CHARACTERISATION OF THE DIVERGENCE OF THE ELSENBURG MERINO RESOURCE FLOCK

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ABSTRACT

The Elsenburg Merino flock has been divergently selected for the ability of ewes to rear multiple offspring since 1986. Updated genetic trends for reproduction are reported for the Elsenburg Merino resource flock. The objective was to determine whether genetic trends estimated previously for the Elsenburg Merino Resource flock changed significantly with the introduction of genetic material from the industry to the high (H) line. All analyses included the full pedigree file, consisting of 6547 individuals. Heritability estimates were 0.08 ± 0.02 for number of lambs weaned and 0.11 ± 0.02 for corrected weight of lamb weaned. The ewe permanent environment variance was estimated at 0.09 ± 0.02 and 0.11 ± 0.02 for number of lambs weaned and for corrected weight of lamb weaned, respectively. Genetic trends for the H and low (L) lines were divergent ($P < 0.05$) for all reproduction traits during the period prior to the observed breakpoints. Progress for number of lambs weaned in the H line stabilised after 1999 while a decline in response for weight of lamb weaned in the H line occurred after 2003. The change points may result from reduced selection intensity during the formation of reciprocal crossbred lines, or the introduction of unrelated industry sires in the H line.

The pedigree was analysed and inbreeding trends computed for the H and L lines with the aim to test the significance of inbreeding within the lines. The software packages used for the statistical analyses were ENDOG v4.8 and POPREP web analysis software. The average inbreeding coefficients (F) were 1.47% and 0.73% for the divergently selected H and L lines. The rate of inbreeding (ΔF) per generation was 0.5% for the H line and 0.6% in the L line. The overall rates of inbreeding per generation were different in the H and L lines but within acceptable levels. The L line, however, showed an unwanted recent increase in inbreeding that will need to be considered in future.

Since 2003, part of the Elsenburg Merino breeding flock was subjected to structured reciprocal within-breed crossing. Lamb survival traits and ewe reproductive performance of purebred (H and L) and reciprocal crosses (HxL and LxH) were evaluated using least squares analyses. Levels of heterosis were also assessed. The mean survival of the two crossbred lines was notably superior to the midparent value in absolute terms, although the contrast did not reach statistical significance ($P = 0.098$). Further research is required to establish whether this within breed heterosis for lamb survival can be exploited to decrease lamb losses. Reproduction, number of lambs born (NLB) and number of lambs weaned (NLW) in the H line was higher than in the L line ($P < 0.05$) while the two crossbred lines were intermediate and different from both the H line and the L line ($P < 0.05$) from the analyses of annual reproduction and overall "lifetime" reproduction across three lambing opportunities. Individual heterosis for annual reproduction was estimated at 2.2% for NLB, 13.8% for NLW and 8.5% for corrected weight of lamb weaned (TWW), with the estimate for NLW reaching significance ($P < 0.05$). Corresponding estimates for total production over three lambing opportunities were 8.7% for TNLB, 19.1% for TNLW and 13.8% for TTWW, with the estimate for NLW reaching significance ($P < 0.05$).

Ten RAPD markers were used to study molecular divergence between the H and L lines. Phenotypic data on the lifetime reproduction of ewes born in 1999 and 2000 indicated that reproduction in the H line ewes was markedly higher than that of L line contemporaries ($P < 0.01$). The RAPD assay, conducted on 15 ewes from each line, used eight primers and produced 87% polymorphic loci. The mean coefficient of genetic differentiation between lines (G_{ST}) was estimated to be 0.25. In conclusion, the H and L lines were shown to be divergent for genetic trends and levels of inbreeding. The derived estimates of heterosis may also be used to infer divergence between the lines and significant molecular divergence proven using RAPD assays.

PREFACE

The experimental work described in this dissertation was carried out part-time at the Institute for Animal Production, Research and Technology Development Services, Department of Agriculture, Western Cape Government under the supervision of Professor SWP Cloete and Mr EF Dzomba of the University of Kwa-Zulu Natal.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1 - PLAGIARISM

I, **Pavarni Naidoo** declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

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DECLARATION 2 – PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis:

1. Naidoo P, Cloete S, Fossey A (2005) South African Merinos divergently selected for multiple rearing ability: A preliminary study of divergence based on RAPD markers. In '16th Proceedings of the Association for the Advancement of Animal Breeding and Genetics'. Noosa, Queensland, Australia, **16**, 254-257.

The peer-reviewed article was published in the proceedings of the 16th Association for the advancement of Animal Breeding and Genetics (AAABG) Conference, 2005.
P Naidoo did research work, analysis and writing of the article. Prof SWP Cloete and Prof A. Fossey were involved in the planning of the research and revision of the article.

2. Naidoo P, Cloete S, Dzomba, E (2012) Evaluation of reciprocal crosses and pure lines of reproducing Merino ewes divergently selected for their ability to rear multiples. In 'Proceedings of the 45th National Congress of the South African Society for Animal Science'. East London, Eastern Cape, South Africa. (Poster)

The poster was presented at the 45th National Congress of the South African Society for Animal Science, 2012. P Naidoo did analysis and writing of the article. Prof SWP Cloete was involved in the planning of the research, analysis and revision of the article.

Signed: ……Date: ……

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CHAPTER 1

General Introduction

1.1 Introduction

Sheep are an important livestock species in developing countries such as South Africa. There is extensive small stock production in large arid areas of SA contributing 8.1% to the total gross value of animal products (Schoeman *et al*. 2010). The Abstract of Agricultural Statistics (2012) publishes the number of sheep as 21.325 million and the number of goats as 2.033 million in South Africa. Since the vast majority of small stock comprises of sheep, it can be assumed that the bulk economic contribution is from sheep production. An estimated 11 million of sheep farmed in South Africa are Merino-types (DAFF 2011a). The distribution of sheep per province in South Africa is represented in Fig. 1(A) and Fig. 1(B) is a map of South Africa showing the areas were Merinos are farmed. The Merino industry produces wool for the international market and meat for local consumption, with mutton usually providing 60 to 70% of gross income to a commercial Merino enterprise (Olivier 1999) depending on fluctuations in the meat: wool price ratio. The result of this dual purpose enterprise is that South African Merino breeders have adopted a strategy that enhances both wool and meat production to increase overall profitability.

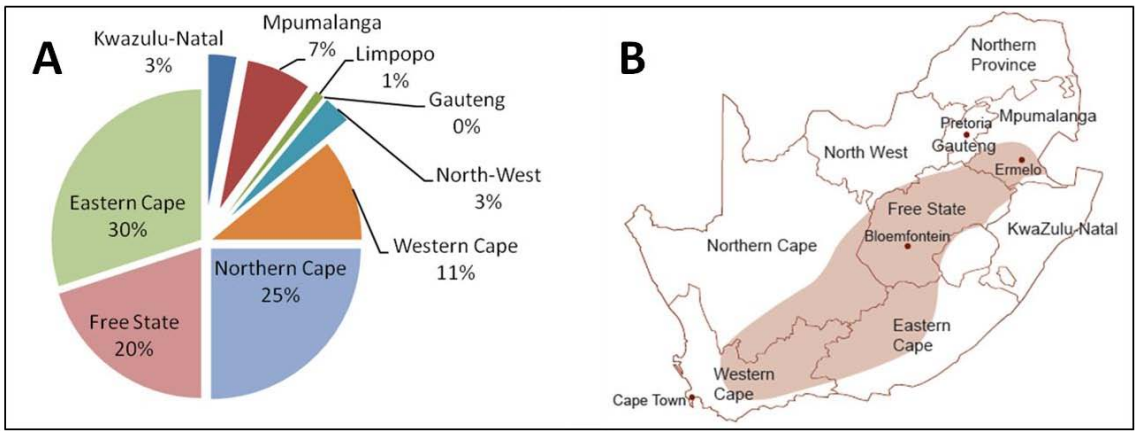

Figure 1.1 (A) Percentage distribution of sheep per province in South Africa 2011 (NDA 2011) and (B) Map of South Africa showing the geographical distribution of the Merino breed (NDA 2008)

However, a steady decline in the number of Merinos from 25 million in 1970 to the current 11 million sheep (DAFF 2011a) combined with the rapid expansion of the human population in South Africa has led to shortages of mutton in the local market (DAFF 2011b). The production of meat is determined by fertility thus good reproduction rates (≥90% lambs weaned per ewe mated) is a critical consideration for the Merino industry to remain lucrative (Olivier 1999).

1.2 Motivation

In response to a low lambing percentage of 74% for South African Merinos reported by de Klerk (1983) a divergent selection experiment for and against the ability of ewes to rear multiples (net reproduction rate) was initiated in 1986 (Cloete and Scholtz 1998). Genetic responses for reproduction were marked but slightly asymmetric, with responses in the High (H) line about twice those in the Low (L) line (Cloete *et al.* 2004). A landmark finding was that lamb survival was improved in the H line relative to the L line as a result of the improved survival of multiples (Cloete and Scholtz 1998; Cloete *et al.* 2005; Cloete *et al.* 2009b). This observation is important, as increased lamb mortality rates are expected with an increase in the proportion of multiples (Haughey 1989). The study of the divergent selection of the Elsenburg Merino flock determined that sustained genetic progress in lamb survival is possible if directed selection is applied to a correlated trait such as the ability of ewes to rear multiples (Cloete *et al.* 2009a).

1.3 Study objectives

This dissertation contributes to knowledge of the well-established Elsenburg Merino resource flock. In Chapter 2, a literature review presents the background to this study. Updated genetic trends for reproduction traits in the Elsenburg Merino resource flock are reported in Chapter 3. Chapter 4 is a study of inbreeding and a pedigree analysis of the selection lines. Chapter 5 is an evaluation of the reciprocal cross and pure lines of the divergently selected Elsenburg Merinos. An investigation of the molecular divergence between the H and L lines using random amplified polymorphic DNA (RAPD) assays is detailed in Chapter 6. The general discussion, conclusions and recommendations of the study are stated in Chapter 7.

Against this background, the present study aims to characterise the divergence of the Elsenburg Merino resource flock by:

(i) updating genetic trends for reproduction traits in the Elsenburg Merino resource flock;
(ii) estimating inbreeding within the lines;
(iii) evaluating the reciprocal cross and pure lines of the divergently selected Elsenburg Merinos;
(iv) performing an investigation of the molecular divergence between the H and L lines using RAPD assays.

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CHAPTER 2

Literature Study

2.1 Introduction

The domestic sheep *Ovis aries* (karyotype 2n=54 (Bunch and Foote 1977; Maddox and Cockett 2007)) has adapted to thrive in a diverse range of environments as a result of their domestication and subsequent selection over several millennia. The RBG-banded domestic sheep karyotype and the taxonomic classification of domestic sheep is presented in Fig. 2.1 and Table 2.1.

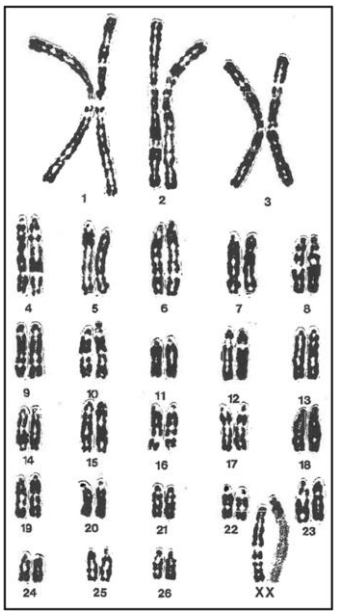

Table 2.1 Taxonomic classification of the domestic sheep. *(Adapted from Franklin (1997))*

Kingdom:	Animalia
Phylum:	Chordata
Class:	Mammalia
Order:	Artiodactyla
Suborder:	Pecora (Ruminantia)
Family:	Bovidae
Subfamily:	Caprinae
Genus:	*Ovis*
Species:	*aries*

Figure 2.1 Karyotype of a female domestic sheep *Ovis aries* (reprinted from Broad *et al.* 1997)

The Merino is one of the oldest known domesticated sheep breeds. It is accepted that Merinos were derived from an ancestor *Ovis aries vignei* in Phoenicia and Carthage. The breed reached Spain through the Moors, with the earliest prehistoric recordings of the Merino breed found in the South of Spain ca. AD 700 – 1200, although the exact origin of the breed is unknown (McKee 1913; Hanekom 1957; Maijala 1997; Diez-Tascon *et al.* 2000; Chessa *et al.* 2009; Kijas *et al.* 2012). South Africa became the first country outside Europe to obtain Merino sheep in 1789 (Hanekom 1957). Merinos are still numerically the largest sheep breed in the country.

The genetic improvement of meat production i.e. net reproduction rate, defined as total weight of lamb weaned per breeding ewe, has thus become imperative to the Merino industry (Cloete and Olivier 1998; Ingham and Ponzoni 2001; Duguma *et al.* 2002; Apps *et al.* 2003; Cloete *et al.* 2004; Olivier and Cloete 2007).

2.2 Reproduction in sheep

The profitability of the sheep industry is highly dependent on the level of lamb production, thus the study of reproduction is fundamental to the improvement of the industry. Research into reproduction in sheep has been prolific and literature on the various components involved abundant. There are large differences between breeds, and between ewes and between rams within breeds, and within flocks for measures of reproductive efficiency (Purvis and Hillard 1997; Snyman *et al.* 1998). Although this variation seems to allude to the possibility for changing reproductive processes by selection, a relatively small part of the phenotypic variation is attributed to genetic differences, thus achieving permanent change of reproduction by genetic selection has proven to be a challenge. Some specific challenges to the genetic improvement of reproduction include gender-limited expression, computational difficulties owing to the discrete nature of data and low levels of genetic variation (Purvis & Hillard 1997). Reproduction is also expressed relatively late in the life of an animal.

The efficiency of lamb production is determined by two important factors: reproduction and survival rate (Olivier *et al.* 2001). The reproductive rate in the ewe is complex and influenced by several components, each with genetic variation: puberty (defined as the time at which reproduction first becomes possible); the combined effect of oestrus expression, seasonality and fertility (which defines the fertility level of a flock at a particular time); ovulation rate (a fundamental polygenic component of prolificacy and reproductive efficiency in any flock); embryonic mortality; and litter size (lambs born per ewe lambing) (Purvis and Hillard 1997). After birth of the offspring, lamb survival also becomes an important component of net reproduction rate and will be discussed in detail later.

Table 2.2 Literature sources for studies of the components of reproductive rate

Reproductive rate component trait	Literature source
Puberty	Schott *et al.* 1939; Dickerson and Laster 1975; Hare and Bryant 1985; Kinder *et al.* 1995; Sunderland *et al.* 1995; Smith and Clarke 2010
Oestrus expression, seasonality and fertility	Thimonier 1981; Aboul-Naga *et al.* 1985; Foote 2003*; Notter 2002; Notter and Cockett 2005*; Rosa and Bryant 2003*
Ovulation rate	Davis and Kelly 1983; Webb and Gauld 1985; Carrick 1990; Montgomery *et al.* 2001; Davis 2005; McNatty *et al.* 2005; Fabre *et al.* 2006
Embryonic mortality	Dutt 1963; Thwaites 1967; Gunn and Doney 1975; Wilmut *et al.* 1985; Thatcher *et al.* 1994; Kaulfuss *et al.* 1997
Litter size	Bradford 1985; Hanrahan and Quirke 1985; Bradford *et al.* 1986; Gama *et al.* 1991; Bittante *et al.* 1996; Analla *et al.* 1998; Everett-Hincks *et al.* 2005

* Reviews

2.2.1 Introduction to QTLs and a review of major genes for fecundity in sheep

Production traits in domestic livestock are the result of multiple genes acting in an additive manner and are the basis of many breeding strategies. Genetic progress in reproduction traits is expected to be slow, as these traits have low heritabilities. Whereas traditional quantitative genetics uses phenotypic information to identify animals with superior genes; its extension to include information from molecular genetics techniques aims to find and exploit gene loci which have major effect on quantitative traits (Falconer and Mackay 1996; Crawford *et al.* 2000; Snustad and Simmons 2000; Weller 2009). These quantitative trait loci (QTL) have typically been targeted by their linkage to major genes of interest. In contrast to conventional breeding where the genetic basis is irrelevant to genetic progress, marker assisted selection relies on the existence of QTL of major effect (Franklin and Mayo 1998; Mayo and Franklin 1998; Dekkers 1999; Dekkers and Van der Werf 2007). The identification and use of specific major genes may facilitate an increased rate of genetic improvement in production traits (Davis *et al.* 2001c). QTL mapping in crosses between genetically diverse lines has detected several QTL for traits of economic importance in livestock species (Rothschild *et al.* 1996; Heyen *et al.* 1999; Rohrer *et al.* 1999; Crawford 2001; Walling *et al.* 2002; Notter and Cockett 2005; Purvis and Franklin 2005; Raadsma *et al.* 2009). However, Dekkers (2004, 2007) argues that although industry programs increasingly use molecular genetic information, the extent of use has not lived up to initial expectations.

The high genetic variation in ovulation rate in sheep is consistent with segregating major genes (Hanrahan *et al.* 2004). The search for major genes in the pathway controlling ovulation rate in sheep has been successful (Table 2.3) and has been comprehensively reviewed by Montgomery *et al.* (2001); McNatty *et al.* (2004); Davis (2004); Davis (2005) and Fabre *et al.* (2006).

The first observations of a putative major gene for prolificacy in relation to inheritance patterns and DNA testing were made in a Booroola Merino flock, notable for its exceptional litter size at birth (Piper and Bindon 1982; Fogarty 2009). They hypothesised that a major gene affecting litter size contributes to the enhanced fecundity of the Booroola Merino; which was indicated by the Mendelian pattern of inheritance with additive effects on ovulation rate and dominance for litter size. They concluded that the basic reproductive biology of the Booroola Merino differed significantly from other high fecundity breeds and that the increased fecundity may be attributed to a different genetic basis (Piper and Bindon 1982).

These speculations were confirmed by three independent groups of researchers almost simultaneously in 2001 (Mulsant *et al.* 2001; Souza *et al.* 2001; Wilson *et al.* 2001). The research they published announced the discovery that the inheritance of prolificacy observed in the Booroola Merinos was the result of a mutation in the bone morphogenetic protein 1B receptor (*BMPR-1B*) and increased ovulation rate in both heterozygous and homozygous ewes. A DNA test developed to detect the gene led to the finding that the Booroola gene originated in dwarf Garole sheep in northeast India and was imported into Australia in 1792; the highly prolific Booroola Merino strain is highly likely to be a direct descendant of the Garole sheep (Davis *et al.* 2002b).

Table 2.3 A summary of major genes for fecundity that affect ovulation rate that have been identified in sheep

Gene Name	Gene (chromosome)	Allele	Founder breed	References
Booroola	*BMPR-1B* (6)	*FecB*^{B}	Merino, Garole, Javanese, Hu, Han	Davis *et al.* 1982; Piper and Bindon 1982; Montgomery *et al.* 1994; Mulsant *et al.* 2001; Souza *et al.* 2001; Wilson *et al.* 2001; Davis *et al.* 2002b; Davis *et al.* 2006; Fogarty 2009; Polley *et al.* 2010; Chu *et al.* 2011
Inverdale	*BMP-15* (X)	*FecX*^{I}	Romney	Davis *et al.* 1991; Galloway *et al.* 2000; Davis *et al.* 2001a
Hannah	*BMP-15* (X)	*FecX*^{H}	Romney	Galloway *et al.* 2000; Davis *et al.* 2001a; McNatty *et al.* 2001
Belclare	*BMP-15* (X)	*FecX*^{B}	Belclare	Owen *et al.* 1990; Hanrahan 1991; Hanrahan *et al.* 2004
Galway	*BMP-15* (X)	*FecX*^{G}	Belclare, Cambridge	Hanrahan *et al.* 2004
Lacaune X-linked	*BMP-15* (X)	*FecX*^{L}	Lacaune	Bodin *et al.* 1998; 2002; 2003
High Fertility	*GDF-9* (unknown)	*FecG*^{H}	Belclare, Cambridge	Hanrahan *et al.* 2004
Thoka	*GDF-9* (5)	*FecI*^{I}	Icelandic, Olkuska, Bell-Ile	Jonmundsson and Adalsteinsson 1985; Martyniuk and Radomsa 1991; Mahler and Le Chere 1998; Nicol *et al.* 2009
Woodlands	(X)	*FecX2*^{W}	Coopworth	Davis *et al.* 2001b; Davis *et al.* 2002a

The Booroola gene has been introgressed from Merino sheep to various other breeds around the world, including the Dohne Merino in South Africa (Davis 2008). A gene marker is available for the Booroola gene using molecular markers linked to the favourable allele to identify carriers to increase the accuracy of selection of breeding animals compared to phenotypic selection alone. However, marker-/gene-assisted selection (GAS/MAS) have not been widely applied in the commercial sheep industry (Dominik *et al.* 2007; Van der Werf 2007). The reason for this is the fact that the high levels of fecundity in the homozygous carrier ewes become very difficult to manage as prolific ewes require intensive care during lambing. This is not practical in extensive systems thus resulting in high lamb losses (Dekkers and Van der Werf 2007).

Along with the investigations of the Booroola *FecB BMPR-1B* gene; other researchers published results of putative prolificacy major genes in other flocks. A mutation in the oocyte-derived growth factor bone morphogenetic protein 15 gene (*BMP15*) and the closely related growth differentiation factor 9B (*GDF9B*), belonging to the transforming growth factor-β (TGFβ) superfamily, was found to be responsible for increased ovulation rate and infertility in a dosage-sensitive manner in New Zealand Inverdale sheep carrying the X chromosome linked *FecX*^{I} gene (Galloway *et al.* 2000; McNatty *et al.* 2004).

The high ovulation rate with extreme variation among individuals that characterise the United Kingdom's Cambridge and Belclare sheep is consistent with segregating major genes (Owen *et al.* 1990). Mutations of the *BMP15* and *GDF9* genes, different to the Inverdale *FecX*^{I}, are present in the Cambridge flock, designated *FecX*^{G} and *FecG*^{H}, respectively (Hanrahan *et al.* 2004).

An exceptionally high litter size of greater than 4 lambs, as well as the high heritability coefficient of 0.4 indicated the strong possibility of the presence of a major gene resulting in prolificacy in the French Lacaune sheep breed (Bodin *et al.* 1998). Another sheep breed from France, Belle-Ile with high ovulation rate of 2.54; a high litter size of 2.23; as well as highly repeatable performance, characterised the particular flock as one in which a major gene was segregating (Bodin *et al.* 2002; 2003; Davis 2005).

The Thoka gene is a single gene found in Icelandic sheep, with an effect of two genetic standard deviations on the number of lambs born (Jonmundsson and Adalsteinsson 1985). Prompted by studies of the major gene segregating in Booroola sheep the latter authors searched the records of a prolific line of Icelandic sheep. The authors traced five rams from the Borgarhoefn flock that appeared to carry the high fecundity gene back to an individual ewe named Thoka, the first known carrier of the Icelandic high fecundity gene (Jonmundsson and Adalsteinsson 1985). A single base-pair mutation in *GDF9* has been linked to increased fecundity in heterozygous ewes and infertility in homozygotes (Nicol *et al.* 2009), a pattern similar to that observed by the Inverdale gene.

