

Table 2.2 A non-comprehensive summary of genomic studies on the characterization of Merino sheep populations worldwide

| Breed | Article title | Markers used | Reference |
|-------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|-----------------------------------|
| 20 SA breeds (Including SA Merino) | Genetic characterization of South African Sheep breeds using DNA markers | 31 Microsatellite markers | (Buduram, 2004) |
| 29 European breeds (including Merino) | Genetic structure of European sheep breeds | 23 Microsatellite markers | (Handley <i>et al.</i> , 2007) |
| 5 Australian breeds (including Merino) | Genome-wide linkage equilibrium and genetic diversity in five populations of Australian domestic sheep. | Illumina® OvineSNP50 BeadChip | (Al-mamum <i>et al.</i> , 2015) |
| SA Merino | Identification of SNPs associated with robustness and greater reproductive success in the South African Merino sheep using SNP chip technology | Illumina® OvineSNP50 BeadChip | (Sadenbergh, 2015) |
| 37 Merino or Merino-derived breeds | Merino and Merino-derived sheep breeds: a genome-wide intercontinental study | Illumina® OvineSNP50 BeadChip | (Ciani <i>et al.</i> , 2015) |
| 11 Merino or Merino-derived breeds | A combined multi-cohort approach reveals novel and known genome-wide selection signatures for wool traits in Merino and Merino-derived sheep breeds | Illumina® OvineSNP50 BeadChip | (Megdiche <i>et al.</i> , 2019) |
| 14 SA breeds (including SA Merino) | Runs of homozygosity analysis of South African sheep breeds from various production systems investigated using OvineSNP50k data | Illumina® OvineSNP50 BeadChip | (Dzomba <i>et al.</i> , 2021) |
| 63 global breeds (including Merino) | The pattern of runs of homozygosity and genomic inbreeding in worldwide sheep populations | Illumina® OvineSNP50 BeadChip | (Nosarati <i>et al.</i> , 2021) |
| 14 SA and Australian sheep breeds | The genomic structure of isolation across breed, country, and strain for important South African and Australian sheep populations | Illumina® OvineSNP50 BeadChip | (Nel <i>et al.</i> , 2022) |
| 403 Spanish Merino sheep | Genomic population structure of the main historical genetic lines of Spanish Merino sheep | Axiom™ Ovine 50K SNP Genotyping array | (Granero <i>et al.</i> , 2022) |
| 10 SA Mutton Merino, 39 Australian and Chinese Merino | Detection of selection signatures in South African Mutton Merino sheep using whole-genome sequencing data | Whole Genome sequencing (WGS) | (Liu <i>et al.</i> , 2022) |
| 42 Merino and Merino-derived sheep breeds | A comprehensive analysis of the genetic diversity and environmental adaptability in worldwide Merino and Merino-derived sheep breeds | Illumina® OvineSNP50 BeadChip | (Ceccobelli <i>et al.</i> , 2023) |

2.6 Genomic parameters for the estimation of autozygosity levels in sheep populations

Genetic diversity and the maintenance thereof play a crucial role in populations as it allows for adaptive flexibility within a population (Ovaska *et al.*, 2021). In populations with little genetic variability or where multiple alleles are no longer present, or are present in low frequencies, adaption to a shift in selection pressure may be more difficult (Oldenbroek & van der Waaij, 2015). In addition, inbreeding (i.e., increased homozygosity levels), results in an increase in the frequency and/or fixation of alleles with deleterious effects causing recessive disorders to become expressed (Oldenbroek & van der Waaij, 2015). Maintaining genetic diversity can be achieved by maximizing the effective population size (N_e) or by minimizing the consequences of inbreeding (de Cara *et al.*, 2011; Gómez-Romano *et al.*, 2016).

