produce and

reproduce for long period of time (Scherf, 2000). The conservation of these low-productions breeds could contribute to current or future traits of interest and therefore it should be considered essential for maintaining future breeding options (Groeneveld *et al*., 2010).

The Food and Agricultural Organisation of the United Nations (FAO) have reported that about 690 (9%) of the world's 7500 documented breeds of livestock have become extinct within the past 150 years (FAO, 2007). Furthermore Groeneveld *et al.* (2010) reported that about 20% breeds of domestic animals worldwide are currently at risk. Specifically with regard to domestic sheep, about 14% of sheep breeds worldwide have already been lost (Taberlet *et al*., 2008). In Table 2.1 the population size, current number and number of extinct sheep breeds in Africa are shown.

Table 2.1 Total population size of all recorded African breeds, current number of breeds and number of extinct breeds in Africa (Taberlet *et al*., 2008)

African sheep	**Number**
Population size	127 440 000
Current number of breeds	147
Number of extinct breeds	8

In order to prevent this rapid loss of farm animal genetic diversity the Convention on Biological Diversity (CBD, 1992) has put the need to conserve farm animal genetic diversity on the agenda. This has resulted in the establishment of a program for Global Management of Farm Animal Genetic Resources by the Food and Agricultural Organization with the main objective to stimulate conservation activities and create an awareness of possible losses of genetic resources on an international scale (Scherf, 1995; Gandini & Oldenbroek, 1999, Shresta, 2005). Means to conserve animal genetic resources for food and agriculture include: conservation through utilisation and improvement, *In-situ* conservation and *Ex-situ* conservation. Conservation through utilisation and improvement is a long process and setting up the breeding programs takes a long time to develop. In South Africa, the Damara sheep is an example of an indigenous breed that has commercial value and is farmed with on a commercial scale. It can thus be argued that conservation of indigenous breeds should be focused towards commercialisation of these breeds (Snyman, 2011).

In-situ conservation is the maintenance of live populations of animals in their adaptive environment or as close to it as possible. Geerlings *et al*. (2002) suggested that the *in-situ* conservation of live populations is the most accurate way of conserving local adapted breeds of livestock, especially if the

production systems in which the breeds evolved can also be maintained. Indigenous breeds originate from specific ecological and cultural environments, and if they are removed from their original context their genetic make-up and integrity will be affected. The *ex-situ* conservation involves the collection and freezing of animal genetic resource in the form of living ova, semen or embryos. It may also be the preservation of DNA segment in frozen blood or tissues (FAO, 2002; Fadlaouia *et al*., 2005).

Genetic diversity is a prerequisite for genetic improvement and environmental adaptation of any species or breed (Boettcher *et al*., 2010b). Since it is not possible to conserve genetic diversity of all farm animal genetic resources, prioritisation is imperative (Boettcher *et al*., 2010b). Farm animal genetic resources that are endangered should be prioritised for conservation (Hanotte & Jianlin, 2005). The degree of the endangerment of farm animal genetic resources can be determined by its numerical scarcity, geographical concentration and genetic erosion (Alderson, 2010). Numerical scarcity is measured in many programs by the number of breeding females and effective population size (Ne). The categories for endangerment status of domestic populations according to the numerical scarcity are shown in Table 2.2.

The geographic distribution of a breed around the country, is one of the primary indicators of the breed endangerment. This is because the distribution of a breed is inversely correlated to its vulnerability in the event of a disease epidemic where death or slaughter is the expected outcome (Alderson, 2010). Furthermore, the genetic erosion of a breed that results from high rate of inbreeding, genetic drift, introgression and other impacts on the gene pool is also an important indicator of endangerment (Alderson, 2010).

In order to effectively manage farm genetic resources it is important that comprehensive knowledge of the breed's characteristics is documented (Groeneveld *et al.,* 2010). These include data on the population size, structure, geographic distribution, production environments and within and between breeds' genetic diversity (Groeneveld *et al.,* 2010). Molecular based methods such as microsatellites markers has been used for the capturing of information to estimate the genetic diversity of farm animal genetic resources. These markers give an insight into breed history and provide information regarding both the distinctiveness (across-breeds) and the (within-breeds) diversity of a breed (Boettcher *et al.,* 2010b).

Table 2.2 Categories for endangered status of domestic populations (Bodo, 1989)

Status	Number of breeding females	Description
Extinct	<0	No possibility of restoring the population, no purebred males or females can be found.
Critical	<100	Close to extinction, genetic variability reduced to below that of the ancestral population, action to increase the population size is essential if it is to survive.
Endangered	100–1,000	In danger of extinction because the effective population size (Ne) is too small to prevent genetic loss through inbreeding which will result in a reduction in the viability of the breed. Preservation must be prioritised.
Insecure	1–5,000	Population numbers decreasing rapidly.
Vulnerable	5–10,000	Some disadvantageous affects endanger the existence of the population and some precautionary measures should be taken to prevent further decline.
Normal	>10,000	Population not in danger of extinction can reproduce without genetic loss, no visible changes in population size.

South Africa has established a biological reserve bank for small stock research and conservation purposes. This was an expansion of the DNA bank for Angora goats established at GADI in 2005 as a collaborative effort between GADI, the Department of Animal and Wildlife Sciences at the University of Pretoria and the Angora goat producers. The establishment of the Biological Reserve for South African sheep and goat breeds at Grootfontein Agricultural Development Institute addressed not only the issue of conservation of biodiversity of South Africa's small stock breeds, but also serves as a reserve for genomic research on sheep and goats (Snyman, 2011). The above-mentioned program comprise of three projects, which respectively deal with the establishment and maintenance of live herds of animals (conservation and research), cryopreservation bank (primarily conservation, secondarily research) and Blood and DNA bank (genomic research) (Snyman, 2011). Three flocks of Namaqua Afrikaner sheep are already part of the South Africa Biological Reserve at GADI. The flocks include Carnarvon Experimental Station, Karakul Experimental Station and one private farmer from Welgeluk farm.

The South African Department of Agriculture, Forestry and Fisheries (SADAFF) together with the Agricultural Research Council (ARC) have also established a program for the conservation and development of indigenous livestock that combine traditional conservation approaches with modern biotechnology techniques. These valuable indigenous breeds are currently identified through livestock village surveys and germplasm are conserved through cryopreservation technology for the maintenance of gene pool diversity, for breeding-line restoration, global genetic trading and for the rescue of rare and endangered breeds for future breeding program (Nedambale *et al*., 2010).

2.3 DNA markers as indicators of genetic diversity

The development of molecular tools for the analysis of DNA that has taken place in the last few decades has made an important contribution to characterise variation within and between breeds (Toro *et al*., 2009). Molecular characterisation provides reliable information for assessing, among other factors, the amount of genetic diversity, the structure of diversity in samples and populations, the rates of genetic divergence among populations and the distribution of diversity in populations found in different locations (Hanotte & Jianlin, 2005; Toro *et al*., 2009). Molecular characterisation also helps the understanding of gene flow, the movement of alleles within and between populations of the same or related species, and its consequences (Toro *et al*., 2009). It can also serve as an aid in the genetic management of small populations, to avoid excessive inbreeding. As a result, information from molecular markers or DNA sequences offers a good basis for improving conservation approaches (Hanotte & Jianlin, 2005).

DNA markers are generally classified into single and multi-locus markers; both of these marker types have been used for breed characterisation in both plants and animals (Toro *et al.,* 2009). Multi-locus markers include Variable Tandem Repeat (VNTR) or minisatellites, Random Amplified Polymorphic DNA fragment (RAPD) and Amplified fragment length polymorphisms (AFLP). Minisatellite markers were the first tandem repeat markers with multiple alleles to be developed (Jeffreys *et al*., 1985) and were the first marker to be sufficiently informative to reveal a unique genotype in each individual. A few highly informative single loci minisatellites were identified in livestock, and have been used in genetic diversity studies (Georges *et al.,* 1990). However, single locus microsatellites are now preferred due to their co-dominant nature. Amplified fragment length polymorphism (AFLP) markers are the multilocus marker of choice, being more reproducible than RAPDs. Both these markers are easily generated, but have the disadvantage of being dominant (Crawford *et al*., 2000). RAPD markers have been used in the genetic characterisation of the Nguni sheep (Kunene *et al.,* 2009) while AFLP markers have been used to assess the genetic diversity within and between Italian

goat populations (Ajmone-Marsan *et al*., 2001). Single locus markers have the advantage of being co-dominant and have high reproducibility. They can allow the analysis of only one locus per amplification and are more informative because of the allelic variations that can be distinguished (Beaumont & Bruford, 1999). These include Restriction fragment length polymorphism (RFLP), microsatellite markers and SNP. RFLP markers general refer to the differences in banding pattern obtained from DNA fragments, after digestion with restriction enzymes. RFLP require large amount of DNA and are technically demanding. RFLP have however been applied successfully in the assessment of genetic diversity in chickens (Smith *et al.*, 1999).

Single nucleotide polymorphism (SNP) is the variation in DNA sequence occurring when a single nucleotide - A, T, C, or G – has been substituted by another. SNP technology allows the simultaneous analysis of thousands of parameters within a single experiment, thus generating large amounts of genomic data within a single experiment (Templin *et al.,* 2002). SNP are attractive because they are abundant, genetically stable, and adaptable to high-throughput automated analysis. To date, most work relating to characterisation and conservation has been done with microsatellites markers. SNP are however, becoming the marker of choice and could also be used for conservation purposes (Fan *et al*., 2010). For genome scanning, high-density SNP chips have been developed for the most major livestock species (cattle, pigs, chicken and sheep) (Fan *et al.,* 2010). These chips allow for the simultaneous typing of tens to hundreds of thousand SNP and could improve the accuracy of characterisation (Boettcher *et al*., 2010a).

Microsatellites are the markers responsible for the development of genetic linkage maps of farm animals. They are multi-allelic tandem repeats like minisatellites, but are single-locus, spread abundantly throughout the genome. They require only small amounts of template DNA, are relatively easy to find and characterise and are variable and exhibit a high level of allelic variation (Beaumont & Bruford, 1999). These characteristics enable to estimate within and between breeds genetic diversity. Potential disadvantages of microsatellites include that for certain groups of animals they are difficult to isolate and that data generated in different laboratories using different methods have proved difficult to integrate (Beaumont & Bruford, 1999). A large number of microsatellite markers are however available for most farm animals including sheep (Toro *et al.*, 2009).

Microsatellite markers have been successfully applied in population genetic studies on different sheep breeds. They have revealed a high degree of genetic variation within and between sheep breeds. They have also elucidated the relationships between breeds and identified the genetically most distinct

breeds (Boettcher *et al*., 2010a). Microsatellite markers have therefore provided useful molecular information which could assist in the future management of the small stock breeds (Boettcher *et al*., 2010a). In Table 2.3 a summary is provided of genetic characterisation studies performed on a number of sheep breeds.

Table 2.3 Genetic characterisation studies of sheep breeds world-wide

Sheep breeds	**Title of study**	**Reference**
SA Mutton Merino, Pedi, Namaqua Afrikaner, Nguni, Swazi (South Africa)	Genetic characterisation of South African sheep breeds	Buduram, 2004
Alpine sheep breeds (Italy)	Genetic diversity and variability in Alpine sheep breeds	Dalvit *et al.*, 2008
Jalauni sheep (India)	Genetic variability in Jalauni sheep	Arora *et al*., 2008
Madras Red sheep (India)	Molecular genetic characterisation of Madras Red sheep	Selvam *et al*., 2009
Red Maasai sheep (Kenya)	Genetic characterisation of Kenya Sheep populations	Muigai *et al.*, 2009
Ganjam sheep (India)	Morphological and genetic characterisation of Ganjam sheep	Arora *et al*., 2010
Chinese indigenous sheep breeds (China)	Genetic diversity of Chinese indigenous sheep breeds	Zhong *et al*., 2010

Goat populations have also been characterised to determine the level of genetic diversity within and between populations using microsatellite markers. In studies performed by Visser *et al*. (2004), Visser & van Marle-Koster (2009) and Ramamoorthi *et al*. (2009) microsatellite markers were used to study the genetic diversity and relationship amongst different goats populations for conservation applications. Microsatellite markers are of benefit in the conservation and management of endangered species as they can be amplified via the polymerase chain reaction (PCR) using non-invasive samples, which is important for the study of endangered species (Arranz *et al*., 2001; Kim *et al*., 2004).

Microsatellite markers were chosen for the current study, as a set of markers for diversity recommended by ISAG is available and used in various studies that make comparison possible.

2.4 Statistical analysis of genetic diversity

Genetic diversity is usually expressed as the frequencies of genotypes and alleles, the proportion of polymorphic loci and the observed and expected heterozygosity. To measure diversity within populations, the expected heterozygosity (He) or gene diversity is the most widely used parameter (Nei, 1973).