In 1999, the inheritance pattern of another X chromosome linked, maternally imprinted (that is, only expressed from the paternally inherited allele) gene influencing ovulation rate was discovered in New Zealand Coopworth sheep, the Woodlands prolificacy gene (*FecX2*^{W}) (Davis *et al.* 2001c). One copy of the Woodlands gene increases litter size by about 0.25 lambs per ewe lambing.

In summary, mutations that increase ovulation rate have been discovered in the *BMPR-1B*, *BMP15* and *GDF9* genes. Sheep are now considered a model species to identify genes involved in mechanisms controlling ovulation rate (Fabre *et al.* 2006). Vast differences in reproductive capacity between the Elsenburg Merino H and L lines can conceivably be attributed to the influence of one or more QTL's with a moderate influence on reproduction and fitness. The detection of a DNA marker that is linked to the trait will facilitate the long-term goal of developing a probe for the detection of these putative QTL's. The initial step towards this objective is to determine molecular divergence between the H and L lines using Randomly Amplified Polymorphic DNA (RAPD).

2.3 Lamb survival and maternal effects

Reproduction is one important factor that determines the efficiency of sheep production; the other critical factor is that of the lamb survival rate, with aspects of reproduction and lamb survival being inter-related. Lamb mortality is regarded as a major constraint to efficient sheep production, which is not accounted for by enhancing

prolificacy alone (McGuirk 1982; Alexander 1988; Haughey 1991). The benefit of increasing the number of lambs born (net reproduction rate) by improving fertility may be negated by the reduction in survival due to an increase in multiple births (Everett-Hincks and Cullen 2009; Hatcher *et al.* 2010b). Thus strategies that aim to increase net reproduction rate should be accompanied by strategies to improve lamb survivability (Brien *et al.* 2010).

Lamb survival is has two components: the lamb's own capacity to survive (direct genetic) and the rearing ability of the dam which has both genetic and environmental components (Piper *et al.* 1982; Safari *et al.* 2005a). Furthermore, factors that affect lamb survival include birth weight, birth type (single, twins, triplets), dam age, year effects and sex (Hatcher and Safari 2009). It is widely documented that as litter sizes increase, the average birth weight declines and lamb survival decreases (Dwyer 2008; Everett-Hincks and Dodds 2008; Hatcher and Safari 2009).

Several publications have reported that the heritability of lamb survival in sheep is effectively zero (Fourie and Cloete 1993; Fogarty and Gilmour 1998; Snyman *et al.* 1998; Safari *et al.* 2005b; Hatcher *et al.* 2010a). This, as well as the binomial distribution of survival traits and generally low levels of genetic variation (Cloete *et al.* 2009b) has led some researchers to the conclusion that the improvement of lamb or litter survival through selection would be ineffective (Morris *et al.* 2000; Everett-Hincks and Cullen 2009) and suggest that modifications to the environment via management practices during lambing contributed more to lamb survival. Studies have thus attempted to improve lamb survival by enhancing the environmental effects through management instead of breeding for better maternal genotypes (Brand *et al.* 1985). Nevertheless, the optimisation and intensification of management during the perinatal period as a possible means of improving lamb survival and ewe rearing ability is unfeasible due to the expense and lamb losses that still occur despite these practices (Cloete and Scholtz 1998; Dwyer 2008).

However, besides heritability, genetic gain also depends on selection intensity, generation interval and genetic variation (Brien *et al.* 2010; Hatcher *et al.* 2010b). Investigations using threshold models have reported considerable genetic variation on the underlying liability scale for lamb survival (Welsh *et al.* 2006; Riggio *et al.* 2008) which may compensate for the low heritability of lamb survival on the normal scale. The genetic selection of sheep to improve lamb survival can provide a better solution, with previous studies illustrating realised successes to selection for correlated traits like ewe rearing ability (Haughey 1983; 1991; Cloete and Scholtz 1998; Gudex *et al.* 2005).

Maternal behaviour or maternal genetic effects (defined as the influence of the mother on her offspring apart from through the direct effect of the genes transmitted (Bradford 1972)) have long been recognized to influence the survival of the offspring. Alexander (1988) described the influence of maternal effects on lamb survival and growth as observed in the dam's nesting, parturition, grooming of the newborn, suckling behaviour, bonding, spatial association with the offspring, defence against predators (or predator substitutes, like a sheep dog) and care of multiple births. Maternal influences are most evident during the lamb's early development and decline as the lamb matures (Snyman *et al.* 1995; Cloete *et al.* 2003b). The variation between

females in maternal performance may arise from either genetic or environmental causes (Bradford 1972).

Improvement of maternal behaviour should be associated with an increase in the composite trait litter weight weaned. The selection on a composite trait rather than on component traits such as lamb survival to improve overall production and ewe productivity in sheep has been advocated by Snowder and Fogarty (2009). The improvement of maternal performance has been identified as a further, largely untapped, resource to exploit for breeders to benefit from the genetic merit of superior ewes (Fogarty *et al.* 2000).

2.4 The Elsenburg Merino Resource flock

Since 1986, prompted by a low reproduction rate observed in the South African Merino industry i.e. lambing percentages of 74% (de Klerk *et al.* 1983), two lines of Merino sheep were established by divergent selection from the same base population using maternal ranking values for number of lambs reared per joining (Cloete and Scholtz 1998; Cloete *et al.* 2004; Cloete *et al.* 2009a). The lines were derived from ewes descended from a Merino line selected for increased wool secondary: primary follicle ratio (Heydenrych and Vosloo 1984). The selection procedures for the lines has been described in detail by Cloete *et al.* (2004; 2009a). The flock was initially kept at the Tygerhoek experimental farm near Riviersondereind (34°8´S and 21°11´E) from 1986 to 1992. The lines were transferred to the Elsenburg experimental farm near Stellenbosch (33°51´S and 18°50´E) at the end of 1992 where they are currently maintained (Cloete *et al.* 2004).

Several major studies have been published by Professor SWP Cloete and associates on the two lines divergently selected for (High line, H) and against (Low line, L) ewe multiple rearing ability (Cloete and Durand 1994; Cloete and Olivier 1998; Cloete and Scholtz 1998; Cloete *et al.* 2003a; 2003b; 2004; 2009a). Literature estimates for lamb traits and ewe reproduction traits are presented in Table 2.4. Multiple rearing ability has been defined as the total weight of lamb weaned per breeding ewe (net reproduction rate) but has also been suggested as number of lambs reared (Olivier 1999; Cloete *et al.* 2004; 2009a).

A seminal finding on lamb survival in relation to lambing and neonatal behaviour in the divergently selected lines found an improvement in the H line mostly attributed to the improved survival of multiples (Cloete and Scholtz 1998). It was noted that selection for ewe multiple rearing ability did not negatively affect the survival of lambs in spite of the expectation that an increase in multiple births generally result in higher mortality rates. The sustained genetic improvement of lamb survival in the H line has been ascribed to a correlated response to directed selection for the ability of ewes to rear multiples in the Elsenburg Merino resource flock (Cloete and Scholtz 1998; Cloete *et al.* 2004; 2009a; 2009b).

Behavioural observation trials have also been conducted on the H and L Merino lines. Behavioural differences beneficial to lamb survival in the H line, were reported by

(Cloete *et al.* 1998; Cloete and Scholtz 1998; 2001; Cloete *et al.* 2002; 2005), which supports the idea that behavioural adaptations may play a role in selection responses for lamb survival (Cloete *et al.* 2002; 2005) (Table 2.4).

Table 2.4 Summary of literature estimates for lamb traits and ewe reproduction traits in the H and L lines of the Elsenburg Merino resource flock.

Trait	H Line	L Line	Reference
Lamb traits			
Survival to weaning	0.776	0.690	(Cloete *et al.* 2009a)
Lamb birth weight (kg)	3.90 ± 0.09	3.80 ± 0.09	(Cloete *et al.* 2003b)
Birth coat score	3.20 ± 0.12	3.24 ± 0.14	(Cloete *et al.* 2003b)
Mortality to weaning (singles)	0.173	0.199	(Cloete and Scholtz 2001)
Mortality to weaning (multiples)	0.251	0.404	(Cloete and Scholtz 2001)
Reproduction traits			
No. of lambs born	1.50 ± 0.06	1.32 ± 0.07	(Cloete *et al.* 2004)
No. of lambs weaned	1.16 ± 0.05	0.91 ± 0.05	(Cloete *et al.* 2004)
Av. weight of lamb weaned (kg)	23.9 ± 1.2	18.0 ± 1.3	(Cloete *et al.* 2004)

In conclusion, the study of selection responses to divergent selection for the ability of ewes to rear multiples has revealed a marked, slightly asymmetric response in overall reproduction in the lines selected (Cloete *et al.* 2004; 2009a). Two distinct lines were established: the H line composed of Merinos with desirable and the L line with undesirable reproduction traits. The reciprocal crosses of the lines have been available for evaluation since 2003. This offers a unique opportunity to study heterosis based on within breed crosses of distinct lines for which literature is scarce. Non-additive variation is of particular importance for lowly heritable fitness traits which may benefit from heterosis and is described in detail later. It would also be advantageous to explore the divergence between the selection lines further in order to potentially identify superior alleles linked to desirable reproduction traits.

This study contributes to previous investigations of this flock by updating genetic trends for reproduction traits, conducting an analysis of the pedigree and inbreeding within the lines, evaluating the reciprocal crosses for lamb survival and ewe reproduction traits and finally, by assessing the divergence between the lines at a molecular level using a preliminary RAPD assay. A review of published literature pertinent to the present study of inbreeding, heterosis and molecular divergence assays follows.

2.5 Inbreeding

Inbreeding and its deleterious effects have been of concern since studies by Darwin in the 18th century (Charlesworth and Charlesworth 1987; Hedrick 1994). Inbreeding is defined as the mating between animals that are related by descent from a common ancestor, subsequent to a defined baseline population (Lacy 1995).

The inbreeding coefficient (*F*) was described independently using different arguments by the pioneers of population genetics: Wright (1921), Haldane (1937), Malecot (1948) and Fisher (1949) (reviewed by Cockerham and Weir 1968; Crow 2010; Nagylaki 1989). However, the fundamental concept of identity by descent which is the basis of the current understanding of the concept of inbreeding was independently discovered by Cotterman and Malecot in the 1940s and in recent years it has become standard practice to measure *F* by Malecot's probability analysis method (Crow 2010). Two genes that have originated from the replication of one single gene in a previous generation may be called identical by descent. Homozygotes of identical genes are described as identical homozygotes or autozygous. Thus, the inbreeding coefficient is defined as the probability that the two genes at any locus in an individual are identical by descent (i.e. autozygous) (Van Vleck *et al.* 1987; Falconer and Mackay 1996).

The inbreeding coefficient (*F*) can be calculated through knowledge of the pedigree or estimated by determining allele frequencies after the detection of genetic polymorphisms. It is important to note that the degree of relationship expressed in the inbreeding coefficient is a comparison between the study population and a defined base population which contain all available genetic variation heritable by descendants (Falconer and Mackay 1996). The average inbreeding coefficient of a population is commonly used as a measure of its level of homozygosity (Gutierrez *et al.* 2003).

The genetic improvement of livestock involves a balance between intensive selection of a small number of parents in the current generation and the maintenance of quantitative genetic variation to facilitate response in future generations (Weigel 2001). The practice of maximizing selection responses using animal model best linear unbiased predictors (BLUP) of breeding values may lead to more closely related individuals being chosen for mating with the possibility of inbreeding, especially in a small population (Weigel 2001; McParland *et al.* 2007; Swanepoel *et al.* 2007; Ceyhan *et al.* 2011). Since most experiments on selection involve relatively small base populations, it is accepted that a certain level of inbreeding may occur during the progress of selection. Heterozygosity and allelic diversity in small, closed, selected populations may be lost at a rapid rate (Falconer and Mackay 1996). It is essential to maintain genetic variation and to avoid inbreeding depression in the long term (Bijma *et al.* 2001).

An outcome of efficient selection programmes is that inbreeding is accumulating rapidly in most commercial livestock species (Weigel 2001). The level of inbreeding is influenced by the ratio of males to females, reproduction ability, mating systems and population size (Norberg and Sorensen 2007; Ceyhan *et al.* 2011). It is therefore important to have knowledge of the effective size of a population (N_e) and the rate of inbreeding (ΔF), which is the relative increase in inbreeding by generation (Boichard *et al.* 1997). Wright (1923) defines the effective size of a population as the size of an idealised population which would give rise to the rate of inbreeding (Boichard *et al.* 1997; Gutierrez *et al.* 2003).

A concerning consequence of inbreeding is the phenomenon of inbreeding depression. Inbreeding depression occurs because inbreeding increases the probability that an individual will be homozygous for segregating deleterious recessive alleles and

homozygous at loci exhibiting overdominance. This may result in a reduced production or fitness of inbred animals. In the presence of inbreeding depression, asymmetry in the rate of response of the selected character would be expected (Falconer and Mackay 1996; Bijma *et al.* 2001; Slate *et al.* 2004; Norberg and Sorensen 2007).
Inbreeding depression in sheep has been found for several traits and has been reviewed by Lamberson and Thomas (1984). Inbreeding studies of highly inbred lines found inbreeding depression for lamb weights and litter size (Erckanbrack and Knight 1991; Analla *et al.* 1998). Inbreeding depression has been estimated for birth weight and weaning weight (Norberg and Sorensen 2007; Van Wyk *et al.* 2009).

Inbreeding has been studied in the Dohne Merino breed in South Africa (Swanepoel *et al.* 2007) as well as the Elsenburg Dormer sheep stud (Van Wyk *et al.* 1993; Van Wyk *et al.* 2009). The average inbreeding coefficients in the H and L lines were estimated as below 2.5% in a preliminary analysis (Cloete 2002). However, a complete pedigree analysis of the Elsenburg Merino flock will be valuable in properly characterising the divergently selected lines in terms of the level and rate of inbreeding, as well as determining the effective population size of this important genetic resource.

2.6 Heterosis

Heterosis, a term coined by Shull in 1914 (Shull 1948), describes the observation that when sufficiently divergent lines are crossed the performance of their F1 offspring is often notably superior to the average of the parents (Sheridan 1981; Whitlock *et al.* 2000). This description does not account for the origin of the observed heterosis, for which there are several hypothesised mechanisms (Crow 1948; Willham and Pollak 1985). The earliest scientific investigations of heterosis were conducted by East (1908), Shull (1908), and Bruce (1910); with developments on the topic reviewed by Jones (1917) and East (1936) and recently by Birchler *et al.* (2010). This study defines heterosis as the difference between the average performance of the crossbred individuals and the mean of the two parent populations which is commonly known as midparent heterosis and expressed as a percentage of the midparent (Falconer and Mackay 1996; Mavrogenis 1996).

The genetic basis of crossbreeding arises from additive effects due to the averaging of merit in the parental stocks and non-additive effects observed as heterosis (Kinghorn and Atkins 1987). This is the amount by which merit in crossbreds deviates from the additive component (Kinghorn 1987). Heterosis is commonly attributed to genetic interactions within loci (dominance) and interactions between loci (epistasis) (Swan and Kinghorn 1992). Levels of heterosis are often higher in traits with lower heritability, such as reproductive fitness, which makes crossbreeding a potentially useful tool to achieve commercial benefits in such traits by exploiting non-additive variance (Willham and Pollak 1985).

Nitter (1978) reviewed literature values for heterosis in crossbred meat sheep. This review indicated that crossbred ewes had an advantage in weight of lamb weaned reared per ewe exposed mainly due to improved fertility and prolificacy. Several other

studies have also estimated heterosis for reproduction traits in sheep breed crosses. Literature estimates for individual heterosis are summarised in Table 2.5.

Table 2.5 Literature values for heterosis in number of lambs born (NLB), number of lambs weaned (NLW) total weight of lamb weaned (TWW) and average lamb weaning weight (AWW) of sheep, as derived from crosses among breeds or from crosses among lines within breeds

Type of study and resources used	Trait			
	NLB*	NLW*	TWW	AWW
Across breeds				
5 Pure breeds, 20 crosses[1]	n.a.	n.a.	24.7 (-24 to 54)	n.a.
3 pure breeds and crosses[2]	n.a.	n.a.	6.7	n.a
3 pure breeds, reciprocal crosses[3]	1.8 (0.6 to 3.2)	n.a.	13.5 (11.6 to 16.7)	n.a.
Merino and Border Leicester, reciprocal cross[4]	4.6	n.a	n.a.	n.a.
Review of literature[5]	11.5	14.7	n.a.	6.3
5 pure breeds, various crosses[6]	17.9 (11.0 to 21.5)	40.2 (35.5 to 44.6)	39.1 (34.7 to 43.4)	n.a.
3 pure breeds, reciprocal crosses[7]	3.3 (3.3 to 3.4)	n.a.	13.8 (9.2 to 17.9)	2.8 (-0.1 to 6.0)
Finnish Landrace and Ile de France, reciprocal cross[8]	9.3	14.7	n.a.	1.4
Finnish Landrace and Lamon, reciprocal cross[9]	n.a.	n.a.	4.4	0.6
Chios and Awassi, reciprocal cross[10]	-10.5	-4.2	4.1	n.a.
3 pure breeds, 6 crosses[11]	6.2	11.6	4.1	n.a
Between bloodlines within breeds				
Merino bloodlines[12]	7.0	10.4	n.a.	2.8
6 Merino bloodlines[13]	< 1	n.a.	n.a.	n.a.

References – breed crosses: [1]Sidwell and Miller (1971), [2]Bradley *et al.* (1972); [3]Hohenboken *et al*, (1976); [4]McGuirk and Bourke (1978) [5]Nitter (1978); [6]Fogarty *et al.* (1984); [7]Long *et al.* (1989); [8]Van Handel and Visscher (1995); [9]Bittante *et al.* (1996); [10]Mavrogenis (1996); [11]Boujenane and Kansari (2002); – within breed crosses: [12]Kinghorn and Atkins (1987); [13]Analla *et al.* (1998)

* Also referred to as litter size at birth and weaning (or in the latter case at a specific lamb age, i.e. 70 days) respectively in some references

Long term selection studies are used for measuring rate of cumulative change in selection response. The response to selection is achieved by exploiting additive genetic variance. A diversity of gene frequency for selected and correlated traits is expected among different lines. Therefore, crosses among selected lines after long term divergent selection can provide information on non-additive genetic effects for quantitative traits (Yang *et al.* 1999). Yet, there is a paucity of information about within sheep breed crosses in the literature. Kinghorn and Atkins (1987) evaluated the use of crossbreeding between strains or bloodlines within the Merino breed and concluded that there is considerable heterosis worth exploiting, especially for traits related to

survival and prolificacy. Analla *et al.* (1998) concluded that the six Spanish Merino lines they studied could be crossed to take advantage of some heterotic effects. One of the aims of this study is to evaluate reciprocal crosses between the H and L lines in terms of reproductive performance and to estimate heterosis for the within breed crosses.

2.7 Molecular marker technology

The development of DNA-based markers, after the advent of the polymerase chain reaction (PCR) (Mullis *et al.* 1986), has had a revolutionary impact on gene mapping, and on animal genetics in general (Dodgson *et al.* 1996). Molecular markers are genetic tools that allow the examination of differences or polymorphisms between individuals at many randomly spaced positions across an entire genome. DNA markers preferably have the following desirable properties: high levels of polymorphic alleles; co-dominant inheritance (homozygous and heterozygous states of diploid organisms can be distinguished); frequent occurrence and distributed evenly throughout the genome; neutrality; genetic independence of markers and easy and fast assays that are reproducible (Vignal *et al.* 2002; Arif *et al.* 2010).

There are numerous molecular techniques for identifying such markers: protein electrophoresis (isozymes), restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD) amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR or microsatellites), non-nuclear markers (mitochondrial), single nucleotide polymorphisms (SNP) and a host of others (Dodgson *et al.* 1996; Montaldo and Meza-Herrera 1998; Parker *et al.* 1998; Crawford *et al.* 2000; Vignal *et al.* 2002; Van Marle-Koster and Nel 2003; Avise 2004). DNA markers can be grouped into clone/sequence based (CSB) markers and fingerprint (FP) markers. Clone or sequence based markers requires the isolation of a cloned DNA fragment and often determination of some, if not all, of its DNA sequence. The CSB markers include microsatellites and RFLP among others. The fingerprint markers require no prior knowledge of the sequence of the polymorphic region or isolation of a cloned DNA fragment, and they include RAPD and AFLP assays (Dodgson *et al.* 1996).

Most of these anonymous markers recognise non-coding, or non-gene, sequences and have been traditionally used because the position and sequence of genes were mostly unknown. In the more studied species, genomic knowledge is such that gene sequences are starting to be compared directly between individuals, instead of through the intermediary of genetic markers, for example whole genome sequencing (Magee *et al.* 2010) and single nucleotide polymorphisms (SNPs) (Brookes 1999). However, in most species, this approach is not yet possible and anonymous markers remain indispensable.

2.7.1 A brief overview of developments in ovine genomics

Genomics is defined as the scientific study of structure, function and inter-relationships of both individual genes and the genome in its entirety (Fadiel *et al.* 2005). The success of the pioneering Human Genome Project, established in 1990, inspired the

mapping of the genomes of other species (Snustad and Simmons 2000; Baltimore 2001).

There are two general categories of genome maps than can be constructed for a species; physical maps and genetic or linkage maps. Physical maps are based on the physical assignment of loci on chromosomes. Genetic or linkage maps are based on chromosomal recombination events and are developed by genotyping animals and using linkage analysis or linkage disequilibrium to construct maps. The information from both the physical and genetic techniques is then integrated into combined genome maps for the species (Maddox and Cockett 2007).

Initial work by Crawford *et al.* (1995) produced the first extensive ovine genetic linkage map. This initial linkage map of the sheep genome has since been revised (de Gortari *et al.* 1998; Maddox *et al.* 2001) with the most recent version presented by Maddox and Cockett (2007).

The impetus to map the sheep genome has been spurred by the formation of the International Sheep Genomics Consortium (ISGC) (McEwan 2007) which combined the mapping endeavours of AgResearch, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), the National Centre for Biotechnology Information (NCBI), Australian Sheep Genome Mapping Institute, the Children's Hospital Oakland Research Institute (CHORI) and the Centre for Genetics and Molecular Medicine (CGeMM). Sheep genomic resources are available through the ISGC website (http://www.sheephapmap.org/) (McEwan 2007).

