

**Studies on genetic responses and genomic characterisation in
South African and Australian sheep**

by

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated) that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: April 2022

ABSTRACT

The current scope of small stock breeding in South African (SA) Merinos is constrained. In contrast to Australia (AUS) and New Zealand, no traits indicative of animal resilience or fitness are currently being recorded. Furthermore, the use of genomic selection (GS) could be a valuable tool to widen the scope of breeding objectives, but has not been formally explored on local populations. SA hosts multiple Merino resource flocks that are well recorded for a series of difficult to measure fitness traits, which can be exploited to the benefit of evaluating similar traits in commercial Merinos. The Elsenburg Merino flock is a resource flock that has been divergently selected for reproductive success on number of lambs weaned (NLW) that separates the H-Line (positive selection) from the L-Line (negative selection). The first aim of this study was to elaborate genetic parameters and realized genetic trends obtained in the Elsenburg flock as a demonstration of responses to genetic selection on a lowly heritable, sex-limited trait recorded late in an animal's lifetime. Apart from a focus on production and reproduction traits, the genetics and factors influencing lamb survival received special attention. A second aim of this study was to use marker data to better define the population genetic architecture of important SA and AUS sheep breeds, with a focus on the across country compatibility of SA and AUS ovine genetic resources. A third aim of this study was to assess the benefits of genomic information in genetic prediction of SA Merinos. Genetic trends in the Elsenburg lines showed that long term divergent selection for NLW did not have severely detrimental effects on the genetic change in other production traits, with a possible exception of clean fleece weight and staple strength. Despite the low heritability, favorable genetic trends (~1% of the overall mean) per year were reported for NLW in the H-Line. Genetic change was more moderate, but also worthwhile for component traits, namely conception rate and litter size. Survival to weaning, as a trait of the lamb, also showed a rate of gain close to 1% in the early years of the experiment, which contradicted the premise that lamb survival is not amenable to genetic selection. As measured by rectal temperature, H-Line lambs also performed better during cold stress conditions, and the fitness of H-Line lambs was strongly linked to increased cold stress adaptation. Population genetic parameters such as linkage disequilibrium and effective population size reiterated the fact that the genetic diversity of sheep can be high both across and within breeds, especially for Merinos. According to across country imputation, there is opportunity to combine SA and AUS Merino databases. This was supported by parameters of divergence (F_{ST}), principal component analysis and relatedness, but a narrow spectrum approach of specific populations is most likely to deliver the best results. The outcomes of GS were promising when a single-step GBLUP was used to predict genetic merit in unvalidated candidates for a series of production and reproduction traits. However, results varied across flocks and traits, and more research is needed to optimize these results. The study provided a foundation for further research on these and related topics.

OPSOMMING

Die huidige omvang van kleinveeteling is beperk in Suid-Afrika (SA). In teenstelling met Australië (AUS) en Nieu-Seeland, word geen eienskappe aanduidend van die fiksheid of veerkragtigheid van tans in SA aangeteken nie. Genomiese seleksie (GS) kan waardevol wees om die omvang van teeltdoelwitte uit te brei, maar dit is nog nie voldoende in plaaslike populasies skaappopulasies ondersoek nie. SA beskik oor etlike Merino hulpbronnkuddes wat omvattend aangeteken word vir 'n reeks moeilik-meetbare eienskappe. Hierdie hulpbron kan teoreties benut word om soortgelyke eienskappe in kommersiële Merinos te ondersoek. Die Elsenburg Merinokudde is so 'n hulpbronnkudde wat uiteenlopend vir reproduksiesukses in terme van getal lammers gespeen (NLW) geselekteer is, om twee lyne, naamlik die H-Lyn (positiewe seleksie) en die L-Lyn (negatiewe seleksie) te vorm. Die eerste oogmerk van die studie was om opgedateerde genetiese parameters en verhaalde genetiese tendense in dié kudde te vermeld om vordering in 'n laag-oorerflikke, geslagsgekoppelde eienskap wat laat in die dier se lewe gemeet word, te demonstreer. Benewens aandag aan produksie- en reproduksie-eienskappe word daar spesifiek op die genetika van lamoorlewing gefokus. 'n Tweede oogmerk was om genetiese merkers te gebruik om die populasiestruktuur van belangrike SA en AUS skaaprasse te ontrafel, met fokus op die oor-land verenigbaarheid tussen SA en AUS genetiese hulpbronne. 'n Derde oogmerk was om die voordele van genetiese inligting vir voorspellings in SA Merinokuddes te ondersoek. Genetiese tendense in die Elsenburglyne het daarop gedui dat langtermynseleksie vir NLW nie genetiese verandering in ander produksie-eienskappe benadeel het nie, met die moontlike uitsondering van skoonvaggewig en stapelsterkte. 'n Gunstige genetiese tendens (~1% van die algehele gemiddelde) per jaar (pj) is vir NLW in die H-Lyn gevind, ten spyte van 'n lae oorerflikheid. Genetiese verandering was stadiger (maar steeds noemenswaardig) vir besetting en meerlinggeboortes as komponenteieenskappe van NLW. Lamoorlewing tot speen het ook aanvanklik 'n genetiese toename van na aan 1% pj getoon, in teenstelling met die uitgangspunt dat lamoorlewing nie deur genetiese seleksie verbeter kan word nie. Soos aangedui deur rektale temperatuur, het H-Lynlammers beter tydens koue-strestydperke gevaar. Die beter fiksheid van H-Lynlammers het grootliks met verbeterde aanpassing by koue-stres verband gehou. Populasie-genetika parameters soos skakelingskoppeling en effektiewe populasiegrootte het bevestig dat genetiese diversiteit van skape binne en oor rasse hoog kan wees, veral in Merinos. Imputasie oor lande het daarop gedui dat die SA en AUS Merino databasisse kombineerbaar is. Hierdie resultaat is ondersteun deur die maatstaf van uiteenlopendheid (F_{ST}), hoofkomponentanalise en verwantskappe, wat getoon het dat 'n nosppektrumbenadering van die uiteenlopende populasies moontlik die beste resultate sal lewer. Die uitkomst van GS was belowend in gevalle waar 'n eenstap GBLUP gebruik is om genetiese meriete in ongevalideerde kandidate vir 'n reeks produksie- en reproduksie-eienskappe te evalueer. Resultate het egter oor kuddes en eienskappe verskil, tot so 'n mate dat meer navorsing benodig word om resultate te optimeer. Die studie het 'n basis vir verdere navorsing op hierdie en aanverwante onderwerpe verskaf.

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NOTES AND PUBLICATIONS

Chapters in this thesis have either been published or prepared for publication as a journal article, and were thus written as an individual entity. Consequently, some repetition occurs across selected chapters. Language, style and referencing are in accordance with the specifications of the journal *Animal Production Science*.

Results from this dissertation have been published in or submitted for publication in the following journals/conference proceedings:

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General Introduction

Small stock production contributes only a modest portion (8 - 10%) of the animal product income of South Africa (SA), but maintains preference for being one of the few viable enterprises suited to the large semi-arid and arid zones found in the country (Cloete and Olivier 2010). Traditionally, mutton or lamb, was seen as a by-product of the wool industry, but the income from meat has grown to 65-88% in woolled sheep (eg. Merino or Dohne Merino), with larger margins for dual-purpose (e.g. South African Mutton Merino or Dorper) or meat (e.g. Dorper or Meatmaster) sheep (Hoon *et al.* 2000).

The SA Merino was imported from Spain in the early 1700s and has been a major breed within the local sheep industry. The importance of the breed declined after the textile industry became less dependent on wool, but the breed maintained relative popularity into the modern era. Cloete and Olivier (2010) ranked breeds by the number of weaning weights submitted to the National Small Stock improvement scheme (NSIS) in the 2005 - 2008 period and reported a large majority (~55%) of records stemming from the major wool breeds (Merino and Dohne Merino), followed by the Dorper meat breed (~24%) and the dual-purpose South African Mutton Merino (~6.1%). The review by (Cloete *et al.* 2014) noted that the Dohne Merino (28%) had surpassed the Merino (22%) by the time of the updated 2010 – 2011 records, thus replacing the Merino as SA's most popular breed according to the NSIS.

Traditional sheep breeding in the commercial sector proved slow to align itself with more sophisticated developments and often subscribed to uncorrected and often biased methods of visual appraisal of 'overall excellence' according to subjective classing. While these traditionalist methods resulted in moderate improvements in hogget live weight and clean fleece weight, the absence of a well-developed selection index also resulted in unfavourable responses in fibre diameter (Erasmus and Delport 1984). Despite the establishment of the NSIS as early as 1964, functional recording in commercial flocks was only initiated by the early 1990s for wool and meat sheep breeds (Cloete and Olivier 2010; Schoeman *et al.* 2010). The demand for a more profitable yield from lamb production also influenced the selection objectives of Merinos, and the objectives of the NSIS aimed to improve income per animal by increasing reproduction, growth, and optimizing fibre traits in woolled sheep (Olivier 1999). The genetic trends for Merinos subsequently showed useful gains in liveweight, fleece weight, a reduction in fibre diameter, and a moderate gain in total weight weaned (Olivier and Cloete 2007).

As outlined by Schoeman *et al.* (2010), the traits considered by the major breeds are currently limited to the production traits such as bodyweight at weaning or yearling age, wool traits such as fibre diameter, fleece weight and staple length, with some attention to reproduction output such as number of lambs born, number of lambs weaned or total weight weaned per ewe lambled. The limited focus on production, or 'output' traits is in stark contrast with the developments at Sheep Genetics Australia which, at the earlier time of reporting by Brown *et al.* (2007) included up to 46 traits into the national evaluation targeting growth, carcass yield, wool, fitness and fertility. Similarly, the analysis of Sheep Improvement Limited in New Zealand (Newman *et al.* 2000, 2010) includes extensive trait groups for the output trait complexes growth, meat, wool, and reproduction, but also includes lamb survival, parasite resistance and resilience and examples such as facial eczema tolerance. Besides reproductive output in the form of number of lambs weaned, no such traits indicative

of animal resilience or fitness are currently recorded by the National Small Stock Improvement (NSIS) in SA (Cloete and Olivier 2010).

However, SA hosts various Merino resource flocks recorded for a variety economically important as well as difficult to measure traits (Schoeman *et al.* 2010). Since their establishment, the respective resource flocks have played an important role in leading the development of breeding methods and objectives for SA Merinos (see reviews: Cloete and Olivier, 2010; Schoeman *et al.* 2010; Cloete *et al.* 2014). An important example includes the demonstrable benefits of genetic selection for reduced fibre diameter while increasing liveweight in the Grootfontein Merino stud (Olivier *et al.* 1995; Cloete *et al.* 2007b), which promoted the use of selection indices to counteract unfavourable genetic correlations.

A population of particular relevance to this thesis is the Elsenburg Merino resource flock, a selection experiment that evaluated the gain from divergent selection for reproductive performance based on number of lambs weaned per ewe mated. The flock was established in 1986 by randomly screening ewes from another resource flock that selected ewes for secondary to primary (S:P) wool follicle ratio (Heydenrych *et al.* 1984). Limited to about 120 to 200 ewes in the H-Line (positive selection) and 80 - 100 ewes in the L-Line (negative selection) the flock is a numerically small resource, but to date stretches back 36 years since the onset of the selection program and presents the only experiment of this design and longitudinal depth. Selected results from the Elsenburg selection lines includes divergent responses for number of lambs weaned, at an upwards trend of 2% (of the mean) per year for the H-Line and 1% per year downward in the L-Line (Cloete *et al.* 2004, 2017). At the time, demonstrable results for genetic selection for reproductive performance were scarce and this finding had a major impact on farmers and breeders who had previously subscribed to more traditional views such traits were not amenable to genetic selection. Subsequent responses in the National Merino flock for number of lambs weaned (NLW) (Cloete *et al.* 2007b; Schoeman *et al.* 2010) could be directly linked to the demonstration within the H- and L-Lines. However, favourable selection line differences were not limited to the criterion for selection (i.e. number of lambs weaned). Within 12 years from the onset of the selection experiment, the H- and L-Lines had separable rates of lamb survival on the phenotypic level (Cloete and Scholtz 1998). In latter studies, divergent genetic trends for lamb survival were reported (Cloete *et al.* 2009) which also contradicted the wide held notion that survival rates cannot be improved by selection. Divergent trends were also reported for live weight and total fold score (Cloete *et al.* 2005) and maternal behaviour conducive to better lamb survival rates (Cloete *et al.* 2003, 2021). The H-Line also had a higher resistance to breach blowfly strike compared to the L-Line (Scholtz *et al.* 2010). In more empirical studies, animals originating from the H-Line appeared to have developed greater sensitivity in the function of the hypothalamic-pituitary-adrenal (HPA) axis in reaction to insulin induced hypoglycaemic stress tests (Hough *et al.* 2010), suggested to be a better capacity to cope with stressful situations (Hough *et al.* 2013). From these studies, it is evident that the Elsenburg lines present a valuable example of selection of animal fitness and resilience. There is thus an opportunity for further elaborating on the previous results obtained from this flock to contribute unique insight into the feasibility and strategies for selection of fitness or 'hard to measure' traits.

Genetic selection within a limited scope, such as placing the sole emphasis on production or 'output' traits, risks detrimental effects in metabolic, reproductive, metabolic or behavioral traits in farm animals (Rauw

et al. 1998). In turn, genetic selection is a valuable tool to be applied to the benefit of animal health and welfare (Jensen *et al.* 2008; Rauw 2016) and can also deliver indirect economic benefits by reducing input costs and reproductive wastage. Besides the need to minimize the current incidence of stress and early mortality of farm animals, current patterns of climate change indicate that production environments will become increasingly challenging, with direct relevance to small stock production (Rust and Rust 2013).

Most facets of the agricultural industry have a circular relationship with global warming patterns by both contributing to the progress of climate change as well as being affected by it (Aydinalp and Cresser 2008). An important objective in breeding programs should thus be to reduce the contribution to greenhouse gas emissions. Genetic selection for increased productivity and/or efficiency has been suggested as a valid option for reducing the carbon emissions of livestock per unit of production (Capper and Bauman 2013). However, overemphasizing output traits could risk an unwanted increase in feed intake (Pickering *et al.* 2015) in addition to the risks to animal health and welfare already noted above. More direct methods, such as the recording and quantifying of methane release (Pickering *et al.* 2015) is being explored, but would not be a realistic objective given the already limited capacity of widespread recording in SA, especially in the case of pastoral animals. The second objective of small stock breeding programs should thus be to consider animals' capacity to adapt to increasingly harsh environmental circumstances. Besides an increase in the cost of feed grains and forage crop production, effects brought about by climate change are likely to include an increase of parasite and disease challenges, and an increase in the severity and incidence of harsh climatic conditions (Smit *et al.* 1996). In the winter rainfall regions of SA, mean temperatures are expected to increase, and while episodes of cold, wet and windy weather are expected to become more sporadic, they could occur at a higher intensity (Midgley *et al.* 2005). Elsewhere, the need to adapt to unpredictable and varying stressors has appropriately prompted an increased interest in breeding animals more robust to harsh environmental conditions (Hayes *et al.* 2013; Misztal 2017; Berghof *et al.* 2019).

This could become particularly relevant to small stock that are generally maintained under extensive conditions where there are often little options to seek shelter from wind and rain in winter or shade in the summer. In fact, the exposure of sheep to challenging circumstances might increase as they are preferred for their perceived adaptability to more extreme environments (Rust and Rust 2013). If this is to be the case, it becomes increasingly important that input, or 'hard to measure' fitness traits should be considered in selection objectives. Such development should allow the genetic improvement of Merino sheep to be better equipped for adaption to a challenging landscape.

However, targeting the improvement of hard to measure traits is challenging. Traits associated with fitness or resilience very commonly have a low h^2 , are expensive or time consuming to measure, and in some cases are sex limited. In spite of the useful genetic variation locally demonstrated for fitness traits such as, for example, lamb survival (Cloete *et al.* 2009) or worm egg count (Cloete, *et al.* 2007a; Matebesi-Ranthimo *et al.* 2014), phenotypes to capture performance needs to be recorded on a large scale for genetic analysis. An attractive strategy to address these issues would be to exploit the initiatives already established by the research sector (Schoeman *et al.* 2010) to the benefit of development to a larger platform (Cloete *et al.* 2014). However, to date the contribution by the research sector has generally been by demonstration. In turn, there are only

limited examples of studies including data of industry animals (Olivier 1999; Olivier and Cloete 2007). In these findings, suboptimal genetic trends were reported compared to observations in research flocks. In addition to recording fewer traits, the reliability and completeness of industry data is considered compromised compared to that of research flocks, which would reflect in the accuracy of genetic selection, and thus delivered gain. It could thus be challenging to replicate or superimpose the results of research experiments in the commercial sector, and this requires further investigation. It is thus pertinent that an across sector analysis is explored, but the first consideration is the isolated basis in which resource flocks tend to operate. Population substructures can be highly influential in quantitative genetic analyses (Swan *et al.* 2016), and could be exacerbated where there is limited linkage by pedigree.

Modern developments in animal breeding provide multi-faceted opportunities to enhance genetic prediction where traditional pedigree-based best linear unbiased prediction (BLUP) has proved limiting. Genome wide marker data in the form of single nucleotide polymorphism (SNP) genotypes capture genetic variation at the molecular level. Meuwissen *et al.* (2001) proposed the use of these genome wide markers in genetic prediction as an estimate of ‘total genetic merit’, a concept that has broadly become known as genomic selection (GS). From the relatively widespread availability of SNP genotyping platforms, there remains an interest in functional annotation and exploiting of sequence data for the identification of ‘major genes’ (Georges *et al.* 2019). However, the basic theory of GS does not account for the specific identity of quantitative trait loci (QTL) which, at least conceptually, resembles a reversion to the assumptions of the infinitesimal model that formed the theoretical backbone within BLUP mixed models (Henderson 1976). The first application of GS in prediction models were in the form of genomic BLUP (GBLUP) models that replaced the numerator relationship matrix **A** with the genomic relationship matrix **G** (VanRaden 2008). Where **A** was derived from the pedigree, **G** was based on genetic covariance of SNP allele counts, scaled to be analog to **A**. The simplicity of GBLUP is attractive, but the method was limited by depending on all animals in the analysis to be genotyped. There was thus great interest combining both pedigree and genomic information and simultaneously evaluating both genotyped and non-genotyped animals. First, a two-step blending method was proposed that estimated SNP effects based on pseudo-observations derived from traditional BLUP analysis (VanRaden *et al.* 2009) and subsequently combining both pedigree and genomic predictions. However, the two-step method required an adjustment for double counting since the parent average, as by pedigree, is partially reflected in the genomic estimate. Alternatively, Misztal *et al.* (2009) suggested that genotype and pedigree information should be simultaneously considered in a single relationship matrix, and the methodology for a combined matrix (**H**) was presented by Legarra *et al.* (2009) and the inverse (**H**⁻¹) by Aguilar *et al.* (2010) and Christensen and Lund (2010):

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

Termed ‘single-step’ GBLUP (ssGBLUP), this implementation of genomic selection based on replacing **A** with **H** compared favorably with two-step GBLUP (Aguilar *et al.* 2010). As of 2021, single step methodology was still a very active topic of research (Misztal *et al.* 2020) with a variety of emerging issues (Misztal *et al.* 2021) and remains under development.

Sometimes considered a ‘revolution’ in animal breeding, GS has been applied in a variety of both livestock (Van Eenennaam *et al.* 2014; Boichard *et al.* 2016) and plant species (Crossa *et al.* 2017). The accuracy of GS depends on the h^2 of the trait, the genetic diversity and the size of the reference population (Goddard and Hayes 2009a). The more diverse populations of interest are; the lower the linkage disequilibrium (LD) between markers; the higher the effective population size (N_e); and the larger the sample size (N) needed to attain a set level of accuracy (Goddard and Hayes 2009b). Besides the direct application in GBLUP or ssGBLUP models, marker data has delivered unprecedented insight into these population genetic parameters, including in sheep (Kijas *et al.* 2009, 2012, 2014). Knowledge of these parameters could facilitate breeding program design or predict the expected benefits available from investment in genomic data. Studies have subsequently shown that these predictors of the accuracy of GS is highly variable across species. For example, the level of LD in sheep at a 50 kilo-basepair (kb) distance observed for most sheep breeds (r^2 : 0.08 to 0.12) (Kijas *et al.* 2014) was considerably lower than that observed for Holstein dairy cattle (0.18 – 0.3) (Habier *et al.* 2010) or pigs (0.36 to 0.46) (Badke *et al.* 2012). Another important observation was the highly variable estimates of genetic diversity across breeds within the sheep species. Compared to some low N_e estimates of 150 for breeds such as the Border Leicester, Kijas *et al.* (2012) reported N_e estimates for breeds such as the Australian Merino to be particularly high (850) and a generally high level of genetic diversity for the breed has also been well reported elsewhere (Al-Mamun *et al.* 2015; Ciani *et al.* 2015; Prieur *et al.* 2017).

For these reasons, it is important not to superimpose the predicted benefits of GS from commonly reported examples such as dairy cattle (eg. García-Ruiz *et al.* 2016; Wiggans *et al.* 2017) onto sheep breeding programs, especially in the case of a breed of high genetic diversity such as the Merino. Also, for a large, or industry wide population to contribute to and benefit from the same reference population, a sufficient level of connectivity, or genetic linkage, is important across boundaries that might lead to genetic group structures within the population (Clark *et al.* 2012; Pszczola *et al.* 2012; Van der Werf *et al.* 2015). In this regard, population genetic studies remain a valuable tool for efficient breeding program design.

Despite the challenges and different breeding structures associated with small ruminants, the use of genomic information in sheep genetics became an active area of research for meat or dual purpose sheep, as well as dairy sheep and goats (see review: Rupp *et al.* 2016). With a reference population of 2000, Shumbusho *et al.* (2013) predicted that annual genetic gains could be up to 18% greater for combined meat and maternal traits in sheep. Accordingly, Van der Werf (2009) noted that the expected gain from GS in sheep would be less compared to the above species, but could rather deliver opportunities for including hard to measure traits. In this regard, methods of GS should be considered as a tool to enlarge the scope of genetic improvement of SA Merinos. Some traits that capture animal resilience or fitness (Brito *et al.* 2020) could be more cumbersome to measure or invasive. However, promising results obtained by the implementation of GBLUP or ssGBLUP models suggested that there is potential to limit these measurements to the reference population (Pickering *et al.* 2013, 2015; Brito *et al.* 2017; De Oliveira *et al.* 2021).

Similar to the situation regarding recording of difficult to measure traits, the objectives of the research sector (Cloete *et al.* 2014) has provided the only example of a dedicated genotyping strategy for SA Merinos (or any SA sheep breed). This was not restricted to resource flocks, but samples originating from these flocks

contribute to the current bulk data that would form the genotypic reference population is SA. Similar to the initial developments for Australian Merinos (Swan *et al.* 2012, 2014), the research sector could play a valuable role in establishing the development of GS methods for SA Merinos. This would include the commonly recorded production traits but should also serve with a dual objective of building a foundation for a wider range of selection objectives.

In the context of the discussion above, the objectives of this thesis consider a range of topics that aims to elaborate on options for genetic selection of Merino sheep in SA. The first five chapters delivers an in-depth account of the information generated by the Elsenburg resource flock as a unique example of selecting for improved fitness while maintaining economic value. This includes the realised responses in production (Chapter 1) and reproduction traits (Chapter 2) following 32 to 36 years of divergent selection. The subsequent chapters (3, 4 and 5) decompose the apparent correlated benefits of greater resilience of new-born lambs in the flock. To address the contrast of local breeding strategies relative to the high level of development within the international landscape, Chapters 6 and 7 explores the compatibility of local genetic resources with the well-established structures seen in Australia, with important comments extended to both within and beyond the Merino breed. Finally, Chapter 8 considers the first application of genomic selection methods for SA sheep including both resource and commercial flocks in a single analysis. This research is limited to production and reproduction traits but serves as a useful guide for initiating developments towards more inclusive breeding goals for SA sheep.

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CHAPTER 1:

Genetic parameters and trends for production traits in South African Merino lines divergently selected for number of lambs weaned per mating

Abstract

The Elsenburg Merino flock is a South African resource flock that was divergently selected for number of lambs weaned per joining and recorded for an array of production traits. The flock consisted of the H-Line (positive selection) and L-Line (negative selection) divergently selected from 1986. The study aimed to contribute to genetic parameters of production traits as well as observe subsequent trends as a response to selection for NLW. Genetic parameters were estimated for weaning weight (WW), yearling weight (YW), greasy fleece weight (GFW), clean fleece weight (CFW), fibre diameter (FD), clean yield (CY), staple length (SL), staple strength (SS), coefficient of variation of fibre diameter (CVFD) and total skin wrinkle score (SWS). Genetic and environmental (co)variance components and ratios as well as genetic trends were computed. The heritability (h^2) of weight traits were moderate (WW: 0.15; YW: 0.28) and wool traits were generally highly heritable between 0.34 (CFW) to 0.60 (CY) while h^2 of SWS was 0.50. Maternal genetic (m^2) components were significant for WW, YW, GFW, CFW and SWS, but the largest m^2 estimate was 0.10 for GFW. The direct-maternal covariance (r_{am}) was high and positive for YW (0.57), but negative for GFW (-0.35). Genetic correlations were generally favourable or negligible, with the exception of positive relationships of FD with CFW and SS as well as that of CFW with SWS. Genetic trends involving the H- and L-Lines were significantly ($P < 0.05$) divergent for all traits except SL and CVFD. However, annual gains expressed as a percentage of the phenotypic mean were generally low, with the notable exception of SWS, which increased by 1% per annum in the L-Line. The traits considered in this study can be improved by genetic selection, but antagonistic relationships with FD should be accounted for. Except for an unfavourable trend in SS, selection for reproduction had little detrimental effects on traits of economic importance. Emphasis on reproduction traits can be recommended.

1.1 Introduction

Useful genetic variation has been observed in traits related to wool and meat production in Merino sheep (Safari *et al.* 2007a; Greeff *et al.* 2008; Huisman *et al.* 2008), but exploiting genetic components depends on robust and reliable genetic parameters for economically important traits. A review of earlier studies reported means of genetic parameter estimates affecting wool and meat production in a variety of sheep breeds (Safari *et al.* 2005). Model selection, such as accounting for maternal effects, is key to deriving accurate genetic parameters. There is a risk of inflated direct heritability estimates when significant maternal components are not considered (Maniatis and Pollott 2003; Fozi *et al.* 2005) and slower genetic gains from indices that ignore such effects (Fozi *et al.* 2005). Also, substantial negative direct-maternal genetic covariance estimates have been reported which further risks affecting measures of direct heritability (Maniatis and Pollott 2003; Safari *et al.* 2006; Huisman *et al.* 2008; Swan *et al.* 2008). Wool and dual purpose sheep breeding aims to increase yield in both wool and meat traits while reducing fibre diameter (Olivier 1999; Olivier and Cloete 2007). In managing relationships deemed unfavourable (Swan *et al.* 1995), accounting for genetic correlations between traits of economic importance is also necessary. In the review of genetic parameters by Safari *et al.* (2005), correlations were not reported with the same level of confidence as the heritability estimates, and both accurate genetic parameters are essential for worthwhile genetic gains to accrue in response to multi-trait indices (Safari *et al.* 2006).

The Elsenburg Merino flock is an experimental research flock divergently selected for reproduction through maternal performance in terms of number of lambs weaned per mating. The flock is well recorded for a wide variety of traits including those of economic importance, making it suitable to contribute the estimation of genetic parameters as well as observe genetic trends during the extended period of the selection program. Observed responses in the Elsenburg flock have allowed various studies to demonstrate the feasibility of genetic progress in reproductive traits (Cloete *et al.* 2004; 2007; 2009). The importance of reproductive performance to overall production output motivated inclusion into South African Merino breeding objectives as a key trait (Olivier 1999). However, the current state of the market reflects a relatively promising and stable outlook for both wool and meat prices (Abstract of Agricultural Statistics 2019) and the performance of economically important traits should not be neglected in the objective of improving reproductive performance. In a previous analysis, Matebesi-Ranthimo *et al.* (2017) reported generally favourable or negligible genetic correlations between reproductive traits and objectively measured wool traits, but some unfavourable correlations were reported for fibre diameter, staple strength and clean yield. It is thus worthwhile to elaborate on the genetic change of economically important traits subject to selection for reproductive performance.

Against this background, the first objective of this study was to estimate direct and maternal genetic parameters and correlations between economically important traits. The second objective was to investigate the long-term responses of the same production traits in a selection environment defined by divergent selection for number of lambs weaned.

1.2 Materials and Methods

1.2.1 Flock description and selection history

The Elsenburg Merino flock consists of two selection lines separated by divergent selection for reproduction. The experiment commenced in 1986 by sourcing a base population from a Merino line selected for an increased secondary to primary (S:P) wool follicle ratio (Heydenrych *et al.* 1984). Selection evaluation of replacements of both sexes was based on maternal rankings for number of lambs weaned per joining according to a ranking table for number of lambs weaned per ewe joined, as proposed by Turner (1977). As maternal performance was used to select progeny of the following generation, no selection was thus based on the performance of the current flock. Ranking values were derived as follows (see Turner 1977):

$$(\hat{G} - G_o) = (0.5h^2) \left(\frac{k}{[1 + (k - 1)t]} \right) (P_D - P_\mu)$$

Where $(\hat{G} - G_o)$ is the ranking value for the individual considered as replacement, h^2 is the heritability of number of lambs weaned per joining, k is the number of times the dam has been joined, t is the repeatability of number of lambs weaned per joining, $P_D = K/k$ where K is the total number of lambs weaned by the dam and P_μ is the mean number of lambs weaned per joining for ewes up to and including the present age of the dam. Initial parameter values were obtained from the Tygerhoek Merino flock, credited to be a good representative of a typical South African Merino Flock at the time (Cloete 1986). In practice, the implementation of the ranking values boiled down to selecting both of male and female replacements from the descendants of ewes that have reared more than one lamb per joining (i.e. reared twins at least once) within the High Line (H-Line). In contrast, progeny of ewes that have reared fewer than one lamb per joining (i.e. were barren or lost all lambs born at least once) were preferred for selection within the Low Line (L-Line). Flock replacement occasionally required that maiden ewes descended from dams that reared one lamb per joining be screened into both lines to ensure that 30-36 replacements were added to the ewe flock annually (i.e. a replacement rate of 25-30%). Replacement ewes remained in the breeding flock for at least 5 joinings, bar death as well as severe udder or teeth malfunction. Roughly 120 ewes represented each line at the onset of the experiment. In later years, the H-Line was allowed to increase to 130-200 breeding ewes while the L-Line decreased to about 40-80 breeding ewes. As the experiment commenced, it became easier to find an adequate number of replacements in the H-Line and more challenging to find replacement ewes in the L-Line, as evident in divergent genetic trends for reproductive output (Cloete *et al.* 2004). The H-Line included 28 ewes born as part of a multiple ovulation and embryo transfer (MOET) programme in 1991 and 1992 (Cloete *et al.* 1998a). A second MOET programme was launched during 2009-2014, primarily to replenish ewe numbers in the L-Line (Burger 2019). This resulted in the addition of another 66 ewes to the H-Line and 58 ewes to the L-Line from 2011 to 2016. Progeny born in the MOET programmes were excluded from analyses but retained in the pedigree. Selection of rams was predominantly based on the progeny of dams with at least 3 joinings. Rams used from 1986 to 1989 in both lines were screened from external Merino lines selected for increased fleece weight and a control line (Cloete *et al.* 1998b). Five rams were initially used for each line and a ram was used for only a single year until 1992, from which point one to three rams per line were carried over to provide sire

links across years. From the mid 1990's the rams used for the H-Line increased to six to eight, while that for the L-Line decreased to four to six. This was needed to ensure that the number of ewes allocated to individual sires remained within reasonable limits. Ram replacements were selected to represent all sires within their year cohort wherever possible. Replacement within sire lines was expected to curb inbreeding by ensuring that no single sire contributed disproportionately to the next generation. If no suitable replacement for a specific ram was available, a candidate sire from another sire group was selected. Within-flock selection from 2003 was based on the best linear unbiased prediction (BLUP) breeding values derived from a single-trait repeatability model fitted to the annual reproduction records of the ewes in the flock (Cloete *et al.* (2004)). Initially, the ranking values described previously were considered in tandem by ensuring that replacements of both sexes still met the requirements noted above in terms of ranking values of their dams, but the derived breeding values of animals available for replacement were the sole selection criterion since 2010. Ram selection for the H-Line included some external sires since 2008, and since 2013 for the L-Line. Care was taken to use rams screened from their flocks of origin based on either national or within-flock breeding values for number of lambs weaned. There was a variable amount of selection for conformation and type in the H-Line during this period but all viable animals in the L-Line were selected irrespective of visual appearance.

The flock was originally maintained at the Tygerhoek research farm from 1986 to 1992. Tygerhoek is situated near Rivieronderend at 34°8'S and 21°11'E at an altitude of 425 m. Long-term rainfall for the site averaged 429 mm, with 60% of the annual precipitation recorded between the months of April and September. At the end of 1992, the flock was moved to the Elsenburg research farm. The site is located roughly 10km north of Stellenbosch with coordinates 33°51'S and 18°50'E. The elevation of the site is 177 m and average long-term (1995-2017) rainfall is 604.4 mm. The climate is considered Mediterranean and 77% of the rainfall is recorded between the months of April and September. The two selection lines were maintained as a single flock throughout most of the year. Exceptions include joining in single sire groups at Elsenburg. Following parturition in the 10-20 lambing paddocks of approximately 0.4-0.5 ha each, ewes and their lambs were moved into randomly allocated smaller mobs of 20 to 40 for a period post-lambing (Cloete and Scholtz 1998). Further information on management and nutrition can be found elsewhere (Cloete *et al.* 2004). All weaner lambs were maintained in a single flock until the age of about 6 months, at which stage sexes were separated. These single-sex groups were then managed separately until hogget age at Tygerhoek and yearling age at Elsenburg, when they were assessed for wool and liveweight traits. No deliberate culling or marketing, apart from natural attrition, affected the number of hoggets/yearlings assessed.

1.2.2 Data and Statistical analysis

Data recorded over a 31-year period from 1986 to 2016 included records of weaning weight (WW), yearling weight (YW), greasy fleece weight (GFW), clean fleece weight (CFW), fibre diameter (FD), clean yield (CY), staple length (SL), staple strength (SS), coefficient of variation of fibre diameter (CVFD) and total skin wrinkle score (SWS). Lambs were shorn just after weaning (not recorded) and the wool data were subsequently recorded with YW at roughly 12 months of age. These recordings were made on a maximum of 5092 individuals, born from the commencement of selection in 1986 to 2016. The identities of the dams of all the

progeny were known, while five animals had unknown sires. The ASREML® program (Gilmour *et al.* 2015) was used for the estimation of the fixed effects and variance components for the respective traits in univariate analyses. All analyses included the full pedigree file, consisting of 5146 individuals, the progeny of 267 sires and 1550 dams. The environmental factors that were tested as fixed effects included year of birth (1986-2016), selection line (H-Line or L-Line), sex (male or female), age of dam (2 to 7+ years) and birth type (singles or pooled multiples). Random effects tested for significance were the direct and maternal genetic effect, their covariance, the maternal permanent environmental variance, and residual effects. Analysis commenced by the fitting of various combinations of fixed effects and possible interactions in order to obtain a fixed effects model. Effects observed as significant ($P < 0.05$) by conditional Wald tests were retained in this model for subsequent testing of random effects. In the analysis used for the estimation of variance components and their respective ratios and correlations, selection line and its interactions with other fixed effects were included as fixed effects (if significant). This procedure was followed to avoid inflated estimates of genetic variances relative to observed phenotypic variances and was prompted by genetic divergence in certain traits reported in earlier studies (Cloete *et al.* 2003; 2005).

Random terms were then added to the operational model in a step-wise basis that resulted in either of the following genetic models used for analysis (in matrix notation):

$$\text{Model (i)} \quad y = Xb + Z_1a + e$$

$$\text{Model (ii)} \quad y = Xb + Z_1a + Z_3m + e$$

$$\text{Model (iii)} \quad y = Xb + Z_1a + Z_2c + e$$

$$\text{Model (iv)} \quad y = Xb + Z_1a + Z_3m + Z_2c + e \quad [CoV_{am} = 0]$$

$$\text{Model (v)} \quad y = Xb + Z_1a + Z_3m + e \quad [CoV_{am} = A\sigma_{am}]$$

$$\text{Model (vi)} \quad y = Xb + Z_1a + Z_3m + Z_2c + e \quad [CoV_{am} = A\sigma_{am}]$$

In the preceding equations, y represented the vector of observations of the respective traits, b the vector of fixed effects, a represented the vector of direct genetic variances, m the vector of maternal genetic effects, c the vector of maternal permanent environmental variances and e the vector of residuals. The corresponding incidence matrices of each effect was respectively represented by X , Z_1 , Z_2 , and Z_3 . A represented the numerator relationship matrix, and CoV_{am} the covariance between direct and maternal genetic effects.

It was assumed that:

$$V(a) = A\sigma_a^2; \quad V(m) = A\sigma_m^2; \quad V(c) = I\sigma_c^2; \quad V(e) = I\sigma_e^2$$

with A representing the numerator relationship matrix, I representing identity matrices and σ_a^2 , σ_m^2 , σ_c^2 and σ_e^2 the direct genetic variance, maternal genetic variance, maternal permanent environmental variance and environmental (residual) variance, respectively. These analyses yielded estimates of genetic and permanent environmental variances, which were used to compute (co)variance ratios for direct additive genetic (h^2), maternal genetic (m^2), the direct-maternal genetic correlation (r_{am} ; where applicable) as well as maternal

permanent environmental (mc^2) variances as a proportion of the total observed phenotypic variance. A log-likelihood ratio test (LRT) was used to test for significant differences in model likelihood:

$$LRT = -2 \log_e \left[\frac{L_s(\theta)}{L_g(\theta)} \right] = 2 |LogL_g - LogL_s|$$

where $L_g(\theta)$ and $L_s(\theta)$ represents the likelihood of the general (full) and the simpler (nested) model, respectively. The log-likelihood ratio test is a chi-squared distributed random variable, with degrees of freedom (df) equal to the difference in the number of random effects. However, the two-sided LRT statistic was adjusted for being bound above 0, i.e. on the boundary of the parameter space, by considering a critical value of 1.36 (0.5×2.71) instead of 1.92 (0.5×3.84) for a chi-square variable with 1 degree of freedom. This adjustment did not apply when testing for the direct-maternal covariance component (Model (v) vs. (ii) or model (vi) vs. (model (iv))), where 1.92 was used. The models so derived were used as the operational model for the estimation of variance components and subsequently for multi-trait analysis. The multi-trait models that were fitted allowed the estimation of direct, maternal, environmental (residual) and phenotypic correlations as well as their respective standard errors. Multi-trait analysis was explored by considering various combinations of traits or trait groups, as and assessed by criteria of convergence of log-likelihood and stable parameters across analyses. A maximum of two traits were used when both traits were observed as maternally influenced in preceding uni-trait analyses. Analysis reached convergence for several 5-trait-models of which only one of the 5 traits included a maternal effect and the direct-maternal covariance (where significant). Correlations between the relevant traits were reported from these 5-trait models where possible, or otherwise from respective 2-trait models.

In order to assess possible genetic divergence between selection lines, breeding values were obtained from a separate analysis that excluded selection line and interactions with selection line from the model. The retention of selection line effects as fixed within the model would deflate possible genetic differences that could have resulted from selection. These breeding values were used to predict means for each selection line for each birth year, which was used to plot genetic trends over the 30-year selection period. Linear regression lines were fitted for both trends representing the H- and L-Lines and tested for significance ($P < 0.05$) of their regression coefficients from zero and difference in regression slopes by t-tests using standard errors (SE) and the pooled SE of the regression coefficients, respectively. Coefficients were derived using animals that did not originate from external sires and grand-sires, and the mean breeding values for the animals with the 'external' parentage (up to grandparents) were plotted separately. The intercepts for linear trends involving the divergent lines were fixed at zero as divergent selection commenced (year 0) from a common pool of animals born in 1986. Maternal genetic trends were calculated accordingly.

1.3 Results

1.3.1 Fixed effects and random variance components

The effects of birth year were not tabulated, as birth year was involved in significant ($P < 0.05$) interactions with selection line and/or sex for most of the evaluated traits. Animals within the High Line (H-Line) selection

group were on average heavier ($P < 0.01$) than the Low Line (L-Line) group both for weaning weight (WW) and yearling weight (YW, Table 1.1). Greasy fleece weight (GFW) of L-Line progeny was heavier than in the H-Line, but the effect only approached significance ($P < 0.051$). This tendency is supported by the remaining wool output traits, as the L-Line group had a higher clean yield (CY) ($P < 0.01$) as well as a higher clean fleece weight (CFW) ($P < 0.01$). In turn, the H-Line group displayed a longer staple length (SL) ($P < 0.01$), a lower fibre diameter (FD) ($P < 0.01$) and a lower staple strength ($P < 0.01$). The coefficient of variation of fibre diameter (CVFD) was not affected by selection line and the total skin wrinkle score (SWS) was higher in the L-Line ($P < 0.05$), i.e. the L-Line was more wrinkly. Rams were heavier than ewes for both WW and YW ($P < 0.01$), and ewes displayed a higher ($P < 0.01$) GFW, CY, CFW, SL, FD and CVFD than rams. Sex did not affect SS or SWS. Compared to multiples, single-born progeny displayed a higher ($P < 0.01$) WW and YW as well as higher ($P < 0.01$) GFW, CFW and SWS. Singles also displayed a lower ($P < 0.05$) FD, while CY, SL, SS and CVFD were all unaffected by birth type. The age of dam significantly ($P < 0.01$) influenced WW, YW, GFW and CFW and SWS. In all cases, progeny 2-year-old (first lambing) ewes displayed the lowest values, while performance tended to peak at dam ages 3 to 5 with slight decreases in the 6th (or subsequent, but commonly last) reproduction year.

The addition of a random direct genetic component (Model (i)) improved ($P < 0.05$) the log likelihood ratio for all traits, while a random maternal genetic component (Model (ii)) only proved significant ($P < 0.05$) for WW, YW, GFW, CFW, FD and SWS (Table 1.2). The addition of the maternal permanent environmental variance (σ_{pe}^2) significantly ($P < 0.05$) increased log-likelihoods of WW and FD. It is worthwhile to mention that model selection was conflicting in the current assessment of FD, as both models (ii) (including m^2) and (iii) (including c^2) provided a significant ($P < 0.05$) improvement over model (i), but the inclusion of both m^2 and c^2 (model (iv)) did not provide a significant ($P < 0.05$) further improvement over either model (ii) or (iii). The improvement of the log likelihood ratio for models (ii) and model (iii) were also of a similar magnitude (Table 1.3), suggesting that the fitting of these random effects rivalled for the same maternal variation, thus compromising their statistical partitioning. Given that both models (ii) and (iii) had the same number of random effects, model (iii) should be preferred for having a higher log-likelihood. The addition of the direct-maternal covariance component (Model (v)) led to significant ($P < 0.05$) improvements of the log-likelihood in YW and GFW. The direct-maternal genetic correlations (r_{am}) were estimated as 0.57 ± 0.21 for YW and -0.35 ± 0.11 for GFW. Heritability (h^2) estimates for the various fibre traits exceeded 0.5, ranging from 0.56 for FD and CVFD to 0.60 for CY (Table 1.3). Corresponding h^2 estimates for the other wool traits ranged from 0.34 for CFW to 0.48 for GFW, with the estimates for SS and SL being intermediate at 0.37. Heritability estimates for weight traits were lower than those observed for the wool traits, being 0.15 for WW and 0.28 for YW. Subjectively assessed SWS was highly heritable at 0.50. Estimates of maternal genetic components (m^2) were lower or equal to 0.10 for the traits where it reached significance, ranging to 0.03 for SWS to 0.10 for GFW ($P < 0.05$).

Table 1.1

Predicted means (\pm SE) for weight and wool traits for all significant ($P < 0.05$) fixed effects considered. Relevant effects were estimated at an average dam age. NS denotes not significant

Fixed Effects	WW [kg]	YW [kg]	GFW [kg]	CFW [kg]	FD [μ m]	CY [%]	SL [mm]	SS	CVFD	SWS
Number of records	4811	4714	4558	4546	4721	4714	3892	1992	3152	4715
Overall mean	21.49 \pm 0.07	37.75 \pm 0.13	3.19 \pm 0.02	2.34 \pm 0.02	19.19 \pm 0.02	74.3 \pm 0.07	74.58 \pm 0.4	47.21 \pm 0.25	20.61 \pm 0.06	7.85 \pm 0.04
Selection Line										
High Line	21.93 \pm 0.07	38.38 \pm 0.09	3.23 \pm 0.01	2.36 \pm 0.01	19.16 \pm 0.02	74.32 \pm 0.07	81.02 \pm 0.17	45.98 \pm 0.26	NS	7.45 \pm 0.04
Low Line	19.42 \pm 0.15	34.45 \pm 0.19	3.24 \pm 0.02	2.39 \pm 0.01	19.63 \pm 0.05	75.37 \pm 0.15	80.25 \pm 0.28	46.42 \pm 0.58	NS	9.66 \pm 0.09
Sex										
Male	21.27 \pm 0.11	39.41 \pm 0.13	3.21 \pm 0.01	2.35 \pm 0.01	19 \pm 0.03	74.59 \pm 0.11	78.33 \pm 0.16	NS	20.77 \pm 0.1	NS
Female	20.08 \pm 0.1	33.42 \pm 0.13	3.26 \pm 0.01	2.41 \pm 0.01	19.8 \pm 0.03	75.10 \pm 0.10	82.94 \pm 0.16	NS	21.37 \pm 0.1	NS
Birth Type										
Single	22.86 \pm 0.1	37.51 \pm 0.13	3.42 \pm 0.01	2.51 \pm 0.01	19.35 \pm 0.03	NS	NS	NS	NS	9.14 \pm 0.06
Multiple	18.5 \pm 0.11	35.32 \pm 0.14	3.05 \pm 0.01	2.25 \pm 0.01	19.44 \pm 0.03	NS	NS	NS	NS	7.97 \pm 0.06
Age of Dam										
2	19.46 \pm 0.15	35.7 \pm 0.18	3.13 \pm 0.01	2.31 \pm 0.01	NS	NS	NS	NS	NS	8.08 \pm 0.08
3	20.93 \pm 0.13	36.77 \pm 0.16	3.24 \pm 0.01	2.38 \pm 0.01	NS	NS	NS	NS	NS	8.41 \pm 0.07
4	21.28 \pm 0.13	36.93 \pm 0.17	3.29 \pm 0.01	2.42 \pm 0.01	NS	NS	NS	NS	NS	8.72 \pm 0.08
5	21.35 \pm 0.14	36.99 \pm 0.18	3.28 \pm 0.01	2.42 \pm 0.01	NS	NS	NS	NS	NS	8.69 \pm 0.08
6	21.23 \pm 0.17	36.75 \pm 0.21	3.29 \pm 0.01	2.42 \pm 0.01	NS	NS	NS	NS	NS	8.82 \pm 0.10
7+	19.81 \pm 0.27	35.34 \pm 0.34	3.18 \pm 0.01	2.33 \pm 0.01	NS	NS	NS	NS	NS	8.6 \pm 0.15

Table 1.2

Log Likelihoods for weight and wool traits for considering additions of applicable random effects. The chosen model is shown in 'bold' font type. 'FE' row presents the Fixed Effects only model

Model	WW	YW	GFW	CFW	FD	CY	SL	SS	CVFD	SWS
(FE)	-8900.65	-9663.16	277.739	1623.08	-3567.71	-8682.29	-10316.2	-5623.59	-4997.11	-6070.18
(i)	-8809.66	-9509.91	452.938	1757.38	-3237.76	-8342.68	-10201.4	-5577.53	-4693.91	-5790.31
(ii)	-8747.66	-9491.68	461.406	1767.72	-3231.83	-8342.68	-10201.3	-5577.47	-4693.64	-5786.94
(iii)	-	-	-	-	-3231.16	-8342.63	-10201.4	-5577.52	-4693.39	-
(iv)	-8735.71	-9490.01	461.597	1767.72	-3229.85	-	-	-	-	-5786.71
(v)	-8747.27	-9487.33	463.973	1768.42	-3230.89	-	-	-	-	-5785.37
(vi)	-8735.09	-9486.7	-	-	-	-	-	-	-	-

Table 1.3Variance components and direct genetic (h^2) as well as maternal (m^2) variance ratios (\pm SE) for weight and wool traits assessed in divergently selected Merino progeny

Variance Components	WW [kg]	YW [kg]	GFW [kg]	CFW [kg]	CY [%]	SL [mm]	FD [μ m]	SS	CVFD	SWS
σ_e^2	10.17	14.22	0.16	0.1	6.37	49.99	0.68	72.53	3.82	2.36
σ_a^2	2.3	6.85	0.16	0.06	9.7	29.88	0.99	43.02	5.07	2.56
σ_m^2	1.19	1.45	0.03	0.01	-	-	-	-	-	0.16
σ_{am}	-	1.81	-0.03	-	-	-	-	-	-	-
σ_{pe}^2	1.58						0.07			
σ_p^2	15.24	24.34	0.33	0.18	16.08	79.87	1.74	115.54	8.89	5.08
Variance Ratios										
h^2	0.15 ± 0.03	0.28 ± 0.04	0.48 ± 0.06	0.34 ± 0.03	0.60 ± 0.02	0.37 ± 0.03	0.56 ± 0.03	0.37 ± 0.05	0.56 ± 0.03	0.50 ± 0.03
m^2	0.07 ± 0.02	0.05 ± 0.01	0.10 ± 0.02	0.06 ± 0.01	-	-	-	-	-	0.03 ± 0.01
mc^2	0.10 ± 0.02						0.03 ± 0.01			
Correlation Coefficients										
r_{am}	-	0.57 ± 0.21	-0.35 ± 0.11	-	-	-	-	-	-	-

1.3.2 Genetic, environmental, and phenotypic correlations

The correlations between the maternally influenced traits (WW, YW, CFW and SWS) were derived from pairwise two trait analysis. The results of three 5-trait analyses are also reported by separately analysing YW, CFW, and FD with the wool traits CY, SL, SS and CV. The correlations between the latter group of traits were derived from the 5-trait analysis with FD (Table 1.4). The genetic correlation between YW and WW was significant ($P < 0.05$) and high, suggesting that animals genetically predisposed to higher weaning weights would also be heavy at yearling age (Table 1.4). Yearling weight was genetically correlated to the wool traits except FD and SS, with heavier yearlings tending to also have a higher CFW and CY as well as longer SL and a reduced CVFD. In general, phenotypic and environmental correlations mostly reflected significant genetic relationships in sign but were of a smaller magnitude. An exception was the relationship between YW and SWS where the negative genetic relationship suggested that heavier yearlings would be plainer. In contrast, an environment conducive with a higher YW would also sustain an increased SWS. Also, the environmental and phenotypic correlation between YW and CFW was of considerably larger magnitude compared to the genetic relationship. Very high maternal genetic correlations of 0.90 ± 0.06 (between YW and WW) and 0.94 ± 0.10 (between YW and CFW) suggested a maternal genetic relationship of unity between the maternal traits. In contrast, the maternal genetic relationship between YW and SWS of 0.18 ± 0.26 was low and not significant. The genetic relationships of CFW with the wool traits (FD, CY, SL, SS and CVFD) were favourable, bar the positive correlation with FD and a negligible correlation with CVFD. These relationships were also reflected in the environmental and phenotypic relationships which were in the same direction and of moderate magnitudes. The genetic relationship between CFW and SWS was positive, suggesting that improving fleece weight in plainer animals could be problematic. This unfavourable relationship was also present at the environmental and phenotypic levels. A positive and moderate maternal genetic correlation of 0.48 ± 0.21 was also observed between CFW and SWS. The unfavourable genetic correlation between FD and SS was notably high and moderately reflected in the phenotypic correlation. The remaining significant ($P < 0.05$) correlations of FD with SL, CV and SWS were also unfavourable, but moderate to low on both the genetic and phenotypic levels. Clean yield had a favourable, but moderate relationship with SL both on the genetic and phenotypic levels, suggesting that fleeces with longer staples would have a better CY. CVFD had favourable (negative) genetic relationships with CY, SL, and SS.

Table 1.4

Genetic, environmental and phenotypic correlations (\pm SE) amongst live weight and wool traits in Merino progeny. Significant coefficients are indicated as 'bold' type

Trait		Additive genetic	Environmental	Phenotypic
<i>Yearling Weight X</i>				
Weaning Weight	2-T	0.71 \pm 0.06	0.57 \pm 0.02	0.69 \pm 0.02
Clean Fleece Weight	2-T	0.2 \pm 0.09	0.49 \pm 0.03	0.42 \pm 0.02
Fibre Diameter	2-T	0.08 \pm 0.07	0.3 \pm 0.03	0.18 \pm 0.02
Skin Wrinkle Score	2-T	-0.36 \pm 0.08	0.14 \pm 0.04	-0.06 \pm 0.02
Clean Yield	5-T	0.17 \pm 0.07	-0.01 \pm 0.04	0.07 \pm 0.02
Staple Length	5-T	0.39 \pm 0.09	0.15 \pm 0.03	0.24 \pm 0.02
Staple Strength	5-T	-0.18 \pm 0.12	0.21 \pm 0.05	0.07 \pm 0.03
Coefficient of Variation	5-T	-0.32 \pm 0.08	-0.05 \pm 0.04	-0.17 \pm 0.02
<i>Clean Fleece Weight X</i>				
Fibre Diameter	2-T	0.26 \pm 0.06	0.26 \pm 0.04	0.24 \pm 0.02
Skin Wrinkle Score	2-T	0.31 \pm 0.07	0.26 \pm 0.03	0.29 \pm 0.02
Clean Yield	5-T	0.25 \pm 0.06	0.22 \pm 0.04	0.34 \pm 0.03
Staple Length	5-T	0.38 \pm 0.07	0.25 \pm 0.03	0.3 \pm 0.02
Staple Strength	5-T	0.31 \pm 0.11	0.23 \pm 0.05	0.39 \pm 0.04
Coefficient of Variation	5-T	0.07 \pm 0.08	0.05 \pm 0.04	0.09 \pm 0.04
<i>Fibre Diameter X</i>				
Skin Wrinkle Score	2-T	-0.12 \pm 0.06	0.06 \pm 0.04	-0.06 \pm 0.03
Clean Yield	5-T	-0.03 \pm 0.05	0 \pm 0.04	-0.02 \pm 0.02
Staple Length	5-T	0.35 \pm 0.06	0.14 \pm 0.04	0.23 \pm 0.02
Staple Strength	5-T	0.51 \pm 0.07	0.19 \pm 0.05	0.33 \pm 0.02
Coefficient of Variation	5-T	-0.18 \pm 0.06	0.09 \pm 0.05	-0.07 \pm 0.02
<i>Clean Yield X</i>				
Staple Length	5-T	0.22 \pm 0.06	0.13 \pm 0.04	0.17 \pm 0.02
Staple Strength	5-T	0.16 \pm 0.08	0.18 \pm 0.05	0.17 \pm 0.03
Coefficient of Variation	5-T	-0.21 \pm 0.06	-0.21 \pm 0.05	-0.21 \pm 0.02
<i>Staple Length X</i>				
Staple Strength	5-T	0.17 \pm 0.11	0.2 \pm 0.05	0.19 \pm 0.03
Coefficient of Variation	5-T	-0.26 \pm 0.08	-0.17 \pm 0.05	-0.21 \pm 0.03
<i>Staple Strength X</i>				
Coefficient of Variation	5-T	-0.4 \pm 0.08	-0.17 \pm 0.05	-0.27 \pm 0.02

5-T and 2-T indicate correlations estimated in 5-Trait analysis and 2-Trait analysis, respectively.

1.3.3 Genetic trends

The regressions of averaged estimated breeding values on birth years, as indicative of genetic trends, were significant ($P < 0.05$) for both lines in all traits except for FD and CY and SL within the H-Line. (Table 1.5). Divergent genetic trends for CVFD was not investigated since there was no evidence for line differences on the phenotypic level (Table 1.1). Divergence between the lines was significant ($P < 0.05$) for all traits except SL. Expressed as a percentage of the overall trait means, the group breeding values increased by respectively 0.23 and 0.18% per annum (p.a) for WW and YW in the H line. Corresponding declines, amounting to respectively 0.28 and 0.33% p.a were observed in the L-Line (Fig. 1.1). Both lines displayed an upwards (positive) trend for CFW, but the gain of 0.17% p.a in the L-Line was faster compared to the 0.05% p.a in the H-Line. For FD, the trend observed in the H-Line was non-significant and negligible, while an annual increase of 0.17% was found in the L-Line (Fig. 1.2). The L-Line displayed an upward genetic trend of 0.15% p.a for CY while the trend in the H-Line was negligible. Similar responses between lines were observed for SL, with slightly downward trends of respectively 0.07% and 0.04% p.a for the H- and L-Lines. The regression coefficient was, however, not significant in the H-Line in relation to the standard error ($P > 0.05$). Staple strength decreased by 0.20% annually in the H-Line. The L-Line, in turn, displayed an increase of 0.21% p.a in SS, which was divergent ($P < 0.01$) from the H-Line. For SWS, the H-Line became plainer at 0.56% p.a, while the L-Line regressed upwards at 0.94% p.a in a divergent ($P < 0.01$) response (Fig. 1.3). The maternal genetic trend for WW in the H-Line amounted to 0.09% p.a in the upward direction, while WW in the L-Line regressed downwards at 0.19% p.a. The coefficients for the remaining maternal trends can be seen in table 1.5, but were negligibly low besides the possible exception of the downward trend of 0.20% for WW in the L-Line.. The breeding values of animals that descended from 'external' sires or grand-sires were not included in these genetic trends and were plotted separately in Fig. 1.1 to 1.3. For WW, these external trends ranged about the mean of the internal H-Line, but external trends were higher compared to pure L-Line ewes (Fig. 1.1). As for YW, the descendants of external sires with H-Line ewes were generally heavier than H-Line progeny. The progeny and grand-progeny of external sires on L-Line ewes resembled the H-Line rather than the L-Line for YW. Descendants of external rams with ewes of both selection lines mostly resembled the genetic trend in the H-Line for FD (Fig. 1.2) and SWS (Fig. 1.3).

Table 1.5Regression coefficients (\pm SE) of direct and maternal genetic breeding values for 'High' and 'Low' line selection lines

Trait	Coefficients	Significance	R ²	% of mean	Divergence
Direct genetic					
<i>WW</i>					
H-Line	0.048 \pm 0.003	**	0.90	0.23%	**
L-Line	-0.059 \pm 0.0039	**	0.89	-0.28%	**
<i>YW</i>					
H-Line	0.062 \pm 0.0053	**	0.82	0.18%	**
L-Line	-0.116 \pm 0.0094	**	0.83	-0.33%	**
<i>CFW</i>					
H-Line	0.001 \pm 0.0004	**	0.18	0.05%	*
L-Line	0.003 \pm 0.0006	**	0.43	0.17%	
<i>FD</i>					
H-Line	-0.003 \pm 0.0023	N/S	0.05	-0.01%	**
L-Line	0.038 \pm 0.0038	**	0.77	0.20%	**
<i>CY</i>					
H-Line	0.01 \pm 0.0068	N/S	0.07	0.01%	**
L-Line	0.113 \pm 0.0083	**	0.86	0.15%	**
<i>SL</i>					
H-Line	-0.022 \pm 0.014	N/S	0.20	-0.07%	-
L-Line	-0.023 \pm 0.0094	**	0.16	-0.04%	
<i>SS</i>					
H-Line	-0.094 \pm 0.0092	**	0.78	-0.20%	**
L-Line	0.1 \pm 0.0121	**	0.69	0.21%	**
<i>CVFD</i>					
H-Line					
L-Line					
<i>SWS</i>					
H-Line	-0.04 \pm 0.0044	**	0.73	-0.55%	**
L-Line	0.078 \pm 0.0074	**	0.78	1.07%	**
Maternal genetic					
<i>WW</i>					
H-Line	0.19 \pm 0.001	**	0.87	0.09%	**
L-Line	-0.04 \pm 0.002	**	0.94	-0.20%	**
<i>YW</i>					
H-Line	0.03 \pm 0.002	**	0.91	0.08%	**
L-Line	-0.03 \pm 0.002	**	0.85	-0.08%	**
<i>CFW</i>					
H-Line	-0.0006 \pm 0.0002	**	0.08	0.01%	**
L-Line	0.0002 \pm 0.0001	N/S	0.33	-0.02%	
<i>SWS</i>					
H-Line	-0.05 \pm 0.0003	**	0.93	-0.07%	**
L-Line	-0.002 \pm 0.0007	**	0.27	-0.02%	**

Asterisks (**) and (*) indicate significance levels ($P < 0.01$) and ($P < 0.05$), respectively.