Alternatively the genetic diversity can be measured by the allelic diversity (number of alleles segregating in the population); this parameter is of key relevance in conservation programs. A high number of alleles imply more genetic variation (Nei, 1987). The mean number of alleles detected depends on sample size of the population because of the potential presence of unique alleles in a population that may occur at low frequencies. The number of detected alleles may increase with an increase in population size. Therefore it is important to sample population sizes that are more or less equal for comparison. Allelic diversity is also important from a long-term perspective, as the limit of selection response is determined by the initial number of alleles. It is more sensitive to bottlenecks than expected heterozygosity as it reflects past fluctuations in population size more accurately (Toro *et al.*, 2009).

In a structured population with *n* breeds/populations, the total gene diversity is partitioned into a component within breeds/populations and another between breeds/populations (Toro *et al*., 2009). Therfore in order to illustrate the partition of gene diversity into components the analysis of molecular variance (AMOVA) can be used (Excoffier *et al*., 2005).

The population subdivision entail an inbreeding-like effect, therefore it is convenient to measure the effect in terms of decrease in the proportion of heterozygous genotypes (Hartl, 1988). The effects of population subdivision are measured by a quantity called the fixation index (Wright, 1978). These include: F_{ST} which measures the reduction in heterozygosity of a subpopulation due to random genetic drift; F_{IS} which is the inbreeding coefficient concerns with inbreeding in individual (I) relative to the total subpopulation (S) to which they belong and F_{IT} which measures the reduction in heterozygosity of an individual relative to the total population (T) (Hartl, 1988). In Table 2.4 the Wright's fixation index formula are summarised.

Table 2.4 Formula for fixation index (Hartl, 1988)

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

The genetic relationship between populations can be measured by determining the genetic distance between populations. This difference measured between two populations provides a good estimate of how divergent they are genetically. Nei's (1978) unbaised genetic distance estimate is one of the common measurements of genetic distance.

The computer program Structure (Pritchard *et al*., 2000) is currently one of the most frequently used statistical tools for describing population structure. It implements a model-based clustering method for inferring population structure using genotypice data. It is suitable for the assignment of populations. Structure (Pritchard *et al*., 2000) assumes a model in which there are K populations (where K may be unknown), each of which is characterised by a set of allele frequencies at each locus. Individuals are assigned to populations according to their membership confidence for each cluster which is interpreted as a probability of membership. This program may be used to assign individuals correctly to a population or a breed, especially when the phenotypic differentiation between breeds/ populations is difficult to detect or when genealogical information is absent. Molecular markers can detect whether introgression or crossbreeding occurred (Pritchard *et al*., 2000).

2.5 Phenotypic characterisation

Livestock breeds have genetically distinct physical characteristics, such as coat colour, performance and other commercial important traits. These characteristics make different breeds of livestock valuable in their own right and also for the particular genes they may possess (Blott *et al*., 2003). Molecular techniques have aided in the identification and characterisation of individuals. However,

for the formulation of appropriate utilisation and conservation strategies phenotypic characterisation of animal genetic resources is also required (FAO 2005; FAO, 2010). Phenotypic characterisation of a breed includes the description of physical characteristics, productive parameters and adaptive characteristics, including a description of environmental conditions under which performance has been measured (Rege & Lipner, 1992). The status of the populations/breed needs to be documented and the full range of existing production and management systems in order to compare breeds with respect to the mean and phenotypic variance of their performance and characteristics (Yakubu *et al*., 2010).

Morphological measurements of breeds/populations provide useful information on the suitability of animals for selection (Yakubu *et al*., 2010). Examples of usually measured morphological traits in sheep are presented in Table 2.5. Also the morphological decsription of a breed such as the description of a coat colour, size, shape of horns, conformation of a tail and also information on historical origin assist in breed description and setting breed standards, thus allowing distinction between breeds and strains (McManus *et al*., 2010).

Performance traits associated with productivity and adaptation provide a basis for variation between and within livestock breeds, which could be utilised for selection purposes (McManus *et al*., 2010). This knowledge may also influence priority for conservation (Boettcher *et al*., 2010b). The recording of production performances for farm animal genetic resources through recoding schemes have been identified by the FAO (1998) as one of the prerequisites for the proper management of farm animal genetic resources. The data collected on the individual animals (pedigree and performance records) could be used for selection and also in management programs that may lead to an improvement of the productivity and profitability of the sheep farms (Gabina, 2002).

In South Africa, the Agricultural Research Council (www.arc.agric.za) is responsible for the documentation of production performance data for all sheep breeds participating in the National Small Stock Performance and Recording Scheme. Production performances commonly recorded in sheep include: male and female birth weight, age at sexual maturity, average age of breeding males, age at first parturition, length of productive life, litter size, carcass weight, dressing percentage and fleece weight (www.arc.agric.za).

Table 2.5 Traits of morphological measurements in small stock breeds (McManus *et al*., 2010)

Traits	Description
Body length (BL)	Distance from the external occipital protuberance to the base of the tail on the dorsal line; distance between tip of scapula and ischium, measured as the distance between the point of shoulder and the pinbone.
Dorso-sternal distance (DD)	From the point of the shoulder to the sternum; distance between dorso and sternum
Eye distance (ED)	Inter orbital distance
Ear length (EL)	From central point of the base to the vertix; from the base of the Notch To the most distant point of the margin of the pinna (external ear)
Head length (HL)	From the external occipital protuberance to tip of nasal bone
Heart girth (HG)	Total distance around the animal (circumference) measured directly behind the front leg; total distance around the animal (circumference) measured directly behind the front leg;
Length of hip (LH)	From the external iliac tuberosity to the point of the pin bone; from the external angle of the ileum to the isquiatic tuberosity
Longitudinal distance (LD)	From point of the shoulder to the point of the pin bone
Shoulder height (SH)	From the surface of a platform to the top of the shoulder
Shoulder length (SL)	From the superior border of the scapula to the carpus
Snout length (SL)	Tip of the nasal bone to coronal suture; From the frontal-nasal suture to the point of the snout
Tail length (TL)	From insertion of the tail to the tail tip

The definition and study of adaptive traits of indigenous breeds is an important field for improved utilization in sustainable farming systems, especially in developing countries (Rege, 2006). Characterisation of the adaptive traits of selected African sheep is also part of the FAO (1998) agenda. Studies to assess variation in resistance to gastro-intestinal parasites in Red Maasai and Dorper sheep in coastal Kenya have shown that Red Maasai breed, which is an indigenous breed, is more resistant to these parasites (Rege, 2006). A large number of African indigenous small stock breeds have been characterised on phenotype and in Table 2.6 a few of these studies are presented.

Table 2.6 Phenotypic characterisation of small stock breeds

Indigenous breeds	**Title of study**	**Reference**
Nguni sheep (South Africa)	Genetic and Phenotypic diversity in Nguni sheep populations	Kunene *et al*., 2009
Indigenous Tswana goats and sheep (Botswana)	Phenotypic characterisation of Indigenous Tswana Goats and sheep breed	Nsoso *et al*., 2004.
Sahelian goats, Mossi goats and Djaloonke goats (Burkina Faso)	Multivariate analyses on morphorlogical traits of goats in Burkina Faso	Traore *et al*., 2008
Ganjam sheep (India)	Morphological and genetic characterisation of Ganjam sheep	Arora *et al*., 2010
Mauritius local goats breed (Maurituis)	Phenotypic characterisation of the local goats in Mauritius	Geerjanand, 2010
West African dwarf sheep Type (West Africa)	Application of categorical traits in the assessment of breed and performance of sheep in a humid tropic	Oke & Ogbonnaya, 2011a
West African dwarf sheep Type (West Africa)	Application of Physical body traits in the assessment of breed and performance of West African Dwarf sheep in a humid tropic	Oke & Ogbonnaya, 2011b

These phenotypic characterisation studies have progressively used body measurements, production and reproduction performance records to describe the breed's characteristics. Thus providing phenotypic information for small stock breeds, which could be useful for the proper improvements, conservation and utilisation of farm animal genetic resources.

2.6 Conclusion

The utilisation and conservation of farm animal genetic resources require both genetic and phenotypic characterisation of a breed. Although modern technologies have focused on molecular techniques, most breeds originally were described on the basis of phenotypic characteristics. In this study the Namaqua Afrikaner sheep breed was characterised for both genetic and phenotypic information that could be useful in the future management of this indigenous breed.

Chapter 3

Materials and Methods

3.1 Introduction

The genetic and phenotypic characterisation of South African Namaqua Afrikaner sheep was carried out using animals of two Namaqua Afrikaner flocks maintained at the Carnarvon and Karakul Experimental Stations respectively and from a third Namaqua Afrikaner flock kept by a private owner, on his farm Welgeluk in the Carnarvon district. These flocks are part of the biological reserve for small stock research and conservation at Grootfontein Agricultural Development Institute.

The three Namaqua Afrikaner sheep populations were characterised using a set of 22 microsatellite markers recommended by both ISAG and the FAO to determine genetic variation and genetic differentiation within this breed. Production and morphological data of the Carnarvon flock were included to describe the phenotypic characteristics of the breed.

3.2 History and management of the flocks

Namaqua Afrikaner flock at Carnarvon Experimental Station

The Department of Agriculture bought one of the last purebred Namaqua Afrikaner flocks from Mr. P. J. Maas from Namies, Springbok during 1966 and since then this flock has been kept at the Carnarvon Experimental Station. Currently the flock comprises of approximately 120 ewes.

The flock is run on the veld continuously and no supplementary feeding is given at any time. A system of one breeding season per year has been followed since 1966, with a 34-day mating period during April. The lambs are weaned at 4 months of age. All lambs are retained until the age of 18 months, when ewe and ram replacements are picked at random. No selection for any specific production or reproduction traits is carried out. However, animals with physical deformities and those which do not conform to the general breed appearance are culled. Ewes are replaced at a rate of 20%, while all rams are replaced annually. In an effort to keep the inbreeding level as low as possible the ewes in the flocks are divided into three groups. The old ewes are replaced by young ewes from the same group. The rams are used on a rotational basis between the groups (cyclic mating). Four rams per group are used in a group mating system, where each group of rams is run with their group of 35 to 40 ewes (Snyman *et al.,* 1993; Snyman *et al.*, 2005c). No outside rams have been introduced to the Carnarvon Namaqua Afrikaner flock since 1980.

Namaqua Afrikaner flock at Karakul Experimental Station

In March 1985, 30 ewes and 5 rams from the Carnarvon Namaqua Afrikaner flock were transferred to the Tarka Conservation area near Hofmeyer in the Eastern Cape Province. Their numbers were allowed to increase to approximately 100 breeding ewes. In 1991 this flock was transferred to the Grootfontein Agricultural Development Institute (GADI). At Tarka and Grootfontein, a free mating system was followed, where the rams were run with the ewes throughout the year. No supplementary feeding was given nor was any drenching and inoculation programme followed. Once a year, replacement rams and ewes were picked at random and the surplus culled. Old ewes were culled when they had virtually no teeth left and started losing condition as a result.

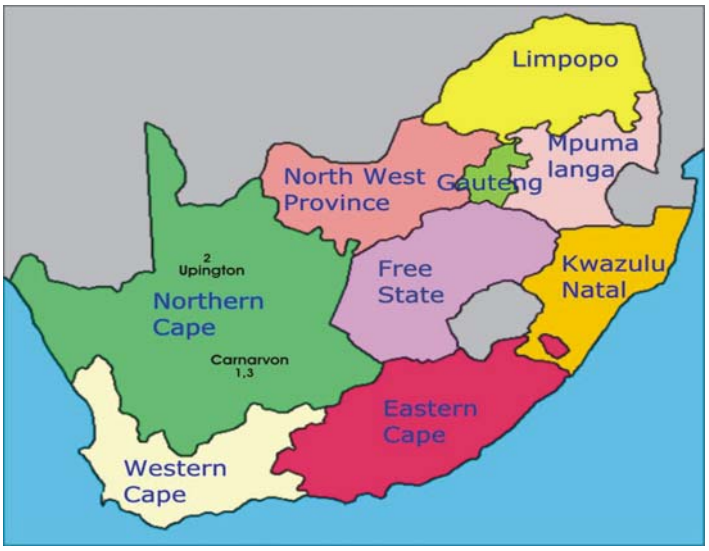
In August 1995, the flock was transferred to the Karakul Experimental Station near Upington, where it is still maintained. The ewe flock comprises of ± 120 ewes, which are run on the veld continuously, and no supplementary feeding is given at any time. A system of one breeding season per year has been followed since 1997, with a 34-day mating period during September. As is the case at Carnarvon, these lambs are weaned at 4 months of age. All lambs are retained until the age of 18 months, when ewe and ram replacements are picked at random. No selection for any specific production or reproduction traits is carried out. However, animals with physical deformities and which do not conform to the general breed appearance are culled. Ewes are replaced at a rate of 20%, while all rams are replaced annually. The same cyclic mating system as used on Carnarvon, is practised in the Karakul Namaqua Afrikaner flock. No outside rams have been introduced to this flock since 1985.

