A genome wide association study of body weight and reproduction traits in two South African sheep breeds

By

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DECLARATION

I, Sunika Süllwald declare that the thesis, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Breeding and Genetics 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: ……..

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ABSTRACT

This study aimed to identify genomic regions of significance that are associated with body weight traits and reproduction traits in sheep by using estimated breeding values in a genome wide association study (GWAS). The following traits were investigated: body weight recorded at selection age of 14 months (BW), number of lambs born (NLB), number of lambs weaned (NLW) and total weight of lamb weaned (TWW). A total of 411 animal were genotyped using the Illumina® Ovine SNP50 BeadChip, and consisted of 152 Afrino, 129 Cradock Merino and 130 Grootfontein Merino sheep. Quality control (QC) were implemented using PLINK v1.07 where the parameters were set as 90% for individual call rate, 95% for SNP call rate, minor allele frequency of less than 2% and P <0.001 for Hardy-Weinberg equilibrium. Population parameters were calculated per dataset. The average MAF values estimated for the populations were 0.252, 0.372 and 0.372 for the Afrino, Cradock Merino and Grootfontein Merino, respectively. Inbreeding coefficients were estimated at - 0.025 (Afrino), - 0.025 (Cradock Merino) and 0.002 (Grootfontein Merino). The expected heterozygosity was 0.363 for the Afrino and 0.369 for both Merino populations. All three sheep populations had low inbreeding levels and moderate genetic variation. The population genetic substructure, ancestry proportion and genetic relatedness between the populations were investigated via principal component analysis (PCA) and admixture plots. These plots corresponded to the populations' selection practises and breeding programs as well as to the geographical locations where the individuals were kept. The GWAS was applied to each dataset separately and per trait using the efficient mixed model association eXpedited (EMMAX) software and visualised by Manhattan plots. Nine suggestive SNPs were identified to be in possible association with the traits. Of these nine, seven SNP were identified to be in close proximity or linked to previously annotated genes. Seven genes were identified which were in association with growth and reproduction traits. The genes *SIX6*, *C14orf13* and *TRPS1* showed the most promise for body weight and growth traits. For reproduction and fertility traits the genes *LIG1*, *CABP5*, *GRIK3* and *HDAC9* warrants further investigation.

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
Ave	Average
BLUP	Best linear unbiased prediction
BW	Body weight
CFW	Clean fleece weight
CV	Cross validation
DNA	Deoxyribonucleic acid
EBV	Estimated breeding value
EMMAX	Efficient mixed model association eXpedited
FD	Fibre diameter
F_{IS}	Inbreeding coefficient
GADI	Grootfontein Agricultural Development Institute
GCTA	Genome-wide complex trait analysis
GWAS	Genomic wide association studies
H_e	Expected heterozygosity
H_o	Observed heterozygosity
HWE	Hardy-Weinberg equilibrium
h^2	Heritability
IBD	Identical by descent
IBS	Identical by state
ISGC	International Sheep Genomics Consortium
LD	Linkage disequilibrium
MS	Microsoft Software
MAF	Minor allele frequency
MAS	Marker assisted selection
Max	Maximum
Min	Minimum
MSats	Microsatellites
N_e	Effective population size
NLB	Number of lambs born per ewe
NLW	Number of lambs weaned
OAR	*Ovis Aries* chromosome
PCR	Principal Component Analysis
QC	Quality control

QTL	Quantitative trait loci
REV	Relative economical value
REV-Rep	Relative economical value including reproduction
RNA	Ribonucleic acid
ROH	Runs of homozygosity
SA Merino	South African Merino
SE	Standard error
SNP	Single nucleotide polymorphisms
TWW	Total weight of lambs weaned per ewe lifetime

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

The sheep industry is considered a crucial sector in South African agriculture (Cloete *et al.*, 2014; Waldner *et al.*, 2017). Most agricultural land in South Africa that is unsuitable for crop production can be utilised by small stock (Cloete & Olivier, 2010). Sheep production is practised all over South Africa but is mainly situated in the arid regions of the country (Cloete *et al.*, 2014; DAFF, 2017a). The South African sheep industry consist out of three main enterprises namely mutton, wool and karakul pelts (Louw, 2013; DAFF, 2016a). Mutton production is the main contributor to the South African sheep industry and generates a gross value of R 4.1 billion per annum (DAFF, 2017a). The Department of Agriculture, Forestry and Fisheries estimated that commercial sheep farmers in South Africa employed up to 35 000 workers in 2017 (DAFF, 2017a).

The success and profit of any sheep enterprise is dependent on reproduction rate and body weight (Zishiri *et al.*, 2013). Growth and reproduction has been extensively studied in both South African (Zishiri *et al.*, 2013; Olivier, 2014; Nemutandani *et al.*, 2018; Molotsi *et al.*, 2017a) and global sheep populations (Safari *et al.*, 2005; Wolc *et al.*, 2011; Boujenane *et al.*, 2013). Heritability estimates range from low for reproduction traits (Cassell, 2009; Glaze, 2011; Matebesi-Ranthimo *et al.*, 2017) to medium for growth traits (Safari & Fogarty, 2003; Safari *et al.*, 2005; Zishiri *et al.*, 2013). Genetic progress in especially the reproductive traits are slower due to the low heritability and the relatively bigger contribution of the environment to the phenotype (Keats & Sherman, 2013; Khatib & Bormann, 2015). Reproduction traits are also sex-limited and expressed later in life, which makes selection for these traits difficult and results in slow genetic progress (Rosati *et al.*, 2002).

Natural and artificial selection have resulted in genetic differentiation between sheep breeds (Fariello *et al.*, 2013; Makina *et al.*, 2015). These genetic differences are seen as variable regions on the genome (Gurgul *et al.*, 2014; Gutiérrez-Gil *et al.*, 2017), and can harbour important functional mutations or candidate genes that could be favourable for economically important traits of interest (Gurgul *et al.*, 2014; Makina *et al.*, 2015). Genome wide association studies (GWAS) is a useful genomic tool that detects genetic variants that are associated with the expression of a complex trait (Blasco & Toro, 2014; Molotsi *et al.*, 2017a).

In this study, GWAS was used to identify single nucleotide polymorphisms (SNPs) that were associated with growth and reproduction traits. The genomic regions were further investigated to identify putative / candidate genes by comparing them to existing gene databases, such as http://pantherdb.org/about.jsp (Mi et al., 2019) and https://www.ensembl.org/ index.html (Zerbino et al., 2018). By identifying regions that are under directional selection for growth and reproductive traits, insight will be gained into the underlying biological processes and mechanisms involved in these traits (Fariello *et al.*, 2013; Makina *et al.*, 2015; Zwane *et al.*, 2016; Gutiérrez-Gil *et al.*, 2017). Regions associated with reproduction and body weight traits can then be used in selection strategies to increase genetic gain and this should result in faster genetic progress (Blasco & Toro, 2014; Fleming *et al.*, 2018; Hay & Roberts, 2018).

1.2 Aim and objectives

The broad aim of the study was to identify genomic regions associated with body weight and reproduction in two South African sheep breeds. To achieve this aim, the following objectives were set:

- Principal component analysis (PCA) to investigate population diversity and population differentiation between and within the breeds.
- Perform a genome wide association study (GWAS) on both breeds for each phenotypic trait separately.
- Identify markers associated with the traits under investigation and compare these to genomic databases to identify putative / candidate genes associated with the traits.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

During the Neolithic revolution human-kind changed from being hunter-gatherers to practising agriculture (Horsburgh & Rhines, 2010). One method of acquiring sufficient food was through domestication of animals (Horsburgh & Rhines, 2010). Sheep was one of the first livestock species to be successfully domesticated 10 500 to 9 000 years ago during the Neolithic period in the Fertile Crescent (Rocha *et al.*, 2011; Florian *et al.*, 2018). The Asiatic mouflon (*Ovis orientalis*) is widely recognized as the common ancestor of modern domestic sheep (Horsburgh & Rhines, 2010).

In Southern Africa, the earliest archaeological evidence found of domesticated small stock dates back to 2 000 to 2 500 years ago (Pleurdeau *et al.*, 2012). Sheep arrived in Africa from the Fertile Crescent through two geographical routes into sub-Saharan African via either the Horn of Africa or Egypt (Goldblatt, 2011). From Egypt sheep were further distributed by hunter-herders southwards along the Atlantic seaboard trade routes to the southern tip of Africa (Sadr, 2015). Sheep from the Horn of Africa were dispersed south-easterly over the Zambezi and Limpopo River Basins into southern Africa (Sadr, 2015). Sheep were essentially bred for the production of meat and milk, which became dependable food sources and were only later selected for secondary products such as wool, skins and dairy products such as yogurt and cheese (Rocha *et al.*, 2011; Larson & Fuller, 2014).

The aim of this literature review is to give a brief overview of the South African sheep industry, including the wool industry. The review will give a detailed description of the two South African sheep breeds included in the study and provide an overview of the reproduction and body weight traits under investigation, as well as their importance in selection strategies.

2.2 The South African sheep industry

Sheep production is an important sector of the South African economy. Up to 80% of land in South Africa is unsuitable for crop production and can only be utilised for extensive livestock production systems (Cloete & Olivier, 2010; Schoeman *et al.*, 2010). Most of this land can be used effectively by small stock. In periods of drought and/or crop failure, pastoral and crop farmers rely on small stock for income (Cloete & Olivier, 2010). Semi-extensive farming practises provides financial stability to regions where cropping practises are precarious (Cloete *et al.*, 2014).

Most sheep farming practises in South Africa are concentrated in the arid regions of the country extending from the Karoo in the Northern Cape Province and Western Cape Province and further eastwards towards the lower part of the Free State (DAFF, 2017b). Figure 2.1 illustrates the distribution of sheep production in South Africa, relative to other main agricultural sectors.

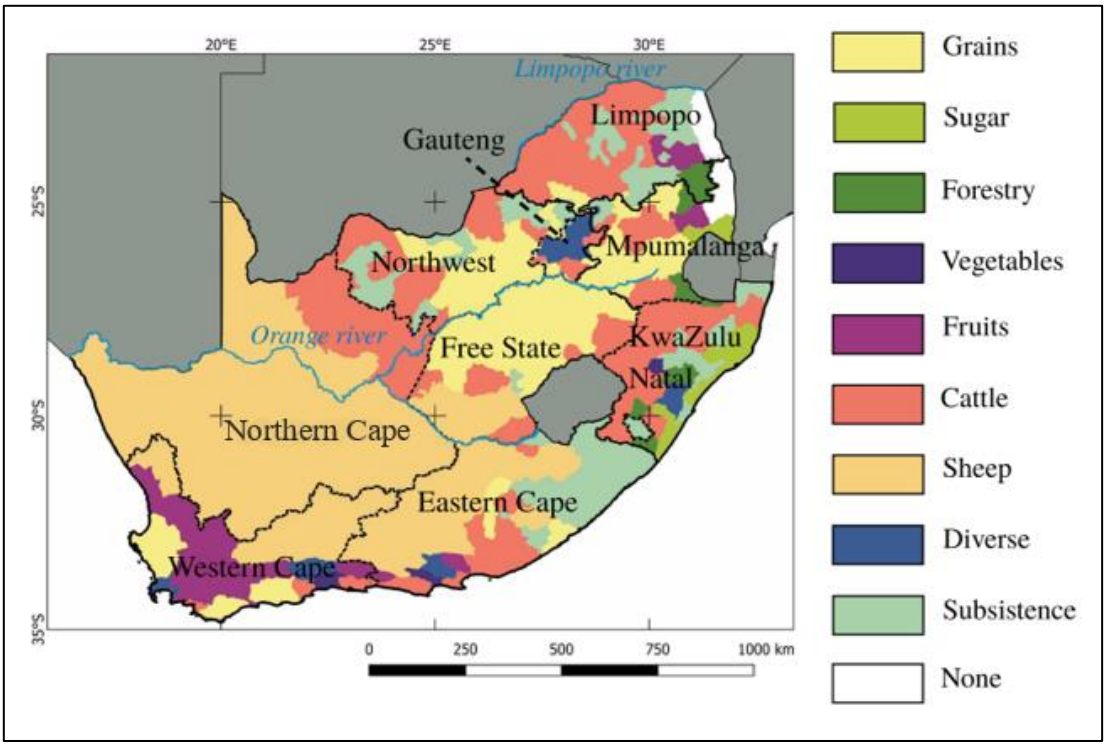

Figure 2.1 Distribution of agricultural sectors in South Africa (adapted from: Waldner *et al.*, 2017)

Primary Agriculture is important for economic growth in South Africa and provides substantial employment opportunities (Cloete & Olivier, 2010; DAFF, 2015). The small stock sector contributes 8 to 10% to the revenue obtained by animal products in South Africa (Cloete & Olivier, 2010). The majority of the profit is generated from mutton (60.6%) and wool (31.4%) production. Milk and karakul pelts contribute less than 1%, while the remainder of the profit is generated from mohair and other products (Cloete & Olivier, 2010; DAFF, 2016a, 2017a). The total sheep population of South Africa in 2018 was estimated at ±22.6 million animals (Netshifhefhe, 2018), of which more than 70% were located in three provinces. Figure 2.2 illustrates the sheep populations per province.

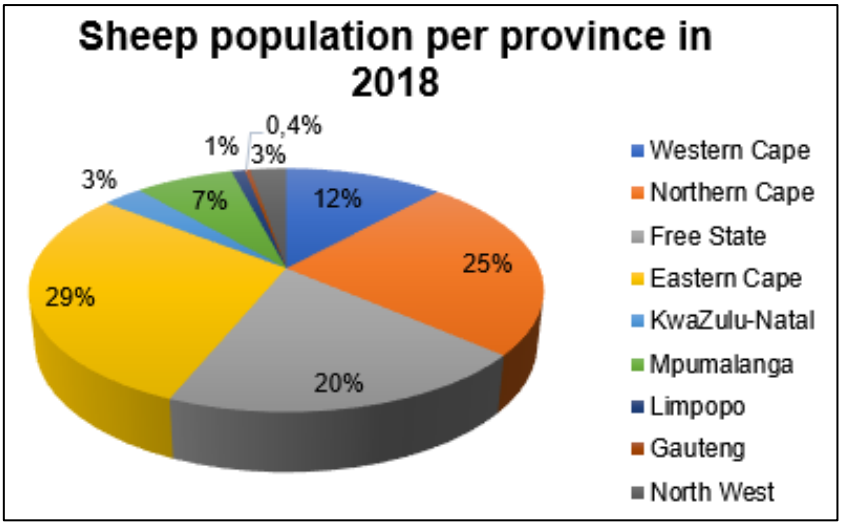

Figure 2.2 The distribution of South African sheep per province in 2018 (adapted from: Netshifhefhe, 2018)

The mutton farming industry (including woolled sheep breeds) consists of approximately 8 000 commercial farmers and 5 800 communal farmers (DAFF, 2017b). In South Africa on average ± 184 600 tons of mutton are produced per annum and the gross value of mutton produced for 2017 was estimated at ± R 7 008 million (DAFF, 2016b, 2017b). Over the period of 2014 to 2017, more mutton was consumed than produced in South Africa, making South Africa a net importer of mutton (DAFF, 2017b). The major countries from which South Africa import mutton are Namibia (50%), Australia (37%) and New Zealand (13%) (DAFF, 2016b, 2017b).

The wool industry is an important industry in the South African economy as a foreign exchange earner (DAFF, 2016a; 2016b). The wool industry's gross value for 2016/17 was estimated at ± R4 158 million (DAFF, 2016a). Average wool production for the years 2013 and 2016 was 50 506 and 49 788 tons respectively (DAFF, 2016a). South Africa is a net exporter of wool and the major importing countries include China (61%), the Czech Republic (22%), India (7%) and Italy (7%) (DAFF, 2016b). South Africa only contributes 2.3% to the total world wool clip and therefore is regarded as price takers (DoA, 2007). The Australian market determines the wool prices internationally as they are the largest contributors to the world wool clip (www.iwto.org/wool-production). Approximately 90% of the South African wool clip is produced primarily by four provinces, namely the Eastern Cape, Free Sate, Northern Cape and Western Cape as illustrated in Figure 2.3.

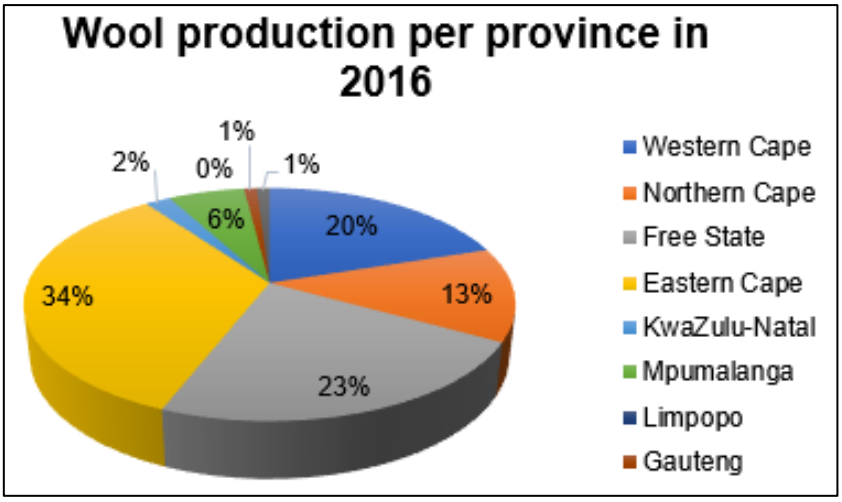

Figure 2.3 Wool production per province in 2016 (adapted from: DAFF, 2016a)

The South African human population is increasing rapidly and the declining sheep population and increased demand for red meat leads to shortages of mutton in the supply chain (DAFF, 2017a). In order to satisfy the demand, sheep production nationally must increase in terms of more productive animals retained in farming practises, as larger sheep flocks are generally not feasible (Goldblatt, 2011; DAFF, 2016a, 2017a). The two sheep breeds used in the current study will be discussed in more detail.

2.3 The South African breeds included in this study

The two breeds that were used in this study is the South African Merino and the Afrino.

2.3.1 The Afrino sheep breed

The Afrino is a dual purpose, composite breed that was developed in South-Africa in the 1960's (Louw, 2013). The breed was originally developed due to a request made by the South African Agricultural Union for a dual purpose mutton sheep breed that could produce white-wool and could be used in extensive grazing systems (Stud Book, 2004; Snyman, 2014a). The South African Agricultural Union requested that the breed should be free of kemp and coloured fibres, have good reproductive ability, yield early slaughtering lambs of good quality, should be hardy and adapted to the extensive grazing systems in the South African environment (Stud Book, 2004; Snyman, 2014a). The first breeding project was launched in 1969 and was located in the North-Western Karoo at the Carnarvon Experimental Station (Snyman, 2014a). The project aimed to develop a breed with the mutton producing abilities and reproductive performance of the South African mutton sheep breeds, combined with the superior wool quality of the Merino. In 1976 the first successful cross for this white wool mutton breed was made and consisted of 25% Merino, 25% Ronderib Afrikaner and 50% South

African Mutton Merino (Schoeman *et al.*, 2010; Snyman, 2014a). Figure 2.4 illustrates a typical Afrino ram and ewe.

Figure 2.4 A typical SA Afrino ewe (left) and ram (right), (Snyman, 2014a)

This cross was retained for upgrading and to further develop the breed (Louw, 2013). The Afrino Sheep Breeders' Society was formed on 5 February 1980 (Stud Book, 2004). On this day the white-wool mutton breed was named the Afrino and breed standards was compiled. The modern Afrino is defined as a dual purpose large framed, white-wool breed (Louw, 2013). Afrino ewes are polled whereas rams are either polled or scurred. The body and belly should be covered in a fair amount of good quality wool (Snyman, 2014a). Afrino fleeces should have a fibre diameter of less than 23 microns and should be free of kemp, and white, yellow or chalky white fleeces are unacceptable (Snyman, 2014a). Afrino ewes have good mothering abilities and fertility as shown in Table 2.1.

Table 2.1 Summary of the Afrino ewe wool production and reproduction norms (adapted from: Snyman, 2017)

Wool production norms	
Trait	**Average (±SE)**
Greasy fleece weight (kg)	2.72 ± 0.09
Clean fleece weight (kg)	1.74 ± 0.04
Fibre diameter (µm)	21.0 ± 0.1
Clean yield (%)	63.7 ± 0.6
Staple length (mm)	74.9 ± 1.0
Number of crimps/25 mm	14.3 ± 0.3
Coefficient of variation of fibre diameter (%)	17.9 ± 0.02
Standard deviation (µm)	3.51 ± 0.04
Comfort factor (%)	99.1 ± 0.1
Staple strength (N/Ktex)	31.5 ± 0.7
Creeping belly score	34.0 ± 1.1

Reproduction production norms	
Trait	**Average (±SE)**
Total weight of lamb weaned/year (kg)	36.13 ± 0.06
Number of lambs born/year	1.33 ± 0.10
Number of lambs weaned/year	1.17 ± 0.05
Number of lambing opportunities	3.34
Total weight of lamb weaned/lifetime (kg)	130.8 ± 2.5
Number of lambs born/lifetime	4.69 ± 0.06
Number of lambs weaned/lifetime	4.22 ± 0.05

[SE] Standard error

In Afrino farming flocks, 80% of the income is generated by meat production and 20% by wool production (Stud Book, 2004; Snyman, 2014a). The Afrino breed's dual-purpose ability allows farmers to produce good quality wool and good quality lambs (Louw, 2013; Snyman, 2014a). Table 2.2 summarises production norms of Afrino lambs. This breed allows farmers to market their lambs at a later stage and at heavier body weights, as the Afrino breed is a late maturing type (Stud Book, 2004; AGTR, 2010a; Snyman, 2014a). Afrino sheep have good carcass quality when finished in feedlots and produce carcasses with an even fat cover and high meat quality (AGTR, 2010a).

Table 2.2 Summary of Afrino lamb production norms (adapted from: Snyman, 2017)

Growth production norms (±SE)		
Trait	**Ram lambs**	**Ewe lambs**
Birth weight (kg)	4.85 ± 0.01	4.58 ± 0.01
42-day body weight (kg)	16.0 ± 0.2	14.9 ± 0.2
100-day weaning weight (kg)	32.4 ± 0.3	29.9 ± 0.3
5-month body weight (kg)	33.6 ± 0.4	30.6 ± 0.4
6-month body weight (kg)	38.3 ± 0.4	35.0 ± 0.4
7-month body weight (kg)	41.0 ± 0.4	37.5 ± 0.4
8-month body weight (kg)	45.9 ± 0.4	40.9 ± 0.4
9-month body weight (kg)	49.6 ± 0.4	43.8 ± 0.4
10-month body weight (kg)	53.1 ± 0.5	46.8 ± 0.5
11-month body weight (kg)	56.5 ± 0.5	49.2 ± 0.5
12-month body weight (kg)	60.8 ± 0.5	52.7 ± 0.5

[SE] Standard error

2.3.2 The Merino sheep breed

The Merino is a white-wool sheep breed that originated in Spain and first arrived in the Cape colony in 1789 (Snyman, 2014b).The Dutch Government donated two Spanish Merinos rams and four Spanish Merino ewes to the Cape colony military commander, Colonel Jacobs Gordon in 1789 (Louw, 2013). By the 1830s there were well-established wool producers in the Western and South-Western Cape. The “Groot Trek” of the Voortrekkers in 1834 played an important part in disseminating the breed further across South Africa, especially towards the North and North East and by the mid-1800s the breed was dispersed over South Africa (Louw, 2013; https://merinosa.co.za/history/). In 1891 other Merino-type breeds, such as the American Vermont Merino and the Australian Wanganella and Peppin Merino were imported to improve and develop the local Merino flocks (AGTR, 2010b; Louw, 2013).