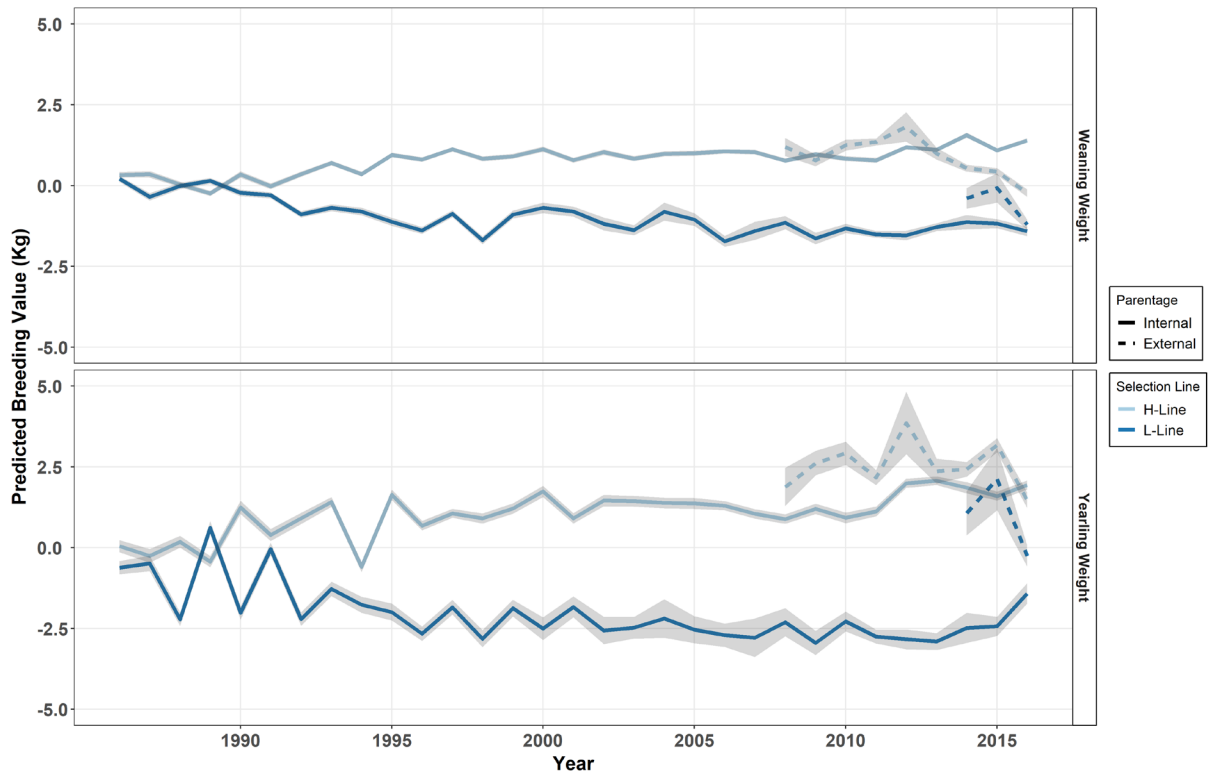


Fig. 1.1 Predicted direct genetic trends of the H- and L- Lines for Weaning and Yearling Weight from 1986 to 2016. Shaded areas represent standard errors around the mean. Dotted lines represent means of progeny and grand-progeny of external sires.

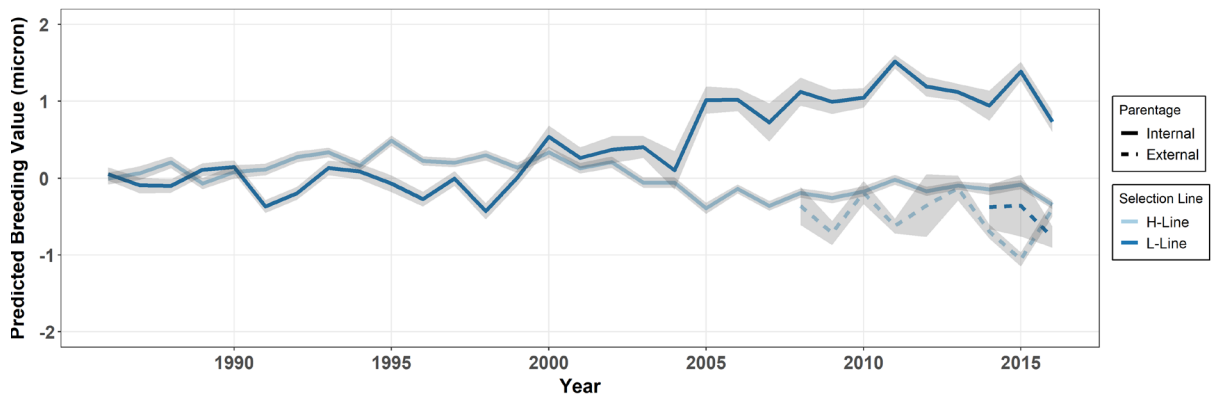


Fig. 1.2 Predicted genetic trends of the H- and L- Lines for Fibre Diameter from 1986 to 2016. Shaded areas present standard errors surrounding the mean. Dotted lines represent means of progeny and grand progeny of external sires.

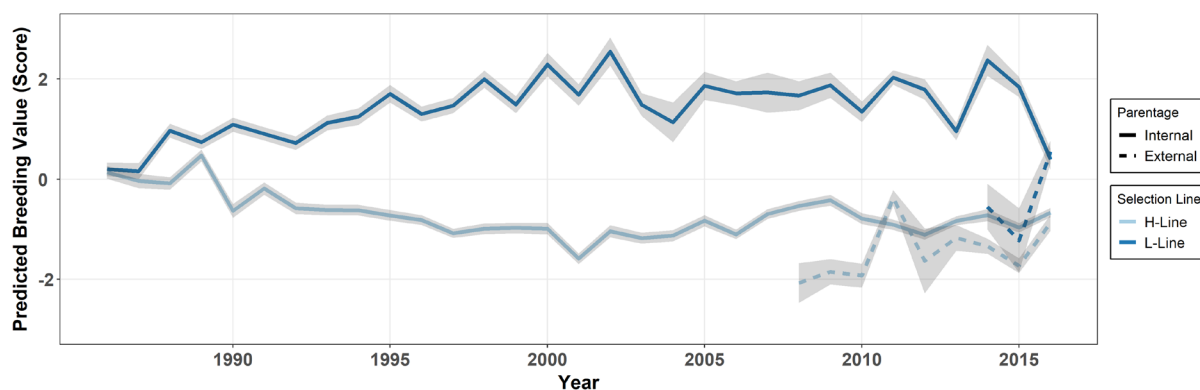


Fig. 1.3 Predicted genetic trends of the H- and L- Lines for Skin Wrinkle Score. Shaded areas represent standard errors surrounding the mean. Dotted lines represent means of progeny of external sires.

1.4 Discussion

1.4.1 Fixed Effects

The main effects and interactions of selection line with birth year were considered to be associated with genetic trends and are discussed below. It was found that rams and singles were heavier than ewes and twins respectively, but ewes and singles had higher fleece weights. The progeny of 2-year-old dams were lighter, plainer and with lower fleece weights than descendants of 3- to 6-year-old dams. These observed effects accorded with expectations from the literature and are not discussed.

1.4.2 Variance ratios

The moderately low heritability (h^2) for WW suggested only under limited direct genetic influence and susceptible to environmental changes (Table 1.3). Previous estimates ranged from 0.12 to 0.15 for South African and Australian Merinos (Erasmus *et al.* 1990; Cloete *et al.* 2001; Mortimer *et al.* 2017) as well as for Turkish Merinos (Ozcan *et al.* 2005), which concur well with the current finding. Other studies suggested a h^2 of slightly larger magnitude for WW. From various breeds, Safari *et al.* (2005) reported a mean h^2 of 0.21 ± 0.02 for 9 studies that included maternal effects, and 0.23 ± 0.02 from 15 with only additive effects. More recently, Ciappesoni *et al.* (2013) used Bayesian methods to estimate h^2 at 0.19 ± 0.025 (\pm Posterior Standard Deviation or PSD) for Uruguayan Merino studs. Swan *et al.* (2008) reported a similar value of 0.21 ± 0.03 for an Australian Fine Wool institutional breeding flock. In an extensive across flock study using Australian Merino research flocks, Safari *et al.* (2007a) derived an overall h^2 ratio of 0.29 ± 0.01 and Huisman *et al.* (2008) reported a h^2 estimate of 0.40 ± 0.03 , thus defining a considerably wide range for the heritability of WW. Considering the nutritional dependence on the dam, it was expected to observe significant maternal genetic components in determining WW, but m^2 was low and roughly half the magnitude of the direct genetic effect (Table 1.3). This m^2 ratio was in the same range (0.04 – 0.09) as some previous estimates (Duguma *et al.* 2002; Cloete *et al.* 2003; 2005; Ozcan *et al.* 2005), but lower than a more moderate range between 0.11 and 0.12 (Erasmus *et al.* 1990; Safari *et al.* 2007a; Ciappesoni *et al.* 2013). Swan *et al.* (2008) reported a moderate

m^2 which coincided well with the mean 0.16 ± 0.04 reported by Safari *et al.* (2005) from 9 studies, while a high m^2 estimate of 0.23 ± 0.02 reported by Huisman *et al.* (2008) suggested a strong influence of maternal genetics for WW.

The maternal permanent environmental variance ratio (mc^2) of 0.10 was slightly larger than genetic effects of the dam, but also of a small magnitude. The estimate is well aligned with reports of mc^2 between 0.08 and 0.09 (Ozcan *et al.* 2005; Swan *et al.* 2008; Ciappesoni *et al.* 2013) while slightly lower observations (Duguma *et al.* 2002; Cloete *et al.* 2003) were similar to the mean value of 0.06 ± 0.01 reported by Safari *et al.* (2005).

The addition of the direct-maternal genetic covariance (σ_{am}) in model (v) did not lead to a significant ($P < 0.05$) improvement in the log-likelihood for WW (Tables 1.2 and 1.3). Of the 9 studies reviewed by Safari *et al.* (2005), only 3 similarly excluded σ_{am} from the operational model. The literature provided support for models including σ_{am}^2 estimates, but the range for r_{am} of WW is extensive. A high and positive r_{am} of 0.57 has been reported in an older study on the Klerefontein Merino flock (Erasmus *et al.* 1990), whereas Safari *et al.* (2005) reported a mean r_{am} of 0.35 ± 0.11 . The r_{am} derived from the currently unused Model (vi) ($r_{am} = 0.30 \pm 0.28$) was similar to Swan *et al.* (2008) and Duguma *et al.* (2002) who reported positive, but non-significant r_{am} estimates. Contradictory reports of r_{am} ranged from moderately high negative estimates of -0.21 ± 0.03 (Safari *et al.* 2007b) and -0.37 ± 0.07 (Huisman *et al.* 2008), as well as an intuitively improbable -0.92 (Ozcan *et al.* 2005).

The total maternal effect ($m^2 + mc^2 = 0.17$) was similar in magnitude to the direct genetic effect of the lamb ($h^2 = 0.15$). This observation was supported by several studies (Erasmus *et al.* 1990; Cloete *et al.* 2003; Swan *et al.* 2008; Ciappesoni *et al.* 2013; Mortimer *et al.* 2017) that suggest the importance of both genetic and non-genetic maternal influence at weaning. However, in Safari *et al.* (2005), the number of studies that reported estimates for mc^2 ($n = 7$), m^2 ($n = 9$) and r_{am} ($n = 6$) as (co)variance ratios for WW were notably fewer than the number that reported direct heritability alone ($n = 15$) and it is possible that this affect was not appropriately accounted for in earlier literature.

Weight at yearling age was observed almost two-fold as heritable as WW (Table 1.3). A similarly moderate h^2 was reported by Safari *et al.* (2005) from 8 studies including maternal effects (0.30 ± 0.03) and 18 studies that only included additive effects (0.33 ± 0.02). Comparable studies on Merinos also reported a moderate range between 0.22 and 0.38 (Lee *et al.* 2002; Cloete *et al.* 2005; Swan *et al.* 2016; Mortimer *et al.* 2017), but numerous studies suggested higher h^2 estimates (0.36 to 0.44) for Merino sheep (Wuliji *et al.* 2001; Khusro *et al.* 2004; Pollott and Greeff 2004; Safari *et al.* 2007a; Huisman *et al.* 2008; Ciappesoni *et al.* 2013). Similar to WW, however, the range for h^2 is wide with several studies suggesting a high h^2 larger than 0.5 (Cloete *et al.* 2002; Swan *et al.* 2008; Dominik and Swan 2016; Brown and Swan 2016). The maternal heritability for YW is consistently estimated as low, with examples below 0.05 (Snyman *et al.* 1995; Khusro *et al.* 2004) and below or equal to 0.10 (Safari *et al.* 2007a; Huisman *et al.* 2008; Swan *et al.* 2008; Mortimer *et al.* 2017) and 0.12 (Wuliji *et al.* 2001). The comparative results of WW and YW were consistent with a decreasing trend of maternal influences with age, which is expected (Mortimer *et al.* 2017).

The positive direct maternal covariance of YW suggested a favourable relationship between the YW of ewes and their offspring, but the estimates for r_{am} in YW also lack concurrence between studies. The

moderately high covariance ratio of 0.57 reported here far exceeded the mean r_{am} estimate of 0.18 ± 0.24 from 4 studies (Safari *et al.* 2005) in an interval ranging between -0.29 (Vaez Torshizi *et al.* 1996) and 0.86 (Erasmus *et al.* 1990) for live weight at 6 months of age (Safari and Fogarty 2003). Safari *et al.* (2007a) accordingly estimated a positive r_{am} value of 0.25 ± 0.08 for YW. In turn, a low negative r_{am} of -0.16 ± 0.09 was reported by Swan *et al.* (2008). Huisman *et al.* (2008) found a highly negative r_{am} for YW at -0.43 ± 0.06 , but also reported another estimate of -0.25 ± 0.10 from a repeated analysis with more stringent criteria to completeness of pedigree structure. For both WW and YW, it is likely that the r_{am} parameter influenced estimates of remaining variance components, as discussed below.

The high h^2 for GFW in the (Table 1.3) agreed with some previous estimates in close range (0.43 – 0.46) (Cloete *et al.* 2002; Safari *et al.* 2007b; Huisman *et al.* 2008; Swan *et al.* 2008; Dominik and Swan 2016) indicating a high potential for genetic gain. However, more moderate values between 0.26 and 0.32 have also been reported (Khusro *et al.* 2004; Ciappesoni *et al.* 2013; Swan *et al.* 2016). Older literature can be represented by the mean of 0.34 ± 0.03 from 25 studies in the principal review by Fogarty (1995). Safari *et al.* (2005) reported a h^2 of 0.37 ± 0.02 from 20 studies and a mean of 0.25 ± 0.06 for 5 studies that also included m^2 . The estimated m^2 (Table 1.3) in the present study agreed with individual estimates of 0.07 ± 0.02 (Swan *et al.* 2008), 0.08 ± 0.01 (Huisman *et al.* 2008) and the mean 0.08 ± 0.01 (Safari *et al.* 2005) while an estimate of 0.16 ± 0.01 (PSD; Ciappesoni *et al.* 2013) indicated a slightly larger maternal genetic component. Combining the direct and maternal genetic effects suggested that GFW is under high total genetic control, but the potential for exploiting both direct and maternal effects is hindered by the negative r_{am} of -0.35 ± 0.11 for GFW. Other studies have also reported this evidence of antagonism with substantial magnitudes of -0.44 ± 0.09 (Swan *et al.* 2008) to -0.60 ± 0.02 (Safari *et al.* 2007a; Huisman *et al.* 2008).

Besides the biological implications, the inclusion of relatively large negative r_{am} estimates inflates h^2 and m^2 (Fozi *et al.* 2005; Safari *et al.* 2007a). Maniatis and Pollott (2003) showed that negative r_{am} coefficients depended heavily on adequate data structure: a poorly structured population with only one or two progeny per dam and less than 10% dams with progeny resulted in r_{am} estimates striving towards -0.99. Data structure was pointed out as a concern by Huisman *et al.* (2008), whom also reported some of the largest negative r_{am} estimates for GFW, CFW and FD. In contrast, Safari *et al.* (2007b) used a data set with good structure (roughly 4 progeny per dam and > 40% dams with records themselves) and fitted models similar to those of Huisman *et al.* (2008) and derived positive r_{am} estimates for the same wool traits. The Elsenburg flock consisted of a relatively small database, but with a high degree of connectedness consisting of an almost complete pedigree, > 95% of dams with own records and between 2.94 to 3.10 progeny per dam for WW, YW, GFM, CFW and FD. Except for GFW, the current results did not support the substantial negative r_{am} estimates reported for various production traits in the literature (Safari *et al.* 2007a; Huisman *et al.* 2008; Swan *et al.* 2008) and it is likely that the magnitude or absence of the r_{am} parameter contribute significantly to discrepancies across studies, such as current examples in WW, YW and GFW and CFW.

Surprisingly, the parameters derived for CFW differed markedly to GFW, principally related to different model choice (model (ii) vs (v)) (Table 1.2). As noted above, the lower h^2 of CFW is likely to be related to the absence of a negative direct-maternal component in the operational model (Table 1.2; the h^2 derived from

model (v) was 0.40 ± 0.06). Some previous results ranged slightly lower (0.20 to 0.28) (Erasmus *et al.* 1990; Khusro *et al.* 2004; Cloete *et al.* 2005), but numerous studies reported h^2 estimates between 0.30 and 0.41 (Pollott and Greeff 2004; Huisman *et al.* 2008; Swan *et al.* 2008, 2016; Ciappesoni *et al.* 2013) similar to the current results. However, several studies reported a high heritability for CFW similar to that observed here for GFW with h^2 estimates between 0.42 and 0.52 (Cloete *et al.* 2002; Dominik and Swan 2016; Mortimer *et al.* 2017). Similar to GFW, a low but significant m^2 (Table 1.3) was observed for CFW. Previous estimates of 0.03 ± 0.01 (Safari *et al.* 2007a) and 0.05 ± 0.02 (Khusro *et al.* 2004) were similarly low but a range between 0.08 and 0.17 (Cloete *et al.* 2005; Huisman *et al.* 2008; Swan *et al.* 2008; Ciappesoni *et al.* 2013) were also reported. The use of model *ii* for CFW analysis disagreed with several recent estimates which included r_{am} and reported fairly large negative covariance ratios ranging between -0.49 to -0.55 (Safari *et al.* 2007a; Huisman *et al.* 2008; Swan *et al.* 2008), which could have been similarly influential in inflating estimates of direct h^2 in these studies.

The estimated heritability for FD was high (Table 1.2), but at the lower end of the range observed in the literature. Similar results included h^2 estimates of 0.53 ± 0.04 (Khusro *et al.* 2004; Cloete *et al.* 2005 on the same resource flock) and the mean ($n = 8$) of 0.59 ± 0.04 derived by Safari *et al.* (2005) from literature values. Higher h^2 estimates of 0.66 ± 0.02 and 0.68 ± 0.01 have been reported by Swan *et al.* (2008) and Safari *et al.* (2007a), respectively. Various examples from the literature suggest FD has a very large genetic influence with h^2 between 0.70 and 0.77 (Cloete *et al.* 2002; Huisman *et al.* 2008; Ciappesoni *et al.* 2013; Dominik and Swan 2016; Swan *et al.* 2016). Multiple sources in the literature included an r_{am} component in the analysis of FD, but these estimates were observed as highly negative, ranging between -0.41 and -0.51 (Safari *et al.* 2007a; Huisman *et al.* 2008; Swan *et al.* 2008). Estimates of mc^2 for yearling FD is scarce and inconsistent with the literature, and no comparable studies were found.

Generally, the remaining wool quality traits had high potential to respond to genetic selection and observed to be of lower complexity with the direct genetic effect being the only random term retained. The high h^2 of CY (Table 1.3) was in good agreement with previous h^2 estimates above 0.6 (Cloete *et al.* 2002; Swan *et al.* 2008) as well as the mean ($n = 15$) of 0.56 ± 0.03 (Safari *et al.* 2005) derived from older literature.

Parameters for CVFD (Table 1.3) showed the trait to also be highly heritable and in strong agreement with estimates of 0.50 ± 0.05 (Swan *et al.* 2016) and the mean of 15 studies amounting to 0.52 ± 0.04 (Safari *et al.* 2005). A notable group of studies reported h^2 to be slightly lower, but above 0.40 (Safari *et al.* 2007a; Huisman *et al.* 2008; Swan *et al.* 2008; Ciappesoni *et al.* 2013; Dominik and Swan 2016). In some cases, significant maternal effects were also reported for CVFD. Mortimer *et al.* (2017) found that combined ($m^2 + mc^2$) maternal effects significantly affected yearling CVFD, while Safari *et al.* (2007a) and Huisman *et al.* (2008) included m^2 estimates and moderately high negative estimates of r_{am} in CVFD analyses.

The moderate h^2 of SL (Table 1.2) was similar to the 0.39 ± 0.02 (\pm PSD) observed by Ciappesoni *et al.* (2013), but higher h^2 estimates are common in the literature. Safari *et al.* (2005) reported a mean h^2 estimate of 0.45 ± 0.04 from 15 literature studies, slightly higher than the current estimate. Various studies estimated a h^2 above 0.5 (Pollott and Greeff 2004; Huisman *et al.* 2008; Swan *et al.* 2008) including values as high as 0.66

± 0.06 (Swan *et al.* 2016). A small, but significant maternal genetic effect of 0.06 ± 0.01 was also reported by Ciappesoni *et al.* (2013).

The h^2 of SS (0.37 ± 0.05) was in good concurrence with multiple sources in the literature (Huisman *et al.* 2008; Swan *et al.* 2008; 2016; Dominik and Swan 2016), reporting a narrow range between 0.35 and 0.37 with overlapping standard errors. All these estimates were also very similar to the mean h^2 of 0.34 ± 0.03 reported by Safari *et al.* (2005) for 11 studies.

A moderately high heritability of 0.50 was derived for SWS (Table 1.2). Comparable South African estimates included a direct h^2 of 0.39 ± 0.03 for the Tygerhoek research flock (Matebesi-Ranthimo *et al.* 2009a) and values around 0.53-0.54 (Cloete *et al.* 2005; Matebesi-Ranthimo *et al.* 2018) from the same resource flock. Australian estimates agree with the moderate to moderately high heritability of wrinkle score with estimates that range between 0.34 and 0.42 (Mortimer *et al.* 2009; 2017; Swan *et al.* 2016). In contrast with the present study, a maternal influence on SWS was not significant in the literature. Model (ii) provided the best likelihood ($P < 0.05$) for SWS, but the maternal genetic effect (0.03 ± 0.01) was very small.

1.4.3 Genetic correlations

The genetic correlations and trends of the H- and L-Lines are evaluated against traditional Merino breeding objectives (Olivier 1999) that aim to optimise out by increasing liveweight (WW and YW), fleece weight (CFW) and yield (CY) while decreasing SWS towards plainer sheep. Desired wool characteristics are targeted by reducing FD and CVFD while increasing SL and SS. Being within the same trait group and being part-whole, a strong genetic correlation (r_g) between WW and YW (Table 1.4) was expected. In cases where only one of WW or YW is available, the other should be a useful indicator trait for simultaneous improvement. Previous estimates of 0.77 ± 0.03 (Ciappesoni *et al.* 2013) and the mean 0.85 (Safari *et al.* 2005) also suggested a strong genetic relationship. More recent estimates of 0.61 ± 0.05 (Swan *et al.* 2008) and 0.51 ± 0.12 (Huisman and Brown 2008) were lower in comparison, but still generally high. The maternal genetic correlation (0.90 ± 0.06) suggested that the maternal genetic influence on WW and YW were the same trait. Estimates of maternal genetic correlations from other studies were moderately high with values of 0.43 ± 0.09 (Huisman and Brown 2008) and 0.54 ± 0.08 (Swan *et al.* 2008), but substantially lower than the current observation near unity. The phenotypic correlation (r_p) was also highly positive and in close accordance with the mean ($n = 9$) of 0.70 reported by Safari *et al.* (2005), representing an evidently favourable relationship between the body weight traits at the different ages.

Breeding programs contain multiple objectives such as increasing yield in both wool and meat traits while reducing fibre diameter (Olivier 1999; Olivier and Cloete 2007). In this regard, it is critical for breeding programs to consider unfavourable relationships (Swan *et al.* 1995) between these difference traits groups. The low positive genetic correlation between YW and CFW (Table 1.2) did not suggest difficulty in improving both meat and wool output parameters. The result agreed with a range of estimates between 0.13 and 0.26 (Cloete *et al.* 2005; Huisman *et al.* 2008; Swan *et al.* 2008) as well as the mean of 0.24 (Safari *et al.* 2005) from 10 studies. There are examples of r_g of 0.3 (Ciappesoni *et al.* 2013) and above 0.5 (Swan *et al.* 2016; Mortimer *et al.* 2017) that further suggested a strong and favourable connection between YW and CFW. A

very high and favourable maternal genetic correlation between YW and CFW suggested genetic unity between these maternal traits. Swan *et al.* (2008) reported a lower but substantive positive maternal genetic correlation between YW and CFW of 0.54 ± 0.08 , but other maternal genetic correlations were generally scarce in the literature.

An unfavourable relationship between FD and YW is well known and well supported in the literature. Significant r_g estimates ranged between moderate but notable values of 0.17 to 0.26 (Cloete *et al.* 1998b; 2002; 2005; Safari *et al.* 2005; Huisman and Brown 2008; Swan *et al.* 2008; Ciappesoni *et al.* 2013). Recent estimates reported by Mortimer *et al.* (2017) and Swan *et al.* (2016) were higher at respectively 0.37 ± 0.08 and 0.39 ± 0.09 . It was thus unexpected to observe a non-significant genetic relationship between YW and FD in the current study as it contradicts the majority of the literature. Also, YW and FD were unfavourably correlated on a phenotypic level with a similar estimate to Huisman and Brown (2008) ($r_p = 0.12 \pm 0.01$), likely related to the influence of nutrition on both traits.

The negative genetic relationship between YW and SWS (Table 1.2) is favourable as present breeding objectives in South Africa tend towards plainer sheep (Matebesi-Ranthimo *et al.* 2009a). The r_g estimate of -0.35 ± 0.13 reported by Swan *et al.* (2016) is in close concordance, while other studies reported a more moderate negative relationship around -0.20 (Mortimer *et al.* 2009; Brown *et al.* 2010) and -0.26 ± 0.08 (Cloete *et al.* 2005, using less data from the same flock). Selection for increased skin folds resulted in a significant ($P < 0.01$) reduction in LW amounting to -0.25 % p.a, with a realised genetic correlation of -0.22 (Crook and James 1991). However, the environmental relationship was in a positive direction and it is possible that animals on a high level of nutrition also became heavier and more developed in SWS compared to their contemporaries on a low feeding level, as suggested by Herselman and King (1993).

Genetic relationships of YW with the remaining wool traits were generally low and favourable and generally in accordance with the literature with respect to SL (Swan *et al.* 2008; Ciappesoni *et al.* 2013; Mortimer *et al.* 2017) (Swan *et al.* 2016) and CVFD (Mortimer *et al.* 2017), (Swan *et al.* 2016) while the lack of a relationship with SS was in accordance with the findings of Huisman and Brown (2008) and Mortimer *et al.* (2017). Selection for increased weight is thus not expected to be detrimental to these wool quality traits.

The unfavourable r_g (Table 1.2) between CFW and FD would have implications for genetically improving both wool quality and quantity. This unfavourable relationship is well known, but there are notable differences in the magnitude estimated. Swan *et al.* (2008) reported r_g as significant, but low at 0.15 ± 0.06 . Other literature sources supported the present r_g with a range of 0.19 to 0.32 (Fogarty 1995; Cloete *et al.* 1998b; Safari *et al.* 2005; Ciappesoni *et al.* 2013; Swan *et al.* 2016). Cloete *et al.* (2005) reported a substantially higher r_g of 0.45 ± 0.08 in a previous analysis on the same flock. The results support the requirement that the antagonistic relationship is appropriately considered in breeding programs, especially since both traits are of high economic importance.

Selection for increased fleece weight could also result in higher SWS (Table 1.2). Similar estimates of 0.22 ± 0.02 and 0.36 ± 0.11 have been reported for the r_g between fleece weight and body wrinkle score by Mortimer *et al.* (2009) and Brown and Swan (2016), respectively. These r_g estimates were of notable magnitude and the unfavourable relationship was also reflected on the phenotypic level. This relationship is

important when considering the emphasis applied to conformation traits by South African stud breeders (Snyman and Olivier 2002) and the economic value of CFW.

The r_g estimates between CFW and the remaining wool traits were favourable (Table 1.2), with genetic gains in CFW associated with corresponding gains in CY, SL and SS, in order of increasing magnitudes from 0.25 to 0.38. The literature supports strong positive relationships of CFW with quality traits such as CY (Safari *et al.* 2005), SL (Safari *et al.* 2005; Swan *et al.* 2008; 2016; Ciappesoni *et al.* 2013) and SS (Safari *et al.* 2005) and selection for CFW appears to have little effect on wool quality, with the exception of FD.

In contrast to CFW, improving FD could be more problematic to maintaining other quality traits. The unfavourable relationship with SL (Table 1.2) was supported by other estimates above 0.19 (Safari *et al.* 2005; Swan *et al.* 2016). Selecting for lower FD is also likely to reduce SS, as similarly suggested by r_g of 0.26 ± 0.05 (Swan *et al.* 2008) and the mean of 0.37 (Safari *et al.* 2005). These estimates suggested multiple unfavourable responses were likely to result from intense downward (favourable) selection on FD. Similar correlated responses were indeed demonstrated in the Tygerhoek Fine Wool selection line (Cloete *et al.* 2013).

The favourable and negative r_g between SS and CVFD was in accordance with previous estimates (Safari *et al.* 2005; Swan *et al.* 2008). This result reinforced previous assertions that the more readily available CVFD could be used as a proxy for the less commonly recorded SS in genetic improvement programmes (Greeff *et al.* 1995). Directly considering CVFD in indexes could, however, provide a balancing effect for maintaining SS while reducing FD.

1.4.4 Genetic Trends

The trends displayed for both WW and YW were favourable and in agreement with an earlier assessment of genetic trends for the same flock up to 2003 (Fig. 1.1; Table 1.5; Cloete *et al.* 2005). H-Line animals became heavier at both weaning and yearling ages, the correlated response for YW over 30 years of selection accumulating to 2.1 kg compared to the base flock, while the L line regressed downward to be about 3.3 kg lighter. Positive genetic correlations between live weight and reproduction have been reported previously (Snyman *et al.* 1998; Cloete *et al.* 2004; Safari *et al.* 2007c; Dominik and Swan 2016). The low magnitude of the response in YW was expected since weight traits were not directly included in the selection objective. In a previous selection experiment that emphasised live weight, genetic gains between 0.86% to 1.0% (of the mean) p.a was observed (Cloete *et al.* 2007), at least three-fold the rate observed here. In cases where YW was also not directly included into the selection objective, a response of 0.34% p.a was realized in selection for “overall excellence” based on conformation (Olivier *et al.* 1995) and 0.4% p.a in a programme selecting for CFW in the Tygerhoek Merino flock (Cloete *et al.* 2007).

The selection lines traded rankings in the consideration of favourable genetic trends for CFW and FD (Fig. 1.2; Table 1.5). The superior gain in CFW of the L-Line was one of only few examples where the selection program of the Elsenburg flock did not benefit the H-Line. The regression coefficient reported for the H-Line was significant ($P < 0.01$), but the realized gain (as a % of the mean) was negligible. The trends for CFW should be considered an indication that the genetic component of the trait can remain stable in the presence of selection pressure for reproduction, rather than be considered as gains *per se*. In genetic trends reported by

Cloete *et al.* (2007), CFW responded with 1.2% p.a. as a direct response in the Cradock Merino stud and 0.6% in the Tygerhoek flock when subjected to positive selection, useful points of reference when observing the magnitudes of current responses. The favourable ranking of the L-Line for CFW was also reflected on the phenotypic level ($P < 0.05$; Table 1.1) and was supported by Olivier (2014) and Dominik and Swan (2016) who reported an unfavourable genetic relationship between wool weight and NLW. Other results, however, did not suggest a relationship in the negative direction. Previous results from the Elsenburg flock did not report a significant correlation between adult greasy wool weight and reproduction (Cloete *et al.* 2004). More recently, Matebesi-Ranthimo *et al.* (2017) reported CFW to be positively correlated to number of lambs weaned after the first parity, which does not concur with the observed direction in trend. Further research into the relationship of fleece weight and reproduction is merited.

The non-significant genetic trend in FD in the H-Line (Fig. 1.2; Table 1.5), suggested that selection for number of lambs weaned failed to influence this trait. FD was positively correlated to total weight weaned after 3 parities (Matebesi-Ranthimo *et al.* 2017) and had a 0.30 genetic correlation with number of lambs born (Safari *et al.* 2005). The lack of a positive (unfavourable) genetic trend for FD in the H-Line could possibly be ascribed to the selection opportunities therein because of the higher number of potential replacements to select from. However, Dominik and Swan (2016) reported that FD was negatively (thus favourably) correlated to number of lambs born, in a report that the authors challenged the conventional wisdom regarding the higher mortality rates associated with fine wool sheep. With respect to the results of Dominik and Swan (2016), an increase in FD in the L-Line could be expected. However, this trend could also be readily explained by a lack of replacements in this line owing to the lower number of lambs weaned. Replacements conforming to the downwards selection objective were commonly selected despite unfavourable phenotypes for other production traits. In other selection experiments, FD responded by -0.67% of the mean p.a. (Cradock Merino flock) and -0.33% (Grootfontein Merino stud) p.a because of downward (favourable) selection pressure. FD also unfavourably increased by 0.19% p.a during a period of selection for “overall excellence” in the Grootfontein stud (Olivier *et al.* 1995). Although this trend was consistent with observations for the L-Line it is debatable whether the underlying cause for the observed trends was the same.

The magnitude of divergence for SS breeding values was low, but it is another example where the L-Line was observed with the more favourable trend. Dominik and Swan (2016) reported a moderately high negative correlation between SS and number of lambs weaned, which supported these observations. The trends for CY and SL were small in magnitude with little biological implications to be expected from the significant slopes observed in the L-Line.

It was interesting to observe the trends of SWS as the most pronounced and consistent compared to the wide variety of traits included in this study (Fig. 1.3; Table 1.5). The L-Line became more wrinkly at slightly more than 1% p.a and the H-Line trended downwards at 0.55 % p.a., which could both be considered responses of considerable magnitude. Accordingly, a study of the same resource flock reported reproduction to be negatively correlated to SWS (Matebesi-Ranthimo *et al.* 2018). The direction of trends in SWS are especially interesting in comparison to the earlier selection experiment reported by Atkins (1980). In this earlier study, selection was based on divergent selection for skin folds by establishing the ‘Folds Plus’ and ‘Folds Minus’

flocks. Following 19 years of divergent selection, pronounced differences were reported for net reproductive rate (the number of hogget ewe hogget replacements produced by a ewe in her lifetime, 1.248; Folds Plus vs 2.270; Folds Minus) and which included better fertility and lamb survival for the Folds Minus group (Atkins 1980), a finding supporting the current results. Furthermore, the Folds Plus groups were considered to have benefited ‘only slightly’ in increased CFW, which also accords well with that seen in the H- and L-Lines of the present study. Considering further benefits of plainer sheep are lower incidence of blowfly strike (Scholtz *et al.* 2010), a downwards trend in SWS is generally desirable besides the unfavourable relationship to CFW discussed above. The South African Merino industry commonly associates plainer sheep with increased growth rates and lambing percentages (Olivier and Cloete 1998). These expected correlated responses are both borne out by the trends reported in Table 1.5 and Fig. 1.1 and 1.3.

The magnitude of regression coefficients, while significant, was often low as is reflected in the small % gains/losses relative to overall means. It is assumed that the genetic correlations with reproduction mostly drove these changes as correlated responses. Correlated responses are, in part, a function of the products of the heritabilities of the traits and it is well known that reproductive traits, especially number of lambs weaned, has a low heritability (Cloete *et al.* 2004; Dominik and Swan 2016). These results should nevertheless still be considered as favourable, the results suggesting that intensive selection for number of lambs weaned should not have obvious detrimental effects on the genetic merit of animals for these traits, with the possible exception of CFW and SS.

It was furthermore evident that descendants of external sires generally resembled the H-Line rather than the L-Line in Figs 1 to 3 with 95% confidence intervals for the H-Line and progeny of external sires commonly overlapping. This result seemed to suggest that the selection pressure in the H-Line was more closely aligned with industry selection practices than in the L-Line. This was not unexpected as the sires originated from flocks with more traditional selection objectives that could have favoured the production traits considered in this study.

1.5 Conclusions

This study contributes to the available body of knowledge for the accurate estimation of (co)variance parameters for use in genetic evaluation of economically important traits. Investigations into the effects of data structure on the accuracy of estimates could prove fruitful as breeding objectives are bound to increase in complexity. Nevertheless, sufficient genetic variation was available for exploitation in all economically important traits that were considered. Genetic correlations observed in this study supported previous estimates that identified antagonistic genetic mechanisms among some important traits, most notably with FD. It is important to consider these relationships in indices to maintain a balance in trait responses to selection. The trends have shown promising indications that, in the long term, intensive selection for reproduction resulted in few unfavourable responses for traits of economic importance, the most notable exceptions being for CFW and SS. Emphasis on reproductive performance can be recommended.

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CHAPTER 2:

Genetic trends in components of reproduction in Merino ewes following 36 years of divergent selection for multiple rearing ability

Abstract

Selection for composite traits such as the number of lambs weaned or total weight weaned risks unpredictable outcomes in genetic responses. It is possible that considering the underlying components of composite traits, such as fertility, fecundity, or rearing ability, could deliver improved gains in reproductive performance. However, the use of component traits in South African Merinos requires further evaluation. This study thus investigated the genetic parameters and trends of component and composite reproduction traits in the Elsenburg Merino flock that has been divergently selected in favour (H-Line) or against (L-Line) performance in number of lambs weaned. Genetic parameters and trends were derived for component traits number of ewes lambing per ewe joined (EL_{EJ}), number of lambs born per ewe lambing (NLB_{EL}), as well as ewe rearing ability per ewe lambing (ERA_{EL}). Additionally, the composite traits number of lambs born per ewe joined (NLB_{EJ}), number of lambs weaned per ewe joined (NLW_{EJ}) and the average lamb weaning weight per ewe reared (AWW_{ER}). All traits were heritable, but h^2 was low with the highest value observed for NLB_{EL} . All traits were affected by animal permanent environment with variance ratios ranging from 0.05 for ERA_{EL} to 0.12 for EL_{EJ} . All traits except for NLB_{EL} were affected by service sire, but this effect was also small with the highest estimate of 0.04 for EL_{EJ} . All traits were observed as divergent between the H- and L-Lines, with an upward rate of gain of nearly 1% for NLW_{EJ} , 0.3% for EL_{EJ} , 0.45% for NLB_{EL} , and 0.15% for ERA_{EL} .

2.1 Introduction

Reproductive performance is a key facet of small stock production. A higher performance in reproductive output allows for more marketable yearlings while still satisfying replacement rates; can support meeting market demands in the face of declining ewe numbers (Cloete and Olivier 2010); or facilitate the rebuilding of flock numbers following drought conditions (Apps *et al.* 2003). The prospects of improving reproductive output of ewes by genetic selection had already been positively treated in much earlier studies (Turner 1978), but initial developments had been hindered by the challenges of selection for traits with low heritability, a narrow distribution of variance and which is sex-limited (Purvis and Hillard 1997).

Selection for reproduction, both in Australia and South Africa, in the past relied on composite traits such as the number of lambs born and weaned per ewe mated (Brien *et al.* 2014). The latter authors reported that selection for reproduction in New Zealand was based on lamb records, including ewe litter size and lamb survival as separate traits. It has been suggested that the number of lambs weaned as a trait is in close correspondence with the breeding objective strived for in South African Merinos (Olivier 1999). Snowden and Fogarty (2009) argued that selection for such a composite, or the total weight weaned (TWW) in particular, would promote a balanced response in underlying component traits.

However, sole emphasis on a composite trait could risks unpredictable outcomes (Bunter *et al.* 2020). According to Bunter and Brown (2015), considering underlying components contributing to reproductive output and direct and maternal effects to weaning weight as part of a balanced selection index could deliver improved and more predictable genetic gains. A pipeline for the evaluation of components has since been developed (Bunter *et al.* 2020), and incorporated into the national genetic evaluation of Sheep Genetics Australia (Bunter *et al.* 2019, 2020).

Compared to the developments of the leading sheep producing countries such as Australia and New Zealand, there is a clear opportunity for improvement in the South African sector, which remains limited to the use of the number of lambs born and weaned per ewe lambing (Cloete *et al.* 2021a). Information derived from a well-known South African Merino resource flock (Cloete *et al.* 2004; Schoeman *et al.* 2010) was used to address the following objectives: 1. To extend the existing body of knowledge of local analyses beyond composite traits to include their underlying components; 2. To update and revisit the genetic trends in the population and simultaneously assess the impact of 36 years of divergent selection for number of lambs weaned on the component traits that were not considered previously.

2.2 Materials and Methods

2.2.1 The Elsenburg Merino Flock

The Elsenburg Merino flock is a resource flock managed as a selection experiment for divergent selection based on NLW since 1986 (see review by Schoeman *et al.* 2010). The flock is separated by the High (H-Line; positive selection) and Low (L-Line; negative selection) lines that originated from the same base population. At the beginning of the experiment, roughly 120 ewes were assigned to each selection line. The H-line has since grown to 130 - 200 ewes, while numbers in the L-Line dwindled to about 40 - 80 breeding ewes. Selection

of rams was predominantly as the progeny of dams that have been recorded for at least 3 joinings. Following the results reported by Cloete *et al.* (2004), selection from 2003 was also guided by the use of best linear unbiased prediction (BLUP) breeding values derived from a single-trait repeatability model fitted to annual NLW records of ewes. During this period, the original ranking values were still considered in tandem to BLUP derived breeding values, but BLUP-values have been the only selection criterion since 2010. In the latter years of the selection experiment, breeding management required the use of external sires to link this genetic resource with the broader South African Merino industry and also to curb inbreeding that was expected in any isolated population. From 2008 to 2019, 349 H-Line lambs and 151 L-Line lambs were born as the progeny of external sires. These animals were also considered for selection and 740 H-Line and 118 L-Line lambs were subsequently born as grand-progeny of external sires between 2010 and 2019. The flock was initially (1986 to 1992) maintained on the Tygerhoek Research Farm near to Riviersonderend in the Western Cape. It was moved to the Elsenburg Research Farm near Stellenbosch during 1993. The climate of the experimental site, as well as the pastures grazed by the experimental animals has been described elsewhere (Cloete *et al.* 2004, 2009). An in-depth description of flock selection history can also be seen in Chapter 1 of this thesis.

2.2.2 Data and Statistical analysis

The traits recorded included two composite reproduction traits, namely number of lambs born per ewe joined (NLB_{EJ}) as well as number of lambs weaned per ewe joined (NLW_{EJ}). Added to these traits were three reproduction trait components, namely number of ewes lambing per ewe joined (EL_{EJ}), number of lambs born per ewe lambing (NLB_{EL}) as well as ewe rearing ability per ewe lambing (ERA_{EL} , see Bunter *et al.* 2020; Bunter and Swan 2021). In addition, average lamb weaning weight per ewe reared (AWW_{ER}) were derived from age- and sex corrected individual weaning weights. The fixed effects selection line, lambing year and ewe age group were tested to obtain a fixed effects model. The number of levels assessed was two for selection line (H- or L-Line), 35 for lambing year (1987 - 2021) and five for ewe age group (2 - 6+ years). All possible interactions were tested initially, but it was narrowed down in the final fixed effects models by testing for significance ($P < 0.05$), which varied depending on the specific trait analysed. Random effects considered for each trait were additive animal (ewe), ewe permanent environment (PE; to account for repeated ewe records), as well as the service sire PE (SS), with variance ratios h^2 , PE^2 and SS^2 , respectively. Estimation of fixed effects and subsequent derivation of variance components commenced in ASREML V4.2 (Gilmour *et al.* 2015). Random terms were then added to the operational model in a step-wise basis that resulted in either of the following genetic models used for analysis (in matrix notation):

$$\text{Model (i)} \quad y = Xb + Z_1a + e$$

$$\text{Model (ii)} \quad y = Xb + Z_1a + Z_1pe + e$$

$$\text{Model (iii)} \quad y = Xb + Z_1a + Z_1pe + Z_2ss + e$$

In the preceding equations, y represented the vector of observations of the respective traits, b the vector of

fixed effects, a represented the vector of direct genetic effects, pe the vector of permanent environmental effects, ss the vector of service sire effects and e the vector of residuals. A log-likelihood ratio test (LRT) was used to test for significant differences between models (see Chapter 1) with a difference of 1.355 was regarded as a significant ($P < 0.05$) improvement in the log-likelihood compared to the reduced model tested against. If a model with an additional term did not provide a significant ($P < 0.05$) improvement, the simpler (nested) model was chosen as the operational model for the trait. Genetic responses over the entire period of the selection experiment were derived as the within-line regressions of individual animal solutions (breeding values) on birth year. The breeding values used were derived from single-trait analyses that excluding selection line (as well as its interactions with the other traits). These breeding values were used to predict means for each selection line for each year, which was used to plot genetic trends over the 30-year selection period. The pedigree file that was used in all analyses contained 10903 animals (including base animals only present as sires and dams). The recorded animals were the progeny of 333 sires and 2210 dams.

2.3 Results

2.3.1 Fixed effects and random variance components

Reproduction traits were all highly variable, coefficients of variation ranging from 36 to 72% (Table 2.1). Lamb AWW_{ER} , in contrast, was less variable with a coefficient of variation of 21%. All six traits were affected ($P < 0.01$) by the three main effects of selection line, lambing year and ewe age (Table 2.1). Overall predicted means in the H-Line were significantly higher for all traits, the differences amounting to 14.1% for EL_{EJ} , 14.7% for NLB_{EL} , 29.4% for NLB_{EJ} , 11.0% for ERA_{EL} , 50.0% for NLW_{EJ} and 6.5% for AWW_{ER} when expressed relative to the L-Line (All $P < 0.001$). Selection line interacted with year in the case of EL_{EJ} , NLB_{EJ} , NLW_{EJ} and AWW_{ER} , and also with ewe age in the case of EL_{EJ} , NLB_{EJ} and ERA_{EL} (Table 2.2). The two-factor interaction between lambing year and ewe age was significant ($P < 0.05$) for most traits, with the exception of AWW_{ER} . The three-factor interaction among all three main effects was not significant for any of the traits (Table 2.2).

Table 2.1

Summary statistics and type-III P values of fixed effects for ewes lambed per ewe joined (EL_{EJ}), number of lambs born per ewe lambed (NLB_{EL}), number of lambs born per ewe joined (NLB_{EJ}), ewe rearing ability per ewe lambed (ERA_{EL}), number of lambs weaned per ewe joined (NLW_{EJ}) and average weaning weight per ewe reared (AWW_{ER})

	Trait					
	EL_{EJ}	NLB_{EL}	NLB_{EJ}	ERA_{EL}	NLW_{EJ}	AWW_{ER}
N	6779	5806	6779	5806	6779	5016
Mean	0.86	1.43	1.23	0.79	0.94	22.5
s.d.	0.35	0.52	0.69	0.36	0.68	4.8
CV	40.7	36.4	56.1	48.1	72.3	21.3
Selection line (SL)	***	***	***	***	***	***
Year (Y)	***	***	***	***	***	***
Ewe age (A)	***	***	***	***	***	**
SL x Y	***	0.07	***	0.19	***	***
SL x A	***	0.73	**	*	0.84	0.14
Y x A	***	***	***	***	***	0.06
SL x Y x A	0.08	0.12	0.07	0.08	0.13	0.08

* - $P < 0.05$; ** $P < 0.01$; Actual significance for $P > 0.05$.

However, given the two-factor interactions of selection line with the other main effects (Table 2.1), it is important to consider these first. Predicted means for the component traits EL_{EJ} and NLB_{EL} and the composite trait NLW_{EJ} for each line and year are provided in Fig. 2.1. All three traits were observed to diverge between the selection lines. No distinct line differences were observed during the early years of the experiment, but consistent significant differences in favour of the H-Line for NLB_{EL} were observed from around 1995 ($P < 0.05$). During a later period, no distinct line differences were seen in 2012 and 2013, but the H-Line again outperformed the L-Line in subsequent years. Significant divergence for EL_{EJ} was first observed in 1999 ($P < 0.05$). In subsequent years, no line differences ($P > 0.05$) were also observed in 2009, 2012 and 2016 while 2014 was characterised by a very poor EL_{EJ} in the L-line. It is notable that EL_{EJ} in the H-Line hovered around 0.85-0.90 for most of the past two decades, while performance in the smaller L-Line was more variable. However, it was evident that the H-Line consistently outperformed ($P < 0.05$) the L-line for NLW_{EJ} from 1993. It is notable that this line difference in NLW_{EJ} was found prior to obvious divergence in the component traits EL_{EJ} and NLB_{EL} in Figure 2.1. Although the line difference remained significant ($P < 0.05$), there was a period from 2011 to 2013 that the difference in favour of the H-Line for NLW_{EJ} was reduced somewhat. These graphs are an extension of those reported by Cloete *et al.* (2017), who presented the same phenotypic trends up to 2007. It is thus not surprising that the figures corresponded well for the overlapping period. Considering the smaller advantage in H-Line performance from around 2010, it is appropriate to note that this period coincided with the recording of reproduction of the progeny of external sires introduced in the H-Line from around 2008.

The two-factor interaction of selection line with ewe age for EC_{EJ} indicated that the performance of H-Line ewes was relatively stable at between 0.85 and 0.90 across age groups (Fig. 2.2). In contrast EL_{EJ} increased with age up to 4 years ($P < 0.05$), where it stabilised. The superiority ($P < 0.05$) of the H-Line was therefore more pronounced in younger ewes. When expressed relative to L-Line performance, the better

performance of the H-Line amounted to 28.7% in 2-year-old ewes, 21.9% in 3-year-old ewes and to between 8.3 and 9.8% in 4- to 6-year-old ewes (Fig. 2.2). This trend was transferred to NLB_{EJ} as well (see Table 2.1). In contrast, the improved ERA_{EL} in the H-Line was accentuated in 6-year-old ewes (Fig. 2.1). Expressed relative to the L-Line, H-Line performance was improved by 22.2% in 6-year-old ewes, but only by 7.3 to 11.0% in younger ewes.

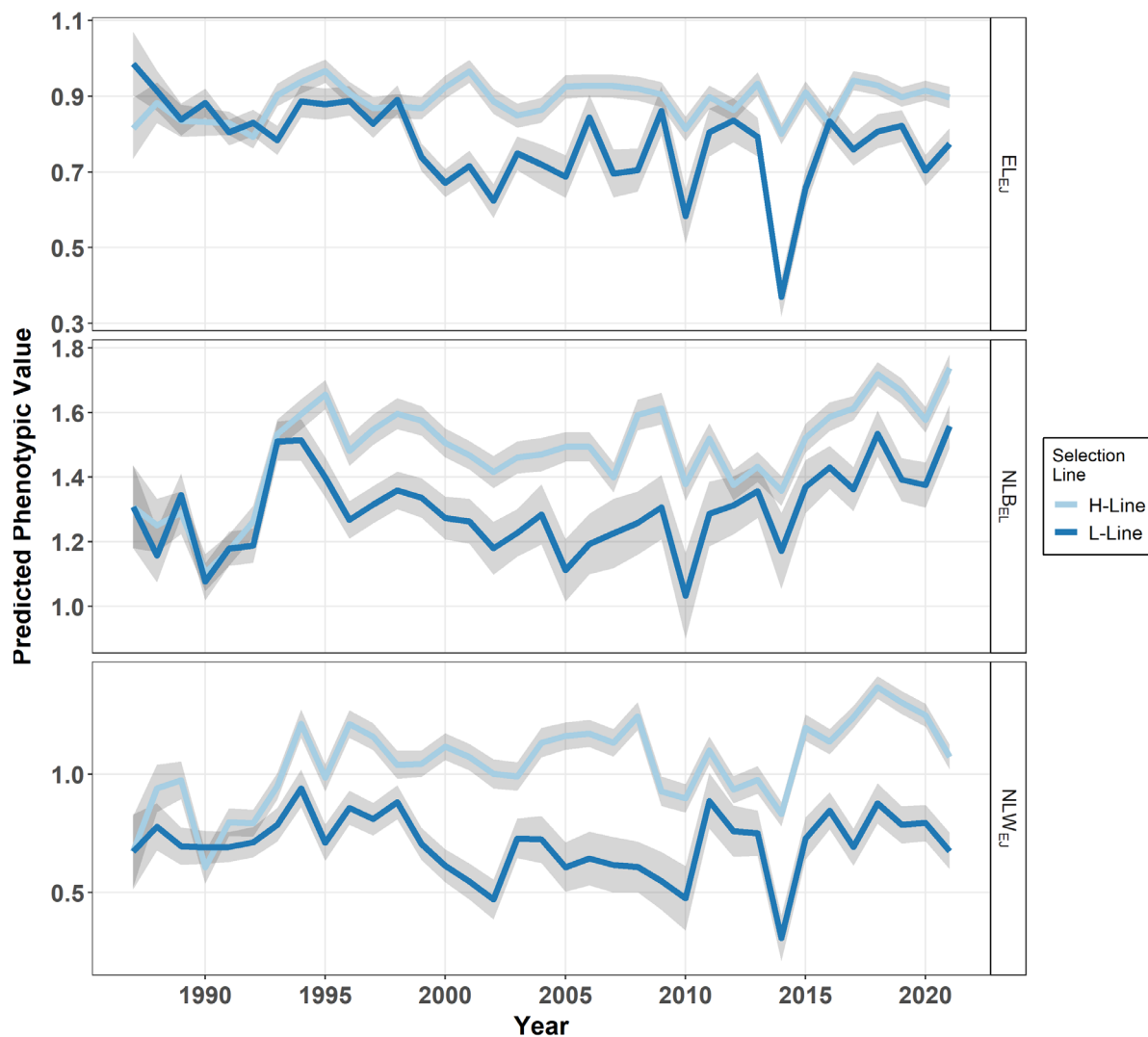


Fig. 2.1 Phenotypic trends as least square means depicting the selection line by year interaction for EL_{EJ} , NLB_{EJ} as well as NLW_{EJ} .

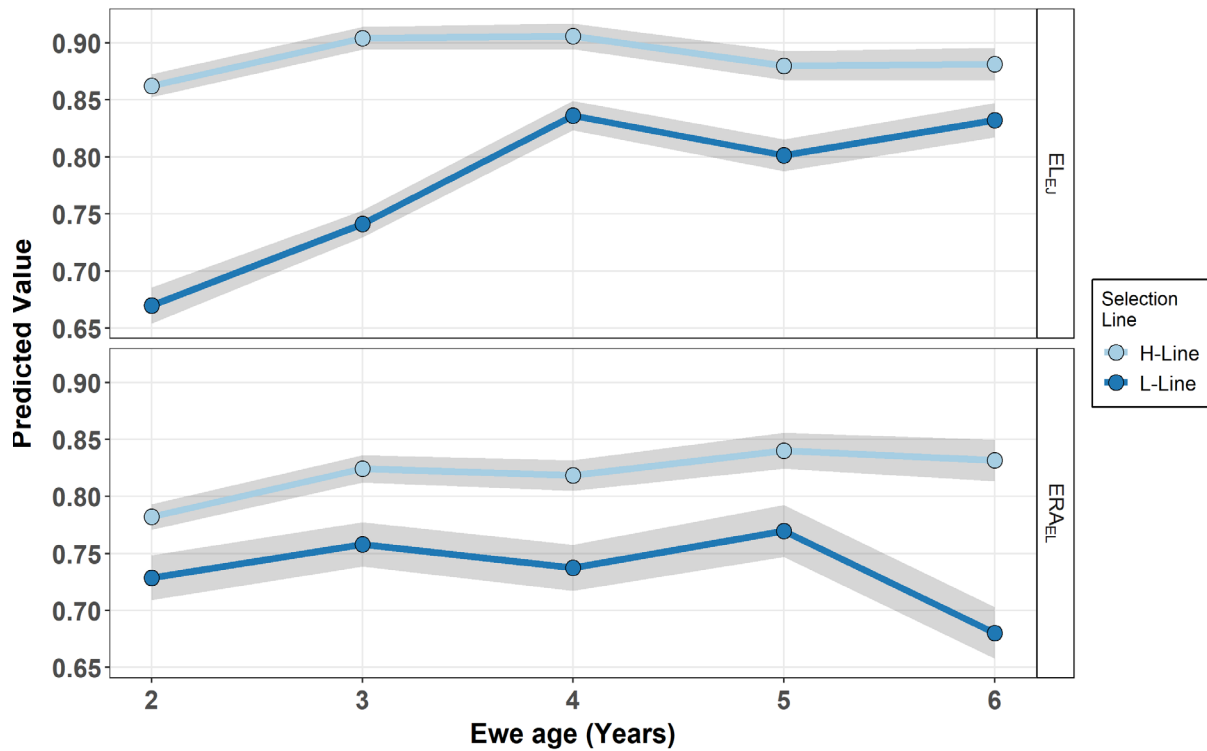


Fig. 2.2 Predicted least square means (\pm SE) depicting the interaction between selection line and ewe age for EL_{EJ} and ERA_{EL}

The introduction of animal additive effects (σ_a^2), animal permanent environmental effects (σ_{pe}^2) and service sire permanent environmental effects (σ_{ss}^2) all resulted in a significant improvement in the log likelihood value when added to fixed effects model (Table 2.2). The only exception was for NLB_{EL}, where the effect of service sire was not significant ($P > 0.05$).

Table 2.2

Log-Likelihood ratios for reproduction traits (see Table 2.1 for abbreviations) for considered additions of applicable random effects. Chosen model represented in 'bold' font type. "FE" row presents the 'fixed effects only' model

Model	EL _{EJ}	NLB _{EL}	NLB _{EJ}	ERA _{EL}	NLW _{EJ}	AWW _{ER}
FE	3463.51	956.36	-786.37	2807.11	-636.6	-9908
(i)	3555.49	1079.55	-648.53	2824.26	-561.28	-9848.24
(ii)	3571.38	1085.48	-637.56	2828.71	-548.78	-9841.28
(iii)	3617.74	1085.7	-625.88	2831.52	-536.34	-9835.64

All reproduction traits were lowly heritable in the single-trait analyses (Table 2.3). All heritability estimates, except for NLB_{EL}, were below 0.10. All traits were affected by animal PE (Table 2.3), the variance ratio ranging from 0.05 for ERA_{EL} to 0.12 for EL_{EJ}. All traits except for NLB_{EL} were affected by SS. These variance ratio estimates were all small at 0.04 for EL_{EJ}, 0.02 for NLB_{EJ}, NLW_{EJ} and AWW_{ER} and 0.01 ± 0.005 for ERA_{EL}.