Namaqua Afrikaner flock at Welgeluk farm, Carnarvon

At Welgeluk farm multi-sire mating was used in the past where rams and ewes were run together. The animals were not subjected to any selection pressure or intentional inbreeding. The owner has however changed to cyclic mating in order to decrease the inbreeding level. During 1994 and 1995 the owner purchased some Namaqua Afrikaner ewes and rams from the Carnarvon Experimental Station flock. He had also acquired some sheep from other private farmers. The ewe flock comprise of ±100 ewes, which are run on the veld continuously, and no supplementary feeding is given at any time (Personal communication: Mr. Johann van der Merwe, Private bag X529, Middelburg 5900, 0498421113).

3.3 Description of the environment

The majority of Namaqua Afrikaner sheep are farmed with in the Northern Cape province of South Africa. The Carnarvon Experimental Station and Welgeluk farms are located in the Carnarvon district (30° 59' S, 22° 9'E) (Figure 3.1). This region is referred to as the north-western Karoo of the Republic of South Africa and lies at an altitude of between 1000 and 1300 meter above sea level. The natural pasture in this region varies from mixed grass and shrub veld to Karoo shrub veld and is described by Acocks (1988) as arid Karoo (veld type 29). The climate is characterised by severe winters and hot summers. The average minimum temperature (July) is 4 °C and the average maximum temperature (January) is 30.5 °C. A frost- free period of 240 days occurs from mid-September to mid-May. The average annual rainfall is 209 mm, with 14% occurring in spring, 34% in summer 41% in autumn and 11% in winter (http://www.southafrica.info/travel/advise/climate.htm).



1. Carnarvon Experimental Station
2. Karakul Experimental Station
3. Welgeluk farm

Figure 3.1 Map of South Africa indicating the locations of Namaqua Afrikaner genotypes sampled in the Northern Cape Province

The Karakul Experimental Station is located in the Upington district, and falls within the Savanna Biome (Figure 3.1). This station is represented by two vegetation types: the Shrubby Kalahari Dune Bushveld and the Karroid Kalahari Bushveld, also called the Kalahari Thornveld (Acocks 16) (Acocks, 1988). The Shrubby Kalahari Dune Bushveld is comprised of gently undulating dunes with pans being scattered throughout this vegetation type, and with an altitude between 1000 m and 1100 m above sea level (Low & Rebelo, 1996). The Karroid Kalahari Bushveld occurs mostly on the flat

and gravelly plains north and north-west of Upington, at an altitude of 1000 m (Acocks, 1988). The average rainfall, which predominantly falls during summer to autumn, is 200 mm.

3.4 Population Sampling

The three Namaqua Afrikaner flocks that were used in this study formed part of the Namaqua Afrikaner sheep conservation projects (AP10/1/1, AP10/2/1 and AP 10/3/2) under the biological reserve programme of GADI and the Department of Agriculture, Forestry and Fisheries (DAFF). Blood samples and phenotypic data stored in the GADI-biobank were used for this study.

A total of 144 animals from the three participating flocks were included (48 from each flock, consisting of 10 rams and 38 ewes each). The blood samples were stored in 2ml Eppendorf tubes at minus 85°C in the blood bank at GADI. No full pedigrees were available for any populations; therefore to include animals that had as little relationship as possible, samples were taken from animals in different groups (cyclic mating groups) and born in different years at the Carnarvon and Karakul experimental stations. Random sampling, within age groups, was conducted on the animals of the Welgeluk farmer.

3.5 DNA isolation and selection of the markers

DNA was extracted from whole blood using the Roche kit (according the Roche protocol) at GADI. A starting volume of 450 µl was used for all extractions. Extracted DNA was quantified by subjecting it to the electrophoresis system in 1% agarose gel stained with ethidium bromide and visualised using a UV transilluminator. The DNA was stored at minus 60 °C until further analysis. The DNA was quantified and qualified through spectrophotometer (Nanodrop ND-1000) at the University of Pretoria, Department of Genetics.

DNA samples were initially amplified with 22 microsatellite markers recommended by the International Society of Animal Genetics (ISAG) and under the FAO's MoDAD program (Table 3.1). Markers were selected on the basis of amplification success, the expected allelic size range and based on the fact that they have been used in previous sheep characterisation studies. Nine out of 22 selected markers were previously used by Buduram (2004) in the genetic characterisation of South African sheep breeds; these markers were included in order to enable the genetic comparison of Namaqua Afrikaner sheep in the present study with some of the sheep breeds previously reported. As stipulated by the Working Group of the FAO, microsatellite loci that can be used on several species such as cattle, sheep and goats are preferable. This aspect was taken into account with the selection of

other markers for this study. A total of nine ovine and nine bovine markers were included in the study.

3.6 PCR and genotyping

PCR and genotyping was performed at the University of Pretoria, Department of Animal and Wildlife Sciences, Animal Breeding and Genetics laboratory. The Polymerase Chain Reaction (PCR) amplification was performed in a total volume of 15 µl. This contained 10x-Buffer (1.5 µl), 10mM dNTPs (0.3 µl), 25mM $MgCl_2$ (0.75 µl), 5U/µl Taq Gold (0.4 µl), 10pmol/µl primers (0.3 µl each), 5 µl extracted DNA of average 90 µg/µl concentration and deionized water (6.45 µl). The amplification was performed using a Perkin-Elmer Gene Amp PCR System 9700 thermocycler. The program was run as follows: 5 minutes at 95 °C, 35 cycles of 45 seconds at 94 °C and 45 seconds at an optimized annealing temperature, 1.5 minutes at 72 °C and a final extension step at 72 °C for 10 minutes.

Following the completion of the PCR cycles, the reaction products were subjected to electrophoresis in 3% agarose gel stained with ethidium bromide to verify the PCR products and the gel was visualized using a UV transilluminator. Samples were prepared for genotpying by diluting the PCR products with distilled water and adding the diluted samples to Formamide and fixed size standard (in this case GeneScan Liz). Samples were denatured at 95 °C for three minutes and immediately put back to minus 4 °C using a Perkin-Elmer Gene Amp PCR System 9700 thermocycler. The data was captured using GeneScan 3.1 software and data analysis was carried out using GeneMarker 1.9 (http://www.softgenetics.com/download/genemarker) to determine the fragment sizes in base pairs. An allelic table was created from this data and converted into a MSToolkit input file format for statistical analysis.

3.1 Characteristics of microsatellite markers: sequences, chromosome number, annealing temperature, fluorescent labels used and observed size range

Primers	Sequences	Chromosome number	Annealing temperature °C	Fluorescent label	Observed Size range	References
BM1824	F: GAGCAAGGTGTTTTTCCAATC	1 (Bovine)	58	Pet®	169-173	Bishop & Kappes (1994)
	R: CATTCTCCAACTGCTTCCTTG					
INRA23	F: GAGTAGAGCTACAAGATAAACTTC	3 (Bovine)	58	Ned®	198-216	Vaiman & Mercier (1994)
	R: TAACTACAGGGTGTTAGATGAACTCA					
ETH225	F: GATCACCTTGCCACTATTTCCT	9 (Bovine)	54	Vic®	134-142	Steffen & Eggen (1993)
	R: ACATGACAGCCAGCTGCTACT					
TGLA53	F: CAGCAGACAGCTGCAAGAGTTAGC	16 (Bovine)	58	Ned®	141-159	Crawford *et al.* (1995)
	R: CTTTCAGAAATAGTTTGCATTCATGCCAG					
MCM527	F: GTCCATTGCCTCAAATCAATTC	5 (Ovine)	60	Ned®	164-182	Hulme *et al.* (1994)
	R: AAACCACTTGACTACTCCCCAA					
INRA005	F: TTCAGGCATACCCTACACCACATG	3 (Ovine)	54	6-Fam®	125-147	Viaman *et al.* (1992)
	R: AAATATTAGCCAACTGAAAACTGGG					
OARCP34	F: GAACAATGTGATATGTTCAGG	3 (Ovine)	60	Ned®	108-122	Ede *et al.* (1995)
	R: GGGACAATACTGTCTTAGATGCTGC					
OARCP49	F: CAGACACGGCTTAGCAACTAAACGC	17 (Ovine)	54	6-Fam®	72-106	Ede *et al.* (1995)
	R: GTGGGGATGAATATTCCTTCATAAGG					
SRCRSP8	F: TGCGGTCTGGTTCTGATTTCAC	(Ovine) unassigend	58	6-Fam®	214-246	Bhebhe *et al.* (1994)
	R: CCTGCATGAGAAAGTCGATGCTTAG					
SRCRSP9	F: AGAGGATCTGGAAATGGAATC	(Ovine) (unassigend)	58	Pet®	113-119	Bhebhe *et al.* (1994)
	R: GCACTCTTTTCAGCCCTAATG					
OARVH72	F: GGCCTCTCAAGGGGCAAGAGCAGG	25 (Ovine)	58	Ned®	121-127	Pierson *et al.* (1993)

	R: CTCTAGAGGATCTGGAATGCAAAGCTC					
CSSM47	F: TCTCTGTCTCTATCACTATATGGC	8 (Bovine)	60	Vic®	128-130	Moore & Byrne (1994)
	R: CTGGGCACCTGAAACTATCATCAT					
BM827	F: GGGCTGGTCGTATGCTGAG	3 (Ovine)	60	Ned®	212-224	Bishop et al. (1994)
	R: GTTGGACTTGCTGAAGTGACC					
INRA63	F: ATTTGCACAAGCTAAATCTAACC	18 (Bovine)	56	6-Fam®	157-189	Vaiman & Mercier (1994)
	R: AAACCACAGAAATGCTTGGAAG					
SRCRSP5	F: GGACTCTACCAACTGAGCTACAAG	18 (Caprine)	56	Ned®	144-146	Arevalo *et al.* (1994)
	R: TGAAATGAAGCTAAAGCAATCC					
INRABERN192	F: AGACCTTTACAGCCACCTCTTC	7 (Bovine)	60	Pet®	181-183	Ihara et al. (2004)
	R: GTCCCAGAAACTGACCATTTTA					
OARHH35	F: AATTGCATTCAGTATCTTTAAACATCTGGC	4 (Ovine)	58	Pet®	114-134	Henry *et al.* (1993)
	R: ATGAAAATATAAAGAGAATGAACCACACGG					
OARFCB11	F: GCAAGCAGGTTCTTTACCACTAGCACC	2 (Ovine)	54	Vic®	121-133	Buchanan & Crawford (1993)
	R:GGCCTGAACTCACAAGTTGATATATCTATCAC					
OARFCB48	F:GACTCTAGAGGATCGCAAAGAACCAG	17 (Ovine)	58	Pet®	144-164	Buchanan *et al.* (1994)
	R: GAGTTAGTACAAGGATGACAAGAGGCAC					
CSRD247	F: GGACTTGCCAGAACTCTGCAAT	(Ovine) (unassigend)	58	Vic®	216-242	Kemp *et al.* (1993)
	R:CACTGTGGTTTGTATTAGTCAGG					
BM7160	F: TGGATTTTTAAACACAGAATGTGG R TCAGCTTCTCTTTAAATTTCTCTGG	7(Bovine)	58	6-fam®	160-160	Stone *et al.* (1995)
ETH10	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC	5(Bovine)	58	6-fam®	203-203	Toldo & Fries (1993)

Markers highlighted in bold were previously used in SA sheep Genetic characterisation study by Buduram, 2004 and the rest of the markers were obtained from the ISAG list.

3.7 Data for comparison

An extract of genotypic data of SA Mutton Merino and Pedi sheep breeds was obtained with permission from the Agricultural Research Council (ARC). These two populations were used for comparison in this study. A total of 35 samples of SA Mutton Merino were included in this study to determine if SA Mutton Merino have been introduced to the indigenous Namaqua Afrikaner sheep through crossbreeding as it has been widely used by farmers due to its superior meat and wool characteristics. The SA Mutton Merino is a crossbred animal that was created from crossbreeding European breeds (Merino and German Merino). The Pedi sheep breed is an indigenous South African sheep breed. Forthy samples of Pedi sheep were also included in order to determine whether the Namaqua Afrikaner has genetic links with this indigenous sheep breed. To compare these breeds, a population structure analysis of the Namaqua Afrikaner sheep population together with SA Mutton Merino and Pedi sheep breeds was conducted based on eight (one was not included in the statistical analysis as it was monomorph) microsatellite markers, which corresponded to the study of Buduram (2004).

3.8 Statistical analysis of genetic diversity

Allele frequencies, polymorphic information content, heterozygosity values and genetic variation estimates were calculated using MSToolkit (Park, 2001). MSToolkit and Convert version 1.3.1 (Glaubitz, 2004) were used to prepare input files for all other genetic software that were used. Alleles were classified as private alleles if they were present in only one particular population. MSToolkit (Park, 2001) were used to calculate the observed heterozygosity (Ho), expected heterozygosity (He) and average number of alleles per locus (Nei, 1987). Population subdivision estimates (fixatation index F_{st}) values per populations were obtained from Arlequin version 3.1 (Excoffier *et al*., 2005) to confirm the expected heterozygosity estimates. Genepop version 4.0 (Raymond & Rousset, 1995) was used for testing deviation from Hardy Weinberg Equilibruim (HWE) at each locus over all populations.