The South African Merino was developed from the following Merino types: Spanish, Saxony, Rambouillet, American and Australian Merino (AGTR 2010b). Proportionally the Australian Merino contributed the most to the South African Merino as Australian Merinos formed the majority of the Merino imports during the 1800s due to the similarity in climate (AGTR, 2010b; Snyman, 2014b). The modern South African Merino (SA Merino) is the result of 200 years of selection for adaptive and functional traits (Louw, 2013; Snyman, 2014b). The SA Merino is defined as a medium to large framed, fine-wool breed (http://dagris.info/node/2491) as shown in Figure 2.5.

Figure 2.5 A typical SA Merino ewe (left) and ram (right), (Snyman, 2014b)

This locally developed breed produces high quality wool that is equivalent to its counterparts around the world (Snyman, 2014b). Merino wool is the most sought-after fine wool globally and also dominates the wool industry in South-Africa (Stud Book, 2004; DAFF, 2016b). Merino wool is characterised as a soft wool that has a uniform crimp which is free of

kemp and other coarse and coloured fibres (DAFF, 2016b). Merinos can produce a clean fleece yield of up to 10 to 15% of its own body weight (AGTR, 2010b; Snyman, 2014b). Merino and Merino-type sheep breeds are found in most extensive sheep farming systems as they tolerate hot and cold temperatures in high and low rainfall areas (Stud Book, 2004; Snyman, 2014b). Table 2.3 summarises production and reproduction performance for adult Merino ewes.

Table 2.3 Summary of the Merino ewe production and reproduction norms (adapted from: Olivier, 2017)

Wool production norms		
Trait	**Cradock average (±SE)**	**Grootfontein average (±SE)**
Mating weight (kg)	60.5 ± 0.9	54.7 ± 0.6
Greasy fleece weight (kg)	4.8 ± 1.1	4.6 ± 1.2
Clean fleece weight (kg)	3.4 ± 1.1	3.1 ± 1.2
Fibre diameter (µm)	19.1 ± 1.2	18.9 ± 1.6
Clean yield (%)	71.3 ± 4.7	67.4 ± 5.2
Staple length (mm)	89.7 ± 9.2	91.5 ± 7.8
Comfort factor (%)	99.5 ± 0.0	98.9 ± 0.8
Staple strength (N/Ktex)	38.7 ± 0.8	34.5 ± 0.9
Number of crimps/25 mm	14.1 ± 1.9	11.5 ± 1.2
Reproduction norms		
Trait	**Cradock average (±SE)**	**Grootfontein average (±SE)**
Number of ewes mated	256	252
Conception rate (%)	74.6	83.3
Lambing percentage (%)	110.5	123
Weaning (%)	84.4	52.8
Total weight of lambs weaned/ ewe (kg)	19.0 ± 5.2	11.7 ± 8.2

[SE] Standard error

Other characteristics of the SA Merino are good fertility and mothering ability (Stud Book, 2004; AGTR, 2010b). Merino carcasses are also in demand as Merinos can produce marketable carcasses over a wide range of grazing and climatic conditions (https://merinosa.co.za/; Stud Book, 2004). In Merino flocks, 60% of the revenue is obtained by meat production and the remaining 40% is from wool production (Stud Book, 2004; Snyman, 2014b). The SA Merino is also a late maturing breed, producing slaughter lambs that could be marketed at a high body weight (Stud Book, 2004). Merinos finished off in feedlots produce carcasses with good conformation and fat cover (Brand *et al.*, 2017). Table 2.4 summarises male and female lamb growth production norms for two different flocks.

Table 2.4 Summary of Merino lamb production norms (adapted from: Olivier, 2017)

Trait	Growth production norms (±SE)			
	Cradock		Grootfontein	
	Ram lambs	Ewe lambs	Ram lambs	Ewe lambs
Birth Weight (kg)	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	4.6 ± 0.1
42-day weight (kg)	18.9 ± 0.6	18.0 ± 0.7	19.1 ± 0.6	19.5 ± 0.5
Weaning weight (kg)	25.2 ± 1.0	23.7 ± 1.1	26.2 ± 1.1	23.4 ± 1.2
6-month weight (kg)	27.5 ± 1.1	29.3 ± 1.1	22.4 ± 1.1	25.0 ± 1.1

[SE] Standard error

2.4 Current selection strategies for growth and reproduction in the sheep industry

The traits that are of importance for most sheep production systems are reproduction, growth and wool traits (Nemutandani *et al.*, 2018; Molotsi *et al.*, 2017a). These are economically important traits and they determine the success and profitability of the production system (Zishiri *et al.*, 2013; Olivier, 2014). Reproduction traits are essential to maintain flock numbers and increase the number of animals that produce meat and wool (Hatziminaoglou & Boyazoglu, 2002; Sandenbergh, 2015). Reproduction is also an indication if animals are adapted; animals that are able to reproduce in a specific environment are adapted and acclimatised to the conditions they are found in (Bailey *et al.*, 2006; Taylor, 2006; Molotsi *et al.*, 2017a). Growth traits are considered important for meat production to increase its quantity (Nemutandani *et al.*, 2018). For the purpose of this study, only reproduction and growth traits will be discussed in more detail.

Current selection strategies for sheep breeds are developed and evaluated by SA Stud Book (http://studbook.co.za), research institutes and the respective breeders' associations. Current selection strategies use selection indices to select and improve breeds. Selection indices use reproduction and production traits and also these traits' economical values in a combined formula (Olivier *et al.*, 2015). Selection indices are available for the Afrino breed but it is not generally applied in breeding programs among Afrino breeders. For the Merino breed, national selection indices are based on profitability (Herselman, 2004; Herselman & Olivier, 2010). Two indices are available, one including reproduction and one excluding reproduction. These selection indices are revised annually in August and are based on the previous five years' average meat and wool prices. The selection indices applicable for August 2017 to 2019 were provided by personal communications from SA Studbook (Dr Bernice Mostert, bernice@studbook.co.za). The explanation and description of the indices are as follows:

August 2017

$$REV = - 613.44 + 1.875EBV_{BW} + 91.87EBV_{CFW} + 0.4EBV_{SL} – 75.34EBV_{FD} +1.5336(20 + EBV_{FD})^2 + 5.5125EBV_{TWW}$$

$$REV_{Rep} = - 613.44 + 0.51EBV_{BW} + 91.92EBV_{CFW} + 0.4EBV_{SL} – 75.34EBV_{FD} +1.5336(20 + EBV_{FD})^2 + 11.025EBV_{TWW}$$

August 2018

$$REV = - 896.68 – 0.94EBV_{BW} + 95.11EBV_{CFW} + 0.50EBV_{SL} – 108.44EBV_{FD} +2.2323(20 + EBV_{FD})^2 + 11.97EBV_{TWW}$$

$$REV_{Rep} = - 892.92 + 0.49EBV_{BW} + 95.23EBV_{CFW} + 0.49EBV_{SL} – 107.99EBV_{FD} +2.2323(20 + EBV_{FD})^2 + 11.97EBV_{TWW}$$

August 2019

$$REV = - 1409.72 – 164.5EBV_{BW} + 108.07EBV_{CFW} + 0.68EBV_{SL} – 161.42EBV_{FD} +3.5243(20 + EBV_{FD})^2 + 6.5925EBV_{TWW}$$

$$REV_{Rep} = - 1403.8 – 1.23EBV_{BW} + 108.02EBV_{CFW} + 0.67EBV_{SL} – 160.75EBV_{FD} +3.5095(20 + EBV_{FD})^2 + 13.185EBV_{TWW}$$

Where,

BW: Body weight

CFW: Clean fleece weight

EBV: Estimated breeding value

REV: Relative economic value

REV_{Rep}: Relative economic value including reproduction

FD: Fibre diameter

SL: Staple Length

TWW: Total weight weaned

2.4.1. History of selection practices followed in the experimental flocks

Selection practises and management of the Afrino, Cradock Merino and Grootfontein Merino flocks were determined by the Grootfontein Agricultural Development Institute (GADI). The institute was the main role player in all three flocks' breeding and selection programs. The history of the selection and breeding practises of the three flocks were obtained via personal communications with GADI (Dr MA Snyman, GrethaSn@daff.gov.za). These practices are discussed in detail, as the selection emphasis differed between the flocks, and should have resulted in genetic differentiation between flocks. The selection practises give background on which traits were selected, and consequently could have influenced the allele frequencies of specific traits. These traits of importance were further investigated for markers and candidate genes associated with these traits.

2.4.1.1. History of selection practices of the Carnarvon Afrino flock

The Afrino animals that are currently at the Carnarvon Experimental Station are the descendants of the first Afrino sheep developed in South Africa. Breed development started at the Carnarvon Experimental Station in 1969. The flock was kept closed since its development and no outside sires were introduced. Early selection was mainly aimed at growth rate of lambs, reproductive performance of ewes and absence of coloured wool fibres and kemp. Animals were excluded based on subjective selection at 18 months of age. Animals that had poor conformation or an excessive amount of coloured wool fibres and kemp were culled. Rams were selected on the basis of post-weaning growth rate. Ewes were not selected for growth performance at the start of the trial to allow for an increase in the breeding flock.

During March 1981, the ewe flock was classed according to breed standards for the first time. Young ewes of 18-month age that had a body weight of 10% or more below average were culled. Since 1985, the breeding strategy changed and allowed mating of all available young ewes, with the exception of those animals that did not attain the conformation and wool standards. Final ewe selection was based on total weight of lamb weaned after the first parity. Rams were selected if they had a below average fibre diameter, as well as an above average index for pre- and post-weaning growth traits. Emphasis was also placed on wool quality and evenness of the fleece, while less emphasis was put on the amount of wool produced. Since 1991, all selection practises in the flock were based on best linear unbiased prediction (BLUP) of breeding values for the specific traits under selection.

In 1991, the following ewe selection procedures were implemented. At 15 months of age, all young ewes were assessed subjectively for breed standards and conformation or wool faults. Animals with defects in this regard were culled. After performance testing of wool traits, only ewes with too high fibre diameters were culled. The remainder of the ewes were mated at 18 months of age and final selection was done after the lambs were weaned. Failure to wean a lamb resulted in automatic culling. Furthermore, three-year-old ewes which had their second lambing opportunity were also evaluated on reproductive performance and those producing below average in terms of total weight of lamb weaned over two lambing opportunities were also culled, depending on ewe numbers in the flock.

Up until 2000, selection was mainly aimed at increasing reproductive performance and body weight, while improving wool quality traits and reducing fibre diameter. The current selection objectives are to improve reproductive performance, maintain body weight, wool

weight and fibre diameter and improve wool quality traits. Selection is done on BLUP of breeding values.

2.4.1.2 History of selection practices of the Cradock fine wool Merino stud

A project aimed at establishing a genetic fine wool Merino stud was initiated in 1988 at the Cradock Experimental Station. For the base stud 520 ewes were bought from the 32 breeders with the finest clips in each wool production area. Four Australian imported fine wool Merino rams were used as breeding sires. Fifty ewes from the Grootfontein Merino stud with a mean fibre diameter of 29 µm were selected and were also mated with the Australian rams. The ewes were then subsequently mated to the rams used in the fine wool strain. Ram and ewe replacements were subsequently selected on fibre diameter, body weight and conformation from within the stud. All animals and their progeny were managed as one stud on irrigated pastures (Olivier, 2014). Data generated in this flock were used for various studies (Olivier *et al.*, 2004; Olivier *et al.*, 2006a, 2006b; Olivier, 2014).

At the initial stages of the project, selection was aimed at improving body weight and body conformation, while fibre diameter was kept constant. In 1996 it was decided to change the objectives and put less emphasis on body weight and more on decreasing fibre diameter. Since 1999, staple length was added as selection criterion. Up until 1999, no selection on the basis of reproductive performance was done in the stud. Since 1999, ewes with poor reproductive performance were culled, as well as all mature ewes with a fibre diameter above 21 µm.

In 2004, selection objectives were changed, and breeding sires and dams were selected on the basis of profitability, according to the selection index for Relative economical value including reproduction (REV_{REP}) described in paragraph 2.4. Animals with the highest profitability values with acceptable breeding values for fibre diameter, body weight, clean fleece weight, staple length and total weight of lamb weaned, were selected as breeding sires (Olivier, 2014). Rams and ewes were thus selected to improve body weight, staple length and reproduction, maintain fleece weight and reduce fibre diameter.

2.4.1.3 History of selection practices of the Grootfontein Merino stud

Olivier (1989) provides a detailed description of the management and selection procedures followed in this stud. During 1955, the basis of the Grootfontein Merino stud was formed and consisted out of 227 ewes bought from local breeders and 63 ewes donated by local breeders (Nemutandani, 2016). An additional 105 ewes formed the remainder of the flock

that were from three selection lines situated at Grootfontein during that time (Nemutandani, 2016). Additional animals outside the stud was brought into the stud and were as follow: four Australian rams imported in 1955 (Olivier, 1989), locally bred animals in 1962 (two rams) and 1964 (two rams and 11 ewes). From 1966 to 2002 only 11 rams were brought into the stud from outside studs. In 2002 linkage was established between the Cradock fine wool Merino stud and the Grootfontein fine wool Merino stud (Nemutandani, 2016). This link was established by bringing in rams and ewes from the Cradock stud into the Grootfontein stud and ewes from the Grootfontein stud into the Cradock stud (Nemutandani, 2016).

Selection strategies followed for the Grootfontein Merino stud changed over the years. The main selection objectives from 1956 to 1968 were good conformation and wool traits, but it were changed in 1985 to include reproduction traits as well (Sandenbergh, 2015; Nemutandani, 2016). The selection objective for good conformation and wool traits led to a favourable increase of breeding values for live weight and clean fleece weight which indirectly led to an unfavourable increase of fibre diameter (Schoeman *et al.*, 2010; Sandenbergh, 2015). Since 1985 animals with definitive conformation and wool faults, as well as animals with low 120-day weight were culled, but final selection was done on animal model BLUP of breeding values. According to Olivier *et al.* (1995), further selection was based on comprehensive supremacy, with the paramount criteria being body size and wool traits. From 1985 onwards the main selection objectives were amended to increase body weight, maintain clean fleece weight and reduce mean fibre diameter and pleat score (Sandenbergh, 2015; Nemutandani, 2016).

In 1999 the Grootfontein Merino stud was divided into two selection lines (Schoeman *et al.*, 2010; Nemutandani, 2016). The one selection line was the control line in which within-line selection to reduce fibre diameter was done, while the other line was a fine wool line in which selection aims were to increase clean fleece weight and improve the live weight of lambs born (Schoeman *et al.*, 2010; Sandenbergh, 2015; Nemutandani, 2016). Cradock fine wool Merino rams were used as breeding sires in the fine wool line.

As with the Cradock Merino stud, selection objectives were changed in 2004, and breeding sires and dams were selected on the basis of profitability, according to the selection index for Relative economical value including reproduction (REV_{REP}) described in paragraph 2.4. Animals with the highest profitability values with acceptable breeding values for fibre diameter, body weight, clean fleece weight, staple length and total weight of lamb weaned, were selected as breeding sires (Olivier, 2014). Rams and ewes were thus selected to improve body weight, staple length and reproduction, maintain fleece weight and reduce fibre diameter.

2.5 Traits of importance

Reproduction efficiency is essential in any farming enterprise as it is directly linked to the profitability of an enterprise (Hatziminaoglou & Boyazoglu, 2002; Zishiri *et al.*, 2013; Yavarifard *et al.*, 2015). Reproduction traits are complex traits that are influenced by multiple genes as well as the environment, resulting in low heritability estimates for these traits (Khatib & Bormann, 2015; Molotsi *et al.*, 2017a). Many reproduction traits are also expressed later in life, contributing to the difficulty in making genetic progress (Rosati *et al.*, 2002; Schoeman *et al.*, 2010; Costa *et al.*, 2015). Genetic progress is typically slow for reproduction traits, but these traits are included in selection programs due to their high economic importance and large genetic variability (Olivier, 2002; Rosati *et al.*, 2002; Glaze, 2011). The larger the genetic variability in a trait, the more genetic progress can be made (Oldenbroek & Van der Waai, 2015b; Hill, 2016).

Although genetic progress can be attained for these lowly heritable traits, it is generally slower and take many generations (Cassell, 2009; Glaze, 2011; Oldenbroek & Van der Waai, 2015b; Hill, 2016). Selecting for correlated traits with higher heritability estimates could result in increased genetic progress in lowly heritable reproduction traits (Cassell, 2009; Zishiri *et al.*, 2013; Khatib & Bormann, 2015). Many individual traits can be selected for to improve reproduction rate in ewes, but not all of the traits are easily measured or observed in practise, which results in slow genetic progress over an extended period of time (Glaze, 2011; Zishiri *et al.*, 2013; Khatib & Bormann, 2015). Number of lambs born (NLB), number of lambs weaned (NLW), weaning weight (WW) and total weight weaned (TWW) per lifetime are traits suitable for selection and will be discussed in more detail.

Number of lambs born per ewe is a trait that is readily measured by farmers and gives a good indication of the fertility of an ewe (Zishiri *et al.*, 2013; Cordero *et al.*, 2019). The most commonly known major gene that contributes to sheep prolificacy is the Booroola gene, but several studies has shown that other major genes also contribute to sheep prolificacy resulting in increased litter size (Gootwine, 2011; Demars *et al.*, 2013; Xu & Li, 2017; Xu *et al.*, 2018). The production system environment will determine if an ewe will be able to support higher litter sizes (Xu & Li, 2017; Xu *et al.*, 2018; Cordero *et al.*, 2019). Inclusion of number of lambs born as a selection criterion should result in an increase in litter size per ewe. This is, however, not always feasible under demanding and unfavourable environmental or managerial conditions (Ekiz *et al.*, 2005; Zishiri *et al.*, 2013; Khan *et al.*, 2017). Number of lambs born per ewe is positively genetically correlated with litter size ($r_g^2 = 0.64 \pm 0.01$) and ewe fertility ($r_g^2 = 0.29 \pm 0.01$) (Zishiri et al., 2013). Similar studies showed high positive correlations of $r_g^2 = 0.93 \pm 0.08$ (Olivier, 2014) and $r_g^2 = 0.999 \pm 0.043$ (Matebesi-Ranthimo *et al.,* 2017) between NLB and

NLW. These high positive correlations between NLB and NLW indicate that selection for NLB will result in indirect selection for higher ewe fertility and bigger litter sizes at weaning, which is favourable (Cloete *et al.*, 2004; Zishiri *et al.*, 2013; Yavarifard *et al.*, 2015). Bigger litter size will, however, influence how the ewe distributes her body reserves and nutrients for growth, pregnancy and wool production (Oldenbroek & van der Waai, 2015a; Al-Atiyat *et al.*, 2016; Xu *et al.*, 2018). Table 2.5 summarises the heritability values for NLB for the Merino sheep breed from previous studies.

Table 2.5 Summary of heritability estimates for number of lambs born in Merino sheep

Heritability	Breed	Reference
0.10 ± 0.02	Merino	Cloete *et al.*, 2004
0.10 ± 0.03	Merino	Matebesi-Ranthimo *et al.*, 2017
0.06 ± 0.01	Merino	Walkom & Brown, 2017

Number of lambs weaned is an easy to record trait and is used to indicate reproductive efficiency in a flock (Duguma *et al.*, 2002; Ekiz *et al.*, 2005; Yavarifard *et al.*, 2015). High number of lambs weaned gives a good indication of an ewe's rearing and maternal ability (Yavarifard *et al.*, 2015; Cordero *et al.*, 2019). The environment is a major contributor to not only the number of lambs weaned but also the quality of the lambs weaned (Zishiri *et al.*, 2013; Khan *et al.*, 2017). Table 2.6 reports the heritability values for NLW from previous studies in the Merino.

Table 2.6 Summary of heritability estimates for number of lambs weaned in Merino sheep

Heritability	Breed	Reference
0.04 ± 0.02	Merino	Cloete *et al.*, 2004
0.07 ± 0.02	Merino	Matebesi-Ranthimo *et al.*, 2017
0.04 ± 0.01	Merino	Walkom & Brown, 2017

Increased litter size at birth and weaning are not the only important contributors to profitability of an enterprise. In farming practises body weight and growth rate of lambs also determine the viability and profitability of an enterprise (Olivier, 2002; Schoeman *et al.*, 2010). Litter size *per se* as a breeding goal is not sufficient as it does not include weaning weight that gives an indication of an ewe's milk producing and mothering ability (Xu *et al.*, 2018; Cordero *et al.*, 2019) or a lamb's growth ability and performance (Ramakrishnappa *et al.*, 2013; Olivier, 2014; Matebesi-Ranthimo *et al.*, 2017). A more sufficient and holistic breeding goal would be to select for TWW (Zishiri *et al.*, 2013; Olivier, 2014; Matebesi-Ranthimo *et al.*, 2017), which is a complex trait used in selection programs as a biological index to measure reproduction potential in ewes (Schoeman *et al.*, 2010; Nemutandani *et al.*, 2018). This composite trait is

the most accurate way to measure an ewes' reproduction rate (Zishiri *et al.*, 2013; Matebesi-Ranthimo *et al.*, 2017) and it is a good measure of a flock's productivity and can be measured as total weight of lamb weaned over an ewe's lifetime (Duguma *et al.*, 2002; Cloete *et al.*, 2014; Olivier, 2014). Total weight of lambs weaned per ewe lifetime do not only include ewe fertility and litter size, but also lamb survival rate and direct growth performance of lambs up until weaning (Ekiz *et al.*, 2005; Zishiri *et al.*, 2013; Yavarifard *et al.*, 2015).

Scientific studies reported the following moderate to high positive genetic correlations between NLB and TWW: $r_g^2 = 0.44 \pm 0.09$ (Zishiri *et al.*, 2013), $r_g^2 = 0.868 \pm 0.105$ (Olivier, 2014), $r_g^2 = 0.98 \pm 0.15$ (Matebesi-Ranthimo *et al.*, 2017). Both Olivier (2014) and Matebesi-Ranthimo *et al.* (2017) reported similar ranges for the genetic correlation between TWW and NLW, which was estimated at $r_g^2 = 0.908 \pm 0.057$ and $r_g^2 = 0.93 \pm 0.22$ respectively. A study done by Zishiri *et al.* (2013) reported a lower correlation value of $r_g^2 = 0.60 \pm 0.07$. Selecting for total weight of lambs weaned will lead to an increase in both NLB and NLW and will in turn result in an increase in body weight of lambs. Table 2.7 summarises the heritability values of TWW in previous studies.