Table 2.3

Variance components and direct genetic (h^2), animal permanent environment (pe^2) and service sire (ss^2) variance ratios (\pm SE) for reproduction traits (see Table 2.1 for abbreviations)

Effect*	Trait					
	EL _{EJ}	NLB _{EL}	NLB _{EJ}	ERA _{EL}	NLW _{EJ}	AWW _{ER}
Variance components						
(σ_a^2)	0.00459	0.02687	0.03956	0.00340	0.02037	1.4362
(σ_{pe}^2)	0.01388	0.01494	0.03649	0.00608	0.03397	1.5886
(σ_{ss}^2)	0.00518	n.a.	0.00851	0.00132	0.00819	0.3350
(σ_e^2)	0.09252	0.19134	0.33854	0.11184	0.34049	16.483
(σ_p^2)	0.11616	0.23316	0.42275	0.12265	0.40302	19.853
Variance Ratios						
h^2	0.04 \pm 0.02	0.12 \pm 0.02	0.09 \pm 0.02	0.03 \pm 0.01	0.05 \pm 0.02	0.07 \pm 0.02
pe^2	0.12 \pm 0.02	0.06 \pm 0.02	0.09 \pm 0.02	0.05 \pm 0.02	0.08 \pm 0.02	0.08 \pm 0.02
ss^2	0.04 \pm 0.01	n.a.	0.02 \pm 0.01	0.01 \pm 0.00	0.02 \pm 0.01	0.02 \pm 0.01

2.3.2 Multi trait analysis and genetic correlations

The derived multi-trait phenotypic variance components, and the h^2 and PE^2 variance ratios were roughly consistent with the single trait estimates (Table 2.3). However, the service sire effect was observed as zero for NLB_{EL}, ERA_{EL} and NLW_{EJ}, but remained significant at 0.023 ± 0.005 for ewe EL_{EJ} and 0.019 ± 0.006 for AWW_{ER}. The composite trait NLW_{EJ} was favourably related to its underlying component traits, with estimates ranging between 0.42 for NLB_{EL} to 0.82 for EL_{EJ} (Table 2.4). The unfavourably negative genetic correlation between NLB_{EL} and ERA_{EL} was also significant and moderately high in magnitude (-0.57). However, the corresponding PE correlation was not significant. Conception rate was also positively related to ERA_{EL} on the PE level, while NLB_{EL} was negatively related to AWW_{ER}. Average weaning weight was negatively correlated to NLB_{EL}, ERA_{EL}, and NLW_{EJ} on the environmental level. Phenotypic correlations were mostly smaller in magnitude but in the same direction as genetic correlations.

Table 2.4

The multi-trait phenotypic variance component (σ_p^2) and (co)variance ratios for the reproduction traits studied (see Table 2.1 for abbreviations)

Effect and trait	Trait				
	EL _{EJ}	NLB _{EL}	ERA _{EL}	NLW _{EJ}	AWW _{ER}
(σ_p^2)	0.11601	0.23650	0.12789	0.40178	20.330
(Co)variance ratios: h^2 in bold on the diagonal, genetic correlations below and phenotypic correlation above					
EL _{EJ}	0.04\pm0.02	0.02 \pm 0.02	0.27 \pm 0.02	0.50 \pm 0.02	0.07 \pm 0.02
NLB _{EL}	0.32 \pm 0.20	0.12\pm0.02	-0.21 \pm 0.02	0.31 \pm 0.02	0.08 \pm 0.02
ERA _{EL}	0.35 \pm 0.24	-0.57 \pm 0.14	0.04\pm0.02	0.48 \pm 0.02	0.02 \pm 0.01
NLW _{EJ}	0.82 \pm 0.11	0.42 \pm 0.12	0.58 \pm 0.15	0.05\pm0.02	0.07 \pm 0.02
AWW _{ER}	0.35 \pm 0.22	0.12 \pm 0.16	0.04 \pm 0.21	0.21 \pm 0.21	0.08\pm0.02
(Co)variance ratios: PE^2 in bold on the diagonal, PE correlations below and environmental correlations above					
EL _{EJ}	0.11\pm0.02	-0.00 \pm 0.02	0.18 \pm 0.02	0.43 \pm 0.02	0.22 \pm 0.02
NLB _{EL}	0.02 \pm 0.19	0.06\pm0.02	-0.13 \pm 0.02	0.45 \pm 0.02	-0.32 \pm 0.02
ERA _{EL}	0.55 \pm 0.16	0.15 \pm 0.26	0.04\pm0.02	0.32 \pm 0.02	-0.08 \pm 0.02
NLW _{EJ}	0.84 \pm 0.08	0.70 \pm 0.15	0.54 \pm 0.12	0.08\pm0.02	-0.31 \pm 0.02
AWW _{ER}	0.18 \pm 0.15	-0.64 \pm 0.18	0.04 \pm 0.21	-0.15 \pm 0.16	0.07\pm0.02

2.3.3 Genetic responses and trends

Genetic responses in all traits were positive in the H-Line, the magnitude ranging from 0.065 to 0.922% per annum (p.a) when expressed relative to the overall trait mean (Table 2.5). Corresponding responses in the L-Line were all negative, with a range of -0.95 to -0.566. The responses in the composite traits (NLB_{EJ} and NLW_{EJ}) were generally faster than for the component traits (EL_{EJ}, NLB_{EL} and ERA_{EL}) in both selection lines. Predicted genetic trends according to selection line by year were also plotted EL_{EJ}, NLB_{EL} and NLW_{EJ} in Figure 3, which supports the observations of divergence contended in Table 2.4.

Table 2.5

Regression coefficients (\pm SE) of estimated breeding values for reproduction traits (see Table 2.1 for trait abbreviations) expressed relative to overall trait means

Trait	Selection Line	Coefficient \pm s.e.	R^2	% of mean
EL _{EJ}	H-Line	0.00254 \pm 0.00001	0.904	0.295
	L-Line	-0.00250 \pm 0.00004	0.779	-0.29
NLB _{EL}	H-Line	0.00636 \pm 0.00004	0.872	0.445
	L-Line	-0.00136 \pm 0.00010	0.271	-0.095
NLB _{EJ}	H-Line	0.00966 \pm 0.00005	0.916	0.785
	L-Line	-0.00410 \pm 0.00013	0.557	-0.333
ERA _{EL}	H-Line	0.00119 \pm 0.00001	0.777	0.151
	L-Line	-0.00179 \pm 0.00003	0.776	-0.226
NLW _{EJ}	H-Line	0.00867 \pm 0.00004	0.928	0.922
	L-Line	-0.00523 \pm 0.00009	0.764	-0.566
AWW _{ER}	H-Line	0.01454 \pm 0.00031	0.502	0.065
	L-Line	-0.05272 \pm 0.00064	0.862	-0.234

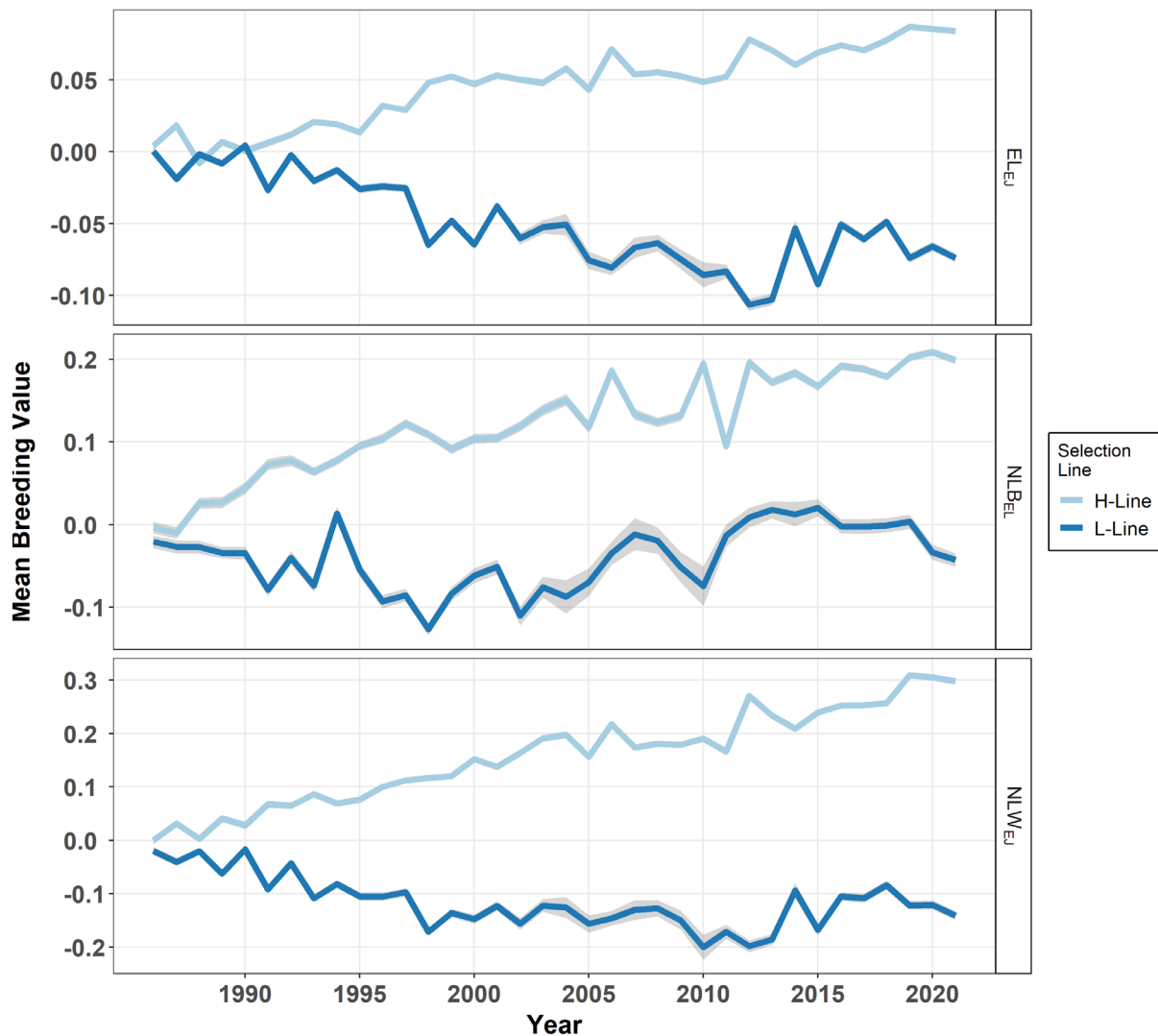


Fig. 2.3 Predicted genetic trends as the least square means of the H- and L-Line breeding values between 1986 to 2021. Shaded areas represent the standard errors surrounding the mean.

2.4 Discussion

2.4.1 Fixed Effects

Results observed for fixed effects and the variability of the traits accorded well with that expected from the literature (Safari *et al.* 2005, 2007a; Newton *et al.* 2014; Bunter and Brown 2015; Cloete *et al.* 2017; Walkom and Brown 2017; Bunter and Swan 2021) and will not be discussed further. Cloete *et al.* (2003) also reported that the two-factor interaction between selection line and ewe age was not significant for the composite trait NLW_{EJ} ($P > 0.05$). Comparable differences for the composite reproduction traits and for lamb weaning weight were also reported in previous research on the flock that utilised part of the data (Cloete and Scholtz 1998; Cloete *et al.* 2004) and Cloete *et al.* (2017) also reported significant line differences for both component and composite traits. The exception was ERA_{ER} , which did not form part of the previous analysis. Interestingly, these results are also comparable to those reported previously by Atkins (1980) for the Fertility flock at Trangie. Despite selection for conception and multiple births, the Fertility flock maintained a better lamb survival than the control line, as for the H-Line in the present study.

Cloete and Heydenrych (1986) reported that EL_{EJ} in the Tygerhoek Merino flock increased from 0.70 to 0.74 in 2-year-old ewes to 0.83 to 0.87 in 3-year-olds, 0.86 to 0.89 in 4-year-olds, 0.83 to 0.87 in 5-year-olds and 0.82 to 0.89 in 6-

year-olds. These trends seem to be comparable with the present results, but seemed to be more pronounced in the L-Line. Cloete *et al.* (2021b) reported a broadly consistent age trend as with the H-Line for ERA_{EL} in extensively managed Dohne Merino flocks, while the trend in the L-Line were more like that of the intensively managed Dohne Merino flocks. Although the lambing year x ewe age interaction was significant for all traits except AWW_{ER}, these results are not considered pertinent to the current study. It will thus not be presented and discussed, although it remained in the operational models in the analyses to estimate genetic parameters.

2.4.2 Variance Components

2.4.2.1 Heritability

In contrast with the previous study on the same flock (Cloete *et al.* 2017), where the heritability of 0.02 ± 0.02 for EL_{EJ} was not significantly different from zero ($P > 0.05$), the present estimate for EL_{EJ} was heritable. At 0.04, this estimate was within the range of literature values, which included estimates of 0.05 (Safari *et al.* 2007b), 0.02 (Bunter and Brown 2015), 0.03-0.21 (Bunter *et al.* 2016), 0.03 (Walkom and Brown 2017), 0.05 (Bunter *et al.* 2020) and 0.09 (Bunter and Swan 2021). At 0.12, the present heritability for NLB_{EL} was in the range of comparable values in the literature, including estimates of 0.07 (Safari *et al.* 2007b), 0.04-0.12 (Newton *et al.* 2014), 0.10 (Bunter and Brown 2015), 0.06-0.10 (Bunter *et al.* 2016), 0.13 (Cloete *et al.* 2017), 0.08 (Walkom and Brown 2017), 0.04-0.06 (Bunter *et al.* 2020) and 0.09 (Bunter and Swan 2021). Accordingly, the estimate of 0.09 for NLB_{EJ} was consistent with values of 0.05 (Safari *et al.* 2007b), 0.09 (Pickering *et al.* 2012), 0.04-0.05 (c), 0.07 (Bunter and Brown 2015), 0.07-0.19 (Bunter *et al.* 2016), 0.10 (Cloete *et al.* 2017) and 0.06 (Walkom and Brown 2017). At 0.03, the heritability of ERA_{EL} was the lowest of all traits. This estimate was nevertheless consistent with comparable values 0.01-0.08 (Bunter *et al.* 2016), 0.04 (Walkom and Brown 2017), 0.04 (Bunter *et al.* 2020), 0.03 (Bunter and Swan 2021) and $< 0.01 - 0.02$ (Cloete *et al.* 2021b). NLW_{EJ} was heritable at 0.05 (Table 2.3), which was just above previous estimates of 0.04 reported by Cloete *et al.* (2004, 2017) on the same flock. Other comparable estimates were 0.05 (Safari *et al.* 2007b), 0.03 (Bunter and Brown 2015), 0.03-0.10 (Bunter *et al.* 2016) and 0.04 (Walkom and Brown 2017). The heritability of 0.07 for AWW_{ER} was consistent with literature values of 0.08 (Bunter and Brown 2015) and 0.06 (Cloete *et al.* 2017).

2.4.2.2 Permanent environmental effect

The estimate of 0.12 for EL_{EJ} was within the range of 0.01 to 0.14 in the literature (Safari *et al.* 2007b; Bunter and Brown 2015; Bunter *et al.* 2016; Cloete *et al.* 2017; Bunter *et al.* 2020; Bunter and Swan 2021). The animal PE estimate of 0.06 was at the higher end of the range of 0.02 to 0.06 for NLB_{EL} in the literature (Safari *et al.* 2007b; Bunter and Brown 2015; Bunter *et al.* 2016; Cloete *et al.* 2017; Bunter *et al.* 2020; Bunter and Swan 2021). The animal PE estimate of 0.09 for NLB_{EJ} was just above comparable estimates of 0.03 to 0.08 in the literature (Cloete *et al.* 2004; Safari *et al.* 2007b; Pickering *et al.* 2012; Bunter and Brown 2015; Bunter *et al.* 2016). The animal PE value of 0.05 for ERA_{EL} in Table 2.3 corresponded with values of 0.05 to 0.11 in the literature (Bunter *et al.* 2016) The derived value of 0.08 for NLW_{EJ} was similarly in correspondence with the range from 0.06 to 0.11 in the literature (Cloete *et al.* 2004; Safari *et al.* 2007b; Bunter and Brown 2015; Bunter *et al.* 2016). The animal PE estimate of 0.05 for AWW_{ER} reported by Bunter and Brown (2015) was the only comparable estimate we could find in the literature.

2.4.2.3 Service sire effect

Safari *et al.* (2007) accordingly reported significant service sire variances for EL_{EJ}, NLB_{EJ} and NLW_{EJ}. They also suggested that the heritability of these traits would be inflated if the effects of service sires were not accounted for. Bunter

and Brown (2015) accordingly reported service sire variance ratios of 0.05 for EL_{EJ} , 0.02 for NLB_{EJ} and 0.02 for NLW_{EJ} . The close correspondence of all the parameter estimates in Table 2.3 with results reported in the literature is reassuring, as it suggests that the present results are robust and consistent with a broad body of existing results.

2.4.2.4 Genetic correlations

The generally favourable correlations between NLW_{EJ} and its underlying components is expected given the part-whole relationship between the composite and component traits. A moderate to high genetic correlation between NLW_{EJ} and EL_{EJ} is supported by the literature with a range of 0.69-0.87 (Newton *et al.* 2014; Bunter *et al.* 2016; Cloete *et al.* 2017). High estimates for the genetic correlation between NLW_{EJ} and NLB_{EL} with a range of 0.76 to 0.95 (Newton *et al.* 2014; Cloete *et al.* 2017), while some estimates reported by Bunter *et al.* (2016) were lower and notably variable between 0.29 and 0.82. NLW_{EJ} and ERA_{EL} had a slightly more moderate genetic relationship, in relative agreement to the estimate of 0.41 reported by Newton *et al.* (2014), and the range of 0.49-0.85 reported by Bunter *et al.* (2016). In the component traits, the moderate genetic relationship (0.35, Table 2.4) between EL_{EJ} and ERA_{EL} falls within a wide range of estimates between 0.07 (Bunter *et al.* 2020) and 0.41-0.56 (Bunter *et al.* 2016), whereas Newton *et al.* (2014) reported a large difference between estimates based on hogget (0.07) and adult (0.79) ewes. Generally, as noted by Bunter *et al.* (2020), genetic correlations below 0.5 suggests the ability to target components individually if desired. The most problematic relationship, however, is the negative correlation between NLB_{EL} and ERA_{EL} , which is well supported elsewhere with a range between -0.21 to -0.82 (Newton *et al.* 2014; Bunter *et al.* 2016; Bunter *et al.* 2020), but is not unexpected given the well-known susceptibility of multiples. The magnitude of this unfavourable relationship, which was also reflected on the phenotypic level (Table 2.4), further motivates the need to consider these components individually.

2.4.2.5 Genetic Trends

As the trait targeted for divergent selection it is not surprising that the greatest responses in both lines were observed for NLW_{EL} . The responses in the composite traits were consistent in direction but smaller in magnitude when compared with previous results on the same resource flock (Cloete *et al.* 2004, 2017). This generalisation also applied to the component traits in common with the results of Cloete *et al.* (2017). Two factors could be involved in these changes in the magnitude of the responses. Firstly, the responses of the earlier studies were initially expressed relative to lower phenotypic figures at the commencement of the study. However, the absolute regression coefficients were also lower when compared using the standard errors ($P < 0.05$). The other factor that could also have contributed is the introduction of external sires in both lines in recent years. The asymmetry in the responses of the H and L-Lines (Fig. 2.3) for the composite traits has previously been attributed to an attempt to select against natural selection in the L-Line, as well as to a reduced selection differential stemming from the reduced lamb output in the latter line (Cloete *et al.* 2004)

2.5 Conclusions

Selection for NLW_{EJ} as a composite trait resulted in favourable genetic responses in the desired direction in the component traits studied. Since previous studies on the resource population did not include a measure of lamb survival, it is reassuring that this generalisation also applied to ERA_{EL} . It can thus be concluded that composite trait selection based on NLW_{EJ} would result in an overall improvement in lamb output when targeted during selection. However, the targeted approach that form the basis of evaluation in Australia at present is absent in such a selection regime. For the time being, it is suggested that NLW_{EJ} continue to be used selection criterion in South Africa. This approach should be revisited as data accrue and more sophisticated statistical models can be applied.

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CHAPTER 3:

Genetic parameters and trends for lamb survival following long term divergent selection for number of lambs weaned in the Elsenburg Merino flock[#]

Abstract

Mortality of new-born lambs is well-known to have important implications for both animal production and welfare. Improving survival rates by genetic selection is very desirable, but the low heritability of survival traits challenges the prospect of useful genetic gain. This study aimed to derive genetic and environmental parameters for lamb survival in the Elsenburg Merino resource flock. It also investigated correlations to possible indicator traits birth weight (BW) and birth coat score (BCS) and reported genetic progress in breeding values for lamb survival following 33 years of divergent selection. The flock was divergently selected for number of lambs weaned and was separated by the High (positive) and Low (negative) selection groups. The pedigree file identified 8138 lambs as the progeny of 273 sires and 2062 dams. The study considered total survival from birth to weaning (Tsv) that was also partitioned into perinatal survival to three days of age (PNsv), and the remaining period (P3sv). Variance components were derived by linear mixed models using the ASREML® program. Genetic trends were derived by predicting mean breeding values for selection groups within each year and evaluated by fitting linear and broken stick regression models. Predicted Tsv of H-line lambs (0.81 ± 0.01) was higher ($P < 0.01$) compared to L-Line lambs (0.68 ± 0.01). Heritability (h^2) was significant but low for survival traits (0.03 – 0.07), moderate for BW (0.16) and high for BCS (0.54). Genetic trends of the H-Line trended divergently ($P < 0.01$) to the L-Line for survival traits, but a changepoint ($P < 0.01$) in trend suggested the H-Line reached a selection plateau following 19 – 22 years of selection. Preceding this period, the rate of genetic change equalled $\sim 1\%$ of the mean for Tsv. Despite the low h^2 of survival, the genetic trends reported in this study contradicted the premise that genetic selection is not a worthwhile method to reduce incidences of lamb mortality. It is recommended that lamb survival phenotypes should be recorded and incorporated into indices where possible, but composite traits are a viable alternative.

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3.1 Introduction

Mortality of new-born lambs is a well-known constraint to small stock production systems (Alexander 1984). A high margin of loss is common with average mortality ranging between 20 - 25% across different breeds (Hinch and Brien 2014). Merinos have been estimated to deliver only two thirds of potential at weaning (Kilgour 1992), or to lose as many as 59 lambs per 100 ewes mated (Kleemann and Walker 2005). Besides the issue of economic wastage, lamb mortality is also a concern from an emotional and welfare perspective which provides further reason that the improvement of lamb mortality should not be neglected.

Survival depends on an interaction between the environment, physiology as well as the behavior of both the ewe and the lamb. Ewes need to facilitate optimal in utero development, give birth without difficulty and provide adequate colostrum along with tactile stimulation and mothering. In turn, the lamb must adapt to the extra-uterine environment, thermoregulate and display favourable vigour in its ability to stand and suckle (see reviews: Dwyer 2008b; Brien *et al.* 2014; Hinch and Brien 2014).

The environment plays an important role in lamb survival (Hinch and Brien 2014). Among other factors, nutrition of the ewe is critical, and malnourishment adversely affects both physical and behavioural performance of the ewe (Dwyer 2008a) which leads to higher lamb mortality rates (Kleemann *et al.* 1993). Intervention at the environmental level can alleviate stress factors that increase risk. Housing and shepherding have been associated with a lower incidence of stillbirths (Binns *et al.* 2002) and early mortality has been reduced by the provision of shelter (Alexander *et al.* 1980; Lynch and Alexander 1980). However, such strategies are expensive, time consuming, and have other associated risks such as increased infection rates (Binns *et al.* 2002). Also, substantial mortality rates of 19% have been shown to occur even under favourable circumstances (Behrendt *et al.* 2011), suggesting room for improvement on a genetic level. Genetic selection for higher survival rates is highly desirable but would depend on practical selection criteria and useful genetic components contributing to the survival outcome.

Survival contributes to composite phenotypes of the ewe such as the total weight weaned (TWW) or total number of lambs weaned per ewe mated (NLW). In addition to survival, these phenotypes are underpinned by other measurable components such as fertility, growth and litter size. Selection for TWW or NLW could provide a balanced approach to simultaneously improving underlying components, including survival (see review by Snowden and Fogarty 2009). A concern is that records such as NLW and TWW do not necessarily account for incidences of mortality unless survival of the lamb or rearing ability of the ewe is expressed explicitly. Multiples are well known to be more susceptible than singles (Slee *et al.* 1991; Kleemann and Walker 2005; Snowden and Fogarty 2009; Bunter *et al.* 2018), and a higher number of lambs born could be accompanied by mortalities not necessarily reflected in a trait such as NLW. Accordingly, studies and reviews have recommended the genetic improvement of lamb survival should be included in index selection individually (Swan 2009; Brien *et al.* 2011, 2014; Hinch and Brien 2014; Bunter and Brown 2015; Bunter *et al.* 2018). In New Zealand, Sheep Improvement Limited (SIL; <http://www.sil.co.nz>) have provided breeding values for lamb survival since 1999 (Newman *et al.* 2000), but other examples of selection for survival as a trait of the lamb are scarce in both the research or industry sectors.

Useful genetic components to survival are supported by differences in lamb mortality across breeds (Alexander 1984; Kleemann *et al.* 2000; Fogarty *et al.* 2005), Merino bloodlines (Mortimer and Atkins 1997), and Romney sire lines (Gudex *et al.* 2005). However, genetic gain by selection within breeds is considered as challenging. A generally low or very low heritability (h^2 ; 0.01 - 0.05) has been reported in Merinos (Brien *et al.* 2009; Hatcher *et al.* 2010) and other breeds (eg. Lopez-Villalobos and Garrick 1999; Morris *et al.* 2000; Riggio *et al.* 2008; Vanderick *et al.* 2015). Studies have reported maternal genetic effects (m^2) as influential, but also low, and often associated with negative and varying estimates of the direct-maternal covariance (r_{am}) (Brien *et al.* 2014). Moreover, survival is a threshold character (Falconer 1989) which may not be amenable to analysis by linear models. Subsequently, studies investigating survival have varied in their approach in analysing lamb survival, causing some complexity in the comparison and interpretation of estimates.

Using threshold analysis, Cloete *et al.* (2009) reported promising genetic trends in lamb survival derived from the Elsenburg Merino flock, a selection experiment subject to divergent selection for NLW since 1986. Genetic gain achieved in the Elsenburg flock proved contradicting to the notion that genetic selection would not be worth-while in improving mortality rates. However, the study by Cloete *et al.* (2009) reported a large negative r_{am} estimate, an unfavourable result that could be symptomatic of analysis rather than a true biological underpinning (Robinson 1996). Also, evaluating survival data on the underlying scale can cause difficulty in deriving accurate and comparable threshold-linear covariance components between survival and continuous traits (Cloete *et al.* 2009). Given the low h^2 of survival, there is interest in identifying favourably correlated traits that could improve realised gain (Brien *et al.* 2010). Such an objective, however would hinge on accurate genetic and environmental correlations. Birth weight has long been known as an important predictor of lamb survival (Smith 1977; Hinch *et al.* 1983; Gama *et al.* 1991), but is complicated by a non-linear relationship to mortality (Dwyer 2008b; Hinch and Brien 2014). A birth coat score that grades the hairy- or woolly-ness of coats could play a part in lamb viability in the early neonatal period (Alexander 1961; Slee 1978; Slee *et al.* 1991). However, this has been lowly correlated to survival in Merinos in a previous study (Brien *et al.* 2010). The objectives of the current study are thus: 1) to revisit genetic parameters for direct and maternal components for lamb survival in the Elsenburg flock with alternative methods to those of Cloete *et al.* (2009); 2) to investigate the relationships of survival with the possible indicator traits birth weight and birth coat score; and 3) to report genetic progress (or lack thereof) in lamb survival following divergent selection that currently extends to 33 years since the onset of the experiment.

3.2 Materials and methods

3.2.1 The Elsenburg Merino flock

The Elsenburg Merino flock is a resource flock managed as a selection experiment for divergent selection based on NLW since 1986 (see review by Schoeman *et al.* 2010). The flock is separated as the High (H-Line; positive selection) and Low (L-Line; negative selection) lines that originated from the same base population. At the start of the experiment, ewes were allocated at random within age groups to each of the two lines. Subsequent selection proceeded by screening ewes according to their ranking for NLW. Besides instances of

death or severe health related problems, replacement ewes remained in the flock for at least 5 joinings. At the beginning of the experiment, roughly 120 ewes were assigned to each selection line. The H-line has since grown to 130 - 200 ewes, while numbers in the L-Line dwindled to about 40 - 80 breeding ewes. Selection of rams was predominantly based on the progeny of dams based on at least 3 joinings. Following the results reported by Cloete *et al.* (2004), selection from 2003 was also guided by the use of best linear unbiased prediction (BLUP) breeding values derived from a single-trait repeatability model fitted to annual NLW records of ewes. During this period, the original ranking values were still considered in tandem to BLUP derived breeding values, but BLUP-values have been the only selection criterion since 2010. In the latter years of the selection experiment, breeding management required the use of external sires to manage levels of inbreeding of the H- and L-Lines. From 2008 to 2019, 349 H-Line lambs and 151 L-Line lambs were born as the progeny of external sires. These animals were also considered for selection and 740 H-Line and 118 L-Line lambs were subsequently born as grand-progeny of external sires between 2010 and 2019.

Details pertaining to management and nutrition are available elsewhere (Cloete *et al.* 2004, 2009) and only a short summary will be repeated here. During this experiment, the flock was maintained at the Elsenburg Research Farm near the town of Stellenbosch (33°51'S and 18°50'E) in the Western Cape Province, South Africa. Besides during joining in single-sire groups within lines, the two lines were maintained as a single flock for most of the year. The flock grazed on irrigated kikuyu grass (*Pennisetum clandestinum*) during the summer (January – February) and during the lambing period in winter (June - July). In the remaining periods, the flock grazed on dryland as well as irrigated lucerne (*Medicago sativa*) and dryland medics (*M. truncatula*). Oats (*Avena sativa*) fodder crops were also available during winter to supplement the slow-growing legume pastures. Ewes were shorn within 3 to 4 weeks prior to lambing. Lambing took place in 10 - 20 kikuyu lambing paddocks of approximately 0.3 to 0.4 ha each, where ewes were set stocked in groups ranging from 15 to 25 depending on the research needs. After a variable period of 3 to approximately 14 days, lambing ewes and their lambs were randomly drafted to somewhat larger (1.0 to 1.5 ha) irrigated lucerne paddocks in groups of 20 to 40. As the irrigated paddocks became depleted, these groups were joined in larger groups on dryland lucerne, medic and oat paddocks.

3.2.2 Data recording

Data were recorded over a 33-year period from 1986 to 2019. The pedigree file identified the line of origin for the vast majority of 8138 lambs as the progeny of 273 sires and 2062 dams. Recording of all traits commenced during daily lambing rounds at 08:00 am that identified all lambs born within the previous 24-hour period with their dams, thus enabling linkage back to the selection line and sire (Cloete *et al.* 2004, 2009). Traits recorded were survival, birth weight (BW) and birth coat score (BCS) for both dead and alive lambs. In rare cases birth weight was unavailable in lambs mutilated by damage-causing animals, mostly crows (*Corvus* spp.). Survival traits were regarded as total survival from birth to weaning (Tsv), survival to three days of age, including stillbirths and ante parturient deaths, as perinatal survival (PNsv), and the remaining period post 3-days to weaning (P3sv). BCS was only recorded since 1994 and included records on 5883 lambs over 25 years. BCS

was subjectively recorded according to a linear 5-point scale graded from the most hairy (1) to most woolly (5) (Cloete *et al.* 2003) with half marks allowed where considered appropriate.

3.2.3 Statistical analysis

3.2.3.1 Variance components

The discrete expression of the survival phenotype as either dead (0) or alive (1) is described as a threshold character (Falconer 1989) that follows a hypothetical normal distribution on the underlying liability scale. Threshold characters violate the assumption of normality on the observed scale, and linear models are theoretically not appropriate for the analysis of binary phenotypes. However, previous examples from the literature analysed survival data on both underlying and observed scale that allowed for the comparison of these methods. Everett-Hincks *et al.* (2014) and Vanderick *et al.* (2015) used cross validation of randomly assigned ‘missing’ phenotypes and reported that linear models were marginally more accurate at predicting missing phenotypes compared to logit transformed alternatives. Also, Matos *et al.* (2000) reported linear and threshold models to be similar according to predictive and goodness of fit parameters. Consequently, in contrast to previous results of survival data from the same flock (Cloete *et al.* 2009), current data were evaluated by linear analysis for benefit of convenient interpretation on the observed scale.

Estimation of fixed effects and subsequent derivation of variance components were undertaken using the ASREML® program (Gilmour *et al.* 2015). The environmental factors that were considered as fixed effects included year of birth (1986 - 2019), selection group (H-Line or L-Line), sex (male or female), age of dam (2 to 6+ years) and birth type (singles vs pooled multiples). The non-linear phenotypic relationship between survival and BW was investigated by fitting BW as a linear (BW) and quadratic (BW²) covariate in a separate analysis of survival traits. These results were reported separately and were identified by Tsv^{BW} , $PNsv^{BW}$ and $P3sv^{BW}$. Otherwise, variance components and genetic trends of survival are reported excluding BW as a covariate.

Random effects considered in the model were direct and maternal genetic effects, the direct-maternal covariance and maternal permanent environment. Analysis commenced by fitting various combinations of fixed effects and possible two-way interactions in order to obtain an operational model. In the model used to derive parameter estimates for random components, selection group was included as a fixed effect to avoid possible inflation of genetic variance that could result from the selection program separating the H- and L-Lines. Fixed effects and interactions were tested for significance according to Wald statistics derived from conditional least square methods. Those observed as significant ($P < 0.05$) were included in the fixed effects model and retained throughout subsequent analysis of testing random effects. Random terms were then added to the operational model on a step-wise basis that resulted in either of the following genetic models used for analysis (in matrix notation):

Model (i)	$y = Xb + Z_1a + e$
Model (ii)	$y = Xb + Z_1a + Z_3m + e$ [$CoV_{am} = 0$]
Model (iii)	$y = Xb + Z_1a + Z_2c + e$
Model (iv)	$y = Xb + Z_1a + Z_3m + Z_2c + e$ [$CoV_{am} = 0$]
Model (v)	$y = Xb + Z_1a + Z_3m + e$ [$CoV_{am} = A\sigma_{am}$]
Model (vi)	$y = Xb + Z_1a + Z_3m + Z_2c + e$ [$CoV_{am} = A\sigma_{am}$]

In the preceding equations, y represented the vector of observations of the respective traits, b the vector of fixed effects, a represented the vector of direct genetic effects m the vector of maternal genetic effects, c the vector of maternal permanent environmental effects and e the vector of residuals. The corresponding incidence matrices of each effect were respectively represented by X , Z_1 , Z_2 , and Z_3 . A represented the numerator relationship matrix, and σ_{am} the covariance between direct and maternal genetic effects.

It was assumed that:

$$V(a) = A\sigma_a^2; V(m) = A\sigma_m^2; V(c) = I\sigma_c^2; V(e) = I\sigma_e^2$$

with A representing the numerator relationship matrix, I representing identity matrices and σ_a^2 , σ_m^2 , σ_{mc}^2 and σ_e^2 the direct genetic variance, maternal genetic variance, maternal permanent environmental variance and environmental (residual) variance, respectively. These analyses yielded estimates of genetic and permanent environmental variances, which were used to compute (co)variance ratios for direct additive genetic (h^2), maternal genetic (m^2), the direct-maternal genetic correlation (r_{am} ; where applicable) as well as maternal permanent environmental (mc^2) variances as a proportion of the total observed phenotypic variance. A log-likelihood ratio test (LRT) was used to test for significant differences in model likelihoods:

$$LRT = -2 \log_e \left[\frac{L_s(\theta)}{L_g(\theta)} \right] = 2 | \text{Log}L_g - \text{Log}L_s |$$

where $L_g(\theta)$ and $L_s(\theta)$ represents the likelihood of the general (full) and the simpler (nested) model, respectively. The log-likelihood ratio test is a chi-square distributed random variable, with degrees of freedom equal to the difference in the number of random effects. However, the two-sided LRT statistic was adjusted for being bound above 0, i.e. on the boundary of the parameter space, by considering a critical value of 1.36 (0.5×2.71) instead of 1.92 (0.5×3.84) for a chi-square variable with 1 degree of freedom. This adjustment did not apply when testing for the direct-maternal covariance component (Model (v) vs. (ii) or model (vi) vs. (model (iv))), where 1.92 was used. If a model with an additional term did not provide a significant ($P < 0.05$) improvement, the simpler (nested) model was chosen as the operational model for the trait. The terms fitted in the operational model were subsequently used for multi-trait models. The multi-trait analyses were limited to 2-trait models that allowed for the estimation of direct genetic (r_g), maternal genetic, (r_m), permanent environmental (r_{mc}), environmental (r_e) and phenotypic (r_p) correlations between the respective traits.

3.2.3.2 Genetic trends

In order to assess genetic divergence between selection lines, individual breeding values were obtained from a separate analysis that excluded selection group (or any interactions with selection group) from the model. This was considered appropriate for trends since the retention of selection group as a fixed effect would deflate possible genetic differences that could have resulted from selection. These breeding values were used to derive means for each selection group (H-Line vs L-Line) for each year (1986-2019) as indicative of genetic trends. In order to assess possible change in genetic trends, two approaches were followed. First, standard linear regression lines were fitted for both trends representing the H- and L-Lines and tested for significance ($P < 0.05$) of their regression coefficients and difference in regression slopes by t-tests using individual standard errors (SE) and the pooled SE of the regression coefficients, respectively. The intercept of linear regressions on genetic trends was fixed at zero (year 0) since the H- and L-Lines originated from the same founding population. Second, within-line trends were investigated for possible selection plateaus, or distinct change in trend, by fitting ‘broken stick’ regression models that delivered an estimate of a possible change point in genetic trends. This was performed with the *segmented* package (Muggeo 2008) in the R environment (R Core Team 2020). A piecewise or segmented relationship between the mean response and explanatory variable is modelled by adding an additional term to the linear predictor:

$$E[Y] = \beta_1 x_i + \beta_2 [(x_i - \psi) \times I(x_i > \psi)]$$

where β_1 is the left (or initial) slope, β_2 is the difference in slopes, ψ is the breakpoint and $I(\cdot)$ is an indicator function equal to one when the statement is true ($x_i > \psi$) and zero when the statement is false ($x_i < \psi$). The *segmented* package evaluates a possible difference in slopes by making use of the Davies (1987) test. This is not the most appropriate test for determining the number of breakpoints (Muggeo 2008), but a maximum of one was assumed for the current analysis. In cases where Davies’ test yielded significant ($P < 0.01$) results, the derived changepoint (CP) and differential slopes/coefficients were reported in addition to standard regression lines. Breeding values, genetic trends and fitted regression lines were plotted with *ggplot2* (Wickham 2016) in R.

3.3 Results

3.3.1 Fixed effects

Trait summaries and least square means of fixed effects can be seen in Table 3.1. Over the period of the study, ~ 10% of animals were lost within the first 3 days after birth, which was a large proportion of the ~ 23% total mortality rate to weaning. The mean BW was 3.85 kg, and mean BCS was 3.2. Year significantly ($P < 0.05$) interacted with selection group for Tsv, PNsv, BW and BCS, and is not tabulated. As the two lines shared the same environment, differences between the H- and L-lines across succeeding years are considered a response to selection and are discussed under genetic trends below. Selection group also interacted ($P < 0.05$) with birth type in all three survival traits. The advantage of the H-Line was more pronounced in multiples compared to singles and predicted that Tsv for L-Line multiples was especially poor (0.57; Fig. 3.1). Generally, lambs born

in the H-Line had higher survival rates ($P < 0.01$) compared to L-Line lambs in PNsv, P3sv and Tsv. H-Line lambs were also slightly heavier ($P < 0.05$) but had a lower BCS ($P < 0.01$), indicating they tended to be hairier and L-Line lambs woollier. Female lambs had a higher ($P < 0.01$) survival rate than male lambs in Tsv and P3sv, but sex was not influential in PNsv ($P > 0.05$). Male lambs were heavier than females ($P < 0.01$) and had a higher ($P < 0.01$) BCS. Birth type was influential in all traits except BCS, with singles having higher ($P < 0.01$) survival rates for all periods, and the significant ($P < 0.01$) difference in BW amounted to nearly 1 kg. The age of the dam also proved influential ($P < 0.01$) in all traits. A non-linear relationship best described this influence as ewes of intermediate age tended to have lambs with better survival rates, a higher lamb birth weight, but lower BCS compared to primiparous or mature ewes.

Table 3.1

Predicted least square means (\pm standard error) of all fixed effects considered. The abbreviation NS indicates effects not significant ($P > 0.05$)

Effect	Tsv	PNsv	P3sv	BW	BCS
No Records	8138	8138	7297	8111	5883
Mean	0.77	0.90	0.86	3.85	3.20
Selection Line					
<i>High Line</i>	0.81 \pm 0.01	0.92 \pm 0.01	0.88 \pm 0.01	3.91 \pm 0.01	3.15 \pm 0.01
<i>Low Line</i>	0.68 \pm 0.01	0.84 \pm 0.01	0.81 \pm 0.01	3.82 \pm 0.02	3.49 \pm 0.03
Sex					
<i>Male</i>	0.73 \pm 0.01	NS	0.84 \pm 0.01	3.99 \pm 0.01	3.45 \pm 0.02
<i>Female</i>	0.76 \pm 0.01	NS	0.86 \pm 0.01	3.74 \pm 0.01	3.19 \pm 0.02
Birth Type					
<i>Single</i>	0.84 \pm 0.01	0.92 \pm 0.01	0.91 \pm 0.01	4.33 \pm 0.01	NS
<i>Multiple</i>	0.66 \pm 0.01	0.84 \pm 0.01	0.78 \pm 0.01	3.40 \pm 0.01	NS
Age of Dam					
2	0.68 \pm 0.01	0.85 \pm 0.01	0.80 \pm 0.01	3.53 \pm 0.02	3.41 \pm 0.03
3	0.76 \pm 0.01	0.89 \pm 0.01	0.86 \pm 0.01	3.85 \pm 0.02	3.38 \pm 0.03
4	0.79 \pm 0.01	0.89 \pm 0.01	0.88 \pm 0.01	3.97 \pm 0.02	3.27 \pm 0.03
5	0.77 \pm 0.01	0.89 \pm 0.01	0.86 \pm 0.01	4.02 \pm 0.02	3.25 \pm 0.03
6 +	0.73 \pm 0.01	0.87 \pm 0.01	0.84 \pm 0.01	3.97 \pm 0.02	3.31 \pm 0.03

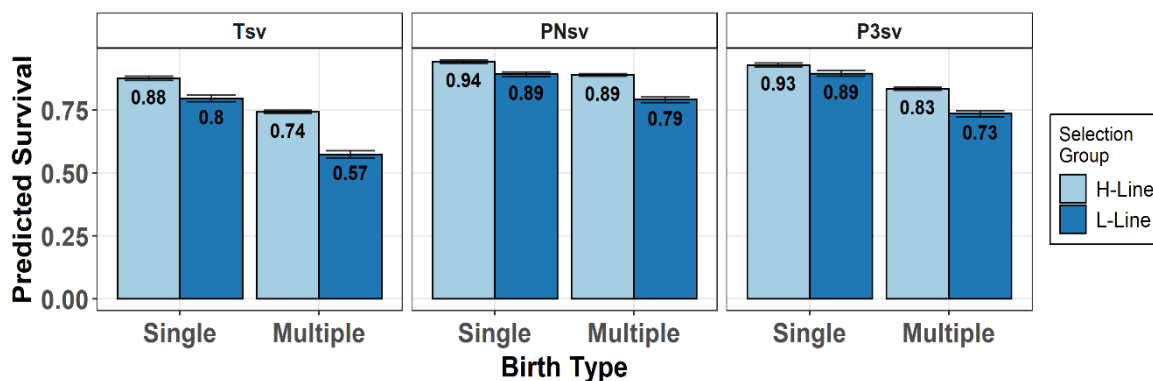


Fig. 3.1 Predicted lamb survival for lambs according to birth type and selection group. Horizontal bars represent standard errors surrounding the mean

When BW was included as linear and quadratic terms in the fixed effects model of Tsv^{BW} , $PNsv^{BW}$ and $P3sv^{BW}$, a non-linear phenotypic relationship of survival and BW was evident (Fig. 3.2), but more pronounced in PNsv compared to P3sv. Very heavy and very light lambs were predicted with a better survival rate in P3sv, suggesting that problems associated with the weight of the lamb were most influential in perinatal period.

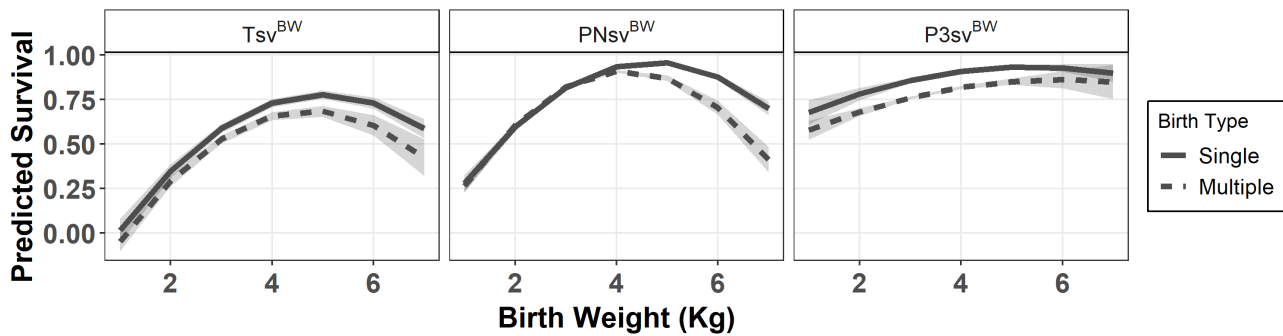


Fig. 3.2 Predicted lamb survival for lambs according to birth weight and type. Shaded areas represent standard errors surrounding the mean. The interaction between birth type and birth weight was only significant ($P < 0.05$) in the case of PNsv^{BW} .

Lambs were predicted to have the best chances of survival according to an intermediate optimum that ranged between 3 to 5 kg. The effect of selection group was tested for a possible interaction with BW and BW^2 , which was not significant ($P > 0.05$). This suggested that advantages of the H-Line lambs were generally independent of BW, making inferences in this respect quite robust. However, BW and BW^2 was in a significant ($P < 0.05$) interaction with birth type in the case of PNsv^{BW} , but not for Tsv^{BW} or P3sv^{BW} . According to Fig. 3.2, the difference between singles and multiples was largest in cases of high BW, but PNsv was independent of birth type for lambs lighter than 4 kg at birth.

3.3.2 Variance components

According to the LRT, the addition of a direct additive genetic effect (σ_a^2) significantly ($P < 0.05$) improved the log-likelihood of all traits (model (i); Table 3.2). In the analysis of Tsv and PNsv , the maternal permanent environmental (σ_{mc}^2) and the maternal genetic (σ_m^2) components appeared to partially compete for the same variance. In preliminary analysis, fitting σ_m^2 improved ($P < 0.05$) log-likelihood (model (ii) vs model (i)), but the further addition of σ_{mc}^2 (model (iv)) deflated σ_m^2 as most maternal variance partitioned to σ_{mc}^2 . Between the model (ii) and model (iii), the best log-likelihood was found in model (iii) which considered only σ_{mc}^2 in addition to σ_a^2 . Similarly, model (iii) also provided the best fit for P3sv In BW, both σ_m^2 and σ_{mc}^2 were retained as components of variance in Model (iv). However, including a component accounting for the direct-maternal genetic covariance (σ_{am}^2 , model (vi)) did not deliver a better log-likelihood compared to the nested model (iv). For BCS, including σ_{mc}^2 proved influential as model (iii) had the best log-likelihood of all models tested. Since σ_{am}^2 was not influential or not applicable in any of the traits, the LRT results of models (v) and (vi) and the correlation (r_{am}) are not shown.

All traits were heritable, but the genetic components of survival traits were generally low to very low (Table 3.3). For Tsv, the direct genetic effect (h^2 ; 0.07) was slightly larger than the permanent maternal environmental component (mc^2 ; 0.04), but h^2 and mc^2 traded ranking across the partitioned periods of survival. In the PNsv , mc^2 was low (0.10), but considerably higher than the very low h^2 (0.03). But for P3sv , the h^2 was

comparatively large (0.07) while mc^2 was very low (0.02), indicating that the variability in dam's influence peaked early on in the life of the lamb. BW was moderately heritable at 0.16, but was more impacted upon by the maternal genetic effect (m^2 ; 0.2). The mc^2 component was also influential, but lower than the genetic effects (0.12). The h^2 of BCS was high (0.54) and the mc^2 ratio was significant, but very low (0.03).

Table 3.2

Log-likelihood ratios derived from models I to IV. Chosen model represented in 'bold' script. 'FE only' represents fixed effects only model

Model	Tsv	PNsv	P3sv	BW	BCS
FE Only	3104.47	5527.59	4220.92	-1008.400	-2040.85
Model (i)	3132.61	5554.80	4236.98	-598.463	-1600.45
Model (ii)	3136.82	5575.50	4237.71	-252.177	-1600.41
Model (iii)	3142.47	5590.62	4238.91	-280.108	-1593.98
Model (iv)	3142.47	5590.62	4238.91	-236.271	-1592.98
Model (v)	-	-	-	-	-
Model (vi)	-	-	-	-235.821	-

Table 3.3Variance components and ratios (\pm SE) of all traits. Empty cells indicate effects not included in final model

Variance Components	Tsv	PNsv	P3sv	BW	BCS
σ^2_a	0.012	0.003	0.007	0.076	0.416
σ^2_m				0.095	
σ^2_{mc}	0.007	0.009	0.002	0.058	0.024
σ^2_e	0.146	0.079	0.103	0.250	0.329
σ^2_p	0.165	0.090	0.112	0.478	0.769
Variance Ratios					
h^2	0.07 \pm 0.02	0.03 \pm 0.01	0.07 \pm 0.02	0.16 \pm 0.02	0.54 \pm 0.03
m^2	-	-	-	0.20 \pm 0.03	-
mc^2	0.04 \pm 0.01	0.10 \pm 0.01	0.02 \pm 0.01	0.12 \pm 0.02	0.03 \pm 0.01

3.3.3 Genetic, environmental and phenotypic correlations

The correlations derived from respective two-trait analyses with BW and BCS did not suggest strong linear relationships with survival (Table 3.4). The genetic correlation (r_g) of the survival traits with BW were all negative in sign, but not significant ($P > 0.05$). Despite the poor precision of the estimate, the magnitude of the negative genetic relationship between PNsv and BW ($r_g = -0.36$) could be a noteworthy suggestion favouring lambs genetically tending to lower BW. In contrast, all non-genetic relationships between BW and survival were positive, moderately in favour of lambs of higher BW. The strongest example was the relationship between Tsv and BW on the maternal permanent environmental level by a moderate magnitude ($r_{mc} = 0.36$), but the benefit of higher BW was lowly reflected on the phenotypic level ($r_p = 0.17$). A similar tendency of environmental and phenotypic relationships was observed between BW and PNsv that also marginally favoured higher BW lambs. For P3sv, the relationship with BW was either insignificant ($P > 0.05$) or low, suggesting that the benefit of a higher BW was almost negligible once the lamb has survived to three days of age.

The genetic correlations of BCS with PNsv and P3sv were opposing in sign, but only significant ($P < 0.05$) in the case of P3sv. Lambs with a genetic tendency for hairy (in contrast to woolly) coats were thus more inclined to survive the post 3-day period to weaning. Negative relationships between BCS and survival were reflected on the phenotypic level for Tsv and P3sv but were negligibly low in magnitude.

Table 3.4

Pairwise additive genetic, permanent maternal, environmental and phenotypic correlations of BW and BCS with all respective survival traits. Significant correlations indicated in bold

Trait	Additive	Maternal Env	Environmental	Phenotypic
Tsv <i>X</i>				
<i>BW</i>	-0.113 ± 0.153	0.363 ± 0.103	0.208 ± 0.018	0.167 ± 0.012
<i>BCS</i>	-0.125 ± 0.124	0.059 ± 0.204	-0.03 ± 0.027	-0.041 ± 0.016
PNsv <i>X</i>				
<i>BW</i>	-0.361 ± 0.230	0.245 ± 0.077	0.232 ± 0.017	0.197 ± 0.015
<i>BCS</i>	0.205 ± 0.174	0.13 ± 0.154	-0.048 ± 0.026	0.004 ± 0.015
P3sv <i>X</i>				
<i>BW</i>	-0.012 ± 0.150	0.36 ± 0.173	0.103 ± 0.019	0.088 ± 0.013
<i>BCS</i>	-0.297 ± 0.125	-0.107 ± 0.294	-0.002 ± 0.028	-0.059 ± 0.016

3.3.4 Genetic trends

Linear regressions of genetic trends were all significant ($P < 0.05$; Table 3.5), indicating all traits responded to selection to some extent. Respective coefficients were not always opposite in sign for H- and L-Line trends but were observed as divergent ($P < 0.01$) in all traits. However, a linear regression proved a poor fit of the genetic trends in the L-Line, with R^2 -values ranging between 0.38 and 0.51 for survival traits. The poor fit of linear regressions of L-Line trends is considered to be due to high levels of year to year variation with little consistency in a directional response to selection (Figs 3.3 and 3.5) with the possible exception of BCS (Fig. 3.6). Means and individual breeding values of the H-Line trended favourably and substantially higher than the L-Line group for all three survival traits (Figs 3.3 and 3.4). The genetic difference between selection groups was also clearly reflected in the raw breeding values which showed favourable ranking for the majority of H-Line lambs for the last 20 years of the experiment, whereas a much more mixed result was observed for the period preceding 1995 (Fig. 3.4). If coefficients of linear regressions are expressed as % of the overall mean per annum (p.a.), the H-Line favourably trended upwards at a rate of nearly 0.8% p.a. for Tsv (Table 3.5). Observed change in the H-Line for PNsv (0.28% p.a.) and P3sv (0.42% p.a.) were of a more moderate magnitude, but this can be expected given that less variation was observed in partitioned periods. Survival in the L-Line also trended upwards for Tsv and P3sv, but downwards for PNsv. Albeit statistically significant ($P < 0.01$), the magnitude of the regression coefficients in the L-Line were small (-0.04% to 0.11%) indicating only marginal net change in L-Line breeding values over the total selection period.

In later years of the selection experiment, the raw values appeared to have a threshold bounding breeding values at a value slightly larger than 0.2 for Tsv and 0.1 for PNsv (Fig. 3.4), and trends showed little further gain in mean breeding values (Fig. 3.3). This apparent selection plateau was evaluated for a possible changepoint (CP) in trend by fitting broken stick regression models as an alternative to single linear regressions in Table 3.5. Davies' test for a difference in slopes proved significant ($P < 0.01$) in H-Line trends for Tsv,

PNsv, P3sv and BW (Table 3.5). For survival traits, a CP in genetic trends suggested that the selection response of the H-Line reached a plateau following 19 – 22 years of selection (Table 3.5), with no further increase in mean breeding values after roughly 2005 (Fig. 3.3). During this period, the initial (A) coefficient suggested a high rate of genetic change equal to ~ 1% of the mean for Tsv. The subsequent (B) coefficient indicated a declining trend but was of a small magnitude with a large SE, indicating little to no genetic change from 2005 to 2019.

Response p.a. in both H- and L-Line breeding values for BW were of a low or negligible magnitude, but it is interesting to note that the regression coefficients of direct and maternal genetic trends were opposite in sign (Table 3.5). In the H-Line, BW divergently ($P < 0.01$) trended downwards in the direct genetic response, but upwards in the maternal genetic response while the opposite was observed in the L-Line. In trends, the H- and L-Lines traded rankings for higher mean breeding values for BW in numerous years with very weak evidence of a divergent response reported in Table 3.5 (Fig. 3.5). In the maternal genetic trends, the selection groups were more distinguishable compared to direct genetic trends and appeared divergent following the 1995 - 2000 period. As deduced from regression coefficients, the responses of direct and maternal trends were in opposite directions. According to the broken stick regression, the H-Line trend changed sign to lower BW following 12 years of selection (year 1998) (Table 3.5). Both initial (A) and subsequent (B) coefficients were, however, of a low magnitude, and both A and B coefficients had a poor fit ($R^2 < 0.45$) compared to those of survival traits. Also, in both the direct and maternal genetic trends, breeding values over the last ~ 5 years of the experiment trended in a relatively narrow margin to the zero-line used to define the originating population, resembling only marginal net change in BW associated with selection for NLW.

Responses observed for BCS were also low, but the slight upwards trend of 0.36% p.a. in the L-Line indicated that L-Line animals slowly tended to higher BCS values with time, describing a tendency toward woollier coats. According to trends, divergence between the lines became more pronounced following the year 2000, and the L-Line persistently had higher scores from 2005 onwards, but Davies' test for a difference in slopes did not indicate a change ($P > 0.01$) in coefficients.

Table 3.5

Linear and broken stick regression coefficients fit to genetic trends of H- and L-Line selection groups. The changepoint (CP) and initial (A) and subsequent (B) slopes reported only in cases where Davies' test significant ($P < 0.01$) for a change in slope

Trait	Group	Coefficient	Linear Regression				Divergence	Group	CP	Broken-Stick Regression		
			P-Value	R ²	% of Mean	Coefficient				R ²	% of Mean	
Tsv	H-Line	0.0061 ± 0.00026	< 0.01	0.94	0.79%	< 0.01	H-Line	20	A	0.008 ± 0.0003	0.99	1.06%
	L-Line	0.0008 ± 0.00015	< 0.01	0.48	0.11%		L-Line	-	B	-0.0008 ± 0.001	0.99	-0.10%
PNsv	H-Line	0.00025 ± 0.0001	< 0.01	0.95	0.28%	< 0.01	H-Line	22	A	0.003 ± 0.0004	0.99	0.35%
	L-Line	-0.0004 ± 0.00008	< 0.01	0.38	-0.04%		L-Line	-	B	-0.0004 ± 0.0005	0.99	-0.04%
P3sv	H-Line	0.0036 ± 0.00018	< 0.01	0.92	0.42%	< 0.01	H-Line	19	A	0.005 ± 0.0002	0.98	0.60%
	L-Line	0.0006 ± 0.00011	< 0.01	0.51	0.07%		L-Line	-	B	-0.0008 ± 0.0007	0.98	-0.09%
BW	H-Line	-0.0009 ± 0.00044	< 0.05	0.12	-0.02%	< 0.01	H-Line	12	A	0.0034 ± 0.0016	0.45	0.09%
	L-Line	0.0015 ± 0.00059	< 0.05	0.17	0.04%		L-Line	-	B	-0.0056 ± 0.0014	0.45	-0.15%
BW ^M	H-Line	0.0052 ± 0.00046	< 0.01	0.79	0.13%	< 0.01	H-Line	-	A	-	-	-
	L-Line	-0.0036 ± 0.00048	< 0.01	0.63	-0.09%		L-Line	-	B	-	-	-
BCS	H-Line	-0.0041 ± 0.00082	< 0.01	0.44	-0.13%	< 0.01	H-Line	-	A	-	-	-
	L-Line	0.0115 ± 0.00123	< 0.01	0.73	0.36%		L-Line	-	B	-	-	-

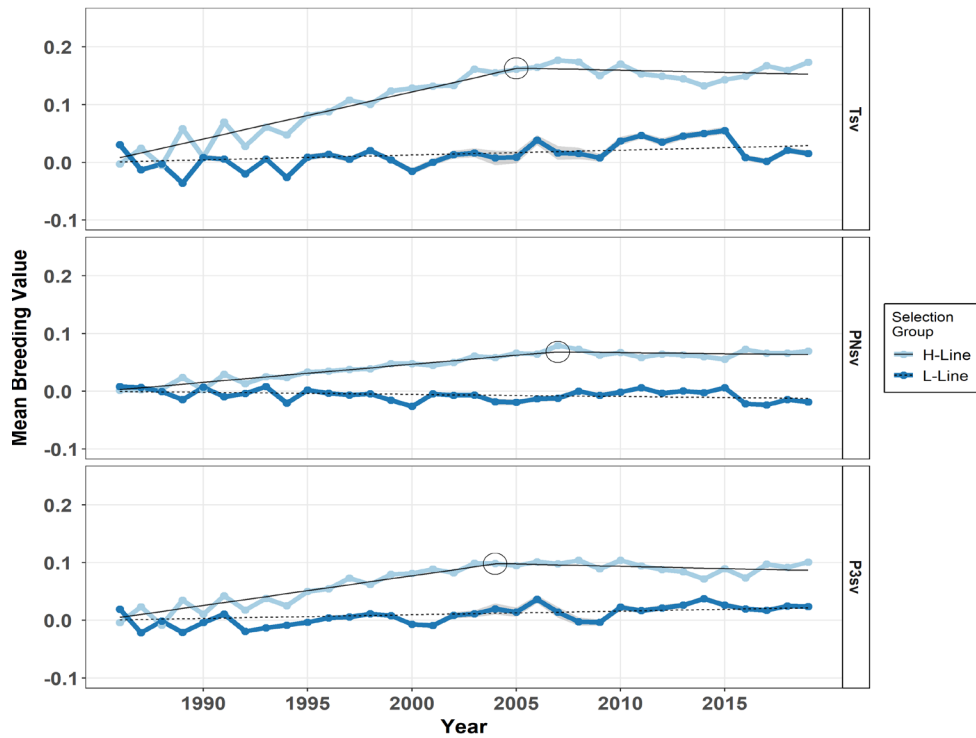


Fig. 3.3 Genetic trends and fitted regression lines for survival traits represented by averaged breeding values by selection group (H- and L-Line) and year. Shaded areas represent the standard error surrounding the mean. ‘o’ represents changepoint between slopes in cases where Davies’ P -value < 0.01 .