The ISGC has produced a virtual sheep genome (Dalrymple *et al.* 2007) by using comparative genomics to reorder the human genome sequence. The OvineSNP50 genotyping beadchip was developed in collaboration with Illumina®, which features over 54,000 evenly spaced single nucleotide polymorphisms (SNP's) across the entire sheep genome (Kijas *et al.* 2009; 2012). The value of this new genomic technology is that DNA markers can provide accurate information on identity, parentage, prediction of an animal's genetic worth and its individual performance (McEwan 2007). The adoption of whole genomic selection (Meuwissen *et al.* 2001) is restricted by the current high cost of genotyping. The ISGC is currently in the process of creating a draft reference genome sequence of the sheep (Archibald *et al.* 2010).

2.7.2 *Random Amplified Polymorphic DNA (RAPDs)*

Randomly amplified polymorphic DNA (RAPD, pronounced "rapid") markers were simultaneously reported by Williams *et al.* (1990) (RAPD) and Welsh & McClelland (1990) (as arbitrarily primed PCR, AP-PCR) and also by Caetano-Anolles *et al.* (1991) (as DNA amplification fingerprinting-DAF) as a DNA fingerprinting technique. A RAPD assay involves the PCR amplification of purified genomic DNA using short single oligonucleotide primers of randomly generated sequence (one primer acts as both forward and reverse primers) with lowered annealing criteria followed by visualization of major fragments using agarose gel electrophoresis and viewed with ethidium bromide staining under an ultraviolet light source (Welsh and McClelland 1990; Williams *et al.* 1990; Micheli *et al.* 1994; Arribas *et al.* 1997; Caetano-Anolles 1997;

Hoelzel and Green 1998; Montaldo and Meza-Herrera 1998; Van Marle-Koster and Nel 2003).

This combination of short oligonucleotide primers (approximately 10 bases) and less stringent annealing criteria will amplify a range of fragments from almost any template DNA yielding RAPD markers (usually due to a single base change in the primer binding site) of which some may be polymorphic. A polymorphism is defined as one of two or more alternate forms (alleles) of a chromosomal locus that differ in nucleotide sequence or have variable numbers of repeated nucleotide units (Snustad and Simmons 2000). Genetic polymorphisms may result in discontinuous genetic variation in traits in individuals within the population of a single species.

The RAPD assays detect fragment length polymorphisms (base changes at primer annealing sites, insertions and deletions) in the absence of specific nucleotide information and the subsequent polymorphisms are inherited in a Mendelian fashion (Hoelzel and Green 1998; Montaldo and Meza-Herrera 1998). The RAPDs are dominant markers (do not distinguish between homozygous and heterozygous loci) with polymorphic alleles defined as the presence or absence of a specific band (Williams *et al.* 1993; Micheli *et al.* 1997; Snustad and Simmons 2000; Avise 2004). The RAPD markers are evenly distributed throughout the genome and are found in both highly variable and conserved regions (Montaldo and Meza-Herrera 1998; Van Marle-Koster and Nel 2003; Avise 2004). Each RAPD primer may potentially amplify up to ten fragments from different loci during the same reaction providing a truly random sample of polymorphic DNA markers (Bardakci 2001; Waugh and Russell 2001).

2.7.3 RAPD assays in ovine investigations

Several studies investigating the ovine genome have used RAPD markers. The RAPD analysis is a cost effective DNA profiling method that may be useful for initial screening of populations for genetic diversity. However, a major problem with RAPD assays is their dependence on the exact PCR conditions employed, which can lead to reproducibility problems (Cushwa and Medrano 1996; Van Marle-Koster and Nel 2003; Avise 2004).

In a study of genetic variation across breeds of sheep and cattle, Kantanen *et al.* (1995) tested the suitability of RAPD markers. They found that sheep had a greater presence of polymorphic RAPD markers, but according to similarity indices, sheep populations showed a higher degree of homogeneity than cattle populations under study. However, Cushwa *et al.* (1996) identified and mapped 53 RAPD markers in the Agresearch international mapping flock (IMF). Their conclusion was that the RAPD assay is powerful approach for identifying polymorphisms that could be used for constructing a sheep genetic linkage map. The current sheep linkage map contains 1374 markers representing 1333 loci and includes 49 RAPD markers (Maddox and Cockett 2007).

Refshauge *et al.* (2000) used RAPD assays for the detection of gene markers for clean fleece weight in medium-wool Peppin Merinos. Their study revealed significant genetic diversity within their fleece plus and minus flocks (Refshauge *et al.* 2000). RAPDs

were used to measure genetic diversity in Egyptian (Ali 2003; Mahfouz *et al.* 2008); Turkish (Devrim *et al.* 2007; Elmaci *et al.* 2007); and Pakistani (Qasim *et al.* 2011) sheep breeds. Kunene *et al.* (2009) used RAPD's to provide genetic diversity data for three populations of indigenous Zulu sheep to evaluate the potential for the conservation and exploitation of locally adapted breeds.

In an investigation of a study that aims to detect putative molecular markers for traits with a definition as broad and complex as fitness and reproduction, the 'shotgun' approach of RAPD markers has some initial benefits as an overview of DNA based divergence between the H and L lines of the Elsenburg Merino resource flock.

2.8 Hypotheses

Against this background, the present study aims to characterise the divergence of the Elsenburg Merino resource flock.

The hypotheses of this work were:

I. Had the genetic trends estimated previously for the Elsenburg Merino Resource flock changed significantly? (Chapter 3)
II. Was inbreeding significant in the H and L selection lines? (Chapter 4)
III. Did the reciprocal crosses perform significantly better than the average of the pure lines (a.k.a. the midparent value)? (Chapter 5)
IV. Was there evidence that the H and L lines are significantly divergent at a molecular level? (Chapter 6)

2.9 References

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CHAPTER 3

Genetic trends for reproduction in a Merino flock divergently selected for their ability to rear multiples

3.1 Abstract

Genetic trends are reported for the Elsenburg Merino resource flock which has been divergently selected for and against the ability of ewes to rear multiples (net reproduction rate) since 1986. The objective was to determine whether genetic trends estimated previously for the Elsenburg Merino Resource flock changed significantly with the introduction of genetic material from the industry to the high (H) line. All analysis included the full pedigree file, consisting of 6547 individuals, the progeny of 211 sires and 1501 dams. Heritability estimates were 0.08 ± 0.02 for number of lambs weaned and 0.11 ± 0.02 for corrected weight of lamb weaned. The ewe permanent environment variance was estimated at 0.09 ± 0.02 and 0.11 ± 0.02 for number of lambs weaned and corrected weight of lamb weaned, respectively. Genetic trends for the H and L lines were divergent ($P < 0.05$) for all reproduction traits during the period prior to the observed breakpoints. Progress for number of lambs weaned in the H line declined after 1999 while a decline in response for weight of lamb weaned in the H line occurred after 2003. After an initial decline, breeding values for both traits appear to be at roughly the same level since 1998 in the L line. This study reinforces and updates previous studies of this resource flock. Previous research that was conducted over a shorter period since the establishment of the lines only reported linear trends as responses to selection, while the present study also reports change points in responses in the lines.

3.2 Keywords: response to selection, genetic trends, reproduction traits, number of lambs weaned, weight of lamb weaned

3.3 Introduction

Reproduction is a key consideration in the enhancement of profitability that it has to receive attention (Olivier 1999). Some of the reservations pertaining to the genetic improvement of reproduction include ewe reproduction being a complex composite trait with gender-limited expression; low heritability; recording at a relatively advanced age; and low levels of genetic variation equates to an extent with a low heritability. These factors contribute to a perception that genetic improvement is unlikely. However, coefficients of variation allow substantial gains based on simple phenotypic selection (Purvis and Hillard 1997; Cloete *et al.* 2004; Scholtz *et al.* 2010).

In response to a low reproduction rate observed in the South African Merino industry (De Klerk *et al.* 1983; Fourie and Cloete 1993), two lines of Merino sheep were established by divergent selection from the same base population using maternal ranking values for number of lambs reared per joining since 1986 (Cloete and Scholtz 1998). The H line and the L line show marked phenotypic differences in terms of number of lambs born and weaned, lamb survival, as well as weight of lamb weaned (Cloete and Scholtz 1998; Cloete *et al.* 2004; 2009). This experiment, based on the

strategy for the improvement of reproduction performance of ewes, showed that sustained genetic progress in lamb survival is possible if directed selection is applied to a correlated trait such as the ability of ewes to rear multiples, net reproduction rate according to Olivier (1999).

Genetic trends in the population under consideration should be monitored to determine the effectiveness of a breeding programme (Van Wyk *et al.* 1993). The last period genetic trends for the lines where estimated for were 1986 to 2002 (Cloete *et al.* 2004). The selection strategy has changed since then, as genetic material from the industry has been introduced to the H line. Trends from 1986 to 2012 will thus provide new insight into the divergent selection strategy. The objective of the study was to derive updated genetic trends for reproduction traits in the divergently selected Merino lines.

3.4 Materials and Methods

3.4.1 Animals, selection procedures and location

Two lines of Merino sheep were divergently selected from the same base population from 1986 to 2011, using maternal ranking values for number of lambs reared per joining (Cloete *et al.* 2004). Ewe and ram progeny of ewes rearing more than one lamb per mating (reared twins at least once) were preferred as replacements in the high (H) line. Replacements in the low (L) line were preferably descended from ewes that reared less than one lamb per mating (barren or lost all lambs born at least once). Initially, progeny of ewes that reared one lamb per mating were occasionally accepted in both lines depending on the average reproduction of the lines and the replacement needs. In contrast, very few female progeny are available in the L line at present as a result of successful downward selection; thus progeny from ewes rearing one lamb per mating often have to be selected to maintain the line. Selection decisions were generally based on more than three maternal matings for rams. Due to the greater replacement needs in females, progeny from ewes with fewer records were also selected as replacements (Cloete *et al.* 2009).

The Elsenburg Merino flock was derived from ewes descended from a Merino line selected for increased wool secondary: primary follicle ratio (Heydenrych and Vosloo 1984). Initially, these ewes were mated to rams from the other selection lines (clean fleece weight and control) maintained at Tygerhoek (Cloete *et al.* 1998a) to curb inbreeding that may have accrued since the beginning of the experiment in 1969. Rams were selected on maternal performance from a line selected for clean fleece weight and a control line during the first three years of the experiment (Cloete *et al.* 1998a), with five rams from each line. Until 1992, rams were used for only one breeding season. During subsequent years, one or two rams in each line were carried over to the next year to provide sire links across years. From the mid-1990s, the number of rams used in the H line was increased from four to six, while only two to four rams were used in the L line. This ensured that the number of ewes joined to each ram remained relatively constant; progeny groups of acceptable size were produced. Ram replacements were selected to represent all of the sires present in their progeny group wherever possible (Cloete *et al.* 2004; 2009).

In the progeny groups up to 2002, both ram and ewe replacements were selected only on ranking values based on the maternal phenotype, and no additional information was used. Once selected, ewes remained in the breeding flock for at least five joinings, except for cases of death and teeth or udder malfunction. Ewes were thus not selected on reproduction in the current flock. From the progeny group born in 2003, information on maternal ranking values used for selection was augmented by breeding values for number of lambs weaned per ewe joined in potential ram and ewe replacements (Cloete *et al.* 2009). These breeding values were derived from a single-trait repeatability model, as described by Cloete *et al.* (2004).

At the onset of the experiment, each line was represented by approximately 120 breeding ewes. The upwardly selected High (H) line was gradually allowed to increase to between 130 and 140 breeding ewes. The relatively poor reproduction in the low (L) line in later years caused a reduction in breeding ewe numbers to between 40 and 80 breeding ewes (Cloete *et al.* 2009). The H line was augmented by 28 ewes, born during 1991 and 1992, derived from a multiple ovulation and embryo transfer programme (MOET) (Cloete *et al.* 1998b).

Initially a 3 generation pedigree was maintained on paper for each animal to aid in the allocation of ewes to specific rams with the lowest levels of inbreeding. However, since 1998 this system was replaced by a system whereby all selected sires were allocated to all available dams within lines prior to mating each year. Individual inbreeding coefficients were computed for hypothetical offspring from each combination using the MTDFNRM algorithm of Boldman *et al.* (1995). These coefficients were used during the allocation of sires to dams in a way that inbreeding was minimised.

During the 1998 to 2002 lambing years, 74 6.5-year-old dams with five lambing opportunities were screened into both the H and L lines from other selection lines maintained at the Tygerhoek research farm (Cloete *et al.* 1998a) to augment numbers especially in the L line. Twenty-eight ewes rearing more than seven lambs and at least one lamb per opportunity were screened into the H line. Forty-six ewes rearing one to three lambs over five lambing opportunities were selected in the L line.

Since 2003, part of the breeding flock was subjected to reciprocal crossbreeding between the two lines, with the intention of forming a genetic resource population for possible future genomic projects (Naidoo *et al.* 2005). All animals were managed in the same flock for most of the time. They were separated on selection line for single-sire mating in January-February each when they grazed similar kikuyu paddocks. At lambing time in June-July, the ewe flock was randomly separated to have more manageable groups of 20-30 ewes at lambing in kikuyu paddocks of 0.3-0.4 ha. Lambed ewes were drifted from the lambing pastures to lucerne pastures within ~3 days of lambing, where they were retained in smaller groups of 30-35 until the tails of their lambs were docked at 3-4 weeks of age (Cloete *et al.* 2009).

A second MOET programme was implemented in 2009, using 5 breeding ewes from each line for 2009, 2010 and 2011. These ewes were laparoscopically inseminated with electro-ejaculated semen from rams within lines. This intervention was

necessitated by a marked decline in ewe numbers in the L line in particular, and was needed to ensure the continued availability of this line.

The resource flock studied was maintained at the Elsenburg research farm from 1993. The climate at the experimental site is Mediterranean, with a winter lambing season (June-July) and pre-lamb shearing in May being practiced routinely. Mature ewes in the breeding flock were shorn in April/May and crutched in springtime (5 – 6 month's wool growth) to reduce the probability of blowfly strike over the festive season (Scholtz *et al.* 2010).

Prior to 1993, the flock was kept at the Tygerhoek research farm near Riviersonderend (Cloete *et al.* 2004). At this location, ewes were mated during October-November of the preceding year to lamb during autumn of the respective birth years of their progeny. Situated about 150 km east of Elsenburg, the climate at Tygerhoek is still Mediterranean, but with a lower expected rainfall in winter (60%).

3.4.2 Recordings

The traits that were assessed included: number of lambs weaned per ewe per parity (NLW) and total weight of lamb weaned per ewe per parity (TWW). Weaning weight of individual lambs was recorded at an age of approximately 3.5 months, and adjusted for age. Individual weaning weights were then corrected for the effect of gender. A previous study investigated total weight of lamb weaned without any correction, and after correction for gender and/or the birth year of the lamb (Cloete 2002). Genetic, ewe permanent environmental, phenotypic and environmental correlations between these measures of lamb output were either unity, or not significantly different from it (Cloete 2002). Against this background, only a gender correction was applied in the present study. Corrected weaning weights were then used to calculate total weight of lamb weaned for a specific parity in individual ewes. Complete reproduction records (i.e. number of lambs born and weaned) were available for individual parities.

In view of the inbreeding in the H line (Cloete 2002) it was decided to also use industry rams in this line. So far 3 rams with ewe progeny that reproduced were introduced in this way. The first of these migrants was ram identity 4.007 from stud 801 that produced progeny in 2008. This ram was followed by 4.043 from stud 2323 and the ram with the popular name 100% from stud 1954. These rams were treated as base population animals in the following analyses.

3.4.3 Statistical analysis

The ASREML programme (Gilmour *et al.* 1999) was used to derive variance components for the respective reproduction and production traits in single-trait analyses. Fixed effects that were considered included year of lambing (1987-2012) and ewe age (2-7+ years). The analyses excluded selection line and its interactions with other traits from the operational model. The inclusion of selection line as fixed would reduce the genetic differences between lines that accrued as a result of selection. The fixed effects that were fitted were significant ($P < 0.05$) in preliminary analyses, and were retained in subsequent analyses. Direct genetic effects and ewe permanent environmental effects were added to the operational model as random

terms, as described in detail by Cloete *et al.* (2004). The following genetic model was used for analysis (in matrix notation):

$$y = Xb + Z_1a + Z_2c_{ewe} + e, \text{ where}$$

y is a vector of observations for ewe production or reproduction traits; b,a and c_{ewe} are vectors of fixed effects, direct genetic effects and ewe permanent environmental effects, respectively; X, Z_1 and Z_2 are the corresponding incidence matrices relating the respective effects to y; and e is the vector of residuals. It was assumed that:

$$V(a) = A\sigma_a^2 ;$$
$$V(c_{PE}) = I\sigma_{ewe}^2; \text{ and}$$
$$V(e) = I\sigma_e^2$$

where A is the numerator relationship matrix, I is an identity matrix and σ_a^2, σ_{ewe}^2 and σ_e^2 are the direct genetic variance, ewe permanent environmental variance and environmental (residual) variance, respectively. All analyses included the full pedigree file, consisting of 6547 individuals, the progeny of 211 sires and 1501 dams.

Direct breeding values of all ewes for the traits were obtained and averaged within birth years to obtain genetic trends. Preliminary findings suggested that the response observed was not linear throughout. Against this background, a broken stick (aka hockey stick, segmented, piecewise or split line) regression model was fitted with change-point (breakpoint) estimated using GenStat V12.1 (Payne *et al.* 2009):

$$Y = \beta_0 + \beta_1 (X) + \beta_2(X - C)^+ + \epsilon$$

Where Y is the response variable, X is the covariate, and C is the change point and β_0 is the intercept, β_1 is the slope before the change point C, and β_2 is the difference in slope after the change point. The slope after the change point is $\beta_1 + \beta_2$. The variable $(X - C)^+$ is a derived variable which takes the value of 0 for values of $X < C$ and the values X - C for values of $X > C$. These genetic trends were tested for divergence between the lines, using standard errors obtained for the regression coefficients.

3.5 Results

Heritability estimates were 0.08 ± 0.02 for number of lambs weaned and 0.11 ± 0.02 for corrected weight of lamb weaned. The ewe permanent environment variance was estimated at 0.09 ± 0.02 and 0.11 ± 0.02 for number of lambs weaned and corrected weight of lamb weaned, respectively.

The breakpoint in the H line occurred earlier for the trait NLW (1999) than for TWW (2003). Breakpoints could not be estimated by GenStat split-line regression function for the L line data for both traits assessed but were approximated by the visual inspection of the data at approximately 1998. The spread of the data indicated multiple breakpoints and the split-line model did not fit the L line data.

Genetic trends for the H and L lines were divergent (P < 0.05) for all reproduction traits during the period prior to the observed breakpoints (Table 3.1). Genetic trends for number of lambs born per ewe joined and corrected weight of lamb weaned per ewe are provided as illustration in Fig. 3.1 and Fig 3.2. Expressed as a percentage of the overall phenotypic means of the respective traits (0.90 for NLW and 18.6 kg for TWW), averaged breeding values in the H line increased annually at 1.9% for number of lambs weaned per ewe joined and by 2.1 % for weight of lamb weaned per ewe joined. Corresponding trends in the L line were –1.0% and –1.4% per annum respectively.

Table 3.1 Details of broken stick regression equations depicting genetic change as reflected by the regression (b ± s.e.) of averaged predicted breeding values on year of birth for ewe reproduction traits. Regressions were forced through the origin.

	Trait and selection line			
	NLW		**TWW**	
Statistical information	**H line**	**L line**	**H line**	**L line**
Slope 1 (b ± s.e.)	0.0171 ± 0.001*	-0.0083 ± 0.001*	0.3846 ± 0.015*	-0.2588 ± 0.027*
r	0.98	0.90	0.99	0.94
R^2	0.96	0.82	0.97	0.88
Breakpoint _X (R^2)	13[a] (0.87)	12[b]	17[c] (0.91)	12[b]
Slope 2 (b ± s.e.)	-0.0032 ± 0.002	-0.0005 ± 0.001	-0.2378 ± 0.069	0.0308 ± 0.034
Intercept 2	0.2618 ± 0.039	-0.0979 ± 0.025	10.54 ± 1.435 (kg)	-3.298 ± 0.622
r	0.43	0.12	0.81	0.26
R^2	0.19	0.014	0.66	0.07

* Denote significant (P<0.05) divergence between lines for a specific trait

[a,b,c] denotes the year of selection relative to the start of the project, thus 0 corresponds to 1986

Explanation: b – regression coefficient; r – correlation coefficient; R^2 - proportion of the variance in predicted breeding values that could be attributed to its linear regression on birth year.