The FSTAT version 2.9.3.2 (Goudet, 1995) program was used to compute Wright's *F*-statistics for each locus; including *F*, Θ and *f* which are analogous to Wright's (1978) F_{IT}, F_{ST} and F_{IS}. F_{IT} is the inbreeding coefficient of an individual (I) relative to the total (T) population, F_{IS} is the inbreeding coefficient of an individual (I) relative to the subpopulation (S) compared to the total population (T) (Hartl, 1988). The Weir & Cockerham (1984) estimation of F_{IT}, F_{ST} and F_{IS} were performed for every locus among populations with a Jack-knifing procedure applied over the loci in deriving their significance levels. An analysis of variance was performed to indicate differentiation within and

between populations using Arlequin version 3.1 (Excoffier *et al*., 2005). To detect genetic relationship among three Namaqua Afrikaner populations, the genetic distance was estimated according to the method of Nei's (1978) unbiased genetic distance using the POPGENE computer program.

A population structure analyses for Namaqua Afrikaner sheep was performed using Structure (Pritchard *et al*., 2000). A Bayesian-based assignment test (Pritchard *et al*., 2000) was used to infer the true number of genetic populations (clusters or K) in the dataset using the structure program. Prior population information was ignored before testing and identifying distinct genetic populations, and assigning individuals to populations. The model used for simulation was based on assumption of admixture ancestry and correlated allele frequencies. To estimate the true number of populations the parameter LnPr (X|K) was applied, where $K \leq 5$. Twelve independent runs for each K were used. The probability value for each K was averaged over 12 runs. All runs were carried out with a burn-in period of 20000 steps followed by 100000 MCM iterations (Pritchard *et al*., 2000). Similarly the population structure analysis was done for three Namaqua Afrikaner sheep populations together with SA Mutton Merino and Pedi where $1 > K \leq 10$.

3.9 Morphological description and performance data

Production and reproduction data collected since 1982 on the animals in the Namaqua Afrikaner flock at the Carnarvon Experimental Station were available. No reliable data were collected on the Namaqua Afrikaner flock during the time that they were kept at the Karakul Experimental Station. Furthermore, no production records were available for the sheep at Welgeluk farm. Therefore, only phenotypic data of between 386 and 2668 animals (depending on the traits) of the Carnarvon Namaqua Afrikaner flock could be used for this part of the study. Table 3.2 shows the traits that were measured, the number of records and the time period.

The available data for each ewe per lambing season included identity of ewe and lamb/s, birth date, birth weight, sex, birth status and 120-day weaning weight of each lamb. From these data, the total weight of lamb weaned per ewe joined for each lambing season (TWW/EJ) was calculated as follows: Firstly, within each lambing season, weaning weight for all lambs was corrected to 120 days, followed by least-squares corrections for sex of the lamb. Secondly, TWW/EJ was calculated by adding the corrected weaning weight of all the lambs weaned by each ewe in that specific lambing season. Subsequently, total weight of lamb weaned by each ewe over her lifetime in the flock (TWW/L) was calculated by adding the TWW/EJ for each year that the ewe was in the flock.

Table 3.2 Traits measured, number of records and time period

Trait	Number of records	Time period
Production:	2668	1993 - 2009
Birth weight of lamb (kg)		
42 day body weight (kg)		
100 day body weight (kg)		
Monthly body weight 5-12 months (kg)		
Reproduction:	2925	1982 - 2009
Body weight of ewes before mating (kg)		
Body weight of ewes after weaning (kg		
Total weight of lamb weaned / year (kg)		
Number of lambs born / year		
Number of lambs weaned / year		
Number of lifetime lambing opportunities		
Total weight of lamb weaned / lifetime (kg)		
Number of lambs born / lifetime		
Number of lambs weaned / lifetime		
Morphological:	386	2007 - 2009
Wither height (cm)		
Body length (cm)		
Heart girth (cm)		
Cannon bone (cm)		
Colour pattern (any colour on body)		
Colour of the head (brown or black)		
Tail circumference at the root of the tail (cm)		
Tail length (cm)		
Twist of tail (none, to the right, to the right)		
Horned or polled		
Teat length		
Testis circumference		

Number of lambs born and number of lambs weaned per ewe per year and over her lifetime in the flock were also calculated. The number of lambing opportunities for each ewe over her lifetime in the flock was also noted. For the analyses of variance of body weight of ewes, total weight of lamb

produced per ewe per year, number of lambs born and number of lambs weaned per ewe per year, fixed effects for year and age of the dam were included in the models. Least-squares means for these traits were obtained with the PROC GLM-procedure of SAS (Littell *et al*., 1991; SAS, 2006). Fixed effects included for lifetime reproduction were year of birth of the ewe and number of lambing opportunities.

The body weight and morphological data were analysed by means of least-square means procedures (Littell *et al*., 1991; SAS, 2006). The following fixed effects were included in the linear model fitted to the data, namely year of birth, sex and birth status of the lamb, age of dam, cyclic mating group, and all significant two-factor interactions.

The following model was fitted to the data

$Y_{ijklmn} = \mu + y_i + f_j + s_k + r_l + a_m + (yf)_{ij} + (ys)_{ik} + (fs)_{jk} + b_1AL + e_{ijklmn}$

Where

Y_{ijklmn} = trait of the n'th animal of the m'th dam age of the l'th birth/rearing status of the k'th sex of the j'th group of the i'th birth year,

μ = overall mean,

y_i = fixed effect of the i'th birth year,

f_j = fixed effect of the j'th group,

s_k = fixed effect of the k'th sex,

r_l = fixed effect of the l'th rearing status (birth status in the case of birth weight),

a_m = fixed effect of the m'th age of dam (years),

$(yf)_{ij}$ = effect of the interaction between the i'th birth year and the j'th group,

$(ys)_{ik}$ = effect of the interaction between the i'th birth year and the k'th sex,

$(fs)_{jk}$ = effect of the interaction between the j'th group and the k'th sex,

b_1 = linear regression coefficient of the appropriate deviation from the mean of age of the lamb (AL) at recording (except for birth weight),

e_{ijklmn} = random error with zero mean and variance $I\sigma^2_e$

Chapter 4

Results

4.1 Genetic characterisation

Allelic frequencies

A total of twenty-two microsatellite markers were tested in the sheep populations of Carnarvon Experimental Station (CES), Karakul Experimental Station (KES) and Welgeluk (WGK) farm. Twenty microsatellite markers adhered to the parameters for studying genetic diversity in all three populations. Two markers (BM7160 & ETH10) were monomorphic in all three populations and were therefore not included in the statistical analysis. Allelic frequencies for this study were attached as an Appendix A. In Table 4.1 the number of detected alleles with the least and most frequent alleles is summarised. A total of 100 different alleles were detected for the twenty two microsatellite markers that were genotyped for the 144 individuals. The number of alleles observed across microsatellite markers varied from one (CSSM47, SRCRSP5) to eight (INRA23, INRA005, CSRD247) over three populations. The mean number of alleles detected across the populations was 5.0 over all loci (excluding monomorphic markers). The WGK population had the highest mean number of alleles (4.2), followed by CES population (3.8) and KES population (3.6).

Alleles unique to certain populations were observed (Table 4.1). Unique alleles with low frequencies were checked for genotyping error and confirmed as read (Table 4.1). WGK population had the highest number of unique alleles, where ten alleles were observed with frequencies varying between 0.010 and 0.19. Five unique alleles were observed in the KES population with frequencies between 0.01 and 0.16 and four in the CES population (0.02 to 0.13).

Genetic diversity

Heterozygosity values and polymorphic information content (PIC) for the twenty markers are presented in Table 4.2. Except for markers SRCRSP05 and CSSM47 that were monomorphic in the KES and WGK populations, the PIC values for the remaining markers varied between 0.094 and 0.75. Overall, the average values for PIC in the tested markers were low.

The microsatellite markers were tested for deviation from HWE for all populations (Table 4.2). Results revealed that most of the loci (16 out of 20) were in Hardy-Weinberg Equilibrium ($P > 0.05$).

Only four loci (SRSRSP05, ETH225, TGLA53 and BMI824) did not show adherence to HWE (Table 4.2).

The heterozygosity values showed large variation between individual markers, ranging between 0.10 (CSSM47) and 0.91 (INRA23) for observed heterozygosity over the populations (Table 4.2). Expected heterozygosity values were marginally lower as expected, with a maximum value of 0.79 (INRA23).

Table 4.1. Number of alleles with most and least frequent alleles (Allele frequency) for microsatellite markers applied

Locus	n	Alleles observed	Most frequent alleles			Least frequent alleles		
			CES	KES	WGK	CES	KES	WGK
OARCP49	6	72, 78, 80, 90, 96, 106	72 (0.42)	80 (0.50)	96 (0.42)	106 (0.1)	106 (0.02)	90 (0.01)
SRCRSP08	7	214, 218, 232, 236, **238**, 244, **246**	214 (0.73)	214 (0.55)	214 (0.94)	244 (0.01)	232 (0.02)	218, 244, 246 (0.01)
CSSM47	2	128, 130	130 (0.69)	130 (1.0)	130 (0.94)	128 (0.31)	0	128 (0.05)
OARCP34	6	108, 110, **112**, 116, 118, 122	116 (0.58)	122 (0.44)	116 (0.71)	108 (0.03)	118 (0.03)	118, 112 (0.01)
SRCRSP05	2	144, ***146***	144 (0.87)	144 (1.0)	144 (1.0)	146 (0.12)	0	0
BM827	5	212, 216, 218, 222, **224**	218 (0.46)	218 (0.47)	216 (0.68)	212 (0.02)	212 (0.045)	224 (0.02.)
SRCRSP09	2	113, 119	113 (0.72)	113 (0.51)	113 (0.89)	119 (0.27)	119 (0.48)	119 (0.1)
INRABERN192	2	181, 183	181(0.68)	181 (0.94)	181 (0.61)	183 (0.31)	183 (0.05)	183 (0.38)
INRA005	8	125, 127, 129, 131, 133, 135, 145, **147**	129 (0.37)	129 (0.61)	129 (0.38)	145 (0.04)	131 (0.01)	147 (0.01)
INRA63	7	157, 159, 167, 171, ***181***, 183, **189**	167 (0.33)	157 (0.40)	171 (0.64)	181, 183 (0.03)	167 (0.04)	183 (0,01)
OARFCB11	5	121, 123, **125**, 131, 133	121 (0.64)	121 (0.45)	123 (0.59)	131 (0.01)	133 (0.03)	125 (0.01)
CSRD247	8	**216**, 220, **222**, 226, 228, 230, 238, 242	226 (0.39)	228 (0.42)	226 (0.36)	220 (0.01)	226 (0.04)	238 (0.01)
OARVH72	3	121, 123, 127	121 (0.91)	121 (0.71)	121(0.95)	127 (0.02)	123 (0.27)	122 (0.01)
MCM527	4	164, 166, 172, ***182***	164 (0.51)	164 (0.41)	166 (0.53)	182 (0.02)	172 (0.24)	125, 127 (0.01)
OARHH35	5	114, 120, **122**, 126, 134	134 (0.42)	134 (0.40)	134 (0.50)	114 (0.09)	120 (0.14)	122 (0.01)
OARFCB48	4	144, 148, 150, 164	148 (0.52)	148 (0.76)	148 (0.72)	164 (0.21)	144 (0.01)	144 (0.01)
ETH225	5	***134,*** **138**, 140, 141, 142	140 (0.61)	140 (0.97)	142 (0.47)	134 (0.02)	138 (0.02)	141 (0.14)
TGLA53	6	141, 151, **153,** 155, 157, 159	155 (0.69)	155 (0.59)	155 (0.71)	141 (0.02)	151, 153 (0.02)	141, 151 (0.01)
INRA23	7	198, **202**, 206, 210, 212, **214,** 216	206 (0.26)	206 (0.63)	216 (0.34)	210 (0.15)	216 (0.08)	202, 214 (0.01)
BM1824	4	**169,** 171, 172, 173	171 (0.62)	171 (0.59)	171 (0.51)	172 (0.09)	172 (0.01)	172 (0.18)
BM7160	1	160	160 (1.0)	160 (1.0)	160 (1.0)	0	0	0
ETH10	1	203	203 (1.0)	203 (1.0)	203 (1.0)	0	0	0
AVERAGE	5*							

n: number of alleles, * Average exclude monomorphic markers, Alleles unique to CES: italic and bold, KES: bold, JFV: bold and underline

Table 4.2 Observed (Hobs) and expected (HExp) heterozygosity, polymorphic information content (PIC) and Hardy Weinberg Equilibrium (HWE)