Table 2.7 Summary of heritability estimates for total weight of lambs weaned in Merino and Afrino sheep breeds

Heritability	Breed	Reference
0.04 ± 0.02	Merino	Cloete *et al.*, 2004
0.10 ± 0.03	Merino	Matebesi-Ranthimo *et al.*, 2017
0.08 ± 0.01	Merino	Walkom & Brown, 2017
0.17 ± 0.07	Afrino	Snyman *et al.*, 1997

Weaning weight is an important trait as it indicates the quality of lambs and it influences the price of a slaughter lamb (Olivier, 2002; Rosati *et al.*, 2002; Schoeman *et al.*, 2010). The weight at which the lamb is weaned also influences the carcass characteristics that in turn influences the marketability of the carcass (Caneque *et al.*, 2001). Age at weaning is less important than the weight at weaning, as weaning age has a lower impact than weight on optimal growth rate in young lambs (Ramakrishnappa *et al.*, 2013). The rate of pre-weaned growth and weight at weaning are determinants of marketing age (Olivier, 2014). Previous studies reported a high positive genetic correlation between TWW and WW which ranged between 0.60 and 0.80 (Zishiri *et al.*, 2013; Olivier, 2014). Selection based on the weight of lambs can result in an increase in meat production and growth performance (Cloete *et al.*, 2003; Nemutandani *et al.*, 2018). A growth trait such as weaning weight is a moderately heritable trait and the heritability of weaning weight for both breeds are presented in Table 2.8.

Table 2.8 Summary of heritability estimates for weaning weight in Merino and Afrino sheep breeds

Heritability	Breed	Reference
0.57 ± 0.03	Merino	Walkom & Brown, 2017
0.31 ± 0.06	Merino	Matebesi-Ranthimo *et al.*, 2017
0.33	Afrino	Snyman *et al.*,1995

2.6 Molecular technology available for genetic improvement of sheep production

Low to medium heritable traits can benefit from genomics and molecular technology to increase their rate of genetic progress (Blasco & Toro, 2014; Cloete *et al.*, 2014). Molecular technology can assist in the estimation of more accurate and reliable breeding values, especially for lowly heritable traits (Duguma *et al.*, 2002; Berry *et al.*, 2014; Molotsi *et al.*, 2017c), which will lead to an increased rate of genetic progress (Blasco & Toro, 2014; Molotsi *et al.*, 2017b; Sheriff & Alemayehu, 2018).

Traditional animal breeding methods were primarily based on estimated breeding values (EBV) which was developed between 1980 and 1990 by the statistician C.R. Henderson (Oldenbroek & van der Waai, 2015a; 2015c). Best linear unbiased prediction (BLUP) is the mathematical methodology to obtain EBVs by using performance testing data and pedigrees (Van Der Werf, 2012; Oldenbroek & van der Waai, 2015c). BLUP was first implemented in the late 1990s in South Africa for sheep selection and breeding programs (Olivier *et al.*, 1995; Olivier, 2002). BLUP resulted in the estimation of accurate EBVs and this provided the first method to rank animals according to their estimated genetic potential (Van Der Werf, 2012; Oldenbroek & van der Waai, 2015b; 2015c). This method has made significant contributions to advance genetic progress in livestock breeding practises, but it only provides rough estimates of genetic variation and cannot describe the source of variation at DNA level (Ewens, 2006; Van Der Werf, 2012). The biggest limitation of a BLUP breeding strategy was that traits measured later in life, are sex-limited, expensive or difficult to physically measure or have low heritability values were difficult to improve and genetic progress was slow (Khatib & Bormann, 2015; Oldenbroek & van der Waai, 2015b).

DNA markers were developed in the early 1970s and are used in modern selection of livestock and population studies to improve the rate of genetic progress (Toro, 2011; Blasco & Toro, 2014). DNA markers include Amplified Fragment Length Polymorphisms, Restriction Fragment Length Polymorphisms, Microsatellites (MSats) and Single Nucleotide Polymorphisms (SNPs) (Fan & Chu, 2007; Gurgul *et al.*, 2014). MSats and SNP have become

the markers of choice in the last decade for molecular research in livestock and will be discussed in more detail (Blasco & Toro, 2014; Gurgul *et al.*, 2014).

Microsatellites are specific DNA sequences that contain mono, di, tri or tetra tandem repeats that are polymorphic and spread throughout the genome (Toro, 2011; Fernández *et al.*, 2013). They are commonly found in non-coding regions of the genome and has complex mutational dynamics which are still poorly understood (Bhargava & Fuentes, 2010; Li *et al.*, 2015). Even though SNPs have largely replaced microsatellites in most molecular applications, microsatellites are still readily available and used in population genetics (Fan & Chu, 2007; Keats & Sherman, 2013; Li *et al.*, 2015). There are multiple studies done on sheep that used MStats, i.e. population diversity studies (Soma *et al.*, 2012; Al-Atiyat *et al.*, 2016; Sheriff & Alemayehu, 2018), identification of Quantitative trait loci (QTL) (Dashab *et al.*, 2012; Marshall *et al.*, 2013; Gutiérrez-Gil *et al.*, 2014; Zeng *et al.*, 2018), association studies for specific traits (Al-Atiyat *et al.*, 2016; Peng *et al.*, 2017; Sheriff & Alemayehu, 2018), research on disease resistance (Marshall *et al.*, 2013; Plastow, 2016), admixture (Peters *et al.*, 2010; Soma *et al.*, 2012; Visser *et al.*, 2016) and conservation studies of endangered indigenous sheep breeds (Kunene *et al.*, 2009; Qwabe *et al.*, 2012; Molotsi *et al.*, 2017c).

The breakthrough of Single Nucleotide Polymorphisms (SNPs) in the 1960s was one of the key discoveries in human biomedical and genetic research (Kassam *et al.*, 2005). SNPs occur when a single nucleotide is altered on the genome. SNPs are bi-allelic and are more abundant in genomes compared to MSats (Beuzen *et al.*, 2000; Fernández *et al.*, 2013). This is one of the reasons why SNPs are the more readily used marker of choice as they can explain more genetic variation than MSats (Toro, 2011; Molotsi *et al.*, 2017c). SNPs are more likely to be located in coding regions or regulation sites (Fan & Chu, 2007; Brenda, 2011; Fernández *et al.*, 2013). SNPs also have simpler mutation mechanisms resulting in lower error rates that gives more reliable results, which is easily reproducible and compatible between labs (Bhargava & Fuentes, 2010; Keats & Sherman, 2013; Al-Atiyat *et al.*, 2016).

The first use of SNPs in livestock genetic studies and genome sequence projects was reported in the 2000s (Buduram, 2004; Goddard & Hayes, 2009; Fernández *et al.*, 2013).The first sheep reference genome sequencing project was launched in 2009 in collaboration with the International Sheep Genomics Consortium (Archibald *et al.*, 2010). The project commenced at two sequence facilities namely the Kunming Institute of Zoology and the BGI Shenzhen (Archibald *et al.*, 2010; Jiang *et al.*, 2014). Two Texel sheep, a ram and ewe, were used to construct the reference genome with a ~150-fold sequence coverage. The virtual sheep genome was released in 2006, while the updated fully annotated genome sequence

was published in June 2014 (Jiang *et al.*, 2014; Consortium International Sheep Genomics, 2018).

After the development of the reference genome, SNP arrays (SNP chips) were developed to enable researchers to screen genetic variability in livestock species (Fan *et al.*, 2010; Tosser-Klopp *et al.*, 2014). SNP chips have revolutionised genomic association studies and marker assisted selection in breeding programs (Tosser-Klopp *et al.*, 2014; Dalrymple *et al.*, 2015). It also contributed significantly to greater genetic progress (Dalrymple *et al.*, 2015). There are currently three commercial ovine SNP chips available: the Illumina® OvineSNP50K BeadChip (released in 2007-2008), the high-density (606K) SNP chip which was developed between 2010-2013 (released in 2013) and the low-density (15K) SNP chip which was released in 2015 (Clarke *et al.*, 2014; Consortium International Sheep Genomics, 2018).

SNP arrays have been used widely in small stock genetic studies i.e. for identification of selection pressures that changed genetic frequencies by identifying selection signals and selection signatures (Kijas *et al.*, 2012; Muchadeyi *et al.*, 2015; Kim *et al.*, 2016; Brito *et al.*, 2017). SNP panels were also used to identify associated SNP and putative QTLs for specific traits (Jiang *et al.*, 2014; Dalrymple *et al.*, 2015; Sandenbergh, 2015). SNP arrays are an effective tool to investigate genetic diversity in populations and study population structure to understand breed differences, adaption and ancestry (Lashmar *et al.*, 2016; Mdladla *et al.*, 2016; Visser *et al.*, 2016).

2.7 Genomic measures of population diversity

The theory of population genetics is to study the change in the genetic make-up of a population over generations which resulted from selection, mutation and other genetic factors (Ewens, 2006). Population genetics explain the genetic diversity with-in and between populations and define the complex changes over time in allelic and genotypic frequencies (Keats & Sherman, 2013). A few population genetic parameters will be discussed, namely: Linkage Disequilibrium, Inbreeding, Runs of Homozygosity, Effective population size, Principal Component Analysis and Admixture.

Linkage disequilibrium (LD) is the non-random association of alleles at two or more loci, therefore the alleles do not undergo independent assortment and are inherited as a unit (Keats & Sherman, 2013; Gurgul *et al.*, 2014). LD gives insight into the history of natural selection, gene conversion, mutations and other factors that evolved or changed gene-frequencies in populations (Gurgul *et al.*, 2014; Al-Mamun *et al.*, 2015a). Linkage Disequilibrium is important

in population studies to identify regions or genes affecting quantitative traits and to establish the number of markers needed for genomic selection (Toro, 2011; Gurgul *et al.*, 2014).

Inbreeding is defined as the mating of genetically related individuals, i.e. animals that share a common ancestor (Falconer, 1960). This leads to a change in the genotypic frequencies of a population and results in increased homozygosity and decreased heterozygosity (Falconer, 1960; Keats & Sherman, 2013). High levels of inbreeding reduces the genetic diversity in a population and may result in inbreeding depression (Santana *et al.*, 2010; Leroy, 2014), which is the gradual decrease in phenotypic performance of fertility, productivity and survivability traits (Falconer, 1960; Santana *et al.*, 2010; Leroy, 2014). Inbreeding is measured on a genomic level using either the inbreeding coefficient (F_{IS}) or runs of homozygosity (ROH). The inbreeding coefficient (F_{IS}) measures the probability that both copies of a gene are inherited from a common ancestor (Falconer, 1960; Leroy, 2014). It describes the proportion of the variance in the sub-population that is contained/inherited by one individual in the population. The formula for inbreeding coefficient is based on Wright's definition:

$$F_{IS} = \frac{(H_{exp} - H_{obe})}{H_{exp}}$$

Where: H_{exp} is expected heterozygosity, H_{obe} is observed heterozygosity.

F_{IS} measures the proportion of heterozygosity in the population and is used as a measure to indicate inbreeding (Nielsen & Slatkin, 2013). The Inbreeding coefficient is expressed as an fraction, a positive F_{IS} indicates high levels of inbreeding (Zhivotovsky, 2015).

ROH is also a measure of inbreeding and is a more effective measure as it can distinguish between identical by descent (IBD) and identical by state (IBS) (Leroy, 2014). ROH are autozygous regions on the genome that are inherited as a unit together by the offspring (Santana *et al.*, 2010; Metzger *et al.*, 2015). The lengths of ROH can also be used to indicate if inbreeding occurred recently (long segments, >5 Mb) or long-ago (short segments <5 Mb) (Weigel, 2010; Metzger *et al.*, 2015).

Principal component analysis (PCA) is a mathematical procedure which is a framework of multivariate analysis (Savegnago *et al.*, 2011; Dadousis, 2012). This procedure uses an orthogonal transformation that reduces the number of originally-correlated variables into

smaller non-correlated variables, while maintaining original variability (Savegnago *et al.*, 2011; Agudelo-Gómez *et al.*, 2015). These smaller non-correlated variables is called principal components which has minimal loss of information (Nascimento *et al.*, 2014; Agudelo-Gómez *et al.*, 2015). The mathematical orthogonal technique was first introduced by Pearson in 1901 and developed further by Hotelling in 1933 (Dadousis, 2012).

PCA reduces dimensionality of data by removing repetitive information and produces patterns of genetic (co)variances that is easily interpreted (Buzanskas *et al.*, 2013; Nascimento *et al.*, 2014; Boligon *et al.*, 2016). With advanced technology PCA has become a popular method in genetic and population studies (Dadousis, 2012; Agudelo-Gómez *et al.*, 2015; Boligon *et al.*, 2016). PCA is used in quantitative genetic studies to visually illustrate patterns of genetic variation (Agudelo-Gómez *et al.*, 2015).

Admixture is observed when isolated populations interbreed and the product of the next generation is a combination of alleles from the different ancestral populations (Skotte *et al.*, 2013). Admixture analysis is the investigation of population structure or substructure based on the proportion of shared ancestral alleles (Alexander *et al.*, 2009, 2015). This analysis allows genetic and association studies to illustrate and describe genetically variable populations and group populations of unknown ancestry into discrete populations (Edea *et al.*, 2015; Makina *et al.*, 2016; Mdladla *et al.*, 2016). Admixture also gives insight into populations' genetic structure and diversification and the origin of genes and alleles (Peters *et al.*, 2010; Soma *et al.*, 2012; Mdladla *et al.*, 2016).

2.8 Genomic regions of significance

Animals have been subjected to natural and artificial selection over centuries (Duguma *et al.*, 2002; Oldenbroek & van der Waai, 2015b). As selection of animals was performed based on variable phenotypes, indirect selection took place for certain favourable alleles and mutations in the animals' genome (Gurgul *et al.*, 2014; Gutiérrez-Gil *et al.*, 2014; Makina *et al.*, 2015).

This resulted in variable regions on the genome that differ between and within animal breeds and populations (Riggio *et al.*, 2013; Gutiérrez-Gil *et al.*, 2014). These regions can be associated with certain genes and traits of importance (Randhawa *et al.*, 2014; López *et al.*, 2015; Gutiérrez-Gil *et al.*, 2017). Molecular signatures of selection are regions in the genome that contain functionally important traits and when these regions are under directional selection

it results in changes in the frequency of variants (Gurgul *et al.*, 2014; Qanbari & Simianer, 2014; Makina *et al.*, 2015).

There are two methodologies or approaches to search for regions on the genome or loci that are associated with genes that control the expression of phenotypic variation of traits of interest (López *et al.*, 2015). One approach is the candidate gene approach, this involves studies on candidate genes, association mapping and identification of QTLs (Gu *et al.*, 2009; Van Marle-Köster *et al.*, 2013; López *et al.*, 2015). This approach has some challenges; prior knowledge and putative identification of genes that influence the trait is needed, as well as family relationship information between individuals and phonotypic records of relatives (Van Marle-Köster *et al.*, 2013; Gutiérrez-Gil *et al.*, 2014; López *et al.*, 2015). This approach requires that the phenotype of interest is well understood and uses genomic tools and genetic analysis to identify genes or causal regions associated with the phenotype (Gu *et al.*, 2009; López *et al.*, 2015).

The second approach is the genomic approach, this involves statistical evaluation of molecular information to identify regions and genomic scans / association studies (GWAS) (Al-Mamun *et al.*, 2015b; López *et al.*, 2015; Molotsi *et al.*, 2017a). This approach relies highly on linkage disequilibrium as it searches for patterns on the genome to identify differences in loci / marker frequencies and genetic differentiation of the genome (Qanbari & Simianer, 2014; López *et al.*, 2015; Sharmaa *et al.*, 2015). GWAS is a powerful genetic tool to detect genetic variants affecting economically important traits and identify candidate genes or quantitative trait loci associated with various phenotypes (Sharmaa *et al.*, 2015; Hay & Roberts, 2018).

GWAS performs association analysis to identify candidate genes or quantitative trait loci that is associated with the traits of interest by using SNPs, pedigree and phenotypic data (Zhang *et al.*, 2012; Ali *et al.*, 2015). In comparison to traditional QTL-mapping, GWAS is a more effective method to detect candidate genes (Hayes & Goddard, 2010; Sharmaa *et al.*, 2015). It is more effective as it can not only detect causal variants with moderate effects but also define narrower genomic regions that harbour causal variants (Zhang *et al.*, 2012; Sharmaa *et al.*, 2015). A main advantage of GWAS is that it considers the additive effect of genes, that multiple genes influences the expression of one or more trait and that each locus contribute a small proportion to the expression of the trait (Van Marle-Köster *et al.*, 2013; Molotsi *et al.*, 2017a). This allows GWAS to explain a larger portion of variation compared to marker assisted selection (MAS) which considers the non-additive effect of genes (Hayes & Goddard, 2010; Van Marle-Köster *et al.*, 2013). The GWAS approach has been followed for this study.

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

The data that was used in this study was obtained from the Biobank at Grootfontein Agricultural Development Institute (GADI). Blood samples from animals of two sheep breeds (Afrino and Merino) have been collected since 2006 and are stored in the GADI-Biobank. Animals were selected for inclusion in this study and subsequent genotyping based on their respective estimated breeding values for reproduction and body weight traits. Genotypes from 411 animals obtained with the Illumina® Ovine SNP50 BeadChip were used for further analyses. SNPs that met the quality control criteria were used to calculate basic population statistics and to illustrate the populations' genetic structures through PCA and Admixture. The data was further investigated to identify genomic regions of significance that are associated with reproduction and body weight traits, using a GWAS approach.

3.2. Resources

Resources from three different flocks were used in the study, namely one Afrino flock and two Merino flocks. The Afrino flock is kept at the Carnarvon Experimental Station, which is located in the Northern Cape Province. The Cradock fine-wool Merino stud is kept at the Cradock Experimental Station (Eastern Cape Province), while the Grootfontein Merino stud is kept at GADI near Middelburg in the Eastern Cape Province.

Blood samples for genotyping were collected under approval numbers AP10/3/3 and AP10/3/4 of the Animal Research Ethics Committee of the Grootfontein Agricultural Development Institute. Approval for the use of external data was granted by the ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria (NAS125/2019).

3.2.1 Locations of experimental stations

The Carnarvon Experimental Station is located in the upper Western Karoo close to Carnarvon in the Northern Cape Province of South Africa. It is situated at a latitude of 31° 00' 96" S and at a longitude of 21° 53' 38.04" E (Du Toit *et al.*, 2014; http://www.earth.google.com, 2019). The Carnarvon Experimental Station is at an altitude of 1314 m above sea level and has an average rainfall of 211 mm and an average temperature of 15.2 ºC (https://deims.org/f980f657-9ebd-4ec7-beca-d930154bb090; http://www.earth.google.com, 2019).

Both Cradock Experimental Station and Grootfontein Agricultural Development Institute are located in the Eastern Cape Province of South Africa. The Cradock Experimental Station is located near Cradock, at a latitude of 32° 13' 10" S and a longitude of 25° 41' 14" E (http://www.earth.google.com, 2019; Olivier, 2014). It is situated at an altitude of 847 m above sea level and has an average rainfall of 366 mm (Olivier, 2014). Maximum temperatures at the Cradock Experimental Station range from 8.1 ºC during winter to 23.5 ºC during summer (Olivier, 2014). GADI is located in the North Eastern Karoo near Middelburg, at a latitude of 31° 28' 16.82" S and at a longitude of 25° 01' 30.72" E (Olivier, 2014; http://www.earth.google.com, 2019). It is located at an altitude of 1318 m above sea level and has an average rainfall of 360 mm (Olivier. 2014). Figure 3.1 illustrates the locations of the experimental stations in South Africa.

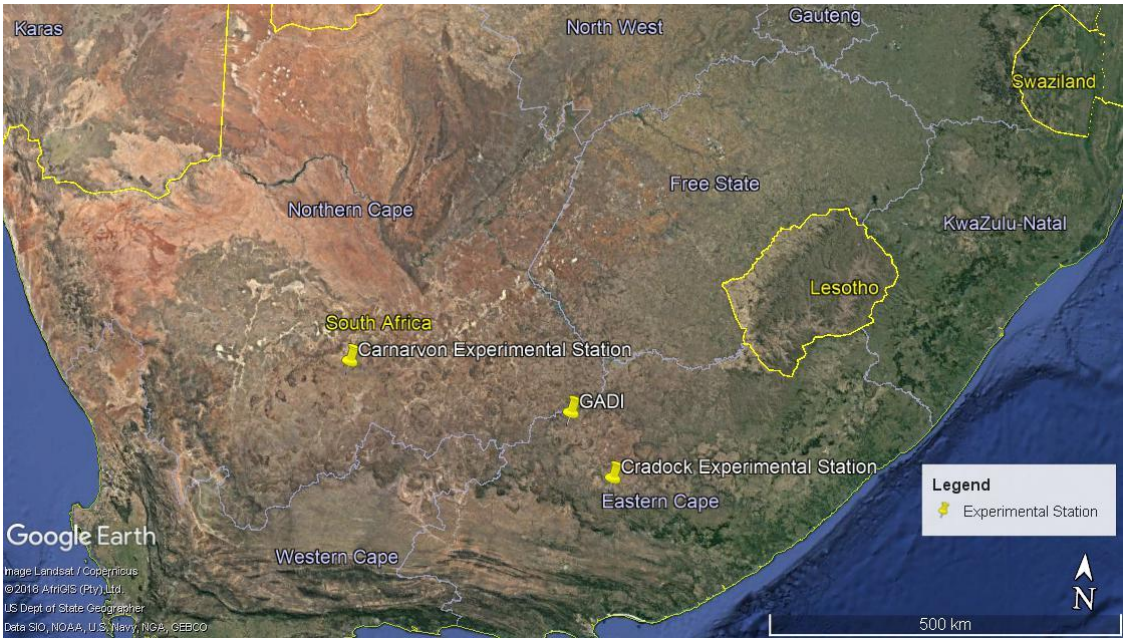

Figure 3.1 Map of South Africa illustrating the location of the three experimental research stations (http://www.earth.google.com, 2019)

3.2.2 Animals selected for genotyping

Estimated breeding values (EBV) were obtained from GADI for all three flocks for the following traits: body weight recorded at selection age of 14 months (BW), number of lambs born (NLB), number of lambs weaned (NLW) and total weight of lamb weaned (TWW) over the ewe's lifetime in the flock. Amongst the animals with available blood samples in the GADI-Biobank, ewes with varying combinations of high and low breeding values for body weight and reproduction were identified for genotyping as summarised in Table 3.1. Animals with the lowest inbreeding coefficients were included for genotyping. In the Afrino flock, animals were

categorised as “High” if their EBV values were in the top 25% for the specific trait, and as “Low” if their EBV values were in the bottom 25% for the specific trait. The corresponding percentage for the Cradock and Grootfontein Merino animals was 20%. This difference was due to the different number of animals available and required for genotyping.

Table 3.1 Number of ewes selected for genotyping in the different estimated breeding value (EBV) combination categories [a]

Combination of EBVs	Number of ewes		
	Afrino	Cradock Merino	Grootfontein Merino
High EBV Body weight + High EBV Reproduction	32	20	20
High EBV Body weight + Low EBV Reproduction	32	20	20
Low EBV Body weight + High EBV Reproduction	32	20	20
Low EBV Body weight + Low EBV Reproduction	32	20	20
Total	**128**	**80**	**80**

[a] These include only the 288 ewes genotyped specifically for this study

Additional genotypic data obtained with the Illumina® Ovine SNP50 BeadChip (Illumina Inc., 2015) from 50 Grootfontein Merino ewes, 49 Cradock fine wool Merino ewes and 24 Carnarvon Afrino ewes were available from previous studies. The genotypes of these animals were also included in the study. These animals were allocated to the high or low reproduction and body weight categories on the basis of their EBVs. Where these animals did not conform to the High or Low category as stipulated for the newly genotyped animals, a Medium category was allocated. A complete summary of all animals included in the project (including those shown in Table 3.1) is given in Table 3.2.