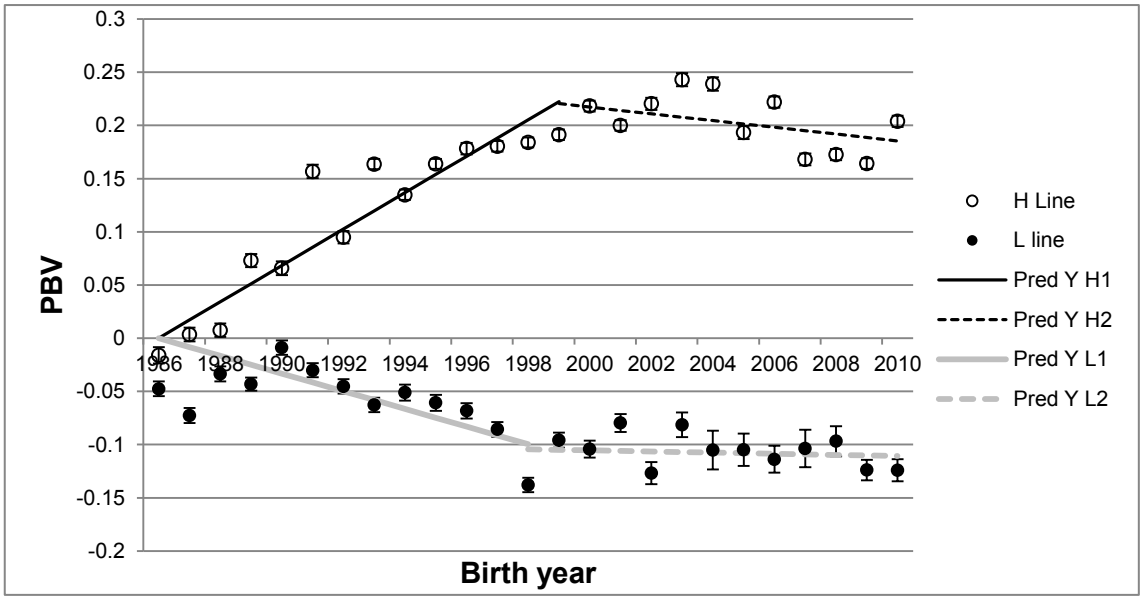

Figure 3.1 Genetic trends depicting averaged predicted breeding values (PBV) within birth year for the H and L lines for number of lambs weaned per ewe joined. Piecewise regressions were forced through the origin. Statistical information is given in Table 3.1

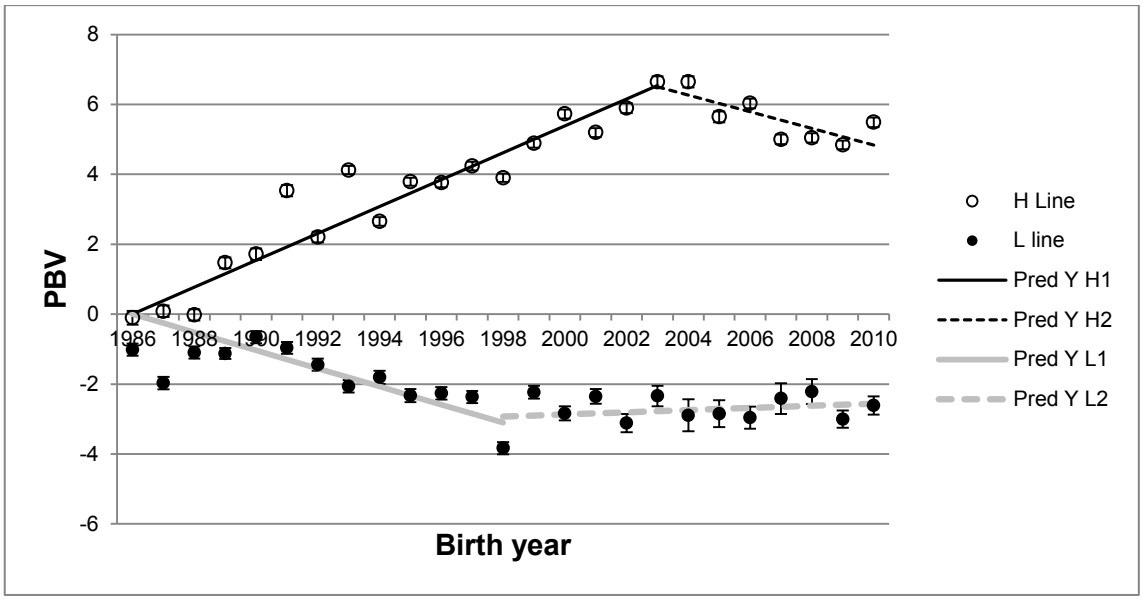

Figure 3.2 Genetic trends depicting averaged predicted breeding values (PBV) within birth year for the H and L lines for corrected weight of lamb weaned per ewe in kg. Piecewise regressions were forced through the origin. Statistical information is given in Table 3.1

3.6 Discussion

Previous estimates of heritability and the ewe permanent environment variance for this resource flock were 0.04 ± 0.02 and 0.11 ± 0.03 for number of lambs weaned and 0.04 ± 0.02 and 0.11 ± 0.03 for corrected weight of lamb weaned, respectively (Cloete *et al.* 2004). More between animal variation has been partitioned towards additive variance (heritability) and less towards the ewe permanent environmental variance compared to the Cloete *et al.* (2004) study. This is highly likely to be the result of a deeper pedigree used in this study.

Genetic trends for reproduction indicate divergence between the H and L lines as shown in Table 3.1. Genetic change per year amounted to 1.9% and 2.1% of the corresponding phenotypic means for number of lambs weaned and corrected weight of lamb weaned per ewe in the H line, respectively. In the L line, change for the same traits in the downward direction were slower, namely -1.0% and -1.4% per year. The earlier study that this work is based on reported similar genetic change per year of 1.5 % and 1.8 % for the H line and -1.0 % and -1.3 % for the L line per year for lambs weaned per ewe born and corrected weight of lamb weaned per ewe, respectively (Cloete *et al.* 2004).

Progress for number of lambs weaned in the H line tended to decline after 1999, although the regression did not differ from zero. The decline of response for weight of lamb weaned in the H line after 2003 can possibly attributed to relaxing selection due the beginning of reciprocal crossing between the lines. Further, commercial rams with lower within-flock breeding values than that of the homebred rams were introduced in 2008 to ensure that the inbreeding in the H line stay in check. In order of introduction,

the derived within-flock estimated breeding values for number of lambs weaned per ewe mated of these rams amounted to -0.007 for 4.007, -0.025 for 4.043 and 0.101 for 100%. Corresponding breeding values for corrected weight of lamb weaned per ewe were respectively 0.99 kg, 0.55 kg and 2.43 kg. Fourteen progeny of 4.007 in 2008 had averaged breeding values of 0.114 for number of lamb weaned and 3.81 kg for total corrected weight of lamb weaned. The corresponding means of 153 progeny of rams from within the flock were respectively 0.178 and 5.16 kg. Corresponding averaged breeding values for 42 progeny of ram 4.043 in 2009 amounted to respectively 0.116 and 3.90 kg, compared to values of 0.178 and 5.10 kg for 150 progeny of within flock rams. Twenty-seven progeny of 100% in 2010 had averaged breeding values of respectively 0.191 and 5.13 kg, compared to respective values of 0.206 and 5.56 for 139 within flock progeny.

It is reasonable to assume that the observed breakpoints could be attributed to specific events in the history of the flock (Figures 3.1 and 3.2). The only specific event that could be associated with breakpoint in the graph for TWW in the H line was the commencement of reciprocal crosses between the lines in 2003. It needs to be said that the breeding values of these industry rams and their progeny are liable to change as more lambing opportunities accrue for their female progeny. Nevertheless, at this stage it seems as if these introductions could have contributed to the downward trend in the H line after the observed breakpoint. Plotting the within-flock values on the graphs in Figures 3.1 and 3.2, however, seemed to have a very small effect on the derived trends.

The response to selection in the L line appears to have reached a plateau, possibly as a result of selection in the downward direction going against natural selection. It could be argued that such selection would limit further progress, while also reducing additive genetic variation (Ecklund and Bradford 1976; Barton and Partridge 2000). There is also the possibility that inbreeding had an effect on these results, since inbreeding depression is known to impair reproduction and fitness traits (Lamberson and Thomas 1984; Van Wyk *et al*. 2009).

When the absolute values of the respective regression coefficients in Table 3.1 were compared, it was clear that genetic change in the H line was generally faster ($P < 0.05$) in the upward direction than that of the L line in the downward direction. Responses were thus asymmetric for the selection lines. If it is assumed that the traits that were considered are fitness traits, this result is not entirely unexpected. Fitness traits are expected to be lowly heritable, to show inbreeding depression and heterosis, while responses to divergent selection in such traits are often asymmetric (Frankham 2009).

3.7 Conclusions

This study reinforces and updates previous studies of this resource flock. The H and L trend lines are still significantly divergent for the traits assessed. The genetic trends show a decline in the response to selection for corrected TWW and NLW in the H line. The effect of inbreeding within the H and L lines should be determined to elucidate its potential impact on these trends.

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CHAPTER 4

Inbreeding trends and pedigree analysis of the Elsenburg Merino resource flock

4.1 Abstract

The pedigree was analysed and inbreeding trends computed for the Elsenburg Merino flock using ENDOG v4.8 and POPREP web analysis software. The complete pedigree data used in this study comprised of 7446 records of the Elsenburg Merino flock collected from 1979 to 2011. The average inbreeding coefficients (*F*) were 1.47% and 0.73% for the divergently selected H and L lines, respectively. Mean average relatedness is higher in the H line than in the L line, 4.17% compared to 2.48%. The generation intervals for males and females were similar in both lines, amounting to 2.3 and 4.4 in the H line and 2.4 and 4.2 in the L line. The rate of inbreeding (ΔF) per generation was 0.5% for the H line and 0.6% in the L line. The effective number of founders (f_e) and effective number of ancestors (f_a) were 48 and 322 in the H line and 90 and 227 for the L line. The overall rate of inbreeding per generation in the H and L lines are within acceptable levels.

4.2 Keywords: rate of inbreeding, effective population size, mean average relatedness, generation interval

4.3 Introduction

The study of inbreeding and pedigree analysis is pertinent to modern livestock improvement programmes; which use intense selection of a small number of individuals or families to greatly improve genetic gains but results in a corresponding increase in rates of inbreeding (Weigel 2001). Although the commonly acknowledged passive inbreeding (inbreeding coefficient <6.25 %) that arises from the unavoidable mating of related animals due to a small effective population size in selection experiments accumulates at a slower rate than active inbreeding, the key concern to farmers is inbreeding depression (Falconer and Mackay 1996; Miglior 2000).

Inbreeding depression has a deleterious effect on additive genetic variance and the phenotypic expression of traits under selection, impairing reproduction and fitness in particular (Lamberson and Thomas 1984; Erasmus *et al.* 1991; Van Wyk *et al.* 2009). However, the loss of heterozygosity and genetic variation in these selected populations due to inbreeding can be mitigated by correctly managed selection programmes (Erckanbrack and Knight 1991; Miglior 2000). Inbreeding is also an important tool used in the development of breeding stock to reveal and possibly eliminate detrimental genes and to increase the frequency of desirable genes in a population if managed correctly (Erckanbrack and Knight 1991; Hedrick 1994).

The net effect of inbreeding in a selection programme will depend on the magnitude of the selection response relative to the possible depression and rate of accumulation of

inbreeding (Weigel 2001; Swanepoel *et al.* 2007; Van Wyk *et al.* 2009). To manage the possible effects of inbreeding in a selection programme, inbreeding coefficients should be considered in setting up mating lists (Van Wyk *et al.* 2009).

The level of inbreeding is influenced by the ratio of males to females, reproduction ability, mating systems and population size (Norberg and Sorensen 2007; Ceyhan *et al.* 2011). It is therefore important to have knowledge of the effective size of a population (N_e) and the rate of inbreeding (ΔF), which is the relative increase in inbreeding by generation (Boichard *et al.* 1997). Wright (1923) defines the effective size of a population as the size of an idealised population which would give rise to the rate of inbreeding (Boichard *et al.* 1997; Gutierrez *et al.* 2003).

An outcome of efficient selection programmes is that inbreeding is accumulating rapidly in most commercial livestock species (Weigel 2001). A recent study of the effect of inbreeding on production and reproduction traits in the Elsenburg Dormer sheep stud, using pedigree data collected over a 62 year period, reported a relatively high mean inbreeding coefficient of 16% for all animals over all years (Van Wyk *et al.* 2009). This level of inbreeding was reached over time despite the flock being developed under planned mating to reduce inbreeding. Genetic trends presented in Chapter 3 show a decline in response to selection in the H line of the Elsenburg Merino Resource flock that may be a result of inbreeding. Further, inbreeding may also have contributed to the selection response in the L line. The Elsenburg Merino resource flock has been divergently selected for 25 years and a retrospective pedigree analysis could provide useful information to conserve these lines in the future.

The objectives of this study were to describe the population dynamics, analyse the pedigree and calculate inbreeding parameters for the two lines of the divergently selected Elsenburg Merino flock described in the previous chapters.

4.4 Materials and Methods

4.4.1 Animals, selection procedures and location

Two lines of Merino sheep were divergently selected from the same base population from 1986 to 2011, using maternal ranking values for number of lambs reared per joining (Cloete *et al.* 2004). Ewe and ram progeny of ewes rearing more than one lamb per mating (reared twins at least once) were preferred as replacements in the high (H) line. Replacements in the low (L) line were preferably descended from ewes that reared less than one lamb per mating (barren or lost all lambs born at least once). Initially, progeny of ewes that reared one lamb per mating were occasionally accepted in both lines depending on the average reproduction of the lines and the replacement needs. In contrast, very few female progeny are available in the L line at present as a result of successful downward selection; thus progeny from ewes rearing one lamb per mating often have to be selected to maintain the line. Selection decisions were generally based on more than three maternal matings for rams. Due to the greater replacement needs in females, progeny from ewes with fewer records were also selected as replacements (Cloete *et al.* 2009).

The Elsenburg Merino flock was derived from ewes descended from a Merino line selected for increased wool secondary: primary follicle ratio (Heydenrych and Vosloo 1984). Initially, these ewes were mated to rams from the other selection lines (clean fleece weight and control) also maintained at Tygerhoek (Cloete *et al.* 1998a) to curb inbreeding that may have accrued since the beginning of the experiment in 1969. Rams were selected on maternal performance from a line selected for clean fleece weight and a control line during the first three years of the experiment (Cloete *et al.* 1998a), with five rams from each line. Until 1992, rams were used for only one breeding season. During subsequent years, one or two rams in each line were carried over to the next year to provide sire links across years. From the mid-1990s, the number of rams used in the H line was increased from four to six, while only two to four rams were used in the L line. This ensured that the number of ewes joined to each ram remained relatively constant; progeny groups of acceptable size were produced. Ram replacements were selected to represent all of the sires present in their progeny group wherever possible (Cloete *et al.* 2004; 2009).

In the progeny groups up to 2002, both ram and ewe replacements were selected only on ranking values based on the maternal phenotype, and no additional information was used. Once selected, ewes remained in the breeding flock for at least five joinings, except for cases of death and teeth or udder malfunction. Ewes were thus not selected on reproduction in the current flock. From the progeny group born in 2003, information on maternal ranking values used for selection was augmented by breeding values for number of lambs weaned per ewe joined in potential ram and ewe replacements (Cloete *et al.* 2009). These breeding values were derived from a single-trait repeatability model, as described by Cloete *et al.* (2004).

At the onset of the experiment, each line was represented by approximately 120 breeding ewes. The upwardly selected High (H) line was gradually allowed to increase to between 130 and 140 breeding ewes. The relatively poor reproduction in the low (L) line in later years caused a reduction in breeding ewe numbers to between 40 and 80 breeding ewes (Cloete *et al.* 2009). The H line was augmented by 28 ewes, born during 1991 and 1992, derived from a multiple ovulation and embryo transfer programme (MOET) (Cloete *et al.* 1998b).

Initially a 3 generation pedigree was maintained on paper for each animal to aid in the allocation of ewes to specific rams with the lowest levels of inbreeding. However, since 1998 this system was replaced by a system whereby all selected sires were allocated to all available dams within lines prior to mating each year. Individual inbreeding coefficients were computed for hypothetical offspring from each combination using the MTDFNRM algorithm of Boldman *et al.* (1995). These coefficients were used during the allocation of sires to dams in a way that inbreeding was minimised.

During the 1998 to 2002 lambing years, 74 6.5-year-old dams with five lambing opportunities were screened into both the H and L lines from other selection lines maintained at the Tygerhoek research farm (Cloete *et al.* 1998a) to augment numbers especially in the L line. Twenty-eight of these ewes rearing more than seven lambs and at least one lamb per opportunity were screened into the H line. Forty-six ewes rearing one to three lambs over five lambing opportunities were selected in the L line.

Since 2003, part of the breeding flock was subjected to reciprocal crossbreeding between the two lines, with the intention of forming a genetic resource population for possible future genomic projects (Naidoo *et al.* 2005). All animals were managed in the same flock for most of the time. They were separated on selection line for single-sire mating in January-February each when they grazed similar kikuyu paddocks. At lambing time in June-July, the ewe flock was randomly separated to have more manageable groups of 20-30 ewes at lambing in kikuyu paddocks of 0.3-0.4 ha. Lambed ewes were drifted from the lambing pastures to lucerne pastures within ~3 days of lambing, where they were retained in smaller groups until the tails of their lambs were docked at 3-4 weeks of age (Cloete *et al.* 2009).

A second MOET programme was implemented in 2009, using 5 breeding ewes from each line for 2009, 2010 and 2011. These ewes were laparoscopically inseminated with electro-ejaculated semen from rams within lines. This intervention was necessitated by a marked decline in ewe numbers in the L line in particular, and was needed to ensure the continued availability of this line

The resource flock studied was maintained at the Elsenburg research farm from 1993. The climate at the experimental site is Mediterranean, with a winter lambing season (June-July) and pre-lamb shearing in May being practiced routinely. Mature ewes in the breeding flock were shorn in April/May and crutched in springtime (5 – 6 month's wool growth) to reduce the probability of blowfly strike over the festive season (Scholtz *et al.* 2010).

Prior to 1993, the flock was kept at the Tygerhoek research farm near Riviersonderend (Cloete *et al.* 2004). At this location, ewes were mated during October-November of the preceding year to lamb during autumn of the birth years of their offspring. Situated about 150 km east of Elsenburg, the climate at Tygerhoek is still Mediterranean, but with a lower expected rainfall in winter (60%).

4.4.2 Analysis of pedigree structure and inbreeding

The complete pedigree data used in this study comprised of 7446 records of the Elsenburg Merino flock collected from 1979 to 2011. Only the relationships between animals belonging to the base population, H and L selection lines were used in the pedigree and inbreeding analysis. The ENDOG v4.8 (Gutierrez and Goyache 2005) and POPREP web application (Groeneveld *et al.* 2009) programmes were used for pedigree and inbreeding analyses.

POPREP and ENDOG v4.8 use the pedigree completeness index (PCI) to measure pedigree completeness by applying the following formulas presented by MacCluer *et al.* (1983), namely: $I_d = \frac{4I_{d_{pat}} I_{d_{mat}}}{I_{d_{pat}} + I_{d_{mat}}}$ *and* $I_{d_k} = \frac{1}{d}\sum_{i=1}^{d} a_i$ *k = pat, mat*

where *k* represents the paternal or maternal line of an individual, a_i is the proportion of known ancestors in generation *i*. The *d* is the number of generations considered in the calculation of the pedigree completeness. This index summarizes the proportion of known ancestors in each ascending generation, thus assessing pedigree quality, and quantifies the probability of detecting inbreeding in the pedigree (Groeneveld *et al.* 2009; Gutierrez *et al.* 2010). Pedigree completeness level was also assessed by

computing the equivalent number of generations and by counting the proportion of known ancestors several generations back (Gutierrez and Goyache 2005; Gutierrez *et al.* 2010).

Generation intervals (GI) were calculated as the average age of the parents at the birth of their progeny that were kept for reproduction (Gutierrez *et al.* 2010).

The inbreeding coefficients were computed for all animals in the database, using the fast tabular method (Groeneveld *et al.* 2009). Average annual inbreeding coefficients within selection lines were derived by averaging individual inbreeding coefficients within selection lines and birth years. The rate of change of the inbreeding coefficient per generation (*ΔF*) is defined as: $\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$ where *t* is the tth generation, F_t is the inbreeding of the individual and F_{t-1} is the average inbreeding of the parents.

POPREP also calculates *ΔF* using the log regression of (1 - F) on birth date, a method suggested by Perez-Enciso (1995). The rate of inbreeding per generation is given by: $\Delta F = (-1)\, bL$, where *L* is the generation interval and *b* the slope of the regression of *ln(1 - F)* on year of birth. When individual inbreeding coefficients are available, as is the case with pedigree data, the rate of inbreeding can be calculated by regressing $ln(1 - F_i)$ on year of birth, where F_i is the inbreeding coefficient the ith individual (Groeneveld *et al.* 2009).

The effective number of founders (f_e), defined as the number of equally contributing founders that would be expected to produce the same genetic diversity as in the population under study (Lacy 1989; Boichard *et al.* 1997), was computed as: $f_e = \frac{1}{\sum_{k-1}^{f} q_k^2}$ where q_k is the probability of gene origin of the k^{th} ancestor.

The effective number of ancestors (f_a), the minimum number of ancestors, not necessarily founders was computed as: $f_a = \frac{1}{\sum_{j-1}^{a} q_j^2}$ where q_j is the marginal contribution of an ancestor *j*, which is the genetic contribution made by an ancestor that is not explained by other ancestors chosen before (Gutierrez *et al.* 2010).

The effective population size can be computed using different methods. POPREP uses two methods to calculate *Ne* based on the number of parents and based on the rate of inbreeding. *Ne* based on the number of parents is calculated as: $Ne = \frac{4N_m N_f}{N_m + N_f} * 0.7$ where N_m and N_f are the number of male and female parents in a population with discrete generations, respectively (Groeneveld *et al.* 2009). A generation of animals was identified as those animals born in the time span of one generation interval (GI) which ended in the reporting year. This means that the number of parents in consecutive years may include some of the same animals. N_e was also calculated as: $N_e = \frac{1}{2\Delta F}$ (Wright 1923; Falconer and Mackay 1996; Groeneveld *et al.* 2009).

In ENDOG v4.8, the effective population size based on family size variance was calculated as:

$$\frac{1}{Ne} = \frac{1}{16ML}\left[2 + \sigma^2_{mm} + 2\left(\frac{M}{F}\right)cov(mm, mf) + 2\left(\frac{M}{F}\right)^2\sigma^2_{mf}\right] + \frac{1}{16FL}\left[2 + \left(\frac{F}{M}\right)^2\sigma^2_{fm} + 2\left(\frac{F}{M}\right)cov(fm, ff) + \sigma^2_{ff}\right]$$

where *M* and *F* are the number of male and female individuals born or sampled for breeding during each time period, *L* the average generation interval, σ^2_{mm} and σ^2_{mf} are the variances of the male and female offspring of the male, σ^2_{fm} and σ^2_{ff} are the variances of the male and female offspring of a female, and *cov(mm, mf)* and *cov(fm,ff)* the respective covariances (Gutierrez *et al.* 2010).

4.5 Results

The average percentage of pedigree completeness using the index developed by MacCluer *et al.* (1983) for the H and L lines is presented in Table 4.1. Fig. 4.1 shows the level of pedigree completeness of the male and female lines up to three generations back for the H and L lines

Table 4.1 Average percentage of pedigree completeness for the H and L lines of the Elsenburg Merino flock 1 to 6 generations from 1986 to 2011

Gen. depth	1	2	3	4	5	6
H Line	95.6%	84.5%	75.0%	66.9%	59.5%	52.8%
L Line	94.8%	86.3%	78.7%	71.7%	65.1%	58.6%

The number of breeding animals at a given time determines the genetic structure of the population in subsequent generations and to a great extent determines the effective population size N_e, a fundamental parameter in population management. Between 1985 and 2011, a total of 171 males and 979 females produced 4659 offspring (4.8 offspring per female) while the offspring of 140 males and 399 females were selected as parents themselves in following generations in the H line. Similarly, in the L line, a total of 122 males and 511 females produced 2286 offspring (4.5 offspring per female) with 111 males and 268 females being selected as parents. The generation intervals for males and females were similar in both lines, amounting to 2.3 and 4.4 in the H line and 2.4 and 4.2 in the L line. The genetic contributions of founders and ancestors and summary statistics from the pedigree analysis of the H and L lines of the Elsenburg Merino resource flock are presented in Table 4.2.