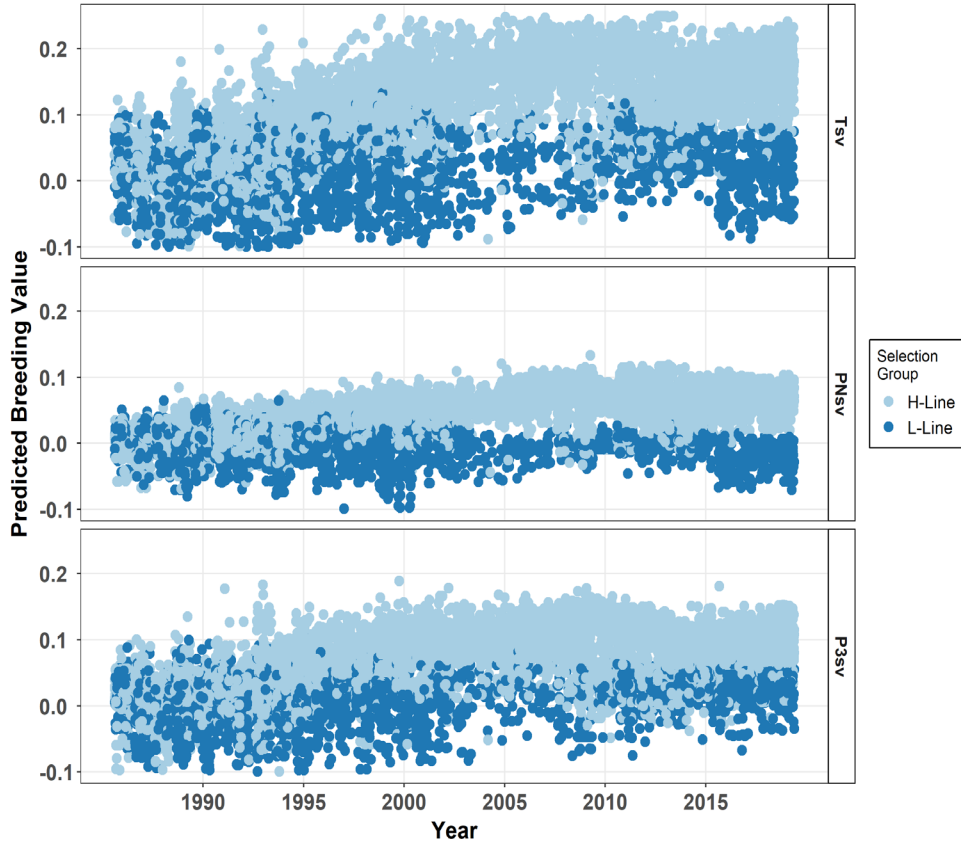


Fig. 3.4 Raw breeding values for survival traits of H- and L-Line animals from 1986 to 2019.

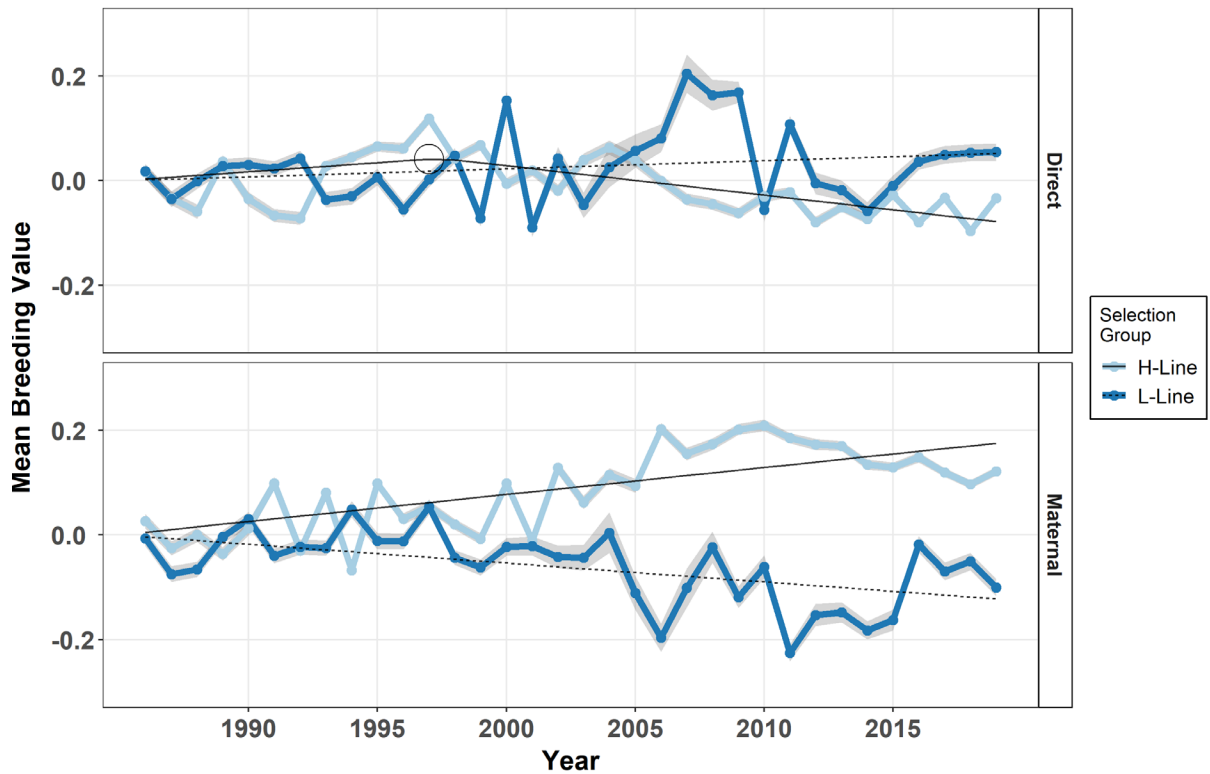


Fig. 3.5 Direct and maternal genetic trends and fitted regression lines for birth weight in High- and Low-Lines. Shaded areas represent standard errors surrounding the mean. 'o' represents changepoint between slopes in cases where Davies' P-value < 0.01.

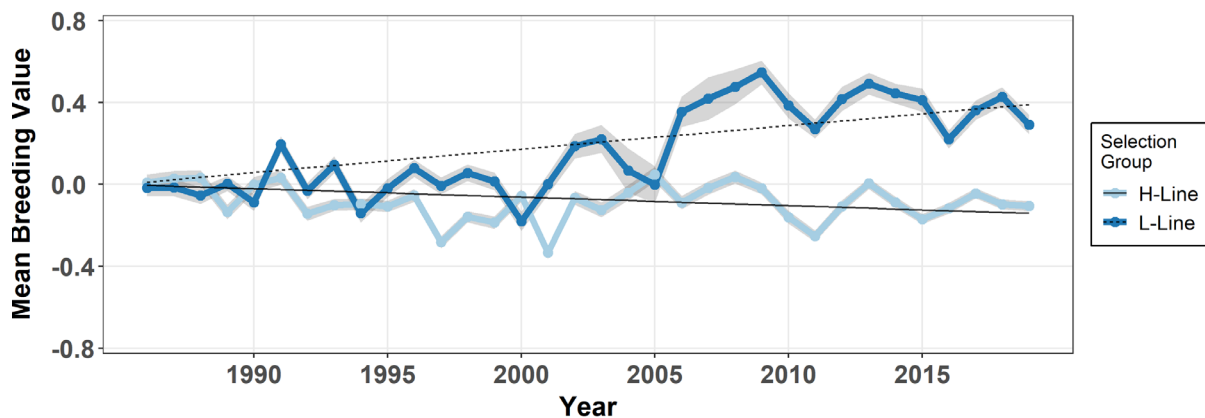


Fig. 3.6 Genetic trends and fitted regression lines for birth coat score in High- and Low- Lines. Shaded areas represent the standard error surrounding the mean.

3.4 Discussion

3.4.1 Descriptive statistics and fixed effects

Mean survival to weaning was consistent with what could be expected according to the literature (Hinch and Brien 2014). In Merinos, survival to 7 days has been reported at 85% and to weaning around 72% (Hatcher *et al.* 2010). Similarly Brien *et al.* (2010) reported an 85% survival rate to three days of age, and 80% survival to weaning from a predominantly Merino population. It is evident that the period around birth and the first days following birth are critical for lambs, as a proportionally high mortality rate coincided with this short period. Female, H-Line and single lambs were less likely to succumb than male, L-Line and multiple lambs (Table 3.1). Males accordingly suffered higher mortality rates in Scottish Blackface (Sawalha *et al.* 2007; Riggio *et al.* 2008), Texel, Shropshire and Oxford Down (Maxa *et al.* 2009), Texel and Texel-cross (Morris *et al.* 2000), Coopworth (Everett-Hincks *et al.* 2005) and Merino lambs (Brien *et al.* 2009), an outcome which accorded with the current findings. The marked difference in survival between the H- and L-Lines is supported by previous experiments that successfully manifested within breed differences by selection on fitness traits (Atkins 1980; Haughey 1983; Knight *et al.* 1988). The interaction between birth type and selection group (Fig. 3.1) supported the earlier findings of Cloete and Scholtz (1998), who suggested that advantage of H-Line lambs was predominantly derived from better survival of multiples. However, the advantage of the ‘pro-fertility’ Merino selection flock reported by Atkins (1980) was consistent across singles and multiples. Also, Romney ewes selected for survival and rearing ability also had a 8.5% higher survival rate compared to the control strain, but the effect of selection line did not interact with birth rank (Knight *et al.* 1988) as in the current study. Generally, an increased litter size is associated with decreased lamb survival, since the survival of twins are lower than that of singles (Slee *et al.* 1991; Kleemann and Walker 2005; Table 3.1) especially in unfavorable circumstances (Hinch *et al.* 1983). Multiples in Merinos maintained a survival rate of 72 - 73% compared to 85 - 89% for singles (Brien *et al.* 2009). The differences in birth type is not entirely explained by birth weight, since heavy twins have also been shown as susceptible (Morris *et al.* 2000). The current results implied that birth type affected survival both during the perinatal and subsequent phases of life (Table 3.1), but some studies have reported other findings. Scottish Blackface lambs born as multiples were more susceptible than singles only during early neonatal periods (up to around 14 days), although multiples had greater chances of surviving birth (Sawalha *et al.* 2007). Interestingly, Maxa *et al.* (2009) reported survival to 24-hours as greater in Texel lambs born as twins compared to triplets and singles, but Riggio *et al.* (2008) found triplets more susceptible than singles or twins across all periods considered. In this study, experienced ewes were found to be better mothers than primiparous ewes (Table 3.1). Lower offspring mortality rates in older dams are common in the literature (Owens *et al.* 1985; Morris *et al.* 2000; Southey *et al.* 2001; Everett-Hincks *et al.* 2005; Riggio *et al.* 2008). However, the best lamb-rearing performance is likely found in ewes of intermediate age since lambs borne by older ewes also have a lower survival rate (Sawalha *et al.* 2007; Brien *et al.* 2009; Maxa *et al.* 2009). Phenotypic results of Tsv^{BW} , $PNsv^{BW}$ and $P3sv^{BW}$ (Fig. 3.2) derived from including BW as a covariate to survival traits supported the consensus of a curvilinear relationship on the phenotypic level (Hall *et al.* 1995; Sawalha *et al.* 2007; Hatcher *et al.* 2009; Maxa *et al.* 2009; Everett-Hincks *et al.* 2014). Both heavy and light

lambs are prone to higher mortality rates, but the symptoms surrounding the cause of death tend to be different between very light or heavy lambs (Refshauge *et al.* 2016). Morris *et al.* (2000) reasoned that heavier lambs have a good chance of survival once they get through the birth process but could be more susceptible to complications close to birth. Current results suggested that both very light and very heavy lambs are more likely to succumb within the early perinatal phase, with birthweight less important to survival at later stages to weaning.

3.4.2 Variance ratios

3.4.2.1 Variance components of lamb survival

Lamb survival has often been associated with low to very low estimates of h^2 , as was also reported in Table 3.3. These results are suggestive of poor prospects of genetic improvement (Brien *et al.* 2014). This study found Tsv lowly heritable at 0.07 but with a h^2 of more than two-fold the mean h^2 estimate of 0.03 derived from earlier literature by Safari *et al.* (2005) as well as more recent results also derived by linear models for Merinos (0.03; Brien *et al.* 2009; Hatcher *et al.* 2010). The method used to derive genetic parameters could be influential in varying estimates across studies. Previous results from the same flock were derived from threshold analysis and yielded estimates that differed by a large margin to the current results (Cloete *et al.* 2009). From that study, a relatively high h^2 of 0.28 was reported for Tsv, with a significant maternal genetic effect of 0.07 as well as a negative direct-maternal genetic correlation of -0.61. Comparing these ratios to the current results suggests that differences in estimates and could be related to analysing survival data on the observed as compared to the underlying scale. Similarly, Matos *et al.* (2000) reported that the h^2 of survival from birth to weaning from a threshold model amounted to about three-fold the magnitude of the value derived by linear models in the same study. However, a moderate h^2 estimate of 0.11 for Tsv has also been derived from threshold models (Welsh *et al.* 2006). Comparatively high h^2 estimates of 0.18 to 0.33 was also reported for postnatal survivability derived from sire models (Sawalha *et al.* 2007).

In numerous instances, however, estimates of h^2 were consistently low across different methods. Using both logit transformed and linear analysis, Morris *et al.* (2000) and Lopez-Villalobos and Garrick (1999) reported h^2 estimates not different from zero or 0.01, respectively. All models, including a logit transformation, estimated h^2 values below 0.01 for survival to weaning for a large study of a range of New Zealand composite breeds (Everett-Hincks *et al.* 2014). For survival to twelve weeks, Riggio *et al.* (2008) reported relatively larger h^2 estimates of 0.05 ± 0.03 and 0.08 ± 0.06 from linear and probit transformed analyses, respectively. While the current h^2 estimate is also low, it coincides with the upper end of these estimates, and the highest thus far reported from linear analysis.

The present study excluded maternal genetic effect from the final model for all survival traits (Tables 3.2 and 3.3). Maternal effects are generally expected to have both genetic and environmental components (Bradford 1972). Similarly, Muller *et al.* (2020) found no evidence of a significant m^2 component in Tsv of Dorset lambs, but this is contradicting to a substantial body of evidence that found m^2 influential to survival, albeit generally low with a range between 0.02 to 0.08 (Lopez-Villalobos and Garrick 1999; Morris *et al.* 2000; Welsh *et al.* 2006; Brien *et al.* 2009; Hatcher *et al.* 2010; Everett-Hincks *et al.* 2014; Vanderick *et al.* 2015)

and a mean of 0.05 ± 0.01 (Safari *et al.* 2005). Also, m^2 is equivalent to rearing ability when survival is evaluated as a trait of the ewe, and a number of studies have also reported the genetic component of ewe rearing ability as low, but significant with h^2 between 0.01 and 0.08 (Safari *et al.* 2005; Afolayan *et al.* 2008; Hebart *et al.* 2010; Bunter *et al.* 2016) which provides further evidence to support maternal genetic effects. The exclusion of m^2 from the current operational model is therefore unexpected and contradicting to the majority of the literature. It is likely that the model choice is an artefact of conflicted partitioning of variance between the m^2 and mc^2 components. In preliminary analysis, the m^2 derived from model (ii) were significant and consistent with the literature cited above, but the maternal genetic variance was deflated to negligibly low values when both mc^2 and m^2 were included in model (iv). The weak support for model (iv) was guided by the LRT but was also preferred by criteria based on the Bayesian Information Criterion (data not shown). Management of the Elsenburg flock maintains breeding ewes for at least 4 to 5 seasons which resulted in an average > 4.3 survival records per dam. This can be considered as a strong structure for partitioning maternal effects in sheep across dam generations, which further makes the contradicting result unexpected. Given the discussion above the obtained results should be accepted until it is possibly refuted in future studies involving more data. The Elsenburg flock currently lacks estimates of ewe rearing ability, but this could deliver an opportunity to revisit the topic in the near future.

Treating early survival as a partitioned period revealed different variance ratios for survival pre- and post-the perinatal (3-days of age) phase at respectively 0.03 and 0.07 (Table 3.3). Genetic variation in lamb survival between birth and weaning is influenced by the age of the lamb (Southey *et al.* 2001; Sawalha *et al.* 2007; Riggio *et al.* 2008). Current findings suggested that success of the new-born lamb was predominantly dependent on the environment with a small direct genetic component. Following the initial 3 days after birth, the influence of the direct genetic effect of the lamb became proportionally more important to survival as h^2 increased in magnitude. Similarly, Hatcher *et al.* (2010) reported a direct h^2 of only 0.02 for survival to 7 days of age, but a larger value of 0.05 for the period 7 to 100 days. In turn, Brien *et al.* (2009) reported declining h^2 estimates associated with increasing lamb age, being highest at birth (0.07). For survival at 24 hours, Riggio *et al.* (2008) reported a comparatively large h^2 estimate of 0.33 ± 0.11 from probit transformed data and an estimate of 0.09 ± 0.03 from a linear analysis. Generally, however, a range between 0.01 to 0.07 (Morris *et al.* 2000; Maxa *et al.* 2009; Everett-Hincks *et al.* 2014) suggests that the h^2 of perinatal survival was low, but m^2 similar or larger than direct genetic effects with a range between 0.02 to 0.10 (Morris *et al.* 2000; Maxa *et al.* 2009; Everett-Hincks *et al.* 2014). However, a recent study compared neonatal mortality and rectal temperature of the H- and L-Line lambs across a cold stress gradient, and reported the differences between lines to be dependent on the severity of cold stress during the neonatal period (Chapter 4: Nel *et al.* 2021). These results were strongly suggestive that a significant genotype by environment interaction could be important to neonatal mortality/survival, which would affect estimates of h^2 by the animal model which assumes genotype are evaluated under equal opportunity.

An influential mc^2 component is well supported in the literature, but it should be considered that the current estimates of mc^2 would likely also reflect the influence of maternal genetic effects, as detailed in the discussion above. The study by Muller *et al.* (2020) similarly reported a mc^2 of 0.03, but also included litter

variance ratio of 0.12 for Tsv in Dormer lambs. Other studies were able to separate maternal genetic effects. For survival to weaning, Barwick *et al.* (1990) and Lopez-Villalobos and Garrick (1999) reported a mc^2 estimate of 0.09, larger than the observed h^2 and m^2 effects combined (0.04; both studies). When considering survival to an early timepoint, Morris *et al.* (2000) also reported a mc^2 component of 0.10 from a linear model for survival up to 24 hours. Comparing the magnitude of estimates between PNsv and P3sv suggested that the effect of mc^2 declined with age of the lamb (Table 3.3). Similarly, Hatcher *et al.* (2010) reported ‘dam repeatability’ as 0.12 between 1 to 7 days, but reported about half this magnitude (0.06) during the period between 7 to 100 days. Observing a relatively high maternal influence in the perinatal period is not surprising given the high dependence of lambs in the neonatal period (Dwyer 2008a), but would further be expected when perinatal survival is defined to include mortalities at birth, which is currently the case.

Since maternal genetic effects were not fitted, the current results also excluded the direct-maternal genetic correlation (r_{am}) as a component of variance affecting lamb survival. This is in contradiction to the negative and large r_{am} estimate of -0.60 previously reported by Cloete *et al.* (2009), an estimate that was similar to the r_{am} of -0.75 also derived from threshold analysis by Welsh *et al.* (2006). The issue surrounding r_{am} is pertinent if found to have a biological significance, since it implies that ewes with a high genetic tendency for survival would tend to be poor mothers. Also, negative r_{am} components inflates estimates of h^2 , m^2 or mc^2 (Fozzi *et al.* 2005) causing discrepancies across studies. Hatcher *et al.* (2010) included σ_{am} but found r_{am} not different from zero. In another study (Maxa *et al.* 2009), estimates varied widely across different breeds (-0.79 Shropshire; -0.52 Texel and -0.05 for Oxford Down) in analyses based on the logit-link function. Other logit transformed models estimated positive r_{am} at values above the theoretically feasible threshold of 1, while results derived from linear analysis in the same study found r_{am} to be negative, but of a more feasible margin (Morris *et al.* 2000). Using linear models, Vanderick *et al.* (2015) reported a low to moderately negative range of r_{am} values between -0.14 to -0.44, while results from logit transformed phenotypes were positive, but very low at 0.02 to 0.03. Other negative estimates varied from -0.23 (Lopez-Villalobos and Garrick 1999) to -0.74 (Everett-Hincks *et al.* 2005) to exceeding -1 (Burfenig 1993) while r_{am} was also reported to be positive between 0.01 and 0.62 (Barwick *et al.* 1990; Matos *et al.* 2000; Sawalha *et al.* 2007). It is evident from the literature cited that it is difficult to pinpoint a reasonable expected range for r_{am} , since the reported range of estimates shows little to no consistency across studies.

3.4.2.2 Birth weight

The h^2 of BW was moderate, and m^2 was the largest component affecting the trait (Table 3.3). The values of respectively 0.16 and 0.20 were quite similar to previous estimates derived from the Elsenburg flock using less data (Cloete *et al.* 2003, 2009). The current estimate of h^2 (0.16) agrees well with values of 0.15 (Riggio *et al.* 2008) and 0.18 (Safari *et al.* 2007), but a slightly higher range between 0.20 and 0.24 has also been reported (Snyman *et al.* 1995; Al-Shorepy and Notter 1998; Safari *et al.* 2005; Hatcher *et al.* 2010). The moderate m^2 estimate of 0.20 coincides well with previous estimates of 0.18 – 0.19 (Safari *et al.* 2007; Riggio *et al.* 2008) or the mean of 0.21 ± 0.03 across studies (Safari *et al.* 2005). However, some previous estimates for m^2 are considerably lower at 0.06 – 0.09 (Snyman *et al.* 1995; Hatcher *et al.* 2010), while a uniquely high value of

0.38 has also been reported by Al-Shorepy and Notter (1998). The observed mc^2 agreed well with a previous estimate of 0.12 (Snyman *et al.* 1995). Safari *et al.* (2007) reported a small mc^2 at 0.07, but the study also included a litter effect of 0.33 that was not considered in the current analysis. Permanent environmental effects of a similar magnitude (0.10 - 0.16) have also been reported in the literature (Safari *et al.* 2005; Riggio *et al.* 2008).

The additional r_{am} component accounting for direct-maternal genetic covariance did not provide a better log-likelihood to the nested model, suggesting no antagonism between high BW ewes and the weight of their new-born lambs. Similarly, Snyman *et al.* (1995) did not find this effect influential, and Hatcher *et al.* (2010) reported that r_{am} did not differ from zero. Other studies found the effect significant and negative at between -0.15 to -0.31 (Safari *et al.* 2005, 2007; Cloete *et al.* 2009). However, if there is a true antagonism underlying these observations, it would not be considered problematic since both high and low BW is not desirable on the phenotypic level (Fig. 3.2). If an ewe is genetically prone to a very high (or low) BW, it could be favourable that the maternal influence on the lamb does not lead to an extreme BW in the same direction or of the same magnitude.

3.4.2.3 Birth coat score

Very few estimates of h^2 of BCS derived from animal models were found in the literature. The current estimate of 0.54 is within the range between very high estimates of 0.65 (Kemper *et al.* 2003) and 0.70 (Cloete *et al.* 2003; from the same flock) and a considerably lower estimate of 0.31 (Brien *et al.* 2010). Despite a considerable range between these estimates, it can be assumed that fast genetic change is possible for BCS. The effect of mc^2 , however, was very small at 0.03. Other than a similar estimate of mc^2 reported by Cloete *et al.* (2003) on the same flock but using fewer records, no other estimates were found in the literature.

3.4.2.4 Correlations

Despite the convenience of two-trait analysis with both traits on the observed scale, derived genetic correlation coefficients were often compromised by a low accuracy as reflected by large standard errors. Birth weight and mortality have long been considered to be linked (Smith 1977), but the curvilinear relationship in Fig. 3.3 complicates the use of BW as a correlated trait to genetically improve lamb survival. In correspondence with results in Table 3.4, previous results in Merinos also reported that the genetic relationship between BW and Tsv was not different from zero (Cloete *et al.* 2009; Brien *et al.* 2010; Hatcher *et al.* 2010), but some studies have found positive genetic correlations of BW with perinatal survival (0.24) and survival to 4 weeks (0.45) (Riggio *et al.* 2008). The current r_g for BW and PNsv, albeit non-significant, was unexpected in its moderate magnitude and the fact that it was opposite in direction to the relationships on all non-genetic levels. This contrast was not consistent with other results since the opposite was reported for Scottish Blackface lambs where neonatal mortality and BW were positively correlated at 0.21 but with a negative phenotypic relationship of -0.25 (Sawalha *et al.* 2007). Generally, the low-positive relationship of BW with survival on the maternal permanent environmental, environmental and phenotypic levels could be due to the very high susceptibility of extremely small lambs (Fig. 3.2) that skews the intermediate optimum slightly higher than the mean of 3.85.

The use of BCS as a correlated trait to improve lamb survival is also limited. A hairy birth coat has been lowly correlated to lamb survival in Merinos (Brien *et al.* 2010), but the current findings only found a significant genetic relationship with BCS for P3sv (Table 3.4). This moderate negative genetic correlation was in favour of hairier lambs. Earlier studies from climate chambers (Alexander 1961) or water bath tests (Slee 1978; Slee *et al.* 1991) suggested that longer or hairier coats could alleviate the rate of heat loss in lambs. This, in turn, was associated with the perinatal period and a reduced mortality stemming from exposure (Geenty *et al.* 2014). In this regard, the genetic correlation with P3sv and not PNsv was unexpected, since thermoregulation is considered most critical in the neonatal period (Plush *et al.* 2016). However, the precision of the genetic correlation in the perinatal period was poor and the 95% confidence interval included 0. It is possible that the benefits of a hairier coat (or low BCS) is restricted to lambs born in severe environmental circumstances (Mullaney 1966), causing difficulty in deriving robust estimates of the genetic (co)variance since the bulk of lambs were born in relatively benign conditions (see Chapter(s) 4 and 5).

3.4.2.5 Genetic trends

The genetic trends reported in this study (Table 3.5) contradicted the premises that the h^2 of lamb survival was too low to support genetic gains in the desired direction (Fig. 3.3). It provides an opposing view to the suggestion that genetic improvement of lamb survival is unlikely to be worthwhile (Everett-Hincks *et al.* 2005; Everett-Hincks and Cullen 2009). Substantial genetic gains were evident in the genetic trends of the H-Line, and selection group differences were also clearly reflected on the phenotypic level (Table 3.1). Assuming an h^2 of 0.03, genetic responses in lamb survival was predicted to only amount to about 14% (Brien *et al.* 2009) and 26% (Fogarty *et al.* 2006) of the rate possible for genetic gain in clean fleece weight (assumed to have a h^2 of 0.42 in both studies). As a point of reference in South African Merinos, a resource flock subject to positive selection for CFW reported gains of 1.2% p.a. (Cloete *et al.* 2007). The current gain in lamb survival of nearly 0.80% per annum (p.a) is about 66% of the rate achieved for CFW, which far exceeds these previous predictions, especially since gain in Tsv hinged on a correlated response to selection for NLW. However, a more conservative rate of gain of 0.68% p.a. could be considered a more appropriate selection response by adjusting for the slight upwards gain observed in the L-Line. However, the genetic trend for Tsv appeared to show that most of the genetic gain was made in the initial years of selection. The initial (A) coefficient derived from broken stick regression suggested genetic change as much as 1% of the mean between 1986 and 2006 (Table 3.5). Accordingly, the H- and L-lines were phenotypically separable by survival performance within 10 years after the onset of the selection experiment (Cloete and Scholtz 1998). The selection plateau indicated by the CP of the H-Line genetic trend could in part be due to the influence of external genetics introduced to the H-Line around the year 2008. Animals born as progeny (50% pure) or subsequent grand-progeny (25% pure) of external sires are likely to have partially counteracted the divergent genetics manifested between the pure H- and L-Lines. However, observed breeding values were likely approaching the boundaries of what is achievable under the current selection regime. The upper threshold of ~ 0.2 observed in breeding values (Fig. 3.4) could be expected since mean Tsv was 0.77 and survival is bounded at below unity (Alexander, 1984). In a scenario where the best ranked animals reach this level, further selection for lamb survival would likely be

met with diminishing gains, especially since positive results from first cross animals suggested influential non-additive genetic components to lamb survival (Fogarty 1972; McGuirk *et al.* 1978).

In turn, the L-Line technically also responded against the direction of selection for Tsv, but a lower-level plateau is also expected since selection for lower levels of fitness directly opposes the influence of natural selection. Also, while the dams of dead lambs could remain in the flock to produce siblings that survive, selection intensity for reducing NLW is limited by the need to perpetuate the numbers of the L-Line. For the partitioned periods of survival, diverging trends were most pronounced in P3sv (Fig. 3.3). The perinatal period was partially masked by the environment provided by the ewe, but a higher h^2 was observed for P3sv which explains direct genetic gains of higher magnitude in the H-Line for this trait compared to PNsv. However, the amount of variation observed for PNsv was high considering it only included mortalities up to three days of age (including stillbirths), and the gains during this period were also favourable and worth-while despite the low h^2 .

The low levels of consistency in genetic trends for BW and BCS (Fig. 3.5 and 3.6) suggested weaker underlying genetic relationships to the NLW phenotype compared to the survival traits. The downwards direct genetic trend of the H-Line for BW was of a very low magnitude in both linear and broken stick regression lines (Tables 3.5 and 3.6; Fig. 3.5) and is better considered as a stabilising response that trended within the optimal range for BW. In turn, it was interesting to see conflicting directions in maternal genetic trends as H-Line dams tended to support slightly heavier birth weights. These opposing responses are suggestive that a negative direct-maternal covariance could exist for BW, but its magnitude was below the threshold for denoting significance in the present analysis. Also, neither direct nor the maternal genetic change p.a. were of a large magnitude, and both H- and L- line trends appeared generally bounded to a moderate margin surrounding the zero-line descriptive of the original founding population. Given the intermediate optimum of BW and the strong phenotypic relationship to survival, it is likely that natural selection also provided a stabilizing effect on genetic trends for BW in the L-Line. This argument is supported by the fact that the phenotypic relationships of lamb survival with BW were robust across selection lines (Fig. 3.2).

Regarding BCS, the persistently higher trend of the L-Line (since 2005) suggested that a genetic tendency toward woollier coats were associated with the selection response of the L-Line to negative selection for NLW (Fig. 3.6) and BCS differences between selection groups were also reflected on the phenotypic level (Table 3.1). However, a genetic relationship of woollier coats with lower survival was only observed in P3sv (Table 3.4). Most correlation coefficients involving BCS were estimated with poor precision, and further investigation is needed to elucidate a possible effect of BCS on survival.

3.4.2.6 Lamb survival in selection indices

Snowder and Fogarty (2009) promoted the use of composite trait selection to increase overall ewe productivity, reasoning that the composite traits could act as an appropriate biological index to balance relationships between underlying component traits. This study has confirmed the general findings of Cloete *et al.* (2009) that favourable and worthwhile responses in a correlated component trait such as lamb survival are possible by selection for NLW. This reasoning is supported by positive genetic correlations between lamb survival and

NLW in Merinos (Swan *et al.* 2001; Afolayan *et al.* 2008; Hebart *et al.* 2010). However, determining an appropriate selection criterion requires multiple factors to be considered. Firstly, composite trait selection is also associated with risks. Selection for NLW also depends on the number of lambs born and would favour ewes bearing multiples. An increase in multiples is important given substantial and negative genetic and phenotypic correlations between survival and litter size (Swan *et al.* 2001; Hebart *et al.* 2010; Bunter *et al.* 2018). Also, litter size, as a trait of the ewe, has a higher h^2 than lamb survival (Afolayan *et al.* 2008; Hebart *et al.* 2010), which could upset the balance assumed by composite trait selection (Snowder and Fogarty 2009). Combined with the higher fertility rates of the H-Line (Cloete *et al.* 2004), this study has shown that survival can be improved along with an increased lambing rate. Phenotypic predictions (Fig. 3.1) suggested most of the gains in the H-Line were made by better survival rates of multiples. However, the greater likelihood of multiples to succumb, including in the H-Line, remained evident. Accordingly, the simulation study by Amer *et al.* (1999) showed diminishing economic returns from genetic gain in reproductive rate if lamb survival is not simultaneously improved. Furthermore, there is evidence that survival as multiples or singles are different genetic traits (Bunter *et al.* 2018) and there could be benefit in targeting survival of multiples specifically. Also, while the response observed in the Elsenburg Merino flock was favourable, NLW would not be treated with the same emphasis in traditional indices, and the underlying response in component traits is unlikely to be of the same magnitude. In fact, Brien *et al.* (2010) predicted a genetic decline in survival if represented only as a component of NLW in a selection index. An alternative to composite trait selection could also be to focus on ewe rearing ability, a trait that is currently being incorporated into Australian Sheep Breeding Values (Bunter *et al.* 2019, 2020). However, greater clarity on maternal genetic components is needed, given the unexpected lack of a significant m^2 for lamb survival the current study (Table 3.3).

For these reasons, it is recommended that lamb survival phenotypes should be recorded and incorporated into indices individually. Direct selection will require large, comprehensive datasets (Brien *et al.* 2010, 2014) which requires the inclusion of dead lambs into pedigrees, which is currently not routine in South African Merinos. However, adequate record keeping of survival is feasible and delivers an opportunity to optimise both breeding and welfare objectives. For example, mortality can be reduced by assistance at birth, but identification of such incidences can contribute (or detract from) the breeding value of relevant animals. Conversely, animals lost to misadventure or predation can be recorded as alive to avoid random, non-genetic events from interfering with breeding values related to fitness. A good example can be seen in the phenotyping protocol outlined by Vanderick *et al.* (2015), a system that should not be excessively difficult or expensive to accommodate since most breeding flocks require early identification and tagging of lambs as part of general management and pedigree recording.

3.5 Conclusions

Lamb survival in the H-Line was markedly higher than in the L-Line for the last two decades. This result confirms previous studies on the same resource flock that selection for NLW benefited lamb survival as a correlated response. This study also confirmed that the direct heritability of survival was low or very low but maintained that worth-while gain in survival traits is achievable by genetic selection. Although survival in the

H-Line plateaued from around 2005, the selection line's favourable status could be maintained despite the introduction of external genetics. The potential benefit of using either BW or BCS as indirect selection criteria was limited, but the significant genetic correlation of BCS with P3sv should be studied further. Wherever possible, survival traits should be recorded and evaluated individually in national ovine evaluation schemes. Although the infrastructure to record Tsv is present in South Africa, it has so far been poorly supported by industry.

3.6 References

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CHAPTER 4:

Long term genetic selection for reproductive success affects neonatal lamb vitality across cold stress conditions[#]

Abstract

Adverse weather conditions are important contributors to mortality in new-born lambs. Previous studies have shown variation between lambs in their ability to cope with circumstances of cold stress, and genetic selection could be a viable option for improving animal robustness. The Elsenburg Merino flock was divergently selected on number of lambs weaned (NLW). This resulted in divergent responses in reproduction and lamb survival. This study evaluated lamb vitality and mortality of positively selected H-Line relative to the negatively selected L-Line in response to cold stress. Traits included lamb rectal temperature (RT), surface temperature (ST), shiver score (SS), lamb vigor score (LVS), breaths per minute (BPM), mortality to three days of age (M3) and to mortality to weaning (TM). Cold stress was described by a chill index derived from daily rainfall, wind speed and ambient temperature, and represented as the mean of the one (CI), two (CI-2) or three (CI-3) days since parturition. Overall, H-Line lambs had a higher neonatal RT and were less likely to succumb than L-Line contemporaries. In a significant ($P < 0.05$) interaction, the predicted RT of L-Line showed a non-linear decline with increased levels of CI-2, while H-Line lambs better maintained their core temperature. M3 was also affected by a significant interaction between CI-3 and selection line, further suggesting that observed lower mortality rates in the H-Line depends on H-Line lambs' improved ability to cope with stressful environments. Long term selection for NLW in the H-Line led to improvements in both adaptations associated with lower lamb losses. The continued recording of viability traits to produce larger datasets amenable to genetic analysis is recommended, specifically for rectal temperature.

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4.1 Introduction

Lambs are often born in challenging circumstances, and adverse weather conditions compromise lamb survival (Geenty *et al.* 2014). Exposure to cold, wet and windy conditions interact to slow lamb progress resulting in lamb deaths with symptoms typical of the starvation-mismothering-exposure complex (Cloete and Scholtz 1998). Stressful conditions during the neonatal phase contribute to an already challenging period for lambs, which must survive birth and readily adapt to the extra-uterine environment.

A critical factor during the neonatal period is the ability of lambs to self-sustain and thermoregulate to maintain core temperature (Plush *et al.* 2016). This period is particularly stressful in sheep, since lambs born under pastoral conditions often have limited options to seek shelter, leaving them exposed to environmental elements. The neonatal period is characterized by a sharp fall in temperature for the lamb. From being equivalent to that of the dam at roughly 39 °C, core temperature may fall to a range below 35 °C within one hour of birth (Alexander and McCance 1958). Lambs have adapted counteracting mechanisms to increase body temperature by maintaining a high metabolic turnover right after birth (Symonds *et al.* 1995). Ambient conditions determine the rate of heat lost to the environment, and thus influence the requirement for heat production (Alexander 1961).

The ability to produce heat is facilitated by cold-induced non-shivering thermogenesis (NST), and diet induced thermogenesis. The former mechanism follows from the mobilization of brown adipose tissue (BAT) that specifically enables heat production (Cannon and Nedergaard 1985). The metabolism of BAT by the stress hormones epinephrine, nor-epinephrine and glucocorticoids is thought to be a key mechanism of thermogenesis in young lambs (Plush *et al.* 2016) and is essential since newborn lambs are not always immediately able to suckle to allow diet induced thermogenesis.

Lambs' capacity for NST depends on synthesis of cortisol toward the end of pregnancy (Mellor 1988; Fowden *et al.* 1998). Previous studies on the Elsenburg selection lines that were divergently selected for number of lambs weaned per joining (NLW) indicated that the hypothalamic-pituitary-adrenal (HPA) axis of animals from the upwards selected H-Line was more active than in the L-Line that was selected in a downwards direction. This resulted in an enhanced reaction to insulin induced hypoglycemic stress by producing higher levels of cortisol to effectively counter this imbalance (Hough *et al.* 2010; Van Der Walt *et al.* 2009). In a subsequent review, Hough *et al.* (2013) suggested that useful genetic gains are possible in increased sensitivity in the function of the HPA axis, resulting in an increased 'robustness' by selecting animals more capable to respond to stressful situations. Variation in the ability to resist cold stress have also been reported across breeds (Dwyer and Morgan 2006), selection lines (Slee and Stott 1986), and individuals (Slee *et al.* 1991), indicating that there are putative genetic components to this response.

To ensure that lactating ewes utilize the peak fodder production of the winter rainfall period, lambing in certain regions often commences during the period from late autumn to midwinter when there is also a higher probability of adverse climatic conditions (Cloete *et al.* 1994). Concerningly, the Merino breed was pointed out as an example of poor performance when resistance to cold stress was tested (Sykes *et al.* 1976; Slee 1978). The need to improve adaptability within the breed is concerning when considering that Merinos and other Merino types constitute the bulk of the South African ovine genetic resource (Cloete *et al.* 2014). The capacity

of lambs to resist hypothermia in field conditions hinges on an interaction of factors determined by both the adaptive capacity of lambs and the level of mothering provided by their dams. A favorable outcome for lambs thus also depends on a successful parturition followed by adequate stimulation and mothering and the necessary supply of colostrum (see reviews: Dwyer 2008b; Brien *et al.* 2014; Hinch and Brien 2014) that will be critical for initiating diet induced thermogenesis.

Since the onset of the selection experiment, lamb mortality was improved in the H-Line relative to the L-Line (Cloete and Scholtz 1998; Cloete *et al.* 2009) contributing to a marked divergent response in NLW for H-Line ewes (Cloete *et al.* 2004). Combined with the mechanisms reviewed by Hough *et al.* (2013), it can be hypothesized that long-term selection for NLW enhanced the ability of H-Line lambs to respond to sub-optimal environments, including cold stress conditions.

Against this background, available weather data were added to biological vitality and survival data to elucidate the physiological performance of neonatal lambs under field conditions. The objective was to apply a stress variable defined by heat loss during the neonatal period and to relate it to selected viability and mortality traits in H- and L-line lambs.

4.2 Materials and Methods

4.2.1 The Elsenburg Merino Flock

The Elsenburg Merino flock is a resource flock managed as an experiment for divergent selection based on NLW since 1986. The flock consists of the High (H-Line; positive selection) and Low (L-Line; negative selection) lines that originated from the same base population. Details pertaining to management and nutrition are available in the literature (Cloete *et al.* 2004, 2009), and only a short summary will be repeated here. At the start of the experiment, ewes were allocated at random within age groups to each of the two lines. Subsequent selection proceeded by screening ewes according to their ranking for NLW. Besides instances of death or severe health related incidences, replacement ewes mostly remained in the flock for at least 5 joinings. At the beginning of the experiment, roughly 120 ewes were assigned to each selection line. The H-line has since grown to 130-200 ewes, while numbers in the L-Line dwindled to 40-80 breeding ewes. Replacement rams were mostly the progeny of dams with at least 3 joinings. Following the results reported by Cloete *et al.* (2004), selection from 2003 was also guided by the use of best linear unbiased prediction (BLUP) breeding values derived from a single-trait repeatability model fitted to NLW records of ewes. In this period, the original ranking values were still considered in tandem to BLUP derived breeding values, but BLUP values have been the only selection criterion since 2010.

The flock was maintained under Mediterranean conditions at the Elsenburg Research Farm near the town of Stellenbosch (33°51'S 18°50'E) in the Western Cape Province, South Africa. Besides during joining in single-sire groups within lines, the two lines were maintained as a single flock for the entire production year. The flock grazed on irrigated kikuyu grass (*Pennisetium clandestinum*) during mating in summer (January – February) and during the lambing period in winter (June - July). In the remaining periods, the flock grazed on dryland as well as irrigated lucerne (*Medicago sativa*) and dryland medics (*M. truncatula*). Oat (*Avena sativa*) fodder crops were also available during winter to supplement the slow-growing legume pastures. Ewes were

shorn within 3 to 4 weeks prior to lambing. Ewes lambed in 10 - 20 paddocks of roughly similar size and including the 10 paddocks of 0.33 to 0.45 ha previously used for lambing by Cloete *et al.* (2002). After lamb-marking ewes and their lambs were randomly allocated into groups of 20 to 40 and grazed on irrigated lucerne. As the irrigated paddocks became depleted, these groups were joined in larger groups on dryland lucerne, medic and oats paddocks.

4.2.2 Data Collection

Data were recorded during the 2017 and 2018 lambing seasons, following 31 - 32 years of divergent selection. Daily handling of new-born lambs is part of annual management of the flock that maintains a pedigree, and the recording of all traits took place during daily lambing rounds from 8:00 am that identified all lambs born within the previous 24-hour period with their dams, thus enabling linkage back to the selection line and sire (Cloete *et al.* 2004, 2009). The traits recorded on lambs included rectal temperature (RT), surface temperature (ST), shiver score (SS), lamb vigor score (LVS), breathing rate per minute (BPM), birth weight (BW), and birth coat score (BCS). An adapted maternal behavior score (MBS) was recorded on ewes during the tagging process (Brien *et al.* 2014; Everett-Hincks *et al.* 2014). A standard medical thermometer was used to determine rectal temperature, while a handheld infrared thermometer (92000-008-ca; AllaFrance®, Chemillié-en-Anjou - France) were used for determining surface temperature in the left axilla of the lamb. Care was taken to measure surface temperature in the shade on sunny days, to ensure that direct solar radiation did not have an effect. SS, LVS and BPM were recorded while the rectal temperature was allowed to calibrate. BPM was represented by counting the number of side movements during a 30 second period and multiplied by 2 to express it per minute. SS, LVS and MBS were scored according to the description provided as Table S1 in the Supplementary Information Chapter. The original 5-point scale used for LVS was subset to a 4-point scale to exclude dead lambs, which were scored as 1. BW and BCS were recorded as described by Cloete *et al.* (2003). Mortalities were identified and linked to date of birth to record the age of lambs into two categories, namely: Lambs having died within 3 days of age (M3) (but alive at birth) and lambs succumbing within any period prior to weaning, termed as total mortality (TM).

4.2.3 Weather Data and Chill Index

The Elsenburg weather station (33°51'S 18°50'E, alt: 171 m) recorded daily data including rainfall, wind speed and ambient temperature. The weather station was in close proximity to the experimental site with a maximum radius of approximately 1000 m to the furthest boundary of the lambing paddocks. The weather station data was regarded as a good indication of the climatic conditions across all paddocks during lambing.

The weather data allowed for the quantification of an environmental stress parameter defined as the chill-index (CI; Nixon-Smith 1972; Donnelly 1984). This index estimated heat loss based on a combination of wind speed, rainfall, and temperature as:

$$CI = [11.7 + 3.1V^{0.5}][40 - T] + 481 + 418(1 - e^{-0.04R})$$

where CI is the estimated heat loss ($\text{KJ m}^{-2} \text{h}^{-1}$), V is the mean daily wind velocity (ms^{-1}), T is the mean daily temperature ($^{\circ}\text{C}$) and R is the total daily rainfall (mm). This was visualized by a 3D surface plot using the *plotly* package in R (Sievert 2020).

The index derived from the weather data was linked to the date of tagging as follows: 1) the chill index for the day prior to the day lambs was tagged and identified with their dams. As lambs were tagged from 8 o'clock in the morning, the previous day was presumed as the date of birth (CI-1, value for one day); 2) the mean of the day before tagging and the day of tagging (CI-2; average over 2 days); 3) the mean of the day before, during, and after tagging (CI-3; average over 3 days).

4.2.4 Data Analysis

Factors considered as fixed effects were selection line (H- or L-Line), year of birth (2017 or 2018), sex (male or female), and birth type (singles or pooled multiples). BW, BCS, MBS, CI and the age of dam (AOD, 2 to 6 years) were considered as linear and/or quadratic covariates in the analyses of all dependent traits. CI was tested as either CI-1, -2 or -3, of which only one definition was retained in the model. Specific regression coefficients were not reported but only significance levels and direction are discussed.

All dependent variables: RT, ST, SS, LVS, BPM, M3 and TM were treated as numeric. Binary traits such as M3 and TM are not generally considered amenable to analysis by linear models, but previous results evaluating lamb survival used cross validation to show that there is little advantage to analysis of such data on the logit transformed scale (Everett-Hincks *et al.* 2014; Vanderick *et al.* 2015). Rectal temperature was skewed left of the normal distribution due to a small number of observations between 32 and 37 $^{\circ}\text{C}$. This data was not transformed, but values below 35 $^{\circ}\text{C}$ were removed from the dataset to improve the distribution of RT.

Analysis commenced by fitting model parameters by restricted maximum likelihood analysis with the ASREML-R (Version 4; Butler *et al.* 2018) package within the R environment (R Core Team 2020). Fixed effects and relevant interactions were tested for significance by deriving Wald Statistics from conditional least squares methods. All significant ($P < 0.05$) factors and covariates were retained in the operational model. Covariates were tested by both linear and second-degree polynomial regressions evaluated under the same critical statistic. Where there was evidence of non-linear trends, cubic splines with various sets of knot points (Verbyla *et al.* 1999) were considered in addition to the 2nd degree polynomial modeled by fitting a quadratic covariate. The addition of the random non-linear component of the spline was evaluated by differences in the log-likelihood ratio. However, this random component was not observed as influential ($P > 0.05$) to the viability traits and only fixed factors and covariates are presented. Pearson's correlations were also determined between all pairwise combinations of dependent variables.

4.3 Results

4.3.1 Summary Statistics

4.3.1.1 Weather

Mean temperatures were similar for 2017 and 2018 at roughly 17 °C, but years differed markedly for total precipitation (Table 4.1). Cumulative rainfall reached a total of 345 mm for 2017, while 613 mm was recorded in 2018. The dates and ranges of weather data recorded for the respective lambing seasons, expressed as daily means, can be seen in Table 4.1. In contrast to the annual figure, the 2017 lambing season had a higher daily rainfall and CI compared to 2018, while means for average temperature and wind speed were quite similar. The surface plot depicting the relationship between wind speed and rainfall in determining the chill index at a constant average temperature of 13 °C can be seen in Fig. 4.1. It is evident that the CI increased from around 800 KJ m⁻² h⁻¹ as windspeed and rainfall inclined. The combined effects of rainfall and wind culminated in a maximum CI of roughly 1300 KJ m⁻² h⁻¹.

Table 4.1

Summary statistics of weather data collected as daily means over the lambing seasons of 2017 and 2018

Year	Period	Range	Temp °C	Rainfall (mm)	Wind (ms ⁻¹)	Chill Index KJ m ⁻² h ⁻¹
2017	31 May to 24 July	<i>min</i>	8.3	0	0.5	811
		<i>mean</i>	13.12	5.2	1.4	952
		<i>max</i>	18.91	22.4	5.5	1258
2018	31 May to 2 Aug	<i>min</i>	8.61	0	0.4	799
		<i>mean</i>	13.82	3.1	1.3	916
		<i>max</i>	20.44	37.1	3.6	1269

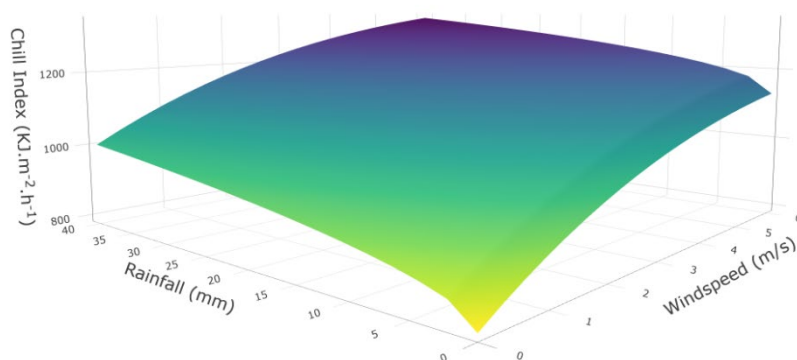


Fig. 4.1 A 3D surface plot depicting the chill index (z-axis) across the observed ranges for windspeed (y-axis) and rainfall (x-axis). The temperature was set constant to the observed mean of 13 °C.

4.3.1.2 Fixed effects and covariates in relation to vitality traits

The operational model of RT involved an interaction ($P < 0.05$) of selection line with CI-2, modeled as linear and quadratic covariates (Fig. 4.2). This interaction suggested that the difference in RT between selection lines also depended on the ambient conditions during the first two days of life. L-Line lambs displayed a non-linear response defined by markedly lower RT values when born in more extreme CI-2 environments. In turn, H-Line lambs were able to maintain homeothermy fairly well across the full range of CI-2 conditions observed in this study.

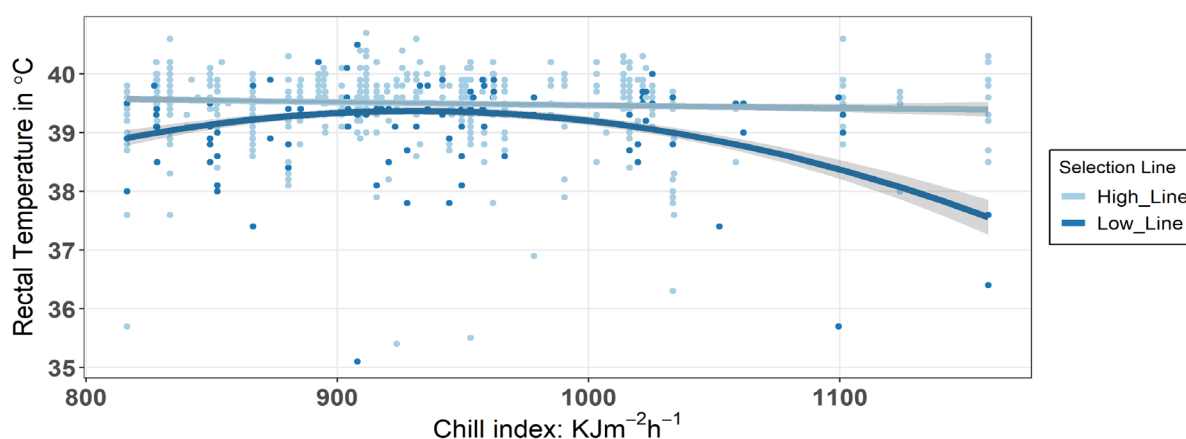


Fig. 4.2 A scatter-plot depicting predicted and observed rectal temperatures for lambs born in the H- and L-selection lines across environmental chill gradients. Shaded areas resemble standard errors (\pm SE) around the mean.

BW and BCS were not explicitly analyzed in this study and were only modeled as covariates to account for size-related effects in the traits studied. However, it is notable that, when treated as a dependent variable (data not shown), BW was independent of a selection line effect ($P > 0.05$), supporting a contention that selection line effects were independent of BW. When the RT of live-born lambs that succumbed before 3 days of age were compared to those that survived, it was evident that the average RT of surviving lambs (39.3 ± 0.03 °C) exceeded ($P < 0.01$) that of those lambs that died (38.7 ± 0.10 °C). The predicted selection line means derived from a linear model without interactions are reported in Table 4.2. RT was significantly ($P < 0.01$) higher in H-Line lambs and in lambs born during 2017 (Table 4.2), while heavier lambs also maintained their rectal temperature better ($P < 0.01$). ST was higher ($P < 0.01$) in heavier and more woolly coated lambs. ST was also impacted on by CI-2 ($P < 0.01$), with lower ST values observed at higher CI-2 levels. Male lambs had a higher ST ($P < 0.05$) than females and predicted means for 2018 were lower ($P < 0.01$) compared to 2017. ST of lambs was independent of selection line ($P > 0.05$). SS was increased ($P < 0.05$) with an increased MBS. Not surprisingly, lambs born in higher chill conditions (CI-2) recorded higher SS values ($P < 0.01$). Also, lambs born in the H-Line ($P < 0.05$) and in 2017 ($P < 0.01$) shivered less compared L-Line lambs and those born in 2018. BW was the only influential covariate determining LVS ($P < 0.01$), with higher scores recorded for heavier lambs. Birth year was also influential, with higher ($P < 0.01$) LVS scores predicted for 2017. Lamb BPM was similarly influenced by birth year and BW. Heavier lambs ($P < 0.05$) or lambs born during 2017 ($P < 0.01$) displayed higher BPM levels. Increased CI-2 levels resulted in a reduced BPM ($P < 0.01$); an effect

not observed on LVS. It is notable that all traits were independent of birth type when BW was modeled. Birth type was only significant ($P < 0.05$) in preliminary analyses on RT, without BW being fitted as a covariate. BW thus majorly accounted for the difference in RT between singles and multiples.

Table 4.2

Predicted least square means (\pm SE) for all significant ($P < 0.05$) fixed effects. For covariates, asterisks (*) and (**) indicate P-values ($P < 0.05$) and ($P < 0.01$), respectively. 'ns' indicates ($P > 0.05$) throughout

Fixed Effects	Viability					Mortality	
	RT	ST	SS	LVS	BPM	M3	TM
N	675	697	700	705	697	741	741
Mean	39.43	29.94	1.33	3.91	69.31	0.06	0.16
Line							
H-Line	39.51 \pm 0.03	ns	1.29 \pm 0.06	ns	ns	0.05 \pm 0.01	0.14 \pm 0.01
L-Line	39.16 \pm 0.05	ns	1.61 \pm 0.13	ns	ns	0.08 \pm 0.02	0.26 \pm 0.03
Year							
2017	39.46 \pm 0.04	31.24 \pm 0.17	1.62 \pm 0.1	4.11 \pm 0.04	71.38 \pm 0.72	ns	ns
2018	39.21 \pm 0.04	28.79 \pm 0.16	1.27 \pm 0.09	3.83 \pm 0.04	67.5 \pm 0.67	ns	ns
Sex							
Female	ns	29.74 \pm 0.16	ns	ns	ns	ns	ns
Male	ns	30.29 \pm 0.16	ns	ns	ns	ns	ns
Covariates							
BW	**	**	ns	**	*	**	**
BCS	ns	**	ns	ns	ns	ns	ns
MBS	ns	ns	**	ns	ns	ns	ns
CI-2	*	**	**	ns	**	ns	ns
CI-3	ns	ns	ns	ns	ns	**	*

Pearson's correlations were significant ($P < 0.01$) and positive for the majority of pairwise relationships among vitality traits, except for pairwise correlations including SS (Table 4.3). However, the correlations were generally low to moderate and ranged from 0.12 (ST x BPM) to 0.25 (RT x BPM). The strongest relationships to mortality traits were that of RT with TM ($r = -0.29$) and M3 ($r = -0.25$).

Table 4.3

Pearson correlation coefficients (upper-triangle) and P-Values (lower-triangle) between all viability and mortality traits recorded

	RT	ST	SS	LVS	BPM	M3	TM
RT	-	0.19	-0.03	0.15	0.25	-0.25	-0.29
ST	< 0.01	-	-0.05	0.16	0.12	-0.13	-0.14
SS	0.43	0.15	-	0.12	-0.03	0.04	0.03
LVS	< 0.01	< 0.01	< 0.01	-	0.2	-0.17	-0.17
BPM	< 0.01	< 0.01	0.5	< 0.01	-	-0.16	-0.14
M3	< 0.01	< 0.01	0.33	< 0.01	< 0.01	-	0.55
TM	< 0.01	< 0.01	0.48	< 0.01	< 0.01	< 0.01	-

4.3.1.3 Fixed effects and covariates in relation to mortality rates

The effects of year or sex did not influence M3 and TM, but BW was influential for both traits ($P < 0.05$; Table 4.2). CL-3 also exerted a significant influence on M3 ($P < 0.01$) and TM ($P < 0.05$). H-Line lambs outperformed their L-Line contemporaries for both measures of mortality, but M3 was also affected by a significant ($P < 0.01$) interaction between selection line and the linear effect of CI-3 (Fig. 4.3.). According to this interaction, the difference between the lines was most pronounced at the high values of CL-3, suggesting that the lower mortality rate in the H-Line could be a related to an ability to better cope with stressful CI-3 environments.

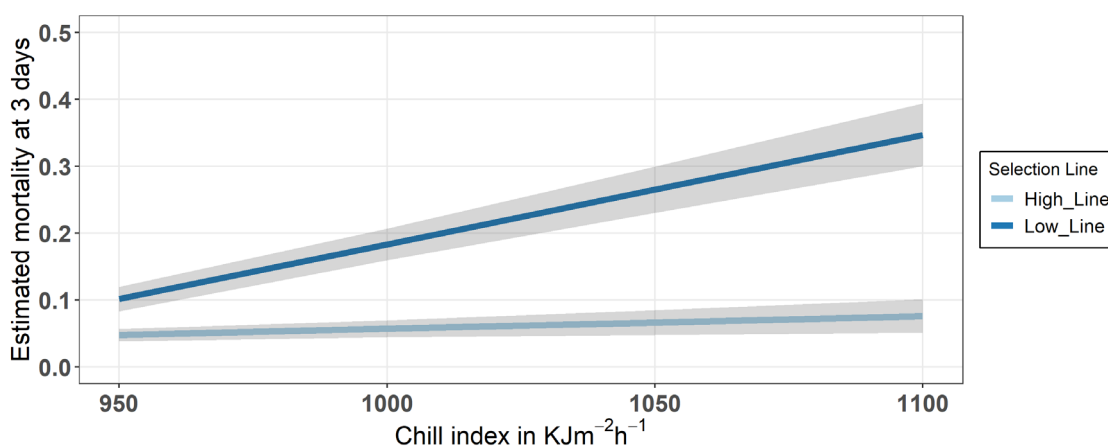


Fig. 4.3. Estimated mortality at 3 days of age for High and Low selection lines across chill index gradient. Shaded areas resemble standard errors (\pm SE) around the mean.

4.4 Discussion

It was clear that the CI was important in predicting cold-stress related conditions in newborn lambs, as reflected by reductions in RT, ST, BPM and an increase in SS (Table 4.2). Predicted lamb mortality also increased markedly with an increase in CI-3, indicating that the physiological burden imposed by cold stress during the neonatal stage constrained lamb survival significantly (Plush *et al.* 2016). Also, the lambing seasons studied were characterized with an array of climatic conditions, resulting in a marked range of CI-values (Table 4.1). The range and influence of the observed CI reflects the challenges of winter lambing in extensive production systems (Cloete *et al.* 1994).