Table 3.2 Number of all available genotyped animals in the High, Medium and Low estimated breeding value categories for body weight and the reproduction traits [a]

EBV category	Number of ewes		
	Afrino	Cradock Merino	Grootfontein Merino
High EBV BW [b]	66	57	53
Medium EBV BW	12	19	27
Low EBV BW	74	53	52
High EBV NLB [c]	72	58	56
Medium EBV NLB	9	16	13
Low EBV NLB	71	55	63
High EBV NLW [d]	72	58	56
Medium EBV NLW	12	14	16
Low EBV NLW	68	57	60

High EBV TWW [e]	72	61	57
Medium EBV TWW	15	11	15
Low EBV TWW	65	57	60
Number of genotypes	**152**	**129**	**130**

[a] These include all 411 genotyped ewes available; [b] BW: Body weight at 14 months;
[c] NLB: Number of lambs born; [d] NLW: Number of lambs weaned; [e] TWW: Total weight of lambs weaned

3.3 Statistical analyses

3.3.1 Phenotypic data

Phenotypic data recorded on these traits since 1982 in the Afrino and Grootfontein Merino flock, and since 1990 in the Cradock Merino flock, were included. EBVs estimated by GADI were made available for inclusion in this study. Estimated breeding values for all traits for the individual animals were obtained with the ASReml program (Gilmour *et al*., 2014). Animal models including direct and maternal additive genetic random effects were fitted for body weight, while only a random direct genetic effect was fitted for the reproductive traits. The minimum, average and maximum values of the EBVs for the High, Medium and Low groups for the different traits for the three flocks were obtained with the PROC MEANS procedure of the SAS statistical package (SAS Institute Inc., 2016).

3.4 Genomic analyses

3.4.1 Quality control (QC)

The individual genomic datasets were updated with Oar v4.0 SNP Chimp that was downloaded from SNPchiMp v.3 database (Nicolazzi *et al.*, 2015). Each dataset's information was updated for individual identification number, breed and sex in PLINK v1.07 software (Purcell, 2017). Sex chromosomes were removed and only the 26 autosomal chromosome pairs were used for analysis.

Quality control was performed on each individual flock's dataset first and thereafter the datasets were merged and analyses performed on the merged dataset. Individual and marker-based quality control (QC) measures were performed using PLINK v1.07 software (Purcell, 2017). All non-informative SNPs and individuals with missing genotypes were removed at the following parameters: individual call rate of below 90%, a SNP call rate lower than 95%, minor allele frequency of less than 0.02 (MAF <2%) and violation of Hardy-Weinberg equilibrium (P <0.001).

3.4.2 Summary statistics reports

The SNP heterozygosity report was generated using PLINK v1.07 software (Purcell, 2017). The heterozygosity report was used to study the genetic variation within each of the flocks separately and also in the merged dataset.

The command *--het* generated the individual heterozygosity report which lists each individual's number of non-missing genotypes and inbreeding coefficient estimates which was used to estimate the populations' inbreeding coefficient (F_{IS}-statistic). The individual inbreeding coefficient was calculated across all polymorphic loci (MAF >2%).

The report also listed each individual's number of observed and expected homozygotic counts, therefore the heterozygosity needed to be calculated. The report was used to calculate observed (H_O) and expected (H_E) heterozygosity for each individual across all populations. The individual heterozygosity report was calculated and plotted in MS Excel with the use of the following formulas:

$$H_0 = \frac{(N(NM) - O(Hom))}{N(NM)}$$

$$H_E = \frac{(N(NM) - E(Hom))}{N(NM)}$$

Where,

H_O: Observed Heterozygosity counts

H_E: Expected Heterozygosity counts

N (*NM*): Number of non-missing genotypes

O (H_{om}): Number of observed Homozygotes

E (H_{om}): Number of expected Homozygotes

The *--hardy* command was used to generate the SNP heterozygosity report. This report lists the genotypic counts (H-values) for each SNP and Hardy-Weinberg test statistics for each SNP. The report was further used to calculate the average H_O and H_E across chromosomes per population.

3.4.3 Flock structure

3.4.3.1 Principal component analysis (PCA)

Principal Component Analysis was performed to investigate the genetic relatedness of individuals in the populations. The genomic relationship matrix and estimated principal components were generated with the use of the Genome-wide complex trait analysis v 1.24 (GCTA) software (Yang *et al.*, 2011). This was done using the following commands:

gcta64 --bfile [file name] --autosome --autosome-num [insert number of autosomes] --make-grm --out [output file name]

and

gcta64 --grm [file name: same as output file name in previous step] --pca [specify number of principal components] –out [output file name].

This produced *.eigenval* and *.eigenvec* files. The *.eigenvec* files were used in MS Excel to generate the scatter plots.

3.4.3.2 Admixture

Admixture plots were used to indicate the population structures that were based on the proportion of shared ancestral SNP genotypes. The model-based clustering ADMIXTURE 1.23 software (Alexander *et al.*, 2009) was used to generate the admixture plots for each population separately and the merged dataset with all three populations together at a K-value of two to five. The cross-validation (CV) procedure was used to identify the optimal number of inferred clusters to enable the identification of a K-value with the lowest cross-validation error estimate (Buchmann & Hazelhurst, 2014). The admixture plots were illustrated and plotted with the use of the Genesis.jar software program for the appropriate K-value (Buchmann & Hazelhurst, 2014).

3.5. Genome-wide association study (GWAS)

For the GWAS the Afrino, Cradock Merino and Grootfontein Merino datasets were analysed separately for each flock and each trait, using EMMAX (Kang *et al*., 2010). The results were illustrated in Manhattan plots according to the trait under investigation for each dataset.

3.5.1. GWAS analysis

The software, efficient mixed model association eXpedited (EMMAX) was used for GWAS (Kang *et al*., 2010). EMMAX software was favoured for the analysis as it controls

genome wide error rate successfully compared to other genomic software (Eu-ahsunthornwattana *et al.*, 2014). PLINK (Purcell, 2017) performs less satisfactory for relationship estimations when estimating kinships (Eu-ahsunthornwattana *et al.*, 2014). PLINK may result in inflated control factors, therefore EMMAX software was favoured to calculate the kinship matrix as it is more suited for smaller populations (Manichaikul *et al.*, 2010; Eu-ahsunthornwattana *et al.*, 2014).

The kinship matrix generated by EMMAX produced transposed Ped and Fam files. Output files from the kinship matrix were **.mibs* and the transposed files were in the **.tfam* and **.tped* format. These files together with the phenotype file gave the family ID, individual ID and respective phenotypes for the different traits. In the phenotype files, the EBVs for each trait were used to draw an association analysis between EBVs for the respective traits and the genotypes of the animals. Significance testing was based on Bonferroni corrected significance thresholds in order to correct for the number of SNP loci tested. To perform the GWAS using EMMAX the following command was used:

emmax -v -d 10 -t [tped filename] -p [phenotype file.txt] -k [kinship file] -o [output file name].

Results from the association analysis was visualised by creating Manhattan plots in R-studio (R Core Team, 2017). A separate GWAS was performed for all SNPs with MAF <0.02 (SNPs that were excluded during routine QC) to ensure that rare, fixated SNPs associated with the traits of interest have not been discounted. None of these SNPs showed association with the traits and therefore, for simplification purposes only the Manhattan plots for SNPs with MAF >0.02 was considered.

From the Manhattan plots the significant ($P < 10^{-7}$) and suggestive ($P < 10^{-5}$) SNP markers were identified and investigated further. Each SNP marker's published name and position was identified via the database SNPchiMp v.3 (Nicolazzi *et al.*, 2015). The databases Ensembl (Zerbino *et al.*, 2018; http://www.ensembl.org), UniProt (The UniProt Consortium, 2019; http://www.uniprot.org), NCBI (https://www.ncbi.nlm.nih.gov) and Panther (Mi et al., 2019; http://www.pantherdb.org) was used to identify putative genes that are linked or closely situated to the SNP marker under investigation. The allele frequencies were calculated for each suggestive SNP identified in the populations. These allele frequencies were calculated for the three EBV groups (High, Medium and Low) to compare the frequencies of the suggestive SNP in the EBV groups.

CHAPTER 4: RESULTS

4.1 Introduction

The genotypic data of two South African sheep breeds were used in combination with their EBVs for four traits to identify possible genomic regions/areas associated with reproduction and body weight. The results of the genomic analyses are reported in this chapter and include quality control, population parameters, within and between population differentiation and suggestive SNPs identified in the genome wide association analyses.

4.2 Descriptive statistics

For the study a total of 411 animals were used from three different flocks. In Table 4.1 the minimum, average and maximum EBV values for each trait according to the High, Medium and Low categories are summarised within population.

Table 4.1 The minimum (Min), average (Ave) and maximum (Max) estimated breeding values (EBV) for each trait per population

EBV category	Afrino			Cradock Merino			Grootfontein Merino		
	Min	Ave	Max	Min	Ave	Max	Min	Ave	Max
High EBV BW (kg)	6.78	10.61	14.4	5.53	7.94	12.51	5.70	8.48	12.87
Medium EBV BW (kg)	5.55	7.26	9.92	3.50	4.40	5.51	2.77	4.21	5.69
Low EBV BW (kg)	0.78	4.80	9.26	-4.03	0.39	3.44	-6.58	1.94	2.75
High EBV NLB	0.63	0.99	1.43	0.33	0.48	0.82	0.27	0.40	0.59
Medium EBV NLB	0.19	0.53	0.71	0.18	0.25	0.33	0.04	0.16	0.23
Low EBV NLB	-0.55	0.19	0.44	-0.38	-0.05	0.16	-0.79	-0.21	0.03
High EBV NLW	0.51	0.82	1.21	0.32	0.46	0.89	0.20	0.30	0.58
Medium EBV NLW	0.21	0.45	0.60	0.18	0.24	0.29	0.02	0.10	0.18
Low EBV NLW	-0.36	0.16	0.37	-0.35	-0.01	0.18	-0.40	-0.14	0.01
High EBV TWW (kg)	13.12	18.59	25.50	8.19	11.96	20.98	8.19	9.05	15.51
Medium EBV TWW (kg)	8.62	10.50	12.32	4.85	5.84	7.52	1.25	4.08	5.79
Low EBV TWW (kg)	-7.46	4.73	9.84	-9.92	-0.32	4.3	-9.01	-2.56	1.07

Animals were allocated to the different categories according to their EBVs. Animals with either high or low EBVs for the respective traits were selected specifically for this study. Additional genotypic data available from previous studies were also included in the study. Some of these animals had to be allocated to a Medium category. From Table 4.1 it is evident that the Medium category EBV values sometimes overlap with the High and Low category values, in contrast with the High and Low categories where there were distinct differences in EBVs between the categories.

4.3 Quality control

The genotypes of all 411 animals were subjected to quality control (QC) analysis. There were no duplicate animals in the data set. A total of five individuals did not conform to the quality control analysis and were excluded from downstream analyses. Table 4.2 contains the number of SNPs removed during marker-based quality control in the three populations.

Table 4.2 Number of SNPs removed during marker-based quality control

Population	SNP call rate (<95%)	SNP MAF (<2%)	HWE (P ≥0.001)	Total SNPs removed
Afrino	490	4170	50	4710
Cradock Merino	631	2634	56	3321
Grootfontein Merino	3172	2166	86	5424

A higher number of SNPs were removed from the Afrino and Grootfontein Merino datasets compared to the Cradock Merino dataset, due to more loci with MAF <0.02 in the Afrino data set and the Grootfontein Merino data set having more SNPs with a call rate below 95%. After basic QC and marker-based QC, the datasets were further subjected to individual call rate to exclude individuals with high proportions of missing genotypes (genotyping rate). The amount of SNP markers before and after QC as well as the number of individuals excluded due to low genotyping rate is summarized in Table 4.3.

Table 4.3 Summary of SNPs and animals remaining after quality control (QC)

AFRINO		
	Number of SNPs	**Individuals**
Total before QC	46827	152
Total after QC	42117	151
Total removed	4710	1
CRADOCK MERINO		
	Number of SNPs	**Individuals**
Total before QC	46827	129
Total after QC	43506	128
Total removed	3321	1
GROOTFONTEIN MERINO		
	Number of SNPs	**Individuals**
Total before QC	46827	130
Total after QC	41403	127
Total removed	5424	3

The Cradock Merino datasets retained more than 90% of their number of SNPs, while both the Afrino and Grootfontein Merino dataset retained just under 90% of its SNPs. The

Grootfontein Merino dataset had the highest number of individuals removed but still retained a genotyping rate above 95%.

4.4 Population parameters

After marker-based and individual QC, the genotypes were subjected to genetic diversity analyses. Figure 4.1 illustrates observed heterozygosity values per chromosome for all three sheep populations.

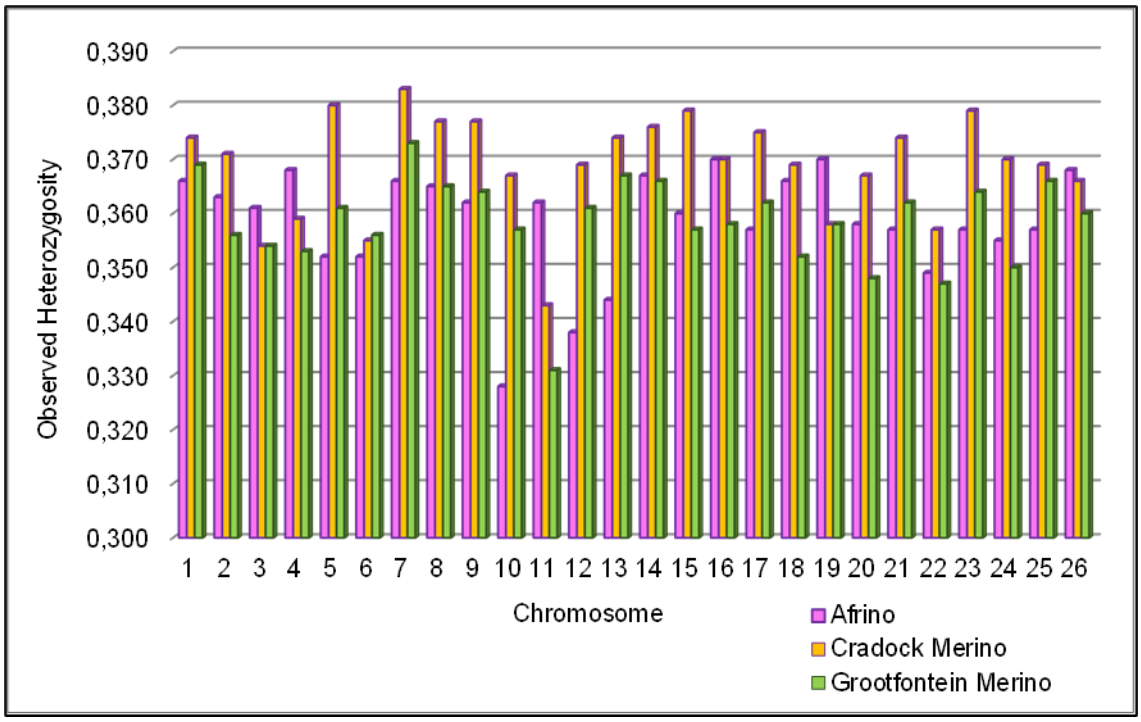

Figure 4.1 Average heterozygosity per chromosome in the Afrino, Cradock Merino and Grootfontein Merino populations

The average observed heterozygosity (H_O) for the Afrino, Cradock Merino and Grootfontein Merino populations were 0.372, 0.379 and 0.369 respectively. In the Afrino populations OAR 16 and 19 had the highest observed heterozygosity (H_O = 0.370) and the lowest was observed on OAR 10 (H_O = 0.328). OAR 7 had the highest observed heterozygosity of H_O = 0.383 and H_O = 0.373 in the Cradock and Grootfontein Merino populations respectively. The lowest observed heterozygosity was observed on OAR 11 for both Cradock (H_O = 0.343) and Grootfontein Merino (H_O = 0.331) populations. The average minor allele frequency (*MAF*), inbreeding coefficient (F_{IS}), average observed heterozygosity (H_O) and average expected heterozygosity (H_E) of the three populations are summarized in Table 4.4.

Table 4.4 Summary of the averages of MAF, F_{IS} and heterozygosity within the populations

Population	MAF	F_{IS}	H_E	H_O
Afrino	0.252	-0.025	0.363	0.372
Cradock Merino	0.267	-0.025	0.369	0.379
Grootfontein Merino	0.269	0.002	0.369	0.369

Both the Afrino and Cradock Merino datasets shows higher proportion of observed than expected heterozygosity ($H_E < H_O$), indicating genetic variability. The Grootfontein Merino data did not present any gain or loss in genetic variability ($H_E = H_O$). The F_{IS} values were generally low in all three populations and the average MAF values of the two Merino populations were identical. Population parameters indicated moderate genetic variation and negligible levels of inbreeding.

4.5 Genetic relatedness within and between populations

Various principal component analyses (PCA) were performed on the available data. PCAs for each individual population representing the High, Medium and Low category animals were done for each of the four traits. These graphs are depicted in Addendum A. However, for all three populations, the animals did not cluster according to the High, Medium and Low EBV categories, and therefore the data were pooled per population for further PCA analyses.

Consequently, three PCAs were performed, one PCA for the Afrino, one for the Merino populations and one for the merged dataset to illustrate the diversity within the populations. The relatedness between individuals within the populations are shown in Figures 4.2 and 4.3, while the between population differentiation is depicted in Figure 4.4.

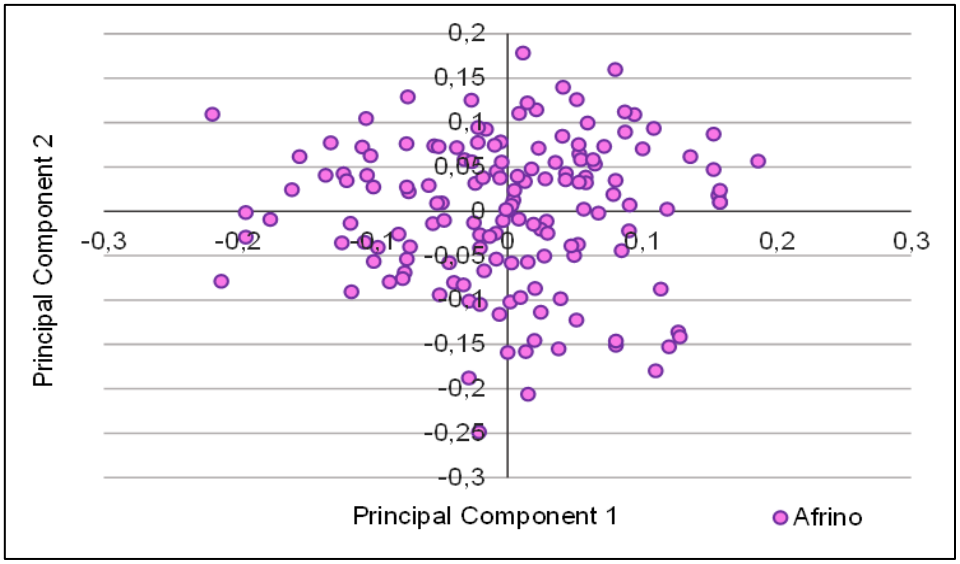

Figure 4.2 Genetic relationships among 151 Afrino sheep for the first and second principal components

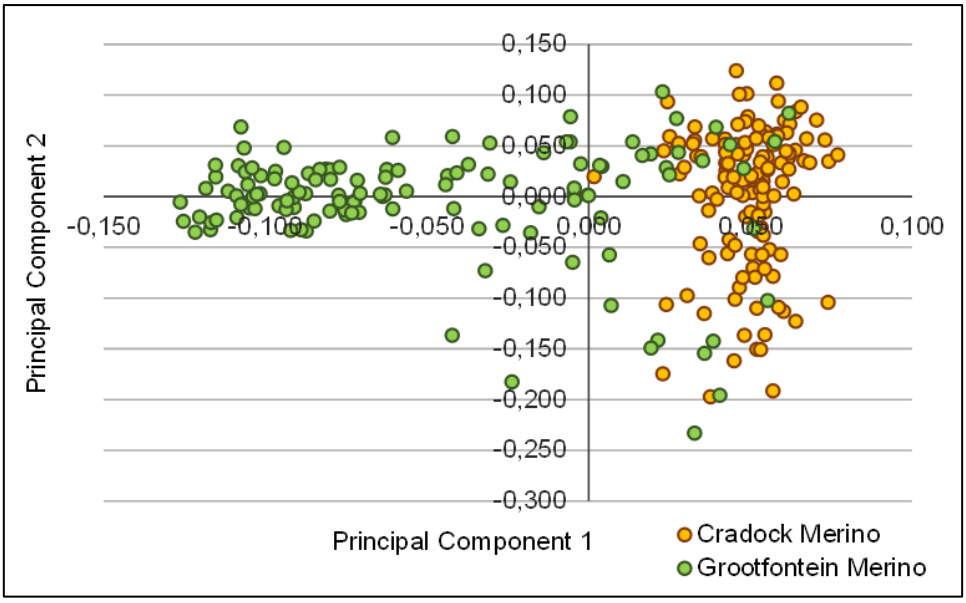

Figure 4.3 Genetic relationships among the 255 Merino sheep for the first and second principal components

The two Merino populations, Cradock and Grootfontein, showed two definite clusters based on their respective populations and geographical origin. Figures 4.3 and 4.4 illustrate some genetic relatedness between the two Merino populations that could be attributed to the use of the same rams between these two populations.

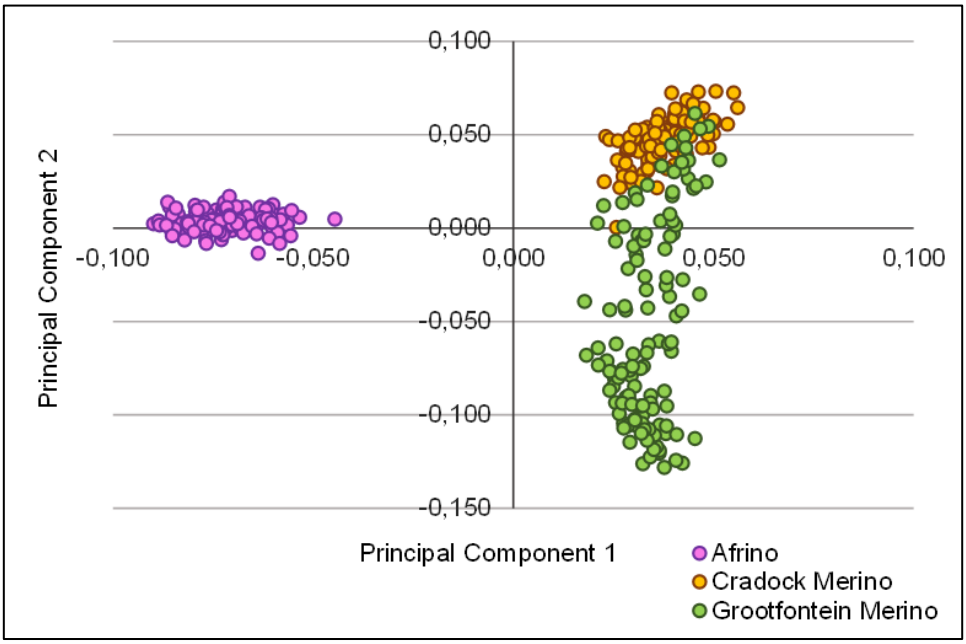

Figure 4.4 Genetic relationships among the 406 sheep for the first and second principal components

There are three definitive clusters illustrated in Figure 4.4. All three populations, namely the Afrino, Cradock Merino and Grootfontein Merino, cluster according to geographical region and show flock structure. The Afrino population forms a tight cluster separate from the two Merino populations. The Cradock Merino and Grootfontein Merino clustered separately, with some individuals that overlap between the two populations.