The quantitative effects of inbreeding can be measured by assessing the autozygosity levels and inbreeding depression of a population (Lashmar *et al.*, 2018). Intense directional selection over several generations, the overuse of a small number of superior sires, and the use of genomic selection practices (through a reduction in the generation interval) may contribute to an increase in the occurrence of inbreeding-related genetic consequences (Nicholas & Smith, 1983; Verrier *et al.*, 1993; Robertson, 2008). Changes in autozygosity levels within a population impact several aspects, for instance, the redistribution of genetic variation within and between populations (Fernández *et al.*, 1995), with potentially decreasing within-population genetic diversity in finite populations (Ferenčaković, 2015). Inbreeding depression affects additive genetics by reducing the population mean for traits closely related to fitness (Charlesworth & Willis, 2009), for example, Selvaggi *et al.* (2010) reported a 20.47% reduction in inbreeding coefficients in in-bred sheep fertility traits compared to non-inbred sheep. Inbreeding depression causes a higher incidence of homozygous recessive defects caused by single genes (Arcos-Burgos & Muenke, 2002; Alvarez *et al.*, 2009), for example, the occurrence of spider-lamb syndrome (Analla *et al.*, 1998) and consequently a decrease in homeostasis in a population (Lerner, 1954). Other impacts of autozygosity include changes in the effective population size and population structure, genetic drift, and deviations from Hardy-Weinberg equilibrium (HWE) (Ferenčaković, 2015).

At present, runs of homozygosity (ROH) are widely used to estimate the level of autozygosity in the genome (Kim *et al.*, 2016; Peripolli *et al.*, 2018). Other statistical parameters currently used for the estimation of homozygosity levels in livestock populations include pedigree-based (F_{PED}) (which will not be discussed in depth in this review) and SNP- and/or ROH-based (F_{SNP} & F_{ROH}) F-statistics, respectively. Proxy indicators of diversity include linkage disequilibrium (LD), observed and expected heterozygosity (H_O and H_E), and effective population size (N_e).

2.6.1 Proxy-indicators of inbreeding

2.6.1.1 Minor Allele Frequency (MAF)

Minor allele frequency (MAF) refers to the frequency at which the less common allele occurs in a population (Keller *et al.*, 2011), and relates to genetic diversity. A higher MAF explains a higher level of polymorphic markers, which corresponds to higher levels of H_E and consequently indicates a greater genetic diversity (Engelsma *et al.*, 2014). The opposite is also true, a low MAF indicates a lower genetic diversity and hence more frequent fixed alleles (Engelsma *et al.*, 2014) and a higher probability of homozygous genotypes (for the fixed allele).

2.6.1.2 Observed and Expected heterozygosity (H_O and H_E)

Mean expected heterozygosity (H_E) and observed heterozygosity (H_O) are both measures of genetic diversity that are estimated from molecular information (marker-based summary statistics) (Eusebi *et al.*, 2020). The H_E parameter is defined as the probability that an individual's two alleles chosen randomly from the population are different at a specific locus (Nei, 1973; Mäki-Tanila *et al.*, 2010). The H_O parameter is defined as a measure of the frequency of heterozygous genotypes in a set of markers (Mäki-Tanila *et al.*, 2010). The H_O value is negatively correlated with inbreeding depression (Mäki-Tanila *et al.*, 2010). In nature one cannot assume that a finite population is in HWE, therefore, the relationship between H_E

and H_o reveals a gain or loss of genetic diversity, which translates to the estimation of a population's inbreeding coefficient (Eusebi *et al.*, 2020). A population with high H_E values is assumed to be more genetically diverse than populations with lower H_E values and thus are capable of adapting to changes and responding to selection pressures (Allendorf *et al.*, 2013). Therefore, genotypes showing low observed heterozygosity may indicate inbreeding (Anderson *et al.*, 2010).