Locus	**Hobs**			**HExp**			**PIC**			**HWE**
	CES	**KES**	**WGK**	**CES**	**KES**	**WGK**	**CES**	**KES**	**WGK**	
OARCP49	0.770833	0.729167	0.75	0.704605	0.658114	0.723246	0.643668	0.599453	0.67029	0.9585 ± 0.0041
SRCRSP08	0.479167	0.520833	0.104167	0.4375	0.619079	0.101754	0.39479	0.560887	0.099329	0.5907 ± 0.0184
CSSM47	0.333333	0	0.104167	0.434211	0	0.099781	0.337372	0	0.093866	0.1171 ± 0.0020
OARCP34	0.574468	0.723404	0.479167	0.605353	0.716312	0.461623	0.556817	0.664131	0.429013	0.4277 ± 0.0149
SRCRSP05	0.162791	0	0	0.225718	0	0	0.198208	0	0	**0.0106 ± 0.0006***
BM827	0.666667	0.477273	0.521739	0.604605	0.628265	0.449355	0.514427	0.547098	0.363169	0.3002 ± 0.0100
SRCRSP09	0.468085	0.439024	0.219512	0.404484	0.505872	0.197832	0.320109	0.374851	0.176325	0.7308 ± 0.0030
INRABERN192	0.382979	0.106383	0.595745	0.439259	0.101807	0.477694	0.340153	0.095652	0.360931	0.7147 ± 0.0039
INRA005	0.75	0.595745	0.708333	0.782237	0.561199	0.740789	0.74532	0.496988	0.691663	0.4693 ± 0.0133
INRA63	0.708333	0.673913	0.604167	0.760965	0.668657	0.516009	0.712825	0.594759	0.449742	0.4388 ± 0.0172
OARFCB11	0.510638	0.595745	0.541667	0.474262	0.592313	0.58114	0.36867	0.498287	0.52229	0.5645 ± 0.0123
CSRD247	0.708333	0.638298	0.73913	0.695175	0.728666	0.781892	0.630299	0.679164	0.741406	0.0820 ± 0.0087
OARVH72	0.173913	0.377778	0.06383	0.163402	0.405743	0.062915	0.153742	0.32074	0.061185	0.5428 ± 0.0041
MCM527	0.625	0.702128	0.520833	0.635746	0.659117	0.568202	0.5655	0.57772	0.472453	0.5064 ± 0.0104
OARHH35	0.71875	0.709677	0.5	0.700893	0.721311	0.602183	0.634562	0.658913	0.512733	0.3567 ± 0.0082
OARFCB48	0.666667	0.382979	0.458333	0.619518	0.38275	0.423026	0.54382	0.337916	0.365598	0.7855 ± 0.0101
ETH225	0.347826	0	0.425532	0.555184	0.042096	0.618623	0.487932	0.040781	0.531541	**0.0002 ± 0.0000***
TGLA53	0.446809	0.531915	0.510638	0.47838	0.579959	0.458476	0.426225	0.503602	0.414068	**0.0002 ± 0.0001***
INRA23	0.913043	0.533333	0.782609	0.798137	0.557054	0.77162	0.75583	0.511018	0.726955	0.6884 ± 0.0117
BM1824	0.608696	0.375	0.6	0.532489	0.519937	0.619975	0.456509	0.415603	0.541001	**0.0120 ± 0.0013***
Average	**0.550817**	**0.45563**	**0.461478**	**0.552606**	**0.482413**	**0.462807**	**0.489339**	**0.423878**	**0.411178**	

*P- Values in bold did not adhere to HWE ($P < 0.05$)

Population differentiation was evaluated using fixation indices (F_{IS}, F_{ST} and F_{IT}) for each of the twenty markers across the three Namaqua Afrikaner sheep populations (Table 4.3). The mean estimates of *F*-statistics obtained by jackknifing over loci were: F_{IS} = 0.019 ± 0.0019, F_{ST} = 0.105 ± 0.013 and F_{IT} = 0.123 ± 0.025. A significant deficit of heterozygotes was observed at a few loci (CSSM47, SRCRSP05 and ETH225). Of the twenty markers, 6 (27 %) showed negative F_{IS} values, while eleven showed low positive values. The average F_{IS} value across the three populations was low positive (0.019), indicating limited inbreeding. The average genetic differentiation between all populations (F_{ST}) was 0.105 (Table 4.3), indicating that 10.5% of genetic diversity can be explained by the genetic differentiation among the populations whereas 89.5% can be explained by differences among individuals within the populations.

Table 4.3 Wright's *F*-statistical for 20 microsatellite loci (F_{IT}, F_{ST} and F_{IS} values) for each locus over all populations

Locus	***F*$_{IT}$ *(F)***	***F*$_{ST}$ *(ø)***	***F*$_{IS}$ *(f)***
OARCP49	0.056	0.125	-0.080
SRCRSP08	0.176	0.135	0.047
CSSM47	0.372	0.232	0.182
OARCP34	0.140	0.137	0.003
SRCRSP05	0.358	0.105	0.283
BM827	0.060	0.056	0.004
SRCRSP09	0.127	0.148	-0.024
INRABERN192	0.089	0.146	-0.066
INRA005	0.105	0.091	0.015
INRA63	0.104	0.122	-0.021
OARFCB11	0.087	0.087	0.000
CSRD247	0.184	0.137	0.054
OARVH72	0.159	0.137	0.026
MCM527	0.051	0.043	0.009
OARHH35	0.079	0.032	0.048
OARFCB48	0.013	0.068	-0.059
ETH225	0.535	0.266	0.366
TGLA53	0.061	0.044	0.018
INRA23	0.059	0.103	-0.049
BM1824	0.055	0.012	0.044
Over all loci (± SD)	**0.122(±0.025)**	**0.105(±0.013)**	**0.019(±0.0019)**

Markers indicated in bold had significant heterozygosity deficit

In Table 4.4 the level of genetic diversity and population subdivision within the three populations are shown. The overall genetic diversity in the populations was low to moderate, with the highest unbaised Hz values in CES (55%) compared to KES (48%) and WGK (46%). With regard to population subdivision, the F_{ST} value for KES (0.112) and WGK (0.113) was marginally higher than the value for CES (0.109), indicating a reduction of heterozygosity in KES and WGK populations, which supported the unbiased heterozygosity estimates (Hartl, 1988).

Table 4.4 Measures of genetic variation in the populations studied

Population	Sample size	Loci typed	Unbiased Hz ±SD	Obs Hz ± SD	No Alleles ± SD	F_{ST}
CES	48	20	0.552 ± 0.0380	0.550 ± 0.0163	3.85 ± 1.42	0.1094
KES	48	20	0.482 ± 0.0554	0.455 ± 0.0166	3.65 ± 1.53	0.1122
WGK	48	20	0.462 ± 0.0549	0.461 ± 0.0165	4.20 ± 1.85	0.1130

An AMOVA analysis was also performed to further explain the partitioning of genetic variation of Namaqua Afrikaner sheep populations. The results obtained by AMOVA analyses were similar to those revealed by F_{ST} estimate, illustrated that 89.4% of genetic diversity occurred within populations and 10.6% between the populations (Table 4.5).

Table 4.5 AMOVA analyses for the three Namaqua Afrikaner sheep populations

Source of Variation	Sum of squares	Variance components	Percentage variation	P-Value
Among Populations	120.011	0.59115	10.56946	0.001
Within Populations	1356.396	5.00181	89.43054	0.001
Total	1476.408	5.59296		

The genetic distance estimates done to determine the relationship between the three populations are presented in Table 4.6. The genetic distance estimate ranged from 0.062 between CES and WGK to 0.160 between KES and WGK, revealing a close relationship between CES population and WGK population.

Table 4.6 Nei‘s unbiased genetic distance between Namaqua Afrikaner genotypes.

Pop ID	CES	KES	WGK
CES	****		
KES	0.0987	****	
WGK	0.0621	0.1596	****

The population structure and level of admixture were estimated using Structure (Pritchard *et al.*, 2000). In structure analysis, the Ln Pr (X|K) increased distinctly from K = 1 to K = 3 and reached a plateau at K = 3, and it did not show significant fluctuation from K = 4 to 5. Therefore K = 3 was taken as the most probable number of inferred populations. In Table 4.7 the proportion of individuals of each of the populations in the three most likely clusters inferred by the Structure (Pritchard *et al.*, 2000) are presented and this corresponded to the three different populations included in the study. The CES population was mainly assigned to cluster 1 (86%), and KES mainly assigned to cluster 2 (89%). The WGK population was divided between a large partitioning in cluster 3 (81%) and a smaller component in cluster 1 (15%). In Figure 4.1 the bar plot showing the proportion of membership of each individual to one or more of the three real clusters were shown.

Table 4.7 Proportion of membership of the analysed Namaqua Afrikaner sheep populations in each of the three clusters inferred in the structure program

Predefined populations	**Inferred clusters**			**n**
	1	**2**	**3**	
CES	0.858	0.075	0.067	48
KES	0.038	0.888	0.074	48
WGK	0.152	0.037	0.810	48

n = number of individuals

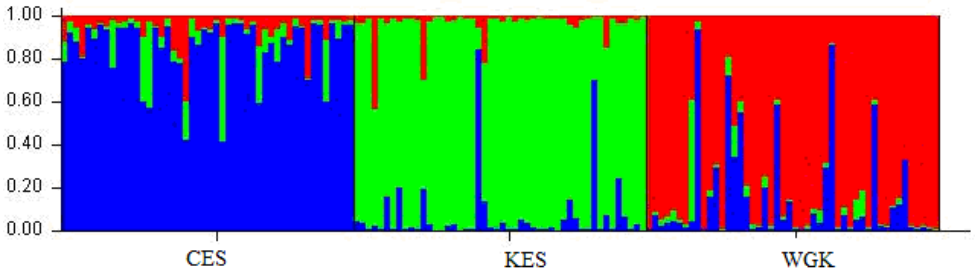


Figure: 4.1 A summary plot of the estimate of *Q*. Each individual is represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the three inferred clusters

The population structure analysis was also conducted for Namaqua Afrikaner sheep populations with SA Mutton Merino (SAMM) and Pedi sheep breeds. Eight markers which corresponded

between the present study and the study of South African sheep by Buduram (2004) were used. The most likely number of clusters (K) was five, as inferred by the Ln Pr (X|K) values. In Table 4.8 the proportion of individuals of each populations in the five most likely clusters inferred by the Structure (Pritchard *et al*., 2000) are presented. Structure analysis revealed that 98 and 95% of SAMM and Pedi populations were mainly assigned to cluster 4 and to cluster 5 respectively. The Namaqua Afrikaner sheep population were mostly divided between the three remaining clusters. Thirty three of CES individuals were assigned to cluster 3 while 60% were assigned to cluster 1. Eighty of KES individuals were mainly assigned to cluster 2 and a small component (16%) to cluster 1. Sixty seven percent of WGK individuals were assigned to cluster 3 and 29% to cluster 1. Figure 4.2 shows the bar plot of the proportion of membership of each individual to one or more of the five real clusters.

Table 4.8 Proportion of membership of the analysed Namaqua Afrikaner, Mutton Merino and Pedi sheep populations in each of the five clusters inferred in the structure program

Predefined populations	**Inferred cluster**					**n**
	1	**2**	**3**	**4**	**5**	
CES	0.609	0.002	0.326	0.058	0.005	48
KES	0.169	0.798	0.029	0.002	0.003	48
WGK	0.288	0.003	0.677	0.028	0.004	48
SAMM	0.003	0.003	0.003	0.986	0.005	35
Pedi	0.004	0.005	0.041	0.006	0.945	40

n = number of individuals

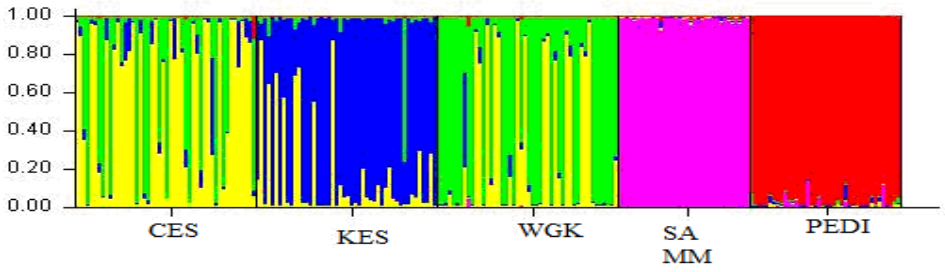


Figure: 4.2 A summary plot of the estimate of *Q*. Each individual is represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the five inferred clusters

Overall the Namaqua Afrikaner had no genetic relationship with the SAMM or Pedi sheep breed. SAMM and Pedi were assigned to their specific clusters, while the Namaqua Afrikaner shared the remaining three clusters indicating the pureness of Namaqua Afrikaner sheep included in this study relative to the other two breeds

4.2 Phenotypic characterisation

Phenotypic characterisation of the breed was limited to the Namaqua Afrikaner sheep from Carnarvon Experimental Station, where production and morphological data has been recorded. Phenotypic characterisation of Namaqua Afrikaner sheep from this Station was conducted to describe the breed's morphological measurements and production performances. Significant levels obtained after fitting the model for each trait are summarised in Table 4.9. From Table 4.9 it is evident that year of birth, sex, birth status of the lamb and age of the dam had a significant ($P < 0.001$) influence on body weight at all ages. Only 120-day weaning weight was significantly influenced by sex x birth status. Year x sex had a significant effect on most traits, with the exception of birth weight and 120-day weaning weight, while age of the lamb significantly influenced all traits with the exception of 11 and 12 mouths body weight. The year of birth significantly influence all morphological traits with the exception of wither height. The sex of the animals had a significant influence on the body length, heart girth circumference, tail circumference and tail length, while the tail circumference at base was significantly influenced by the mating groups of the animal.