Admixture analysis was performed on the merged dataset to illustrate the proportion of shared ancestral SNP genotypes. The cross-validation and k-values are illustrated as a line plot in Figure 4.5. From Figure 4.5 the inflection point indicated by the lowest CV error, was at K=3. Therefore, the population substructure for the three populations for K=3 is illustrated in Figure 4.6.

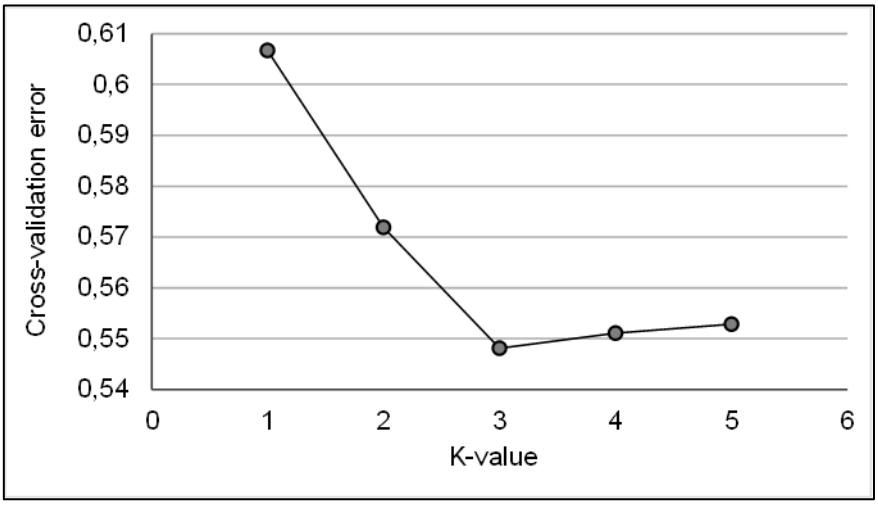

Figure 4.5 K-value plot illustrating lowest cross-validation error

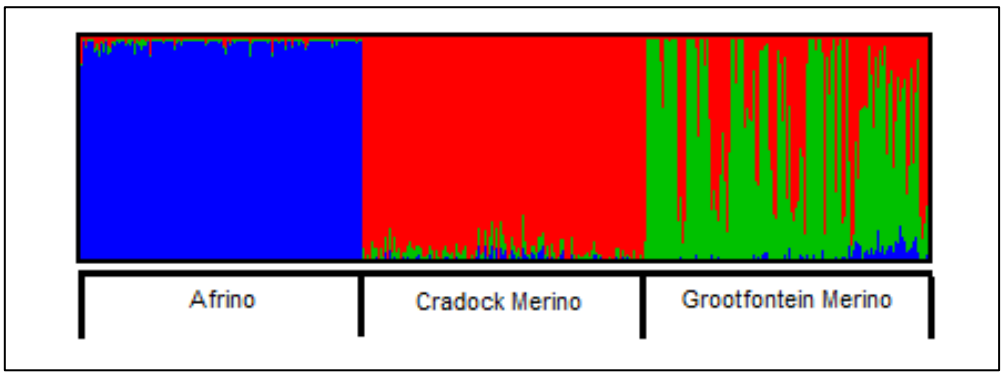

Figure 4.6 Population structure plot (K=3) of the three sheep populations

From Figure 4.6 it is clear that the two sheep breeds have their own distinct ancestral backgrounds and this confirms the results shown in PCA plot Figures 4.3 and 4.4. Although the Cradock Merino population shows some admixture with the Grootfontein Merino

populations, in agreement with the PCA, the two Merino populations largely clustered separately.

4.6 Genome wide association study

Based on the between-population differentiation observed in the previous section of the study, GWAS was performed for the three sheep populations separately. The populations were considered independently for the GWAS analysis due to the populations forming distinct clusters in PCA analyses. GWAS was performed per population on all four traits and Manhattan plots were used to illustrate suggestive SNPs that were associated with the traits under investigation. SNPs were classified as suggestive at a $P < 10^{-5}$ (circled in green in the associated Figures), and as significant at $P < 10^{-7}$. No significant SNP were identified in the analyses, but a number of suggestive SNPs ($P < 10^{-5}$) were identified in all populations. A separate GWAS was performed for all SNPs removed during quality control based on low MAF (<0.02), but no suggestive or significant SNP were identified in this step. Only the Manhattan plots for SNP with MAF >0.02 is reported in this chapter. A total of four suggestive SNPs was identified in the Afrino population, while five were identified in the Cradock Merino, and two in the Grootfontein Merino population.

4.6.1. Body Weight (BW)

For all three datasets, the suggestive SNPs with a putative association with BW are illustrated in Figure 4.7. For the Afrino population three suggestive SNPs ($P < 10^{-5}$) were identified with a putative association with BW (Figure 4.7a). Two SNPs were situated on chromosome three (s10640.1, OAR3_195631696.1) and one on chromosome 14 (OAR14_56900862.1). No suggestive SNPs for this trait were identified in the Cradock Merino population (Figure 4.7b). One suggestive SNP (OAR9_64654880.1) in association with BW was identified for the Grootfontein Merino population on chromosome nine (Figure 4.7c).

4.6.2 Number of Lambs Born (NLB)

The suggestive SNPs with a putative association with NLB are illustrated in Figure 4.8 for all three datasets. No suggestive SNPs were identified for either the Afrino population (Figure 4.8a) or the Grootfontein Merino population (Figure 4.8c). In Figure 4.8b two suggestive SNPs ($P < 10^{-5}$) located on chromosome one (s27280.1; OAR1_10554666.1) were identified for the Cradock Merino population.

4.6.3 Number of Lambs Weaned (NLW)

For all three datasets, the suggestive SNPs with a putative association with NLW are illustrated in Figure 4.9. As illustrated in Figure 4.9a, no suggestive SNPs were identified in the Afrino population that had an association with the NLW. Two suggestive SNPs (P <10^{-5}) were identified in the Cradock Merino population. These two SNPs were SNP number 1 (s27280.1) and number 2 (OAR4_28838482_X.1) which is situated on chromosome one and four respectively (Figure 4.9b). For the Grootfontein Merino population there was also only one suggestive SNP that was identified in putative association with NLW (Figure 4.9c). The SNP (OAR2_150119548.1) was found on chromosome two.

4.6.4 Total Weight Weaned (TWW)

Figure 4.10 illustrates the identified SNPs (P <10^{-5}) that are suggestive with a putative association with TWW. From Figure 4.10a only one suggestive SNP (OAR7_76295917.1) on chromosome seven was identified in the Afrino flock. For the Cradock Merino flock one suggestive SNP located on chromosome one (s27280.1) was identified that had a putative association with TWW (Figure 4.10b). No suggestive SNPs were identified in the Grootfontein Merino population for TWW.

a.

b.

c.

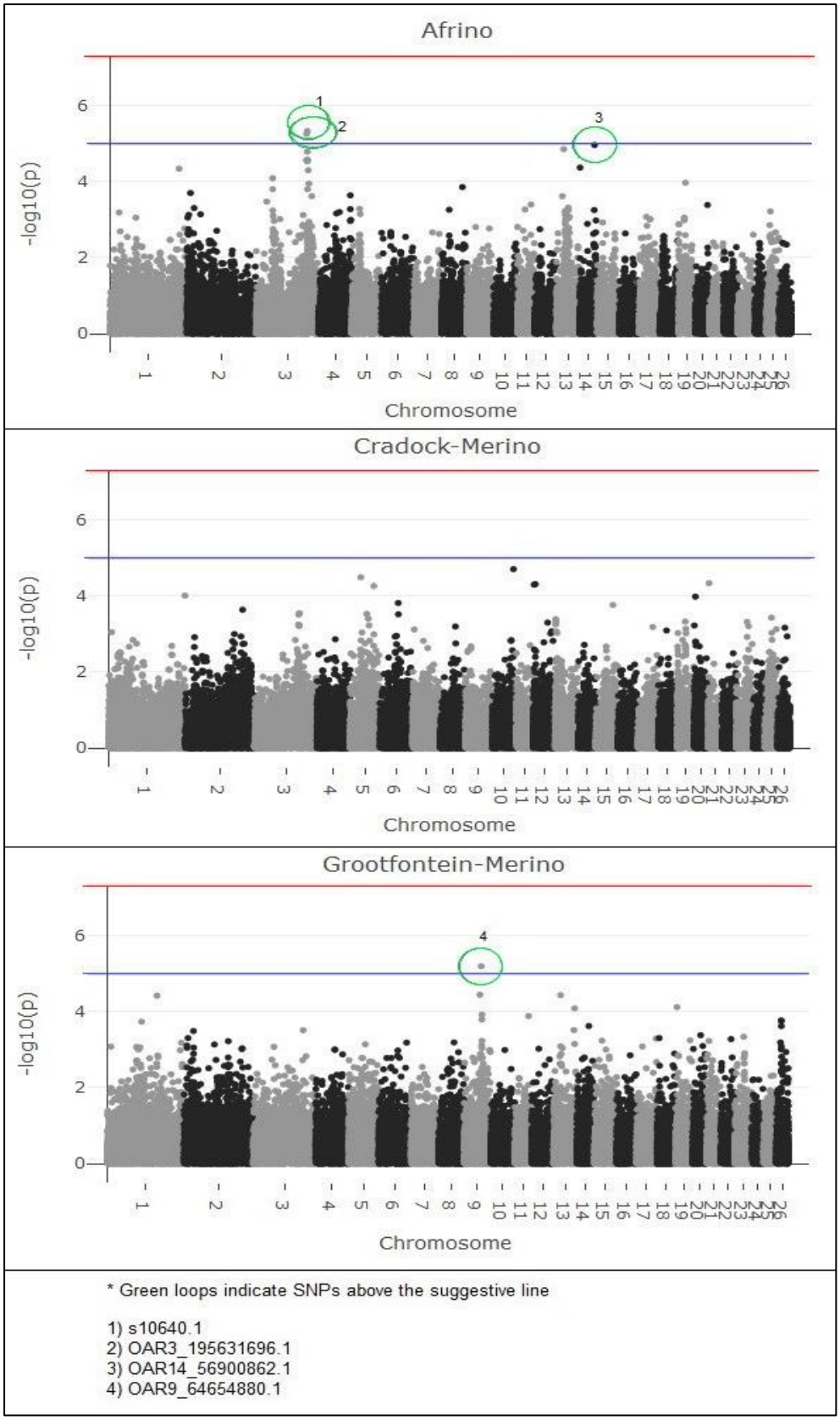

Figure 4.7 Manhattan plot illustrating the results of SNP associated with body weight at 14 months age in a: Afrino, b: Cradock Merino, c: Grootfontein Merino

a.

b.

c.

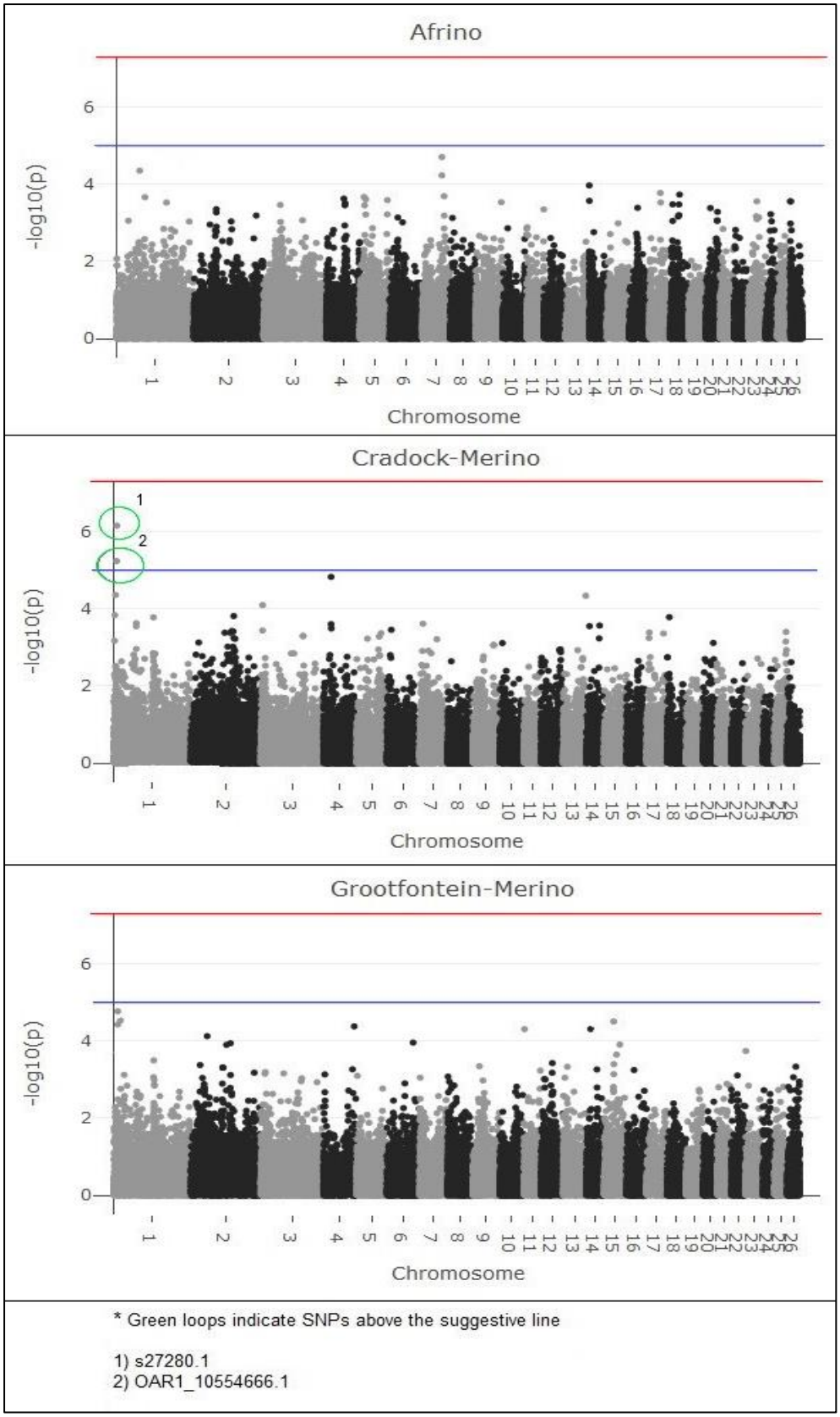

Figure 4.8 Manhattan plot illustrating the results of SNP associated with number of lambs born in a: Afrino, b: Cradock Merino, c: Grootfontein Merino

a.

b.

c.

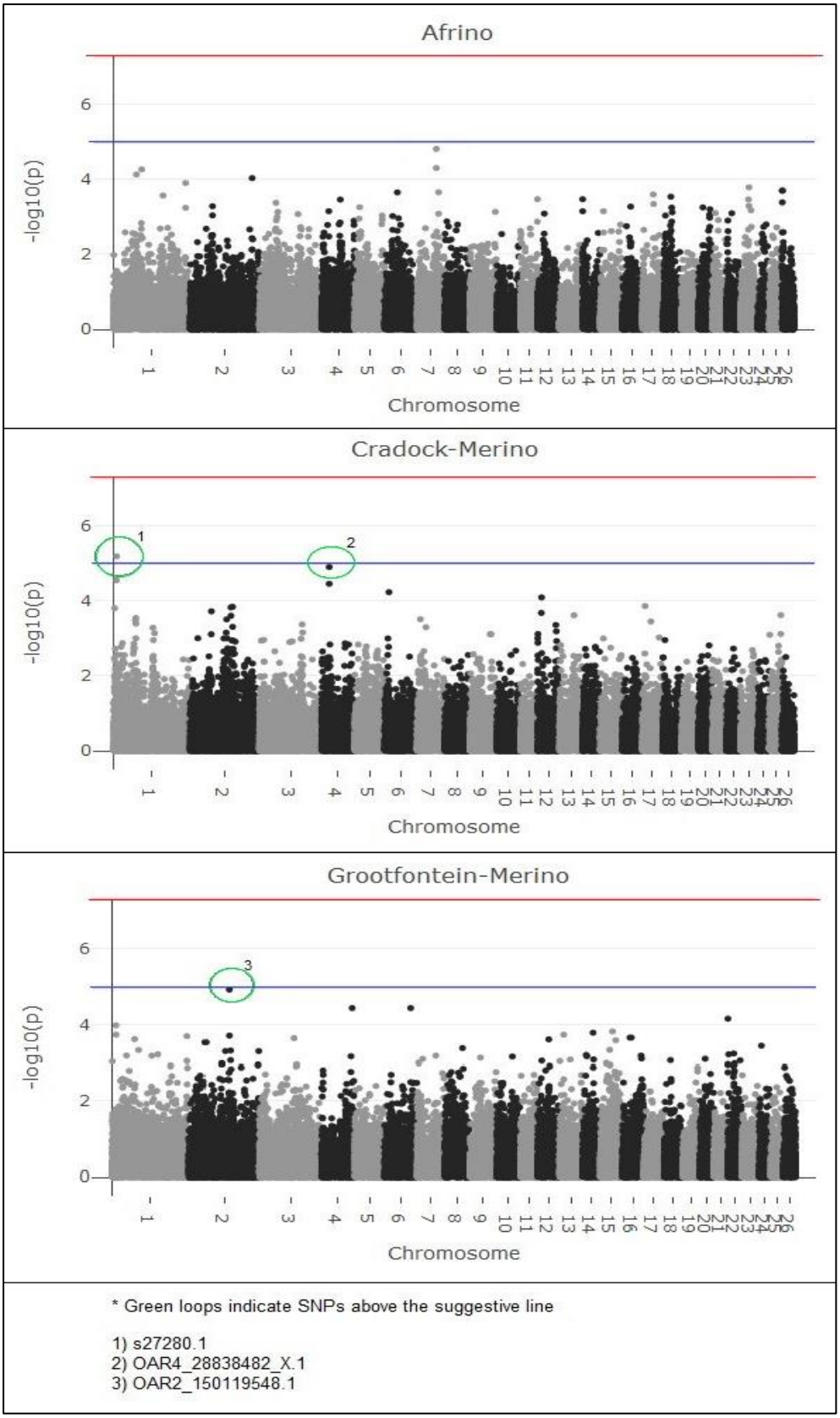

Figure 4.9 Manhattan plot illustrating the results of SNP associated with number of lambs weaned in a: Afrino, b: Cradock Merino, c: Grootfontein Merino

a.

b.

c.

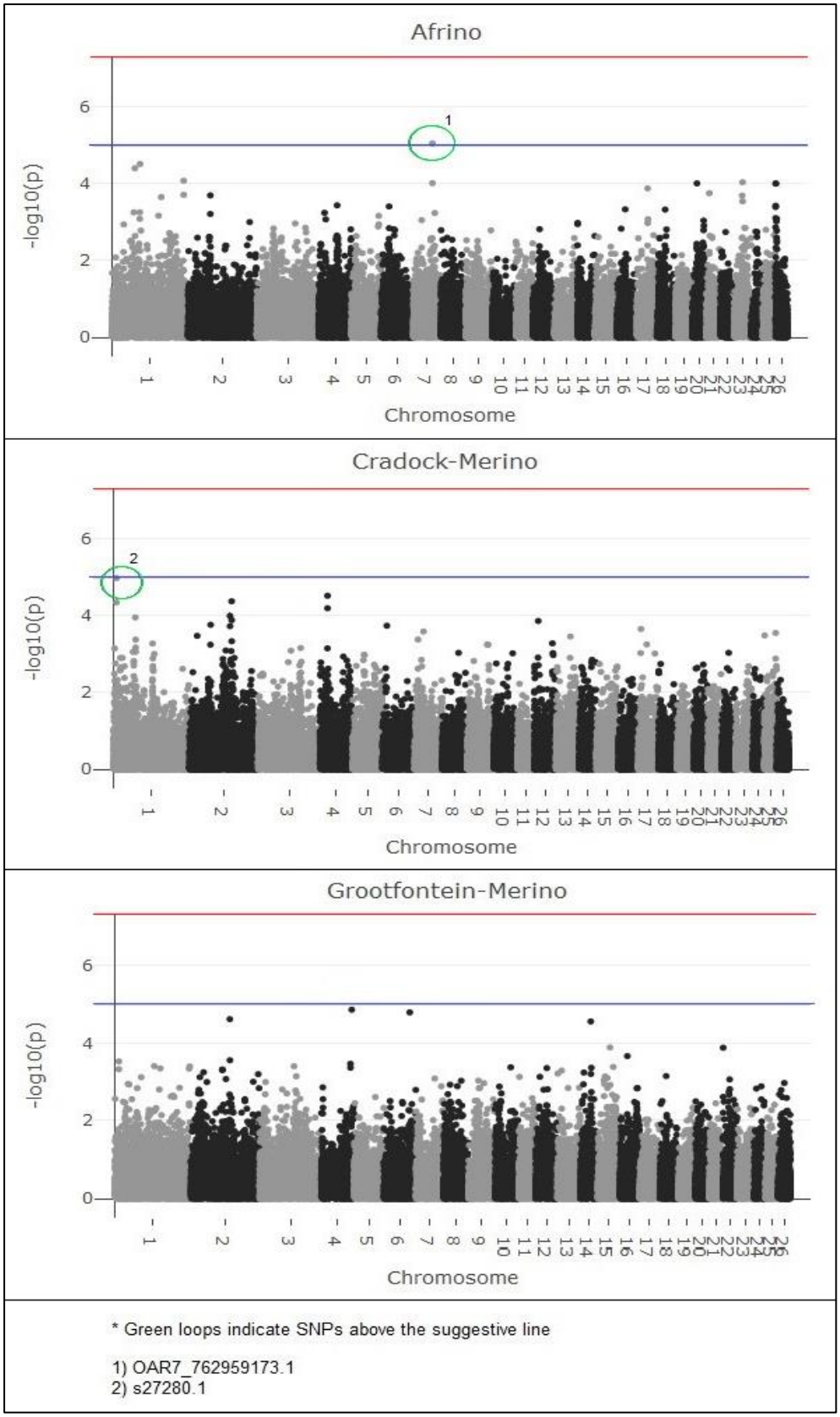

Figure 4.10 Manhattan plot illustrating the results of SNP associated with total weight of lambs weaned in a: Afrino, b: Cradock Merino, c: Grootfontein Merino

Table 4.5 contains the SNP positions of the identified suggestive SNPs and the genes that are possibly associated with these SNP markers. The SNP markers and regions in the table can be observed in detail via the Ensembl (Zerbino *et al.*, 2018; http://www.ensembl.org), UniProt (The UniProt Consortium, 2019; http://www.uniprot.org) and NCBI (https://www.ncbi.nlm.nih.gov/) database. A snapshot was taken from the Ensembl database where the regions were viewed. These snapshots illustrate the regions of the suggestive SNP markers that were identified and the genes possibly associated with them and are contained in addendum B.