2.6.1.3 Linkage disequilibrium (LD)

Linkage disequilibrium (LD) is a genetic diversity parameter that shows the extent of non-random association between two alleles located at different loci within a population (Al-Mamun *et al.*, 2015; Dzomba, 2021). Linkage disequilibrium may be the result of several different genetic forces such as selection, mutation, genetic drift, non-random mating, as well as non-genetic causes (Sabatti & Risch, 2002). The degree of LD within a population is reflective of breeding systems, population history, and the pattern of geographical subdivision (Goddard, 2012).

The degree of LD within a population can be measured by the estimation of $|D'|$, which in small sample sizes, and the presence of rare alleles, is overestimated (Bohmanova *et al.*, 2010). Alternatively, LD can be estimated using r^2 , which is the correlation between two loci and is mostly used in association studies (Bohmanova *et al.*, 2010). The r^2 measure depends on the time and rate of recombination, as well as the population size (Hill, 1974). Due to the correlation between LD and N_e , to avoid LD estimation biases from small sample sizes, a minimum sample size of 55 is required for accurate estimation of r^2 (Bohmanova *et al.*, 2010). The r^2 measure's formula, as proposed by Hill and Robertson (1968), is as follows:

$$r^2_{(P_a, P_b, P_{ab})} = \left(\frac{(P_{ab} - P_a P_b)^2}{P_a(1 - P_a)P_b(1 - P_b)} \right),$$

where P_{ab} represents the frequency of the haplotype consisting of 2 SNPs; P_a and P_b represent the frequency of allele a at the first locus and allele b at the second locus, respectively.

Linkage disequilibrium directly reflects the recombination rate; regions associated with low recombination rates will have a higher extent of LD and the opposite is also true (Zavattari, 2000). Inter-SNP distance is inversely proportional to the level of LD, i.e. SNPs that are near one another are often inherited as a unit and are usually found in high LD (Zhai *et al.*, 2004). Sheep typically exhibit low genome-wide LD due to their origin from a genetically diverse pool (Kijas *et al.*, 2012), i.e. smaller haplotypes are shared within the population because of increased recombination of large haplotypes in the past. Higher LD captured through dense SNP panels allows for increased chances that markers are in LD with quantitative trait loci (QTL) and, therefore, the range of LD affects the power and precision of mapping (Goddard & Hayes, 2007). High levels of LD and more relatedness result in higher levels of specific allelic combinations which are inherited over generations than what can be expected by chance (Eusebi *et al.*, 2020). Therefore, livestock species that make use of genomics-driven selection, indirectly rely on the level of LD within the population (Meadows *et al.*, 2006; Kijas *et al.*, 2012).

2.6.1.4 Effective population size (N_e)

The effective population size (N_e) of an existing population can be defined as the ideal population size that will result in the same amount of genetic diversity as that which is present in the current existing population (Wright, 1974; Gholizadeh & Ghafouri-Kesbi, 2016). It is possible that N_e can be a smaller number than the actual population and this is due to the disproportionate contribution of genes from individuals to the next generation (Goddard & Hayes, 2007). The N_e parameter determines a population's performance based on genetic variation and inbreeding over long periods (Fernández *et al.*, 2005). In the past, N_e has been estimated using pedigree information (Falconer and Mackay, 1996) and this is still used in many cases where there is no or insufficient genomic information available. More recently, SNP data or SNP-based methods can estimate both historical and recent N_e , but these

methods are limited by the extent of LD captured throughout the genome i.e., the SNP panel density and the number of animals genotyped (Goddard & Hayes, 2007; Visser *et al.*, 2023). As the distance between markers increases, the LD among them decreases (Liu *et al.*, 2017), which allows for the estimation of ancient effective population sizes (N_e) using LD at short distances and the evaluation of recent N_e using LD at longer distances (Hayes *et al.*, 2003).