Table 4.9 Fixed effects included in the statistical model fitted for all traits

Trait	Year of birth	Mating group	Sex	Birth status	Age of dam	Year x sex	Sex x Birth status	Year x Birth status	Age of lamb
Birth weight (kg)	**	ns	**	**	**	ns	ns	ns	-
42-day body weight (kg)	**	ns	**	**	**	**	ns	ns	**
120-day weaning weight (kg)	**	ns	**	**	**	ns	**	ns	**
5-month body weight (kg)	**	ns	**	**	**	**	ns	ns	**
6-month body weight (kg)	**	ns	**	**	**	**	ns	ns	**
7-month body weight (kg)	**	ns	**	**	**	**	ns	ns	**
8-month body weight (kg)	**	ns	**	**	**	**	ns	ns	**
9-month body weight (kg)	**	ns	**	**	**	**	ns	ns	**
10-month body weight (kg)	**	ns	**	**	**	**	ns	ns	**
11-month body weight (kg)	**	ns	**	**	**	**	ns	ns	ns
12-month body weight (kg)	**	ns	**	**	**	**	ns	ns	ns
Body length (cm)	**	ns	**	ns	ns	-	-	-	-
Wither height (cm)	ns	ns	ns	ns	ns	-	-	-	-
Heart girth circumference (cm)	**	ns	**	ns	ns	-	-	-	-
Cannon bone (cm)	**	ns	ns	ns	ns	-	-	-	-
Tail circumference at base (cm)	**	**	**	ns	ns	-	-	-	-
Tail length (cm)	**	ns	**	ns	ns	-	-	-	-
Testis circumference (cm)	**	ns	-	ns	ns	-	-	-	-
Teat length left (cm)	**	ns	-	ns	ns	-	-	-	-
Teat length right (cm)	**	ns	-	ns	ns	-	-	-	-

** P<0.001; ns = not significant, - not included

Reproduction traits

Reproduction data are presented in Table 4.10 with the averages for the various traits. The body weight before and after mating showed marginal differences. The Namaqua Afrikaner ewes from the Carnarvon flock had an average of 1.45 lambs born and 1.31 lambs weaned per year with a total weight of 36.4 kg. The average number of lifetime lambing opportunities was 3.10.

Table 4.10 Production and reproduction performance of Namaqua ewes since 1982 in the Carnarvon flock

Trait	Average
Body weight before mating (kg)	50.1
Body weight after weaning (kg)	51.2
Reproduction	
Total weight of lamb weaned / year (kg)	36.4
Number of lambs born / year	1.45
Number of lambs weaned / year	1.31
Number of lifetime lambing opportunities	3.10
Total weight of lamb weaned / lifetime (kg)	112.9
Number of lambs born / lifetime	4.49
Number of lambs weaned / lifetime	4.05

The effect of age of the dam is presented in Table 4. 11. The age of the dam at recording had a significant influence on all reproductive traits analysed, where the older dams gave birth to heavier lambs than younger dams. The heaviest lambs were born from three to six year old dams and the lightest from two and seven year old dams. Total weight of lamb weaned, number of lambs born and number of lambs weaned increased with an increase in age of the dam from two to five years, after which it showed a slight decline. From Table 4.11 it is evident that this influence got smaller with age. Younger dams had a lower body weight before mating and after weaning compared to the older ewes.

Table 4.11 Effects of age of the dam on the reproductive traits of the Namaqua Afrikaner sheep

Age of dam	Total weight of lamb weaned (kg)	Number of lambs born	Number of lambs weaned	Body weight before mating	Body weight after weaning
2	$32.82^{abc} \pm 0.70$	$1.33^{abc} \pm 0.02$	$1.18^{abc} \pm 0.02$	$47.7^{acdef} \pm 0.17$	50.04 ± 0.50
3	$37.41^{a} \pm 0.74$	$1.47^{a} \pm 0.02$	$1.32^{a} \pm 0.02$	$50.07^{abg} \pm 0.18$	51.03 ± 0.49
4	$38.60^{b} \pm 0.79$	$1.49^{b} \pm 0.02$	$1.36^{b} \pm 0.02$	$51.24^{a} \pm 0.20$	51.36 ± 0.51
5	$38.95^{c} \pm 0.93$	$1.54^{c} \pm 0.03$	$1.39^{c} \pm 0.03$	$50.90^{c} \pm 0.23$	52.55 ± 0.60
6	37.38 ± 1.67	1.47 ± 0.06	1.34 ± 0.06	$51.81^{db} \pm 0.42$	52.83 ± 1.19
7	31.72 ± 4.88	1.28 ± 0.18	1.04 ± 0.18	$53.48^{e} \pm 1.23$	50.64 ± 2.45
8	41.83 ± 11.71	1.42 ± 0.43	1.36 ± 0.44	$60.30^{fg} \pm 2.96$	53.64 ± 6.35

values with no superscripts did not differ significantly

Morphological measurements

The morphological measurements of 386 (209 ewes and 177 rams) Namaqua Afrikaner lambs born between 2007 and 2009 included length and height measurements and described tail conformation, colour and horns. The data are presented in Table 4.12.

Table 4.12 Body measurements (± s.e.) at 14 months of age of the 2007- to 2009-born Namaqua ewe (n=209) and ram (n=177) lambs in the Carnarvon flock

Trait	Rams	Ewes
Body length (cm)	71.2[a] ± 0.6	68.7[a] ± 0.5
Wither height (cm)	74.2[a] ± 4.1	67.6[b] ± 4.1
Hearth girth circumference (cm)	100.6[a] ± 1.1	95.8[a] ± 1.1
Cannon bone length (cm)	17.2[a] ± 0.2	16.9[b] ± 0.2
Tail circumference at base (cm)	49.0[a] ± 0.6	37.5[a] ± 0.6
Tail length (cm)	43.7[a] ± 0.7	41.0[a] ± 0.7
Testis circumference (cm)	32.2 ± 0.4	
Teat length left (mm)		20.4 ± 0.7
Teat length right (mm)		19.1 ± 0.7

[a] Values with the same superscripts differ significantly (P<0.05) between sexes

The average body length, wither weight, heart girth circumference, cannon bone length and tail length of rams were 71.2 cm, 74.2 cm, 100.6 cm, 17.2 cm and 43.7 cm respectively, whereas those of ewes were 68.7 cm, 67.6 cm, 95.8 cm, 16.9 cm and 41.0 cm respectively. In general the morphological measurements of the Namaqua Afrikaner revealed that rams exhibited higher estimates for all body measurements.

The morphological traits of Namaqua Afrikaner from Carnarvon flock are presented in Table 4.13. The percentage of animals possessing horns in rams and ewes were 100% and 84.1% respectively. The colour on the body was present in 30.5% of the rams and 31.1% of the ewes. The majority of the animals had their tail twisted to the left (>60%) and had black heads (>65%).

Table 4.13 Morphological description at 14 months of age of the 2007 to 2009-born Namaqua ewe (n=209) and ram (n=177) lambs in the Carnarvon flock

Trait	Percentage of animals (%)	
	Rams	Ewes
Horns	100[a]	84.1[a]
Polled	0[a]	15.9[a]
Colour head: Brown	28.2	34.9
Colour head: Black	71.8	65.1
Colour on body: Yes	30.5	31.1
Colour on body: No	69.5	68.9
Tail twist: To left	69.5	61.7
Tail twist: To Right	27.7	29.2
Tail no twist - straight	2.8	9.1

[a] Values with the same superscripts differ significantly (P<0.05) between sexes

Productive performance

Least-squares means for body weight of lambs from birth until 12-months of age from 1993 to 2009 are presented in Table 4.14. All values differ significant at P <0.001 between sexes. Ram lambs had significantly higher body weight than ewe lambs in all age groups. The 4.43 kg recorded at birth increases to 28.78 kg at weaning. Thereafter it increased progressively to 53.59 kg at 12-months body weight (Table 4.14), indicating that rams remain heavier throughout the 12 months period.

Table 4.14 Least-squares means for body weight of ram and ewe lambs (± s.e.) since 1993 in the Carnarvon Namaqua Afrikaner flock

Trait	Ram lambs	Ewe lambs
Birth weight (kg)	4.43 ± 0.05	4.21 ± 0.05
42-day body weight (kg)	15.50 ± 0.19	14.16 ± 0.19
120-day weaning weight (kg)	28.78 ± 0.27	26.23 ± 0.27
5-month body weight (kg)	33.07 ± 0.29	29.52 ± 0.29
6-month body weight (kg)	36.33 ± 0.30	32.02 ± 0.30
7-month body weight (kg)	39.16 ± 0.31	34.62 ± 0.31
8-month body weight (kg)	41.49 ± 0.31	36.1 ± 0.30
9-month body weight (kg)	44.06 ± 0.32	38.05 ± 0.32
10-month body weight (kg)	46.48 ± 0.34	39.94 ± 0.34
11-month body weight (kg)	49.88 ± 0.36	41.70 ± 0.35
12-month body weight (kg)	53.59 ± 0.41	44.26 ± 0.40

All values differ significantly (P<0.001) between sexes

Least squares means for body weight of single, twin and triplet lambs are presented in Table 4.15. All values differ significant at P<0.001 between birth status groups. Multiple born lambs were lighter at birth than single born lambs (Table 4.15).

Table 4.15 Least-squares means for body weight of single, twin and triplet lambs (± s.e.)

Trait	Single	Twin	Triplet
Birth weight (kg)	4.88 ± 0.05	4.32 ± 0.04	3.76 ± 0.06
42-day body weight (kg)	17.82 ± 0.19	13.77 ± 0.17	12.89 ± 0.23
120-day weaning weight (kg)	30.89 ± 0.27	25.23 ± 0.25	26.40 ± 0.33
5-month body weight (kg)	34.23 ± 0.29	28.78 ± 0.27	30.87 ± 0.36
6-month body weight (kg)	36.95 ± 0.30	31.7 ± 0.28	33.89 ± 0.37
7-month body weight (kg)	39.16 ± 0.31	34.4 ± 0.28	36.78 ± 0.38
8-month body weight (kg)	41.23 ± 0.30	36.96 ± 0.28	38.99 ± 0.37
9-month body weight (kg)	43.07 ± 0.32	39.01 ± 0.30	41.09 ± 0.40
10-month body weight (kg)	45.03 ± 0.34	41.27 ± 0.31	43.34 ± 0.42
11-month body weight (kg)	47.59 ± 0.35	43.97 ± 0.32	45.81 ± 0.44
12-month body weight (kg)	50.45 ± 0.40	46.89 ± 0.36	49.43 ± 0.52

All values differ significantly (P<0.001) between birth status groups

At weaning the difference between single born lambs and multiple born lambs were 5.66 kg and 4.49 kg for twins and triplets respectively. These differences decreased gradually to 3.56 kg and 1.02 kg at 12-months of age for twins and triplets respectively, indicating that this effect become smaller with an increase in age.

Overall the sex and the birth status of the lambs significantly affected their body weight. Ram lambs were significantly heavier than the ewe lambs at all ages and lambs that were born and raised as singles were heavier than multiple born lambs.

Chapter 5

Discussion

Genetic and/or phenotypic characterisation of breeds or populations is a primary step in the decision making process for breed conservation (Arora *et al*., 2008; Boettcher *et al*., 2010a). The current study was performed to obtain genetic and phenotypic information for development of appropriate management strategies for the Namaqua Afrikaner sheep breed kept as potential conservation populations at Carnarvon Experimental Station, Karakul Experimental station and Welgeluk farm.

5.1 Genetic characterisation

In this study twenty two microsatellite markers were used to study the genetic diversity within three Namaqua Afrikaner sheep populations. Two markers were monomorphic and the rest adhered to the parameters for studying the genetic diversity. The mean number of alleles (5.0) observed over all populations was low, but corresponded to the mean number of alleles obtained in the South African indigenous sheep breeds by Buduram (2004) for Blinkhaar Ronderib sheep (4.3) and Namaqua Afrikaner (4.9) sheep. However, a higher mean number of alleles was observed for other South African indigenous sheep including Swazi (6.5), Nguni (5.4) and Pedi (7.0) (Buduram, 2004). Slightly higher mean number of alleles was also reported by Muigai *et al*. (2009) in African (Kenyan) fat tailed sheep that include Red Maasia-Mutara (6.2), Kakamega (6.6) and Transmara (6.4). All of these breeds are indigenous to their respective countries and were studied for genetic variation with similar microsatellite markers. Indigenous sheep often are at danger of losing variation due to smaller population sizes. Fourteen of the twenty two markers in this study had at least one unique allele across the populations. Two unique alleles were observed for SRCRSP08, INRA63, CSRD247, ETH225 and INRA23. INRA63, CSRD247 and ETH225 were also used in the genetic characterisation study of South African indigenous sheep (Buduram, 2004), but none of the unique alleles observed in this study were reported. These alleles could be useful for the identification of Namaqua Afrikaners sheep populations.