Table 4.5 Suggestive SNPs identified in the populations with their associated genes (http://www.ensembl.org; http://www.uniprot.org; https://www.ncbi.nlm.nih.gov/)

SNP-Name	OAR[a]	Position	Population	Submitted SNP name	Trait	Gene
s10640.1	3	183769978	Afrino	ss836354448	BW	-
OAR3_195631696.1	3	181430556	Afrino	ss836340318	BW	-
OAR7_76295917.1	7	69590358	Afrino	ss836348202	TWW	SIX6 C14orf39
OAR14_56900862.1	14	53769443	Afrino	ss836327115	BW	BSPH1 LIG1 CABP5 ELSPBP1
s27280.1	1	11378621	C-Merino[b]	ss836357760	NLB NLW TWW	GRIK3
OAR1_10554666.1	1	10806824	C-Merino	ss836319264	NLB	MAP7D1
OAR4_28838482_X.1	4	27440899	C-Merino	ss836342639	NLW	HDAC9
OAR2_150119548.1	2	141206227	G-Merino[c]	ss836332337	NLW	XIRP2 ENSOARG00000022371
OAR9_64654880.1	9	61385044	G-Merino	ss836350783	BW	TRPS1 ENSOARG00000026539

[a] OAR - *Ovis aries* chromosome
[b] C-Merino - Cradock Merino
[c] G-Merino - Grootfontein Merino

In the Afrino population four suggestive SNPs were identified, but only two of these were found to be associated with previously annotated genes. In total, six genes were associated with these two SNP markers in the Afrino population. Three suggestive SNPs were identified in the Cradock Merino population and each SNP was associated with a gene. For the Grootfontein Merino flock two SNPs were identified to be suggestive and both of the SNPs were association with genes.

In the Cradock Merino population two SNPs (s27280.1 and OAR4_28838482_X.1) were associated with more than one trait. SNP Marker s27280.1 was found to be suggestive for all three reproductive traits namely NLB, NLW and TWW, while OAR4_28838482_X.1) was suggestive for NLW and approached the suggestive line for TWW. In the Afrino population SNP OAR7_76295917.1 was above the suggestive line for TWW and approached the suggestive lines for both NLB and NLW.

In each population the suggestive SNPs were further analysed to investigate the proportion of the population that inherited the major and minor allele per EBV group. These percentages are presented in Table 4.6 for the Afrino, Cradock Merino and Grootfontein Merino populations.

Table 4.6 The percentage of the Afrino, Cradock-Merino and Grootfontein-Merino populations that possess the major or minor allele for the suggestive SNPs identified within estimated breeding value (EBV) categories

SNP	Trait	Number of animals (H:M:L)	Allelic frequency (%)					
			High EBV Group		Medium EBV Group		Low EBV Group	
			Major allele	Minor allele	Major allele	Minor allele	Major allele	Minor allele
Afrino								
s10640.1	BW	66:12:73	65%	35%	88%	12%	84%	16%
OAR3_195631696.1	BW	66:12:73	61%	39%	88%	12%	80%	20%
OAR7_76295917.1	TWW	72:14:65	92%	8%	83%	17%	92%	8%
OAR14_56900862.1	BW	66:12:73	86%	14%	88%	12%	98%	2%
Cradock-Merino								
OAR1_10554666.1	NLB	58:15:55	67%	33%	54%	46%	65%	35%
s27280.1	NLB NLW TWW	58:11:59	77%	23%	62%	38%	54%	46%
OAR4_28838482_X.1	NLW	57:14:57	89%	11%	71%	29%	68%	32%
Grootfontein-Merino								
OAR2_150119548.1	NLW	55:15:59	76%	24%	63%	37%	51%	49%
OAR9_64654880.1	BW	51:26:50	84%	16%	61%	39%	61%	39%

[H] High estimated breeding values
[M] Medium estimated breeding values
[L] Low estimated breeding values

The percentage of animals having the major allele in all the categories for all but two of the SNPs in the Afrino population are 80% or above, while this is the case for only one SNP each in the High EBV category in the Cradock and Grootfontein Merino populations. This indicates more variation in the SNPs for the latter two populations than for the Afrino population. The Afrino flock at Carnarvon has been selected for increased body weight and some measure of reproduction since its origin in the early 1970s, which could have contributed to this fact.

Comparing the percentage of animals having the major and minor alleles in the High and Low EBV categories, it is evident that these differed in the Afrino population for SNPs s10640.1 and OAR3_195631696.1, which are both associated with BW. In both cases, the percentage of animals in the High EBV group having the major allele is lower than that in the Low EBV group, which is the opposite for all other SNPs in the table.

The percentages of animals in the High and Low EBV categories of the Cradock and Grootfontein Merino populations varies, and in almost all instances the highest percentage animals had the major allele in the High EBV category, followed by the Medium and the Low categories.

Each SNP that was identified to have an association with a gene was further investigated and the gene's biological process was investigated. Table 4.7 summarises genes that were in association with body weight and Table 4.8 summarizes the genes that were in association with reproduction in this respect.

Table 4.7 Summary of genes and their functions that were identified for body weight traits in this study (http://www.ensembl.org; http://www.uniprot.org; http://www.ncbi.nlm.nih.gov/)

Gene	Molecular function	Biological process
BSPH1 (OAR14_56900862.1)	• Di-sulphide bond in the FN2 domain	• Binder of sperm protein homolog
LIG1 (OAR14_56900862.1)	• DNA ligase (ATP) activity • DNA binding • ATP binding	• Base excision repair • DNA replication • Mismatch repair • Nucleotide excision repair • DNA biosynthetic process • DNA recombination • DNA ligation involved in DNA repair
CABP5 (OAR14_56900862.1)	• Calcium ion binding	• Calcium binding protein 5
ELSPBP1 (OAR14_56900862.1)	• Di-sulphide bond in the FN2 domain • Collagen binding	• Epididymal sperm-binding protein I
TRPS1 (OAR9_64654880.1)	• Zinc ion binding, • protein domain specific binding, • RNA polymerase II regulatory region sequence-specific DNA binding, • DNA-binding transcription factor activity	• Skeletal system development • Protein Hetero-oligomerization • Negative regulation of transcription by RNA polymerase II
ENSOARG00000026539 (OAR9_64654880.1)	• LincRNA uncharacterised	• LincRNA uncharacterised

Table 4.8 Summary of genes and their functions that were identified for reproduction traits in this study (http://www.ensembl.org; http://www.uniprot.org; http://www.ncbi.nlm.nih.gov/)

Gene	Molecular function	Biological process
SIX6 (OAR7_76295917.1)	• DNA-binding transcription activator activity • NA polymerase II distal enhancer sequence-specific DNA binding, • Sequence-specific double-stranded DNA binding	• Eye development • Regulation of transcription • DNA-templated • Multicellular organism development
C14orf39 (OAR7_76295917.1)	• Protein coding for protein SIX6OS1	• Meiotic DNA double-strand break processing involved in reciprocal meiotic recombination • Oogenesis • Synapsis • Spermatogenesis
GRIK3 (s27280.1)	• Adenylate cyclase inhibiting G protein-coupled glutamate receptor activity • Karinate selective glutamate receptor activity • Ligand-gated ion channel activity involved in regulation of presynaptic membrane potential	• Ion Transport, • Ion channel, • Ligand-gated ion channel
MAP7D1 (OAR1_10554666.1)	• Encodes the protein W5QEG2/4	• microtubule cytoskeleton organization
HDAC9 (OAR4_28838482_X.1)	• NAD-dependent histone deacetylase activity (H3-K14 specific) • Histone deacetylase activity (H4-K16 specific) • Histone deacetylase binding • Metal ion binding • Protein kinase Calcium binding • Repressing transcription factor binding	• Cellular response to insulin • Cholesterol homeostasis • Negative regulation of cytokine secretion and lipoprotein lipase activity • Negative regulation of transcription by RNA polymerase II • Peptidyl-lysine deacetylation • Positive regulation of cell migration involved in sprouting angiogenesis
XIRP2 (OAR2_150119548.1)	• Actin filament binding, • Alpha-actinin binding, • Metal ion binding	• Actin cytoskeleton organization • Cardiac muscle tissue morphogenesis • Cell-cell junction organization • Ventricular septum development
ENSOARG00000022371 (OAR2_150119548.1)	• miRNA Uncharacterized	• miRNA Uncharacterized

CHAPTER 5: DISCUSSION

The main aim of this study was to identify genomic regions of significance that were associated with traits of economic importance in the South African sheep industry. To date limited genomic studies have been performed on South African sheep breeds and these mainly aimed to describe population diversity. The study samples were generally small and a wide variety of breeds were investigated for their diversity. Greyvenstein *et al.* (2016) reported on only 43 Damara individuals for the horn phenotype. The study of Molotsi *et al.* (2017b) was a GWAS study that focused on identifying a specific wet-dry phenotype using the following breeds: 222 Dorpers, 47 Namaqua Afrikaner and 26 South African Mutton Merinos. The main focus of the Molotsi *et al.* (2017c) study was the population structure and diversity of specific South African sheep breeds which included 224 Dorper's, 46 Namaqua Afrikaner, 26 South African Mutton Merinos and 11 Dorper x South African Mutton Merinos crossbred individuals. The study conducted by Selepe *et al.* (2018) also reported on genetic population structure and diversity of South African sheep breeds and it included Zulu (207), Damara (29), Dorper (53) and SA Merino (53) sheep breeds. Sandenbergh *et al.* (2016) investigated breed diversity and the possibility whether GWAS could be a selection tool to improve breeding programs in South Africa, including Dorper (20), Namaqua Afrikaner (20), SA Merino (85) and South African Mutton Merino (19) sheep breeds. Dlamini *et al.* (2019) reported genetic variation within and between *Haemonchus contortus* resistant and susceptible Dohne Merino sheep from two different locations, namely Wauldby (192) and GADI (48).

Genomic tools have not yet been used with the aim of genetic improvement in small stock and there is no strategic national program to implement genomic selection in small stock as is the case with both beef and dairy cattle (Van Marle-Köster & Visser, 2018). This study was performed to gain insight into mechanisms that underlie economically important traits involved in body weight and reproduction traits.

5.1 Quality control

Sample-based and marker-based quality control are important steps to assure the quality of SNP chip data (Anderson *et al.*, 2010; Zhao *et al.*, 2018). It is necessary to do quality control before analysis to remove potential bias and genotyping errors that occur during sampling, genotype calling and laboratory procedures (Anderson *et al.*, 2010; Zhao *et al.*, 2018). The number of polymorphic SNPs left in the Afrino, Cradock Merino and Grootfontein Merino populations after quality control were 42 117; 46 196 and 43 655 SNPs respectively. The difference in number of SNPs validated for downstream analysis was largely due to a

higher proportion of missing genotypes in the Grootfontein Merino and a lower number of polymorphic loci in the Afrino population. In the Afrino population, 4 142 SNPs were removed based on a minor allele frequency of less than 2%. This was probably due to ascertainment bias introduced during the development and design of the Illumina® Ovine SNP50 BeadChip (Qanbari & Simianer, 2014; Sandenbergh *et al.*, 2016; Ilori *et al.*, 2018). Very few indigenous or locally developed African sheep breeds were included during SNP chip development and thus limited SNPs that are polymorphic in African breeds were included (Sandenbergh *et al.*, 2016; Edea *et al.*, 2017; Ilori *et al.*, 2018).

For the current study the average MAF of the Afrino population was estimated at 0.252. A study conducted on indigenous Ethiopian and African type sheep populations found lower average MAF values of between 0.20 and 0.21 for Arsi-Bale, Horro, Menz, Adilo, and Blackhead Somali sheep breeds (Edea et al., 2017). The lower average MAF for indigenous breeds in Africa can be due to less intensive artificial selection practices in those sheep populations compared to South African sheep populations. The average MAF of the Afrino is still considered low even though it is higher compared to other indigenous African sheep populations. Lower average MAF can be indicative that these sheep breeds are genetically more distant from the discovery breeds that were included on the assay and lead to the under-representation of rare polymorphic alleles in indigenous sheep breeds (Qanbari & Simianer, 2014; Molotsi *et al.*, 2017c; Ilori *et al.*, 2018). Even though the SNP array showed ascertainment bias, the technology is still regarded as efficient to gain insight in terms of genomic studies in the South African sheep industry.

5.2 Population parameters

Limited genomic studies have previously been performed on the Afrino sheep breed. The population parameters estimated in the current study will be compared to similar parameters of local South African sheep breeds. In the current study the estimated average MAF of the Afrino population was 0.252. High polymorphic alleles are defined as SNPs with an average MAF that ranges between 0.3 to 0.5 and therefore the Afrino population's average MAF is considered low (Grasso *et al.*, 2014; Zhang *et al.*, 2018). A study conducted on indigenous Southern Africa sheep breeds by Molotsi *et al.* (2017c) reported that the average MAF ranged between 0.218 to 0.279 for the Dorper, Namaqua Afrikaner, South African Mutton Merino and Dorper x South African Mutton Merinos breeds, which is comparable to the current study's 0.252 average MAF.

For both the Cradock and GADI Merino populations an average MAF value of 0.372 was estimated, which was higher than that reported by Sandenbergh *et al.* (2016) for the SA Merino (0.26). Similar lower MAF values were estimated by Dlamini *et al.* (2018) for the Wauldby Dohne Merino (0.2811 ± 0.1345) and GADI Dohne Merino (0.2780 ± 0.1355) populations. The current study's value was comparable to the average MAF estimates reported by Grasso et al. (2014) for the fine woolled Merino (0.4) in Uruguay and by Ciappesoni *et al.* (2018) for an Australian Merino nucleus flock (0.33) situated at the National Research Institute of Agricultural of Uruguay.

The observed heterozygosity values in the current study were 0.372, 0.379 and 0.369 for the Afrino, Cradock Merino and Grootfontein Merino populations respectively. These results are comparable to local studies conducted by Sandenbergh *et al.* (2016), Molotsi *et al.* (2017c) and Dlamini *et al.* (2019). In the study conducted by Sandenbergh *et al.* (2016), observed heterozygosity values between 0.28 and 0.35, were estimated for Dorper, Namaqua Afrikaner, SA Merino and South African Mutton Merino sheep. Molotsi *et al.* (2017c) reported average observed heterozygosity levels ranging from 0.30 to 0.33 in the Dorper, Namaqua Afrikaner and South African Mutton Merino breeds. Two different Dohne Merino sheep populations were investigated by Dlamini *et al.* (2019), and similar observed heterozygosity values of 0.373 for the Wauldby Dohne Merino population and 0.374 for the GADI Dohne Merino population were reported. Despite continuous and sustainable artificial selection of the three populations, all three still maintained high average heterozygosity within the populations. This indicates that the populations are diverse and contain high genetic variation within the populations, therefore the populations can still be subjected to intense artificial selection to obtain genetic improvement (Qanbari & Simianer, 2014; Molotsi *et al.*, 2017a; Ilori *et al.*, 2018).

The F_{IS} values were used to interpret the level of inbreeding in the three sheep populations. The Afrino population in this study had negligible inbreeding levels (F_{IS}= - 0.025). The inbreeding coefficient of the current study for the Afrino was lower than reported by Molotsi *et al.* (2017c). The Molotsi *et al.* (2017c) study, however, reported relatively low inbreeding coefficients for the Dorper (0.074 ± 0.047) and Dorper x South African Mutton Merino (0.034 ± 0.042) breeds, and a higher level for the Namaqua Afrikaner (0.237 ± 0.05). Low inbreeding coefficient values are indicative of diverse and outbred populations. A lower F_{IS} value in artificial selected populations also indicate that a holistic genetic selection approach and sustainable selection practise were followed, which ensured that not too much focus was placed on one specific entity or trait at the expense of another. For the Merino populations the current study estimated the inbreeding coefficient at F_{IS} = - 0.025 for the Cradock Merino

population and F_{IS} = 0.002 for the Grootfontein Merino population, indicating low inbreeding levels.

This is consistent with previous studies that found commercial South African Merino types to be highly diverse and outbred populations (Sandenbergh *et al.*, 2016; Dlamini *et al.*, 2019). Both the Afrino and Cradock Merino populations had low negative inbreeding coefficients. According to PLINK software manual (Purcell, 2017) a low negative inbreeding coefficient value is as a result of individuals in the population that are less related than expected, while a strong negative inbreeding coefficient could be as a result of contamination or sampling error. Over all, all three populations had negligible inbreeding levels, but these were not indicative of the inbreeding level of the respective flocks or breeds, as animals with low inbreeding levels were selected for the study.

5.3 Genetic relatedness within and between populations

The PCA methodology was used to illustrate relatedness between individuals within and across populations (Anderson *et al*., 2010). Four PCA plots were created for each population (one per trait) to investigate possible clustering according to the High, Medium and Low EBV groups. The expectations were that within each population some distinction would be observed at a genomic level between the High and Low EBV groups within each trait. However, no clustering based on EBV phenotype was observed and the three sub-groups (H, M and L) clustered together within populations. As all animals in the different EBV groups clustered together, the populations were pooled together for further PCA analyses. A tight, separate cluster was observed for the Afrino population, indicating that this population had a separate ancestral background from the two Merino populations.

The Cradock and Grootfontein Merino populations formed two clusters with a few individuals overlapping between the two clusters. This suggested that there was some genetic relatedness between the two Merino populations and that they possibly have some shared ancestral background. This relatedness between the two Merino populations is due to the use of Cradock rams as sires in both populations, as well as the use of Cradock ewes as dams in the Grootfontein population. The most probable number of ancestral populations for the data set was estimated, to assign a portion of each ancestral population to the individuals within the data set (Alexander *et al.* 2009). Based on the PCA plots, it was expected that the admixture plot would illustrate a pattern of some shared ancestry and some genetic relatedness between the populations. The three populations illustrated substructure of shared ancestral SNP genotypes at the lowest cross-validation error (K=3).

The current study's admixture results were generally in agreement with the PCA plots and separated the individuals by geographical region and flock which is similar to the findings reported in previous literature (Edea *et al.*, 2017; Molotsi *et al.*, 2017b; Selepe *et al.*, 2018). Based on the admixture and clustering results, the populations were separated for three distinct GWAS analyses. The separation of the populations was warranted as the populations were expected to have different underlying genetic substructures and genotypes, which in turn could result in varying candidate genes and markers for traits of economic importance.

5.4 Genome wide association study

Nine suggestive SNPs were identified in the GWAS performed on the sheep populations, of which four were associated with body weight and five with reproduction traits. Of the four suggestive SNPs associated with growth, two were situated close to or within candidate genes. The SNP OAR14_56900862.1 identified in the Afrino population was situated close to the genes *BSPH1*, *LIG1*, *CABP5* and *ELSPBP1*. Gene ontology databases UniProt (The UniProt Consortium, 2019; https://www.uniprot.org) and Panther (Mi et al., 2019; http://www.pantherdb.org) reported *BSPH1* to be involved in spermatid development, more specifically in the binding protein that binds sperm *in vitro* and promotes sperm capacitation (Fan *et al.*, 2006). Although this gene is present in the cattle, horse and pig genomes, no previous literature gives evidence that it is associated or involved with BW or growth of animals.

The *LIG1* gene was found to be primarily involved in DNA ligase activity and DNA/ RNA repair and replication (https://www.uniprot.org; http://www.pantherdb.org). A GWAS conducted by Cole *et al.* (2014) investigated calf birth weight in Holstein cattle and identified *LIG1* in the pooled SNPs that could possibly have an association with BW. A study conducted by Da Costa *et al.* (2004) also identified *LIG1* as a novel, possibly growth-related gene. Both studies could not directly link the *LIG1* gene to BW or growth but both reported the gene to be involved in the underlying mechanisms of growth (Da Costa *et al.*, 2004; Cole *et al.*, 2014). The current study adds to this body of evidence and found that a high percentage of individuals in all three EBV categories for BW possessed the major allele for the suggestive SNP (OAR14_56900862.1). The High, Medium and Low category had 86%, 88% and 98% of individuals possessing the major allele for the associated SNP, respectively. Selection should possibly be towards the minor allele, as the frequency of this allele was 14% in the High EBV group, and only 2% in the Low EBV group. These findings and previous literature support further investigation into the gene and its role in body weight.

The *CABP5* gene has been reported to be *a* regulator of neurotransmitter vesicles and could be involved in the organization of neurite networks (https://www.uniprot.org; http://www.pantherdb.org). Studies conducted by Rieke *et al.* (2008) and Sokal & Heaseleer (2011) linked the gene to playing a role in transmission of light signals and photoreceptor synaptic functioning in the retina. Neither of the previous studies reported the involvement of *CABP5* genes in BW mechanisms or pathways (Rieke *et al.*, 2008; Sokal & Haeseleer, 2011).

ELSPBP1 is an epididymal sperm-binding protein that has phosphorylcholine-binding activity and binds to spermatozoa upon ejaculation (http://www.pantherdb.org; https://www.uniprot.org). Previous literature found the gene to be involved in the controlling mechanisms of epididymal maturation of sperm and sperm capacitation during ejaculation (Fan *et al.*, 2006; Song *et al.*, 2011). No literature supports the gene's direct involvement in BW or growth.

The second SNP (OAR9_64654880.1) associated with growth, was identified in the Grootfontein Merino population and was situated within the *TRPS1* gene and in close proximity of the lincRNA gene *ENSOARG00000026539*. *TRPS1* is a transcriptional repressor protein that influences GATA binding (https://www.uniprot.org). It is further reported that the gene is a negative regulator of RNA polymerase II transcription and involved in the development of the skeletal system (http://www.pantherdb.org; https://www.uniprot.org). Maas *et al.* (2019) reported a mutation in the *TRPS1* gene resulting in Trichorhinophalangeal Syndrome in humans that affects the formation of skeletal bone structures, resulting in stunted skeletal growth and misalignment of joints. Several studies reported the involvement of *TRPS1* in economically important traits such as mammary gland morphogenesis and development in dairy cattle (Do *et al.*, 2017) and hair growth in cashmere goats (Guan *et al.*, 2016). The current study found an association between BW and the suggestive SNP, which was linked to the *TRPS1* gene. Upon further investigation in the different EBV category groups, 84% of individuals in the High EBV category for BW possessed the major allele for the suggestive SNP vs. only 61% in the Low EBV group. Previous literature also reported the association of the *TRPS1* gene with carcass weight and eye muscle area in beef cattle (Hay & Roberts, 2018), and with growth and meat traits in sheep (Zhang *et al.*, 2013).

From the five suggestive SNPs associated with reproduction traits, two were in association with NLB (s27280.1 and OAR1_10554666.1), three with NLW (s27280.1, OAR4_28838482_X.1 and OAR2_150119548.1) and two with TWW (s27280.1 and OAR7_76295917.1). One SNP s27280.1 was found in the Cradock Merino population and

was located close to the *GRIK3* gene. SNP OAR1_10554666.1 was also located in the Cradock Merino population and located in close proximity with the *MAP7D1* gene.