The SNeP software tool, for example, uses genome-wide SNP data to estimate recent N_e trends as proposed by Barbato *et al.* (2015), with the following formula employed:

$$N_{T(t)} = (4f(c_t))^{-1} (E[r^2_{adj} | c_t]^{-1} - \alpha)$$

where, $N_{T(t)}$ is the effective population size t generations ago calculated as $t = (2f(c_t))^{-1}$ (Hayes *et al.*, 2003); c_t is the recombination rate for a specific physical distance between SNPs estimated using Sved and Feldman approximation (Sved & Feldman, 1973), r^2_{adj} is the LD value adjusted for sample size and $\alpha = \{1, 2, 2.2\}$ is a correction for the occurrence of mutations (Ohta & Kimura, 1971).

Most livestock species have small effective population sizes which can mainly be attributed to the intense selection of a small number of animals for the parent generation (Leroy, 2014). As for small stock breeds, larger N_e estimates are possible due to less intense selection in certain breeds (Groeneveld *et al.*, 2010). Frankham *et al.* (2002) suggested that to avoid inbreeding depression in the short term, a N_e of at least 50 should be maintained whereas other studies have suggested a N_e of 500 should be maintained (Holt *et al.*, 2005; Meuwissen, 2009). Falconer & Mackay (1996) noted that the number of years that have passed since a common ancestor can be calculated depending on the length of the generation interval (L) as the rate of recombination is proportional to the number of generations.

2.6.1.5 Runs of Homozygosity (ROH) and Heterozygosity-rich areas (HRR)

Runs of homozygosity (ROH) are long stretches of homozygous regions throughout the genome that reflect autozygosity due to consanguineous mating (Ferenčaković, 2015).

Broman & Weber (1999) were the first to recognize that these long stretches of homozygous segments in a human population most likely reflect autozygosity and may have major consequences for human health (Ku *et al.*, 2011; Ferenčaković, 2015). Thereafter, the concept was extrapolated to various livestock species as illustrated in Figure 2.3.

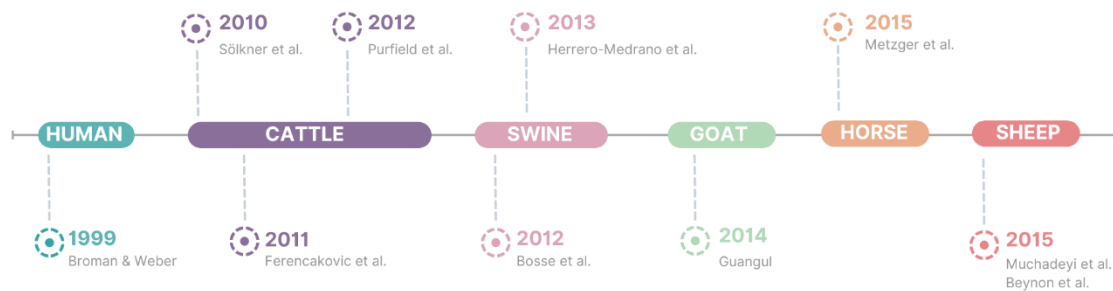


Figure 2.3. Timeline showing the extrapolation of the ROH concept from humans to different livestock animals.

The availability of dense SNP panels has allowed for determining the genome-wide ROH profile of animals (Mastrangelo *et al.*, 2018). Pemberton *et al.* (2012) characterized ROH lengths in humans, showing that the length of the ROH segment is determined by recombination events i.e., the number of generations from a common ancestor. Longer segments (~ 10Mb) translate to more recent inbreeding whereas shorter segments (~ 1Mb) translate to ancestral inbreeding (Pemberton *et al.*, 2012; Ferenčaković, 2015).

Contrary to ROH, runs of heterozygosity (ROHet), more appropriately referred to as heterozygosity-rich regions (HRRs), are consecutive stretches of heterozygous SNP genotypes (Williams *et al.*, 2016). An animal's HRR profile provides information regarding a population's evolutionary history and diversity (Samuels *et al.*, 2016). A population undergoes a continuous process of loss and creation of heterozygous-rich areas due to the selection changes in the population (Mulim *et al.*, 2022). Biscarini *et al.* (2020) showed that HRR can be utilized to detect specific genome segments where preserving greater genetic diversity could be more advantageous. Therefore, the estimation of HRR within a population is important for the identification and maintenance of sufficient genetic diversity (Purfield *et al.*, 2012; Peripolli *et al.*, 2017).