Birth year affected most viability traits in the current study. Year effects are generally transient, climate dependent and not repeatable. Since the detailed discussion of these effects does not contribute to the central theme of the paper, it was generally omitted from the following discussion. There were also no interactions of other effects with birth year that contributed to the discussion below.

4.4.1 Rectal Temperature and Lamb Viability Score

4.4.1.1 Selection line

It was evident that selection for NLW in the H-Line of the Elsenburg flock has led to improvements in the ability of H-Line lambs to withstand cold stress conditions as reflected by a higher RT and a reduced SS. It is

conceivable that such adaptations would have contributed to the reduced mortality rates of H-Line lambs (Table 4.2), as well as to previous results in the literature (Cloete and Scholtz 1998; Cloete *et al.* 2009). The advantage in favor of H-Line lambs was particularly noticeable during periods of increased cold stress (Fig. 4.2 and 4.3). These line differences accord with results from the literature supporting significant genetic components for RT or similar traits. Comparing core temperatures in pens (Dwyer and Morgan 2006) and in the field (Sykes *et al.* 1976; Slee and Springbett 1986) or assessing observations from climate chambers (Slee 1978) and water bath tests (Samson and Slee 1981) all provided strong evidence for differences between breeds to thermoregulate when subjected to cold stress. Merino lambs were characterized by their poor thermoregulatory ability compared to other breeds, suggesting a need for improvement in this breed (Slee 1978; Sykes *et al.* 1976). A few studies have estimated the heritability (h^2) of traits relating to cold resistance in sheep. Results in the literature are inconsistent and predominantly derived from outdated parent-offspring regression methods. Slee and Stott (1986) reported significant differences between short term (~ 4 years) Scottish Blackface selection lines screened for cold resistance in water bath tests. A moderate h^2 of 0.30 ± 0.14 was derived for cold resistance in this study. A moderately high h^2 estimate of 0.44 ± 0.18 (Wolff *et al.* 1987), and a very high h^2 estimate of 0.70 ± 0.25 (Slee *et al.* 1991) have also been reported. However, the h^2 estimate in the latter study was lower in absolute terms at 0.52 ± 0.23 when BW was included as a linear covariate.

The review by Mormède *et al.* (2011) suggested that between-animal variation in HPA function could be exploited to improve robustness of farm animals. The review by Hough *et al.* (2013) similarly considered this prospect by reporting a more effective HPA-axis in the H-Line compared to the L-Line (Van Der Walt *et al.* 2009; Hough *et al.* 2010; Hough 2012). Hough *et al.* (2013) suggested that the selection program of the Elsenburg lines could have manifested in an improved ability to cope with short-term stress, contributing to overall animal 'robustness' or 'fitness'. However, since the nutritional status of individual lambs was not known during the early morning lambing rounds, the measurement of RT is unlikely to only reflect the capacity for NST, or the metabolism of BAT. Additionally, the literature provides several line differences that could all be potentially related to a reduced lamb mortality in the H-Line. Among these were genetic trends showing that selection in H-Line ewes resulted in shorter births, better cooperation with the first suckling attempts of their lambs and remaining for a longer time on or near their birth sites (Cloete *et al.* 2021). In the aforementioned study, genetic trends in the L-Line were all in the opposite direction. On the phenotypic level, H-Line lambs have also shown to progress faster from first standing to first suckling, while H-Line ewes were less likely to frustrate the first suckling attempts by their lambs (Cloete and Scholtz 1998). H-Line ewes were more attentive to their lambs during the neonatal phase and less likely to start grazing early after lambing (Cloete *et al.* 2002), while they also had a 15% advantage in milk production over their L-Line contemporaries (Cloete *et al.* 2011), albeit measured later in lactation. Moreover, H-Line ewes were quicker to reach a neonatal lamb tethered a distance of 20 m away and also quicker to establish contact with all litter members in multiple lambs (Cloete *et al.* 2005). Also, tethered H-Line lambs were more likely to bleat and tug on the tethering rope than L-Line contemporaries in the latter study. Moreover, results from a multi-ovulation and embryo transplantation study established that H-Line lambs had a better survival to weaning when reared by randomly chosen surrogate dams (Burger 2019). All these results support a contention that long-term selection for NLW

affected various characteristics that would be favorable to managing and surviving cold stress conditions. However, it is highly likely that at least some of these relationships are interdependent. Successful transition to diet induced thermogenesis would also depend on NST, since lambs with higher core temperatures are more likely to successfully reach the udder and feed (Slee and Springbett 1986; Dwyer 2008b).

In a design similar to the current study, Brien *et al.* (2010) evaluated RT measured during lambing rounds in the Australian information nucleus flock that comprises of 80% Merinos. Derived from more modern animal model methodology, they reported a low h^2 estimate of 0.10 ± 0.02 , suggesting that genetic selection for RT would deliver only moderate gains. It should be considered that the latter study, in line with the present study, included both inclement and benign lambing conditions, whereas previous studies use contrived situations that were more consistently stressful to lambs. Brien *et al.* (2010) reported moderate phenotypic correlations of RT with survival to 3 days ($r = 0.37 \pm 0.01$) and overall survival ($r = 0.32 \pm 0.01$). Considering that the present study analysed lamb mortality, the corresponding correlations of -0.25 for M3 and -0.29 for TM are in reasonable correspondence with those of Brien *et al.* (2010). The genetic correlation of RT with overall survival in the Brien *et al.* (2010) study was of a larger magnitude at 0.56 ± 0.33 but associated with a large standard error. It thus seems reasonable to contend that benefits pertaining to M3 and TM resulting from selection for NLW in this study would potentially also affect RT.

An interesting result in predicted RT means is the interaction between the chill gradient and selection line differences (Fig. 4.2). The quadratic trend in L-Line animals suggested that they were particularly susceptible at elevated levels of cold stress, an observation not so evident in the H-Line. The selection line trends across the chill gradient suggested that the critical level of cold stress is higher for the H-Line lambs compared to those in the L-Line. If this is borne out in future studies, genetic variation in RT may depend on lambs being subject to circumstances outside a range where they can comfortably maintain homeothermy. Thus, a genotype by environment interaction could be important in explaining variation in RT and could affect h^2 estimates such as those reported by Brien *et al.* (2010). The initial increasing trend of the predicted mean for the RT of L-Line lambs at lower levels of the CI was unexpected (Fig. 4.2). It is not clear why L-Line lambs born under slightly higher chill conditions ($\sim 900 \text{ KJm}^{-2}\text{h}^{-1}$) was initially modeled to exhibit an increased RT compared to lambs born in the minimum chill range of $\sim 800 \text{ KJm}^{-2}\text{h}^{-1}$. The first consideration could be that the initial increase in the predicted mean is an artefact of fitting a second-degree polynomial that to fit the data best in the extended measures of the CI. However, the cubic spline considered in a preliminary analysis showed a similar pattern. An alternative consideration could be biological. The initial range of the chill index were still within the capacity of the average lamb in the L-Line to maintain body temperature, but it is plausible that slightly increased stress levels imposed by a slightly higher CI stimulated an increase in heat production by NST or suckling in L-Line lambs compared to their contemporaries born in the 'low stress' environment below $900 \text{ KJm}^{-2}\text{h}^{-1}$. Previous studies that observed temperature changes in the same animal over time supported such a theory. For instance, an 'initial rise' in rectal temperature was observed by Alexander (1961) in lambs exposed to ambient temperatures below freezing point. Lambs subjected to water bath tests also displayed an initial rise in temperature, and the group characterized as a being cold susceptible responded with increased temperatures sooner than the 'cold resistant' selection line (Slee and Stott 1986). Interestingly, McCutcheon

et al. (1983) reported that wet lambs displayed a higher RT than dry lambs, and reasoned that wet lambs overcompensated to perceived heat loss by increasing metabolic rates despite being in non-stressful ambient conditions between 25 to 30 °C.

4.4.1.2 Birth weight

The emergence of BW as a significant covariate in estimating RT was expected from existing literature (Table 4.2). For instance, temperature regulation in multiple cold stress tests was positively related to BW (Alexander and McCance 1958; Slee *et al.* 1991; Slee and Stott 1986). More recent results by Dwyer and Morgan (2006) also reported that field-born lambs of lower BW tended to have lower RTs than heavier lambs 1, 24 and 72 hours post-parturition. However, the study by Sykes *et al.* (1976) reported that body weight or ambient temperature had significant effects on the body temperature of ‘poorly adapted’ Merino and Finnish Landrace lambs. In contrast, the body temperature of lambs of the ‘well adapted’ Soay sheep breed was not affected by ambient temperatures despite their comparatively low birth weights. Sykes *et al.* (1976) postulated that the lack of an effect of BW in certain breeds was due to a better metabolic ability to maintain body temperature, since BW only appeared a determining factor when heat loss approached the boundary of the metabolic capacity of the lamb for heat production. This outcome supports the current results in suggesting that genetic progress within a known ‘poorly adapted’ breed such as the Merino could possibly be achieved independent of increasing BW. It is also clear that BW was not involved in the reported line differences reported in this study. Apart from the lack of a line effect in BW, means reported for RT in Table 4.2 were controlled for BW by its inclusion as a covariate.

4.4.1.3 Sex and birth type

The present results suggested that RT was independent of sex or birth type if the effect of BW was accounted for by inclusion as a covariate. According to Samson and Slee (1981), litter size influenced cold resistance. In accordance with current results, Wolff *et al.* (1987) also reported that RT were independent of sex and birth type when the effects of bodyweight or size was accounted for. In climate chambers, basal metabolic rates were independent of sex (McCutcheon *et al.* 1983). Other results suggested that birth type effects on thermoregulation were only observable close to birth. Multiples have had lower temperatures than single or twin lambs immediately after birth and at 24 hours, but not at 72-hours (Dwyer and Morgan 2006). Triplet and quadruplet lambs had higher temperatures than twins and singles at birth, but this effect was no longer observable within 6 hours of birth (Stafford *et al.* 2007).

4.4.1.4 Birth coat score

It is of interest to note that birth coat score did not influence lambs’ ability to maintain core temperature, as reflected by RT in this study (Table 4.2), since it would have been expected that animals with better insulation could be less susceptible to heat loss. Some studies have supported coat characteristics as influential to lamb thermoregulatory ability, but results have been mixed. Alexander (1961) reported that hairy-coated Merino lambs produced less heat in cold environments compared to woolly-coated lambs while maintaining similar RT

levels. In turn, Slee (1978) reported significant differences to clipped, short and long coats in cold resistance during water bath cold resistance tests, but that breed and bodyweight effects remained significant even among clipped lambs. Birth coat score in Merino lambs subject to a water bath test was positively and significantly correlated with cold resistance on the phenotypic level (Slee *et al.* 1991). The field study by Sykes *et al.* (1976) reported that ‘poorly adapted’ Merinos ranked lowest according to birth coat length, but suggested that coat characteristics contributed only partially to thermoregulatory ability. In better agreement with the current results, Samson and Slee (1981) found no association between cold resistance and BCS in a study that evaluated cold resistance across 10 breeds. It is possible that BCS could be more influential in extreme circumstances where heat loss transpires over a steep gradient between the animal and the environment. Since the present data set had few observations collected under such extreme conditions it is impossible to confirm or refute these results from the literature. This issue is pertinent if lambs with hairy coats risk being culled in mass selection programs where they are considered undesirable. Also, coat structure may play an influential role in the thermal exchanges of lambs, as discussed below.

4.4.1.5 Relationship between RT and ST

The moderate Pearson’s correlation of 0.19 between RT and ST indicated that ST would have limited use in predicting animal core temperature. This result was supported by a similarly weak relationship between RT and various ST measurements previously reported in neonates (Vicente-Pérez *et al.* 2019), but stronger relationships have been reported elsewhere. Surface temperatures estimated from thermography images of the eye in adult sheep yielded a correlation of $r = 0.504$ with rectal temperature (Pérez de Diego *et al.* 2013). In cattle, correlations between surface and rectal temperature was as high as 0.75 when measured at the less exposed thigh site (Yadav *et al.* 2017). However, these studies measured animals maintained in climate-controlled circumstances, and surface temperatures could be expected to be susceptible to ambient environmental conditions. In an outdoor setting, recordings are likely to be affected by numerous other interacting effects, such as direct solar radiation, wind, and wetness. For instance, Alexander (1961) reported a close to linear relationship of ambient temperature with skin temperature, a relationship which persisted in unfed or partially fasted lambs. However, wetness and air speed interacted to produce much lower skin temperatures at a higher air speed in wet lambs, but not in dry lambs (McCutcheon *et al.* 1983).

Furthermore, it is important to consider that, although measurements were taken on the axilla as a potential wool-free area with direct access to bare skin, lambs differed in their amount of hair cover on the site. Although this was not recorded explicitly, it is plausible that such differences could be underlying the observed effect of BCS on surface temperature. It is likely this effect was not only related to the extent to which different coats isolate the skin, but rather that coat characteristics could have influenced the accuracy of measurements. Infra-red thermographs (IRT) or thermometers (IRTM) depend on infra-red radiation emitted by objects as a function of their temperature. However, the accurate estimation of the temperature of the object depends on knowledge of and calibration for the innate ability of the object to emit radiation energy, otherwise known as the emissivity. It is very likely that this may vary between a hairy surface compared to bare skin (Soerensen *et al.* 2014).

Moreover, coat structure is also important in determining the extent to which coats are able to conduct or resist radiation across temperature gradients. Fibre density, diameter and angle to the plane of the skin (Cena and Clark 1978), combined with hair length and coat depth (Turnpenny *et al.* 2000), are all important predictors for resistance to radiative transfer in sheep. It is thus feasible that IRT measurements would be sensitive to the coat characteristics defining BCS. If hairy coats with longer and thicker fibres at a higher density tend to have a lower emissivity, surface temperatures of animals with a low BCS would tend to be underestimated which could partially explain the positive effect of BCS on ST observed in current study. However, Labeur *et al.* (2017) reported that ST measured by infrared thermography is independent of coat length. Using different methods, Alexander (1961) reported that trunk temperatures of hairy lambs were higher than that of fine-coated lambs, which contradicts the direction of the present relationship between BCS and ST. The results of Alexander (1961) could likely be a better indication of the influences of BCS. If hairy lambs have lower emissivity levels, they would also putatively be better insulators by being less prone to radiative heat loss and better at maintaining a higher surface temperature on the skin of lambs.

4.4.2 Lamb Vitality

Alexander and Williams (1968) and Clarke *et al.* (1997) reported that shivering thermogenesis tends to be recruited only if NST is functioning at a maximum. Supporting this relationship is that the H-Line lambs, considered as better homeotherms based on the results in Table 4.2 and Fig. 4.2, shivered less than their L-Line contemporaries. This also suggested that the improved core temperature of H-Line lambs were associated with NST, curtailing a need to resort to shivering to maintain heat production. However, this was not supported by a significant correlation between SS and RT and lambs with low RT values could have been expected to be more prone to shiver. The cause of the positive influence of MBS on SS is also unknown.

Results furthermore suggested that the improved performance of H-Line lambs in RT, SS, M3 and TM were not related to line differences in LVS (Table 4.2). A previous study indicated that H-Line lambs were also more likely to follow their dams next to their front legs or side than L-Line contemporaries when tested at three days of age (Cloete *et al.* 2005). However, it is not clear if this behaviour would be related to LVS closer to birth. The observation that heavier lambs were less lethargic, as indicated by a higher LVS, however, agrees with previous results indicating that low BW lambs showed the slowest behavioral progress (Dwyer 2003; Dwyer and Morgan 2006). A positive correlation between LVS and RT also agreed with results from Dwyer and Morgan (2006) that lambs that were slower to stand had lower RTs at birth.

None of the measures of chill impacted on LVS in the current study. In contrast, Slee *et al.* (1991) found that animals with higher cold resistance displayed more struggling movements during their water-bath test. Alexander and Williams (1966) showed that teat seeking behavior in Merino lambs declined with an increase in cold stress. It is likely that lambs approaching a hypothermic state would be more lethargic, especially since such lambs would also have higher difficulty feeding and be increasingly at risk of starvation. It is possible that too few lambs in current study were measured in circumstances inducing hypothermia to observe such an

effect. Also, the most severe cases, i.e. lambs that succumbed to hypothermia prior to recording, were not included in the LVS and RT analyses.

4.5 Implications and conclusions

The design of the current study is limited in identifying the exact mechanisms responsible for the improved performance observed in H-Line lambs, and more intensive studies are needed in this regard. However, recording during early morning lambing rounds can be practically adopted by breeding programs, but more intensive studies would not be practical on the large scale needed for genetic selection. Furthermore, the results provide strong empirical evidence that genetic selection for a correlated trait such as NLW is a viable option for improving the extent to which lambs can cope with hypothermic stress, as proposed by Plush *et al.* (2016). Animal robustness is increasingly relevant as climate change has detrimental effects on livestock production, specifically for extensive systems common in South Africa (Rust and Rust 2013). While mean temperatures are predicted to increase for the winter rainfall regions of South Africa, rainfall could become more sporadic, but occur at higher intensities (Midgley *et al.* 2005). More extreme weather fluctuations would have direct implications for cold stress as quantified here, increasing the need to consider an animal's resilience to challenging conditions.

While the present results in favor of the H-Line, backed by evidence in the literature, are promising, a multivariate genetic analysis is needed to separate genetic and environmental influences observed in these traits. The genetic parameters for RT, as well as possible genotype by environment interactions across cold stress gradients determining RT and M3 should be of specific interest. The continued recording of viability traits to produce larger datasets amenable to genetic analysis is recommended, specifically for RT.

4.6 References

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CHAPTER 5:

Factors affecting neonatal lamb mortality across a cold stress gradient in the Elsenburg Merino selection lines

Abstract

The first period post-parturition is particularly challenging when lambs are born into cold, wet, and windy conditions. The level of cold stress during this period can be quantified from weather data in the form of a chill-index (CI), which has been shown to be an important predictor in neonatal mortality rates. The Elsenburg Merino selection lines are separated by divergent selection (H-Line: positive selection, L-Line: negative selection) for multiple rearing ability since 1986. The objectives of this study were to investigate if the favourable rates of survival reported for the H-Line could be linked to cold stress adaption by comparing neonatal mortality (M3, between 1 and 3 days of age) rates of H- and L-Lines as a function of CI during the neonatal period. Additionally, this study also evaluated the use of CI as a covariable in the genetic evaluation of M3. The binary phenotype of M3 was evaluated on the underlying scale according to a logit-link function. The factors considered were sex, birth type, age of dam, selection line (SL), birthweight (BW) and CI. Random genetic effects were derived using the sire model. M3 for the L-Line (0.055) was more than 60% higher ($P < 0.01$) than for H-Line lambs (0.034). Very light lambs (< 3 Kg) were also susceptible ($P < 0.01$) with M3 between 0.10 to 0.20. M3 increased ($P < 0.01$) with CI with M3 of 0.15 during challenging conditions being 5-fold the M3 of 0.02 when conditions were mild. However, evaluation of these factors was complicated by the fact that they tended toward ($P < 0.07$) a three-way (SLxBW \times CI) interaction, the most susceptible lambs being born in the L-Line, of low BW, and during severe cold stress. The h^2 of M3 was low, but limited benefits were observed when fitting CI as a covariate. This study provided strong additional evidence that the improved survival rate in the H-Line is linked to resilience to cold stress. It is recommended that the relationship between CI and M3 be explored further on the genetic level by random regression analysis of larger datasets.

5.1 Introduction

The incidence of lamb mortality is of great importance from both an economical and welfare perspective (see reviews: Brien *et al.* 2014; Hinch and Brien 2014). There has thus been considerable interest in improving lamb survival by targeting additive genetic effects. However, this approach has been discouraged by the low heritability (h^2) for survival traits reported for Merinos (Brien *et al.* 2009; Hatcher *et al.* 2010) and other breeds (eg. Lopez-Villalobos and Garrick 1999; Morris *et al.* 2000; Riggio *et al.* 2008; Vanderick *et al.* 2015).

The first days after birth have been identified as particularly critical with the highest mortality rates (Hatcher *et al.* 2009), and a possibly higher level of genetic variation determining mortality outcomes (Sawalha *et al.* 2007; Riggio *et al.* 2008; Hatcher *et al.* 2010). The neonatal period often delivers a stringent test to the adaptive capacity of lambs, including the dramatic change in environmental temperature upon entering the extra-uterine environment. The ability to quickly respond by inducing its own mechanisms of thermoregulation has been emphasized as a key mechanism contributing to lamb viability (see review: Plush *et al.* 2016). In extensive production systems, this period can be particularly challenging when lambs are born into cold, wet and windy conditions which interact to exacerbate the level of cold stress. Exposure to a high rate of heat loss slows lamb progress by prompting a group of interrelated factors, termed the starvation-mismothering-exposure (SME) complex (Haughey 1991; Cloete and Scholtz 1998). The expected rate of heat loss has been quantified as a function of ambient temperature, rainfall and windspeed as an environmental stress variable termed the chill index, abbreviated as CI (Nixon-Smith 1972; Donnelly 1984). Cold stress represented by the CI has been strongly linked to high mortality rates in new-born lambs (Donnelly 1984; Oldham *et al.* 2011; Geenty *et al.* 2014), emphasizing the unpredictable and detrimental impact of erratic climates upon lamb mortality.

The ability to tolerate a high rate of heat loss, or cold stress, varies across individuals. Differences in the ability to resist cold stress have been reported across breeds (Dwyer and Morgan 2006), selection lines (Slee and Stott 1986), sire lines (Gudex *et al.* 2005), and individuals (Slee *et al.* 1991). It could thus be expected that genotypes better adapted to high levels of cold stress should be associated with a lower incidence of mortality in environments of severe cold exposure.

The Elsenburg Merino selection lines have been divergently selected for reproductive fitness from 1986 to 2020. Despite the low h^2 for survival traits, worthwhile and divergent genetic trends for lamb survival were reported for the H- (positive selection) and L-Lines (negative selection) as a correlated response to selection for number of lambs weaned (Cloete *et al.* 2009; Chapter 4: Nel *et al.* 2021a). In a phenotypic comparison of lamb viability between H- and L-Line lambs over two winter seasons, H-Line lambs coped better with stressful levels of cold exposure (Chapter 4: Nel *et al.* 2021a), suggesting that the better survival rate of H-Line lambs could partially be linked to better cold stress adaptation. The history of the Elsenburg selection lines includes the use of the same lambing paddocks for both the H- and the L-Lines since 1993. Given a very close proximity to a weather station that delivered the data needed to calculate a CI (Nixon-Smith 1972; Donnelly 1984), it was possible to quantify the measure of cold stress during lambing for the majority of the selection experiment. There is thus an opportunity to elaborate on the results of Nel *et al.* (2021a) (Chapter 4) by comparing line performance across a much larger dataset recorded over 28 years, but with an adjusted focus on neonatal lamb

mortality in particular. Furthermore, if line performance is in fact linked to the level of cold stress at birth, a genotype by environment interaction (GxE) is likely to be an important component of neonatal lamb survival, particularly in extensive conditions where there is little protection against environmental elements. If lambs born under high stress conditions are not tested under conditions similar to candidates born when conditions are mild, it would affect the accuracy of h^2 estimates which are evaluated assuming equal opportunity. It should thus also be worth-while to investigate if including CI information into the linear mixed model could deflate the influence of the GxE component by accounting for this difference across records and potentially improve the accuracy of genetic evaluation.

The aim of this study was to elaborate on the observations reported by Nel *et al.* (2021a) (Chapter 4) by evaluating the effect of the CI as a cold stress variable on the respective mortality rates of H- and L-Line lambs across an extended period of the history of the selection lines. This study also aimed to compare the genetic parameters of neonatal mortality in models both including and excluding the environmental covariable accounting for cold stress challenge during the first days of age.

5.2 Materials and Methods

5.2.1 The Elsenburg Merino Flock

The history of the selection lines is divided into two periods defined by the location of the flock. From 1986 to 1992, the flock was maintained at the Tygerhoek Research Farm near to the town of Rivieronderend (34°8'S 21°11'E), Western Cape, South Africa. In 1992, the flock was moved to Elsenburg Research Farm near to the town of Stellenbosch (33°51'S 18°50'E) in the same province (see Cloete *et al.* 2004). Lambing at Tygerhoek generally commenced in the Autumn (March-April) whereas lambing at Elsenburg was during winter (June - July). This study focuses on data collected at Elsenburg, where the ewes lambed during winter.

Besides joining, the two lines were maintained as a single flock for most of the year. At Elsenburg, parturition commenced on 10 - 20 kikuyu lambing paddocks of approximately 0.3 to 0.4 ha each. During this period, ewes were randomly divided in small groups of 15-25 pregnant ewes. Lambing ewes spent 3-15 days on their lambing paddocks, before being drifted off and allocated at random to groups of 20 to 40 ewes and their offspring. These groups grazed on nearby lucerne paddocks of 1-2 ha for a period post lambing (Cloete and Scholtz 1998). Other details pertaining to management and nutrition are available elsewhere (Cloete *et al.* 2004, 2009) and will not be repeated here.

The H- and L-Lines were screened from the same base population before the commencement of the selection experiment in 1986. After the flocks were generated by random sampling within age groups, selection proceeded by screening ram and ewe replacements according to their rankings for number of lambs weaned per joining (Cloete *et al.* 2004). Besides instances of death or severe health related incidences, replacement ewes remained in the flock for at least 5 joinings. At the beginning of the experiment, roughly 120 ewes were assigned into each selection line. The H-line has since grown to 130-200 ewes, while numbers in the L-Line decreased to about 40-80 breeding ewes. Selection of rams was predominantly based on the progeny of dams based on at least 3 joinings. Five rams were initially used for each line and a ram was only used for a single year until 1992, after which two to three rams per line were used across years to provide sire links. Following

the results by Cloete *et al.* (2004), selection from 2003 was also guided by the use of best linear unbiased prediction (BLUP) breeding values derived from a single-trait repeatability model fitted to ewe reproduction records. In this period, the original ranking values were still considered in tandem to BLUP derived breeding values, but BLUP-values have been the only selection criterion since 2010. From 2008 to 2019, 349 H-Line lambs and 151 L-Line lambs were born as the progeny of external sires. These animals were also considered for selection and 740 H-Line and 118 L-Line lambs were subsequently born as grand-progeny of external sires between 2010 and 2019.

5.2.2 Data and Recording

The recording of incidences of mortality and birth weight (BW) took place during daily lambing rounds from 8:00 am that identified all lambs born within the previous 24-hour period with their dams, thus enabling linkage back to the selection line and sire (Cloete *et al.* 2004, 2009). Data for lamb survival was recorded as lambs having succumbed at disjointed intervals starting with mortality at birth (MB) and subsequently before three days of age (M3), tailing (MT) and weaning (MW), which sum to total mortality (TM) at weaning. Birth weight was also recorded to the nearest 0.1 kg. These data were recorded for 6925 lambs born between 1993 and 2020. The pedigree file identified the sires (N = 282) and dams (N = 2141) for the vast majority of these lambs.

5.2.3 Environmental Descriptor

Climatic data were available from weather stations in close proximity to lambing paddocks. Due to random technical errors, climate data were missing in some seasons, resulting in only 5724 of the original 6925 mortality records being linked to the environmental descriptor. At Elsenburg, the weather station (33°51'S 18°50'E, alt: 171 m) was within an 1800m radius to the furthest boundary of the lambing paddocks used after 1992. The weather station recorded daily data that included windspeed, rainfall and ambient temperature. These data allowed for the quantification of an environmental stress variable defined as the chill-index, abbreviated as CI from here (Nixon-Smith 1972; Donnelly 1984). This index estimates heat loss per unit surface area per hour as:

$$CI = [11.7 + 3.1V^{0.5}][40 - T] + 481 + 418(1 - e^{-0.04R})$$

where CI is the estimated heat loss ($\text{KJm}^{-2}\text{h}^{-1}$), V is the mean daily wind velocity (ms^{-1}), T is the mean daily temperature ($^{\circ}\text{C}$) and R is the total daily rainfall (mm).

5.2.4 Statistical Analysis

Given the discrete expression of the survival phenotype as either dead (0) or alive (1), it could be beneficial to consider analysis as a threshold character (Falconer 1989) that considers a hypothetical normal distribution on the underlying scale. Theoretically, linear models are not ideal for the analysis of threshold or binary phenotypes. Examples of analysis of survival data on both underlying and observed scale (Matos *et al.* 2000; Everett-Hincks *et al.* 2014; Vanderick *et al.* 2015) have shown that linear models have very similar predictive properties compared to transformed or threshold models. Linear analysis was initially attempted and delivered

feasible results in simpler fixed effect models, but became erratic when fitting interactions described below. This was likely due to limited observations present within the range or class of interreacted fixed effects and the very low incidence of neonatal mortality that considers only incidence between birth and 3 days of age. The analysis of fixed effects and genetic parameters was thus subsequently considered on the transformed scale by fitting generalized linear mixed models by use of the logit transformation:

$$n_i = \ln \left[\frac{\pi_i}{1-\pi_i} \right], \text{ where } \pi_i \text{ is the probability of mortality.}$$

The level of cold stress during birth has been observed as an important predictor to M3, but with little to no relationship to other periods of mortality (Chapter 4: Nel *et al.* 2021a). Consequently, only M3 was subject to analysis in this study and only summary statistics of MB, MT, MW are reported. Estimation of fixed effects and the subsequent derivation of genetic parameters used the ASREML® program (Gilmour *et al.* 2015). The factors that were considered as fixed included selection line (H-Line or L-Line), sex (male or female), age of dam (2 to 7+ years), birth type (singles vs pooled multiples), while BW was also considered as a linear and quadratic covariate. Fixed effects and relevant interactions were tested for significance by deriving Wald Statistics from conditional least-squares methods. The covariates (CI and BW) were rescaled to a mean of zero prior to analysis, but covariates are represented on the original scale in results. All significant ($P < 0.05$) factors and covariates were retained in the ‘operational model’ that provided the basis for estimating random variance components. Least square means estimated on the logit scale were back transformed to the observed scale by the inverse link function $\pi = \exp(x\beta) / [1 + \exp(x\beta)]$ where x represent the matrix of fixed effects and β the vector of solutions for fixed effects.

5.2.5 Random Variance Components

Year of birth was fitted as a random effect in the operational model. The influence of a genetic effect was derived by building the genetic relationship matrix according to a sire model. The average sire had 30 progeny. With variable climatic conditions, it was expected that sire genotypes could be evaluated by progeny performance at varying levels of the environmental trajectory described by the CI. The operational model was altered to investigate two objectives. The first was to test if including CI as a covariable could partially account for the varying stress level of progeny born on different days. The second objective was to derive genetic parameters without the use of selection line as fixed effect, since accounting for genetic group could deflate the genetic variation observed across the population due to the general superiority of H-Line sires as reported by Nel *et al.* (2021b) (Chapter 3). Random terms considered were the sire genetic variance (σ_s^2) and the total maternal variance (σ_{ewe}^2). The dam permanent environmental variance (σ_{mc}^2) was derived as $\sigma_{ewe}^2 - \sigma_s^2$. The obtained estimates were used to compute the variance ratios for additive genetic variance (h^2) as σ_a^2 / σ_p^2 and the dam permanent environmental variance as $\sigma_{mc}^2 / \sigma_p^2$ where $\sigma_a^2 = 4\sigma_s^2$ and $\sigma_p^2 = \sigma_s^2 + \sigma_{mc}^2 + 3.29$. Subsequently, the heritability of M3 was evaluated by three different models that varied by their fixed effect terms ((i), (ii) and (iii)). Model (i) included all significant fixed effects except CI, (ii) was the full model including CI and its relevant interactions, and (iii) excluded selection line and its relevant interactions. The h^2 was also transformed back to the binary scale as $h^2 z^2 / p(1-p)$, where p was the general mean of M3. The use of

likelihood-based tests (AIC, BIC or LRT) is not appropriate for generalized linear models or for investigating changes to the fixed effects. The inclusion of sire and permanent maternal effects was guided by their significance in a preliminary analysis on a linear scale (data not shown) as determined by a likelihood ratio test. A difference in log-likelihood of 1.36 between the full or nested model was considered as significant ($P < 0.05$) according to a chi-square distribution.

5.3 Results

5.3.1 Summary statistics and weather data

In total, 22% of lambs succumbed before weaning. Of this total mortality rate, 19% were recorded dead at birth, 26% died between birth and 3 days of age (M3), 25% before tailing and the remaining 30% before weaning. Lambs were thus particularly susceptible in the 3-day period defined by M3, since it contributed a comparatively large proportion of the total mortality rate of roughly 100 days to weaning. The mean rate of heat loss during the first 3 days of life across all observations was $963 \text{ KJm}^{-2}\text{h}^{-1}$, ranging between a minimum of 713.8 and a maximum of $1298 \text{ KJm}^{-2}\text{h}^{-1}$. The cold stress gradient represented as the chill index (CI) generally fitted a normal distribution (Fig. 5.1) after 42 erratically high records above $1300 \text{ KJm}^{-2}\text{h}^{-1}$ were set to missing.

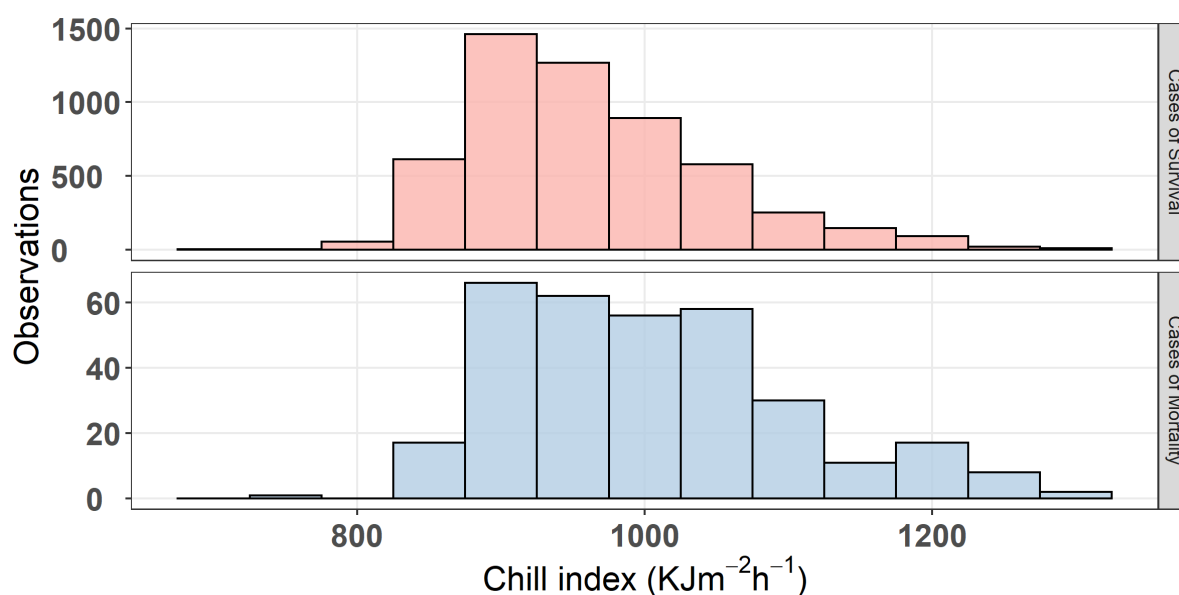


Fig. 5.1 Frequency of observations of lamb survival or mortality (M3) across levels of the chill index (CI) for the period from 1993 to 2020.

5.3.2 Fixed effects on trait M3

All significant effects were retained in the fixed effects model, except in the case of model (i) and (iii) which excluded CI or selection line regardless of significance. The effects of sex, birth type or the age of dam were not significant ($P > 0.05$), but the status of sex and birth type depended on BW being included as a covariate or not. Both effects were significant ($P < 0.05$) when BW was excluded. BW ($P < 0.01$) but not BW^2 ($P > 0.05$) was a significant covariate determining M3. During 1993 and 2020, the selection lines differed ($P < 0.01$) substantially in their incidence of neonatal mortality, with M3 for the L-Line (0.055 ± 0.008) more than 60% higher compared to the expected rate for H-Line lambs (0.034 ± 0.004) according to the retransformed values.

Mean BW of lambs was 3.8 kg. BW was an important predictor ($P < 0.01$) of neonatal lamb mortality and the curvilinear relationship suggested that very light lambs were particularly susceptible to 3-days of age (Fig.5.2).

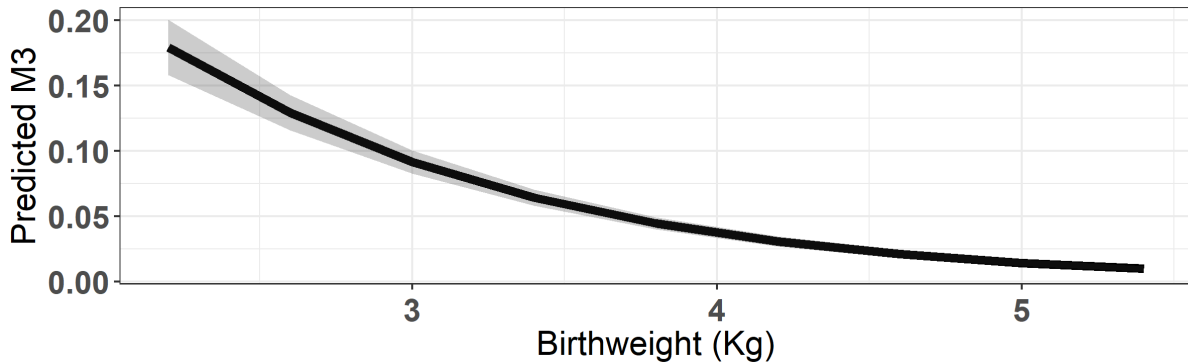


Fig. 5.2 Predicted lamb mortality within 3 days from birth (M3) according to lamb birthweight. Shaded areas resemble standard errors surrounding the mean.

Across the whole population, a higher CI caused higher rates of neonatal mortality ($P < 0.01$), with a predicted mortality rate of nearly 15% at challenging conditions ($\sim 1200 \text{ KJm}^{-2}\text{h}^{-1}$) being 5-fold the low rate of 2% when conditions were mild ($\sim 800 \text{ KJm}^{-2}\text{h}^{-1}$; Fig. 5.3). The data suggested that the factors of M3 tended towards a three-way interaction ($P < 0.07$) between selection line and BW and CI as covariates, with the most susceptible lambs being those born in the L-Line, of low BW, and during severe cold stress (Fig. 5.4). For lambs fitting this description, the mortality rate was predicted to approach and exceed 50% in the case where CI was recorded above $1100 \text{ KJm}^{-2}\text{h}^{-1}$. While low BW ($\sim 2.2 \text{ kg}$) H-Line lambs were also susceptible (20%) at CI levels exceeding $1100 \text{ KJm}^{-2}\text{h}^{-1}$, these circumstances constituted the largest predicted difference between the lines in M3. Lambs with an average (3.5 kg) or high (5.4 kg) BW were much better able to cope with cold stress conditions, and L-Line lambs tended to have a similar performance in M3 than H-Line lambs. However, it is clear from Fig. 5.4 that the predictions of low BW lambs were also estimated with the poorest accuracy,

particularly for L-Line lambs. However, this was expected, owing to few observations available for L-Line lambs within this weight and CI range.

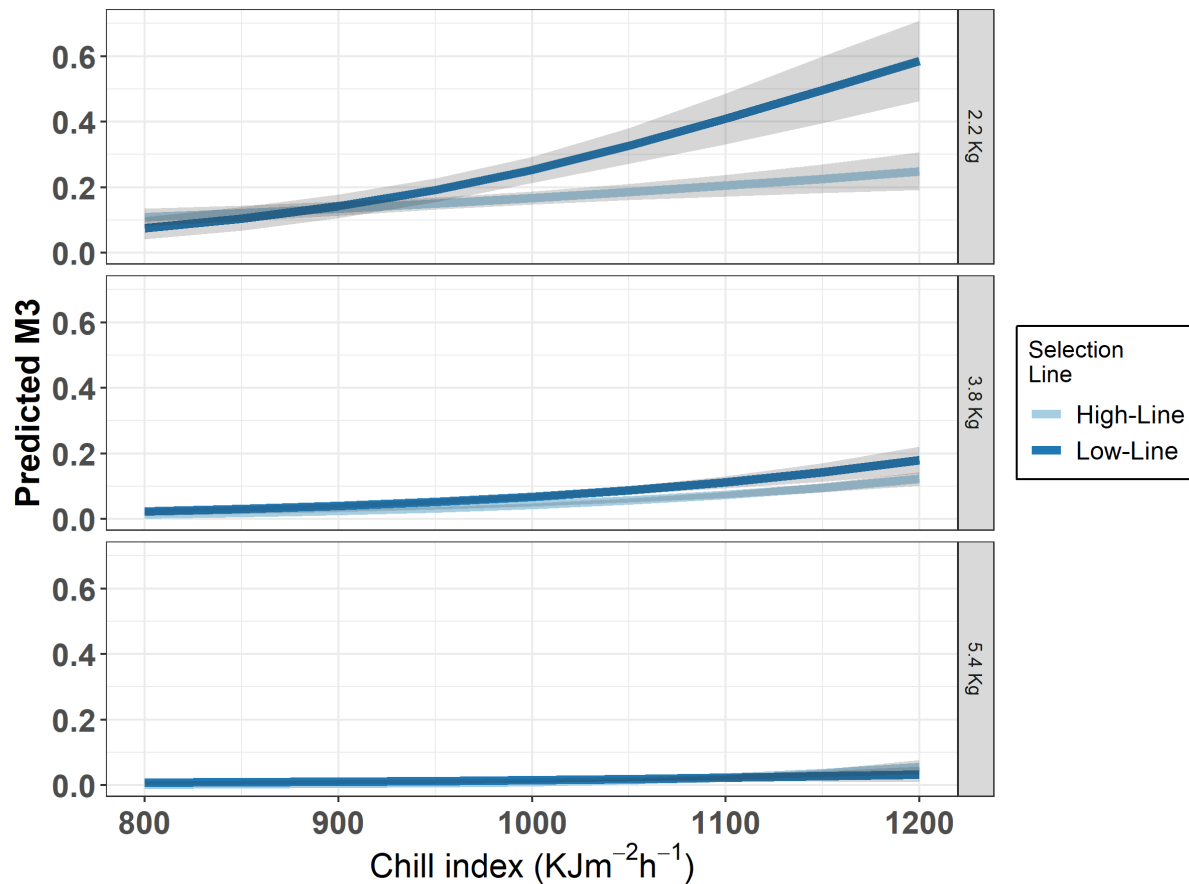


Fig. 5.3 Predicted lamb mortality within 3 days from birth (M3) across the cold stress gradient (CI) according to selection line and BW. Represented weights (2.2, 3.8 and 5.4 kg) represent -2, 0 and +2 SD of BW, respectively. Shaded areas represent the standard errors surrounding the predicted mean.

5.3.3 Heritability of M3 with and without cold stress information

In the conventional analysis by model (i), where CI and interactions with CI was excluded, the estimated sire variance was small ($\sigma_s^2 = 0.07 \pm 0.07$) and estimated with poor precision on the logit scale. According to model (ii) that included CI in fixed effects, the estimated sire variance was slightly higher with a better ratio ($\sigma_s^2/SE = 1.21$) compared to that of analysis (i) ($\sigma_s^2/SE = 1$). Model (iii), which excluded selection line and interactions with selection line, sire variance was markedly higher ($\sigma_s^2 = 0.15 \pm 0.08$) and with stronger evidence of a significant influence ($\sigma_s^2/SE = 1.85$). The year effect was constant ($\sigma_y^2 = 0.10 \pm 0.07$) between models (ii) and (iii), but considerably larger in the case of model (i) ($\sigma_y^2 = 0.28 \pm 0.13$). This would be expected, since the use of CI would have accounted for some of the variance across years due to selected seasons being particularly cold, wet and windy. The maternal permanent environmental variance (after subtracting σ_s^2 from total maternal variance) was observed to be considerably larger in magnitude compared to sire genetic effects, and ranged between 0.76 ± 0.17 (Model (ii)) to 0.80 ± 0.18 (Model (i)). On the logit-transformed scale, the h^2 of M3 was: Model (i): 0.07 ± 0.07 ; Model (ii): 0.09 ± 0.07 and Model (iii): 0.14 ± 0.08 , indicating a slight benefit of

including the CI in the model and also suggesting that the inclusion of selection line accounted for a notable proportion of the genetic variance in M3. Transformed back to the binary scale, however, proportional differences between the analysis remained visible, but all the estimates of h^2 were low to very low ((i): 0.017, (ii): 0.021 and (iii): 0.034), suggesting very little genetic influence in the outcome of M3.

5.4 Discussion

5.4.1 Selection line mortality across the cold stress gradient

In relation to the time segment from birth to 3 days, the proportion of 26% of all lamb deaths prior to weaning was substantial. The first week of life indeed presents a very susceptible period for lambs. As a percentage of total mortality to weaning, Hatcher *et al.* (2009) reported 48% of lamb deaths to occur in the first 7 days of age (excluding lambs dead at birth). In other studies, even higher proportions were reported where neonatal mortality constituted up to 83% of total mortality to marking (Obst and Day 1968) and 90% to weaning (Oldham *et al.* 2011). The incidence of lamb mortality associated with dystocia and starvation mismothering have been reported to be responsible for 72% of all mortality records (Geenty *et al.* 2014), and it is expected that these symptoms would manifest very soon post parturition. In this study, 45% of all lamb deaths occurred from the periparturient period to within 3 days of birth.

BW is well known to be an important factor in early lamb survival (Hinch and Brien 2014), but the definition of neonatal mortality is also important when considering the influence of BW. The current study, for example, excluded lambs found dead at birth from the definition of M3. The incidence of dystocia associated with a high BW (Geenty *et al.* 2014; Refshauge *et al.* 2016) is thus not reflected in Fig. 5.2, given that such lambs are commonly found dead at birth (MB), which also contributed a notable proportion (19%) to total mortality. In wider descriptions of mortality, the risks of not only very light but also very heavy lambs is well reflected in the curvilinear BW-lamb survival relationship (Dwyer 2008; Hinch and Brien 2014), with an optimum weight for Merinos suggested to be around 4.5 kg (Atkins 1980). Twin lambs are lighter at birth than singles and female lambs lighter than males (Knight *et al.* 1988). This generalisation also applied to the Elsenburg flock (Chapter 3: Nel *et al.* 2021b). However, accounting for BW as a covariate explained the entire influence of birth type and sex with regards to M3 in the current analysis. Differences in birth type and sex at a fixed birth weight has been reported elsewhere (Oldham *et al.* 2011).

It was clear that the level of cold stress during the neonatal period was also very important predictor in early mortality rates with a very low incidence rate of M3 when conditions are mild (Fig. 5.2). The moderate to potentially severe implications of cold exposure to early lamb mortality has been well described (Alexander 1962; Obst and Day 1968; Alexander *et al.* 1980; McCutcheon *et al.* 1981) including studies that quantified cold stress by the same or a similar CI (Donnelly 1984; Gudex *et al.* 2005; Oldham *et al.* 2011; Geenty *et al.* 2014).

The tendency of an interaction between BW and CI is feasible, given the better performance of heavier lambs in cold stress tests (Alexander and McCance 1958; Slee and Stott 1986; Slee *et al.* 1991) or rectal temperature recorded in the field (Dwyer and Morgan 2006). Fitting a 3-way interaction complicated the comparison of selection lines since predictions hinged on relatively few observations in low BW L-Line lambs

born during high CI-periods, as reflected by wide confidence intervals for such lambs in Fig. 5.3. The predicted M3 exceeding 0.50 for such lambs appeared extreme relative to the remaining treatment groups of mean and high BW or at low CI. Nevertheless, similar high mortality rates have been reported from previous studies investigating the effect of chill, albeit as a function of birth type instead of BW currently reported. At certain sites, lamb losses were as high as 24% during periods of chill that exceeded $1000 \text{ KJm}^{-2}\text{h}^{-1}$ (Geenty *et al.* 2014). According to Donnelly (1984), mortality rates can range from 0.10 to 0.20 for singles and from 0.30 to 0.40 for multiples at $1000 \text{ KJm}^{-2}\text{h}^{-1}$, but can reach 0.51 to 0.69 for multiples when CI exceeds $1100 \text{ KJm}^{-2}\text{h}^{-1}$. Similarly, Oldham *et al.* (2011) reported a neonatal mortality rate to 48h exceeding 50% for multiples as CI ranged above $1200 \text{ KJm}^{-2}\text{h}^{-1}$. Some earlier studies did not define a chill index per se, but also reported mortality rates of as high as 64% during instances of “bad weather” (Alexander *et al.* 1980) or even a very high incidence rate of 91% for Merinos in circumstances defined by an interaction between high wind and rainfall (Obst and Day 1968). From these results it seems as if the results reported in Fig. 5.4 are not unreasonable.

According to the Fig. 5.4, the lower mortality rate of H-Line lambs mainly hinged on improved resilience of low BW H-Line lambs during events of high chill. This reasoning supports previous observations of lamb viability in the H-Line, as reflected by a higher rectal temperature across the same cold stress gradient, after accounting for BW (Chapter 4: Nel *et al.* 2021a). It is also important to note that while the overall BW of H-Line lambs was heavier, the difference was of a small margin, and that there was no clear evidence of a direct genetic trend of H-Line lambs towards a higher BW (Chapter 3: Nel *et al.* 2021b). In accordance with results across breeds (Sykes *et al.* 1976; Samson and Slee 1981), these selection line differences suggested that it is possible to improve the relative resistance of lambs to severe cold stress without depending on an increase in BW. This is an important result, given the risks of higher BW lambs outlined above. It is also notable that previous results indicated that part of the improved performance of H-Line lambs stemmed from easier births (Cloete *et al.* 2021) and an increased survival of birth (Cloete and Scholtz 1998).

The apparent resilience to cold stress observed in the H-Line hinged on indirect selection for higher survival rates by applying traditional animal model methods to a correlated trait (Cloete *et al.* 2004), without considering the CI environmental descriptor. With a mean CI estimate of $964 \text{ KJm}^{-2}\text{h}^{-1}$ across the winter lambing seasons at Elsenburg, selection pressure during this period probably drove selection responses separating the H- and L-Lines. A contention that useful genetic variation in lamb mortality could be linked to cold stress adaption is well supported by the fact that cold stress resistance was affected by sire lines (Gudex *et al.* 2005) and separable performance has been reported in short term (4 yr.) selection experiments (Slee and Stott 1986). The few estimates of the h^2 of resistance to cold stress were estimated with a low accuracy and by outdated methods, but has been reported as moderate (0.44 ± 0.18 ; Wolff *et al.* 1987) to high (0.52 ± 0.23 ; Slee *et al.* 1991) after adjusting for BW. Given the clear link between neonatal mortality and the CI (Figs 5.3 and 5.4), it was expected that genetic adaptations favouring cold tolerance should benefit lamb survival, or in turn, selecting for survival necessitates animals well adapted to cope with cold stress. In this regard, it is interesting to point out that the initial (1986-1992) period of the study on Tygerhoek made use of an autumn lambing season where high CI-values were scarce, and that the H- and L-Line genetic trends for perinatal

survival visibly stabilised and diverged only from 1993-1995 and onwards (see Fig. 3.3 of Chapter 3: Nel *et al.* 2021b).

5.4.2 Genetic parameters for neonatal lamb mortality

Ranging between 0.02 and 0.03, the heritability for M3 was low in this study irrespective of the modelling approach. Derived from logit models, the heritability of perinatal survival to three days of age did not differ from zero (Everett-Hincks *et al.* 2014). Morris *et al.* (2000) reported another comparable estimate of 0.029 when also including BW and/or BW² as a covariate. A slightly higher range between 0.05 and 0.07 was reported by Maxa *et al.* (2009) for survival to 24 h, which was the largest literature estimates from logit analysis. From linear models, however, the overall h^2 of 0.03 ± 0.01 for perinatal survival (to 3 days of age, but also including survival at birth) derived from the animal model from the same flock was also low (Chapter 3: Nel *et al.* 2021b). A difference change in the observed σ_s^2 between models (ii) and (iii) was expected, given the divergent genetic trends for varied survival phenotypes between the H- and L-Lines (Cloete *et al.* 2009; Chapter 3: Nel *et al.* 2021b). The parameters derived from model (iii) are likely inflated estimates of σ_s^2 and h^2 compared to traditional flocks, but conversely, that derived from model (ii) would be deflated by accounting for genetic variance as genetic group effects. A true value from an unselected flock would likely range between the estimates derived from models (ii) and (iii).

A base level of mortality can always be expected (Alexander 1984), but in some incidences the genetic make-up of the lamb will have no influence (e.g. misadventure, predation). These phenotypes will be of little value or even decrease the accuracy of genetic evaluation. However, in environments defined by significant levels of cold stress (e.g. $1000 \text{ KJm}^{-2}\text{h}^{-1}$ or above), the selection line differences suggested that M3 could in fact be significantly determined by the genetic make-up of the lamb (Fig. 5.4) with phenotypes reflecting a test of the ability to cope with challenging levels of the CI. Expanding the fixed effects model to include CI information (Model (ii) vs (i)) slightly increased the estimate of genetic effects (σ_s^2 : 0.10 vs 0.07). Albeit by a small margin, taking the level of cold stress into account could be partly effective in standardizing the stress level and mortality rate across phenotypes measured at different CI-levels. The small increase in h^2 likely stemmed from accounting for the environmental influence that would otherwise have been covered by the year or residual random components. Also, the use of CI as a covariate cannot account for a GxE component that could be important to M3. It is evident from the current (Fig. 5.4) and previous (Chapter 4: Nel *et al.* 2021a) results that the differences between the H- and L-Lines is at least partially dependent on the CI being sufficiently high. If the expression of genetic merit depends on an environmental trajectory (such as CI) which varies across records, there will be a GxE component. However, the weighted merit of performance in moderate and stressful environments is considered equal in traditional models. This describes the general issue of GxE, which reduces responses to selection when breeding programs do not account for these effects (Mulder and Bijma 2005). If the environment at recording can be reliably quantified, the presence of GxE can be investigated by the 'reaction norm' approach (De Jong 1995; De Jong and Bijma 2002). Reaction norm models define breeding values as continuous functions of an environmental descriptor by random regression of genetic

effects across the environmental trajectory, such as a gradient of cold stress. This approach was investigated in preliminary analysis, but failed to converge on the logit-scale. In linear analysis, the low incidence of M3 caused scaling issues due to a very large proportional change in the mean mortality rate, from around 0.02 when conditions were mild to 0.15-0.20 at high CI-levels. These results were considered unreliable and were not reported. It would be of great interest to elucidate the H- and L-Line performance in cold stress resilience on the genetic level, since the potential of exploiting a GxE component in the selection experiment could at least provide a partial explanation for the divergent genetic trends reported for the H- and L-Lines despite the low h^2 reported here and in previous studies on the same flock (Cloete *et al.* 2009; Chapter 3: Nel *et al.* 2021b). The use of threshold models (e.g. Tsuruta *et al.* 2009) could be a viable alternative for future investigations.

5.4.3 Future perspectives

More elaborate studies are important, since the results reported here are relevant to the growing interest in selecting animals more resilient to the extreme environments anticipated from climate change (Hayes *et al.* 2013; Misztal 2017; Berghof *et al.* 2019). Studies investigating traits similar to M3 is complicated by low incidence, but climate data are commonly available and large-scale studies linking climatic stress to neonatal lamb performance should be feasible. Success on a large scale has been achieved elsewhere, as seen by examples estimating the effects of heat stress in dairy cattle (e.g. Ravagnolo and Misztal 2000, 2002; Bohmanova *et al.* 2008; Cheruiyot *et al.* 2020).

While the current study benefited from observations across the temporal axis of variation on the same site, studies across sites would be more susceptible to interactions between the stress gradient and characteristics of the site. The provision of shelter, for example, lowers mortality rates as much as 50% during severe cold exposure (Alexander and Lynch 1976). In turn, multiple improvements on the current design are also possible. A better phenotyping protocol was proposed by Gudex *et al.* (2005) that eliminates obvious causes of death not related to starvation-exposure-mismothering. Also, additional recording of phenotypes indicating animal well-being (Brito *et al.* 2020), while more expensive than survival records, could deliver multiple benefits in that they would be more articulate in indicating resilience; be more amenable to analysis by random regression models compared to M3 which is binary and of low incidence; and could benefit from the gains in accuracy of including genomic information (Mulder 2016). For example, rectal temperature (Chapter 4: Nel *et al.* 2021a) has been linked to the response of H-Line lambs under cold-stress conditions, and can be easily adopted into the lambing rounds that is standard protocol for breeding flocks which maintain a pedigree (Brien *et al.* 2010).

5.5 Conclusions

This study confirmed and expanded on previous findings on the same genetic resource and provided strong additional evidence that the improved survival rate in the H-Line is linked to resilience to cold stress. The evidence of a three-way interaction complicated findings and traits of very low incidence would benefit from greater statistical power enabled by very large datasets. The use of a cold stress covariable slightly improved the estimate of h^2 . It was recommended that the relationship between genetic merit and cold stress should be

further elucidated by applying a random regression model that would be capable of explicitly modelling GxE interactions.

5.6 References

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CHAPTER 6:

The accuracy of genotype imputation in selected South African sheep breeds using Australian reference panels[#]

Abstract

The cost of genotyping is becoming increasingly affordable but remains an influential factor for determining the SNP-density at which genotyping can proceed. Compared to Australian breeding programs, the South African wool sheep industry represents parallel objectives within similar environments but presently lacks the necessary infrastructure to exploit modern technologies such as genomic selection. The aim of the study was to determine the feasibility of across country imputation as an alternative to high density genotyping on a local basis. Following imputation from a 15K to 50K density, mean imputation accuracy levels of 0.87 and 0.85 were observed in the Merino and Dohne Merino breeds, while the highest levels of accuracy of 0.88 and 0.90 was observed in the Dorper and White Dorper breeds, respectively. The extent of genetic relationships was considered amongst the key factors that limit the ability to impute at an accuracy above 90%, but the observed results suggest that across country imputation could remain useful. Imputation from reference panels genotyped at densities higher than 50K and research into across country prediction is recommended.

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6.1 Introduction

Genomic prediction and Genome Wide Association Studies (GWAS) depend on the size of the reference population as well as the density at which informative individuals were genotyped. Even though medium and high-density genotyping options are becoming more affordable, cost remains a restricting criterion for the choice of a genotyping platform. Economic restrictions are likely to be more severe within a developing infrastructure as is currently experienced in South Africa (Van Marle-Köster and Visser 2018). There could be potential to exploit similarities in South African and Australian ovine breeds and environments through the compilation of genotypic resources. Imputation of un-typed markers of animals genotyped at a lower density has proven a reliable and affordable alternative to widespread genotyping on high density platforms (Browning and Browning 2007; Berry and Kearney 2011; Hickey *et al.* 2011; Huang *et al.* 2012; Moghaddar *et al.* 2015). The objective of this study is thus to investigate the potential of across country imputation of South African datasets from Australian reference populations from low (15K) to medium (50K) densities.

6.2 Materials and Methods

6.2.1 Data Structure and Distribution

The South African sample set was selected from multiple breeds within respective resource flocks (Schoeman *et al.* 2010) as well as a smaller proportion of animals originating from the industry sector. Genotyping of the South African (SA) sample set was performed with the OvineSNP50 (Illumina Inc., CA, USA) beadchip at GeneSeek Inc. (Lincoln, NE, USA) and subjected to quality control measures (> 0.25 GenCall score, > 0.5 GenTrain score, > 0.01 MAF, > 0.95 call rate, > 0.95 sample call rate). Following imputation of randomly missing SNPs, 986 samples with 50095 SNPs remained available for further analysis. Animals were grouped by breed type, namely Merino (552), Dohne Merino (60), Dorper (59), South African Mutton Merino (57), Dormer (42), Meatmaster (39) and White Dorper (27) while the hardy native breeds Damara (30), Pedi (29) and Afrikaner-type (13) animals were grouped together as 'Indigenous' (72). The Australian reference set constituted a database of ~ 84 000 samples from multiple breeds that serve as respective reference populations in genomic prediction programs. The major proportions of the dataset were classified as Merino, maternal (Border Leicester and Coopworth) and terminal (Poll Dorset and White Suffolk) groups. The same OvineSNP50 genotyping platform was used in generating the Australian database and 48 599 SNPs were available for analysis following quality control.