SNP s27280.1 is of great interest as it was associated with all three reproductive traits. This specific SNP is in close proximity to the *GRIK3* gene. The *GRIK3* gene is a glutamate ionotropic receptor that is involved in multiple pathways and biological processes and found in several livestock species such as cattle, chickens, pigs and horses (http://www.pantherdb.org; https://www.uniprot.org). This gene was described to be involved in nervous system processes, regulation of membrane potential, synaptic transmissions and also played a role in the signalling pathway of the glutamate receptor (http://www.pantherdb.org; https://www.uniprot.org). Neural psychosis studies performed in humans found the *GRIK3* gene to be involved in dopamine, serotonin and glutamate pathways which are fundamental to understanding behaviour in psychotic disorders like schizophrenia and depression (Lerma & Marques, 2013; Mas *et al*., 2016). Further studies done on livestock found the *GRIK3* gene to be associated with age at first egg in chickens (Yuan *et al.*, 2015) and with temperament and behavioural traits in cattle (Qanbari *et al*., 2014; Garza-Brenner et al., 2017). It was further postulated that serotonin-receptors could be a mediator in lactation and influence calcium concentrations in blood of lactating animals (Harrelson *et al.*, 2018; Jin *et al.*, 2019). This supports the suggestion that *GRIK3* needs further investigation, specifically into the mechanism that plays a role in maternal behaviour and stress behaviour during and post parturition. In the current study, the SNP major allele had a frequency of 77% in the High EBv group, while the major and minor alleles were almost equally present in the Low EBV group. This indicates that selection for the major allele might have benefits regarding reproductive efficiency.

The *MAP7D1* gene encodes for a microtubule-associated protein involved in microtubule cytoskeleton organization and is also a binding protein for non-motor microtubules (http://www.pantherdb.org; https://www.uniprot.org). Involvement of *MAP7D1* in microtubule organization and mechanism have been investigated but its association with gene expression and gene regulating mechanisms to specific phenotypes and traits is still unknown (Yadav *et al.*, 2014; Tymanskyj *et al.*, 2017; Kikuchi *et al.*, 2018). No current studies directly link *MAP7D1* gene to NLB or other reproductive-type traits.

Of the additional two SNPs associated with NLW, one was identified in the Cradock population (OAR4_28838482_X.1) and one in the Grootfontein Merino population (OAR2_150119548.1). The OAR4_28838482_X.1 SNP was found to be linked to the *HDAC9*

gene, while SNP OAR2_150119548.1 was in close proximity with the gene *XIRP2* and the miRNA gene *ENSOARG00000022371* respectively.

Gene-ontology databases identified the *HDAC9* gene as a histone deacetyl protein that plays an important role in regulating transcription and cell cycle events like progression and development (http://www.pantherdb.org; https://www.uniprot.org). In this study, the major allele frequency of OAR4_28838482_X.1 was 89% in the High EBV group for NLW, vs. only 68% in the Low EBV group. The current study concurs with previous literature, suggesting that the gene could be involved in reproduction, fertility and weight or growth *in vivo* (Du *et al.*, 2011; Udomchanya *et al.*, 2019). Several studies done on livestock associated the *HDAC9* gene with skeletal muscle development (Mei *et al.*, 2019) and possibly with carcass and meat traits (Hagen *et al.*, 2005; De Vos, 2018). Zhang *et al.* (2014) found that *HDAC9* influenced individual birth weight of piglets during embryogenesis and development. In cattle it was reported that the gene was important in sperm quality in Holstein bulls to maintain spermatogonia of stem cells during cell differentiation and ageing (Hering *et al.*, 2014). Further investigation into the *HDAC9* gene specifically for weight and litter number is warranted.

The SNP OAR2_150119548.1 was associated with NLW and in close proximity with the gene *XIRP2.* The *XIRP2* gene encodes a cytoskeletal protein that is part of the actin family, which is involved in cellular and intra-cellular processes (http://www.pantherdb.org; https://www.uniprot.org). A recent study by Mathes *et al.* (2019) revealed that *XIRP2* gene is involved in type one skeletal muscle fibres that are oxidative fibres that fatigue slowly. The *XIRP2* gene is a fibre type-1 costamere gene, which is involved in the control of muscle fibre characteristics (Mathes *et al.*, 2019). Further studies found the gene in pigs to be associated with pork quality (Piórkowska *et al.*, 2017), meat quality in goats (Wei *et al.*, 2019) and meat quality and feed efficiency in cattle (Seabury *et al.*, 2017; Wei *et al.*, 2019). In the current study the gene was associated with NLW, however no other studies linked the gene to reproduction or prolificacy in livestock.

Regarding TWW, SNP OAR7_76295917.1 was identified in the Afrino population to be closely situated to two genes namely *SIX6* and *C14orf39*. The *SIX6* gene is involved in eye development, transcription regulation, sensory system and anatomical structure development (http://www.pantherdb.org; https://www.uniprot.org). In previous literature the *SIX6-Box* has been directly linked to growth in cattle and cashmere production in goat breeds (Huai *et al.*, 2011; Pan *et al.*, 2011). More recent studies performed on cattle and goats concur that the *SIX6* gene is involved in pituitary gland development, which influences downstream hormone production involved in puberty and growth of animals (Cánovas *et al.*, 2014; Fortes *et al.*,

2016; Dias *et al.*, 2017; Ma *et al.*, 2017). The studies conducted in cattle by Cánovas *et al.* (2014) and Fortes *et al.* (2016) both reported that the gene influences the expression and regulation of gonadotropin-releasing hormone. This hormone is an important growth hormone that affects puberty and reproduction in livestock species (Cánovas *et al.*, 2014; Fortes *et al.*, 2016). The current study also found that in all three (H, M and L) EBV category groups for TWW, 80 to 90% of individuals possessed the major allele for the suggestive SNP. Further investigation into the *SIX6* gene is justified.

The final gene identified was the *C14orf39* gene that has also been identified in chicken and pigs (http://www.pantherdb.org; https://www.uniprot.org). It has been linked to biological processes of meiosis such as reciprocal recombination and the process of breaking double-stranded DNA (https://www.uniprot.org). Only one study in mice reported the gene to be linked to fertility, and no other literature links the gene to weight or fertility traits in livestock (Gomez *et. al.*, 2016). The current study found that the major allele of the suggestive SNP (OAR7_76295917.1) was present at a frequency of 92% in both the High and Low EBV categories for TWW. These results indicate that the *C14orf39* gene is possibly involved in reproduction efficiency in livestock.

It is interesting to note that two of the genes (*BSPH1* and *ELSPBP1*) associated with body weight in the Afrino population in fact are involved in the regulation of reproductive processes. Similarly, three of the genes associated with reproduction (*SIX6, XIRP2* and *HDAC9*) seem to also affect growth and carcass traits. In the case of *SIX6* this is understandable, as TWW comprised both the number of lambs as well as the weight of the lambs. Comparable results have been reported for the *LCORL* gene. This gene encodes a transcription factor that function in spermatogenesis. The *LCORL* gene have also been identified in associated with growth and carcass traits in sheep (Al-Mamun et al., 2015b; Bolormaa et al., 2016) and cattle (Han et al., 2017).

The question could be asked if a similar situation applied here or did continued simultaneous selection for body weight and reproduction in the flock caused genes, that are located near to each other but affecting different traits, to become fixed?

During the investigation of SNPs associated with traits of interests, additional SNPs were identified which approached significance at a suggestive level. These SNPs were sometimes in close proximity of candidate genes. In some cases, these candidate genes were involved in mechanism and biological processes that pertained to the traits under investigation. These SNPs were, however, not included in the results of the current study or for further discussion,

as they did not meet the statistical threshold for significance. It must, however, be stated that larger sampling and more discreet groupings (larger differences between the Low and High EBV groups) could result in some of these SNPs showing clear associations.

After investigation into the biological processes of the genes associated with the suggestive SNPs, five genes were identified that showed the most promise. These are *LIG1*, *TRPS1, HDAC9, GRIK3,* and *SIX6.* The associations found in the current study support previous findings and makes biological sense in terms of the metabolic pathways involved. Further research is necessary to confirm the importance of these SNP and to validate the candidate genes.

CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The aim of this study was to perform a GWAS to identify genomic regions associated with body weight and reproduction in two South African sheep breeds. Within the genomic regions, markers were identified that were associated with the traits under investigation. These markers were compared to genomic databases to identify putative / candidate genes associated with the traits under investigation. To conduct the genome-wide association study, the Illumina® Ovine SNP50 BeadChip was used to genotype 288 animals specifically for this study and an additional 123 animals' genotypic data were available from a previous study. The basic population parameters of the three populations were reported. The population parameters estimated in the current study's populations showed low levels of inbreeding (possibly influenced by the study's sampling strategy) and indicated genetic differentiation between the populations. The degree of differentiation was consistent with the history of each of the population's breeding program.

Seven SNP markers were identified in the current study that are associated with body weight and reproduction traits in the three flocks. Gene ontology of the identified chromosomal regions identified six genes that were putatively related to body weight and seven genes to reproduction traits. After investigation into the biological processes of the genes, five genes were identified that showed the most promise. Before recommendations regarding the use of these genes in breeding programs could be made, further investigation into the specific genes linked to body weight and growth traits, namely *LIG1, TRPS1, HDAC9* and *SIX6* is needed. For reproduction and fertility traits the following genes warranted further investigation: *GRIK3, HDAC9* and *SIX6*. A more comprehensive GWAS incorporating more genotyped animals should be done to verify these results.

In conclusion GWAS is a useful tool to detect associations between SNPs and body weight and reproduction traits. The findings in the current study improved the understanding of the genetic mechanisms regulating body weight and growth as well as reproduction and fertility. Using GWAS to identify candidate genes for use in selection and breeding programs is warranted and is an efficient way to identify functional genes and genetic variants associated with economically important traits.

6.2 Recommendations

In the current study the population parameters and genetic substructure results for the sheep populations indicated high genetic diversity and negligible inbreeding levels. All three populations were under good management and selection practises that ensured minimal inbreeding in the population. The current study could use GWAS successfully in identifying regions of significance and drawing an association with specific traits.

The current study had limited animal genotypes available due to funding. A bigger sample size would probably have identified more significant SNPs in association with the traits under investigation. Animal genotypes from previous studies were included in the current study, which proved challenging to categorise animals in the High, Medium and Low EBV category groups. This in turn also influenced the distribution of animals in the PCA plots for the different groups per trait.

Before MAS could be considered as a genomic tool in breeding programs in South Africa, further investigation into the genes identified in the above study is required. It could be advantageous if the GWAS study could be replicated in the same sheep breeds, but using different and bigger population sizes. A national genomic program, similar to those in the beef and dairy cattle industries, should be implemented in small-stock to advance the sheep industry.

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ADDENDA

Addendum A: PCA plots illustrating each population clustering according to High, Medium and low EBV groups

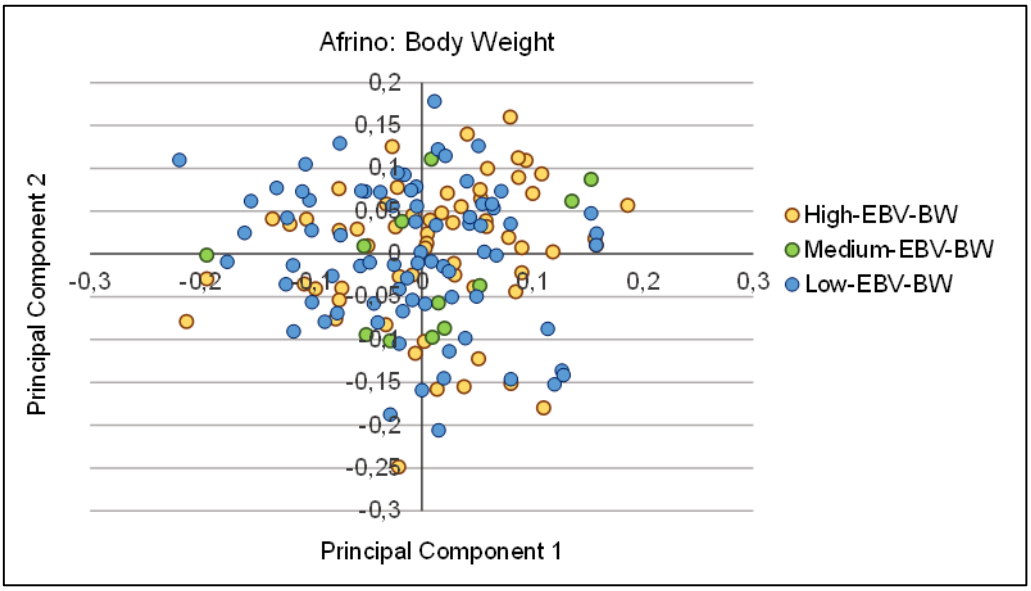

Figure A1 Genetic relationships among 151 Afrino sheep, which illustrate estimated breeding value groups for body weight

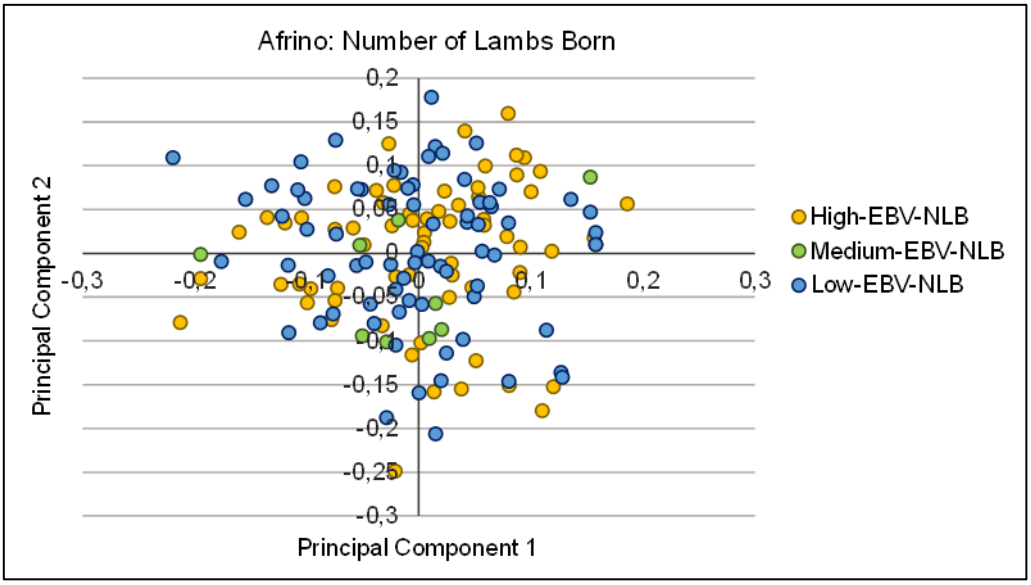

Figure A2 Genetic relationships among 151 Afrino sheep, which illustrate estimated breeding value groups for number of lambs born

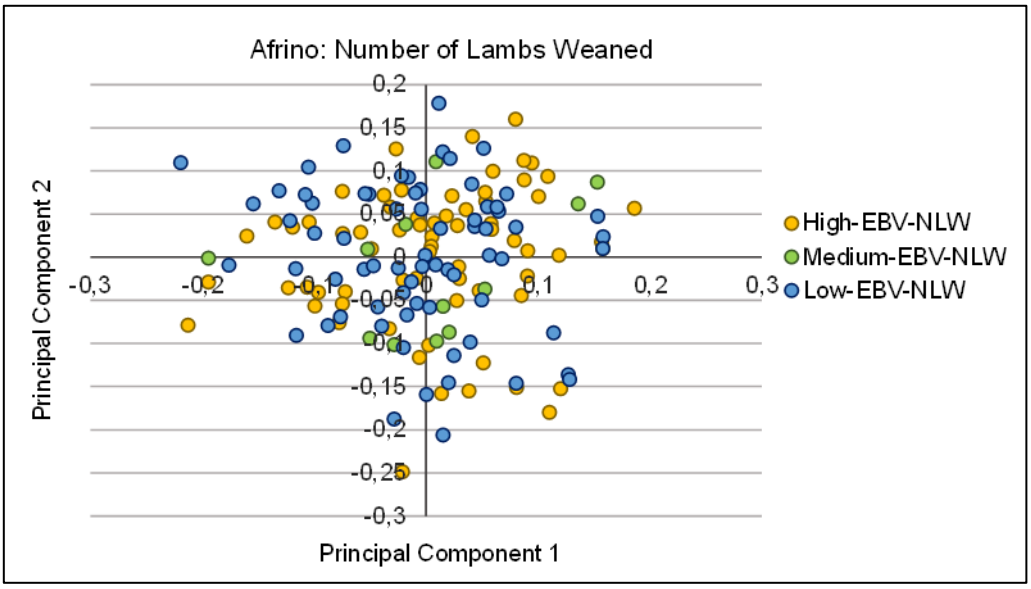

Figure A3 Genetic relationships among 151 Afrino sheep, which illustrate estimated breeding value groups for number of lambs weaned

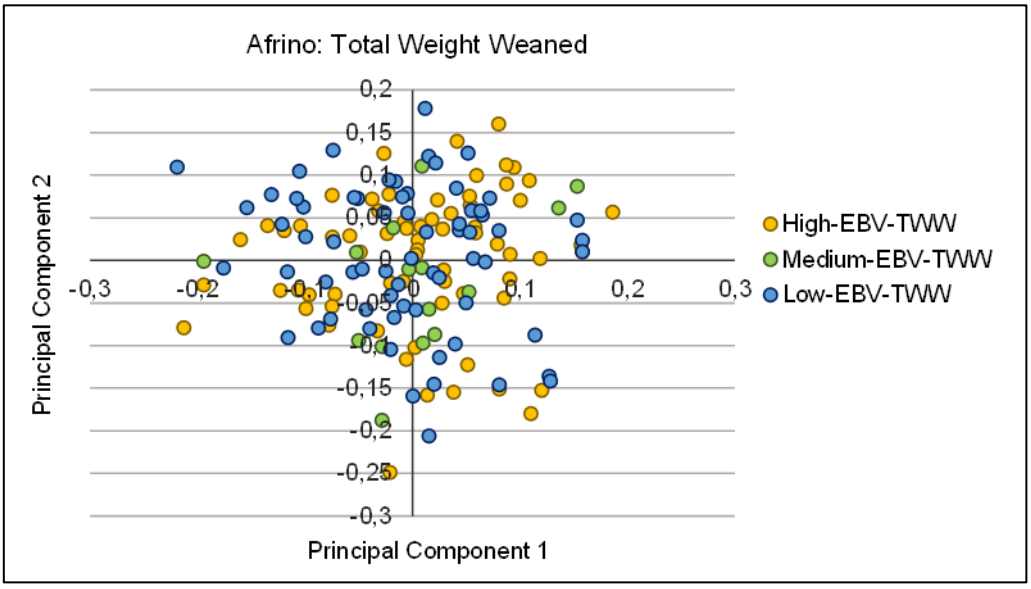

Figure A4 Genetic relationships among 151 Afrino sheep, which illustrate estimated breeding value groups for total weight of lambs weaned

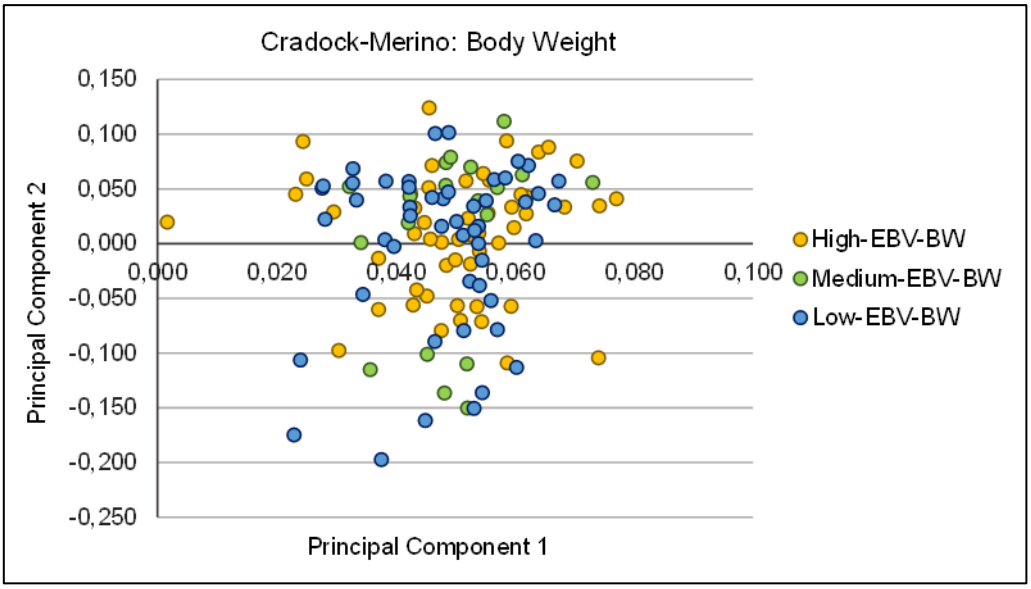

Figure A5 Genetic relationships among 128 Cradock Merino sheep, which illustrate estimated breeding value groups for body weight

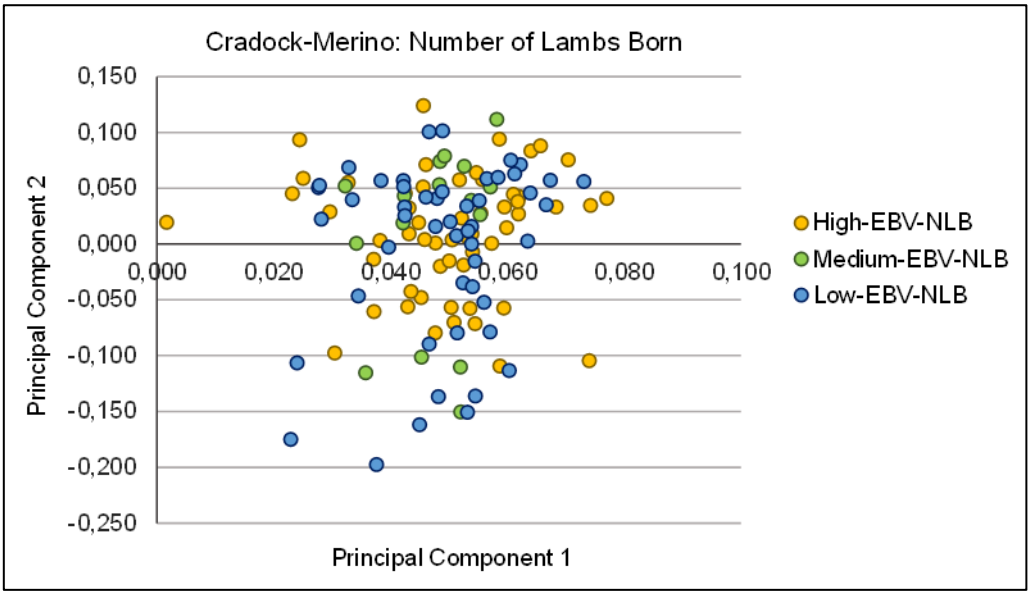

Figure A6 Genetic relationships among 128 Cradock Merino sheep, which illustrate estimated breeding value groups for number of lambs born