The polymorphic information content (PIC) is used as a general measure of how informative a marker is (Ramamoorthi *et al*., 2009). The mean PIC values for CES (0.48), KES (0.42) and WGK population (0.41) indicated a low level of information for the markers used. The markers

INRA63, INRA23, CSRD247 and OARCP49 had greater than 0.5 PIC value over all populations, indicating their usefulness for genetic diversity studies.

The results from this study revealed that 16 out of 20 markers were in HWE, indicating that the populations under investigation were not subjected to any evolutionary forces such as selection, migration, genetic drift and mutation and therefore were able to maintain their relative allele frequencies (Falconer & Mackay, 1996). This was expected as no known selection or exchange of animals have taken place in these populations for the past fifteen years.

The unbiased heterozygosity is the most widely used parameter to measure genetic diversity within populations (Toro *et al*., 2009). In this study the genetic diversity estimates were low to moderate with the unbiased heterozygosity estimates varying between 46% for WGK and 55% for CES. These estimates were similar to the values reported by Buduram (2004) in the Namaqua Afrikaner (49%) and Blinkhaar Ronderib Afrikaner (52%) sheep. If compared to other indigenous breeds in South Africa, the genetic diversity in Namaqua sheep was lower than the values reported for Pedi (67%), Nguni (65%), Swazi (69%) and Karakul (67%) sheep breeds (Buduram, 2004). These were also lower than the values reported for the other indigenous sheep breeds that include Muzzafarnagri sheep (69%) by Arora & Bhati (2004); Red Maasia-Mutara (61%) and Maasia-Olmagogo (58) by Muigai *et al*. (2009) and Ganjam sheep (68%) by Arora *et al.* (2010). The low to moderate observed genetic variation in the Namaqua Afrikaner sheep population was expected as they have been kept as a closed population for more than fifteen years.

The population subdivision estimate supported the unbiased heterozygosity where KES and WGK populations had a marginally higher F_{ST} value than CES, indicating a reduction in heterozygosity in KES and WGK populations (Hartl, 1988). Positive F_{IS} estimates indicate either the presence of inbreeding and/or a Wahlund effect (presence of population substructure within breeds (Pariset *et al*., 2003; Peter *et al*., 2007). The inbreeding estimate (F_{IS} = 0.019) in this study was low positive, indicating limited inbreeding across the populations. This could be attributed to good management practices where cyclic mating has been used in the herds at the different locations. In literature, reports of inbreeding vary for different breeds, from as low as F_{IS} = 0.087 for Ganjam sheep (Arora *et al*., 2010) and F_{IS} = 0.058 for Muzzafarnagri (Arora & Bhatia, 2004) to as high as F_{IS} = 0.294 for Vembur sheep, an Indian sheep breed that is conserved by a non-governmental organisation (Pramod *et al*., 2009).

The F_{ST} (0.105) value and the AMOVA analyses revealed a moderate genetic differentiation amongst the Namaqua Afrikaner sheep populations. There was more genetic differentiation within the populations (89.5%) as compared to between the populations of Namaqua Afrikaner sheep. The fact that no known selection for or against any specific traits has been carried out in these populations, might contribute to the genetic variations observed within the populations.

Nei's unbiased genetic distances estimates revealed a close relationship between CES and WGK. The close relationship between CES and WGK may be explained by the fact that the owner of WGK farm purchased some of his sheep from CES flock in 1994 and 1995. It was interesting to note that KES population was little related to CES population, as animals from KES originated from CES 26 years back. In 1985, 30 ewes and five rams from Carnarvon flock were transferred to Tarka conservation area near Hofmeyer in the Eastern Cape Province. In 1995 these animals were taken to KES to form another breeding flock of Namaqua Afrikaner sheep in the Upington district.

The results of genetic differentiation were confirmed by the population structure analyses. A possible gene flow between the CES and WGK population was observed, confirming the close relationship between these populations. Overall the population structure analysis suggested a true genetic structure with significant differentiation among all three Namaqua Afrikaner sheep populations. Some levels of admixture observed between CES and WGK populations could be attributed to the fact that some of the sheep at WGK farm were bought from CES as stated above. Limited levels of admixture in the KES population might be attributed to the fact that this population is geographically separated from the other two populations.

The Structure (Pritchard *et al*. 2000) analysis of the three Namaqua Afrikaner populations together with the SA Mutton Merino and Pedi sheep as an outgroup revealed that the inclusion of pure breeds (SA Mutton Merino and Pedi) did not affect the Namaqua Afrikaner population structure. The Namaqua Afrikaner sheep maintained its population structure where a some level of admixture was observed between CES and WGK and a limited level of admixture in the KES population. The results suggested that there was no crossbreeding or introgression of SA Mutton Merino genes in the studied Namaqua Afrikaner sheep. This indicated the pureness of this breed as it does not have genetic links with SA Mutton Merino that have been widely used for crossbreeding by South African farmers. This was expected as no known exchange of animals has occurred in these populations. The results also showed no genetic links between Namaqua

Afrikaner sheep and Pedi sheep indicating that the Pedi and Namaqua breeds are distinct breeds with no admixture even though these breeds are both fat-tailed indigenous breeds. Since Pedi sheep was clearly distinct from the Namaqua Afrikaner it is advisable to conserve this breed in the small stock biological bank as a separate breed. The population structure analysis indicated that SA Mutton Merino and Pedi are two different pure breeds with a clearly defined population structure.

The results of this study can be used as a benchmark for the Namaqua Afrikaner sheep kept at CES, KES and WGK farm. Genetic diversity of farm animal genetic resources allows for the sustained ability of a breed or population to respond to selection to increase productivity and for adaptation to changing environmental conditions (Boettcher *et al*., 2010a). It is therefore important that the level of genetic diversity within the Namaqua Afrikaner sheep populations should be increased. An exchange of rams from the different genetic pools is advisable to increase the genetic variance within these populations. It is also important that the Namaqua Afrikaner sheep that still exist should be kept as pure as possible. Therefore crossbreeding with rams of suspected breeds should be avoided. However, crossbreeding with unrelated genetic material may be an option for decision makers to serve the Namaqua Afrikaner sheep when the number of the Namaqua sheep left in the country is critical.

A study based on the management of diversity of rare breeds in France has revealed that in order to maintain or increase population size while also managing within population genetic diversity, the rare breeds need to be valorised by being associated with development initiatives (Lauvie *et al*. 2011). Lauvie *et a*l. (2011) reported that development program initiatives for rare breeds have been shown to increase the number and the genetic diversity of rare breeds in France. Commercialisation of indegenous breeds encourages the breeders to keep them, as it changes the way rare breeds are considered: from being regarded as a genetic resource to be conserved, to a resource for local livestock production and local development (Lauvie *et al*. 2011). The same approach could be applied in the Namaqua Afrikaner sheep in order to increase their numbers while also managing their genetic diversity.

The genetic data from this study will now form baseline data for the three populations kept at CES, KES and WGK farm. A follow-up study should be performed every five years to ensure that the genetic variation remains and inbreeding are kept to a minimum. It is recommended that a development program for Namaqua sheep should be considered with a commercial incentive to

increase their numbers. There should be a strategy in place by the GADI-biobank to sample as unrelated as possible and also include samples from Namaqua sheep kept by small-scale farmers.

5.2 Phenotypic characterisation

In this study the morphological data, production and reproduction performance of Namaqua Afrikaner sheep kept at the Carnarvon Experimental Station (CES) were used to characterise the breed phenotypically. The reproduction performance indicated that the Namaqua Afrikaner sheep compare favourable with the Afrino sheep kept under similar conditions at the CES (Snyman, 2010). The Afrino sheep is a commercial breed reared extensively at the CES. Its total weight of lambs weaned (32.59), average number of lambs born (1.31) and average number of lambs weaned (1.16) per year (Snyman, 2010) corresponded to the values observed for the Namaqua Afrikaner sheep, which were 36.4, 1.45 and 1.31 respectively. Snyman *et al.* (1996) compared the reproduction performance of the Namaqua Afrikaner sheep to that of Dorper sheep reared extensively at the CES under drought conditions. During the year following the extreme drought conditions, total weight of lambs weaned for Namaqua sheep (39.0 kg) was higher than that weaned by Dorper sheep (32.0 kg). This gave an indication that the Namaqua Afrikaner sheep breed has a relatively high reproduction performance under extensive conditions and could be suitable for mutton production under harsh conditions.

The age of the ewe significantly influenced the weights of the lambs, the number of lambs born and the number of lambs weaned. The six years old ewes recorded higher number of lambs born (1.47) and weaned (1.34) than the two-year-old ewes, which had 1.33 and 1.18 respectively. The six-year-old ewes also recorded higher body weight before mating (51.18 kg) when compare to the two-year-old ewes (47.7 kg). The similar influence of age on the production performance of the ewes were reported for other sheep breeds incluing Nguni sheep by Kunene *et al*. (2007), Mehraban sheep by Gamasaee *et al*. (2010) and different sheep breeds by Safari *et al*. (2005).

Morphological characterisation of a breed assists in breed description and setting breed standards, thus allowing distinction between breeds and strains (McManus *et al*., 2010). Similarly, the results obtained for the morphological characterisation in this study could be useful to describe the breed type and set the breed standards of Namaqua Afrikaner sheep. Morphological characterisation revealed that the Namaqua Afrikaner sheep is a fat-tailed breed with long slender body conformation. This breed has long legs and large ears. The neck is long and slender and the shoulders are sharp and prominent. The outer fleece covering consist of a fairly long silky and

relative coarse fibre while the wool fibre of the inner fleece are considerably shorter. This study showed that over 65% of the Namaqua sheep were white with no colour on the body while 30% were white with a black or brown colour. Over 65% of the Namaqua sheep had black heads while about 28% had brown heads. All rams had strong and well developed horns while approximate 84% of the ewes had small light horns.

This study showed that over 60% of the sheep had their tail turned to the left, about 27% turned to the right and only less than 10% had no twist (straight tail). The morphological traits were measured in both sexes of Namaqua Afrikaner sheep at 14 months of age. The morphological measurements revealed that rams exhibited higher estimates for all body measurement traits. A similar influence of sex on morphological estimates was reported for other indigenous sheep including Mecheri sheep (Karunanithi *et al*., 2005), Nguni sheep (Kunene *et al*., 2007) and Ganjam sheep (Arora & *et al*., 2010). Both Mecheri and Ganjam sheep are indigenous sheep of India. The average body length and wither height estimates reported for the Mecheri rams corresponded to the values observed for Namaqua rams; they both had slender body conformation with body length of 70.9 cm and 71.2 cm and wither height of 74.2 cm and 71.1 cm respectively (Karunanithi *et al*., 2005). Comparing the Namaqua rams to the Ganjam rams the Ganjam rams had smaller body length (60.7 cm) and wither height (67.5) (Arora *et al*. 2010). Comparing the Namaqua sheep with the Nguni sheep studied by Kunene *et al.* (2007), both breeds had fat tail, but the Nguni's tail was straight down. The average wither height (61.68 cm) and height girth (75.46 cm) of the Nguni rams reared extensively at Kwamakhathini were also lower than the wither height (74.2 cm) and height girth (100.6 cm) of Namaqua rams.

The body weight estimates of the Namaqua Afrikaner ram lambs at birth (4.43 kg) compared favourable with the body weight estimates for Afrino sheep (4.85 kg) (Snyman, 2010) recorded at the CES. This gave an indication that this breed is capable of producing lambs that are suitable for commercialisation. It was interesting to note that the body weight estimates at birth observed for the Namaqua Afrikaner lambs were higher than the estimates observed for the other indigenous sheep such as the Mecheri sheep (2.88 kg) breed reared extensively in villages of India (Karunanithi *et al*., 2005) and Mehraban sheep (3.38 kg) reared extensively in Iran (Gamasaee *et al*., 2010). The results of this study also showed that the Namaqua sheep have the ability of maintaining a high pre-weaning growth rate under extensive farming conditions.

The body weight estimates for ram lambs were significantly higher than those obtained for ewe lambs at birth and they remain heavier throughout their lives. This effect might be explained by the differences in sex chromosomes, physiological characteristics and endocrinal system between rams and ewes lambs (Gamasaee *et al*., 2010). Similar influences of sex were reported in various breeds of sheep (Snyman *et al*., 1995; Safari *et al*., 2005; Kunene *et al* 2007; Gamasaee *et al*., 2010). The birth status of the lamb also significantly influenced the body weights of lambs. Single born lambs were heavier than the multiple born lambs for all ages. This effect might be caused by the limited uterine space during pregnancy and competition for milk suckling between multiple birth lambs during birth to weaning (Gamasaee *et al*., 2010). The similar influences of birth status on the body weight estimates of lambs has been well established in most breeds including Nguni (Kunene *et al*., 2007) and Mehraban (Gamasaee *et al*., 2010).