6.2.2 Design

All 986 SA samples were subset to ~ 15K SNPs using Illumina map information to simulate a commercial 15K beadchip. Analysis proceeded by the subsequent imputation back up to the 50K density using an Australian reference. The accuracy of imputation was evaluated by Pearson correlation coefficients between the imputed and observed SNP genotypes identified according to the 0/1/2 scoring system. To reduce computation time and increase accuracy (Moghaddar *et al.* 2015), the Australian reference set was screened by assigning an animal in the sample set with the top 50 highest values of animals in the reference set according to a genomic relationship matrix (GRM) that included all the animals in the study. Thus, animals from the

reference set not meeting this criterion for any of the animals in the sample set were not used for imputation.

6.2.3 Software

Genotype imputation was performed using FIMPUTE (V2.2) (Sargolzaei *et al.* 2014). The program assumes a level of relatedness between all individuals and phases reference sets with overlapping sliding windows that are shrunk in successive increments with each chromosome sweep. The initial larger window sizes aim to capture the long-range haplotypes expected from highly related individuals, while the subsequent sweeps aim to capture relationships between more distant individuals. The inclusion of pedigree information is an optional addition to FIMPUTE, but it was not supplied in the current analysis. Summary statistics and visual analyses were performed in R (R Core Team 2016, Vienna, Austria)

6.3 Results

The accuracy of imputation varied considerably both between and within breeds. The accuracy of imputation for indigenous breed group was very low (mean = 0.68) and is not represented in subsequent figures and tables. A low accuracy is to be expected considering their heterogeneous nature and poor representation within the Australian reference set. Moreover, concerns have been raised surrounding an underrepresentation of indigenous breeds in the design of commercial bead chips (Sandenbergh *et al.* 2016). Table 6.1 shows the summary statistics for imputation accuracy (correlation coefficients) for the remaining breed groups.

Table 6.1

Summary statistics for the imputation accuracy of all South African breed groups in the sample set

	Merino	Dohne Merino	Dorper	SAMM	Dorper	Meat - master	White Dorper
(n)	552	60	59	57	42	39	27
Min.	0.82	0.82	0.85	0.81	0.76	0.72	0.87
1 st Quartile	0.86	0.85	0.87	0.83	0.78	0.74	0.89
Mean	0.87	0.85	0.88	0.85	0.79	0.75	0.90
3 rd Quartile	0.87	0.86	0.89	0.86	0.79	0.76	0.91
Max.	0.90	0.88	0.90	0.87	0.81	0.78	0.92

The accuracy for Merino samples was moderate, as Pearson's correlations ranged from 0.82 to 0.90. This is considerably lower than correlation coefficients of 0.93 to 0.96 reported by Moghaddar *et al.* (2015) for 1000 purebred Merinos imputed from smaller proportions of the same reference set. Hayes *et al.* (2012) reported considerably lower values of accuracy (71%) for imputing Merino samples from 5K to 50K densities, but with a reference set confined to ~ 5000 animals. Moderate accuracies were also observed for Dohne Merino and the South African Mutton Merino (SAMM) individuals, while the imputation accuracy for Dormers and Meatmasters were below 0.80.

The Dorper and White Dorper breeds achieved moderately-high to high imputation accuracies (Fig. 6.1). The Dorper originates from South Africa, and it is possible that the animals that represent them in the

Australian database have not drifted extensively from the ancestral lines or share relatively recent parental links. Considering the size of the Australian reference set and the large proportion of Merinos included, it could be considered somewhat unexpected that none of the Merino test samples attained an imputation accuracy of above 0.90. However, both the number of reference samples available as well as their relatedness to the sample population are considered essential factors in the accurate phasing of haplotypes for the imputation of un-typed markers.

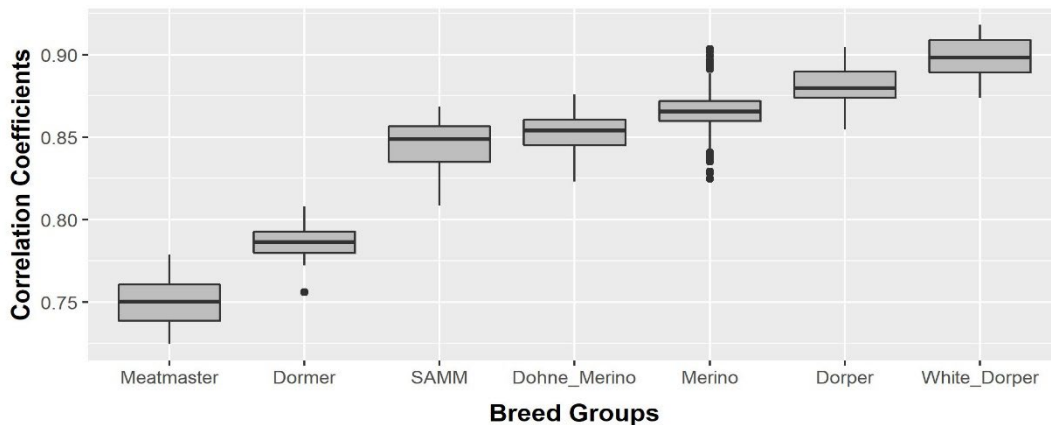


Fig. 6.1 Box plots for the imputation accuracies for all South African breeds in the sample set

Analyses that characterize haplotypes using population linkage disequilibrium (LD) based methods do not utilize a pedigree, but indirectly capture patterns associated with identity by descent (IBD), the accuracy of which is complemented by the indication that there is little benefit in including pedigree data if the reference set is large enough (Browning and Yu 2009; Larmer *et al.* 2014; Moghaddar *et al.* 2015). Hayes *et al.* (2012) proposes that haplotypes are not necessarily shared across breeds and that 50K genotyping platforms do not capture LD to an adequate level for across breed application. With markedly less family linkage, the proportion of genomic regions possibly considered IBD should be markedly smaller when attempting across country imputation. Thus, a similar argument to that proposed by Hayes *et al.* (2012) could be extended to the current results, resulting in poor imputation accuracy despite being within breed analysis. It is possible the denser 500K platform could provide improved phasing of the reference set that is less dependent on long range haplotypes and more appropriate for capturing linkage disequilibrium observable over distant populations.

6.4 Conclusions

Genotype imputation of un-typed markers in a population depends on the representation of that population within a reference set. There is little benefit in the addition of genetically dissimilar animals. Across country imputation will likely be limited by a lack of direct genetic links, but moderately high levels of accuracy can still be achieved within breeds. A slightly higher accuracy would be desired for reliable implementation of this design. Research into across country genomic prediction for shared breeds is recommended.

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CHAPTER 7:

The genomic structure of isolation across breed, country and strain for important South African and Australian sheep populations[#]

Abstract

South Africa and Australia share multiple important sheep breeds. For some of these breeds, genomic breeding values are provided to breeders in Australia, but not yet in South Africa. Combining genomic resources could facilitate development for across country selection, but the influence of population structures could be important to the compatibility of genomic data from varying origins. The genetic structure within and across breeds, countries and strains was evaluated in this study by population genomic parameters derived from SNP-marker data. Populations were first analysed by breed and country of origin and then by subpopulations of South African and Australian Merinos. Mean estimated relatedness according to the genomic relationship matrix varied by breed pair (-0.11 to 0.16) and bloodline pair (-0.08 to 0.06) and depended on historic co-ancestry as well as recent genetic links. Measures of divergence across bloodlines (F_{ST} : 0.04 – 0.12) were sometimes more distant than across some breeds (F_{ST} : 0.05 – 0.24), but the divergence of common breeds from their across-country equivalents was weak (F_{ST} : 0.01 – 0.04). According to mean relatedness, F_{ST} , PCA and Admixture, the Australian Ultrafine line was better connected to the SA Cradock Fine Wool flock than with other AUS bloodlines. Levels of linkage disequilibrium (LD) between markers 55kb apart was generally low, but also varied across breeds (r^2 : 0.14 – 0.22) as well as bloodlines (r^2 : 0.15 – 0.19). Patterns of LD decay were also unique to breeds, but much more uniform for bloodlines. Estimates of effective population size (N_e) showed genetic diversity to be high for the majority of breeds (N_e : 128 – 418) but also for bloodlines (N_e : 137 – 369). This study reinforced the genetic complexity and diversity of important sheep breeds, especially the Merino breed. The results also showed that implications of isolation can be highly variable and extended beyond breed structures. However, knowledge of useful links across these population substructures allows for a fine-tuned approach in the combination of genomic resources. Isolation across country rarely proved restricting compared to other structures considered. Consequently, research into the accuracy of across-country genomic prediction is recommended.

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7.1 Introduction

In South Africa (SA) and Australia (AUS), sheep production is dominated by a few major breeds. In SA, wool- and dual-purpose breeds such as the Merino, Dohne Merino and the SA Mutton Merino (SAMM) contributed nearly 70% of all weaning weight records submitted to the National Small Stock Improvement Scheme between 2010 and 2011 (Cloete *et al.* 2014). In AUS, Australian Sheep Breeding Values are predominantly defined by three major branches as Merino, maternal (majority Border Leicester and Coopworth) and terminal (majority Poll Dorset and White Suffolk) breed groups (Brown *et al.* 2018). Several breeds are in common between the SA and AUS sheep industries. The AUS and SA Merino share a distant relationship from the original Merino thought to have originated from Spain around the 1700s (Diez-Tascón *et al.* 2000) and the AUS Merino was originally a composite of European, Asian and African strains (Meadows *et al.* 2008). Besides this ancestral relationship, a SA Merino resource flock has had intermittent genetic links to the AUS Merino by using sires originating from AUS (Schoeman *et al.* 2010), but this is the only known genetic exchange in recent years. Additional breeds in common include the South African Meat Merino (SAMM) pure breed and composites like the Dohne Merino and the Dorper meat breeds (Schoeman *et al.* 2010) which originated in South Africa and were exported to Australia.

The use of genomic information to increase the accuracy of Australian Sheep Breeding Values has been well established (Brown *et al.* 2018), but is still to be developed for South African sheep (Van Marle-Köster and Visser 2018). By establishing well-recorded reference populations, genomic tools could facilitate the inclusion of hard-to-measure traits by predicting the genetic merit of animals without phenotypes. This is of specific interest in the challenging environment underlying SA sheep production (Cloete *et al.* 2014). With breeds in common across countries, there is potential to facilitate further development by combining genomic resources, but this would hinge on the compatibility of genomic pools of varying origin.

Population genetic parameters such as linkage disequilibrium (LD) and effective population size (N_e) describe the diversity of populations and are important factors in determining the accuracy of genomic selection (Goddard 2009). The more diverse populations are, the higher the sample size required to reach set levels of accuracy (Goddard and Hayes 2009). The level of LD, in turn, can be directly related to the level of inbreeding (Hill and Robertson 1968) and studies have emphasized the positive effect of relatedness between sample animals and reference populations (Clark *et al.* 2012; Van der Werf *et al.* 2015). Livestock breed formation is associated with restricted gene flow and different breeds are generally connected by weak, distant relationships. Results from multi-breed reference populations (Moghaddar *et al.* 2013) suggest that additional genotypes might not be beneficial, or could even be detrimental if they originate from a distant group. This is of interest currently, since the implications of isolation ‘within breed’ - such as separation across country, strain, or flock - could be analogous to the ‘across breed’ situation, depending on the extent to which gene flow is restricted. However, the complicated genetic background of sheep (Kijas *et al.* 2012) implies that genetic connectedness could be highly variable and rely on the unique population histories of each case of isolation between two groups.

Given the influential role of the Merino breed in both AUS and SA, a combined reference population for Merino sheep is of specific interest, but requires consideration of population structure. This breed has been

characterised by a diverse genetic foundation using marker data (Kijas *et al.* 2012; Al-Mamun *et al.* 2015; Ciani *et al.* 2015; Prieur *et al.* 2017). Specifically, the AUS Merino consists of strong substructures, termed ‘lines’ or ‘strains’, originally classified partially by performance in key economic traits, including body weight, fleece weight, and wool fibre diameter (Swan *et al.* 2014). Historically, they were separated by production environments and selection objectives, but the relatively recent implementation of technologies like artificial insemination has facilitated across population genetic links (Swan *et al.* 2016). Genetic variation across these AUS Merino lines derived by both quantitative analysis (Swan *et al.* 2016) and genomic data (Brown *et al.* 2013; Swan *et al.* 2014; Gurman *et al.* 2017) suggested an important role of these lines/strains in the genetic architecture of the AUS Merino. In turn, South Africa hosts multiple Merino resource flocks with widespread recording for hard-to-measure traits (Schoeman *et al.* 2010; Cloete *et al.* 2014) that could be a valuable contribution to a reference population enhancing prediction for SA industry animals. However, these flocks tend to be managed as selection experiments with a low genetic exchange with the wider industry. Also, since only selected flocks have recent links to specific bloodlines within the AUS Merino, the across-country relationships would be variable across subpopulations within SA and AUS Merinos.

The SA and AUS sheep populations are thus important examples of segregated genetic groups with varying histories that determine their linkage. While exact breeding histories are not known, modern genotyping platforms that make use of Single Nucleotide Polymorphisms (SNP) allow for informative estimates of genetic variation and relatedness across and within population groups, including examples related outside of pedigrees.

The objective of this study was to quantify a range of population genomic parameters from a medium density (50K) SNP panel to characterize genetic variation between and within subpopulations defined by different hierarchical structures such as breed, country, bloodline or flock of origin. The results should contribute to the knowledge of the genetic make-up underlying these sheep populations and could also be used to facilitate the combining of genomic resources between AUS and SA, with a particular interest in building toward across country genomic prediction of Merinos.

7.2 Materials and methods

7.2.1 Sample populations

This study examined 3509 genotypes, which are summarised by breed and country in Table 7.1. The selected AUS genotypes in this study were a group of samples used as training animals for supervised ADMIXTURE (Alexander *et al.* 2009) analysis in a previous study (Gurman *et al.* 2017). The genotypes selected by Gurman *et al.* (2017) were intended to capture the diversity in the Australia sheep population. The SA samples were obtained from resource flocks maintained by institutions across the Western Cape and Eastern Cape provinces of South Africa (Schoeman *et al.* 2010) as well as industry animals from commercial lines whose records are managed by the National Small Stock Improvement Scheme. The latter group of commercial South African genotypes are herewith referred to as ‘Industry’. For this study, the non-Merino SA breed groups originated from either industry or resource flocks: Dorper (Nortier = 20, Industry = 66), Dohne Merino (Industry = 39, Langgewens = 12, Elsenburg = 9), SAMM (Industry = 36, Nortier = 21), and Dormer (Industry = 32, Elsenburg

= 10) were pooled under their breed name. Additionally, SA and AUS samples within the Merino breed were subset according to bloodline and/or the flock of origin and separately analysed as a set of Merino subpopulations (Table 7.1). The SA Merinos were separated on a resource flock level and the AUS Merinos by strain (or line), but the subpopulations from both SA and AUS are henceforth collectively referred to as ‘bloodlines’ for simplicity.

The AUS Merinos are historically grouped into the bloodline of origin, partly by performance in fibre diameter, but also by region, with the ‘fine’-type strain popular in high rainfall zones, the ‘medium’ in cropping zones and the ‘strong’ more common in drier pastoral areas (Swan *et al.* 2016). The Elsenburg Merino flock is a resource flock that is divergently selected for reproductive performance by the number of lambs weaned per ewe mated. The flock is subset into two lines, the H-Line (positive selection) and the L-Line (negative selection) (Cloete *et al.* 2004), and was maintained as an isolated group from 1986 until the first inclusion of external sires in 2008 (Cloete *et al.* 2017). The Grootfontein Merino stud is a research flock managed according to commercial objectives and is the most traditional representation of South African commercial lines by a resource flock. It was subjectively selected for ‘overall excellence’ from 1968 to 1985 (Olivier *et al.* 1995) whereafter objectives changed to increasing live weight and decreasing fibre diameter while maintaining fleece weight (Snyman *et al.* 1998). The Cradock fine wool Merino stud was established in the 1980s when Merino ewes of different flocks were screened into a central flock and mated to four AUS fine wool sires for two years. This was followed by the using two more AUS sires in 1996 and another two ‘Ultrafine’ rams in 2002-2003 (Olivier 2014). Selection objectives were first defined by increased live and fleece weight from 1988 to 1996 followed by an increased emphasis on reducing fibre diameter since 1996.

7.2.2 Experimental design

Analyses were firstly conducted for ($n = 13$) subpopulations defined by breed and country of origin (for common breeds), with combinations examined. Second, analyses were repeated only for Merinos with populations defined by the ($n = 9$) bloodlines as described above. However, the ‘Langgewens’ group was often excluded from analyses due to the small number of genotypes, and the fact that this small subgroup was not pertinent to the outcome of the study as representing SA Merinos.

7.2.3 Genotyping and quality control

Genotyping was performed using the OvineSNP50 Chip (Illumina Inc., CA, USA). Quality control measures were applied to SNPs (GenCall score > 0.25 , GenTrain score > 0.5 , MAF > 0.01 , call rate > 0.95) and samples (call rate > 0.95). Only SNPs on autosomal chromosomes were retained. Randomly missing SNPs were imputed with ‘FIMPUTE’ (Version 2.2) (Sargolzaei *et al.* 2014) and ‘Beagle’ (Version 4.0) (Browning and Browning 2016) software for SA and AUS samples, respectively. Following quality control and imputation, the combined dataset consisted of 47,789 markers on 3,509 animals, with an average marker spacing of roughly 55 kilo-basepairs (KB). Genotypes were called according to the AB system and numerically coded according to B-Allele content (i.e., 0, 1 or 2) unless other formats were required.

7.2.4 Genomic analysis

Expected and observed levels of heterozygosity were reported as summary statistics of overall gene diversity. Proportional differences in frequencies between subpopulations were represented by F_{ST} statistics as measures of divergence. Relationships based on the genomic relationship matrix (GRM) (VanRaden 2008) and decomposition of the GRM by principal component analysis (PCA) were used to investigate relatedness between individuals across and within population groups. Additionally, ‘ADMIXTURE’ (Alexander *et al.* 2009) was used to estimate the extent to which individuals identify to the same ancestry based on shared chromosome segments. Linkage disequilibrium (LD) was used to express the correlation between markers in a discrete population and was also used to estimate recent and historical effective population size (N_e). Relationships between more closely linked markers tend to reflect deeper histories since it would require an increasing number of generations for recombination to segregate markers more closely linked (Hill 1981). Furthermore, Hayes *et al.* (2003) showed that the relationship between markers at a given distance can be used to estimate N_e at a given number of generations ago, which can be used to deduce the population history by trends in the effective population size. Unless stated otherwise, plots were prepared in the ‘ggplot2’ ‘R’ package (Wickham 2016).

7.2.5 Heterozygosity and F -statistics

The expected (H_{exp}) and observed heterozygosity (H_{obs}) were calculated for each marker. The mean of all markers and the respective standard deviations across markers were reported for all population groups. H_{exp} was calculated as expected under Hardy-Weinberg equilibrium: $H_{exp} = 2p_i(1 - p_i)$; where p_i is the frequency of the i^{th} allele in a defined population. Genetic distances between populations were determined by pairwise F_{ST} statistics which was calculated by deriving a , b , and c according to Weir and Cockerham (1984) and summing variance components across $i=1, \dots, n$ markers separately:

$$F_{ST} = \theta_W = \frac{\sum_i a_i}{\sum_i (a_i + b_i + c_i)}$$

All of these analyses were performed using custom scripts in ‘R’ (Version 3.6.1; see Supplementary Information – R Scripts) (R Core Team 2020).

7.2.6 Genomic relationships, inbreeding and principal component analysis

Genetic relationships were examined by calculating the genomic relationship matrix (GRM) in R according to VanRaden (2008):

$$\mathbf{G} = \frac{(\mathbf{M} - 2\mathbf{P})(\mathbf{M} - 2\mathbf{P})'}{2 \sum_j (\mathbf{p}_j)(1 - \mathbf{p}_j)}$$

where \mathbf{M} is the genotypic matrix of marker counts with dimensions $m \times n$ (number of genotyped animals, m , by number of markers, n) and \mathbf{P} is the corresponding matrix ($m \times n$) where the j th column of \mathbf{P} is a vector of m replicates of the allele frequency p_j within all the animals in the analysis. The diagonal of the GRM was

used to calculate genomic inbreeding coefficients (F_{VR}) as $G_{ii} - 1$, with the population inbreeding reported as the mean of the inbreeding coefficients. The overall relatedness between or within populations was calculated as the mean of the off-diagonal of the GRM for the two groups of animals. For breed group comparisons, a GRM was calculated with all 3509 animals as G , while Merino bloodline comparisons were derived from a GRM that only included the 1535 animals of Merino origin as G_M . These values were visualized using the ‘corrplot’ R package (Wei and Simko 2017) with the diagonal of the plot replaced by mean relatedness to all other animals in the GRM, but excluding internal relatedness to animals of the same group. A visual analysis of genetic relationships between and within populations was also performed by visualization of the results of a Principal Component Analysis (PCA) of the GRM calculated using the ‘irlba’ ‘R’ package (Baglama *et al.* 2019). The first 40 principal components were calculated for both GRMs. In both cases, the first fourteen components (PC1 to PC14) were visualized to investigate between and within-group clustering. The cumulative proportion of variance (POV) explained by the first 40 principal components was also plotted.

7.2.7 ADMIXTURE analysis

Genetic group proportions and ancestry for each of the samples were estimated by unsupervised runs of ADMIXTURE with a varying number of groups, K . ADMIXTURE produces a cross-validation statistic, ‘CV error’, as an indication of accuracy relative to the choice of the number of groups, K . The lowest CV error is considered the ‘best’ choice of K for determining proportions of shared chromosome segments from co-ancestry. ADMIXTURE analysis was repeated for $K = 3$ to $K = 20$ and results of $K = 3, 5, 7$ and 10 were chosen for visualization by stacked bar plots. This entailed plotting the proportions of each genetic group (Q) within an individual animal by a stacked bar graph. The CV error statistic for K between 3 and 20 was also plotted.

7.2.8 Linkage disequilibrium and effective population size

Linkage disequilibrium (LD) between any two markers was calculated by:

$$r^2 = \frac{(p_{AB} - p_A p_B)^2}{p_A(1 - p_A)p_B(1 - p_B)}$$

where subscripts ‘A’ and ‘B’ is used to distinguish between the two pair-wise markers of a single r^2 estimate (i.e. p_A is the B-allele frequency of the first marker, ‘A’, and p_B is the B-allele frequency of the second marker, ‘B’) and p_{AB} is the frequency of the two B-alleles of the two pair-wise SNPs inherited together in a population. Pairwise LD was calculated at a depth of 400 markers using the ‘snpStats’ R package (Clayton 2019) (for each marker LD was calculated for the 400 markers that succeed it in genome order).

For the syntenic LD, the original genotypes involving the initial 47,789 markers were subset to only contain those needed to compute syntenic pairwise comparisons, leaving 400 pairwise LD estimates each for 37,789 markers. Subsequently, the means for pairwise LD estimates were used to determine mean LD for adjacent markers and subsequent LD decay using LD from SNP pairs of greater depth (gaps of 1 to 400 SNPs). A physical distance was assumed for LD decay by multiplying the depth of pairwise SNPs with the mean

distance between adjacent SNPs (~ 55 KB). No attempt was made to estimate LD at intervals shorter than ~ 55 KB spacing, as this would rely on much fewer SNPs that are only sporadically associated by shorter distances. The LD procedure was repeated with a more stringent filter on MAF (> 0.1), but effects were negligible (results not shown). The pattern of LD decay over distance was plotted for breed and bloodline groups. For non-syntenic LD, results were subset to include only pairwise comparisons from different chromosomes and a single mean was calculated as there was no need to observe non-syntenic decay over distance.

To investigate the effect of sample size on LD estimates, the procedure for non-syntenic LD was repeated for a range of sample sizes ($N = 2^n$ where $n = 1$ to 11.75) where a given population at given sample size N is sampled from the full genotype matrix at random.

LD estimates were used to calculate historical effective population size (Ne_t) as:

$$Ne_t = \frac{(1 - r^2)}{4 c r^2}$$

where Ne_t is the effective population size at t generations ago and r^2 is the calculated LD at each value of c , the recombination rate in Morgans. The values of Ne_t were calculated on the assumption of 1 Morgan = 100 million base pairs (MB) and $t = \frac{1}{2c}$ (Hayes *et al.* 2003).

7.3 Results

7.3.1 Heterozygosity and inbreeding

The average expected (H_{exp}) and observed (H_{obs}) levels of heterozygosity were moderately variable across breed groups, but across-country differences of common breeds were small (Table 7.1). H_{exp} ranged from 0.33 for the Border Leicester to 0.38 for the AUS Merino. H_{obs} ranged between 0.32 for the Border Leicester and 0.37 for the Coopworth, Corriedale and White Suffolk. Across Merino bloodlines, H_{exp} ranged between 0.34 for Fine-Medium-2 to 0.37 for the Ultrafine and Grootfontein lines, a narrower range than that across breed groups. Bloodlines displayed similar H_{obs} with all means between 0.36 to 0.37.

The mean level of inbreeding (F_{VR} ; derived from the diagonal of \mathbf{G}) varied considerably across breed groups (Table 7.1). The F_{VR} of AUS and SA Merinos and SA Dohne Merino was lowest (0.09). In turn, the SA Dorper and SA Dormer displayed the highest F_{VR} (0.21), while F_{VR} -values of the AUS Border Leicester (0.20) and Poll Dorset (0.19) were also high. A narrower range (0.02 to 0.08) of generally low F_{VR} (derived from the diagonal of \mathbf{G}_M) values was observed across the Merino bloodlines (Table 7.1). However, most groups consisted of a wide range of individual F_{VR} estimates indicating that animals lowly inbred (or outbred) and highly inbred is common in most populations.

Table 7.1

Marker heterozygosity (H_{exp} and H_{obs}) ($\pm SD$) and genomic inbreeding ($F_{VR} \pm SD$) for breed and Merino bloodline groups for respective countries

Population		<i>N</i>	$H_{exp} (\pm SD)$	$H_{obs} (\pm SD)$	F_{VR}		
<i>Breed</i>	<i>Country</i>				<i>Min</i>	<i>Mean ($\pm SD$)</i>	<i>Max</i>
Merino		918	0.38 ± 0.12	0.36 ± 0.12	-0.15	0.09 ± 0.04	0.25
Dohne Merino		30	0.36 ± 0.14	0.36 ± 0.16	0.06	0.11 ± 0.03	0.20
Dorper		276	0.35 ± 0.14	0.35 ± 0.14	0.10	0.17 ± 0.04	0.30
SAMM		14	0.36 ± 0.14	0.36 ± 0.17	0.02	0.17 ± 0.06	0.28
Border Leicester	AUS	542	0.33 ± 0.15	0.32 ± 0.15	0.14	0.20 ± 0.03	0.32
Coopworth		114	0.35 ± 0.14	0.37 ± 0.15	0.07	0.13 ± 0.04	0.26
Corriedale		26	0.37 ± 0.13	0.37 ± 0.15	0.06	0.11 ± 0.03	0.18
Poll Dorset		400	0.34 ± 0.15	0.35 ± 0.15	0.10	0.19 ± 0.03	0.31
White Suffolk		247	0.37 ± 0.13	0.37 ± 0.14	0.02	0.12 ± 0.04	0.32
Merino		697	0.37 ± 0.13	0.36 ± 0.13	0.00	0.09 ± 0.04	0.26
Dohne Merino		60	0.37 ± 0.13	0.37 ± 0.14	0.06	0.09 ± 0.02	0.14
Dorper	SA	86	0.35 ± 0.14	0.34 ± 0.14	0.15	0.21 ± 0.03	0.31
SAMM		57	0.35 ± 0.14	0.35 ± 0.15	0.13	0.18 ± 0.03	0.29
Dorner		42	0.34 ± 0.15	0.34 ± 0.16	0.15	0.21 ± 0.03	0.27
<i>Bloodline</i>	<i>Country</i>						
Ultrafine		270	0.37 ± 0.13	0.37 ± 0.13	-0.17	0.08 ± 0.03	0.19
Fine-Medium-1	AUS	224	0.35 ± 0.14	0.37 ± 0.15	-0.17	0.07 ± 0.04	0.24
Fine-Medium-2		133	0.34 ± 0.15	0.37 ± 0.17	-0.01	0.05 ± 0.05	0.22
Strong		291	0.35 ± 0.14	0.36 ± 0.15	-0.16	0.07 ± 0.03	0.17
Elsenburg		400	0.36 ± 0.14	0.36 ± 0.14	-0.04	0.03 ± 0.04	0.16
Langgewens		14	0.36 ± 0.13	0.39 ± 0.18	-0.03	0.02 ± 0.05	0.15
Grootfontein	SA	115	0.37 ± 0.13	0.37 ± 0.14	-0.01	0.06 ± 0.04	0.22
Industry		41	0.36 ± 0.14	0.36 ± 0.15	0.00	0.07 ± 0.04	0.16
Cradock		127	0.36 ± 0.13	0.37 ± 0.14	0.02	0.06 ± 0.02	0.14

7.3.2 Pairwise F_{ST} statistics

The F_{ST} measure of population divergence also varied considerably across pairwise comparisons of breed groups (Table 7.2). The genetic distance of breed groups to their across-country counterparts was small with F_{ST} values of 0.01, 0.02, 0.03 and 0.04 for the Dorper, SAMM, Dohne Merino and Merino, respectively. Across breeds, low F_{ST} values were observed between Merino and Dohne Merino breeds in both SA and AUS (0.05-0.06). With consistently high F_{ST} estimates, the Border Leicester and Poll Dorset breeds diverged highly from all SA populations reported here, but a similar pattern was observed in relation to AUS breed groups (Table

7.2). The Coopworth and White Suffolk breeds were also distant across most comparisons, but to a lesser extent.

Table 7.2

Genetic divergence as pairwise F_{ST} estimates between breed groups across and within Australia and South Africa

<i>Across Country</i>		M	DM	DR	SAMM	BL	COO	COR	WS	PD
Australian										
Merino		0.04	0.07	0.13	0.09	0.19	0.13	0.08	0.11	0.17
Dohne Merino	SA	0.05	0.03	0.13	0.07	0.20	0.13	0.08	0.11	0.18
Dorper		0.11	0.14	0.01	0.15	0.23	0.16	0.12	0.12	0.16
SAMM		0.08	0.09	0.16	0.02	0.23	0.16	0.11	0.13	0.20
Within Country										
Merino			0.06	0.11	0.08	0.17	0.12	0.06	0.09	0.15
Dohne Merino			-	0.15	0.09	0.22	0.15	0.09	0.12	0.19
Dorper			-	-	0.16	0.23	0.17	0.13	0.12	0.16
SAMM			-	-	-	0.23	0.16	0.10	0.13	0.20
Border	AUS		-	-	-	-	0.12	0.14	0.18	0.24
Leicester			-	-	-	-	-	-	-	-
Coopworth			-	-	-	-	-	0.09	0.12	0.19
Corriedale			-	-	-	-	-	-	0.09	0.16
White Suffolk			-	-	-	-	-	-	-	0.11
South African										
Merino			0.05	0.12	0.10					
Dohne Merino	SA		-	0.13	0.07					
Dorper			-	-	0.15					

Pairwise F_{ST} values (Table 7.3) showed that the Merino bloodlines were defined by a strong structure. However, evidence of a pattern of higher divergence across bloodlines from different countries was weak, such that the Strong and both 'Fine-Medium' lines were as diverged from their AUS contemporaries as from the SA groups. The largest F_{ST} distance was between the Strong and Fine-Medium-2 lines (0.12) and the smallest between the Industry and Grootfontein lines (0.04). All the comparisons within SA-only bloodlines were below 0.08. Of note was the low across-country estimate observed between the Cradock and Ultrafine lines (0.05). Also, most of the within-country AUS relationships between bloodlines were between 0.08 and 0.11, a magnitude similar to some F_{ST} values observed across breeds (Table 7.2).

Table 7.3Genetic divergence as pairwise F_{ST} estimates between Merino bloodline groups

Country		SA				AUS		
		Grn	Industry	Cradock	Ultrafine	FM-1	FM-2	Strong
SA	Elsenburg	0.06	0.06	0.08	0.07	0.10	0.11	0.11
	Grootfontein	-	0.04	0.05	0.06	0.09	0.10	0.10
	Industry	-	-	0.06	0.06	0.09	0.10	0.10
	Cradock	-	-	-	0.05	0.09	0.10	0.11
AUS	Ultrafine	-	-	-	-	0.08	0.09	0.09
	Fine-Medium-1	-	-	-	-	-	0.11	0.10
	Fine-Medium-2	-	-	-	-	-	-	0.12

7.3.3 Genomic relatedness

Internal and across population relatedness was only reported for a subset of breed groups. Preference was given to include Merino and Merino types, breeds in common across countries and groups that represent the other major branches of AUS terminal and maternal types. Groups varied considerably in their levels of internal relatedness, determined by the mean (excluding diagonal) genetic relationship of animals to their contemporaries in the same breed or bloodline group (Table S2). The AUS Merinos, on average, were lowly related (0.09), which was in strong contrast to the Border Leicester group (0.44). In the mean relatedness across groups, most relationships ranged around 0. Exceptions were the cross-country comparisons for the Dohne Merino, SAMM and Dorper breeds where mean levels of 0.14, 0.27 and 0.28 were observed, the latter two results being particularly high. Measures involving the Border Leicester made up both the highest (0.16, Coopworth) and lowest (-0.11, SA Merino) result across different breeds. Discernable structures, or across breed ‘families’ were present within the relationships across breeds (Fig. 7.1). A lowly positive block separated Merino and Merino-like breeds, but the close relationships between some dual-purpose breeds were particularly apparent. The SA Dormer terminal sire breed bridged the Merino related groups to the pure meat-type Dorper, which grouped with the Poll Dorset and White Suffolk.

According to G_M , animals in the Ultrafine (0.10) and the SA lines (0.11 to 0.14) were lowly related within their populations (Table S3). The Fine-Medium-2 line had the highest internal relatedness at 0.25, while high values of 0.18 and 0.20 were also observed for Fine-Medium-1 and the Strong line, respectively. The mean relationships across bloodlines in G_M are presented in Fig. 7.2. The only positive relationships are amongst SA groups with the notable exception of a positive relationship between the AUS Ultrafine and SA Cradock lines. Notably, this across-country relationship is the only positive estimate observed for the Ultrafine group. The remaining relationships, as well as the comparisons among AUS lines in general, were all lowly negative.

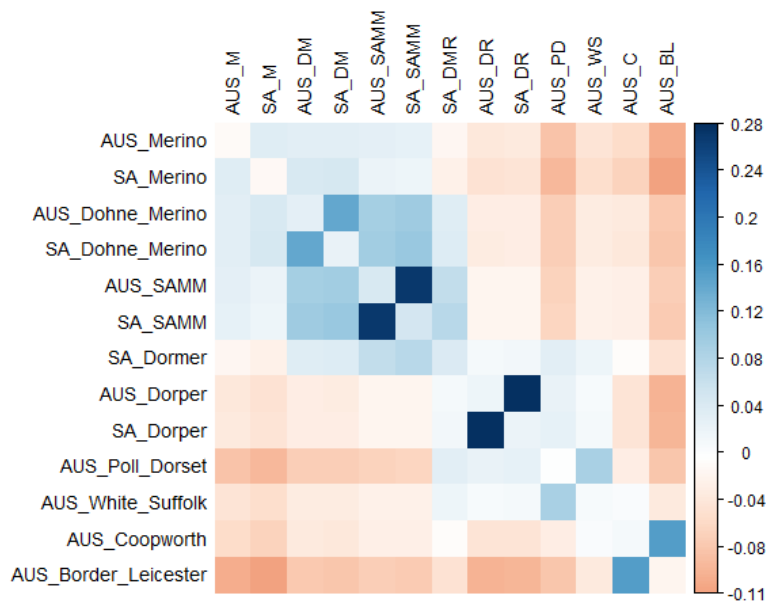


Fig. 7.1 Pairwise estimates of mean relatedness of breed groups according to. The diagonal of the Fig. was replaced by a vector containing the mean relatedness to all other groups. Column abbreviations are a concatenation of country and breed as M: Merino, DM: Dohne Merino, DMR: Dorper, DR: Dorper, PD: Poll Dorset, WS: White Suffolk, C: Coopworth, BL: Border Leicester.

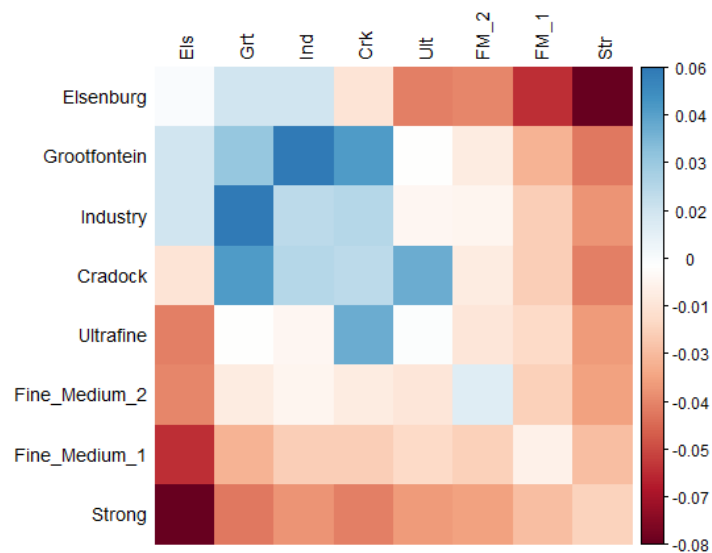


Fig. 7.2 Pairwise estimates of mean relatedness of Merino bloodline groups according to G_M . The diagonal of the Fig. was replaced by a vector containing the mean relatedness to all other groups. Column abbreviations of bloodlines are Els: Elsenburg, Grt: Grootfontein, Ind: Industry, Crk: Cradock, Ult: Ultrafine, FM_2: Fine_Medium_2, FM_1: Fine_Medium_1, Str: Strong.

7.3.4 Principal component analysis and ADMIXTURE

A decomposition of the GRM into its initial principal components provided a clear distinction between animals by breed structure (Fig. 7.3). Plotting against the first (PC1) and second (PC2) principal components separated all breeds except the Merino and Merino-like breeds, with no discernable separation by country for any breed groups in common between SA and AUS. In both PC1 and PC2 the Merino, Border Leicester and Poll Dorset were the most distant breeds. PC1 separated the Merino and Poll Dorset from the Border Leicester group, and PC2 separated the Merino, Corriedale and Border Leicester groups, from the remaining meat breeds with the largest distance observed between the Merino and Poll Dorset group. The proportion of variation (POV) explained by PC1 and PC2 (7.82% and 5.63%, respectively) is small, and the cumulative POV explained by principal components 1 to 40 showed an asymptotic trend at a low percentage (Fig. S5a). The first 20 principal components accounted for roughly 28% and the first 40 for 32% of the total observed variation. In succeeding principal components (PC3 to PC14; Fig. S2), breeds generally remain in group clusters, but the Merino groups are an exception and were observed in multiple clusters from PC3 onwards. However, there was little indication of across-country groups separated by different clusters within these components.

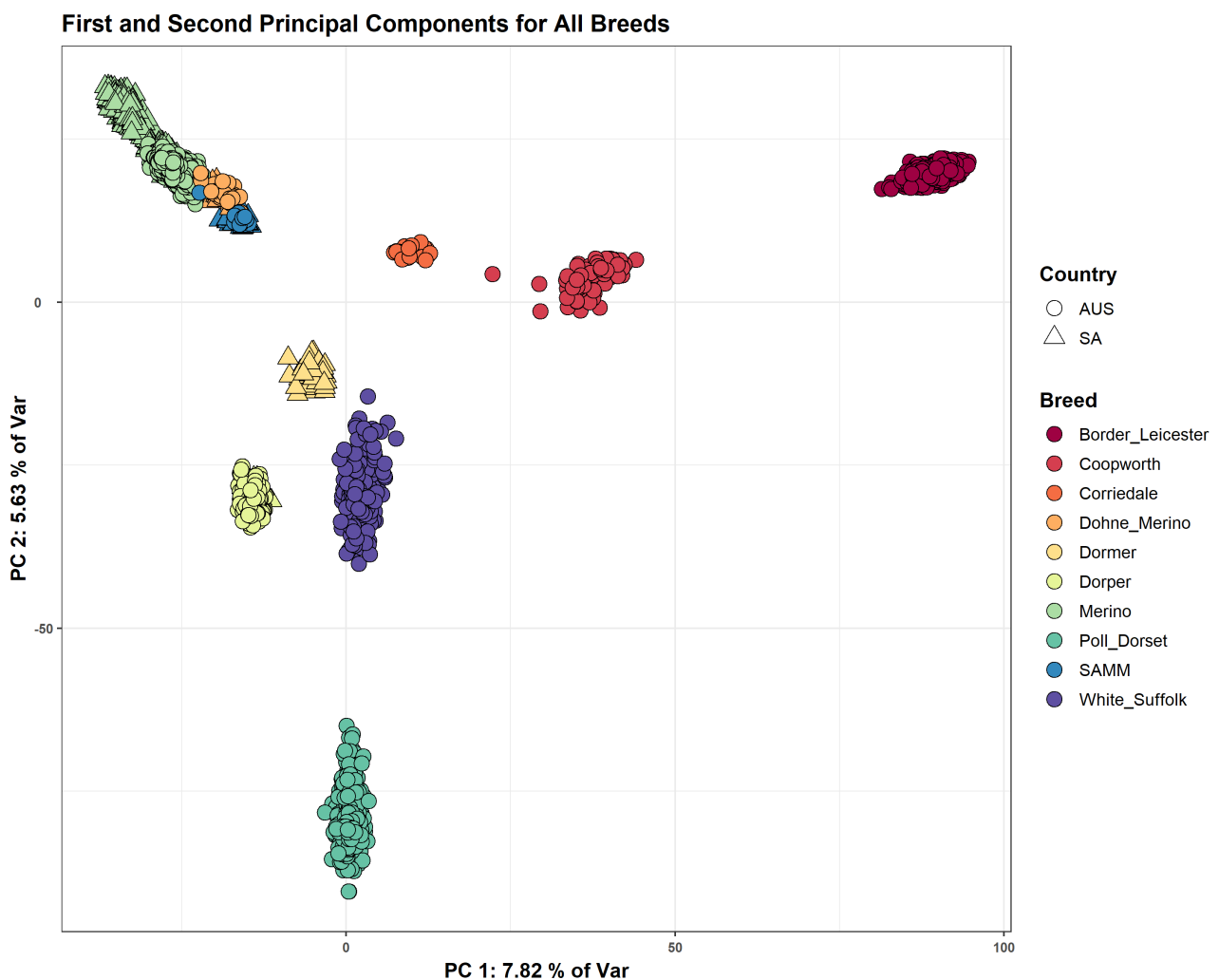


Fig. 7.3 the first (PC1) and second (PC2) principal components of animals identified by breed and country of origin.

PCA of the Merino bloodlines (G_M ; Fig. 7.4) showed less discrete clustering compared to the breed groups (Fig. 7.3) but were still clearly discernable across PC1 and PC2. PC1 separated bloodline populations by country of origin, but to varying degrees.

All animals with positive values of PC1 were from AUS, whereas those with negative values of PC1 were all linked to SA. In PC1, the Elsenburg and Strong lines were separated the most. The Cradock line clustered near the Ultrafine and Fine-Medium-2 lines. PC2 separated the Elsenburg line and Strong lines from the Fine-Medium-1 line and an overlap between the Cradock and Fine-Medium-2 and the Ultrafine lines was observed. In general, the lines of AUS origin had more distinct clusters compared to the SA lines and the SA lines varied more within groups on PC1 and PC2. The POVs explained by PC1 and PC2 (4.97% and 3.28%, respectively; Fig. S5a) of Fig. 7.5 were smaller than those observed by PC1 and PC2 that separated breeds (Fig. 7.3) but not by a large margin.

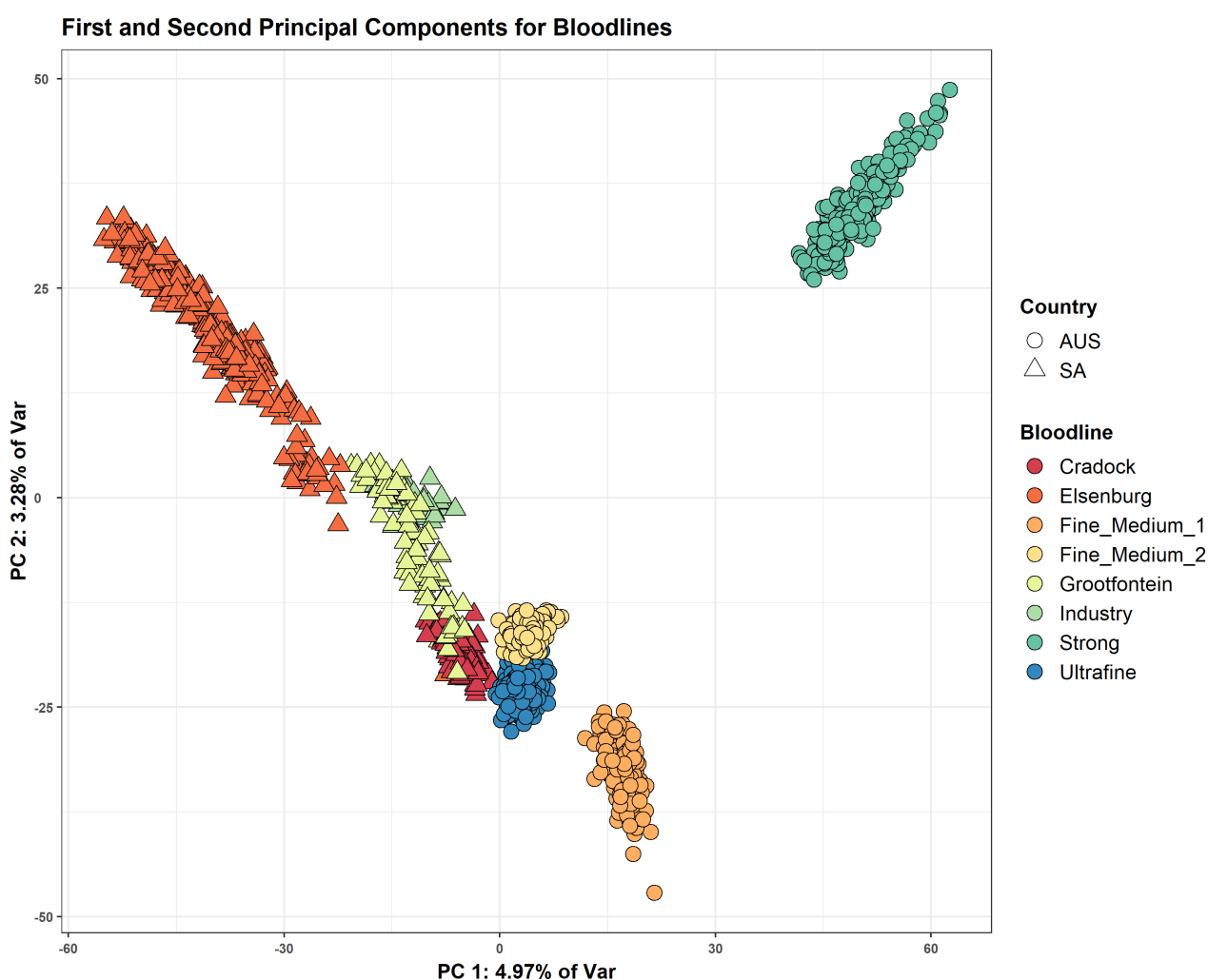


Fig. 7.4 the first (PC1) and second (PC2) principal components of Merinos identified by bloodline and country of origin.

Model-based clustering by ADMIXTURE into genetic groups generally revealed genetic compositions unique to the respective population groups (Fig. 7.5). Assignment of genotypes at $K = 3$ had a definite separation of Merino and Border Leicester groups (Fig. 7.5a).

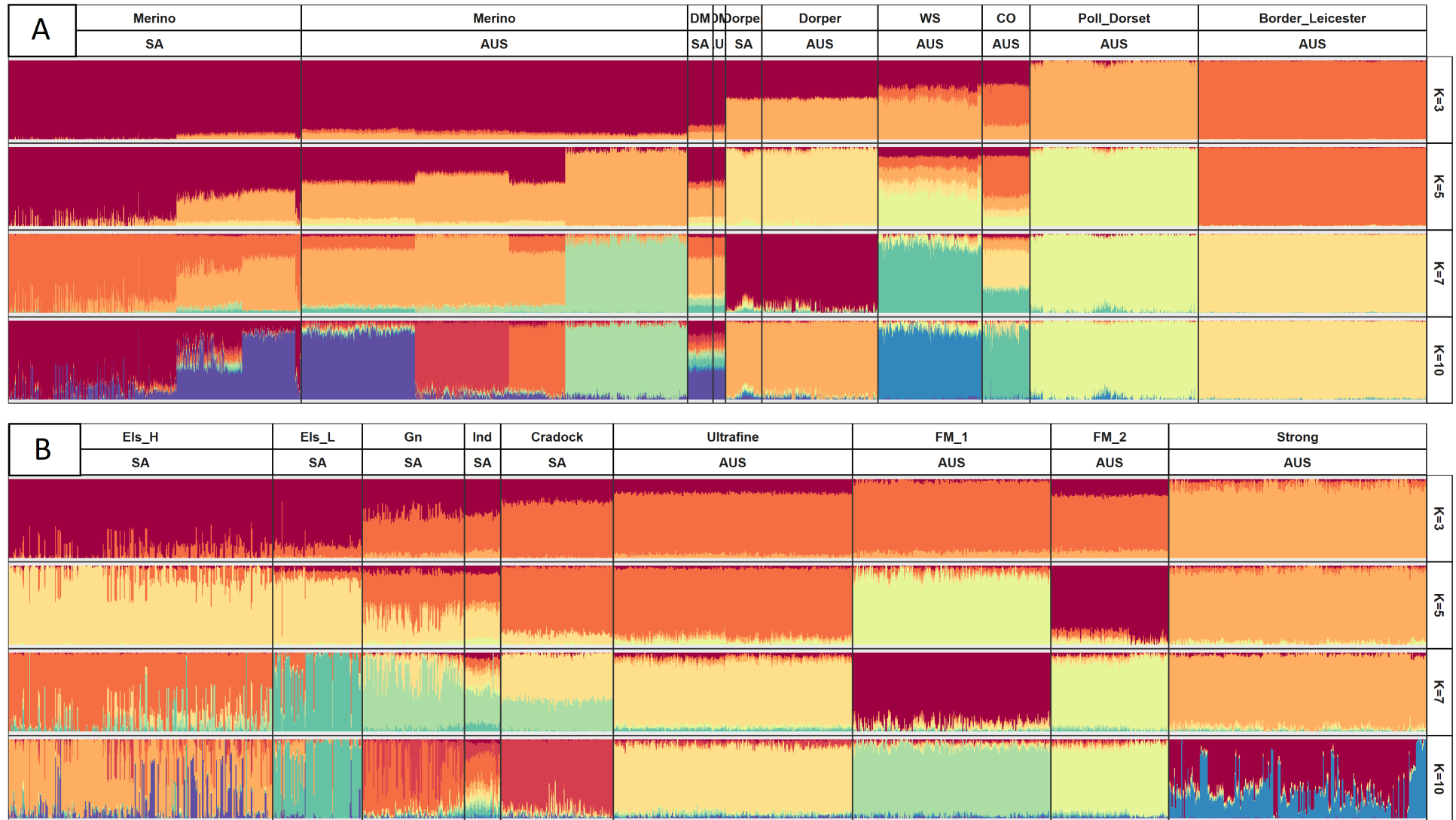


Fig. 7.5 Stacked bar plot where each colour represents the proportion of a genetic group (Q) of an animal's genome resulting from ADMIXTURE analysis for (A) breed groups and (B) Merino bloodlines. Breed labels of (A) are, in order: Merino, Dohne Merino, Dorper, White Suffolk, Coopworth, Poll Dorset and Border Leicester. Merino bloodline labels of (B) are, in order: Elsenburg High, Elsenburg Low, Grootfontein, Industry, Cradock, Ultrafine, Fine-Medium-1, Fine-Medium-2 and Strong

The dual-purpose Dohne Merino groups clustered with the Merino groups, while the Dorper and White Suffolk clustered with mixed composition of both Merino and Poll Dorset co-ancestry. The Coopworth group was separated into almost equal proportions of all three groups. At $K = 5$, within-group substructures became distinct in both AUS and SA Merino groups and were increasingly defined when analyzed at higher values of K . The Dorper, Poll Dorset and Border Leicester breeds appeared distinct as individual groups, while the admixed history of the Dohne Merino, White Suffolk and Coopworth groups was reflected by assignment to multiple genetic groups. At $K = 7$, the Coopworth appeared admixed with links to White Suffolk, Merino, and Dorper, but predominantly remained clustered with the Border Leicester. At $K = 10$, most breed groups were genetically distinct except for the Dohne Merino, whose composition coincided with multiple genetic groups within the Merino cluster of breeds.

An additional set of runs of ADMIXTURE for Merino only genotypes are also reported (Fig. 7.4b). At $K = 3$, bloodlines appeared similar except for the Elsenburg and Strong lines which showed no apparent co-ancestry. The genetic composition of Elsenburg line displayed low levels of heterogeneity, which increased with subsequent values of K . At $K = 5$, the Elsenburg, Fine-Medium and Strong lines were predominantly divided into distinct groups. The group proportions of the Ultrafine line were very similar to the Cradock line, which as a group, shared notable genetic proportions with the Grootfontein and Industry lines. At $K = 7$, the shared genetic composition between the Ultrafine and Cradock lines persisted. However, this was not the same group that linked the three SA lines - Grootfontein, Industry and Cradock, suggesting that different pathways of gene flow connected these bloodlines. At $K = 10$, the Elsenburg, Grootfontein and Strong lines appeared considerably more heterogenous in contrast to the remaining groups. The Fine-Medium, Ultrafine and Cradock lines were grouped into distinct groups with small proportions of contradicting ancestry. For both the full (by breed) and subset (by bloodline) analyses, the ADMIXTURE cross-validation statistic for $K = 3$ to 20 is reported in Fig. S5b. For this range, increasing numbers of clusters (i.e. level of K) resulted in an increased accuracy (lower CV statistic), however, a minimum value of CV was not observed for the 3 to 20 K values investigated.

7.3.5 Linkage disequilibrium and effective population size

From the non-syntenic LD of randomly sampled groups, a strong relationship between non-syntenic LD (and thus LD) and $1/N$ was observed, indicating that measures of LD is rapidly inflated as the sample size decreased below $N = 64$. This trend can be seen in Fig. S1, which also shows the correction factor suggested by Hill and Robertson (1968). Alternatively, a simple correction: $r^2 - \frac{1}{N}$ could have been effective but would inevitably lead to negative r^2 estimates at very small sample sizes and low levels of r^2 . Consequently, LD and effective population size (N_e) is only reported for groups consisting of samples sizes larger than 64, but an exception was made for the SA 'Industry' group ($N = 41$), and results for this group should be treated with caution.

The estimates of average LD between adjacent markers (separated by ~ 55 KB) showed substantial variation in the breed and bloodline groups. The lowest LD values were estimated for the AUS Merino (0.138) and second-lowest for the SA Merino (0.156). The highest LD values were estimated for the Poll Dorset (0.224) and Border Leicester (0.226) breeds. The persistence of LD over physical distance was different for

each breed group (Fig. 7.6). The AUS Poll Dorset and Border Leicester had similarly high estimates of LD at the shortest interval of 55 KB, but a sharp distinction was observed in the patterns of LD decay between these two breeds. LD in the Poll Dorset group displayed a comparatively slow rate of decay and retained the ranking of highest LD to roughly 15000 KB, at which point LD decay had stabilized for most other groups. The Border Leicester, in turn, was observed to have a rapid rate of decay between adjacent LD to a distance of 5000 KB, as its comparative ranking changed from the highest values of r^2 at 55 KB (adjacent) to close to being the lowest at 5000 KB, and persistently the lowest after 7500 KB. Across-country comparisons for the Merino and Dorper groups appeared to have a generally similar pattern of LD-decay, but with small differences at the absolute level. The AUS and SA Merino displayed the sharpest initial rate of decay trending noticeably lower than the remaining groups at initial distances.

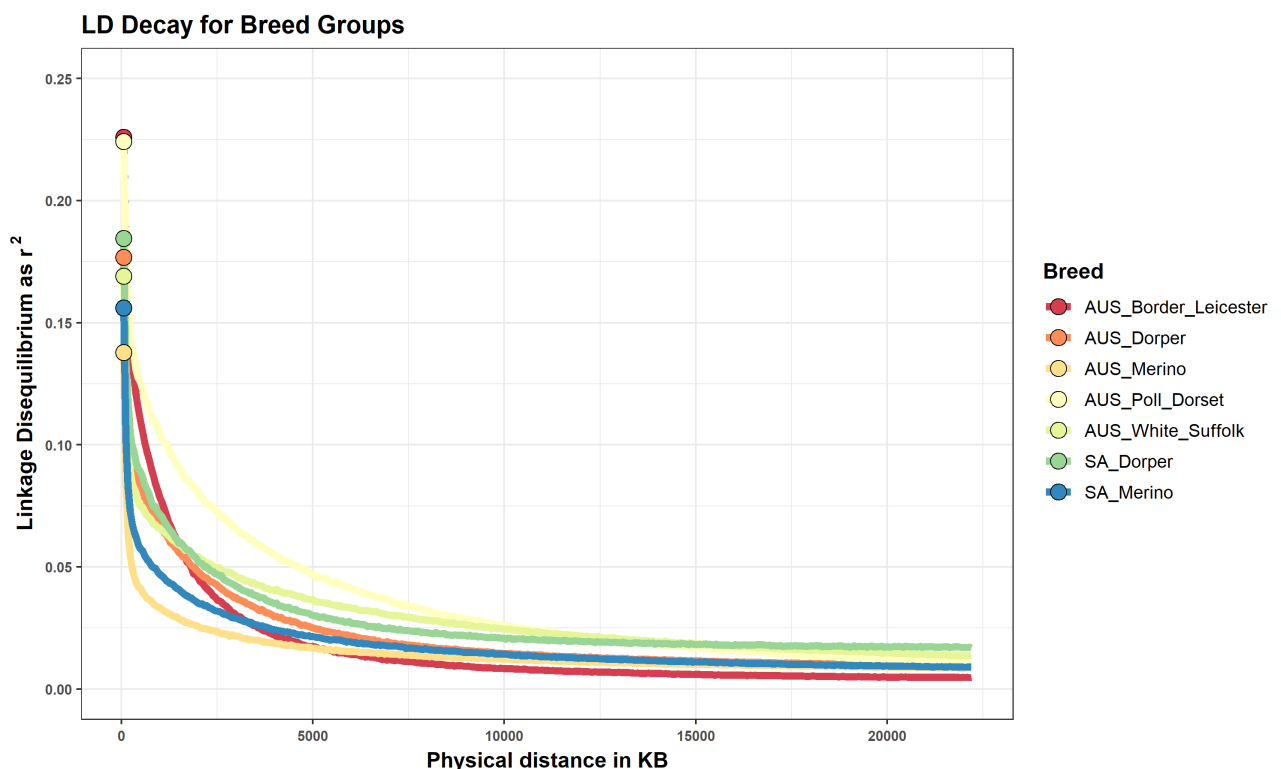


Fig. 7.6 LD decay as levels of mean r^2 over physical distance for selected breed groups. Dots represent the mean adjacent LD at ~ 55 KB.

Patterns of LD decay were generally uniform between Merino bloodlines (Fig. 7.7), except for the rapid LD decay observed in the Ultrafine line, which was a persistent anomaly compared to the other bloodlines across all distances. In general, the split of Merino lines into sub-population by bloodlines had marked effects on LD trends. Excluding the Ultrafine line, the adjacent r^2 estimate for bloodlines were all above 0.17, which was noticeably higher than the values observed for both SA and AUS Merinos. Observing the pattern of decay (Fig. 7.7), the lowest bloodline estimate of r^2 was roughly 0.05 at a distance of 2500 KB, which was two-fold that observed for the AUS Merino at the same distance, and if excluding the Poll Dorset, higher than most breed groups at the same distance (Fig. 7.6).