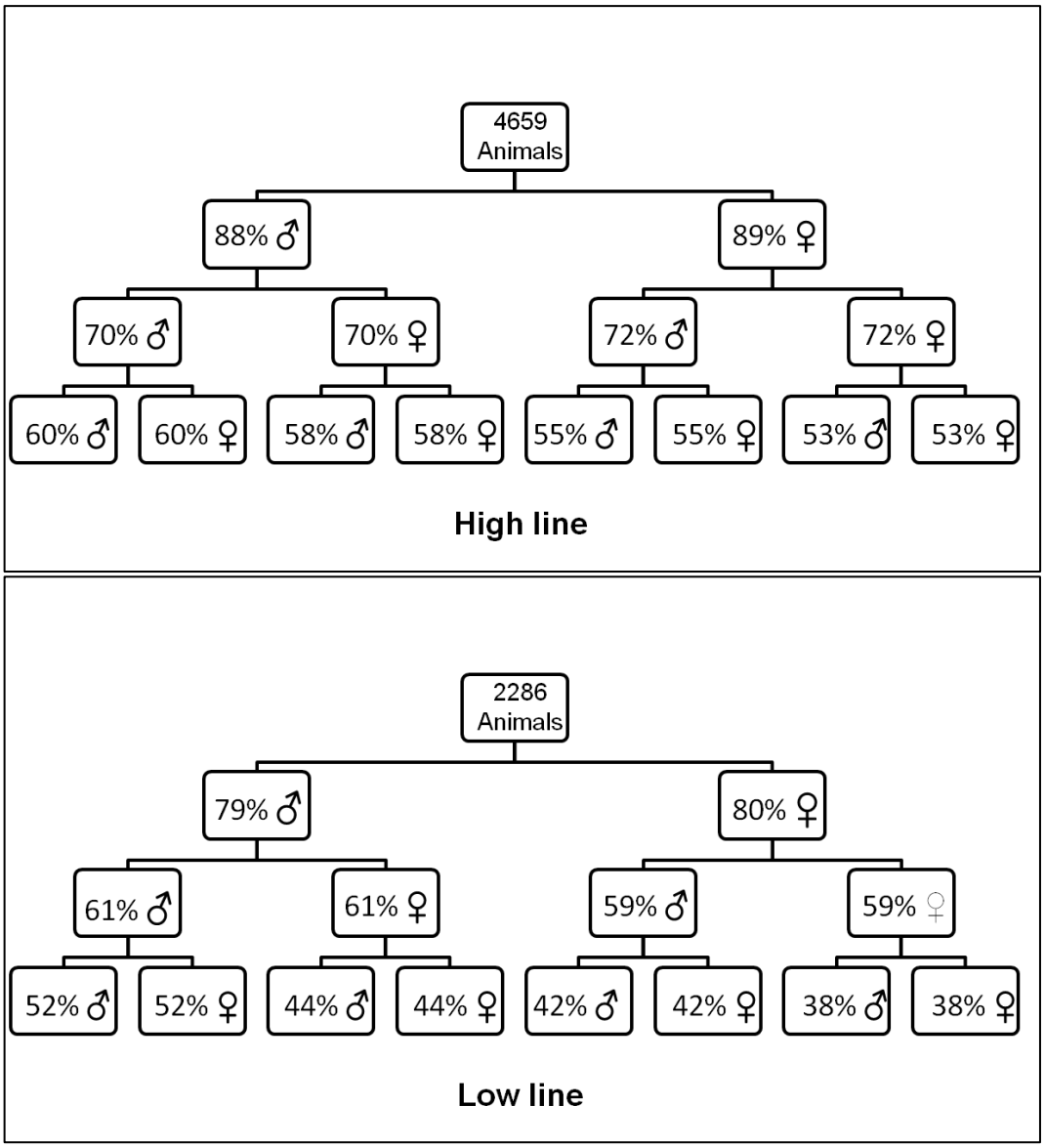


Figure 4.1 Completeness of pedigree information up to 3 generations for the High and Low lines of Merinos from the Elsenburg resource flock

Table 4.2 Summary statistics of the pedigree analysis of the H and L lines of the Elsenburg Merino resource flock

Variable	H line	L line
Size of population	4659	2286
Base population (1 or more unknown parents)	548	475
Effective population size (*Ne*)*	302	290
Effective population number of founders (f_e)	48	90
Number of ancestors contributing to the reference population (f_a)	322	227
Number of ancestors explaining 50% of the gene pool	12	19
f_e/f_a ratio	1.08	1.19
Expected inbreeding caused by unbalanced contribution of founders (%)	1.03	0.56
Average F_i (%)	1.47	0.73
Mean average relatedness (%)	4.17	2.48
Generation Interval	3.27	3.32

* Effective population size based on family size variance

The average inbreeding coefficients of the H and the L lines are presented in Fig. 4.2. The average inbreeding in both lines increased with time, as is expected in any population of finite size. However, the average inbreeding of the L line increased at a fast rate in the most recent years. In contrast, average inbreeding in the H line peaked at 4.2% in 2007, with a subsequent decline. Fig. 4.3 shows maximum inbreeding coefficients in the H and L lines for the period from 1979 to 2011. The maximum individual inbreeding coefficients in both lines over the period from 1990 onwards oscillated between approximately 5% and 15% in most birth years (Fig. 4.3). However, the maximum individual inbreeding in the H line reached 25% during 1992.

The rate of change of the average inbreeding coefficients based on the slope of the regression between 1986 and 2011 amounted to 0.00180 ± 0.01 for the H line and 0.00217 ± 0.01 for the L line, which represents a ΔF per generation of 0.00558 and 0.00675, respectively. The effective population sizes (*Ne*) for the H and L lines based on ΔF were 90 and 74, respectively.

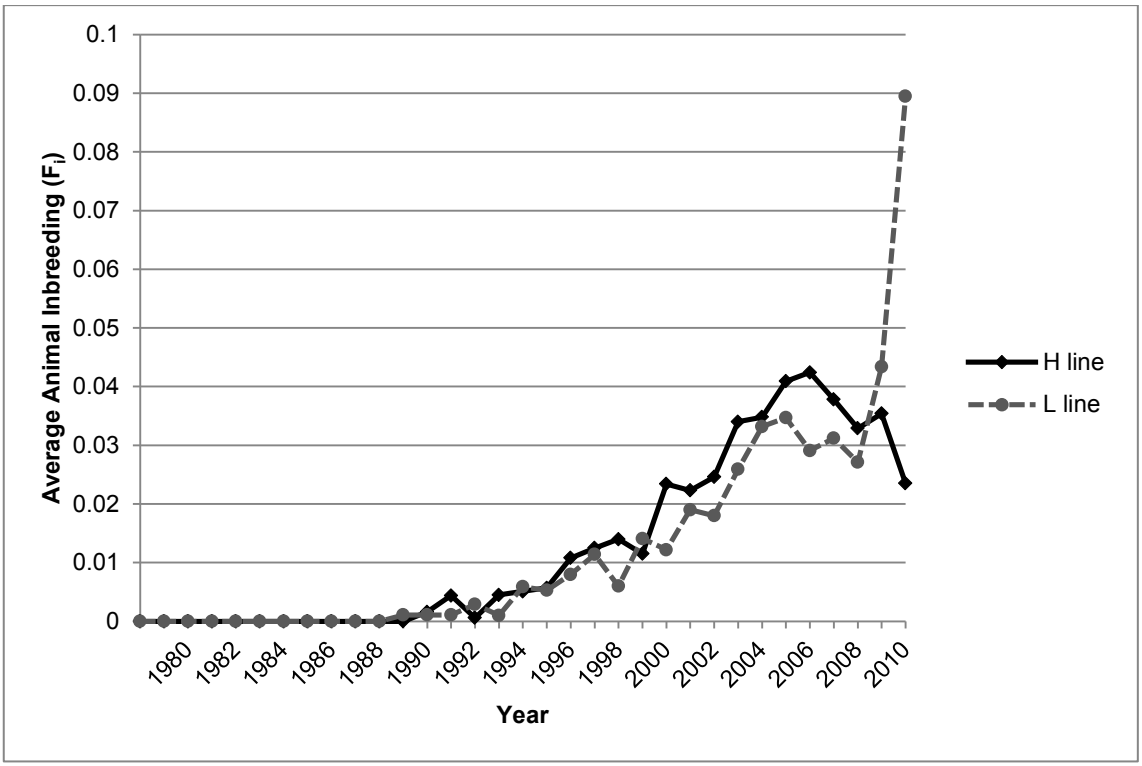


Figure 4.2 Average annual inbreeding coefficients for all animals in the H and L lines for the period from 1979 to 2011

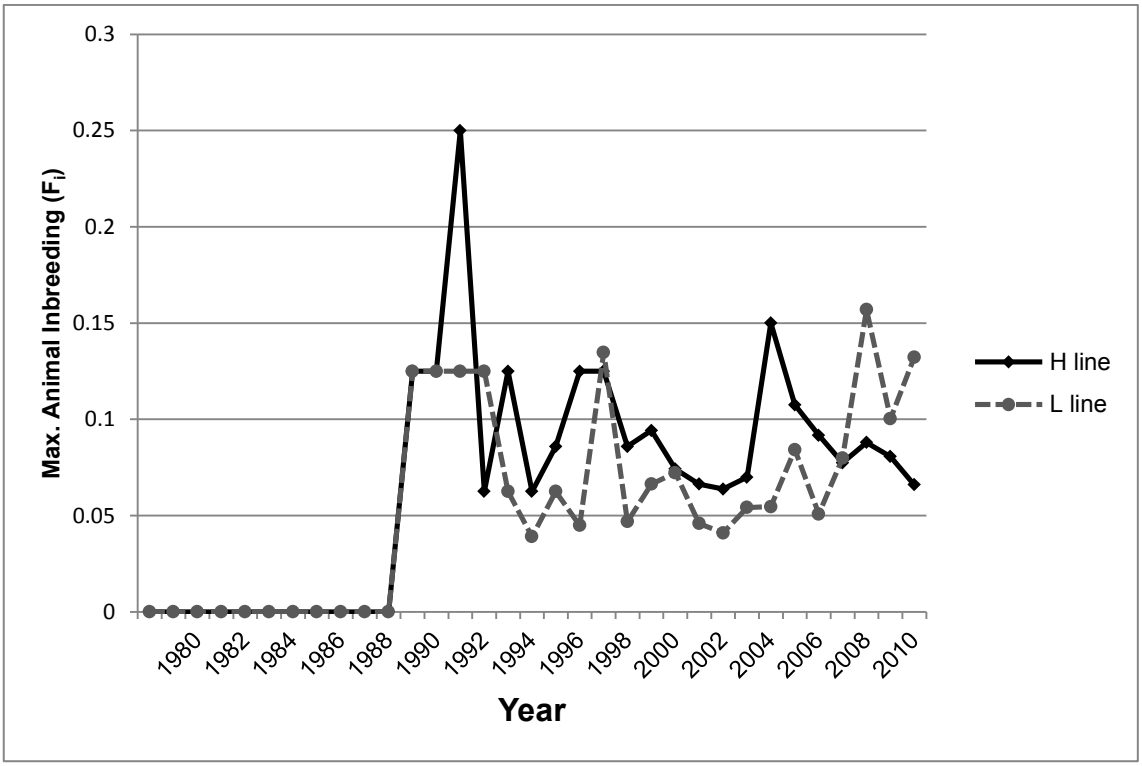


Figure 4.3 Maximum inbreeding coefficients in the H and L lines for the period from 1979 to 2011

The rate of inbreeding in the H and L lines for the period from 1979 to 2011 is depicted in Fig. 4.4. The gradual accrual of inbreeding is evident in Fig. 4.4, followed by a decline in the rate of inbreeding from 2006 to 2008 in both lines. This decline continued in the H line. In contrast, the rate of inbreeding of the L line increased notably from 2008. The total numbers of births and inbred births of animals in the H and L lines of the Elsenburg Merino resource flock are presented in Fig. 4.5.

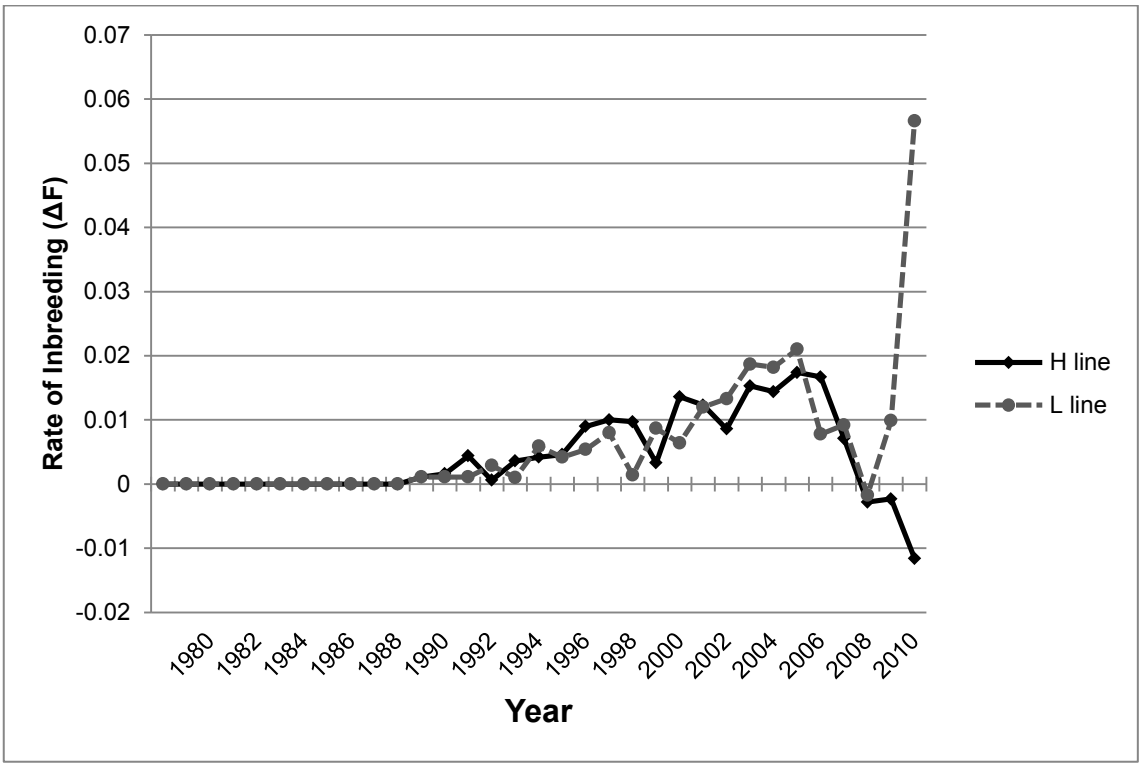
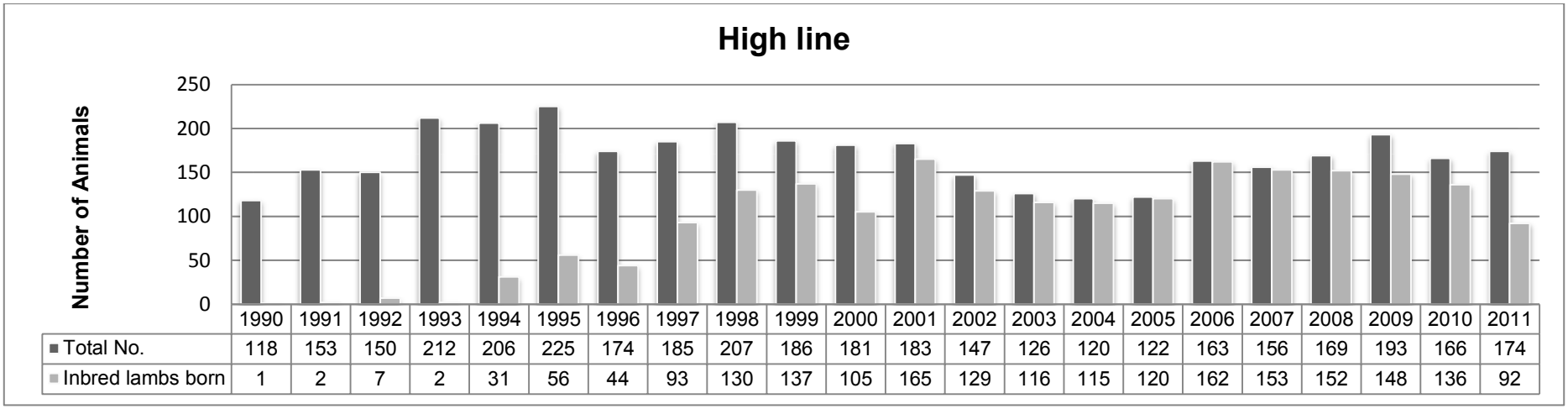
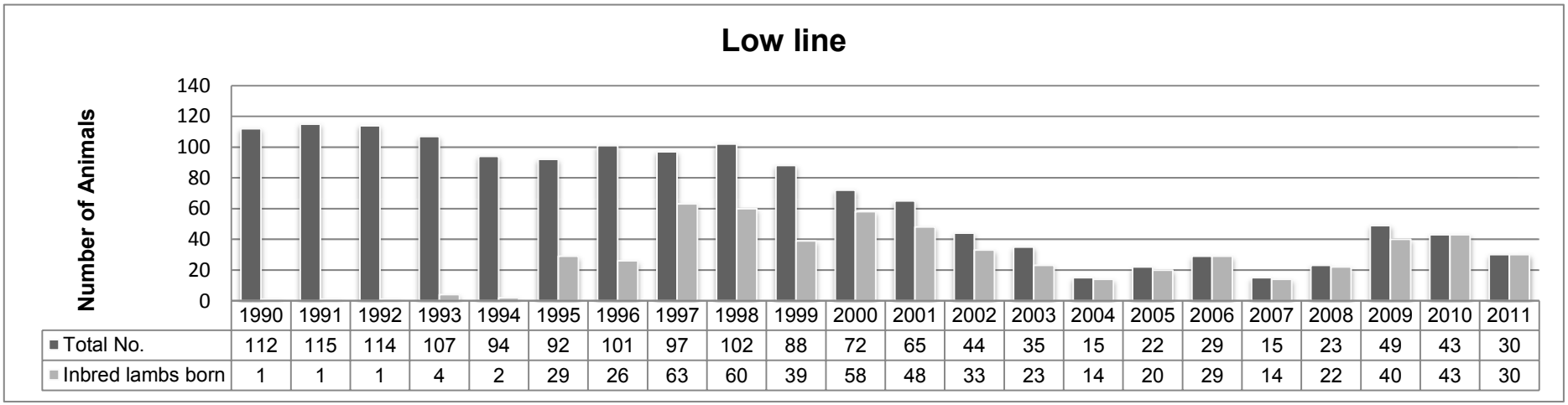


Figure 4.4 Rate of inbreeding in the H and L lines for the period from 1979 to 2011



	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Total No.	118	153	150	212	206	225	174	185	207	186	181	183	147	126	120	122	163	156	169	193	166	174
Inbred lambs born	1	2	7	2	31	56	44	93	130	137	105	165	129	116	115	120	162	153	152	148	136	92



	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Total No.	112	115	114	107	94	92	101	97	102	88	72	65	44	35	15	22	29	15	23	49	43	30
Inbred lambs born	1	1	1	4	2	29	26	63	60	39	58	48	33	23	14	20	29	14	22	40	43	30

Figure 4.5 Comparison of total numbers of births and inbred births of animals in the H and L lines of the Elsenburg Merino resource flock

The number of all sires and dams and inbred sires and dams by year are shown in Table 4.3.

Table 4.3 Total number of sires and dams as well as inbred sires and dams in the H and L lines of the Elsenburg Merino resource flock

Year	High line					Low line				
	*Ne**	Sire		Dam		*Ne**	Sire		Dam	
		Total	Inbred	Total	Inbred		Total	Inbred	Total	Inbred
1986	48	20	-	97	-	25	9	-	80	-
1987	84	18	-	95	-	44	9	-	78	-
1988	117	16	-	101	-	72	13	-	84	-
1989	94	7	-	95	-	64	4	-	91	-
1990	73	7	-	104	-	60	7	-	95	-
1991	47	4	-	103	-	45	6	-	90	-
1992	48	7	-	107	-	53	8	-	88	-
1993	46	7	-	136	-	55	8	-	68	-
1994	66	9	-	127	2	59	8	-	61	-
1995	56	8	-	136	2	60	9	-	66	-
1996	58	7	1	117	4	57	8	-	74	2
1997	72	8	1	120	8	48	6	-	75	8
1998	51	7	2	130	14	37	4	1	74	9
1999	49	5	3	117	18	33	4	1	65	13
2000	61	7	3	119	35	40	4	2	55	14
2001	41	6	4	126	52	35	4	-	51	16
2002	51	6	5	103	44	30	4	3	36	15
2003	59	6	5	92	64	37	4	4	29	11
2004	39	6	6	80	64	24	3	3	9	6
2005	33	6	6	87	74	29	4	4	17	15
2006	43	6	6	108	94	22	2	2	24	16
2007	38	6	5	110	103	16	3	2	12	10
2008	39	6	5	108	102	21	3	2	16	13
2009	46	7	6	103	102	25	4	3	16	14
2010	41	10	7	105	103	19	5	5	10	10
2011	51	7	4	118	104	20	3	3	23	23

* Effective population size (*Ne*) based on the number of parents

4.6 Discussion

An estimate of the inbreeding coefficient of an individual depends on the extent to which its parentage is known to some defined generation in the past. A more reliable estimate of individual inbreeding relative to a defined base population is provided by a more complete knowledge of pedigree (Groeneveld *et al.* 2009). Pedigree completeness was analysed by using two methods: pedigree completeness using the indexing method and by male and female lines up to three generations back. Pedigree completeness using the indexing method for the H and L lines has pedigree quality decreasing from 95% to approximately 50% by the sixth generation back. Pedigree completeness of the male and female lines shows a similar pattern. It is unlikely that inbreeding will be underestimated in either the H or L lines due to the quality of pedigree information used. Mean average relatedness was higher in the H line than in the L line. This may be the result of pairs of twins being more likely to be retained as breeding animals in the H line than in the L line. The GI was similar in both lines at 3.3.

Inbreeding increased with time, as is expected in any population of finite size. The increase in inbreeding is also evident from the increase in the number of inbred animals compared to the total number of animals in both lines over time. Inbreeding in the H line initially appeared to accrue somewhat faster that in the L line, until the rapid increase in the L line in 2011 (F_i = 9%). The rate of change of the average inbreeding coefficients based on the slope of the regression between 1986 and 2011 amounted to 0.00180 ± 0.01 for the H line and 0.00217 ± 0.01 for the L line.

The implementation of MOET in the H line in 1991-1992 and in both lines in 2009 served to mitigate the effects of inbreeding. It was possible to curb inbreeding in the period 2003-2009, when structured crossbreeding was practiced, as ewes where all ram-ewe combinations resulted in levels of inbreeding exceeding 6% were simply allocated to the other line. From 2008 onwards inbreeding in the H line could be controlled by introducing external unrelated sires from industry into the line. External sires were not introduced into the L line as it was difficult to find suitable candidates for introgression at that stage. All subsequent matings were thus within the L line, which accounts for the high levels of inbreeding in the L line past 2009.

The average inbreeding coefficient (F_i) was 1.47% for the H line and 0.73% for the L line. According to Miglior (2000) inbreeding coefficients of below 6.25% are an indication of passive inbreeding which accumulates slowly over time. Natural and/or artificial selection can therefore eliminate most deleterious genes under such conditions. A preliminary analysis by Cloete (2002) reports the average inbreeding coefficients in the H and L lines as below 2.5%. The additional pedigree data in this study allowed these estimates of F_i to be updated.