From the results of this study it was clear that the Namaqua Afrikaner sheep is a hardy and prolific breed, as was evident from the relatively high reproductive performance recorded under extensive conditions. The body weight estimates and reproduction perfomance of Namaqua Afrikaner sheep revealed that this breed can compete with other South African commercial sheep breeds. It is therefore important that appropriate conservation and utilisation strategies for the Namaqua Afrikaner sheep should be developed in order to make sure that this farm animal genetic resource is conserved. The conservation of farm animal genetic resources through utilisation is one way of ensuring that indigenous breeds are successfully conserved (Boettcher *et al*., 2010b). Therefore by combining the conservation effort with a commercial application, the future existence of the Namaqua Afrikaner sheep could be ensured.

The phenotypic data documented for the Namaqua Afrikaner sheep kept at the CES was useful to describe and assess the breed in terms of reproduction and production performance. This information could be useful for the future management and conservation of this indigenous breed.

Chapter 6

Conclusions and recommendations

This study was the first attempt to contribute genetic information on the Namaqua Afrikaner sheep kept at Carnarvon Experimental Station (CES), Karakul Experimental Station (KES) and Welgeluk farm (WGK). The information obtained will contribute in developing appropriate strategies for the improvement, utilisation and conservation of this indigenous breed, and it will also form part of the biological reserve bank at GADI.

A total of twenty-two microsatellite markers were tested in three populations at CES, KES and WGK. Two markes were monomorph and the rest adhered to the parameters for studying genetic diversity. The genetic diversity was measured in terms of the expected heterozygosity and the mean number of alleles. The genetic diversity in the studied Namaqua sheep was low (46%) for WGK to moderate (55%) for CES. The study therefore indicated insufficient heterozygosity in the Namaqua Afrikaner populations included in this study. Crossbreeding with unrelated individuals could increase genetic diversity within the Namaqua Afrikaner. However, it is of paramount importance that the Namaqua Afrikaner sheep that still exists, be kept as pure as possible. Therefore, crossbreeding with rams of suspect descendency should be avoided at all cost. Crossbreeding with unrelated indigenous material should only be considered if the number of Namaqua sheep left in the country is critical. In order to increase the number of Namaqua Afrikaner sheep, while also mananging its genetic diversity, it is recommended that the Namaqua Afrikaner sheep be valorised by associating it with development initiatives.

With regard to the inbreeding estimates, the average F_{IS} was low positive indicating low levels of inbreeding across the three populations. Therefore it is suggested that the current system of cyclic mating should be continued. Assessment of the inbreeding level should be done every five years to determine any unfavourable change in inbreeding level early, so that appropriate steps could be taken to prevent further increases in inbreeding.

The result of both fixation index (F_{ST}) and AMOVA revealed that most of the genetic variation occurred within populations, rather than between populations. The fact that no selection for or against any specific production trait has been carried out in the flocks, contributed to the genetic variation within the flocks. It is therefore proposed that the current system of random selection of

replacement ewes and sires, where only animals with physical deformities and which do not conform to the general breed appearance are culled, should be continued.

As already discussed, the closer distance of CES and WGK could most probably be ascribed to the fact that the owner of WGK bought some of the CES animals in 1994 and 1995. The possibility of natural selection to adapt to the specific environment should also be kept in mind. The distinct clusters of the Carnarvon and Karakul animals are indicative of genetic differences between the two groups. It is proposed that the Carnarvon as well as KES flocks should be maintained as part of the biological conservation flock.

As far as the ex situ conservation of the breed is concerned, 280 embryos obtained from Namaqua Afrikaner ewes of the Carnarvon Experimental Station have already been cryopreserved and are kept in the biological reserve at GADI. It is planned to freeze at least another 250 embryos from this same flock over the next two or three years. Keeping in mind the genetic relationship between the CES and KES flocks, it is recommended that embryos from the KES flock should also be cryopreserved.

Since the start of this study, another two Namaqua Afrikaner flocks have been included in the conservation programme under the maintenance of the live flock project. These flocks are also available for the cryopreservation and blood and DNA bank projects. One of the flocks is kept near Calvinia in the Northern Cape Province, and the other at Barkly East in the Eastern Cape Province. The Barkly East flock has genetic ties with the CES flock, as the owner bought some animals from the CES flock during 1994 to 1996, and again in 2010. It is recommended that the same set of microsatellites used in this study, be used to genetically characterise animals from these two new flocks, in order to determine their genetic diversity and distance from the three flocks already characterised. The molecular data provided by this study will serve as a reference for management and mating strategies of the endangered Namaqua Afrikaner sheep breed.

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Appendix

Appendix A

Allele frequency over populations

Key to Population Names:
Pop1 Carnarvon experimental station
Pop2 Karakul experimental station
Pop3 Welgeluk

Table 6.1 Allele frequencies of the three Namaqua Afrikaner sheep populations

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
OARCP49	1	72	0.4167	0.0833	0.2396	0.2465
OARCP49	2	78	0.0000	0.0312	0.2083	0.0799
OARCP49	3	80	0.1979	0.5000	0.0312	0.2431
OARCP49	4	90	0.0000	0.0729	0.0104	0.0278
OARCP49	5	96	0.2812	0.2917	0.4167	0.3299
OARCP49	6	106	0.1042	0.0208	0.0938	0.0729
# Samples:			48	48	48	.144

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
SRCRSP08	1	214	0.7292	0.5521	0.9479	0.7431
SRCRSP08	2	218	0.1042	0.2708	0.0104	0.1285
SRCRSP08	3	232	0.0000	0.0208	0.0208	0.0139
SRCRSP08	4	236	0.1562	0.0625	0.0000	0.0729
SRCRSP08	5	238	0.0000	0.0625	0.0000	0.0208
SRCRSP08	6	244	0.0104	0.0312	0.0104	0.0174
SRCRSP08	7	246	0.0000	0.0000	0.0104	0.0035
# samples:			48	48	48	144

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
CSSM47	1	128	0.3125	0.0000	0.0521	0.1215
CSSM47	2	130	0.6875	1.0000	0.9479	0.8785
# samples:			48	48	48	144

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
OARCP34	1	108	0.0319	0.1702	0.0521	0.0845
OARCP34	2	110	0.1809	0.1277	0.0729	0.1268
OARCP34	3	112	0.0000	0.0000	0.0104	0.0035
OARCP34	4	116	0.5851	0.2340	0.7188	0.5141
OARCP34	5	118	0.0532	0.0319	0.0104	0.0317
OARCP34	6	122	0.1489	0.4362	0.1354	0.2394
# samples:			47	47	48	142

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
SRCRSP05	1	144	0.8721	1.0000	1.0000	0.9513
SRCRSP05	2	146	0.1279	0.0000	0.0000	0.0487
# samples:			43	36	34	113

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
BM827	1	212	0.0208	0.0455	0.0000	0.0217
BM827	2	216	0.4271	0.3864	0.6848	0.5000
BM827	3	218	0.4583	0.4659	0.2935	0.4058
BM827	4	222	0.0938	0.1023	0.0000	0.0652
BM827	5	224	0.0000	0.0000	0.0217	0.0072
# samples:			48	44	46	138

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
SRCRSP09	1	113	0.7234	0.5122	0.8902	0.7093
SRCRSP09	2	119	0.2766	0.4878	0.1098	0.2907
# samples:			47	41	41	129

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
INRABERN192	1	181	0.6809	0.9468	0.6170	0.7482
INRABERN192	2	183	0.3191	0.0532	0.3830	0.2518
# samples:			47	47	47	141

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
INRA005	1	125	0.1458	0.0000	0.1458	0.0979
INRA005	2	127	0.2083	0.0000	0.0521	0.0874
INRA005	3	129	0.3750	0.6064	0.3854	0.4545
INRA005	4	131	0.0729	0.0106	0.2917	0.1259
INRA005	5	133	0.0521	0.0213	0.0938	0.0559
INRA005	6	135	0.1042	0.2553	0.0000	0.1189
INRA005	7	145	0.0417	0.1064	0.0208	0.0559
INRA005	8	147	0.0000	0.0000	0.0104	0.0035
# samples:			48	47	48	143

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
INRA63	1	157	0.1562	0.4022	0.0625	0.2042
INRA63	2	159	0.1562	0.1739	0.0000	0.1092
INRA63	3	167	0.3333	0.0435	0.2604	0.2148
INRA63	4	171	0.2917	0.3804	0.6458	0.4401
INRA63	5	181	0.0312	0.0000	0.0000	0.0106
INRA63	6	183	0.0312	0.0000	0.0104	0.0141
INRA63	7	189	0.0000	0.0000	0.0208	0.0070
# samples:			48	46	48	142

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
OARFCB11	1	121	0.6383	0.4574	0.2396	0.4437
OARFCB11	2	123	0.3511	0.4468	0.5938	0.4648
OARFCB11	3	125	0.0000	0.0000	0.0104	0.0035
OARFCB11	4	131	0.0106	0.0638	0.1146	0.0634
OARFCB11	5	133	0.0000	0.0319	0.0417	0.0246
# samples:			47	47	48	142

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
CSRD247	1	216	0.0000	0.1596	0.0000	0.0532
CSRD247	2	220	0.0104	0.0000	0.1304	0.0461
CSRD247	3	222	0.0000	0.0000	0.1957	0.0638
CSRD247	4	226	0.3958	0.0426	0.3587	0.2660
CSRD247	5	228	0.1875	0.4255	0.0217	0.2128
CSRD247	6	230	0.0312	0.1596	0.1739	0.1206
CSRD247	7	238	0.0312	0.0000	0.0109	0.0142
CSRD247	8	242	0.3438	0.2128	0.1087	0.2234
# samples:			48	47	46	141

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
OARVH72	1	121	0.9130	0.7222	0.9681	0.8696
OARVH72	2	123	0.0652	0.2778	0.0213	0.1196
OARVH72	3	127	0.0217	0.0000	0.0106	0.0109
# samples:			46	45	47	138

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
MCM527	1	164	0.5104	0.4149	0.3854	0.4371
MCM527	2	166	0.2396	0.3404	0.5312	0.3706
MCM527	3	172	0.2292	0.2447	0.0833	0.1853
MCM527	4	182	0.0208	0.0000	0.0000	0.0070
# samples:			48	47	48	143

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
OARHH35	1	114	0.0938	0.2742	0.0469	0.1368
OARHH35	2	120	0.1875	0.1452	0.0469	0.1263
OARHH35	3	122	0.0000	0.0000	0.0156	0.0053
OARHH35	4	126	0.2969	0.1774	0.3906	0.2895
OARHH35	5	134	0.4219	0.4032	0.5000	0.4421
# samples:			32	31	32	95

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
OARFCB48	1	144	0.0000	0.0106	0.0104	0.0070
OARFCB48	2	148	0.5208	0.7660	0.7292	0.6713
OARFCB48	3	150	0.2604	0.0426	0.2188	0.1748
OARFCB48	4	164	0.2188	0.1809	0.0417	0.1469
# samples:			48	47	48	143

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
ETH225	1	134	0.0217	0.0000	0.0000	0.0071
ETH225	2	138	0.0000	0.0213	0.0000	0.0071
ETH225	3	140	0.6087	0.9787	0.3830	0.6571
ETH225	4	141	0.1087	0.0000	0.1489	0.0857
ETH225	5	142	0.2609	0.0000	0.4681	0.242
# samples:			46	47	47	140

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
TGLA53	1	141	0.0213	0.0000	0.0106	0.0106
TGLA53	2	151	0.0000	0.0213	0.0106	0.0106
TGLA53	3	153	0.0000	0.0213	0.0000	0.0071
TGLA53	4	155	0.6915	0.5638	0.7128	0.6560
TGLA53	5	157	0.0851	0.3191	0.0957	0.1667
TGLA53	6	159	0.2021	0.0745	0.1702	0.1489
# samples:			47	47	47	141

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
INRA23	1	198	0.1630	0.1667	0.1304	0.1533
INRA23	2	202	0.0000	0.0000	0.0109	0.0036
INRA23	3	206	0.2609	0.6333	0.1739	0.3540
INRA23	4	210	0.1522	0.1111	0.2500	0.1715
INRA23	5	212	0.1739	0.0000	0.0761	0.0839
INRA23	6	214	0.0000	0.0000	0.0109	0.0036
INRA23	7	216	0.2500	0.0889	0.3478	0.2299
# samples:			46	45	46	137

Locus	Allele#	Size	Pop1	Pop2	Pop3	Overall
BM1824	1	169	0.0000	0.0250	0.0000	0.0076
BM1824	2	171	0.6196	0.5875	0.5111	0.5725
BM1824	3	172	0.0978	0.0125	0.1889	0.1031
BM1824	4	173	0.2826	0.3750	0.3000	0.3168
# samples:			46	40	45	131