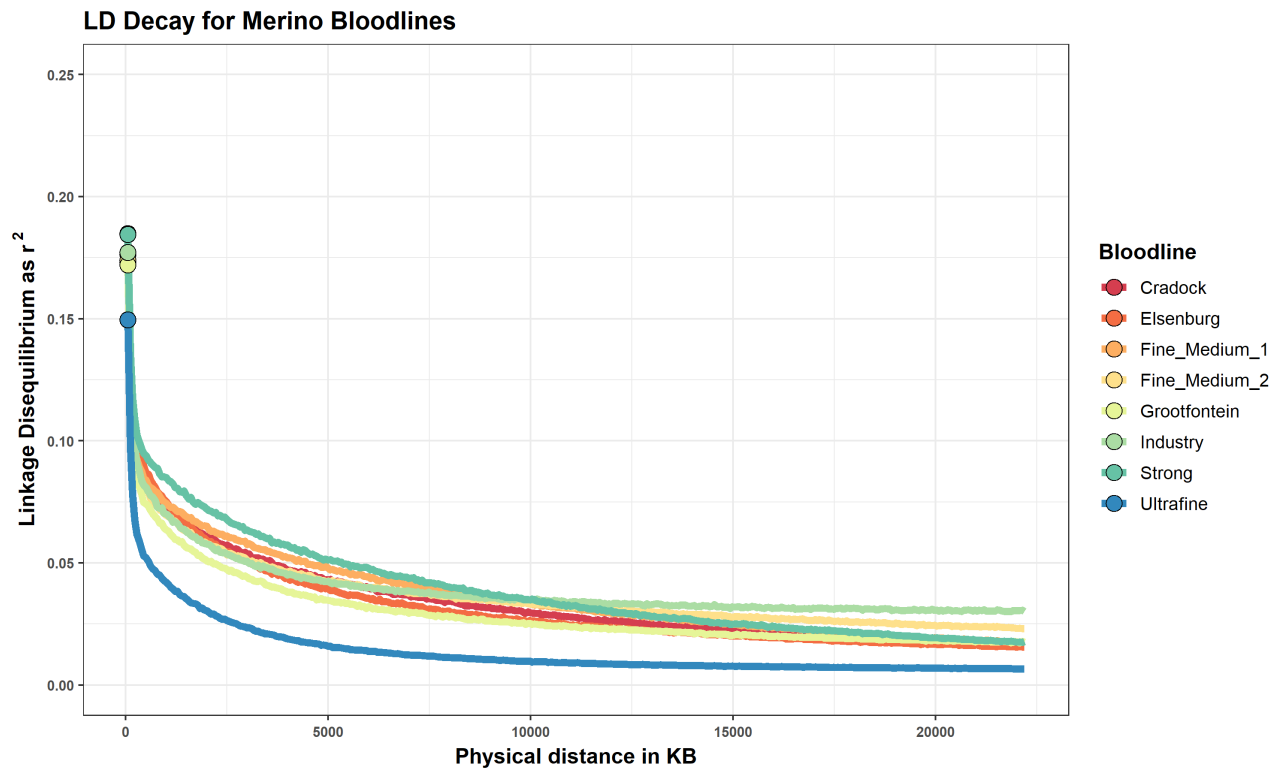


Fig. 7.7 LD decay as levels of mean r^2 over physical distance for selected Merino bloodline groups. Dots represent the mean adjacent LD at ~ 55 KB.

The plotted estimates reflected N_e from 2 to 500 generations ago (Fig. 7.8). Uncharacteristic trends of N_e were observed for some breeds in the recent generations, and the ‘current’ effective population size was estimated at ~ 20 generations ago to represent diversity that was putatively considered a more stable time point (Table 7.4, Fig. 7.8). At this point, the AUS Merino was the most diverse population group with a N_e of 418, more than 3 times the N_e of 128 observed for the Poll Dorset group. The levels of diversity were more uniform across bloodlines, except for the Ultrafine line which had a large N_e estimate of 369 (Table 7.4). According to the estimates at this timepoint (in generations), the Ultrafine group has an effective size larger than multiple breed groups. In the historical trends of N_e for breed groups (Fig. 7.8) and bloodlines (Fig. S4) groups were generally consistent in their ranking across generations. The AUS Merino had a large historical population, but also the steepest rate of decline, especially from 200 generations ago. The SA Merino and Dorper groups were intermediate while the Poll Dorset and Border Leicester had relatively low historical population sizes with an N_e of just over 1000 at 400 generations ago. A notable exception in the N_e trends was observed over recent generations. From 20 generations ago, the Border Leicester group unexpectedly increased in N_e . For the Merino bloodlines, the Ultrafine line was a recurring exception and maintained a persistently high N_e across the estimated time scale. The bloodlines displayed levels of diversity comparable to that observed for breed groups.

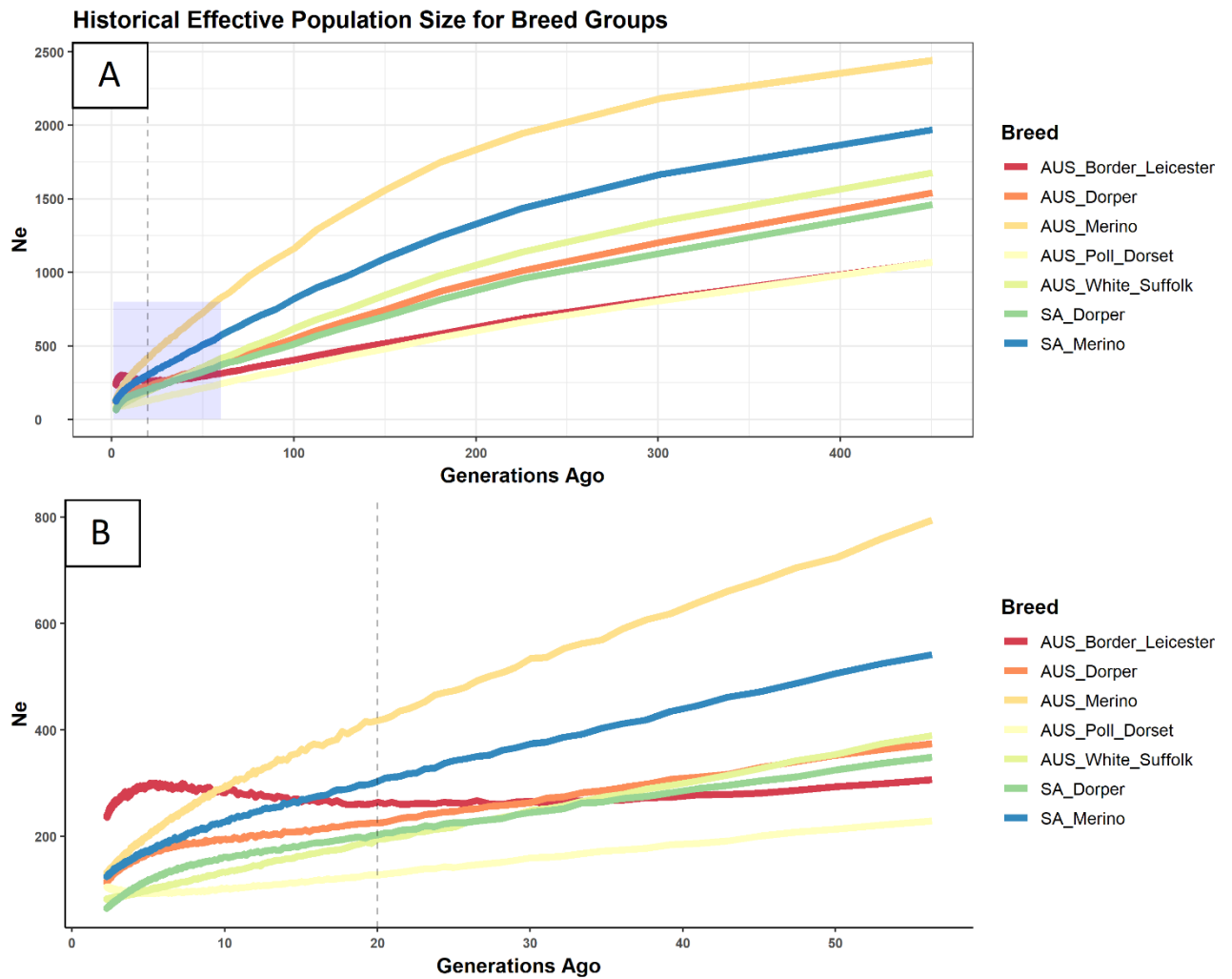


Fig. 7.8 Historical effective population size (N_e) for selected breed groups from (A) 2 to 400 generations ago and (B) 2 to 60 generations ago. In A and B, the vertical-line represents the timepoint used for ‘current’ N_e . The transparent square on A represents the relative boundaries of B

Table 7.4

Linkage Disequilibrium (LD) (Adjacent (~55 KB) and Non-syntenic) and Effective Population Size (N_e ; determined 20 generations ago) for the selected breed and bloodline groups

Population		N	$LD_{Adjacent}$	$LD_{Non-Syntenic}$	N_e 20 Gen
<i>Breed</i>	<i>Country</i>				
Merino		918	0.138	0.006	418
Dorper		276	0.177	0.008	225
Border Leicester	<u>AUS</u>	542	0.226	0.003	265
Poll Dorset		400	0.224	0.005	128
White Suffolk		247	0.169	0.006	196
Merino		697	0.156	0.006	304
Dorper	<u>SA</u>	86	0.184	0.016	203
<i>Bloodline</i>	<i>Country</i>				
Ultrafine		270	0.150	0.005	369
Fine-Medium-1		224	0.173	0.009	154
Fine-Medium-2	<u>AUS</u>	133	0.174	0.016	178
Strong		291	0.185	0.009	137
Elsenburg		400	0.185	0.010	173
Grootfontein		115	0.172	0.013	201
Industry	<u>SA</u>	41	0.177	0.028	177
Cradock		127	0.176	0.011	165

7.4 Discussion

7.4.1 Heterozygosity and inbreeding

The current results for H_{obs} were consistent with previous findings for the AUS Merino (Kijas *et al.* 2012; Al-Mamun *et al.* 2015; Ciani *et al.* 2015), SA Merino (Sandenbergh *et al.* 2015), Coopworth (Prieur *et al.* 2017), Poll Dorset and White Suffolk (Kijas *et al.* 2012) (Table 7.1). Current values were marginally higher than those previously reported for the African Dorper (Kijas *et al.* 2012) and Border Leicester (Kijas *et al.* 2012; Al-Mamun *et al.* 2015), but the general ranking of the Merino, Poll Dorset and Border Leicester as breeds of respectively high, intermediate and low gene diversity agreed with previous results from marker data (Kijas *et al.* 2012, 2014; Al-Mamun *et al.* 2015). A similar range of 0.33 to 0.38 has been reported for mean heterozygosity of New Zealand pure and composite populations (Brito *et al.* 2017; Prieur *et al.* 2017) that originated from programs that included high levels of crossbreeding. Diversity of ‘pure’ breeds currently investigated could be expected less diverse compared to highly crossbred populations, suggesting either particularly high diversity for current breed groups or that H_{exp} and H_{obs} has limited sensitivity as a measure of genetic diversity.

In deriving estimates of inbreeding by marker-based methods, it is important to account for the original definitions of inbreeding described as the correlations between homologous genes within a haploid individual (Wright 1922), or the probability of identity by descent (Malécot 1948). The former definition accommodates a negative F-value (Wright 1965). The latter does not as probabilities, by definition, are bounded between 0 and 1. Also, estimates of inbreeding derived from the diagonal of the GRM are sensitive to the extent that

breed effects influence allele frequencies. The mean (1.13) of the diagonal of \mathbf{G} suggested the multi-breed composition of this GRM slightly inflated estimates of F_{VR} across all groups. This is supported by the fact that F_{VR} estimates of Merino bloodlines, derived from \mathbf{G}_M (mean diagonal of 1.05), are slightly lower than the mean F_{VR} for Merinos according to \mathbf{G} . These estimates of F_{VR} could thus represent slightly inflated measures of individual inbreeding, and this effect would be more pronounced for breed groups presented in low numbers. However, no clear pattern of high F_{VR} estimates was visible for breeds of smaller sample size.

The high mean F_{VR} suggested relative uniformity within populations such as the Dorper, SAMM, Poll Dorset and Border Leicester groups. The high levels of inbreeding for the AUS Dorper and AUS SAMM could be expected as these breeds were established in Australia from limited importation of genetic material from South Africa followed by grading-up programs using back-crossing. Also, F_{VR} was similar or higher in the SA populations, implying that inbreeding in the AUS populations was also a characteristic of their ancestral lines, rather than only a consequence of across-country isolation. High levels of inbreeding precipitate a decline in quantitative genetic variance (Falconer and Mackay 1996), and detrimental effects associated with excess homozygosity have been reported in sheep (Norberg and Sørensen 2007; Barczak *et al.* 2009; Wyk *et al.* 2009). The overall level of inbreeding in populations observed here was generally low, but the comparatively high values for breeds such as the Border Leicester and Poll Dorset are notable for consideration in their breeding program design.

7.4.2 Pairwise F_{ST} statistics

Given analogous definitions for F_{ST} statistics (Wright 1949; Weir and Cockerham 1984), F_{ST} estimates can be thought of as (1) the correlation between randomly sampled alleles within subpopulations relative to the total population or (2) the proportion of genetic variance that can be attributed to variance in allele frequencies between subpopulations (Holsinger and Weir 2009). With ‘across breed’ estimates ranging from 0.05 to 0.26 (Table 7.2), it was clear that the implications of this segregation depend greatly on the historic admixture between any two distinct breeds. Current F_{ST} values were higher than previous estimates of 0.062 and 0.053 between the Merino and, respectively, the Poll Dorset and Border Leicester groups (Al-Mamun *et al.* 2015), but methods were not similar. The low levels of divergence across countries for the SAMM, Dorper and Dohne Merino breed groups reflects the formation of these breeds in AUS by the importation of genetic material directly from SA. The low estimate for SA x AUS Merino groups suggested that the two Merino populations have not diverged greatly, or that the few recent genetic links were influential in reducing the genetic distance of samples included in this study. Interestingly, some F_{ST} values between bloodlines were comparable or larger than certain across breed estimates (Table 7.3). This further highlights the importance of lines that have already been noted for AUS Merinos (Brown *et al.* 2013), and also suggests that partial restriction of gene flow could have important implications, regardless of being ‘within breed’.

The low level of divergence between the Ultrafine and Cradock groups is somewhat expected since these bloodlines were known to have recent across-country links. However, the F_{ST} estimate in this across-country comparison was as low as pair-wise comparisons between SA lines, which is promising for the prospect of a common genomic evaluation for these populations.

7.4.3 Linkage disequilibrium and effective population size

Overall, the reported LD at ~ 55 KB agreed with previous findings that characterised LD in sheep to be generally low (Kijas *et al.* 2014) compared to other domesticated species such as pigs (Badke *et al.* 2012) and dairy cattle (Hozé *et al.* 2013). Current results for Merinos agreed with earlier reports that characterized this breed by rapid LD decay (Kijas *et al.* 2014; Al-Mamun *et al.* 2015; Liu *et al.* 2017). However, Kijas *et al.* (2014) showed substantial differences in LD measurements when determined at shorter distances such as ~ 10 KB, and higher density platforms could thus provide a more accurate indication of LD decay than currently reported.

LD observed for bloodlines reflected the importance of structuring by subpopulations. When compared to Merino breed groups, which is the pooled bloodlines, the pattern of decay showed relative consistency, but the absolute level of LD was substantially higher when structured by subpopulation (Fig. 7.6 vs Fig. 7.8). Thus, while overall levels remained low, the persistence of LD was noticeably sensitive to the connectedness of set populations. Extrapolating this pattern, it could be speculated that the Ultrafine line consists of influential population substructures not accounted for by the current population assignment, which is supported by the low level of relatedness, but the slightly higher F_{VR} within the Ultrafine line. However, no obvious substructures were observed in either PCA or ADMIXTURE results, as discussed below, and it is thus difficult to explain the uncharacteristic pattern of decay of this bloodline.

Calculating historical N_e by the rate of LD decay (Hayes *et al.* 2003) has numerous examples in sheep (Kijas *et al.* 2012; Brito *et al.* 2017; Prieur *et al.* 2017). Domestic populations, under strong selection programmes with the widespread use of preferential sires, deviates heavily from the assumption of random mating of Wright-Fisher populations (Charlesworth 2009) and the loss of diversity seen in Fig. 7.8 is expected. However, compared to other domestic species, sheep have been characterized by relatively high levels of diversity (Kijas *et al.* 2012), attributed to large founding populations combined with less intense selection compared to other domestic species, such as cattle (The Bovine HapMap Consortium* 2009). Using the same methodology, Kijas *et al.* (2012) reported roughly similar estimates for African Dorper (264) and Border Leicester (242), but considerably higher estimates for the Australian Merino (833), Poll Merino (918) and Industry Merino (853) and Australian Poll Dorset (318) which indicates some inconsistency in N_e estimates of the same breeds.

Despite the relative agreement of the N_e estimates of the Border Leicester group to those reported by Kijas *et al.* (2012) the unexpected increasing trend of N_e over recent generations seen in Fig. 7.8(b) made it difficult to evaluate the breed's comparative ranking across more recent generations. Other studies have also reported increasing N_e estimates in recent generations. Using a similar methodology, Brito *et al.* (2017) reported an increasing N_e for the Primera and Lamb Supreme breeds over the last 5 generations. An increase in N_e was also observed for the Romney breed around 20 generations ago only to decline at around 5 generations ago (Prieur *et al.* 2017). The latter authors ascribe the increase in N_e to an increase in animal numbers following successful management, and the recent implementation of technologies like artificial insemination (Prieur *et al.* 2017). However, non-random mating reduces N_e below census size, N (Charlesworth 2009). Thus, given the high intensity of artificial selection and genetic drift, a population-wide

increase in genetic diversity is unexpected in the absence of crossbreeding. Also, according to Hill and Robertson (1968), LD and fixation have a linear relationship. Given that the Border Leicester group had the highest F_{VR} and within-group relatedness, it was surprising not to observe a pattern of LD, and consequently N_e , more comparable to less diverse breeds such as the Poll Dorset group.

7.4.4 Genomic relatedness, PCA and ADMIXTURE

Estimates of relatedness according to G agreed with F_{ST} estimates in describing a close association between breeds in common between countries. The across-country relationships for the SAMM and Dorper breeds were only marginally weaker than the internal relatedness of individual populations (within country). The lower level of relatedness between the AUS and SA Dohne Merino populations could be due to a more diverse SA population, as the SA Dohne Merino maintained a lower F_{VR} and internal relatedness compared to the SA SAMM and SA Dorper groups.

For the Merino breed, the across-country relationship is known to be connected by both deep ancestral and relatively recent relationships, but the positive relationship was expected to be low for multiple reasons. Both groups were internally diverse, and it can be reasoned that a population cannot be more related, on average, to another population than its internal level of relatedness (than it is to itself). Also, the ancestral relationship between SA and AUS Merinos is defined by a distant linkage in the original development of the AUS Merino (Meadows *et al.* 2008). Lastly, the known recent links are limited to those between the Cradock and Ultrafine lines. Considering the defining influence of structure between bloodlines, this across-country relationship is likely to be isolated within these two lines, a tendency also observed in the ADMIXTURE, PCA and F_{ST} results reported here.

This study also reported across breed relationships that were comparatively high in magnitude. These cannot be attributed to recent links and must thus be prompted only by a deeper co-ancestry. For example, the SAMM and Dohne Merino share origins to the German Mutton Merino (Schoeman *et al.* 2010), and the origin of the AUS Coopworth is linked to the Border Leicester (www.coopworth.org.au).

From G_M , the positive relatedness between SA bloodlines suggested that separation on a flock level was less restricting compared to that of lines or strains, which was expected. The generally weak relationships with the Elsenburg line reflect the initial management on an isolated basis. The positive relationship between the Grootfontein and Industry lines is a good indication of the resource flock's objective to represent commercial SA Merinos. Given the relatively few genetic links between the Ultrafine and Cradock lines, it was surprising to observe this across-country relationship as the only positive relationship in a comparison involving AUS bloodlines, including relationships to other AUS Merino bloodlines within country.

Results from PC- and ADMIXTURE analysis generally indicated very similar clusters of breed and bloodline populations. The discrete breed structures of PC1 and PC2 appeared to capture deep ancestral relationships and very little within-group variation. From ADMIXTURE analysis, the distinct genetic structures of Merino, Border Leicester and Poll Dorset agreed with those three breeds occupying the most distant branches of PC1 and PC2.

The ADMIXTURE and PCA results also agreed well with previous parameters that indicated only small effects of across-country separation. However, a study that imputed SA breeds from AUS reference panels showed a markedly higher accuracy of imputation for Dorpers compared to Dohne Merinos (Nel *et al.* 2019). Although imputation accuracy is not directly related to ADMIXTURE and PCA results, it is thought that the homogenous nature of the Dorpers suggested by Fig. 7.5 is likely to have facilitated the more accurate imputation for this breed.

Generally, breed structures did not dissipate in succeeding principal components as groups remained clustered beyond PC1 and PC2 (Fig. S2), and the genotypes of most breeds were defined by a similar genetic composition in ADMIXTURE analysis (Fig. 7.5a). However, Merino groups were an exception and often segregated into multiple clusters across PC3 to PC14 and substructures of Merinos were also clear from ADMIXTURE analysis that commenced on an ‘across breed’ level. Following the advent of artificial insemination (AI), across bloodline links are considered to have become more common in AUS (Swan *et al.* 2016), but these results strongly suggested that the subpopulation, i.e., bloodline of origin, is an important determinant of the genetic composition of Merinos. This has been demonstrated by high levels of quantitative genetic variance across similar groupings (Ultrafine, Fine/Fine-medium, Medium/Strong) of AUS Merinos for key production traits (Swan *et al.* 2016). Also, markedly different accuracies for genomic breeding values were reported for similar groupings (superfine-, fine- and strong-wool types) following genomic prediction of production traits from the same reference set (Daetwyler *et al.* 2010).

The ADMIXTURE analysis of bloodlines revealed further complexities of these population structures (Fig. 7.5b). The identification of the Ultrafine and Strong lines within opposing clusters have been previously reported (Swan *et al.* 2014). Similar to other metrics presented in this study, close association existed between the Cradock line and the Ultrafine and Fine-Medium-1 lines. However, ADMIXTURE results at $K = 7$ suggested that the remaining SA lines might not directly benefit from the relationship between the Cradock and Ultrafine lines. These results should also be seen in combination with the discussion below with reference to POV and the CV error.

The highly defined population structure of the Ultrafine line is notable considering the high estimates of diversity according to N_e and relatively low internal relatedness. However, this is possibly due to the orthogonal nature of principal components, which separated the ancestral relationships for which the Ultrafine line appears uniform, from within-line variation between sampled individuals. This could also partly explain the homogenous composition observed in ADMIXTURE analysis. If the Ultrafine line did consist of many small substructures as previously speculated in this paper, the level of K was likely too low to capture such structures for a population with a strongly defined ancestry.

Despite the good agreement and accuracy of PCA and ADMIXTURE analysis in identifying the known groups of origin, the low POV explained by the initial principal components in Fig. 7.5a supports previous results that specifically noted the high dimensionality as a characteristic of genetic architecture of sheep (Daetwyler *et al.* 2012; Kijas *et al.* 2012). Also, the lack of a turning point in CV errors from 3 to 20 (Fig. S5b) implied that the model had difficulty estimating an ideal value of K within this range. It is possible that higher levels of K would perpetually identify lower-level structures, such as families or sire groups, as unique genetic

groups. The high diversity of the animals in this study and in sheep in general could exacerbate this problem, causing difficulty in estimating a ‘best’ estimate for K . Thus, further analysis across higher increments of K were not explored, also because a similar pattern has been observed for similar sheep datasets with fewer breeds until $K = 40$ (P. Gurman, unpublished data).

7.4.5 Implications for genomic selection

The combining of populations into the same pool - such as the current scenario of merging bloodlines into respective SA and AUS Merino groups - hopes to benefit prediction by increasing sample size. In the presence of heterogeneity this will be accompanied by an associated increase in N_e , and thus a decrease in LD. This trade-off could be an important determinant in breeding program design, and the more diverse and diverged the populations, the more challenging this trade-off is likely to be. Thus, an alternative consideration is also valid that a population of high diversity could benefit from being subset into smaller groups of better-connected animals. Regarding genomic selection, Van der Werf *et al.* (2015) showed that a small number of highly related individuals could be more informative than large numbers of distant individuals. While this previous study binned relatedness by categories (e.g. groups of half-sibs), it should also be valid across a continuous scale of heterogeneity such as currently seen in comparing combinations of populations. In a narrow spectrum approach, knowledge of important population structures would be essential to identify pockets within the population that could deliver optimal results. For example, results reported here are important for the prospect of across-country amalgamation of Merino genotypes. For a subset of Merino populations such as the Cradock and Ultrafine lines, the mean relatedness was as high as the mean internal relatedness for AUS Merinos, which are all currently evaluated in a single analysis (Brown *et al.* 2018). Further measures of PCA, ADMIXTURE and F_{ST} indicated that these two groups are likely to be the best starting point for an across-country platform. However, these ‘bloodlines’ were the only examples of some, albeit low, linkage by pedigree. A minimum level of genetic exchange is thus likely to remain an important factor unless more distant population structures are better accounted for in future evaluations.

Accounting for population structures derived from the extended pedigree have delivered increased accuracies for predicted genomic breeding values in the AUS Merino (Swan *et al.* 2014). Including eigenvalues from PCA analysis (Daetwyler *et al.* 2012), or group proportions from the ADMIXTURE Q-matrix (Swan *et al.* 2014), has decreased accuracy, but the higher accuracy of non-adjusted values are likely biased by picking up on breed effects rather than individual variation. Initially, adjusting for population gene frequencies showed little benefit (Moghaddar *et al.* 2013), but improved results have recently been reported by Gurman *et al.* (2019) for a multi-breed GRM. Given that both PCA and ADMIXTURE results proved informative and accurate in characterizing populations by known group of origin, further research is needed to make efficient use of this information. However, other problems could persist in the likely case where across country prediction would utilize both pedigree and genomic information in the ‘single-step’ approach (Legarra *et al.* 2009) which is now common for Australian sheep (Brown *et al.* 2018). The assumption of unrelatedness of founder parents in the pedigree could be particularly problematic in the case of across country separation where disjointed pedigrees could in fact be well connected by unknown links. In this regard, the use of so called

‘metafounders’ (Legarra *et al.* 2015) could be a promising approach to better align the pedigree and GRM for both disconnected and highly related base populations, but depends on all genetic groups being well represented in the genotypic dataset.

7.5 Conclusions

These results provide valuable information on the population structures of important sheep breeds in South Africa and Australia. According to multiple parameters, the isolation across some bloodlines was as influential as that observed across breeds and showed that important division into subpopulations can be extended beyond breed structures. The connectedness of SA resource flocks suggested they could be valuable for contributing to a pooled SA reference population in combination with industry animals. In the prospect of across-country amalgamation, isolation by country rarely proved restricting. While the need for genetic links remains important, the generated knowledge delivers potential to maximize the benefit of such relationships by a narrow spectrum approach to combining populations. However, the wide range of characteristics observed across and within breeds, bloodlines and flocks suggests that the optimal approach would be unique to any given set of populations and breeding objectives. Investigating across-country genomic prediction and imputation of these populations is recommended.

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CHAPTER 8:

The benefit of including genomic information in the genetic evaluation of production and reproduction traits in South African Merinos

Abstract

There is a need to expand the development of selection objectives of South African Merinos to adapt to increasing challenges expected to face extensive small stock production. Genomic selection (GS) provides a promising opportunity to facilitate the genetic selection of sex limited or hard to measure traits, but these methods have not been validated in South African Merinos. The objective of this study was to compare the results of single step genomic BLUP (ssGBLUP) to pedigree BLUP (ABLUP) in South African Merinos. The prediction methods were evaluated by the cross-validation method of pruning the phenotypic data of candidate animals in a validation set and comparing the accuracy of prediction by the LR-method (ACC_{LR}). Phenotypes included a maximum of 81 176 records for weaning weight (WW), yearling weight (YW), fibre diameter (FD), clean fleece weight (CFW) and staple length (SL) and a maximum of 58 744 repeated ewe records for number of lambs born (NLB), weaned (NLW) and total weight weaned (TWW) from five genetic resource flocks under institutional control and five commercial Merino flocks. The H-matrix was built from the pedigree of 88 600 identities and 2811 genotypes. The ACC_{LR} was moderate to high, but varied considerably across production traits. Potential benefits, denoted by ACC_{LR} , reflected an increase of 0.28 (ABLUP) to 0.44 (ssGBLUP) for WW and from 0.58 (ABLUP) to 0.63 (ssGBLUP) for FD. Mentionable gains of 0.45 (ABLUP) to 0.53 (ssGBLUP) were also attained for NLW. The prediction of commercial data from resource flock data only was limited, but this was expected. Variation across flocks was also highly variable, emphasising that validation of prediction is essential. The use of GS methods should be approached strategically, but should serve as a valuable tool for the further development of more sophisticated breeding objectives in South African wool sheep.

8.1 Introduction

Genetic selection in South African Merino breeding has been directed by improvement of economically important traits. Traits such as live weight, fibre diameter or fleece weight are considered a priority in selection objectives due to their perceived monetary value at marketing (Olivier 1999). Genetic improvement of these traits is also effective because they can be recorded at an early age, at relatively low cost, *with* relative ease and the targeted traits commonly have a moderate to high heritability (h^2) as well as usable levels of phenotypic variation.

In contrast to the developments of Sheep Genetics Australia (Brown *et al.* 2007, 2018) or Sheep Improvement Limited in New Zealand (Newman *et al.* 2000, 2010), no traits indicative of animal resilience or fitness are currently recorded by the National Small Stock Improvement (NSIS) in South Africa (Cloete and Olivier 2010). This is despite useful components of genetic variation having been reported locally for faecal worm egg count (Cloete *et al.* 2007; Matebesi-Ranthimo *et al.* 2014), absence of breech strike (Scholtz *et al.* 2010), lamb survival (Cloete *et al.* 2009), or maternal behaviour (Cloete *et al.* 2021). Genetic selection within a limited scope, such as sole emphasis on production or ‘output’ traits, risks detrimental effects in metabolic, reproductive, metabolic or behavioral traits in farm animals (Rauw *et al.* 1998). In turn, genetic selection to the benefit of animal health and welfare (Jensen *et al.* 2008; Rauw 2016) can also deliver indirect economic benefits by reducing input costs and reproductive wastage.

Failing to consider animals’ ability to adapt to the production environment is particularly problematic in the extensive setting common in sheep production systems where there is limited opportunity to mitigate the effect of harsh environmental circumstances. Furthermore, the need for adapted animals will also become ever more important given the effects of climate change that presents direct challenges to small stock production systems (Rust and Rust 2013). In this context, it is essential to consider opportunities to widen the scope of breeding and selection objectives in Merino and Merino-type sheep which are commonly subject to challenging production environments in South Africa (SA) (Cloete and Olivier 2010). However, targeting traits indicative of fitness or resilience is not a simple undertaking. These traits are commonly cumbersome and/or expensive to measure, have a low h^2 , or are sex limited and expressed late in life the case of reproduction. Useful genetic gains will thus hinge on strategies that make optimal use of the available information as well as the latest developments in animal breeding.

South Africa hosts multiple Merino resource flocks widely recorded for a range of production, reproduction as well as ‘hard to measure’ fitness traits (Schoeman *et al.* 2010). These flocks have previously already played important roles in guiding the direction of local breeding objectives (Cloete and Olivier 2010; Cloete *et al.* 2014). There exists a valuable opportunity to exploit the information generated by these initiatives to the benefit of more inclusive breeding objectives in South African Merinos, but the realized potential of combining information across the research and commercial sectors has not been evaluated. Up to date studies of the South African Merinos have been largely limited to within flock analyses of resource flocks, and the accuracy of prediction across flocks or sectors could be constrained by the isolated basis on which research flocks were generally maintained. In this regard, the developments in genomic selection (GS) could deliver new opportunities to enhance prediction of breeding values in cases where traditional best linear unbiased

prediction (BLUP) methods prove to be limited. GS capitalizes on the ability to capture genetic variation according to genome-wide single nucleotide polymorphism (SNP) markers as an estimate of total genetic merit (Meuwissen *et al.* 2001). Following the development of relatively affordable and commercially available genotyping platforms, including for sheep (Kijas *et al.* 2009), the application of genomic data to enhance the accuracy of genetic prediction has become commonplace across a wide variety of the major livestock species (Van Eenennaam *et al.* 2014).

Due to the very different breeding structures in sheep, and a much larger effective population size, GS is not expected to complement genetic progress to the same extent as reported for dairy cattle (e.g. Schaeffer 2006). However, more modest benefits to sheep breeding programs are expected in the prediction of difficult to measure traits in flocks without phenotypes (Van der Werf 2009), or increasing the accuracy of selection for reproductive output (Shumbusho *et al.* 2013). Reproduction is not necessarily difficult to measure, but has a low h^2 and is sex limited. In Australia (Brown *et al.* 2018) and New Zealand (Auvray *et al.* 2011, 2014; Dodds *et al.* 2014), the use of genome-wide data has already been extensively incorporated into the evaluation of the major sheep breeds. In Australia, this includes ‘hard to measure’ traits like lambing ease (Li *et al.* 2021) or components of reproduction such as conception rate, litter size or ewe rearing ability (Bunter *et al.* 2020) and it is considered for application to breech strike resistance (Dominik *et al.* 2021). In New Zealand, the application of GS has also been explored to target facial eczema (Phua *et al.* 2014), dagginess and internal parasite resistance (Pickering *et al.* 2015) as well as carcass traits (Brito *et al.* 2017).

This is particularly relevant to the current objective, since GS methods could allow for the resource flocks to contribute a reference population that predicts genetic performance in commercial flocks where difficult to measure traits are not routinely recorded. Following the strategies outlined by Cloete *et al.* (2014), the research sector has been the sole contributor to establishing a reference population for South African Merinos, but uptake of this technology by the wider industry can be facilitated by contributing a foundation from which a more expansive database can be developed.

The objective of this study was therefore to deliver the first evaluation of the use of genomic information in the genetic prediction of SA Merino sheep. This included multiple resource flocks combined with selected commercial Merino breeding studs that currently contribute data to the national evaluation system, the National Small Stock Improvement Scheme (NSIS). The study made use of selected economically important liveweight and wool traits as well as composite reproduction traits, which are currently the only traits indicative of fitness recorded in commercial Merino studs. The results provided here should serve as a valuable guide to the benefit of genomic information and may assist to roadmap further development of more sophisticated breeding programs for South African Merinos.

8.2 Materials and Methods

8.2.1 Sample populations

Animals in this study can be partitioned by sector of origin as 5 ‘resource’ flocks maintained by various research initiatives in South Africa (Schoeman *et al.* 2010) and 5 ‘commercial’ flocks currently contributing records to NSIS. The resource flocks are referred to by flock name and can be shortly described as follows:

The Elsenburg Merino flock has been divergently selected for reproductive performance by targeting number of lambs weaned per ewe mated. The flock is subset into two lines, the H-Line (positive selection) and the L-Line (negative selection; Cloete *et al.* 2004) and was maintained as an isolated group until the first introgression of external sires in 2008 (Cloete *et al.* 2017). The Grootfontein Merino stud is a resource flock managed according to commercial objectives and the most traditional representation of South African commercial lines compared to other flocks maintained by the research sector. It was subjectively selected for ‘overall excellence’ from 1968 to 1985 (Olivier *et al.* 1995) whereafter objectives changed to increasing live weight and decreasing fibre diameter while maintaining fleece weight (Snyman *et al.* 1998). Genetic trends emanating from this selection regime were reported by Olivier *et al.* (1995). The Cradock fine wool Merino stud was established in the 1980s when Merino ewes of different flocks were screened into a central flock and mated to four Australian fine wool rams for two years. This was followed by the using two more Australian sires in 1996 and cryopreserved semen from another two ultrafine rams in 2002-2003 (Olivier 2014). Selection objectives were first defined by increased live and fleece weight from 1988 to 1996 followed by increased emphasis on reducing fibre diameter since 1996 (Schoeman *et al.* 2010). The Tygerhoek finewool experimental flock was initiated as a subflock of the Cradock fine wool flock in 1998 with the objective of reduced fibre diameter, with the foundation animals from the Cradock flock. The same two Australian ultrafine rams used at Cradock also had progeny in this flock. Cloete *et al.* (2013) gave an account of genetic gains in fibre diameter in this flock. The Langgewens resource population was maintained to conduct studies on pure breeding and crossbreeding in Merino types (Cloete and Cloete 2015). The sires used for pure breeding have either been sourced from industry or other genetic resource flocks (and particularly Elsenburg). It was thus considered as a pool where genetic material from all the other participating flocks were admixed. However, since genetic group identities in validation designs (described below) were assigned according to the flock of birth of sires, the Langgewens flock is not reported explicitly in ‘within-flock’ results, as no sires were bred on the farm as such. Five commercial flocks were included in this study to aim for an across flock representation of commercial breeding stock of South African Merinos. It is important to note that the particular flocks were selected by criteria of varying degrees of known sire links to various resource flocks. Flocks from the commercial sector will herewith be referred to as ‘Industry -1, -2, -3, -4, or -5’.

8.2.2 Phenotypic and pedigree data

Phenotypes included in this study were two liveweight traits: weaning weight (WW) and yearling weight (YW); three wool traits: fibre diameter (FD), clean fleece weight (CFW), and staple length (SL); and three reproduction traits: number of lambs born (NLB), number of lambs weaned (NLW) and total weight of lamb weaned (TWW) as repeated records of the ewe. Quality control of phenotypes commenced on per flock batches. All progeny records were screened for having an age at weaning between 50 and 150 days and being within 3 standard deviations from the mean. Hand reared or cross fostered lambs were removed from the analysis. Records for CFW and SL were corrected for the wool growth period prior to analysis. Records of WW were corrected within flock for sex and age of the lamb prior to deriving TWW as a trait of the ewe. In the case of the commercial group, data was available during the 1990’s but had a very high level of missing

records and incomplete pedigrees, and the dataset and pedigree was pruned to only include records following the year 2000. Records of the Grootfontein resource flock were available from 1968, but this was also pruned to 1986, which is the start of the Elsenburg selection experiment (Cloete *et al.* 2004).

Contemporary groups (CG) were derived separately for WW, yearling and reproduction traits. CGs were generally derived from concatenating flock, year and season. For the Elsenburg flock, the divergent genetic trends (Cloete *et al.* 2004, 2017) of the H- and L-Line had a clear influence on results in preliminary analysis, and selection line was additionally included into CGs to account for these effects. In the Langgewens research flock, CGs for the ewe reproduction data included a factor to identify ewes mated to non-Merino terminal sires and thus raised crossbred offspring. For industry data, factors for management groups were additionally included in CG definitions. For all traits, CGs smaller than 20 animals were excluded from the analysis.

8.2.3 Genotype Data

The first contributions to the genomic database were samples genotyped using the OvineSNP50 Chip (Illumina Inc., CA, USA) Version 1 (N = 876) and Version 2 (N = 1106). Subsequent genotyping was performed using the Geneseek Genomic Profiler (GGP) Ovine 50K chip (Geneseek®, Lincoln, Nebraska) Version 2 (n = 986). An amalgamated genomic dataset was thus prepared according to the following phases:

Quality control measures were applied to SNPs (GenCall score > 0.50 or > 0.90 in > 95% of samples, MAF > 0.01, heterozygosity < 3-SD of the mean, and a call rate > 0.95) and samples (heterozygosity < 3-SD and call rate > 0.90) per batch using the ‘snpQC’ package (Gondro *et al.* 2014) in the R environment (R Core Team 2020). Markers that were unmapped or on the X, Y chromosomes, mitochondrial DNA and structural variants (I/D) were also removed from the analysis. Prior to imputation across platforms, the base pair location of each SNP was mapped to the sheep genome build OAR_V3.1.

The samples on the different versions (V1 vs V2) of the OvineSNP50 (OVN) chip was then imputed to the post-QC density (~ 47K) of OVN V2 by using ‘Beagle’ (Version 5.2) (Browning *et al.* 2018), and as a single batch imputed across to the GGP V2 platform from a reference set of ~21k SNP markers in common. In order to validate the imputed genotypes, a total of 208 samples were genotyped in duplicate on both the OvineSNP50 and the GGP platforms, and the accuracy of imputation was determined as the Pearson correlation between the imputed and original genotypes (excluding common markers). The accuracy of imputation ranged from 0.94 to 0.99 with the majority of cases (87%) observed to be 0.98 or higher. The imputation was thus considered accurate and further analyses proceeded on the GGP platform.

Following these procedures and quality control measures, data of 41709 SNP markers were available for 2811 (N = 277 sires and N = 2551 dams) individuals. A total of 1606 genotyped individuals originated from the five resource flocks (Elsenburg = 516, Cradock = 363, Grootfontein = 371, Tygerhoek = 277, Langgewens = 79). A total of 1205 individuals from five commercial flocks (Industry_1 = 272, Industry_2 = 320, Industry_3 = 234, Industry_4 = 255, Industry_5 = 124) were also genotyped. Genotyped animals were born between the year 2000 and 2018, but with the majority (90%) between 2006 and 2016 with an average of about 237 genotyped individuals per year during this period.

8.2.4 Statistical Analysis

8.2.4.1 Population Structure

To identify possibly influential population substructures in the dataset used in this study, an overview of genetic relationships between and within populations was visualized by the results of a Principal Component Analysis (PCA) of the genomic relationship matrix (GRM) calculated using the ‘irlba’ ‘R’ package (Baglama *et al.* 2019). The first (and largest) and second PCs were plotted identified by sector (‘commercial’ vs ‘industry’) and flock (eg. ‘Elsenburg’, ‘Industry-1’ etc.) for PCA analysis of the entire GRM as well as separately according to sector of origin.

8.2.4.2 Linear Mixed Models

The study was designed to compare the predictive abilities (methods described below) of the traditional BLUP (ABLUP) analysis to the enhanced ‘single-step’ genomic BLUP (ssGBLUP) method that combined pedigree, genomic and phenotypic information into a single analysis. The numerator relationship matrix (**A**) was built from the full pedigree of 88600 individuals following pedigree checking with RENUMF90 from the BLUPF90 family of programs (Misztal *et al.* 2014).

The method of ssGBLUP relies on combining information from the pedigree in **A** with relationships in the genomic relationship matrix (**G**) by building the combined **H** matrix (Legarra *et al.* 2009; Christensen and Lund 2010). The required inverse (**H**⁻¹) was derived with the ‘PreGSF90’ software (Aguilar *et al.* 2014) from the ‘BLUPF90’ family of programs. **G** was scaled based on **A**₂₂ so that mean(diag(**G**)) = mean(diag(**A**₂₂)) and mean(offdiag(**G**)) = mean(offdiag(**A**₂₂)) with scaling parameters of $\alpha = 0.95$ and $\beta = 0.05$ (i.e. markers explain 95% of genetic variation):

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & (0.95\mathbf{G} + 0.05\mathbf{A}_{22})^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where **A** is the numerator relationship matrix, **A**₂₂ is the block of **A** that has genotyped animals, and **G** is the genomic relationship matrix, derived according to (VanRaden 2008) as:

$$\mathbf{G} = \frac{(\mathbf{M} - 2\mathbf{P})(\mathbf{M} - 2\mathbf{P})'}{2 \sum_j (\mathbf{p}_j)(1 - \mathbf{p}_j)}$$

where **M** is the genotypic matrix of 0/1/2 B-allele marker counts with dimensions $m \times n$ (number of genotyped animals, m , by number of markers, n) and **P** is the corresponding matrix ($m \times n$) where the j th column of **P** is a vector of m replicates of the allele frequency p_j within all the animals.

The ‘operational model’ was obtained per trait in a standard single-trait BLUP analysis and all fixed and random terms were retained ‘as is’ in the ssGBLUP model, with the only difference between the two analyses being the replacement of the relationship matrix **A** with the **H** matrix. Estimation of fixed effects and the subsequent derivation of variance components commenced in ASREML V4.2 (Gilmour *et al.* 2015). For production traits, the relevant CG at weaning (N = 636), and CG at yearling (N = 458) were fitted as sparse fixed effects for WW or YW and wool traits, respectively. Additionally, weaning and yearling age (in days),

sex (SEX; male or female), age of dam (AOD; 1 to 7+), and rearing status as a concatenation of status at birth and weaning (REAR; 1: Single-single, 2: Multiple-single and 3: Multiple-multiple) were considered as fixed effects. For reproduction data, the relevant CG (N = 561) in which the ewe was weaned and the CG (N = 269) of the lambing record were fitted as sparse fixed effects. The age of the ewe (AGE; 1 to 7+) and the service code (SCODE; 1: natural mating, 2: artificial insemination and 3: artificial insemination with hormonal treatment) were considered as fixed effects. Fixed effects were tested for significance according to Wald statistics derived from conditional least square methods. Those observed as significant ($P < 0.05$) were retained in the fixed effects model throughout subsequent analysis.

In the case of production traits, random effects considered in the model were direct and maternal genetic effects and the maternal permanent environment and the full model could be represented as follows (in matrix notation):

$$y = \mathbf{X}b + \mathbf{Z}_1a + \mathbf{Z}_2m + \mathbf{Z}_2c + e$$

where y represented the vector of observations of the respective traits, b the vector of fixed effects, a represented the vector of direct genetic values, m the vector of maternal genetic values, c the vector of maternal permanent environmental values and e the vector of residuals. The corresponding incidence matrices of each effect were respectively represented by \mathbf{X} , \mathbf{Z}_1 and \mathbf{Z}_2 . For reproduction traits, a single-trait repeatability model was used, and random effects considered were direct genetic (σ_a^2) and ewe permanent environmental effects (σ_{ewe}^2). The full model could be represented as follows (in matrix notation):

$$y = \mathbf{X}b + \mathbf{Z}_1a + \mathbf{Z}_1c_{ewe} + e$$

where y represented the vector of observations, b the vector of fixed effects, a the vector of direct genetic values, c_{ewe} the permanent environmental values of the ewe and e the vector of residuals. The corresponding incidence matrices of each effect were respectively represented by \mathbf{X} , \mathbf{Z}_1 and \mathbf{Z}_2 . It was assumed that:

$$V(a) = \mathbf{A}\sigma_a^2 \text{ or } \mathbf{H}\sigma_a^2; \quad V(m) = \mathbf{A}\sigma_m^2 \text{ or } \mathbf{H}\sigma_m^2; \quad V(c) = \mathbf{I}\sigma_c^2; \quad V(c_{ewe}) = \mathbf{I}\sigma_{ewe}^2; \\ V(e) = \mathbf{I}\sigma_e^2$$

with \mathbf{A} or \mathbf{H} representing the relevant relationship matrix in ABLUP or ssGBLUP, respectively, \mathbf{I} representing identity matrices and σ_a^2 , σ_m^2 , and the direct and maternal genetic variances, σ_c^2 , σ_{ewe}^2 , the permanent environmental variance of the dam or the ewe, and σ_e^2 the environmental (residual) variance, respectively.

Analysis commenced by testing various combinations of fixed effects and possible two-way interactions. Random terms were then added to the operational model on a step-wise basis by comparing the log-likelihood of the current model with the additional term to the simpler (nested) model. A log-likelihood ratio test (LRT) was used to test for significant differences between models. The log-likelihood ratio test is a chi-square distributed random variable, with degrees of freedom equal to the difference in the number of random effects. When one random effect was added at a time, a difference of 1.92 was regarded as a significant ($P < 0.05$) improvement in the log-likelihood compared to the reduced model tested against. If a model with an additional term did not provide a significant ($P < 0.05$) improvement, the simpler (nested) model was chosen as the operational model for the trait.

8.2.4.3 The validation designs

The predictive ability of ssGBLUP and ABLUP analyses were compared by assigning the population into cross-validation sets that aimed to replicate the prediction of selection candidates without records or progeny from being either young or originating from a flock where a particular trait is not recorded. For both ABLUP and ssGBLUP, ‘whole’ datasets (a maximum of 81176 production records or 58 744 repeated ewe records) were analysed (as described above) to derive EBVs (a_w) for all animals in the pedigree. Subsequently, a second ‘partial’ dataset was replicated but partitioned into a reference and validation population according to varying designs described below, and all phenotypes of the validation population were set to missing. Accuracy of prediction was then determined by the breeding values of validation animals in both the whole (a_w) and partial (a_p) dataset, as described below. Different approaches were followed in assigning the target candidates included in the validation set. In the first scenario (I), the overall predictive ability of the ABLUP and ssGBLUP model was tested by ‘forward’ validation of all candidates born after a certain timepoint. However, to avoid animals in the validation set from benefitting from having paternal halfsibs in the reference set, the criterion for inclusion into the validation set was based on the year of birth of sires, which was set to sires born after 2011. In this design, the validation population consisted of 18106 records of which 468 were linked to genotyped individuals. In scenario (II), the ABLUP and ssGBLUP models were compared in terms of the extent to which information can be effectively applied across sector by assigning all the commercial flocks into the validation set and predicting commercial candidates solely from data in the genetic resource flocks. In this design (II), the validation population consisted of 34979 records of which 762 were linked to genotyped individuals. Lastly, a scenario (III) was considered in which three out of the five industry flocks were also included into the reference population. This scenario represented a situation where selected, but not all, commercial flocks would contribute to the recording scheme of a particular trait. In scenario (III), the validation (‘Industry-1 and 4’) population consisted of 22 336 records of which $n = 491$ were linked to genotyped individuals. Similar to Scenario (I), to remain consistent with predicting across sire families, across flock groups (in II and III) were defined by assigning animals according to the flock of birth of their sire rather than the flock where they were recorded. For reproduction traits, the year of birth criterion designating the reference population was adjusted to the progeny of sires born on an earlier date (< 2008) in order to obtain a more adequate size of the validation set. Measures of the accuracy of breeding values in validation population for Scenario I consisted of 14 907 recorded individuals of which 827 were genotyped. Scenario (II) had a reference population of 30 255 records with 794 genotypes and Scenario (III) had 23 271 records with 499 genotypes.

The accuracy of prediction within a particular population was determined according to the ‘LR-method’ (Legarra and Reverter 2018):

$$ACC_{LR} = \sqrt{\frac{cov(a_w a_p)}{(1 + \bar{F} - 2\bar{f})\sigma_g^2}}$$

Where a_p and a_w are the (G)EBVs as described above, \bar{F} is the average inbreeding coefficient, $2\bar{f}$ is the average relationship between individuals, and σ_g^2 the genetic variance derived from the ABLUP analysis of the partial dataset.

8.3 Results

8.3.1 Population Structure by Principal Components

Inspection of the first (PC1) and second (PC2) principal components of the GRM showed some evidence of population structures (Fig. 8.1), but overall evidence of highly distant population groups was only moderate. Most resource flocks tended to occupy higher values of PC1, with the greatest distance between the Tygerhoek/Cradock flocks and the Industry-3/4 flocks. According to PC1 and PC2, the genetic architecture of these more distant populations also appeared better defined with little within-flock variation. In turn, the Elsenburg flock clustered separately with some distance to the remaining flocks across PC2, but were not as tightly clustered. For the remaining flocks, PC1 and PC2 suggested that useful relationships existed across sectors, such as the homogenous grouping of the Grootfontein and Langgewens resource flocks with Industry-1 and Industry-5.

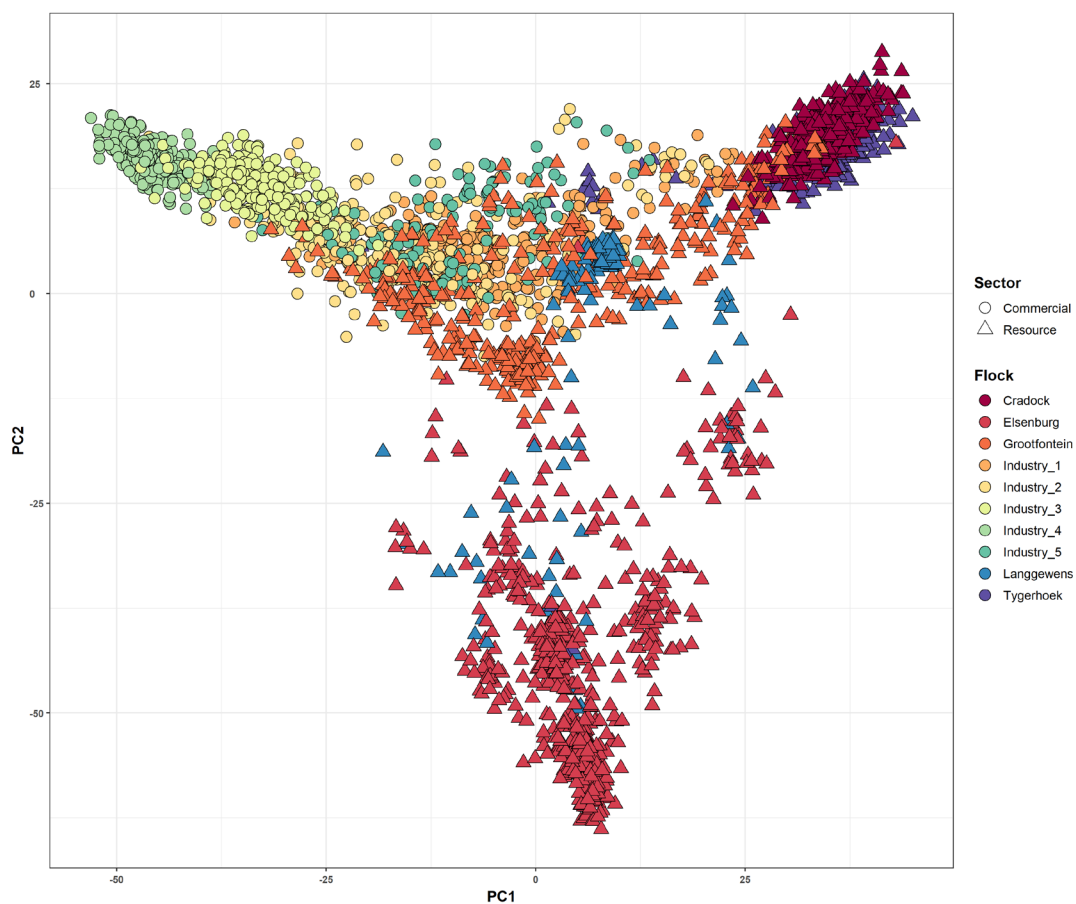


Fig. 8.2 The first (PC1) and second (PC2) principal components of animals identified by breed and sector of origin

8.3.2 Fixed Effects

The effects REAR, SEX and AOD were all significant ($P < 0.01$) predictors for all five production traits considered in this study (Table 8.1). For the majority of traits, lambs born and weaned as singles (S-S) were heavier at weaning and yearling ages, had a greater fleece weight as well as a lower fibre diameter. In terms of SL, the results of the multiple-single (M-S) rearing status group was unexpected, which implied that lambs born as multiples but reared as singles had a higher SL. The reason for this observation is unknown and is not treated in the discussion below. Male lambs were heavier ($P < 0.01$) and had higher CFW ($P < 0.01$). The higher SL and lower FD of males were also significant ($P < 0.01$) according to least square procedures, but the observed differences across sexes were small. A non-linear influence of AOD was observed ($P < 0.01$) for the weight traits WW, YW and CFW, with the best performance seen for the progeny of ewes of intermediate age between 3 and 6 years. Unexpectedly, the progeny of ewes of 4 and 5 years of age appeared to have a slightly lower SL ($P < 0.05$). A more linear trend was observed for FD, as older ewes were associated with progeny of slightly higher FD ($P < 0.01$).

Table 8.1

Summary statistics and predicted least square means (\pm standard error) of the production traits weaning weight (WW), yearling weight (YW), fibre diameter (FD), clean fleece weight (CFW) and staple length (SL) for all the fixed effects rearing status (REAR), sex and age of dam (AOD)

Effect	WW	YW	FD	CFW	SL
No Records	79100	56016	55747	54796	54072
Mean	28.05	50.03	17.83	3.636	98.31
REAR					
S-S	30.5 \pm 0.04	50.8 \pm 0.09	17.6 \pm 0.02	3.62 \pm 0.01	100.5 \pm 0.21
M-S	28.5 \pm 0.07	49.8 \pm 0.12	17.6 \pm 0.02	3.47 \pm 0.01	102.1 \pm 0.29
M-M	26.38 \pm 0.04	49 \pm 0.08	17.7 \pm 0.02	3.41 \pm 0.01	100.8 \pm 0.21
SEX					
M	29.5 \pm 0.04	55.7 \pm 0.09	17.6 \pm 0.02	3.69 \pm 0.01	101.4 \pm 0.23
F	27.4 \pm 0.04	44 \pm 0.09	17.7 \pm 0.02	3.3 \pm 0.01	100.8 \pm 0.23
AOD					
2	27.9 \pm 0.04	49.5 \pm 0.09	17.6 \pm 0.02	3.41 \pm 0.01	101.2 \pm 0.23
3	29.1 \pm 0.04	50.2 \pm 0.09	17.6 \pm 0.02	3.5 \pm 0.01	101.1 \pm 0.22
4	29.3 \pm 0.04	50.4 \pm 0.09	17.6 \pm 0.02	3.53 \pm 0.01	100.7 \pm 0.22
5	29.1 \pm 0.05	50.3 \pm 0.10	17.7 \pm 0.02	3.54 \pm 0.01	100.7 \pm 0.24
6	28.9 \pm 0.06	50.1 \pm 0.11	17.7 \pm 0.02	3.55 \pm 0.01	101 \pm 0.26
7	28.4 \pm 0.11	50.4 \pm 0.18	17.7 \pm 0.03	3.61 \pm 0.02	101 \pm 0.40

For reproduction data, natural mating (SCODE = 1) was associated the lowest ($P < 0.01$) fertility rates, while the use of hormonally induced AI procedures (SCODE = 3) appeared to have the highest as reflected by reproductive output in NLB, NLW and TWW (Table 8.2). Similar to the maternal influence (AOD) reported in Table 8.1, the best performance was observed for ewes of intermediate age ($P < 0.01$) with higher fertility as well as rearing a higher weight of at weaning.

Table 8.2

Summary statistics and predicted least square means (\pm standard error) of reproduction traits number of lambs born (NLB), number of lambs weaned (NLW) and total weight weaned (TWW) for fixed effects service code (SCODE) and age of the ewe (AGE)

Effect	NLB	NLW	TWW
No Records	54256	54256	54256
Mean	1.344	1.177	31.94
SCODE			
1	1.24 \pm 0.02	1.08 \pm 0.03	27.77 \pm 0.47
2	1.28 \pm 0.03	1.12 \pm 0.03	28.18 \pm 0.78
5	1.41 \pm 0.04	1.25 \pm 0.05	31.58 \pm 1.09
AGE			
2	1.21 \pm 0.02	1.04 \pm 0.03	24.47 \pm 0.6
3	1.33 \pm 0.02	1.18 \pm 0.03	29.45 \pm 0.6
4	1.38 \pm 0.02	1.23 \pm 0.03	31.54 \pm 0.62
5	1.39 \pm 0.03	1.24 \pm 0.03	32.06 \pm 0.67
6	1.35 \pm 0.03	1.19 \pm 0.04	31.5 \pm 0.75
7	1.29 \pm 0.03	1.14 \pm 0.04	30.72 \pm 0.89

8.3.3 Variance Components

The variance components and ratios of production and reproduction traits can be seen in Table 8.3 and Table 8.4, respectively. It is clear from Tables 8.3 and 8.4 that the respective variance components (as derived from the ‘whole’ analysis) were very similar between the ABLUP and ssGBLUP methods. This was also reflected in the variance ratios of both the production (Table 8.3) and reproduction (Table 8.4) traits. With regards to variance components, there will thus be no further reference to differentiate between the results of a specific analysis. According to the likelihood ratio test, the additive genetic effect (σ^2_a) was a significant ($P < 0.05$) component in all traits considered. The addition of maternal genetic effects (σ^2_m) also improved the Log-Likelihood ($P < 0.05$) of all production traits (Table 8.3), but the influence of the permanent maternal effect (σ^2_{pe}) was only observed as influential in the case of WW and YW. The permanent environmental effect of the ewe (σ^2_{ewe}) was significantly influential in the case of NLB, NLW and TWW (Table 8.4). Subsequently, all traits considered in this study were heritable, but the heritability (h^2) was low to very low for reproduction traits (0.03-0.04, Table 8.4), low to moderate for WW (0.14), SL (0.23) and YW (0.27) and high for FD (0.57) (Table 8.3). The maternal genetic effect (m^2) was low for WW (0.05), CFW (0.04) and YW (0.03) and significant, but <0.02 for the remaining traits in Table 8.3. The permanent environmental effect of the ewe (c^2) was low (0.05-0.06) for the reproduction traits, but generally larger than the corresponding direct genetic effects.