The rate of inbreeding is of vast importance in small populations (Meuwissen and Woolliams 1994). ΔF per generation was 0.5% for the H line and 0.6% in the L line. Nicholas (1989) suggests a ΔF of <0.5% whereas the FAO (2000) suggest a ΔF of <1%. The rate of inbreeding per generation for both the H and L lines fall within these recommendations. The rate of inbreeding estimated for the Elsenburg Dormer sheep

stud was estimated as 1.53% per generation over 19 generations in a flock developed under planned mating to avoid inbreeding (Van Wyk *et al.* 2009).

Population size, sex ratio, variance of family size and generation intervals are central factors that affect the rate of inbreeding (Santana Jr. *et al.* 2012). The number of inbred sires and dams reached a staggering 100% in the L line in 2011, with only 3 sires used to service the 23 dams. It is common in breeding systems to use unequal representation of the sexes, with fewer males than females used. The shortcoming of this is that small populations with fewer sires limit the gene pool for future generations and have a greater effect on the rate of inbreeding (Falconer and Mackay 1996) as is evidenced in the rapid increase in ΔF in the L line in 2011 to 5.7%.

The effective population size is the number of individuals that would give rise to the observed or calculated rate of inbreeding if bred in the manner of the idealized population (Falconer and Mackay 1996). The effective population size was computed using three different methods. ENDOG v4.8 calculated total *Ne* using the variances of family sizes as 302 and 290 for the H and L lines respectively. The two methods used by POPREP to calculate *Ne* were based on the number of parents and on the rate of inbreeding as 90 for the H line and 74 for the L line. The FAO (2000) guideline advises a minimum effective population size of 50 animals per generation for conserved populations. Meuwissen and Woolliams (1994) suggested a minimum range for *Ne* of between 31 to 250 individuals to maintain population fitness. The effective population size estimated using the variance of family size method places both selection lines safely above minimum requirements. However, the L line has effective population sizes lower than this in several years which may be cause for concern if this selection line is to be conserved. In a study of lines of Spanish Merino sheep, Analla *et al.* (1998) found that lines with small effective population sizes had a higher percentage of inbred animals and a resulting higher average inbreeding coefficient. This finding reflects the inbreeding trend in the L line in recent years.

The concentration of the origin of animals is given by the effective number of founders (f_e), 48 for the H line and 90 for the L line, and the effective number of ancestors (f_a), 322 for the H line and 227 for the L line. The effective number of founders measures how the balance in founder expected contributions is maintained across generations and accounts for selection rate and the variation in family size. The effective number of ancestors is the minimum number of ancestors, not necessarily founders, explaining the complete genetic diversity of of a population (Boichard *et al.* 1997; Gutierrez and Goyache 2005). In both lines, the number of ancestors explaining 50% of the gene pool was comparatively small. The ratio of f_e/f_a was respectively 1.08 and 1.19 for the H and L lines. A ratio larger than one indicates that bottlenecks have played a role in the formation of the population (Fair *et al.* 2012). The H line is close to unity but the L line ratio indicates that bottlenecks may have played a role in the formation of the population.

4.7 Conclusions

The overall rate of inbreeding per generation in the H and L lines are within acceptable levels. However, the low effective population size, high proportion of inbred animals and increase in ΔF in the most recent breeding year is of concern in the L line if this population is to be conserved. The significantly high rate of inbreeding in recent years reported in this chapter as well as the divergent genetic trends in Chapter 3 suggest that the lines are divergent enough to expect some within-breed heterosis for fitness traits in their reciprocal crosses, which is studied in Chapter 5.

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CHAPTER 5

Evaluation of reciprocal crosses and pure lines of Merino lambs and reproducing Merino ewes divergently selected for their ability to rear multiples

5.1 Abstract

Since 2003, part of a breeding flock of Merino ewes divergently selected for their ability to rear multiple offspring (the high and low lines) was subjected to reciprocal crossbreeding. Crossbred progeny in the L line x H line and H line x L line crosses were available from the 2003 year of birth onwards. The objective of this investigation is to evaluate reciprocal crosses between the H and L lines in terms of lamb survival traits, reproductive performance and heterosis. The lamb survival traits subjected to least squares analyses were birth weight, birth coat score, mortality to weaning and weaning weight. Purebred H line lambs and the progeny of H line ewes mated to L line rams were more hairy than L line progeny. The purebred H line lambs were also heavier at weaning than the other three genotypes. The L line lambs had a higher mortality rate prior to weaning compared to both the H line lambs and the crossbred progeny. Complete annual reproduction data for the two divergently selected lines and their reciprocal cross were available from 2005 to 2009. Similar to the analysis on annual reproduction, H line ewes outperformed L line contemporaries for lifetime reproduction across 3 lambing opportunities, from 2 years to 4 years of age ($P < 0.05$). The reciprocal crosses were intermediate and different from both the H and L line ($P < 0.05$) for total number of lambs born. Individual heterosis for annual reproduction was estimated at 2.2% for NLB, 13.8% for NLW and 8.5% for TWW. Corresponding estimates of heterosis for total production over three lambing opportunities were 8.7% for TNLB, 19.1% for TNLW and 13.8% for TTWW. Further research is required to establish whether this between bloodlines within breed heterosis for lamb survival can be exploited to reduce lamb losses and improve annual production.

5.2 Keywords: reciprocal crosses, lamb survival traits, annual reproduction, heterosis

5.3 Introduction

A major limitation to sheep production is perinatal lamb mortality which is influenced by management, the environment and genetic factors (Haughey 1991; Cloete and Scholtz 1998; Morris *et al.* 2000; Everett-Hincks and Cullen 2009). The conventional practice of intensive supervision at lambing time to mitigate obstetric problems in the ewe and to protect neonatal lambs is labour intensive and costly (Dwyer 2008). New methods like the easy care sheep systems with lower inputs have gained popularity, which involve rigorous culling of ewes that require lambing assistance or fail to rear a lamb to weaning, and result in lamb mortalities similar to those achieved through intense management (Alexander 1964; Dwyer 2008; Everett-Hincks and Dodds 2008).

An alternative approach to improve lamb survival and ewe rearing ability under pastoral conditions is through selective breeding (Haughey 1983). A reduction in lamb mortality

through selective breeding for rearing ability has been demonstrated (Haughey 1983; Cloete and Scholtz 1998; Everett-Hincks *et al.* 2005). It has also been shown that selection for the ability of ewes to rear multiples is a heritable trait and does not compromise the survival of lambs even though higher mortality levels are expected with an increase in multiple birth rates (Haughey 1991; Cloete and Scholtz 1998). Further, previous studies of the Elsenburg Merino resource flock divergently selected for the ability of ewes to rear multiples per joining has shown that lamb mortality differed between the selection lines (Cloete and Scholtz 1998; Cloete *et al.* 2009a).

The rate of reproduction (defined as number or total weight of lamb weaned per breeding ewe per year) and the productivity of the ewe flock have the most influence on profitability of a sheep enterprise (Snowder and Fogarty 2009). Thus improved reproduction rates are essential for the financial viability of Merino farmers (Olivier 1999) with the genetic improvement of reproduction a critical consideration. However, ewe reproductivity is a complex composite trait with sex-limited expression. Furthermore, it has a low heritability, which is typical of fitness traits and is recorded relatively late in life. These factors make improvement challenging but not impossible to achieve through composite trait selection (Purvis and Hillard 1997; Cloete *et al.* 2004; Scholtz *et al.* 2010).

Prompted by a low reproduction rate observed in the South African Merino industry (De Klerk *et al.* 1983; Fourie and Cloete 1993), two lines of Merino sheep were established by divergent selection from the same base population using maternal ranking values for multiple rearing ability since 1986 (Cloete and Scholtz 1998). Long term selection studies are used for measuring rate of change in selection response. The response to selection is achieved by using additive genetic variance. A diversity of gene frequency for selected and correlated traits is expected among different lines. Therefore, crosses among selected lines after long term divergent selection can provide information on non-additive genetic effects for quantitative traits (Yang *et al.* 1999). Reciprocal crossbreeding between the high (H) and low (L) lines of a part of this flock has been undertaken since 2003 to create a genetic resource flock for possible future genomic projects (Naidoo *et al.* 2005; van der Walt *et al.* 2009).

Generating heterosis is an important aspect of a crossbreeding strategy with maximum individual heterosis obtainable in the first cross of divergent populations (Leymaster 2002). It may be expected that reciprocal crosses between the H and L bloodlines will exhibit heterosis due to marked differences between the lines divergently selected for almost two decades (~7 generations) reported by previous studies of this flock (Cloete *et al.* 2004). Some heterosis was expected particularly for traits related to reproductive fitness with low a heritability, albeit at lower levels than crosses between breeds or strains (Willham and Pollak 1985; Kinghorn and Atkins 1987; Mortimer 1987).

The genetic basis of crossbreeding arises from additive effects due to the averaging of merit in the parental stocks and non-additive effects observed as heterosis (Kinghorn and Atkins 1987). This study further defines heterosis as the difference between the average performance of the crossbred individuals and the mean of the two parents (Falconer and Mackay 1996) which is commonly known as midparent heterosis and expressed as a percentage of the midparent value. Lamb heterosis is defined as the

performance of crossbred lambs raised by purebred ewes relative to purebred lambs raised by purebred ewes (Leymaster 2002). Heterosis is commonly attributed to genetic interactions within loci (dominance) and interactions between loci (epistasis) (Swan and Kinghorn 1992).

Information about heterosis derived from within sheep breed crosses was scarce in the literature. The purpose of this investigation is to evaluate reciprocal crosses between the H and L lines of the Elsenburg Merino resource flock in terms of lamb survival, lamb weight traits, reproductive performance and heterosis.

5.4 Material and Methods

5.4.1 Animals, management and location

Two lines of Merino sheep were divergently selected from the same base population from 1986 to 2009, using maternal ranking values for number of lambs reared per joining, as described in detail in Chapter 3.

5.4.2 Records

Lamb birth weight was recorded within 24 hours of birth while weaning weight was recorded at an age of approximately 3.5 months. Birth coat score was scored on a linear scale, taking cognizance of halo hair and short, curly, woolly groups of fibres. The scale was as follows: 1- Hairy; 2 – More hairy than woolly; 3 – Equally hairy and woolly; 4 – More woolly than hairy; 5 – Woolly. In cases where it was difficult to judge differences between adjacent classes, half marks were given (Cloete *et al.* 2003; Ponzoni *et al.* 1997).

Complete annual reproduction data for the two divergently selected lines and their reciprocal cross were available from 2005 to 2009. Only maiden ewes in the crossbred lines were present during 2005. This resulted in an interaction with empty cells between year and genotype. Because of this, data of only 832 ewe-year reproduction records of 368 individual ewes mated during the period from 2006 to 2009 were analysed.

The traits that were assessed included: number of lambs born and weaned per ewe mated, total weight of lamb weaned per ewe mated, and average lamb weaning weight at ~100 days of age, treated as a trait of the ewe. For the derivation of the latter records, weaning weight of individual lambs was recorded at an age of approximately 3 months, and adjusted for age. Individual weaning weights were then corrected for the effect of gender. Corrected weaning weights were then used to calculate total weight of lamb weaned for each parity for each ewe. Complete reproduction records (i.e. number of lambs born, number of lambs weaned and total weight of lamb weaned) were available for each parity. Average weaning weight for a parity was derived as age and gender corrected weight of lamb weaned divided by the number of lambs weaned.

Apart from annual reproduction records, reproduction of ewes were also analysed as total figures over three parities from 2 years to 4 years of age. These analyses were conducted to get an indication of crossbred performance over a period approaching the

"lifetime" of animals. To enable this, individual ewe records for the first, second and third lambing opportunity were summed to obtain a single figure for the entire period. "Lifetime" data were obtained for 132 ewes that were born from 2003 to 2005 and lambed from 2005 to 2009. These ewes accordingly belonged to the H line and the L line as well as the reciprocal cross between them.

5.4.3 Statistical analyses for lambs

The data were subjected to least squares analyses to account for uneven subclasses (Harvey, 1990). It is common to analyse continuous data such as birth weight, birth coat score and weaning weight by least squares procedures. However, the analysis of discrete data like lamb mortality is not that common. According to Harvey (1982), such analyses give adequate answers when the number of records analysed are large, and the frequency of success are not extreme (i.e. very close to 1 or 0). Fixed effects included in all analyses were selection line (H line, L line and their reciprocal cross), year (2003-2007), sex (male or female), age of dam (2-7 years) and birth type (single or multiple). Two-factor interactions between main effects were considered at first. They were not significant, and were excluded from the final analyses. The exception was the selection line x birth type interaction, that were retained in the analyses. The linear and quadratic effects of birth weight (mean ± SD = 3.74 ± 0.88) were included in the analysis on lamb survival, whereas weaning age (mean ± SD = 89.9 ± 12.0) was included as a linear covariate in the analysis on weaning weight. Significance levels below the 5% threshold are denoted by asterisks as by convention (*P<0.05; **P<0.01). Actual significance values are provided for P>0.05.

To estimate possible heterosis, linear contrasts were computed between the midparent value (i.e. the mean performance of the H and the L lines) and the mean performance of the reciprocal cross between them according to Harvey (1990).

5.4.4 Statistical analyses for reproducing ewes

The data were unbalanced and least squares procedures were thus used for analysis (Harvey 1982). Repeated records of the same individual were accounted for by fitting the random effect of animal nested within the fixed effect of genetic group (H line, L line and their reciprocal cross), using LSMLMW software (Harvey 1990). Other fixed and interaction effects included in the analyses were year (2006 – 2009), dam age (maiden or mature) as well as two-factor interactions among the fixed effects. Since interactions were not significant, they were excluded from the final runs in all instances. In the case of average weaning weight as a ewe trait, number of lambs weaned was fitted as a linear covariate to account for weaning weight differences between singles and multiples. Between ewe variance components were used to estimate the repeatability of reproduction, as described by Turner & Young (1969).

Total "lifetime" number of lambs born, number of lambs weaned and weight of lamb weaned (all expressed across three lambing opportunities) were analysed by least squares procedures to account for uneven subclasses (Harvey 1990). The fixed statistical model that was used included the effects of genotype (H line, L line and their reciprocal cross) and birth year (2003 or 2005), as well as the interaction between these main effects. As in the case of annual records, number of lambs weaned was

fitted as a linear covariate in the case of average weaning weight, to adjust data for weaning weight differences between multiples and singles.

In both analyses, linear contrasts were fitted to compare average crossbred performance for specific traits with the midparent value for the corresponding trait statistically (Harvey 1990). Individual heterosis (H_I) was then calculated by using the following formula (derived from Mavrogenis, 1996):

$$H_I = \frac{100(CB - MPV)}{MPV}$$

With:
CB = Average crossbred performance
MPV = Midparent value

5.5 Results

5.5.1 Evaluation of reciprocal crosses and pure lines of lambs

Progeny of H line ewes mated to L line rams were between 4.7 and 6.4 % lower (P < 0.05) in live weight than the other three genotypes. The midparent value derived from the two pure lines (the H line and L line) for birth weight were higher (P = 0.037) when contrasted with the two crossbred lines (Table 5.1). This means that heterosis was negative. However, the contrast between the H and L line were not significant (P = 0.53). Purebred H line lambs and the progeny of H line ewes mated to L line rams had lower birth coat scores than L line progeny (P < 0.05), suggesting that lambs from the former genotypes were more hairy than L line progeny. There was no evidence of heterosis as far as birth coat score was considered (P = 0.26).

Table 5.1 Least-squares means and standard errors for birth weight, birth coat score and survival of H and L line lambs, as well as the reciprocal cross between the H and L lines

Sire line	**H line (2)**		**L Line (3)**	
Dam line	**H line (2)**	**L line (5)**	**H line (4)**	**L line (3)**
At birth				
No of observations	677	85	135	113
Birth weight (kg)	3.84 ± 0.03^{a}	3.82 ± 0.08^{a}	3.64 ± 0.07^{b}	3.89 ± 0.07^{a}
Birth coat score (n)	3.27 ± 0.03^{b}	3. 38 ± 0.09^{a,b}	3.24 ± 0.08^{b}	3.53 ± 0.08^{a}
Mortality to weaning	0.108 ± 0.017^{c}	0.179 ± 0.042^{b}	0.131 ± 0.037^{b,c}	0.311 ± 0.036^{a}
At weaning				
No of observations	578	69	108	79
Weaning weight (kg)	21.0 ± 0.2^{a}	18.8 ± 0.5^{b}	18.8 ± 0.4^{b}	18.0 ± 0.4^{b}

^{a,b,c} Means with different superscripts differ (P < 0.05)

In a least-squares analysis where lamb mortality were adjusted for the linear and quadratic terms for birth weight, it was evident that purebred H line lambs, as well as crossbred progeny were less likely to succumb prior to weaning than purebred L line lambs (P < 0.05; Table 5. 1). Purebred H line lambs also sustained fewer (P < 0.05) mortalities than the progeny of L line ewes mated to H line rams. The contrast between the midparent value of the two purebred genotypes and the reciprocal

crossbred lines did not reach significance (P = 0.11), but the mean mortalty of the two crossbred lines (0.155) was substantially better than the estimated midparent value of 0.209 derived from the means of the two purebred lines in absolute terms. Purebred H line progeny were between 11.7 % (crossbred lines) and 16.7 % (L line) heavier than lambs of the other genotypes at weaning (P < 0.05). When the contrast between the two purebred lines and the reciprocal cross were considered, there was a tendency (P = 0.098) for the mean performance of the reciprocal cross to be somewhat below the midparent value of the two purebred lines.

5.5.2 Evaluation of reciprocal crosses and pure lines of ewes

Number of lambs born (NLB), number of lambs weaned (NLW), corrected weight of lamb weaned (TWW) and average weaning weight (AWW) over four lambing years (2006 to 2009), age of ewe at lambing (maiden or mature) and selection line (H line and L line ewes, as well as the reciprocal crosses between the H and L lines) are summarised in Table 5.2.

Table 5.2 Least-squares means (± s.e.) for number of lambs born (NLB), number of lambs weaned (NLW), corrected weight of lamb weaned (TWW) and average weaning weight (AWW) over three lambing opportunities according to lambing years (2006 to 2009), age of the ewe at lambing (maiden or mature) and selection line (H line and L line ewes, as well as the reciprocal cross between the H and L lines)

Effect	Number of observations	Trait			
		NLB	NLW	TWW	AWW
Overall mean	832	1.13 ± 0.04	0.85 ± 0.03	18.9 ± 0.6	21.6 ± 0.3
Lambing year			**	**	**
2006	198	1.06 ± 0.06	0.90^{b} ± 0.06	20.4^{b} ± 1.3	21.9^{b} ± 0.6
2007	218	1.04 ± 0.05	0.91^{b} ± 0.05	22.3^{b} ± 1.0	23.2^{c} ± 0.5
2008	216	1.19 ± 0.05	0.95^{b} ± 0.05	20.6^{b} ± 1.0	21.9^{b} ± 0.6
2009	199	1.22 ± 0.06	0.65^{a} ± 0.06	12.3^{a} ± 1.3	19.2^{a} ± 0.8
Dam age		*	*	**	*
Maiden	217	1.05^{a} ± 0.05	0.76^{a} ± 0.05	16.9^{a} ± 1.1	20.5^{a} ± 0.6
Mature	614	1.21^{b} ± 0.04	0.94^{b} ± 0.04	20.9^{b} ± 0.9	22.6^{b} ± 0.5
Selection line		**	**	**	**
H line	506	1.33^{c} ± 0.04	1.05^{c} ± 0.03	24.4^{d} ± 0.7	23.5^{b} ± 0.3
L line	129	0.90^{a} ± 0.07	0.54^{a} ± 0.06	11.9^{a} ± 1.3	20.1^{a} ± 0.7
L x H cross	99	1.16^{b} ± 0.08	0.91^{b} ± 0.07	18.2^{b} ± 1.4	19.5^{a} ± 0.6
H x L cross	97	1.12^{b} ± 0.08	0.90^{b} ± 0.07	21.2^{c} ± 1.4	23.2^{b} ± 0.7
Heterosis[#]		2.2% $^{(0.67)}$	13.8%$^{(0.03)}$	8.5%$^{(0.13)}$	-2.1%$^{(0.84)}$

^{a,b,c,d} Means with different superscripts differ (P < 0.05)

* (P < 0.05) ** (P < 0.01)

[#] Actual significance levels in bracketed superscripts

Mature ewes performed better than maiden ewes for all traits measured. The H line was superior to the L line and the reciprocal crosses for all traits. The number of lambs weaned in 2009 was notably lower than in other years.

Total number of lambs born (TNLB), total number of lambs weaned (TNLW), total corrected weight of lamb weaned (TTWW) and average weaning weight (AWW) over three lambing opportunities for year of birth and selection line are summarised in Table 5.3.

Table 5.3 Least-squares means (± s.e.) for total number of lambs born (TNLB), total number of lambs weaned (TNLW), total corrected weight of lamb weaned (TTWW) and average weaning weight (AWW) over three lambing opportunities according to ewe birth years (2003 to 2005) and selection line (H line and L line ewes, as well as the reciprocal cross between the H and L lines)

Effect	Number of observations	Trait			
		TNLB	TNLW	TTWW	AWW
Overall mean	132	3.34 ± 0.12	2.76 ± 0.12	61.9 ± 2.4	22.1 ± 0.4
Birth year		0.68	0.09	**	**
2003	47	3.38 ± 0.18	2.98 ± 0.18	69.3^{b} ± 3.6	23.3^{b} ± 0.5
2004	35	3.45 ± 0.22	2.84 ± 0.21	62.8^{b} ± 4.3	22.3^{b} ± 0.7
2005	50	3.21 ± 0.19	2.45 ± 0.18	53.5^{a} ± 3.7	20.8^{a} ± 0.6
Selection line		*	**	**	**
H line	67	3.79^{c} ± 0.15	3.34^{c} ± 0.14	79.9^{c} ± 2.9	25.3^{d} ± 0.5
L line	16	2.62^{a} ± 0.31	1.69^{a} ± 0.29	35.9^{a} ± 6.0	18.9^{a} ± 1.0
L x H cross	25	3.59^{b} ± 0.26	3.07^{b} ± 0.24	63.6^{b} ± 5.0	20.9^{b} ± 0.7
H x L cross	24	3.38^{b} ± 0.25	2.92^{b} ± 0.24	68.2^{b} ± 4.9	23.3^{c} ± 0.7
Heterosis#		$8.7\%^{(0.26)}$	$19.1\%^{(0.04)}$	$13.8\%^{(0.11)}$	$0^{(0.98)}$

^{a,b} Means with different superscripts differ ($P < 0.05$)

* ($P < 0.05$) ** ($P < 0.01$)

Actual significance levels in bracketed superscripts

5.6 Discussion

5.6.1 Reciprocal crosses and pure lines of Merino lambs

The lower birth weight of the progeny of H line ewes mated to L line rams compared to the three other genotypes (including the pure L line) is not obviously explicable. It could perhaps be attributed to the generally poor genetic merit of L line sires after years of downward selection which could not be mitigated by the H line dam. It is also possible that negative epistatic interactions/recombination loss, referred to as outbreeding depression (Mortimer 1987; Lynch 1990) caused by disrupting putative favourable linkages in the L line may account for why progeny of H line ewes mated to L line rams are inferior even to pure L line progeny. Cloete *et al* (2003) reported birth weights of 3.90±0.09 and 3.80±0.09 for the H and L line respectively using 4235

observations. The least squares means for birth weight obtained from the smaller data set in this study accord with the literature (Cloete *et al.* 2003).