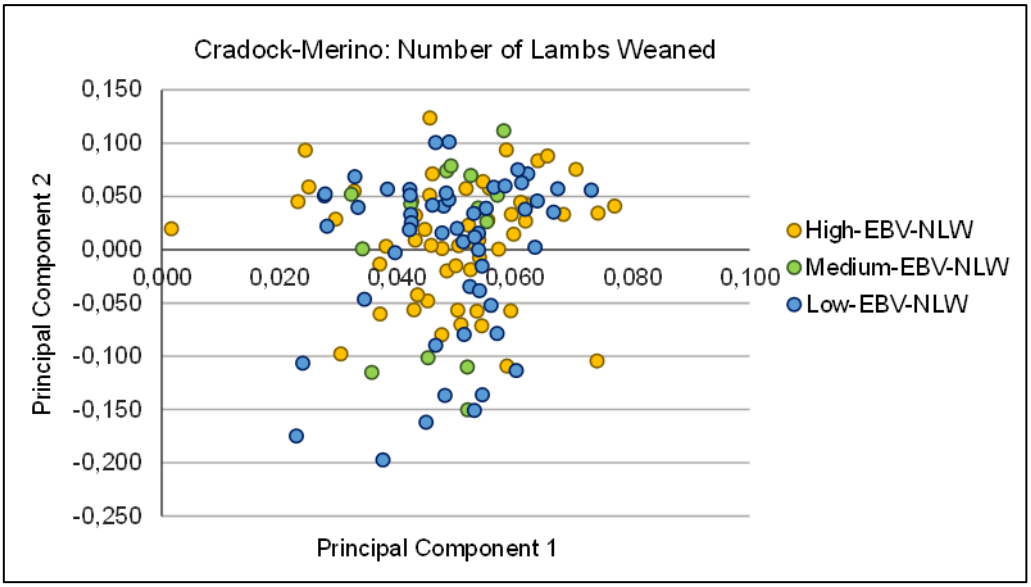

Figure A7 Genetic relationships among 128 Cradock Merino sheep, which illustrate estimated breeding value groups for number of lambs weaned

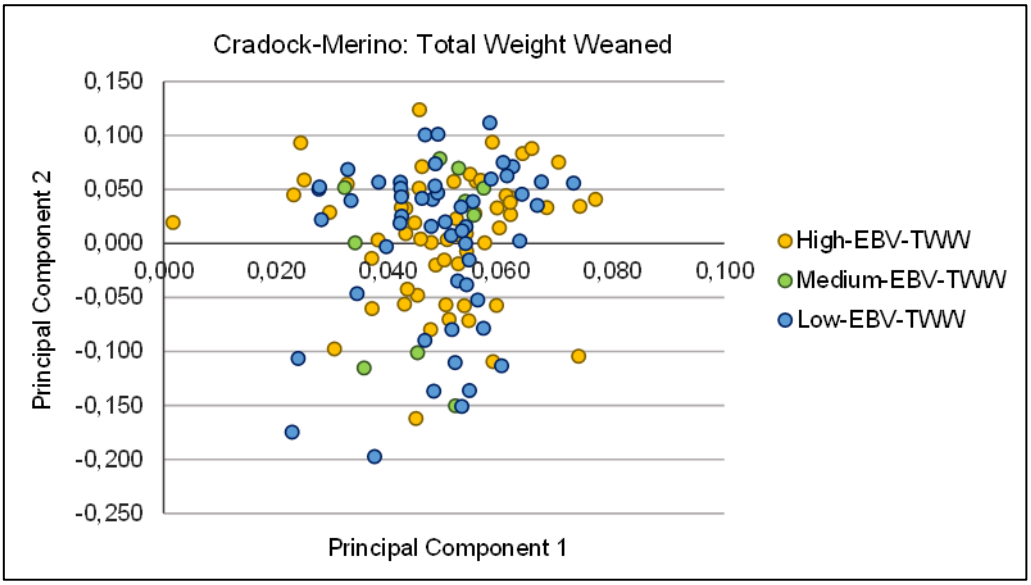

Figure A8 Genetic relationships among 128 Cradock Merino sheep, which illustrate estimated breeding value groups for total weight of lambs weaned

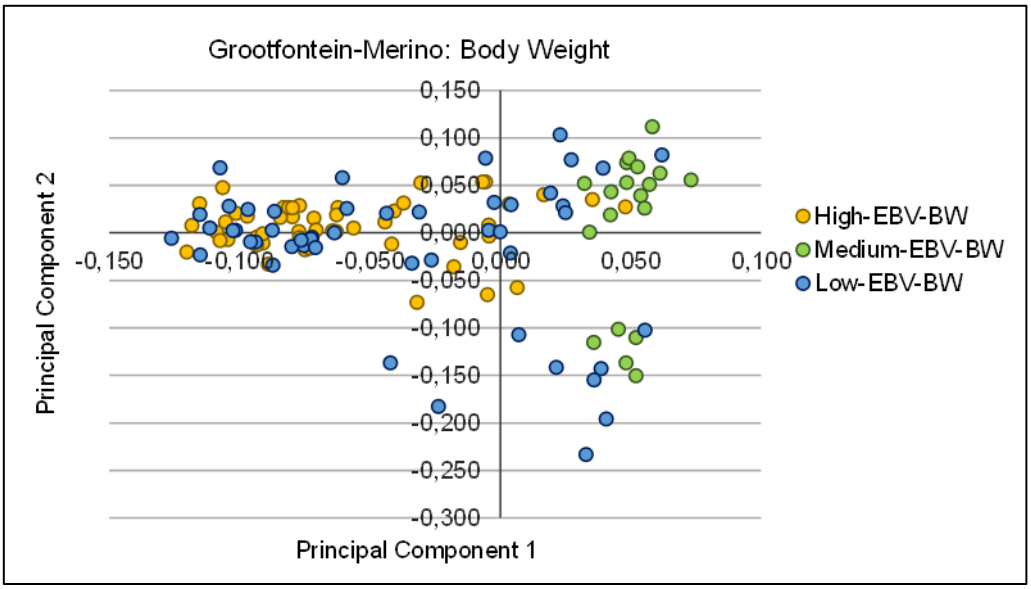

Figure A9 Genetic relationships among 127 Grootfontein Merino sheep, which illustrate estimated breeding value groups for body weight

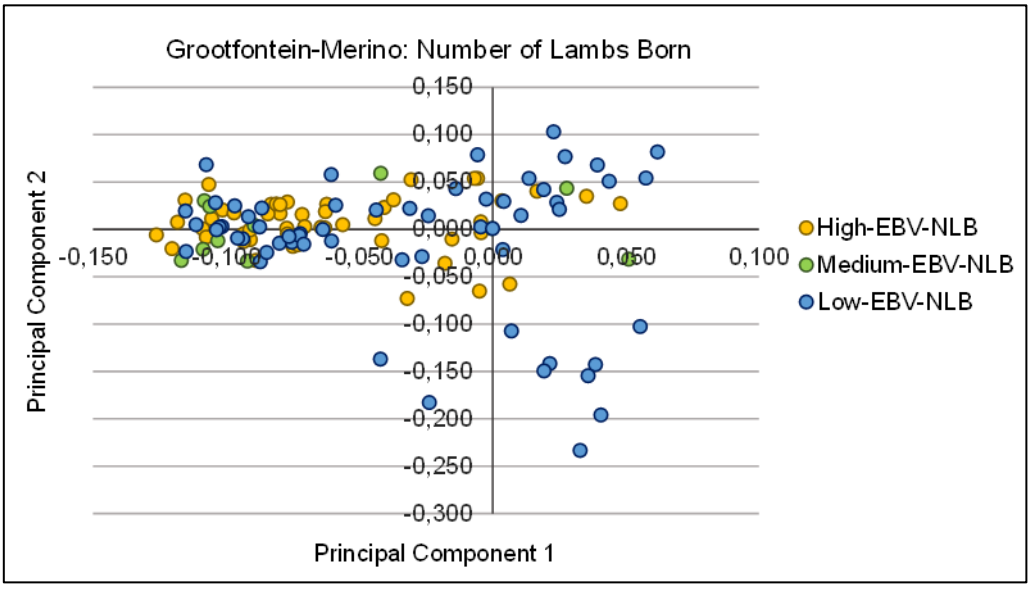

Figure A10 Genetic relationships among 127 Grootfontein Merino sheep, which illustrate estimated breeding value groups for number of lambs born

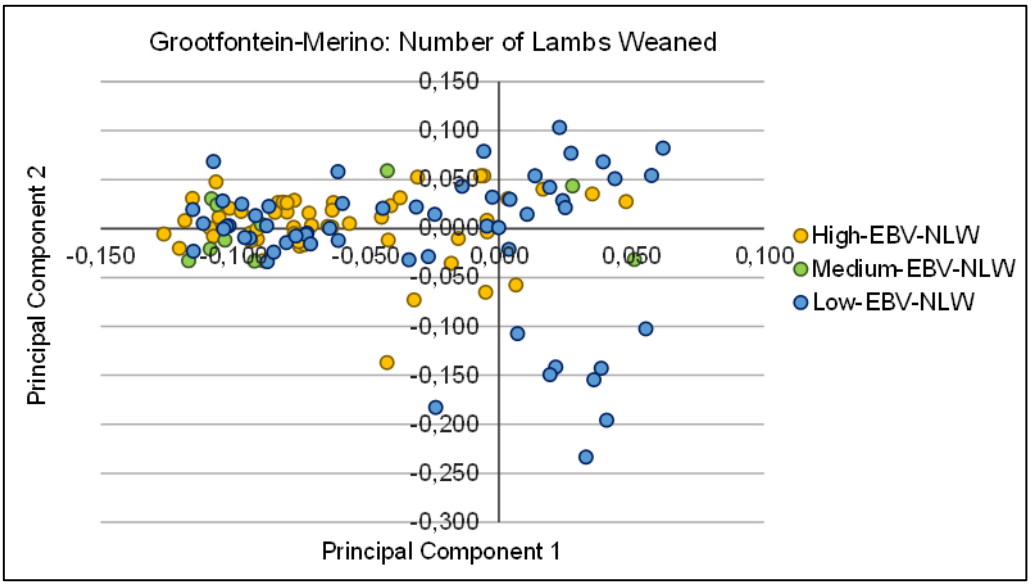

Figure A11 Genetic relationships among 127 Grootfontein sheep, which illustrate estimated breeding value groups for number of lambs weaned

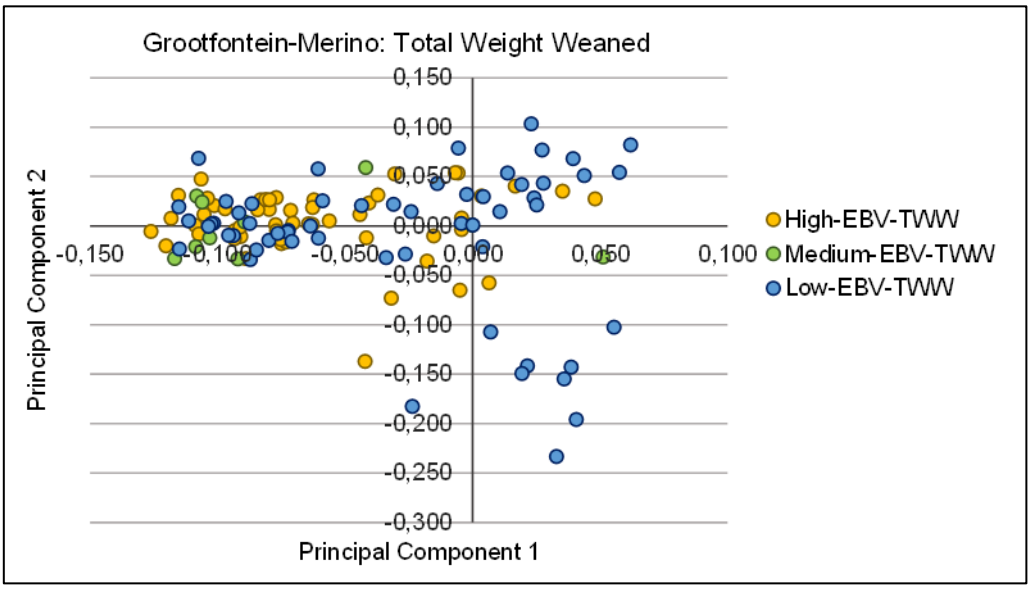

Figure A12 Genetic relationships among 127 Grootfontein sheep, which illustrate estimated breeding value groups for total weight of lambs weaned

Addendum B: Regions of SNP markers identified to be suggestive with associated genes

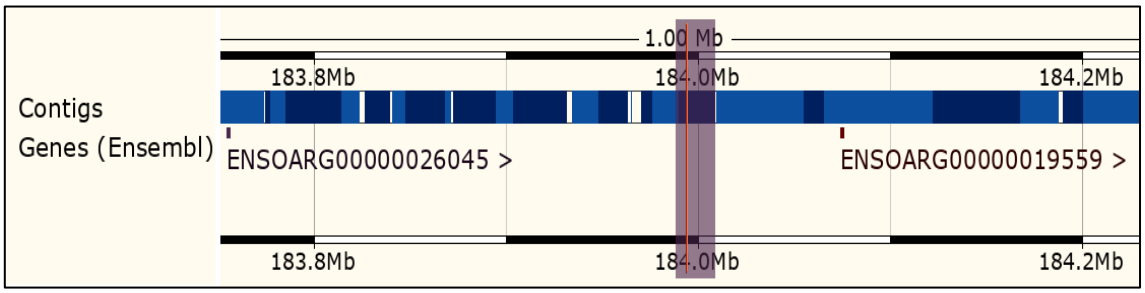

Figure B1 SNP-maker: s10640 with associated genes (orange line marks the location of the SNP marker)

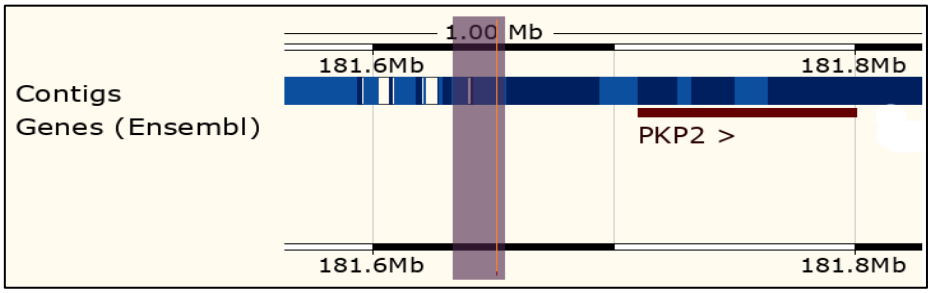

Figure B2 SNP-maker: OAR3_195631696.1 with associated genes (orange line marks the location of the SNP marker)

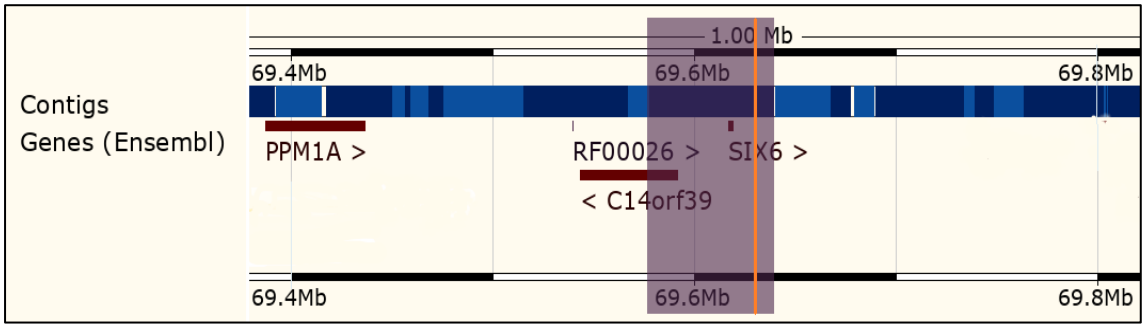

Figure B3 SNP-maker: OAR7_76295917.1 with associated genes (orange line marks the location of the SNP marker)

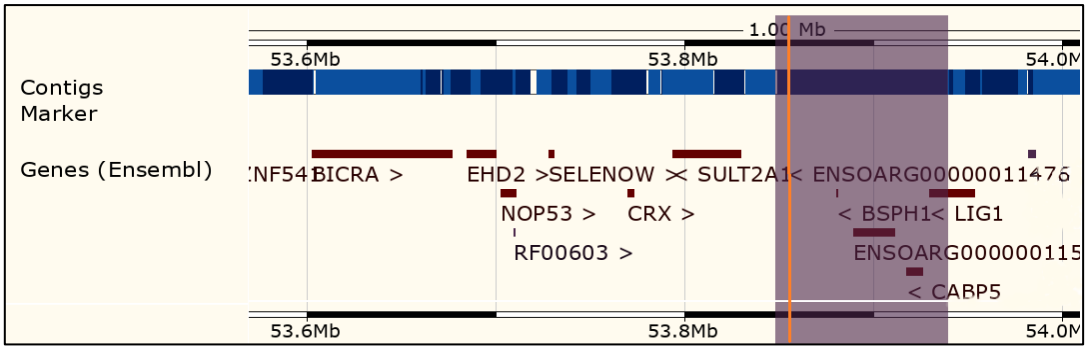

Figure B4 SNP-maker: OAR14_56900862.1 with associated genes (orange line marks the location of the SNP marker)

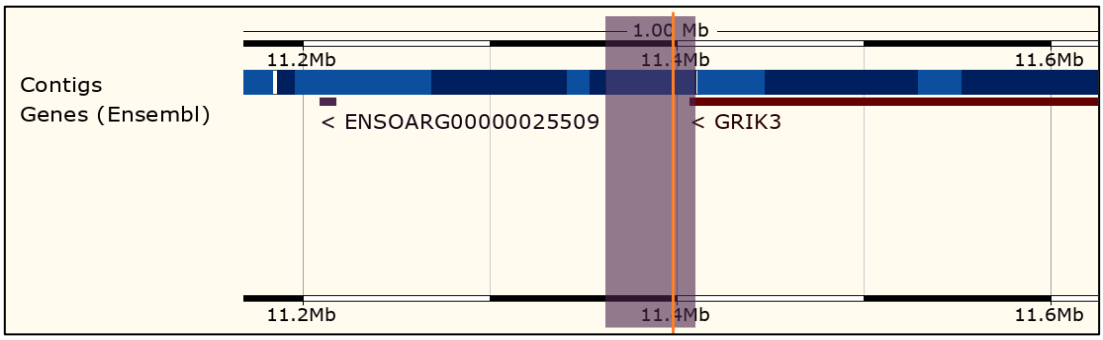

Figure B5 SNP-maker: s27280 with associated genes (orange line marks the location of the SNP marker)

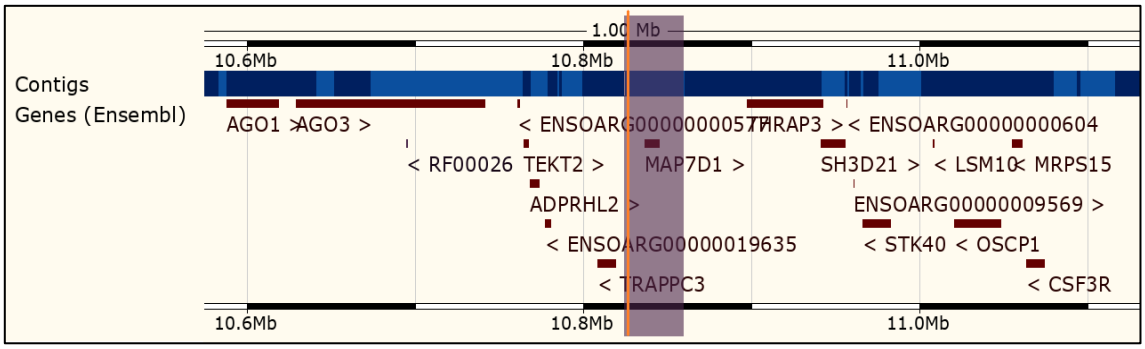

Figure B6 SNP-maker: OAR1_10554666.1 with associated genes (orange line marks the location of the SNP marker)

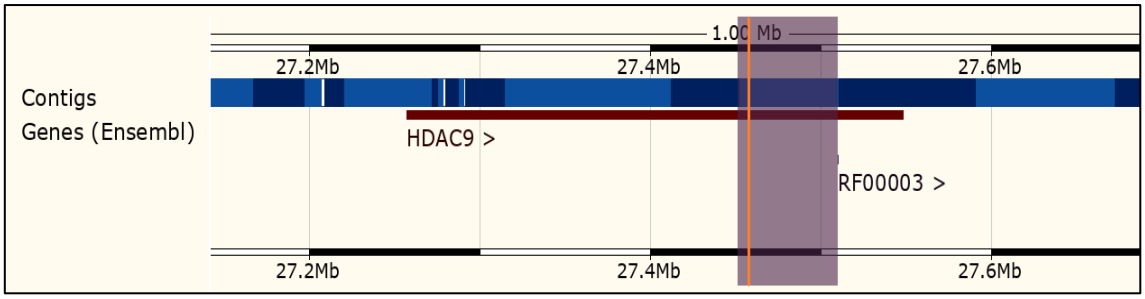

Figure B7 SNP-maker: OAR4_28838482_X.1 with associated genes (orange line marks the location of the SNP marker)

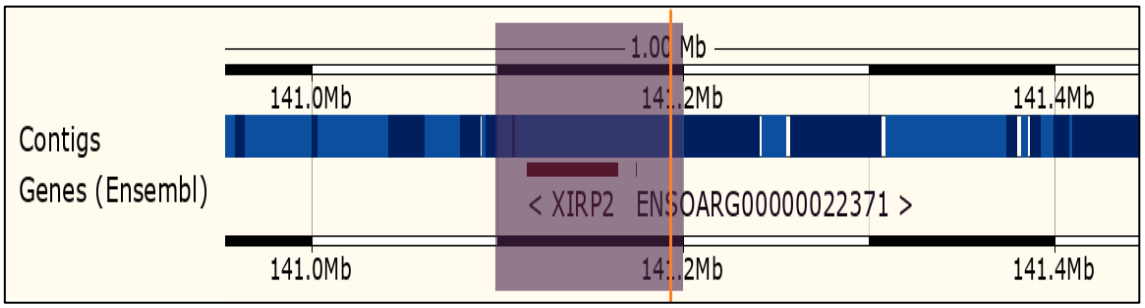

Figure B8 SNP-maker: OAR2_150119548.1 with associated genes (orange line marks the location of the SNP marker)

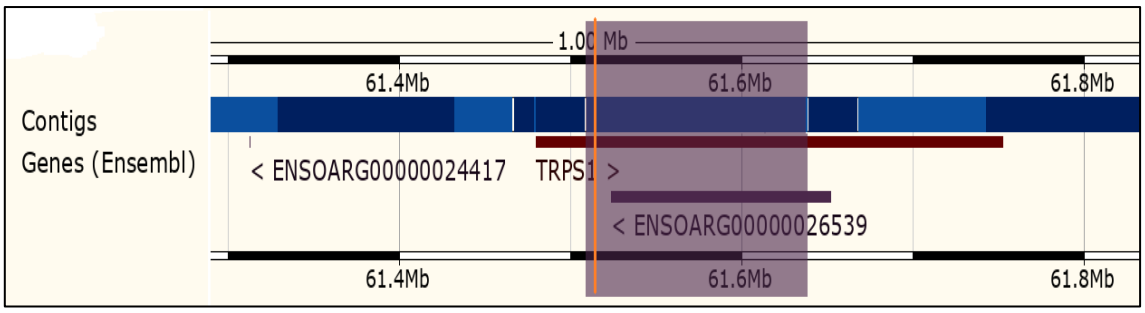

Figure B9 SNP-maker: OAR9_64654880.1 with associated genes (orange line marks the location of the SNP marker)