Table 8.3

Variance components and ratios (\pm SE) of all production traits estimated from both ABLUP and ssGBLUP analysis. Empty cells indicate effects not included in the model

Effects	WW		YW		FD		CFW		SL	
<i>Variance Components</i>	BLUP	ssGBLUP	BLUP	ssGBLUP	BLUP	ssGBLUP	BLUP	ssGBLUP	BLUP	ssGBLUP
σ^2_a	2.84 \pm 0.18	2.84 \pm 0.18	10.15 \pm 0.47	10.69 \pm 0.48	0.78 \pm 0.02	0.78 \pm 0.02	0.12 \pm 0.01	0.13 \pm 0.01	38.24 \pm 1.91	37.59 \pm 1.89
σ^2_m	0.94 \pm 0.11	0.96 \pm 0.11	1.07 \pm 0.19	1.08 \pm 0.18	0.02 \pm 0	0.02 \pm 0	0.02 \pm 0	0.02 \pm 0	2.13 \pm 0.62	2.26 \pm 0.62
σ^2_{pe}	1.67 \pm 0.1	1.64 \pm 0.1	0.44 \pm 0.18	0.39 \pm 0.17						
σ^2_e	14.51 \pm 0.13	14.48 \pm 0.13	26.3 \pm 0.32	25.9 \pm 0.32	0.57 \pm 0.01	0.56 \pm 0.01	0.29 \pm 0	0.29 \pm 0	125.87 \pm 1.38	126.08 \pm 1.38
σ^2_p	19.95 \pm 0.12	19.92 \pm 0.12	37.96 \pm 0.28	38.05 \pm 0.28	1.37 \pm 0.01	1.36 \pm 0.01	0.43 \pm 0	0.44 \pm 0	166.25 \pm 1.18	165.93 \pm 1.17
<i>Variance Ratios</i>										
h^2	0.14 \pm 0.01	0.14 \pm 0.01	0.27 \pm 0.01	0.28 \pm 0.01	0.57 \pm 0.01	0.57 \pm 0.01	0.29 \pm 0.01	0.29 \pm 0.01	0.23 \pm 0.01	0.23 \pm 0.01
m^2	0.05 \pm 0.01	0.05 \pm 0.01	0.03 \pm 0	0.03 \pm 0	0.02 \pm 0	0.01 \pm 0	0.04 \pm 0	0.04 \pm 0	0.01 \pm 0	0.01 \pm 0
mc^2	0.08 \pm 0.01	0.08 \pm 0.01	0.01 \pm 0	0.01 \pm 0						

Table 8.4

Variance components and ratios (\pm SE) of all reproduction traits estimated from both ABLUP and ssGBLUP analysis. Empty cells indicate effects not included in the model

Effects	NLB		NLW		TWW	
<i>Variance Components</i>	BLUP	ssGBLUP	BLUP	ssGBLUP	BLUP	ssGBLUP
σ^2_a	0.02 \pm 0	0.02 \pm 0	0.01 \pm 0	0.01 \pm 0	8.52 \pm 1.28	9.1 \pm 1.29
σ^2_{ewe}	0.02 \pm 0	0.02 \pm 0	0.02 \pm 0	0.02 \pm 0	17.63 \pm 1.52	17.16 \pm 1.51
σ^2_e	0.35 \pm 0	0.35 \pm 0	0.35 \pm 0	0.35 \pm 0	239.13 \pm 1.73	239.12 \pm 1.73
σ^2_p	0.39 \pm 0	0.39 \pm 0	0.39 \pm 0	0.39 \pm 0	265.28 \pm 1.67	265.38 \pm 1.68
<i>Variance Ratios</i>						
h^2	0.04 \pm 0.01	0.04 \pm 0.01	0.03 \pm 0	0.03 \pm 0	0.03 \pm 0	0.03 \pm 0
c^2	0.05 \pm 0.01	0.05 \pm 0.01	0.06 \pm 0.01	0.06 \pm 0.01	0.07 \pm 0.01	0.06 \pm 0.01

8.3.4 Prediction Accuracy

The accuracy (ACC_{LR}) of (G)EBVs for production traits derived from validation scenarios (I) (II) and (III) can be seen in Table 8.5. In the case of the ‘forward’ design (I), individual accuracies within flocks are also presented. The ACC_{LR} was generally moderate to high, but varied considerably across production traits from the ABLUP derived EBV of 0.28 for WW to 0.63 for FD from ssGBLUP analysis. In comparing the methods, the prediction of WW appeared to benefit the most (+0.16) from ssGBLUP analysis, with mentionable gains also for YW (+0.09) and SL (+0.08). In the case of FD, the ACC_{LR} from ssGBLUP was slightly higher (+0.05) than the value of 0.58 derived from ABLUP analysis, representing more marginal gains from the genomically enhanced models. The range of ACC_{LR} also varied considerably across flocks, with evidence of more consistent results for resource flocks, except for the anomaly of very poor prediction of WW in the Cradock flock. In turn, the ACC_{LR} from candidates in the Elsenburg flock was very high compared to most other flocks for YW and WW, with little to no gain by ssGBLUP for these traits. Some flocks with lower initial BLUP accuracies for WW appeared to benefit the most from ssGBLUP (Grootfontein, 0.25 vs 0.12, Industry 5: 0.26 vs 0.14), but this pattern was not consistent across flocks (Industry 1: 0.17 vs 0.16). A similar pattern emerged for YW, and overall, the wool traits had lower gains delivered by ssGBLUP with the most notable within flock difference being also obtained for SL in the Grootfontein flock (0.26 vs 0.16). In the case of design (II), the prediction of candidates in commercial flocks from resource reference populations delivered erratic results for weight traits (Table 8.5), showing little to no capacity for predicting these traits across sectors. This was unexpected, and no similar pattern was observed for wool traits where the ACC_{LR} was moderate for ABLUP with noticeable gains in the case of ssGBLUP. When including some data from the commercial sector (III), the accuracy of ssGBLUP within the validation flocks improved to levels being comparable to ABLUP results observed in scenario (I) for the overall prediction of SL, but only to the lower results of some flocks for FD. For reproduction traits, results from scenario (I) were moderate to moderately high with overall ACC_{LR} of 0.38 to 0.53 with notable gains for NLW (+ 0.08) in ssGBLUP (Table 8.4). Within flock, results derived from the Elsenburg Merino flock similarly skewed the general distribution of accuracies. It is worth noting that the overall result of NLW remained similar after removing the Elsenburg flock from the calculation of ACC_{LR} (but retained in the analysis). Also, similar to results for production traits (Table 8.5), the remaining flocks appeared highly variable in both BLUP and ssGBLUP, ranging between 0.16 (Industry 5, BLUP) and 0.34 (Grootfontein, ssGBLUP) for NLW. The ability to predict reproduction traits in industry flocks (II) solely from resource flock data was limited (0.04-0.10), but better results were obtained from a combined reference

population (III) with an ACC_{LR} of 0.14 for NLW from ssGBLUP. While low, these results presented substantial proportional gains, as shown in more detail for the validation flocks Industry-1 and -4, discussed below.

Table 8.5

The accuracy of estimated breeding values from ABLUP (A) and ssGBLUP (ssG) analyses from validation designs (I), (II) and (III). Cases where the covariance (a_p , a_w) was negative are marked “-”

	WW		YW		FD		CFW		SL	
	A	ssG	A	ssG	A	ssG	A	ssG	A	ssG
SCENARIO (I)										
All	0.28	0.44	0.33	0.42	0.58	0.63	0.25	0.31	0.29	0.37
<i>Cradock</i>	-	0.04	0.45	0.44	0.46	0.45	0.2	0.23	0.2	0.23
<i>Elsenburg</i>	0.44	0.46	0.64	0.64	0.48	0.5	0.2	0.23	0.2	0.24
<i>Grootfontein</i>	0.12	0.25	0.21	0.31	0.37	0.42	0.3	0.35	0.16	0.26
<i>Tygerhoek</i>	0.28	0.29	0.32	0.36	0.28	0.27	0.24	0.27	0.25	0.29
<i>Industry_1</i>	0.16	0.17	0.11	0.15	0.31	0.32	0.21	0.21	0.27	0.29
<i>Industry_2</i>	0.17	0.23	0.15	0.2	0.32	0.33	0.15	0.17	0.16	0.19
<i>Industry_3</i>	0.2	0.23	0.16	0.22	0.22	0.25	0.07	0.15	0.14	0.14
<i>Industry_4</i>	0.24	0.35	0.18	0.27	0.32	0.32	0.22	0.26	0.27	0.3
<i>Industry_5</i>	0.14	0.26	0.14	0.22	0.18	0.17	0.1	0.17	0.17	0.23
SCENARIO (II)										
All	-	-	-	-	0.21	0.27	0.09	0.13	0.121	0.18
SCENARIO (III)										
All	-	-	-	0.11	0.29	0.32	0.13	0.17	0.22	0.27

Table 8.6

The accuracy of estimated breeding values from ABLUP (B) and ssGBLUP (ssG) analyses from validation designs (I), (II) and (III). Cases where the covariance was negative is marked “-”

Flock	NLB		NLW		TWW	
	A	ssG	A	ssG	A	ssG
SCENARIO (I)						
All	0.41	0.44	0.45	0.53	0.38	0.42
<i>Cradock</i>	0.22	0.26	0.23	0.23	0.22	0.24
<i>Elsenburg</i>	0.53	0.50	0.54	0.54	0.48	0.47
<i>Grootfontein</i>	0.31	0.31	0.33	0.34	0.37	0.39
<i>Industry_1</i>	0.17	0.2	0.21	0.22	0.23	0.28
<i>Industry_2</i>	0.23	0.26	0.24	0.26	0.18	0.23
<i>Industry_3</i>	0.19	0.19	0.19	0.2	0.19	0.25
<i>Industry_4</i>	0.18	0.20	0.17	0.2	0.18	0.20
<i>Industry_5</i>	0.1	0.16	0.16	0.18	0.11	0.17
SCENARIO (II)						
All	0.04	0.07	0.04	0.10	0.04	0.09
SCENARIO (III)						
All	0.04	0.09	0.08	0.14	0.1	0.12

8.3.5 Estimated breeding values of Industry-1 and -4 flocks

An example of the EBVs (u_p) compared to the full-data predictions (u_w) for Industry-1 and -4 is plotted individually for NLW for both ABLUP and ssGBLUP across all three (I, II, and III) scenarios in Fig. 8.2. Across both flocks, the advantage of having ancestors in the reference population was visible by the distribution of u_p that best coincided with the validated values u_w . In scenario (II) the low level of genetic connectedness to the reference population was evident, especially in ABLUP, as the majority of breeding values regressed to the zero mean of the base population. The additional information in ssGBLUP appeared to facilitate an improved capacity to distinguish between candidates (see Industry 4, Scenario (II) ssGBLUP vs ABLUP in Fig. 8.2), but also underestimated the EBVs with most u_p being negative. Comparing flocks, the somewhat stronger relationship of resource flocks to Industry-1 compared to Industry-4 (according to Fig. 8.1) appeared to yield better results. The ACC_{LR} for Industry-1 in Scenario (II) was 0.05 (ABLUP) and 0.11 (ssGBLUP) while a very low value of 0.02 (ABLUP) but a notably higher 0.08 (ssGBLUP) was reported for Industry-2. In Scenario (III), better distributions were also evident for both ABLUP and ssGBLUP results (Fig. 8.2), with the ACC_{LR} of 0.13 (Industry-1) and 0.15 (Industry-4) from ssGBLUP analysis close to being comparable to some of the ‘within-flock’ validation results outlined in Table 8.6.

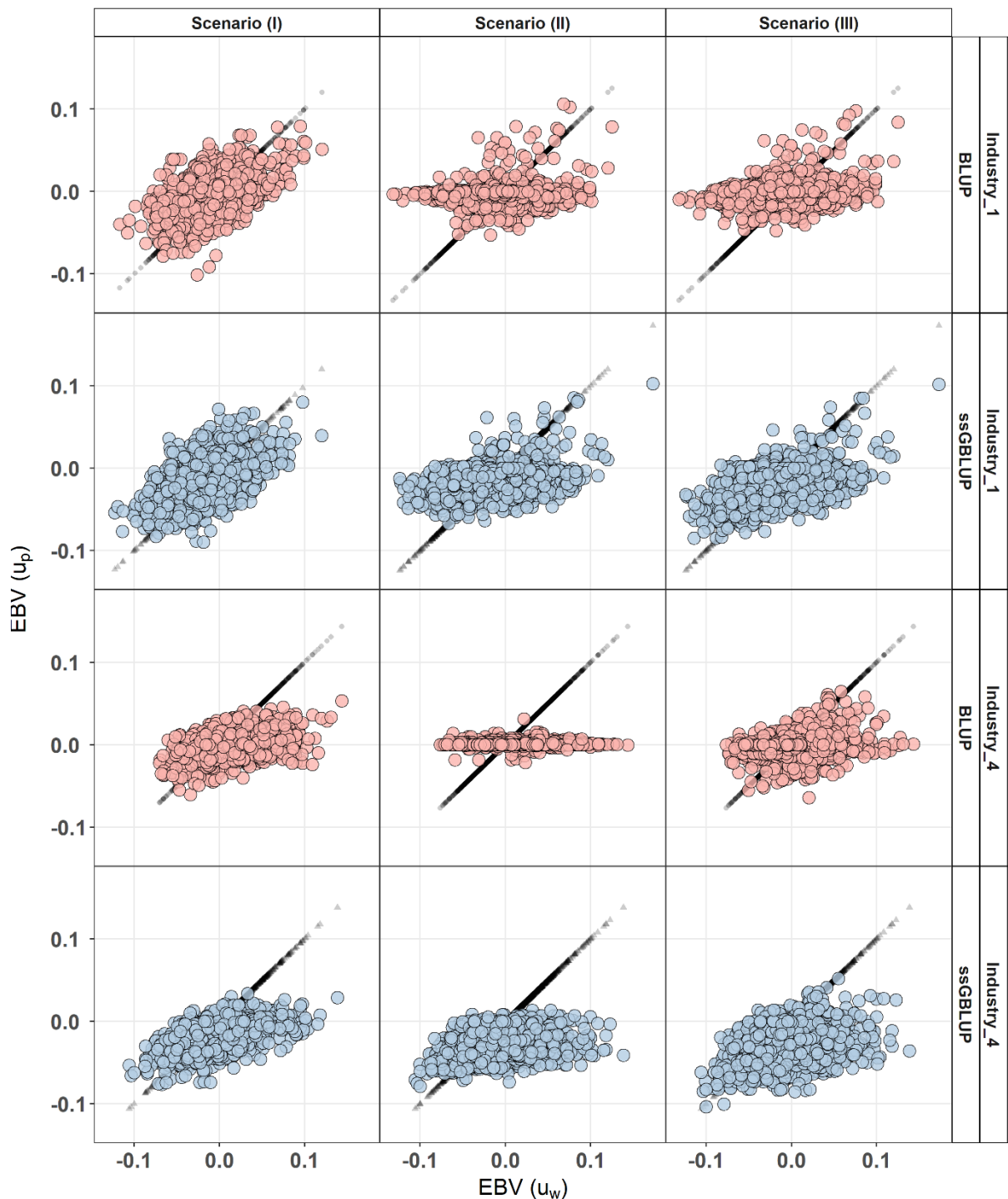


Fig. 8.2 Estimated breeding values (u_p) of Industry-1 and -4 flocks in Scenario (I), (II) and (III) according to EBVs derived from the whole dataset (u_w) for ABLUP and ssGBLUP analyses. The black points are the expected values if $u_p = u_w$.

8.4 Discussion

This study is the first to report on the use of genomic SNP data to enhance the prediction of production and reproduction traits in South African Merinos. It is also the first across-flock analysis of the SA resource flocks (Schoeman *et al.* 2010) and the combination of data originating from both the commercial and resource sectors. The data concatenation of these datasets appeared to deliver reliable results. The least square means of fixed

effects and the estimated variance components were generally in line to what is expected from the literature, and will not be discussed here. For in-depth treatment of the variance components of production or reproduction traits, see Chapter(s) 1 and 2 of this thesis, respectively.

Assuming other factors kept constant, the accuracy of genomic prediction depends on the size of the reference population, the h^2 of the trait, and the level of genetic diversity (Daetwyler *et al.* 2008; Goddard *et al.* 2011). Given the relatively high level of genetic diversity commonly reported for sheep (Kijas *et al.* 2009), and especially Merinos (Al-Mamun *et al.* 2015; Ciani *et al.* 2015; Prieur *et al.* 2017; Chapter 7: Nel *et al.* 2021), it is expected that large reference population sizes are needed for accurate genomic selection. However, despite a relatively modest reference population size, the use of genomic information through the combined H-matrix generally benefited the accuracy of prediction for most traits concurring with the expected range of difference of between +5 to +15 % expected for sheep (Van der Werf *et al.* 2014).

A majority of the previous validation studies for genomic selection production traits in Merinos are earlier studies that were based on genomic BLUP (GBLUP) methods that directly replaced **A** with **G**. From these studies, the accuracy of GEBVs in Australian Merinos for WW (0.52), YW (0.58), CFW (0.65), FD (0.72) and SL (0.56) reported by Moghaddar *et al.* (2014) were slightly higher compared to those reported here (Table 8.4), but ranked in a roughly similar order than in the current study. A notable aspect of the study by Moghaddar *et al.* (2014) was that the accuracy of GBLUP varied considerably across Merino bloodlines, for example between 0.39 ('Ultrafine' line) to 0.50 ('Fine' Line) for FD and between 0.25 ('Ultrafine' line) to 0.61 ('Strong' line) for SL. Very high prediction accuracies for wool traits of 0.79 for CFW and > 0.79 for FD were reported from the first GBLUP results for Merinos (Daetwyler *et al.* 2010), but results for meat traits were lower and highly variable (-0.04 to 0.57). Also from GBLUP analysis, Moghaddar *et al.* (2019) recently reported reasonable accuracies for CFW (0.35) and post-weaning weight (0.40), but a comparatively low estimate for FD (0.38). Using two-step blending, accuracies were also high (0.75 to 0.87) from blended 'multi-step' GEBVs in Merino sires for both weight and wool traits, commonly with gains between 0.05 to 0.07 compared to standard ABLUP analyses, but considerably higher than GBLUP results from the same study (Swan *et al.* 2012).

For reproduction traits, Daetwyler *et al.* (2014) reported GLUP accuracies between 0.06 to 0.12 for NLB, NLW and litter size in Merinos when predicting across sire families, but a much higher range of between 0.21 and 0.40 when the validation sets were assigned at random. A similar pattern was reported by Bolormaa *et al.* (2017), but with a slightly higher GBLUP accuracy (0.32 to 0.54) for reproduction traits when predicting across randomly assigned groups. These results accorded well with the values reported here (Table 8.6), and are similar to the difference between scenario (I), when close relatives were likely present in the validation set, to II and III, where prediction considered only the data from more distant populations. In examples of other sex limited traits, Legarra *et al.* (2014) reported an increase in accuracy of between 0.05 and 0.30 for milk yield, following ssGBLUP in Laxta dairy sheep. They also reported results to be highly variable across breed groups, and in selected instances, better results were obtained from ABLUP.

As with the current results for scenario (II) (Table 8.5 and 8.6), GS was capable of moderate accuracies in relatively unrelated populations when ABLUP predictions were close to zero (Clark *et al.* 2012). This

situation especially applied to the low h^2 of traits such as NLB or NLW, where an increase in the reference population size is expected to improve results (Daetwyler *et al.* 2008; Li *et al.* 2017). However, under these circumstances, a low threshold for the gains made by GS is expected, since it has been shown that the accuracy of genomic prediction has limited penetration across distant populations (Habier *et al.* 2007, 2010). In building reference populations capable of predicting target animals, the value of the additional information relies heavily on the extent to which the additional animals are related to the target animals (Clark *et al.* 2012; Van der Werf *et al.* 2015; Lee *et al.* 2017). Comparing empirical evidence of earlier GBLUP studies in sheep (Daetwyler *et al.* 2010, 2012; Moghaddar *et al.* 2013), the review by Van der Werf *et al.* (2014) identified a general trend of underperformance of realized accuracy compared to the theoretically expected gain in accordance to increased reference population sizes. As stated by Van der Werf *et al.* (2014), it is very likely that population structures not accounted for would influence the capacity for prediction, as could have also been the case in the current study.

The limited results of prediction across sector (Scenario II) are thus likely a function of the somewhat isolated nucleus of the resource populations (Fig. 8.1), especially the Elsenburg flock, which contributed the largest number of genotypes from a single flock in this study. In addition to the results of Moghaddar *et al.* (2014) noted above, Daetwyler *et al.* (2012) reported a compromised accuracy of GEBVs when predicting across different strains (e.g. ‘Ultrafine’, ‘Fine’, ‘Strong’) of the Australian Merino. In a previous study that reported on the population genetic architecture of Merino subpopulations, Nel *et al.* (2021) (Chapter 7) showed that divergence across the South African resource flocks was notable, but considerably less pronounced than that observed across the Australian strains. However, while better results could be expected in the current population, the study by Nel *et al.* (2021) (Chapter 7) also stressed that links to the connectedness of industry sample genotypes tended to vary across resource flocks, and this result was reiterated in Fig. 8.1 of this study.

Despite the limited accuracy observed in the case of Scenario (II) and (III), it was clear from Scenario (I) that the accuracy of prediction varied appreciably across flocks across but also within traits. In terms of population diversity parameters, such as internal relatedness, linkage disequilibrium, or effective population size, there was relative agreement across the SA resource flocks in the results reported by Nel *et al.* (2021) (Chapter 7). It is thus important that the benefits of genomic information are not considered a universal measure and requires validation for a wide variety of particular scenarios. Therefore, implementation and investment should commence strategically. The number of genotypes included in the current study, albeit limited ranging between 128 to 320 for commercial flocks, in fact represent realistic targets within the fiscal scope of seed stock breeders maintaining around ~400 to ~600 ewes. With the cost-effective genotyping rate recommended at around 20% of male candidates (Van der Werf *et al.* 2014; Horton *et al.* 2015), it would still take most flocks numerous years to reach a ‘within-flock’ genotyping rate of about 300 to 400 genotypes. Also, maintaining the reliability of the reference population will require continuous genotyping across generations, since the accuracy of genomic prediction is expected to deteriorate over generations (Habier *et al.* 2010).

These practical limitations to a ‘per flock’ genotyping rate emphasize the need to rely on a combined reference population across sectors and flocks. In this regard, Scenario (II) and (III) become particularly relevant as a general indication of how well information can be beneficially translated across flocks and more

research is needed to optimize these results. From the current results, it can be deduced that a genotyping strategy will be best exploited if combined with dedicated efforts of cross fertilization to ensure that target candidates are linked by relatives to multiple sources. However, it should be noted the objective of this pilot study was to deliver a first assessment of genomic selection for South African Merinos, and has not exhausted all the methodological options to improve on the realized results. Further pruning of the dataset could improve the unexpected and erratic accuracy observed for selected traits, particularly for WW. Also, the scaling parameters α and β heavily weighted information in \mathbf{G} in the ssGBLUP analysis. This could be suboptimal in the current situation where different flocks are genotyped in highly variable numbers and the overall size of the genomic dataset was also small compared to the number of individuals in the pedigree. Alternative scaling parameters could potentially yield better results (McMillan and Swan 2017). The impact of a relatively high proportion of missing pedigree information can be mitigated by considering unknown parent groups (Quaas 1988) but requires particular consideration in ssGBLUP models (Misztal *et al.* 2013). The across sector prediction was likely also inhibited by the distant and entirely unlinked pedigrees. The so called ‘metafounders’ approach (Legarra *et al.* 2015) could therefore prove useful to better align pedigree relationships in \mathbf{A} according to information generated by deriving \mathbf{G} from marker data. Furthermore, there exists opportunity to facilitate development of genomic reference populations by including genotypes from Australian Merinos (Chapter 6 and 7: Nel *et al.* 2019, 2021) for which single step pipelines and reference populations has already been well established (Swan *et al.* 2014; Brown *et al.* 2018).

8.5 Conclusions

The results from this study have shown potential for genomic data to enhance the development of breeding objectives to include a greater scope of traits in South African Merino breeding programs. However, results tended to be variable across flocks, and continued validation of realized results should be a priority in continued development. Prediction across the research and commercial sectors was suboptimal, but larger reference populations and more sophisticated methodologies should improve these results. Continued research will be essential to optimize the use of these resources.

8.6 References

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General Conclusions and Recommendations

Preamble:

The study comprises of two parts, firstly involving responses to selection in a well-known South African ovine genetic resource flock and secondly dealing with genomic tools foreseen to be instrumental in enhancing genetic recording and evaluation in ovine animals. Comparing the first and latter parts of this thesis, a juxtaposition of focus is apparent with the first being very narrow, involving a single resource population. In contrast, the second part drew information from a wide array of commercial and institutional genetic resources with their origin either in South Africa or in Australia.

Conclusions:

The first part of this thesis comprised of an in-depth account of the Elsenburg Merino selection lines as an example of what can be achieved in an ‘alternative’ selection program. The project targeted a divergent selection for and against number of lambs weaned per ewe joined (NLW), a composite trait for which, by traditionalist views of the wider industry, prospects of success were distinctly bleak at the commencement of breeding in 1986. Chapter 1 provided an exhaustive account of genetic parameters and trends involving production traits in these lines. The derived genetic trends showed that realized genetic change associated with selection for number of lambs weaned per ewe joined (NLW) did not have severely detrimental effects for genetic change in economically important traits. In fact, there was evidence of favourable correlated responses in certain traits such as liveweight at weaning and at yearling age. There were some exceptions, such as clean fleece weight and staple strength, but none that should not be manageable within the capacity of an appropriate selection index. This was a very important result for guiding the wider industry into considering alternative selection indices. Chapter 2 emphasized the very favourable direct genetic gains achievable for NLW, as a lowly heritable composite reproduction trait. In retrospect, this should not be too surprising, since NLW boasts considerable phenotypic variation to counter the effect of a low heritability. It should also be remembered that NLW was the primary selection criterion separating the positive and the negative selection lines (referred to as the “High” or H- and the “Low” or L-Lines, respectively). However, the associated divergent trends for conception and multiple birth rates, as well as for ewe rearing ability, resonated the impact of using a composite trait such as NLW as the primary selection criterion. The biggest concern of an increased litter size at birth, however, is the well-known susceptibility of multiples. Because it is a very current and even emotional subject, this topic is treated extensively in Chapters 3, 4, and 5. The results derived for survival (as a trait of the lamb) in Chapter 3 were very favourable on both the genetic and phenotypic levels. A concern is that multiple rearing ability, as based on ewe records, would not be treated with the same emphasis in traditional South African breeding programs. For this reason, it was recommended that survival phenotypes should be considered in selection indices individually. Also, the marked phenotypic differences between the H- and L-Lines suggested that, if a more extensive breeding program were capable of replicating just a portion of the gains made by the H-Line, the realised gain would still be a worth-while reduction in mortality rates. As with previous results (Cloete *et al.* 2009), the realized responses between these lines was surprising, given that the heritability for

both survival (Chapter 3) or mortality (Chapter 5) was consistently low as based on within flock genetic analyses. In this regard, the role of an environmental covariate that described the effect of cold, wet and windy conditions during a typical winter lambing season at the experimental site, strongly suggested that a genotype by environment component was important to early neonatal survival.

The traits considered in Chapters 3, 4, and 5 provided an example of phenotypes that should be feasible for recording by the wider South African industry. Recording survival in a system that does not penalize mortality by non-genetic effects (Vanderick *et al.* 2015); recording date (age) at incidences of mortality; and including dead lambs into pedigrees are all feasible and inexpensive to execute on a large scale. Records of rectal temperature also appeared to be very promising (Chapter 4), but it is moderately invasive and arguably requires more consideration before being recommended on a large scale. The weather data used to derive the cold stress gradient relevant to Chapters 4 and 5 can be supplied by relatively affordable weather data that can be acquired from a network of weather stations throughout South Africa.

An attractive prospect for further studies is to ‘capture the success’ of the H-Line on molecular platforms, such as by using genome wide association studies or signatures of selection to identify markers that could be useful for the prediction of NLW. However, an important point originating from Chapters 1 to 5 stems from the previously observed fact that the Elsenburg selection program benefited multiple facets of animal health and welfare. Each of the noted characteristics of the H-Line (in this thesis or elsewhere), of which examples include growth, survival, viability or thermoregulation of H-Line lambs, as well as conception rate, fertility, rearing ability, flystrike resistance or behaviour of H-Line ewes, represent in themselves a complex trait of a continuous nature. Each of these components could putatively be decomposed into lower-level functions. For example, number of lambs born per ewe lambing (litter size) can be further redefined into underlying components such as ovulation rate and embryo loss. It is therefore likely that the selection program has manifested in a very large number of small favourable changes to overall animal physiology. This contention is further supported by line differences in the activity of the hypothalamic-pituitary-adrenal (HPA) axis in biochemical studies and specifically in the way animals respond to stress (Hough *et al.* 2013). At face-value, this result does not have an obvious link to NLW. From this point of view, it is hard to expect that any particular marker or selected set of markers would prove useful in simultaneously predicting favourable genetics for a composite trait such as NLW, which represents the sum of all these underlying components.

Against this background, it is thus not surprising that the early results of marker assisted selection proved disappointing in other genetic resources and settings, and that the functional annotation of markers are still primarily limited to breed characteristics or deleterious alleles (Georges *et al.* 2019). As such, the overarching concept of ‘many small effects’ extends well to the use of genome wide markers within the theory of the application of genomic selection (Meuwissen *et al.* 2001), which introduces the general theme of Chapters 6, 7, and 8.

Exploring strategies toward building a genomic reference population to predict traits of economic importance for South African Merinos as well as for other common breeds, Chapter 6 provided the first indication of the across-country compatibility of Australian and South African Merinos by imputation across genomic reference sets. The results so obtained were promising, but ultimately suboptimal compared to what

was achieved within the Australian ovine genetic resource (Moghaddar *et al.* 2015). It was concluded from Chapter 6 that higher density panels could potentially capture the limited extension of haplotypes expected to be shared in a diverse breed across countries to a greater extent. However, the results of Chapter 7 suggested that a narrow spectrum design with regards to phasing and imputation panels could be the best approach to deliver the improved results. Results from Chapter 7 supported previous population genomic reports highlighting a generally very high diversity across and within sheep breeds, that was also reflected in a relatively large effective population size, particularly in the Merino-type breeds. Another key result here was that significant population substructures can be extended beyond breeds, and that the extent of relationship was highly variable across populations. Chapter 8 subsequently provided the first validation of genomic selection in South African sheep that we are aware of. Overall, the results were promising, but some unexpected results observed in selected traits, particularly for weaning weight. The variable results across certain flocks also constituted a concern, and continuous validation should be a key strategy during the future implementation of genomic prediction using a suitable genomic reference population. If a larger reference population could be combined with a dedicated effort of cross fertilisation between both the commercial and institutional sectors and flocks, accurate prediction of difficult to measure or fitness traits should be a reality given promising results reported elsewhere (see Pickering *et al.* 2013; Brito *et al.* 2017; Oliveira *et al.* 2021). In Chapter 7, the boundary of across country isolation was rarely associated with strong signs of divergence across common breeds between SA and Australia. The results of this thesis have pointed to valuable opportunities to align the SA small stock selection programs with the progressive trends on the international landscape, as detailed by Dodds *et al.* (2014) and Brown *et al.* (2018). Such an intervention is expected to benefit small stock production as well as the health and welfare of the animals on which it depends.

Against this background, it is recommended that:

- Studies on environmental stressors should continue on the Elsenburg flock, as well as on other local resource flocks. Studies should consider the effects both of hot and dry conditions as well as of cold, wet and windy conditions. The prevalence of both sets of conditions are expected to increase as a result of global climatic instability. The random regression approach that was attempted to model a G x E in Chapter 5 should be refined and applied to larger databases with a greater range of environmental conditions. The principle could also be extended to commercial flocks with suitable data located close to a suitable weather station.
- Genomic approaches to better understand the functional variants underlying the biological changes in the Elsenburg flock should be considered. Although it is very likely that most of these changes would subscribe to principles involving the infinitesimal model, studies such as genome-wide association scans (GWAS) could still contribute to the functional understanding of composite reproduction traits and yield information that could be generalised to other populations in future. Such a project may benefit from a substantial number of animals that were genotyped for other reasons.
- Further studies on imputation are indicated, making use of the presently available genotyping platforms. Within this study, genotyping was based on three platforms, emphasising a need to be able to migrate between these genotyping tools with confidence.

- Chapter 8 merely scratched the surface of what can be achieved in term of genomic selection of South African sheep and needs to be expanded considerably. Further studies should make optimal use both of local and Australian resources. The relatively close correspondence between the South African and Australian ovine genetic resources (Chapters 6 and 7) adds an additional motivation for such studies.
- A limited number of traits form part of formal small stock evaluation in South Africa at present. Consideration should be given to either expand on the number of traits considered in commercial flocks or to consider how the well-phenotyped institutional resource flocks could play a greater part in predicting across flocks and industry sectors by using a genomic approach. Given the need for validation stressed in Chapter 8, recording of hard to measure or fitness traits will have to be initiated for selected, and preferably well connected, commercial flocks.
- This study mostly focused on Merinos. However, there is scope to expand it to other breeds with alternative breeding objectives than wool sheep. Other local breeds may benefit from a smaller effective population size compared to Merinos. A smaller reference population could arguably be needed in such cases. However, most genotyping costs have so far been incurred by research institutions. A substantial portion of genotyping costs should be transferred to industry to broaden the base of local genomic prediction.

It is hoped that this study will provide a foundation for further studies on traits that are hard to measure as well as for the further application of genomic principles to the local ovine genetic resources, both commercial and institutional. Studies of this nature are needed in the face of rapidly changing conditions. New challenges facing the South African sheep industry include aspects like climate change as well as an increased emphasis on traits associated with animal welfare which is not well recorded in South Africa at present. The local industry needs to react to these challenges in a positive and constructive manner, to align itself to international trends as far as ethical and sustainable production practices are concerned.

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Supplementary Information - Tables

Table S1 (Chapter 4)

Definitions characterising subjective scores assigned to lambs and ewes during handling

Definitions characterising subjective scores assigned to lambs and ewes during handling Lamb Shiver Score (LSS)	1 – Lamb does not shiver.
	2 – Lamb shivers slightly, but it is barely perceptible when a hand is rested on its rump.
	3 – Lamb shivers somewhat, the sensation of shivering is distinct when a hand is rested on its rump.
	4 – Lambs shivers distinctly and can be diagnosed without touching it.
	5 – Lamb shivers extremely and the whole body is moving distinctly without the need to resort to touch.
Lamb Vigour Score (LVS)	1 – Lamb is dead.
	2 – Lamb is lethargic and does not get up when approached.
	3 – Lamb gets up when approached but do not attempt to escape.
	4 – Lamb gets up when approached and attempts to run away, but do not struggle/bleat when handled.
	5 – Lamb gets up when approached and attempts to run away and struggles/bleats when handled.
Maternal Behaviour Score	1 – Ewe flees to flock mates (>20 m) and do not return to the lamb(s) and the lamb(s) has to be reunited with her.
	2 – Ewe flees to flock mates (>20 m) but returns to the lamb(s) to be spontaneously reunited within 2-3 minutes.
	3 – Ewe retreats to a distance of <20 m, remains apprehensive but returns to the lamb(s) to be spontaneously reunited within 1-2 minutes.
	4 – Ewe balances staying close to the lamb(s) and retreating to a distance of <5 m and returns to the lamb(s) to be spontaneously reunited within 30 seconds.
	5 – Ewe stays close (<2 m) to the lamb during the marking process and may make a nuisance of herself by putting her head in the bucket used for weighing.

Table S2 (Chapter 7)

Mean relatedness across breed groups according to the genomic relationship matrix (GRM_{BR}). Estimates in bold are the internal relatedness for a breed group calculated without the diagonal of GRM_{BR}

	AUS_M	SA_M	AUS_DM	SA_DM	AUS_SAMM	SA_SAMM	SA_DMR	AUS_DR	SA_DR	AUS_PD	AUS_WS	AUS_C	AUS_BL
AUS Merino	0,09	0,04	0,03	0,03	0,03	0,03	-0,01	-0,03	-0,03	-0,08	-0,04	-0,05	-0,1
SA Merino	-	0,14	0,05	0,05	0,02	0,02	-0,02	-0,04	-0,04	-0,09	-0,05	-0,06	-0,11
AUS Dohne Merino	-	-	0,24	0,14	0,09	0,1	0,04	-0,03	-0,03	-0,07	-0,03	-0,03	-0,07
SA Dohne Merino	-	-	-	0,17	0,1	0,1	0,04	-0,03	-0,03	-0,07	-0,03	-0,04	-0,08
AUS SAMM	-	-	-	-	0,28	0,27	0,07	-0,02	-0,01	-0,06	-0,02	-0,02	-0,07
SA SAMM	-	-	-	-	-	0,31	0,08	-0,02	-0,01	-0,06	-0,02	-0,02	-0,07
SA Dormer	-	-	-	-	-	-	0,38	0,01	0,02	0,03	0,02	0	-0,04
AUS Dorper	-	-	-	-	-	-	-	0,31	0,28	0,03	0,01	-0,04	-0,1
SA Dorper	-	-	-	-	-	-	-	-	0,3	0,03	0,01	-0,04	-0,09
AUS Poll Dorset	-	-	-	-	-	-	-	-	-	0,39	0,09	-0,03	-0,08
AUS White Suffolk	-	-	-	-	-	-	-	-	-	-	0,21	0,01	-0,03
AUS Coopworth	-	-	-	-	-	-	-	-	-	-	-	0,3	0,16
AUS Border Leicester	-	-	-	-	-	-	-	-	-	-	-	-	0,44

Table S3 (Chapter 7)

Mean relatedness across bloodline groups according to the subset genomic relationship matrix (GRM_{BL}). Estimates in bold are the internal relatedness for a bloodline group calculated without the diagonal of GRM_{BL}

	Els	Grt	Ind	Crk	Ult	FM_2	FM1	Str
Elsenburg	0,12	0,01	0,01	-0,02	-0,04	-0,04	-0,05	-0,07
Grootfontein	-	0,11	0,05	0,04	-0,01	-0,01	-0,03	-0,04
Industry	-	-	0,12	0,01	-0,01	-0,01	-0,02	-0,03
Cradock	-	-	-	0,14	0,03	-0,02	-0,03	-0,05
Ultrafine	-	-	-	-	0,1	-0,01	-0,02	-0,03
Fine-Medium-2	-	-	-	-	-	0,25	-0,02	-0,03
Fine-Medium-1	-	-	-	-	-	-	0,18	-0,02
Strong	-	-	-	-	-	-	-	0,2

Supplementary Information - Figures

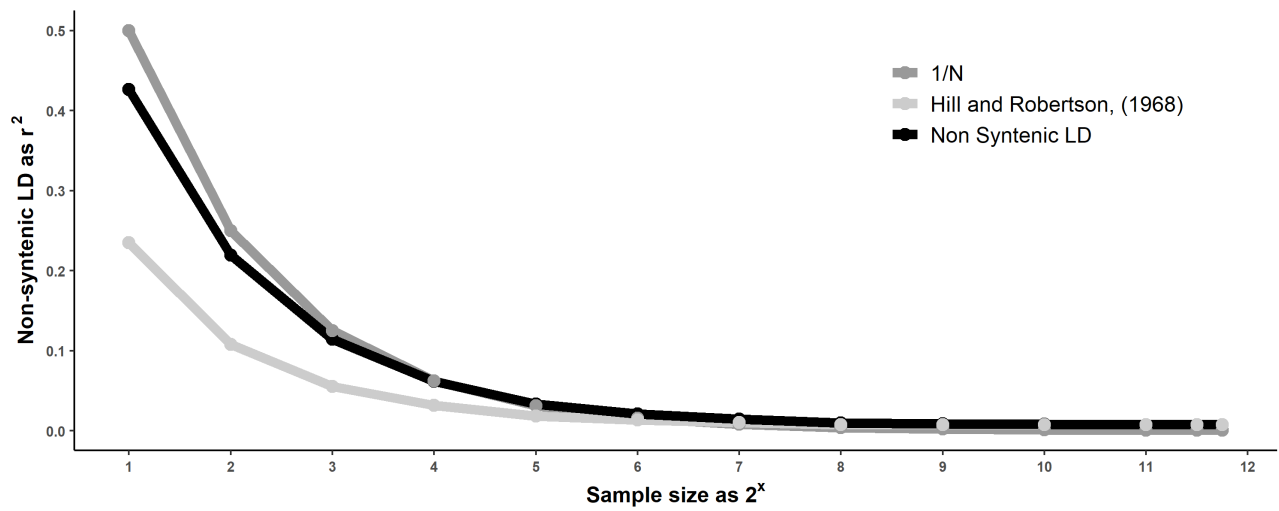


Fig. S1 (Chapter 7). Non-syntenic LD as r^2 calculated from sample sizes of 2^n (where n ranged from 1 to 11.75) with the reciprocal of sample size (N) and the correction factor suggested by Hill and Robertson, (1968)

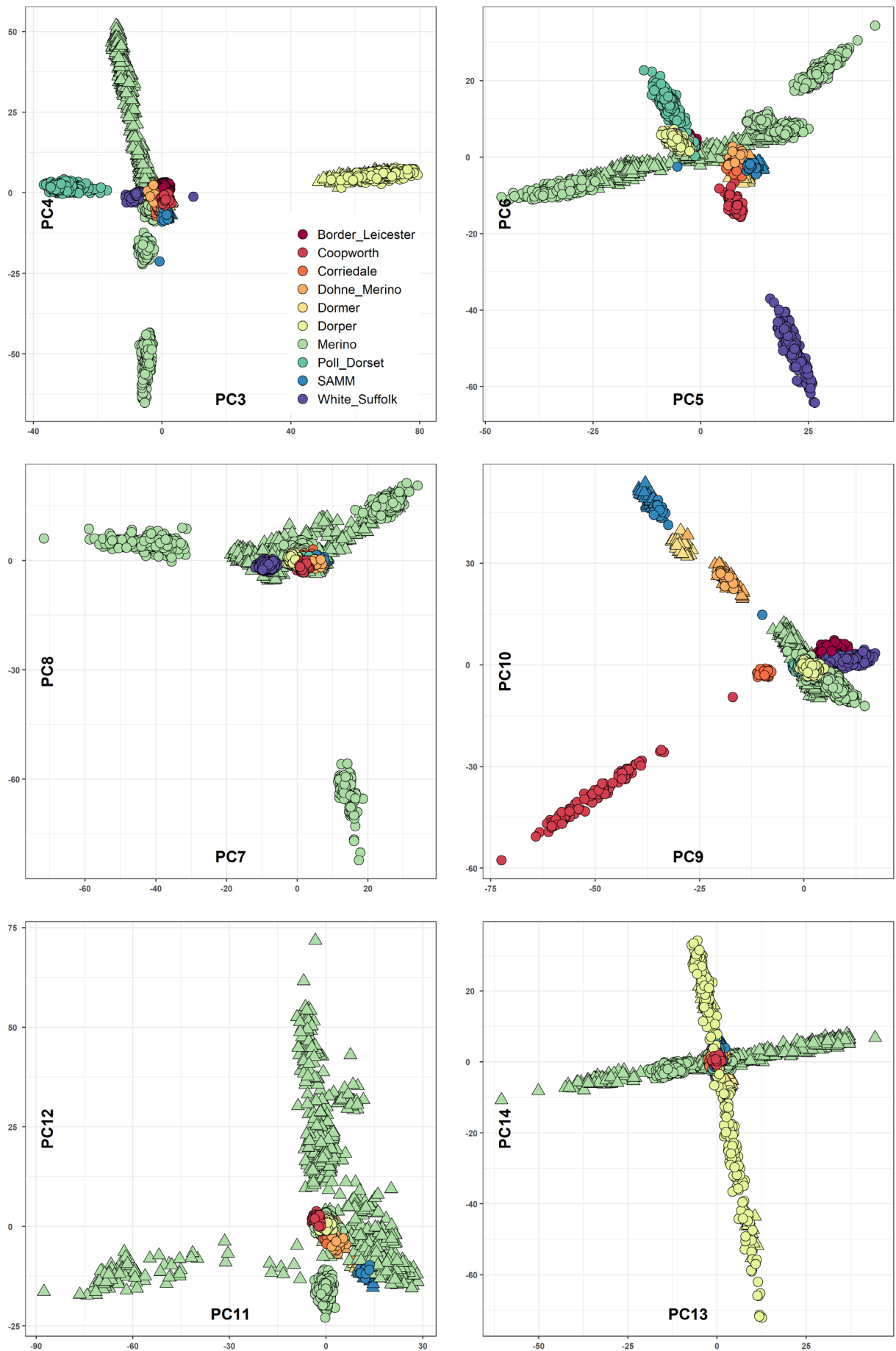


Fig. S2 (Chapter 7). The third (PC3) to fourteenth (PC14) principal components of genotypes identified by breed group

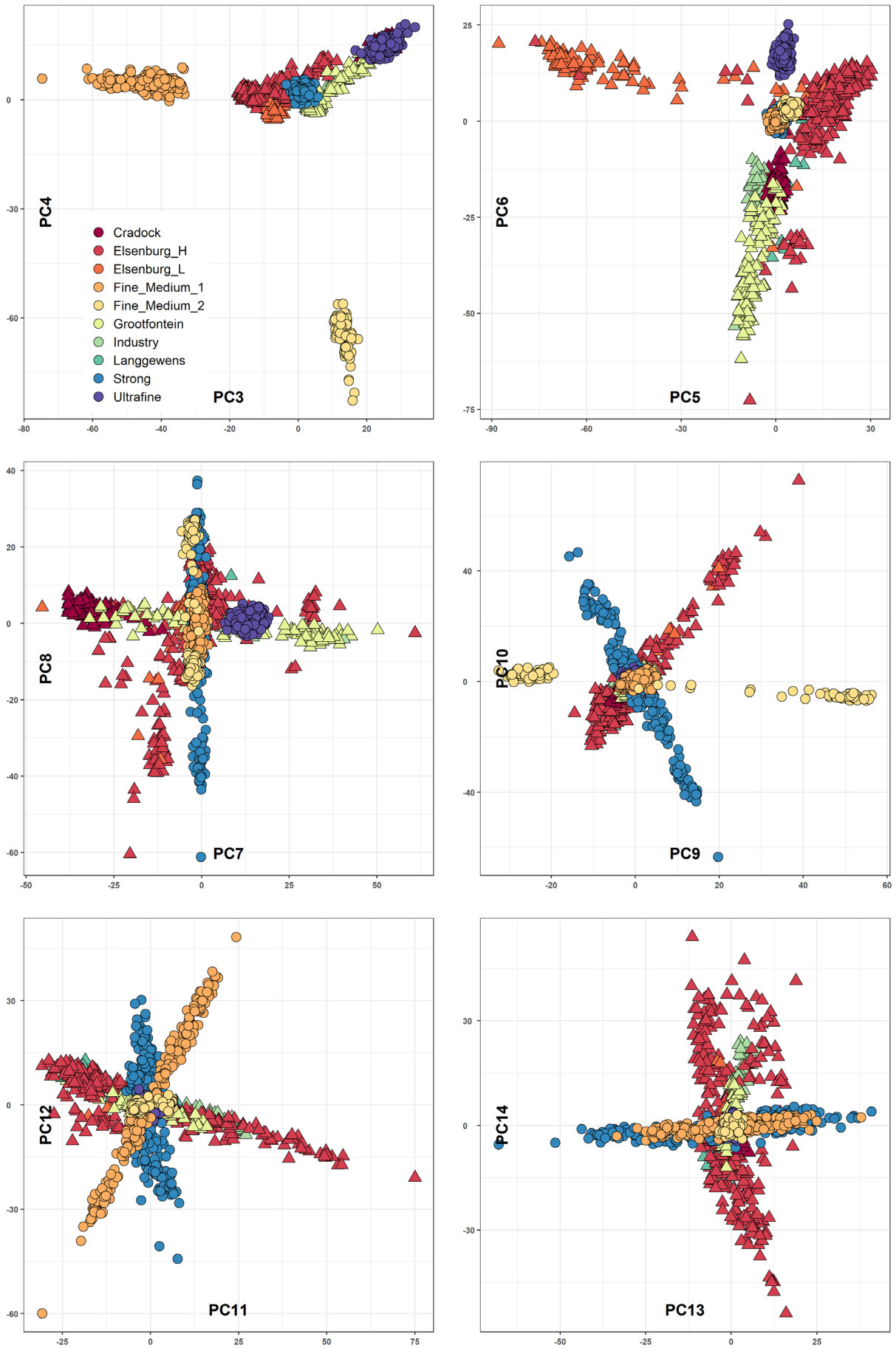


Fig. S3 (Chapter 7) The third (PC3) to fourteenth (PC14) principal components of genotypes identified by bloodline

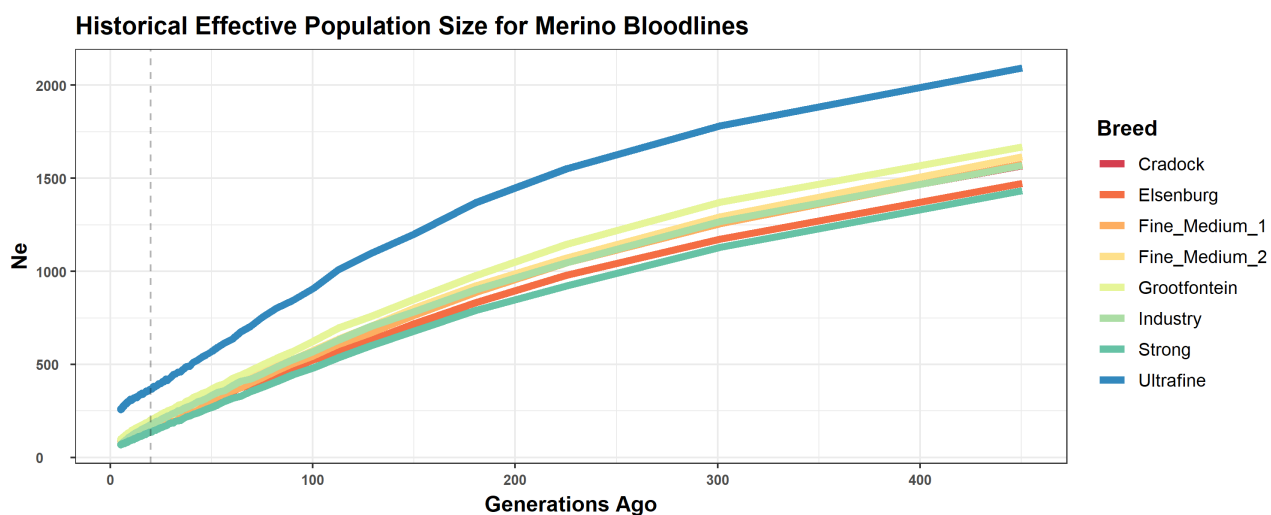


Fig. S4 (Chapter 7) Historical effective population sizes (N_e) for Merino bloodline groups from 2 to 400 generations ago. The vertical-line represents the timepoint used for 'current' N_e

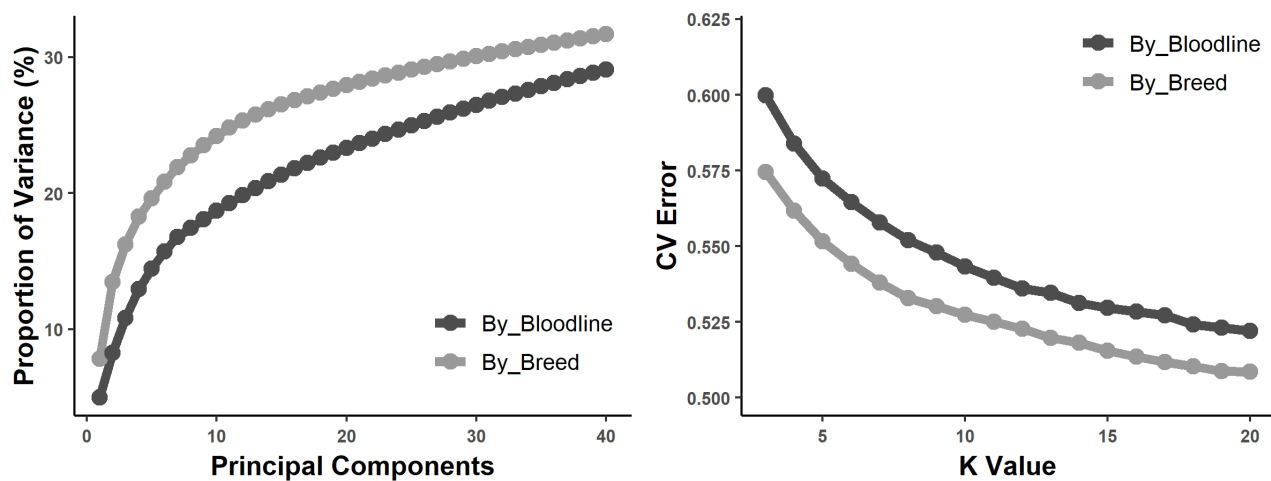


Fig. S5 (Chapter 7) (A) Cumulative proportions of variance (POV) explained by the first 40 principal components of 'by breed' and 'by Merino bloodline' analysis. (B) The "CV-error" statistic resulting from sequential ADMIXTURE runs or $K = 3$ to $K = 20$ of 'by breed' and 'by bloodline' analysis

Supplementary Information – R Scripts

```

#### Weir and Cockerham Fst for all pairwise subpops
# geno = 0/1/2 animals by snps geno file
# spop = list of subpop keys

wcFst_by_pair <- function(geno, spop) {

  N = nrow(geno) # sample size
  if(length(spop) != N) {
    stop("Number of samples not equal to length of spop\n")
  }
  spop <- (as.factor(as.character(spop)))
  unique.spop <- levels(spop)
  nspop <- length(unique.spop)

  n0 <- n1 <- n <- matrix(NA, ncol = nspop, nrow = ncol(geno),
    dimnames = list(NULL, unique.spop))

  for(i in 1:nspop) {
    inds <- which(spop == unique.spop[i])
    n0[,i] <- apply(geno[inds, ] == 0, 2, sum, na.rm = T)
    n1[,i] <- apply(geno[inds, ] == 1, 2, sum, na.rm = T)
    n[,i] <- apply(!is.na(geno[inds, ]), 2, sum, na.rm = T)
  }
  p <- ((2*n0)+ n1) / (2*n)
  Ho <- (n1/n)

  pairwise.wcFst <- matrix(NA, ncol = nspop, nrow = nspop)
  r = 2

  for(i in 1:(nspop-1)) {
    for(j in (i+1):nspop) {

n_bar <- apply(n[,unique.spop[c(i,j)]], 1, sum, na.rm=T)/r

nc <- ((r*n_bar)-(apply((n[,unique.spop[c(i,j)]]*
n[,unique.spop[c(i,j)]])/
(r*n_bar), 1, sum, na.rm=T)))/(r-1)

p_bar <- apply((n[,unique.spop[c(i,j)]]*
p[,unique.spop[c(i,j)]])/
(r*n_bar), 1, sum, na.rm=T)

s_square <- apply((n[,unique.spop[c(i,j)]]*
((p[,unique.spop[c(i,j)]]-p_bar)^2))/
((r-1)*n_bar), 1, sum, na.rm=T)

h_bar <- apply((n[,unique.spop[c(i,j)]]*
Ho[,unique.spop[c(i,j)]])/
(r*n_bar), 1, sum, na.rm=T)

a_hat <- (n_bar/nc)*(s_square-((1/(n_bar-1))*
((p_bar*(1-p_bar))-(((r-1)/r)*s_square)-((1/4)*h_bar))))

b_hat <- (n_bar/(n_bar-1))*((p_bar*(1-p_bar))-
(((r-1)/r)*s_square)-
(((2*n_bar)-1)/(4*n_bar))*h_bar)

c_hat <- h_bar/2

inds <- which(is.finite(a_hat)&is.finite(b_hat)&is.finite(c_hat))

```



```

pairwise.wcFst[i,j]<-sum(a_hat[inds],na.rm=T)/
sum(apply(cbind(a_hat,b_hat,c_hat)[inds,],1,sum,na.rm=T),na.rm=T)

rm(n_bar, nc, p_bar, s_square, h_bar, a_hat, b_hat, c_hat,inds)

}
}

colnames(pairwise.wcFst) <- rownames(pairwise.wcFst) <- unique.spop
return(pairwise.wcFst)

}

##### ----- #####
### LD decay per population
# geno = 0/1/2 animals by snps geno file
# spop = list of subpop keys
# depth is window size
# map is snpMap

LD_decay <- function(geno, spop, depth, map){

require(dplyr)
require(tidyr)
require(snpStats)

nchrom <- length(levels(as.factor(map$Chr)))
print(paste("There are ", nchrom, "chromosomes in map"))
#lastmarker <- matrix( nrow = nchrom, ncol = ncol(map))

# remove last markers from chromosome, the window will pass
# to the next chromorsome

npos <- vector(length = nchrom)
xpos <- vector(length = nchrom)
start <- vector(length = nchrom)
range <- map$chip_1
for(i in 1:nchrom){
  npos[i] <- length(which(map$Chr == i))
  xpos[i] <- map$chip_1[which(map$Chr == i)][npos[i]]
  start[i] <- (xpos[i] - depth)
  range[which(map$chip_1 %in% map$chip_1[start[i]:xpos[i]])] <- NA
}
non_syntenic <- which(is.na(range))
print(paste("Non Syntenic", length(non_syntenic)))

print(paste("Total geno has", nrow(geno), "SNPs with ", ncol(geno), "animals"))

unique.spop <- levels(as.factor(spop))
nspop <- length(unique.spop)
print(paste("Sub populations are ", unique.spop))

decay <- matrix(nrow = nspop, ncol = depth)

for(k in 1:nspop){

inds <- which(spop == unique.spop[k])
print(k)
M <- geno[inds, ]
snpM <- new("SnpMatrix", M + 1)
LDall <- ld(snpM, depth = depth, stats = "R.squared")

```

```

matrix <- summary(LDall) %>% tibble :: as.tibble() %>%
  arrange(i, j) %>% filter(i <= nrow(LDall) - depth) %>%
  getElement("x") %>% matrix(ncol = depth, byrow = TRUE)

print("LD results")
print(matrix[1:5, 1:5])
print(paste("all markers", nrow(matrix)))
matrix <- matrix[-c(non_syntenic), ]
print(paste("non syntenic markers removed with remaining", nrow(matrix)))
decay[k,] <- colMeans(matrix, na.rm = T)

}

print(dim(decay))
print(decay[1:5, 1:5])

decay_df <- data.frame(t(decay))
colnames(decay_df) <- as.character(unique.spop)

decay_df2 <- cbind(Marker = 1:depth, decay_df) %>%
  gather(., key = "Breed", value = "LD", -Marker)

print(head(decay_df2))
return(decay_df2)
  #decay_df <- cbind(Pop = unique.spop, decay_df)
}

##### -----#####

# Pairwise G relationships based on the mean of the G matrix
# geno = matrix of animals by markers
# pop is list of animal ids
# if diag = FALSE, pairwise should be done of subsets of the population
# original pop given by breed
# if m.diag = TRUE, diagonal is replaced by the mean relatedness of that group
# to all other groups

pairwiseG <- function(geno, pop, symm = TRUE, m.diag = FALSE) {

M <- geno
print(paste("Dim of geno is", dim(geno)))
spop <- as.factor(as.character(pop))
unique.spop <- levels(spop)
print(as.character(unique.spop))
nspop <- length(unique.spop)
G_mu <- matrix(ncol = nspop, nrow = nspop)
colnames(G_mu) <- as.character(unique.spop)
rownames(G_mu) <- as.character(unique.spop)

for(i in 1:nspop) {
for(j in 1:nspop) {
  indrow <- which(spop == unique.spop[i])
  indcol <- which(spop == unique.spop[j])
  M_temp <- M[indrow, indcol]
if(i == j) {
  G_mu[i, j] <- mean(M_temp[upper.tri(M_temp)])
} else {
  G_mu[i, j] <- mean(M_temp)
}

}
}

}
# order

```

```
means <- rowMeans(G_mu)
mean_df <- data.frame(group = colnames(G_mu), Mean = means)
mean_df_ordered <- mean_df %>% arrange(desc(means))

print("Ordered")
print(mean_df_ordered)

order <- as.character(mean_df_ordered$group)
G_mu <- G_mu[order, order]

if(symm == FALSE) {
  G_mu <- G_mu[upper.tri(G_mu)]
}
if(m.diag == TRUE) {
  diag(G_mu) <- rowMeans(G_mu)
}
return(G_mu)
}
```