Purebred H line lambs and the progeny of H line ewes mated to L line rams were more hairy than L line progeny. Birth coat scores potentially offer an advantage to lamb survival under extreme inclement conditions (Mullaney 1966). Even though studies have reported a low correlation between birth coat score and lamb survival traits (Kemper *et al.* 2003; Brien *et al.* 2010), it is still considered an important criterion for early age selection for cold resistance (Slee *et al.* 1991). In the Elsenburg Merino resource flock which has been divergently selected for the ability of ewes to rear multiples, a high heritability of 0.70 (Cloete *et al.* 2003) and 0.60 (Cloete *et al.* 2009b) was estimated for birth coat score. However, although Cloete *et al.* (2009 a;b) determined a marked line difference in survival, birth coat score was similar for the H and L lines which do not indicate a marked correlated change. This study found no evidence of heterosis for birth coat score in the reciprocal crosses of the lines.

The L line lambs had a higher mortality rate prior to weaning compared to both the H line lambs and the crossbred progeny. The improved lamb survival in the H line has been mainly attributed to the improved survival of multiples, as well as the behaviour features of H line lambs that improve their chances of survival (Cloete and Scholtz 1998; Cloete *et al.* 2009a). The mean survival of the two crossbred lines was notably superior to the midparent value in absolute terms, although the contrast did not reach significance. In his extensive literature review of crossbreeding and heterosis, Nitter (1978) concluded that lamb survival could be considerably improved by exploiting the individual heterosis of between breed crosses. Lamb survival was found to be highly influenced by heterosis in a study of purebreds and crosses among Merino bloodlines conducted by the New South Wales Department of Agriculture at Trangie (Mortimer *et al.* 1985; Mortimer 1987). This is due to significant variation in lamb survival reported between breeds and bloodlines (Nitter 1978; Mortimer *et al.* 1985; Mortimer 1987; Cloete and Scholtz 1998). Although it could not be demonstrated with a high level of certainty that heterosis in lamb survival was present in the current analysis, the literature suggest that such effects do, in fact, exist. Against this background, the absolute differences in this study appear to be in line with expectations but further studies are needed for confirmation.

The purebred H line progeny were heavier at weaning than the other three genotypes. This result accords with previous studies of the Elsenburg Merino resource flock (Cloete and Scholtz 1998; Cloete 2002).

5.6.2 Reciprocal crosses and pure lines of Merino ewes

Number of lambs weaned and total weight of lamb weaned were substantially poorer during 2009 compared to the other years. Scrutiny of the data for 2009 indicated that mortality from birth to tail-docking were uncharacteristically high at ~24% (67 of 284 lambs that were born alive), compared to a long-term average of 11% (Cloete *et al.* 2009). Two episodes of intense rainfall occurring during peak lambing possibly contributed to this higher than expected postnatal mortality rate. Predation by caracal and domestic dogs also played an important role in reproduction losses after tail-docking during 2009, as 16 lambs of 212 lambs that were tail-docked (7.5%) were

killed during attacks. Minimal lamb losses owing to this cause were recorded in the other lambing years. Lamb losses owing to damage-causing animals in South Africa are unpredictable, and may be extremely high (Herselman 2005). However, the average of 4.5% losses per lamb born in the survey by Herselman (2005) is comparable to the losses observed in the present study.

The reproduction of maiden ewes was, on average, poorer than that of their mature flock mates. This difference was expected, as several literature sources report that maidens are not as reproductive as their mature contemporaries (Sidwell and Miller 1971; Mortimer *et al.* 1985; Long *et al.* 1989; Mavrogenis 1996; Cloete *et al.* 2004).

Reproduction (number of lambs born and number of lambs weaned) in the H line was higher than in the L line. The two crossbred lines were intermediate and different from both the H and L line. Average weaning weight followed a slightly different pattern, being superior in the H line and the H x L line compared to the L line and the L x H line. This resulted in significant differences between all lines for total weight of lamb weaned, the H line being superior to the H x L cross, which was superior to the L x H cross, which in turn was superior to the L line. The markedly improved lamb output in the H line relatively to the L line is consistent with previous studies on the same resource flock (Cloete and Scholtz 1998; Cloete *et al.* 2004).

In the analysis on overall “lifetime” reproduction across three lambing opportunities, no interaction was accordingly observed between genotype and birth year. The combination of a lower average weaning weight and a tendency towards fewer lambs weaned per ewe mated in ewes born during 2005 resulted in a lower value for total weight of lamb weaned in these ewes. This is understandable, as they were the only progeny group assessed during 2009, when overall reproduction and average lamb weaning weight was compromised relative to the other years for reasons listed previously.

The same tendency as in the analysis on annual reproduction was observed, H line ewes outperformed L line contemporaries for lifetime reproduction across 3 lambing opportunities. The reciprocal crosses were again intermediate and significantly different from both the H and L line. When expressed relative to the L line, H line performance was improved with 97% for number of lambs weaned, while relative advantages for the crosses amounted to 82% for the L x H line and 73% for the H x L line. Corresponding values for total weight of lamb weaned were relative advantages of 123% for the H line, 77% for the L x H line and 90% for the H x L line. Previous studies have also demonstrated a marked advantage in terms of overall lamb output in the H line compared to the L line (Cloete *et al.* 2004; Cloete and Scholtz 1998). An Australian study accordingly reported a more than two-fold variation between 14 Merino bloodlines (1 strong-wool, 11 medium wool and 2 fine-wool bloodline), with a range in number of lambs weaned per ewe mated from 0.50 in a fine-wool strain to 1.04 in a medium-wool strain (Mortimer *et al.* 1985).

Individual heterosis for annual reproduction was estimated at 2.2% for number of lambs born per ewe mated, 13.8% for number of lambs weaned per ewe mated and 8.5% for total weight of lamb weaned per ewe mated (Table 6.1). Corresponding estimates for

total production over three lambing opportunities were 8.7% for number of lambs born, 19.1 % for number of lambs weaned (P = 0.04) and 13.8% for total weight of lamb weaned. Overall, the output attained from the two separate analyses was quite consistent. This was not unexpected, as the same data source was used in both instances.

Average values for individual heterosis derived from breed crosses amounted to 5.5% for number of lambs born, to 15.4% for number of lambs weaned and to 13.8% for total weight of lamb weaned (Sidwell and Miller 1971; Bradley *et al.* 1972; Hohenboken *et al*, 1976; McGuirk and Bourke 1978; Nitter 1978; Fogarty *et al.* 1984; Kinghorn and Atkins 1987; Long *et al.* 1989; Van Handel and Visscher 1995; Bittante *et al.* 1996; Mavrogenis 1996; Analla *et al.* 1998; Boujenane and Kansari 2002). Averages for individual heterosis derived from bloodline crosses within breeds were 4.0% for number of lambs born and 10.4% for number of lambs weaned (Kinghorn 1987; Analla *et al.* 1998). These estimates were generally consistent with results from the present study. In contrast to the quantitative reproduction traits, individual heterosis for average lamb weaning weight as a ewe trait was effectively zero. Literature values support this result, as most comparable estimates of individual heterosis for this trait were close to zero, averaging 2.8% for the across-breed analysis. A similar value was reported for the single between bloodline within breed estimate reported by Kinghorn and Atkins (1987).

The H and L lines performed as expected and the line difference accorded with expectation based on literature values. Although the reciprocal crosses did not outperform the parent pure lines in any of the assessed traits, moderate levels of heterosis were observed. Since average inbreeding coefficients were below 5% in both the H and L lines (Cloete 2002), heterosis cannot simply be attributed to the cancellation of the effects of inbreeding depression. Since the amount of heterosis shown by a particular cross depends on the differences of gene frequency between the two populations crossed (Falconer and Mackay 1996), the occurrence of heterosis could conversely be used to infer divergence between the H and L lines. The within line crosses of Merinos divergently selected for 7 generations have lower levels of heterosis than reported from between breed crosses. A few more generations of divergent selection might be required for the lines to become completely differentiated in terms of gene frequency, although the H line might already benefit from the effects of dominance and epistatic interactions between alleles.

5.7 Conclusions

This study contributes to the knowledge of lamb survival, reproductive performance and individual heterosis of crosses between lines within the Merino breed. The purebred H line lambs and the progeny of H line ewes mated to L line rams were generally superior to the L line and L line ewes mated to H Line rams for the traits assessed. A notably inconsistent result was that crossbred H line dams with L line sires had birth weights inferior even to the pure L line. Lamb survival appeared to benefit from individual heterosis, although not so significantly in this study. Total weight of lamb weaned is a good representation of flock productivity and significant levels of

heterosis for this trait where determined in this experiment. Chapter 5 has shown the superiority of the H line compared to the L line in terms of lamb survival and reproductive performance while the preceding chapters have demonstrated the divergence between the H and L lines using quantitative methods. In Chapter 6, both quantitative and molecular analyses were used to investigate the divergence between the lines.

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CHAPTER 6

Molecular divergence of the Elsenburg Merino flock based on RAPD markers

6.1 Abstract

Ten RAPD markers were used to study divergence of South African Merino sheep divergently selected for the ability to rear multiple progeny from the same base population since 1986. Phenotypic data on the lifetime reproduction of ewes born in 1999 and 2000 indicated that reproduction in the high line ewes was markedly higher than that of low line contemporaries ($P < 0.01$). The preliminary RAPD assay, conducted on 15 ewes from each line, used eight primers and produced 87% polymorphic loci. The mean coefficient of genetic differentiation between lines (G_{ST}) was estimated to be 0.25. The RAPD assays indicated the divergence between the H and L lines at a molecular level.

6.2 Keywords: RAPD markers, molecular divergence, reproduction

6.3 Introduction

Over several years, marked fluctuations were observed in the wool: meat price ratio, with the relative monetary value of wool generally declining and mutton becoming more expensive. These trends have resulted in the selection strategy of South African Merinos being adapted accordingly (Olivier 1999). The genetic improvement of reproduction, expressed as total weight of lamb weaned per lambing opportunity, is thus seen as one of the cornerstones of increased productivity.

Prompted by a low reproduction rate observed in the South African Merino industry (De Klerk *et al.* 1983; Fourie and Cloete 1993), two lines of Merino sheep were established by divergent selection from the same base population using maternal ranking values for multiple rearing ability since 1986 (Cloete and Scholtz 1998). Multiple rearing ability is a composite trait affected by the expression of several genetically influenced traits. Variation in these component traits contributes to the phenotypic variation in the composite trait. Multiple rearing ability is a combination of several different aspects of ewe reproduction (fertility and litter size), and offspring growth rate (mothering ability, milking performance, lamb survival, lamb growth rate). Multiple rearing ability is a composite trait that takes into account not only the litter size but also the number of lambs weaned and their survival as reflected in the ability of ewes to rear the multiples born. Lamb survival, and in particular the survival of multiples, were improved in the High line (Cloete *et al.* 2005). The selection strategy based primarily on the maternal phenotype has proven highly successful in the establishment of the two phenotypically distinct Merino lines (Cloete and Olivier 1998; Cloete *et al.* 2002; 2003a; 2004; 2005).

Furthermore, results from this selection experiment have shown a marked, divergent response in total weight of lamb weaned per parity amounting to +1.8% per annum in the H line and -1.3% per annum in the L line (Cloete *et al.* 2004). These distinct differences between lines possibly suggest that one or more putative loci with a marked effect on overall reproduction could be present. Random Amplified Polymorphic DNA (RAPD) markers (Williams *et al.* 1990) could be used to estimate the molecular genetic divergence between the H and L lines in an initial attempt to estimate the genetic distance between lines.

Ovine population diversity studies of various breeds using RAPDs have been undertaken. Refshauge *et al.* (2000) used RAPD assays for the detection of gene markers for clean fleece weight in medium-Peppin Merinos. Their study using RAPDs revealed significant genetic diversity within their fleece plus and minus flocks (Refshauge *et al.* 2000). RAPDs were used to measure genetic diversity in Egyptian (Ali 2003; Mahfouz *et al.* 2008); Turkish (Devrim *et al.* 2007; Elmaci *et al.* 2007); and Pakistani (Qasim *et al.* 2011) sheep breeds. Kunene *et al.* (2009) used RAPD's to provide genetic diversity data for three populations of indigenous Zulu sheep to evaluate the potential for the conservation and exploitation of locally adapted breeds. In a study to detect putative molecular markers for traits with as broad and complex a definition as fitness and reproduction, the 'shotgun' approach of RAPD markers has some initial benefits as an overview of DNA based divergence between the H and L lines compared to using specific co-dominant markers, such as microsatellites (Tapio *et al.* 2010); gene specific markers (van der Walt *et al.* 2009) or single nucleotide polymorphisms (SNPs) (Kijas *et al.* 2009).

The objective of this study is to show phenotypic data that establishes the divergence between the two lines, as well as the first investigation of DNA based diversity using RAPD markers to find evidence that the H and L lines are significantly divergent at a molecular level.

6.4 Materials and methods

6.4.1 Animals, management and location

Two lines of Merino sheep were divergently selected from the same base population from 1986 to 2009, using maternal ranking values for number of lambs reared per joining, as described in detail in Chapter 3.

6.4.2 Records and sample groups

To elucidate the phenotypic divergence between the lines, records of the two most recent ewe groups present for three lambing opportunities (regarded as an approximation of lifetime reproduction for this study) were analysed. Thus only 68 ewes, born in 1999 and 2000, and present in the flock at lambing at 4 years of age were considered. Number of lambs born, number of lambs weaned as well as total weight of lamb weaned were totalled over three lambing opportunities and expressed per ewe joined.

Preliminary RAPD assays were conducted on 30 randomly chosen ewes (15 per line from the H and L lines). Sixteen individuals born during 1999 and fourteen individuals born between 1996 and 1998 were used.

6.4.3 Molecular methods

6.4.3.1 Blood sample collection

Blood samples were collected into 4.5 ml EDTA Vacutainer tubes by jugular venipuncture, mixed by inversion to prevent coagulation, and refrigerated (4 ºC) or frozen (-20 ºC) after collection.

6.4.3.2 DNA purification, verification and quantification

Total genomic DNA, extracted from whole blood samples, was isolated using salt extraction methods. Two DNA purification kits were compared for optimal DNA yield from fresh and frozen blood samples, with an emphasis on frozen blood samples. The Promega Wizard Genomic DNA Purification Kit (Whitehead Scientific) produced optimal DNA yields with fresh blood samples; however the manufacturer's protocol for the isolation of genomic DNA from 300 µl whole blood had to be amended for optimal DNA yield from frozen blood samples. The optimisations to the Promega Wizard Genomic DNA Purification Kit for frozen blood samples were as follows: for optimal white blood cell lysis, the reaction was incubated for 45 minutes at 37 ºC, followed by 15 minutes at room temperature before protein precipitation. Rehydration of the DNA pellet was facilitated by incubation at 60ºC for 10 minutes, followed by overnight incubation at room temperature. The Gentra Puregene DNA Purification Kit (Adcock Ingram) was more effective on the frozen blood samples. It was found that this method produces high DNA yields with frozen blood samples, but rehydration is slow. Care had to be taken to ensure that the DNA pellet was completely dissolved in rehydration solution before using the DNA solution in PCR.

A diagnostic 0.8% agarose gel was run after the purification steps to confirm the presence of high molecular weight DNA. The 0.8% agarose gel was prepared using 0.4 g agarose in 50 ml 1 x TAE and 2.5 µl ethidium bromide (10 mg/ml). Molecular weight marker III (Roche) (0.25 µg/µl) was included in the gel to verify the presence of DNA. Each subsequent well was loaded with 10 µl of the purified sample DNA and 2 µl of loading buffer.

Since 4 µl of MWM III was loaded on the gel; the result was a band with the intensity produced by 1 µg of DNA. Most bands corresponded with the intensity of the MWM III reference concentration band, but some samples were of greater or lower intensity. For the standard sample, it was determined that there was approximately 1 µg of sample DNA per band on the gel, resulting in a concentration of 0.1 µg/µl since 10 µl of each sample was loaded. The approximate DNA yield per sample was 20 µg. Purity of the DNA was evaluated by the colour of the pellet in the rehydration step of the DNA purification procedure. A colourless pellet indicated relatively pure DNA. The PCR amplification in the RAPD assays was not hindered by inhibitors, thus it can be concluded that the DNA was of a high enough quality for these reactions.

6.4.3.3 RAPD assays and genotypic analysis

The RAPD assays conducted involved the selection of RAPD primers; the subsequent amplification of the dominant loci and electrophoresis to generate the RAPD profiles.

Five RAPD primers, selected from a list of 53 RAPD markers submitted to the AgResearch international sheep genetic linkage map collaboration (Cushwa *et al.* 1996), were used in this investigation. The selection was based on the number of polymorphic alleles identified for the marker, as well as the published information on the marker quality type produced by the primer. RAPD primers that produced well-defined and brightly stained fragments as described by Cushwa *et al.* (1996) were selected. Five randomly selected unpublished RAPD primers were included in the study, for the purpose of generating unlinked and neutral fragments for the study of between line divergence. The RAPD primers were named according to the Operon Technologies nomenclature. The RAPD primers and their sequences are listed in Table 6.1. Primers were synthesized at the University of Cape Town and diluted in 10 mM Tris (pH 8.0) to 20 µM working stock solutions.

Table 6.1 RAPD primers selected to test for polymorphic alleles

Primer Name	Sequence 5'-3'	Reference
B08	GTC CAC ACG G	Cushwa *et al.* (1996)
B20	GGA CCC TTA C	Cushwa *et al.* (1996)
C08	TGG ACC GGT G	Cushwa *et al.* (1996)
C19	GTT GCC AGC C	Cushwa *et al.* (1996)
D20	ACC CGG TCA A	Cushwa *et al.* (1996)
F03	CCT GAT CAC C	-
X07	GAG CGA GGC T	-
J09	TGA GCC TCA C	-
P16	CCA AGC TGC C	-
K03	CCA GCT TAG G	-

All RAPD reactions used *Taq* DNA Polymerase in Buffer A (Promega) and PCR Nucleotide Mix (Promega). The RAPD assays followed the protocols of Cushwa *et al.* (1996), but altered the reaction volume to 25 µl. The concentrations of the PCR reagents used are listed in Table 6.2.

Table 6.2 PCR Reagent volumes and concentrations for RAPD assays

Component	Volume per reaction (µl)	Final concentration
10 X PCR Buffer A	2.5	1X
10 mM dNTP mix	0.25	0.1 mM
25 mM $MgCl_2$	2.00	2 mM
RAPD Primer (20 µM)	1	0.8 µM
Taq DNA Polymerase (5u/µl)	0.12	0.6 u
Template DNA	2	
Sterile Millipore H_2O	17.25	
FINAL VOLUME	25 µl	

The constituents of the reactions were made up in a "master mix", mixed vigorously and aliquoted into sterile 0.2 ml PCR tubes on ice in an UV sterilized laminar flow hood. Amplification was effected on the Geneamp PCR System 2700 (Applied Biosystems).

The followed cycling conditions used for all RAPD reactions are represented diagrammatically in Fig. 6.1.

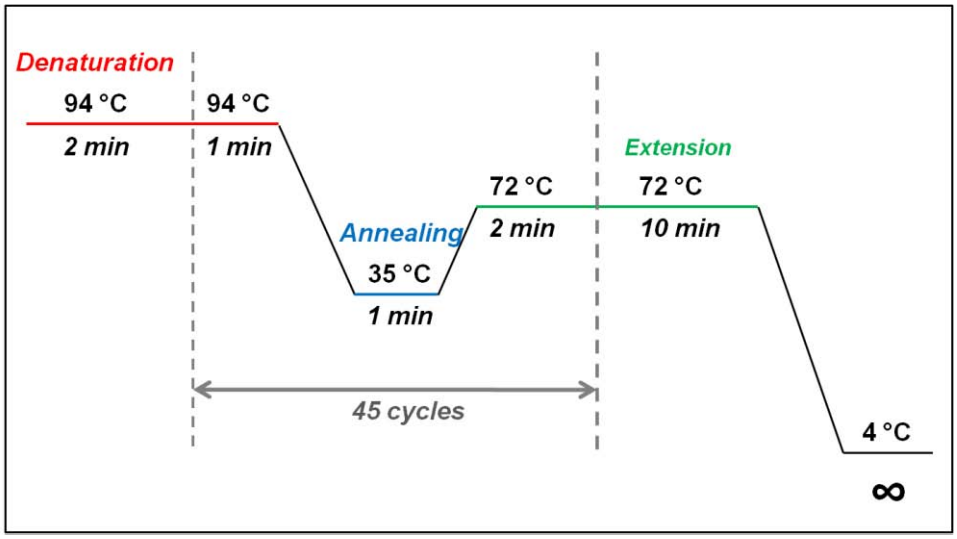

Figure 6.1 Cycling conditions used for all RAPD amplification reactions.

The preliminary RAPD assays were conducted using a single purified DNA sample with all ten RAPD oligonucleotide primers under standard PCR conditions. Subsequent RAPD assays were conducted on the selected sample of 15 high line and low line ewes. All primers were tested for repeatability of amplification patterns under constant reaction conditions.

The RAPD profiles of the 30 individuals of the sample population were generated by separating the RAPD markers amplified by PCR on 1.5% agarose gels by electrophoresis. The gels were run at 80 mA for approximately 90 minutes.

A genotypic analysis of each individual was performed by identifying and scoring the alleles at a RAPD marker locus. The RAPD agarose gel profiles were photographed using an UVIdoc gel documentation system. The captured images were saved using UVIsoft image acquisition and analysis software (UVItec). The UVIdocMW analysis was used to detect the alleles at each RAPD marker locus per individual. The size of each allele was calculated using the reference GeneRuler™ DNA Ladder, Low Range 700 – 25 bp (Fermentas) molecular weight marker. RAPDs are dominant markers with polymorphisms between individuals defined as the presence or absence of a particular band, which will be defined as an allele. For each RAPD primer, polymorphic alleles were scored for their presence or absence in all individuals by visually assessing photographs of the gels. The amplification products of all individuals were listed as discrete character states in a present (1) or absent (0) matrix.

6.4.4 *Statistical analyses*

The population genetic analysis package POPGENE (Yeh *et al.* 1999) was used for the analysis of the RAPD assay data, using the tools for assessing dominant diploid data of single and multiple populations. The Chi-squares analysis of the RAPD assay data was also done using POPGENE. Chi-squares were calculated as:

$$\chi^2 = \Sigma \frac{(Observed\ value - Expected\ value)^2}{(Expected\ value)}$$

The ASREML programme (Gilmour *et al.* 1999) was used for the estimation of fixed effects. Average reproduction records were subjected to analysis of variance, involving the effects of line (H or L) and birth year (1999 or 2000). Least squares procedures were used, to account for uneven subclasses. In the absence of significant selection line X birth year interactions, only line effect means were subsequently computed and tabulated. The following fixed affect model was fitted for all traits:

$$Y_{ijk} = \mu + L_i + BY_j + e_{ijk}$$

where Y_{ijk} is an observation of a reproduction trait (lambs born, lambs weaned or total weight weaned) on the ith animal of the ith selection line and being born in the jth birth year. μ is the overall mean; L_i is the fixed effect of the ith selection line (i= H,L); BYj is the fixed effect of the jth birth year (j = 1999, 2000); and e_{ijk} is the randomly distributed residual variances used as the error term to test the other effects for significance.

6.5 Results

6.5.1 Statistical analysis of reproduction records

Line effect means resulting from the analysis of variance of average reproduction records are shown in Table 6.3. Expressed relative to average L line performance, H line ewes outperform their L line contemporaries by 69% for number of lambs born; 91% for number of lambs weaned; and by 119% for total weight of lamb weaned (all traits are expressed per ewe joined).

Table 6.3 Average (± SE) reproduction of 1999 and 2000 born ewes in the H (n = 40) and L lines (n = 18) over three lambing seasons

Trait	Line		Significance
	H line	L Line	
Lambs born per ewe joined	1.18 ± 0.06	0.70 ± 0.09	**
Lambs weaned per ewe joined	1.07 ± 0.06	0.56 ± 0.08	**
Weight of lamb weaned per ewe joined (kg)	23.3 ± 1.3	10.6 ± 1.9	**

** Denote significant line differences (P < 0.01)

6.5.2 DNA yield

The Gentra Puregene DNA Purification Kit proved to be more effective on frozen blood samples and was subsequently used for the majority of the extractions. The presence of DNA after the isolation protocol was verified by running a 0.8% agarose diagnostic gel, shown in Fig. 6.2 and 6.3.

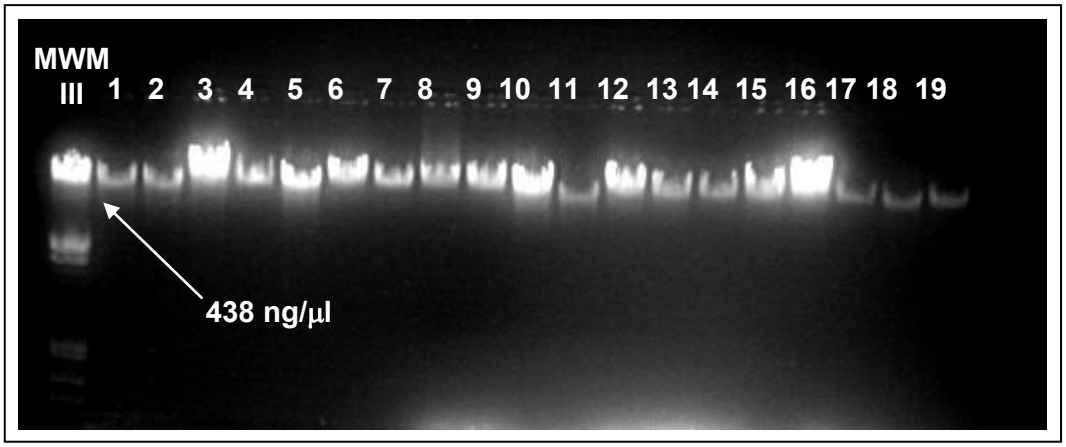

Figure 6.2 Diagnostic gel (0.8% agarose) used for visual DNA quantification by comparison of band intensity of Promega Wizard Genomic DNA kit purified DNA samples (lanes 1-19) to reference MWM III

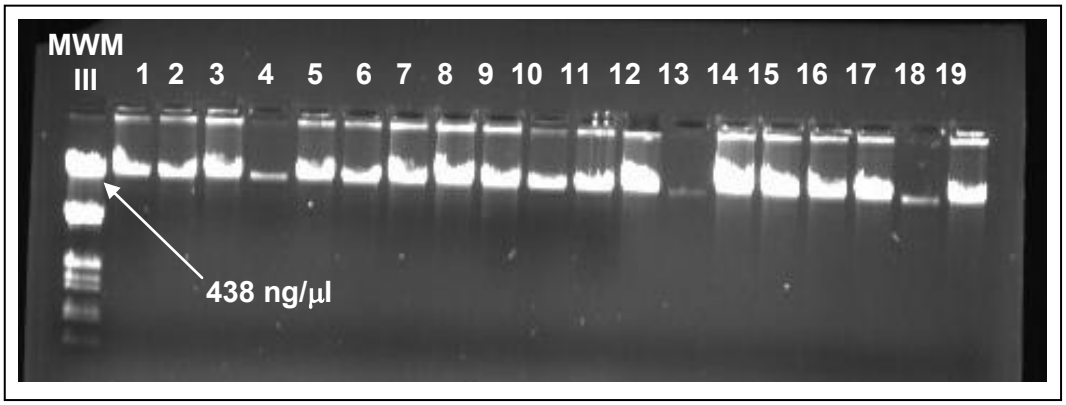

Figure 6.3 Diagnostic gel (0.8% agarose) used for visual DNA quantification by comparison of band intensity of Gentra Puregene DNA Purification Kit purified DNA samples (lanes 1-19) to reference (MWM) III.

6.5.3 RAPD assays and profiles

The ten RAPD primers synthesised were initially tested for optimal PCR amplification; the ability to produce informative polymorphic alleles; as well as repeatability. A preliminary RAPD assay using a standard DNA template with all ten primers was conducted, and repeated. The amplification products were separated on a 1.5% agarose gel. The resulting RAPD profiles are shown in Fig. 6.4.

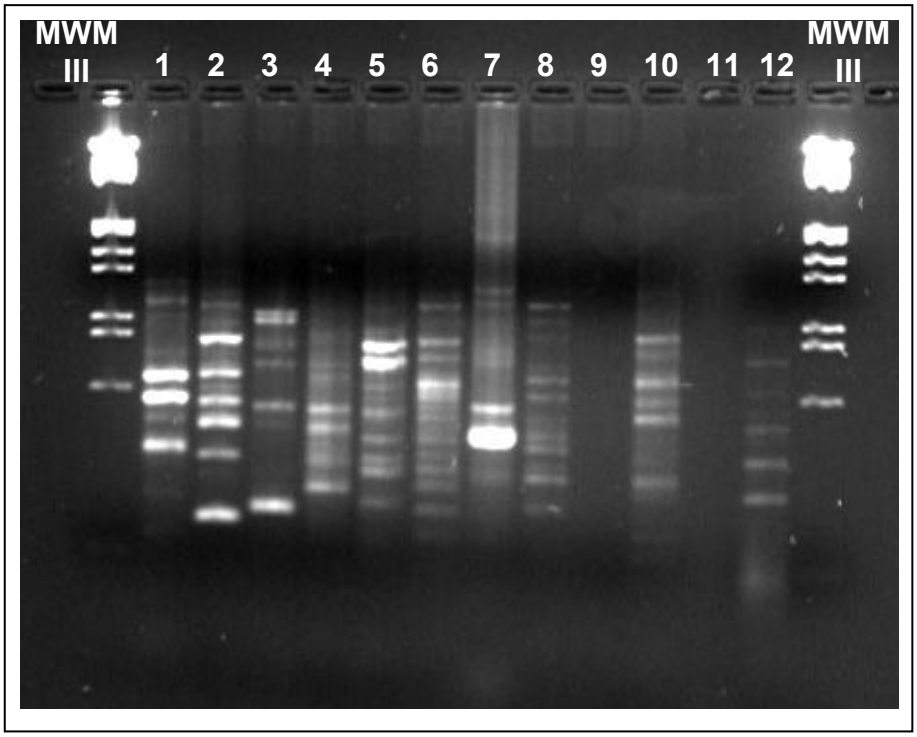

Figure 6.4 Preliminary screening of RAPD primers using a standard template DNA. Lanes 1-9, 10 and 12 contained reaction products of primers B08; B20; C08; C19; D20; F03; X07; J09; K03 and P16, respectively. Lane 9 was empty and lane 11 contained the negative control.

Primer J09 failed to produce repeatable polymorphic alleles and was thus discarded. The other nine primers were used to generate RAPD profiles for the sample population of 15 high line and 15 low line ewes.

The five primers selected from literature (B08; B20; C08; C19 and D20) produced informative polymorphic alleles. However, none of the RAPD bands amplified in this study corresponded to the alleles reported by Cushwa and Medrano (1996). Primer D20 amplified the highest number of significant alleles; the RAPD profile is shown in Fig. 6.5.

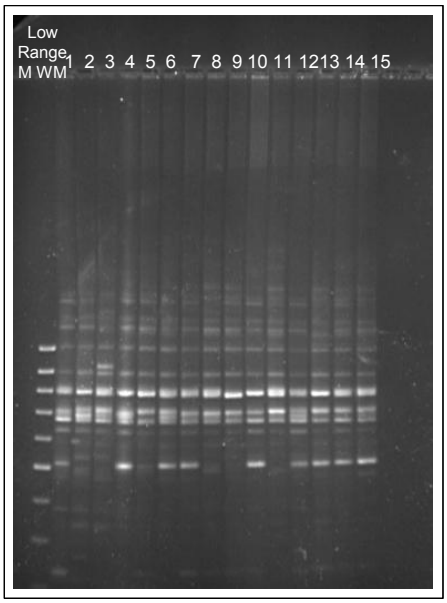

Figure 6.5 RAPD profile generated using primer D20 on high and low line ewes; lanes 1-8 are high line ewes and 9-15 are low line ewes

The RAPD primers selected randomly also performed adequately under the reaction conditions. Primers F03, X07, P16 and K03 produced significant polymorphic alleles. Fig. 6.6 shows the RAPD profiles of the high and low line individuals generated using primer P16.

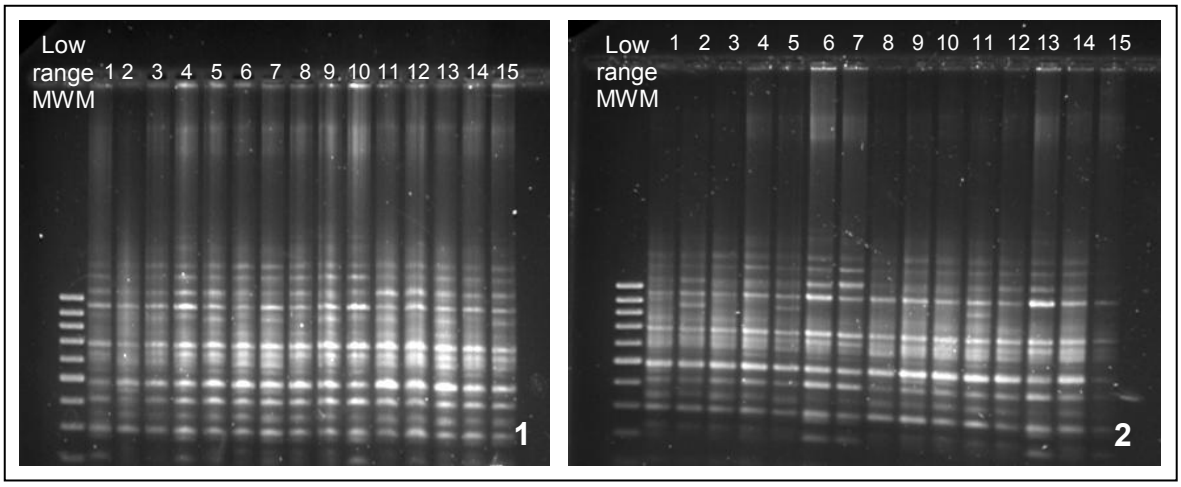

Figure 6.6 RAPD profiles using primer P16 on high and low line ewes; (1) Lanes 1-15 contain RAPD amplifications of P16 on the high line; (2) Lanes 1-15 contain RAPD amplifications of P16 on the low line

6.5.4 Genotypic analysis

The nine RAPD primers used for the analysis produced 51 polymorphic loci in the H line and 48 in the L line. The percentage of polymorphic loci was 69% and 65% for respective lines. The means of the observed number of alleles and the effective number of alleles were 1.69 and 1.36 for the high line, and 1.65 and 1.34 for the low line. The total percentage of polymorphic loci for both lines was 86%.

The mean coefficient of genetic differentiation (G_{ST}) was calculated at 0.25. Nei's genetic distance (Nei 1978) between the two lines amounted to 0.26. Genetic distances within the lines ranged from 0.15 to 0.49 in the high line and from 0.13 to 0.41 in the low line. Table 6.4 shows the significance of the line differences at polymorphic loci as Chi² statistics. While there are several non-significant differences in gene frequencies of the divergently selected lines, there are also a noticeable number of significant ($P < 0.05$) differences in gene frequencies.

Table 6.4 Chi-squares comparisons of gene frequencies between lines at polymorphic loci, derived from the POPGENE analysis

Primer	Allele											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
B08	**	**	ns	ns	ns	ns	**	-	-	-	-	-
B20	ns	**	**	**	**	**	ns	ns	-	-	-	-
C08	ns	ns	ns	ns	ns	**	*	ns	ns	-	-	-
C19	ns	ns	**	ns	**	**	-	-	-	-	-	-
D20	**	**	ns	*	**	ns	ns	**	ns	-	-	-
F03	ns	**	ns	ns	**	**	ns	**	-	-	-	-
X07	ns	**	**	**	ns	**	**	**	*	-	-	-
P16	ns	ns	ns	ns	**	**	ns	*	**	ns	**	ns
K03	**	*	**	ns	**	ns	-	-	-	-	-	-

-Denotes a absence of a locus

ns Denotes non-significance ($P > 0.05$)

* Denotes significant line differences in gene frequencies at polymorphic loci ($P < 0.05$)

**Denotes significant line differences in gene frequencies at polymorphic loci ($P < 0.01$)

6.6 Discussion

Reproduction of the H line ewes was markedly higher than that of L line. Average weight of lamb weaned per joining in the H line was thus more than double that in the L line. This clearly supported marked divergence between the two lines (Cloete *et al.* 2004), presumably resulting from divergent genetic selection since 1986. Means for total weight of lamb weaned accorded very well with earlier figures of 25.1 in the H line and 16.1 in the L line for the period from 1997 to 2002 (Cloete *et al.* 2003b).

The total percentage of polymorphic loci for both lines was 86%. Cushwa *et al.* (1996) correspondingly reported a total percentage of 97% polymorphic loci, when using 131 primers in RAPD assays using the Agresearch international mapping flock, Kantanen *et al.* (1995) generated only 47 fragments using 27 RAPD primers on Finnsheep. In

comparison, the present study produced 51 fragments in the high line and 48 in the low line using only eight RAPD primers. The RAPD markers in this study on Merinos therefore appear to be highly polymorphic.

The mean coefficient of genetic differentiation (G_{ST}) was calculated at 0.25. This indicated that approximately 25% of the total genetic variation was accounted for by differences between lines, while the remaining 75% corresponded to differences among individuals within lines. Nei's genetic distance (Nei 1978) between the two lines amounted to 0.26. Genetic distances within the lines ranged from 0.15 to 0.49 in the high line and from 0.13 to 0.41 in the low line. Despite a relatively small sample size and a low number of markers used in the present study, some indications of genetic differentiation between lines were observed in the RAPD assays. Such an outcome would support the significant phenotypic divergence reported. One of the limitations of the preliminary RAPD study was that the DNA isolated was not of a high enough quality to perform more sensitive and sophisticated assays. It was, however, the first attempt to derive genetic distances between the lines. In a study of the stress coping ability of the H and L lines, van der Walt *et al.* (2009) found no association between the gene specific CYP17 genotype and selection line even though differences in insulin-induced stress coping ability where demonstrated between the lines. Chromosome specific analyses of SNP chip data have identified loci that differ between the two lines (Sandenbergh *et al.* 2012). This finding corroborated the results of the preliminary RAPD assays.

6.7 Conclusions

The RAPD assays indicated the divergence between the H and L lines at a molecular level. It was the first attempt to derive genetic distances between the lines. A more accurate determination of molecular genetic differentiation between the H and L lines could be derived using SNP chip technology, as well as the possible detection of the effect of selection on reproduction traits at the molecular level.

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CHAPTER 7

General Discussion, Conclusions and Recommendations

This dissertation characterizes the divergence of the H and L lines and contributes to the understanding of the well-established Elsenburg Merino resource flock.

The purpose of Chapter 3 was to derive updated genetic trends for reproduction traits in the H and L lines. The first hypothesis postulated: Had the genetic trends estimated previously for the Elsenburg Merino Resource flock changed significantly? (Chapter 2) was tested. While previous research that was conducted over a shorter period since the establishment of the lines only reported linear trends as responses to selection; the present study reports change points in responses in the lines. Further work is required, as the trends that were reported in the current study need to be further explained. The question of whether the lines here have actually reached a selection plateau, or whether the reduction in selection intensity during the crossbreeding phase led to the observed responses needs to be elucidated. Also, the impact of the industry sires is likely to be better understood as more data accrue. It is therefore important for research on the lines to continue in order to expound on the questions generated by the present study. Future work would include the estimation of realized heritabilities as well as using multiple regression analysis to better estimate genetic trends for the lines.

The comprehensive pedigree analysis and assessment of inbreeding in the selection lines were presented in Chapter 4. The second hypothesis posed: Is inbreeding significant in the H and L selection lines? (Chapter 2) was addressed. As expected of any population of finite size, inbreeding in both lines increased with time but average inbreeding coefficients remained within acceptable levels (F_i < 6.25%). The rate of inbreeding *(ΔF)* per generation was also under 1% in both lines. These results indicate that inbreeding has not yet been a significant factor in the development of the H and L lines.

The monitoring of inbreeding in the selection lines and managerial interventions has thus far mitigated the effects of inbreeding, especially in the H line where F_i decreased to 2.3% in 2011. Specifically, the implementation of MOET in 1991-1992 and again in 2009, structured crossbreeding since 2003 that took levels of inbreeding into consideration and the introduction of unrelated commercial sires since 2008 has successfully facilitated the reduction of inbreeding in the H line. Only two of these interventions occurred in the L line: crossbreeding since 2003 and the implementation of MOET in 2009. It is concerning that inbreeding in the L line reached 9% in 2011. The rate of inbreeding in the L line in 2011 increased to 5.7% which is notably above the recommended *ΔF* of between 0.5% and 1%. It is recommended that external sires be introduced to the L line in order to conserve this selection line for future studies. Since increased inbreeding may result in an increase in homozygosity and an accumulation of deleterious recessive alleles especially in fitness traits; it is suggested

that further research might explore the effect of inbreeding depression on reproduction traits in the L line in particular.

Evaluations of the reciprocal crosses and pure lines of the divergently selected Elsenburg Merinos are provided in Chapter 5. The expectation of heterosis arose from the significant genetic divergence estimated between the H and L lines (Chapter 3). Non-additive variance can be exploited when sufficiently divergent populations are crossed, which may be observed as heterosis. Further, it is accepted that traits with lower heritability such as reproduction and fitness may exhibit higher levels of heterosis.

The purebred H line lambs and the progeny of H line ewes mated to L line rams were generally both superior to the L line ewes and L line ewes mated to H Line rams for the traits assessed (Chapter 5). A conspicuously inconsistent result was that crossbred H line dams with L line sires had birth weights inferior even to the pure L line. Lamb survival appears to benefit from individual heterosis, although this result was not significant in this study. However, there appear to be a strong argument from literature cited from between-breed crosses that lamb survival may actually benefit from heterosis. Further investigation is therefore required to determine whether this between bloodlines within-breed heterosis for lamb survival can be exploited to decrease lamb losses.

The evaluation of reciprocal crosses and pure lines of reproducing Merino ewes contributes to the knowledge of reproductive performance and individual heterosis of crosses between lines within the Merino breed (Chapter 5). Number of lambs weaned per ewe mated is a good representation of flock productivity and significant levels of heterosis for this trait where determined in this experiment. Future studies of data from backcrosses could reveal more information on non-additive genetic effects for quantitative traits.

It is conceded that the sample size used in this experiment (Chapter 5) is relatively small and an expanded data set would allow for a more accurate evaluation of the crosses; however it is not expected to change the results obtained significantly. It is also noted that since inbreeding levels were within acceptable levels for the period in which the crosses were evaluated it is unlikely to have played a role in this experiment. Therefore, any heterosis observed is not simply a result of the cancellation of inbreeding depression.

Returning to the third hypothesis posed at the beginning of this study (Do the reciprocal crosses perform better than the average of the pure lines?) it is now possible to state that moderate levels of heterosis were observed for lamb mortality and number of lambs weaned per ewe mated. Total weight of lamb weaned of the reciprocal crosses also tended to be slightly better than the midparent value, but did not reach significance. Although the derived levels of within-breed heterosis did not always reach significance there is ample evidence from the literature on between breed crosses that such effects do occur in sheep.

An attempt to derive genetic distances between the lines using molecular markers is detailed in Chapter 6. In response to the final hypothesis postulated in Chapter 2 (Is there evidence that the H and L line are significantly divergent at a molecular level?) the study yielded evidence that the lines are divergent at the molecular level. The mean coefficient of genetic differentiation between the lines was estimated to be 25%. This study did have several limitations. The infrastructure and funding for the molecular work was severely restricted and influenced the decision to use RAPD assays as a quick assessment of genetic distance. The study was also affected by DNA quality; blood samples drawn from the ewes had been improperly stored prior to the onset of the experiment. The sample size was relatively small but adequate for this type of explorative assay. Despite these constraints, the study was able to determine preliminary estimates of genetic distance between the H and L lines.

This study set out to characterize the divergence of the Elsenburg Merino resource flock. In summary, genetic trends presented indicate divergence between the lines for the traits measured. Levels of inbreeding were shown to be different for the H and L lines. Evaluations of reciprocal crosses and pure lines of the Elsenburg Merino flock do exhibit significant heterosis for some traits. However, the H line has been shown to be superior and different from the L line for all the reproduction traits measured, as was also found in previous studies. Finally, the preliminary RAPD assay estimated significant genetic distance between the H and L lines. This study makes a contribution to the information available regarding the Elsenburg Merino resource flock that was divergently selected for the ability of ewes to rear multiple offspring.

Future studies required to understand the divergence of the H and L lines clearly would include analysis of single-nucleotide polymorphism (SNP) data. The advent of the Illumina® OvineSNP50 genotyping beadchip offers a far more sophisticated approach to genome analysis of the H and L lines. Future research could use this technology to not only accurately determine genetic differentiation between the lines but also possibly detect the effect of divergent selection on reproduction traits at the molecular level.