Two popular software, namely PLINK (Purcell *et al.*, 2007) and the *detectRUNS* R package (Biscarini *et al.*, 2018), are used to calculate ROH, with *detectRUNS* additionally calculating HRR. The *detectRUNS* sliding window approach is comparable to the sliding window software in PLINK (Dixit *et al.*, 2020). This method for the detection of both ROH and HRR uses an algorithm that involves a sliding window size of SNPs that slides along the chromosome (50 as a default), and at every sliding window position, every SNP within that window is determined to be homozygous or not (Purcell *et al.*, 2007; Bjelland *et al.*, 2013). The sum for each SNP in the number of fully homozygous windows and the total number of windows is calculated (Bjelland *et al.*, 2013). An ROH will be called if a specified minimum number of consecutive SNPs are determined to have a homozygous window threshold of more than five percent (Biscarini *et al.*, 2020). In the case of HRR, the characteristics of the sliding window are used to determine if a homozygous SNP is not present in a run (Biscarini *et al.*, 2020).

The *detectRUNS* R package (Biscarini *et al.*, 2018) presents an alternative method for detecting ROH and HRR, namely the consecutive runs method (Marras *et al.*, 2015). This method lacks a specified “window” and rather directly scans the genome on a SNP-by-SNP basis (Marras *et al.*, 2015), in other words, averages over single-point estimates (Dadousis *et al.*, 2022).

2.6.2 Direct measures of inbreeding: F-statistics

The inbreeding coefficient (F) is a measure of an individual’s level of inbreeding and is defined as the probability that two alleles are IBD relative to a base population that segregates following HWE (Wright, 1922). Several different methods to estimate inbreeding coefficients exist, depending on the data type utilized. The F_{PED} estimator is estimated using pedigree data (Malécot, 1948) whereas the F_{IS} estimator uses genomic observed and expected heterozygosity levels to estimate genetic variance within a population (Williams *et al.*, 2016). The level of inbreeding can also be calculated by examining the marker-by-marker identical-by-state (IBS) information based on an SNP-derived genomic relationship matrix (GRM) and estimating inbreeding as F_{GRM} (Van Raden, 2008). This dissertation will only focus on the F_{SNP} and F_{ROH} inbreeding estimators.

2.6.2.1. Single nucleotide polymorphism (SNP) based F_{SNP}

The F_{SNP} inbreeding coefficient estimates the excess in homozygosity using SNP-by-SNP calculations (Rodríguez-Ramilo *et al.*, 2020). The inbreeding coefficients F_{SNP} and F_{ROH} are often highly correlated due to a large proportion of SNPs used to capture F_{SNP} also being present in ROH (Ghoreishifar *et al.*, 2023). In addition, Keller *et al.* (2011) suggested that as the effective population size decreases, the correlation between F_{ROH} and F_{SNP} increases. The formula to calculate F_{SNP} is as follows (Keller *et al.*, 2011):

$$F_{SNP} = \frac{O - E}{N - E}$$

where O is the observed number of homozygous SNPs per individual, E is the expected number of homozygous SNPs under the HWE calculated based on the estimated allele frequencies of the sample, and N is the total number of SNPs.

The level of inbreeding can also be calculated by examining IBS information on a marker-by-marker basis using an SNP-derived genomic relationship matrix (GRM) and estimating inbreeding as F_{GRM} (Van Raden, 2008), which goes beyond the scope of this study. The GRM shows the realized proportion of the genome that is shared by two individuals (Goddard *et al.*, 2011).

2.6.2.2 Runs of homozygosity (ROH) based F_{ROH}

The F_{ROH} coefficient, defined as the proportion of the autosomal genome having ROH above a specific length threshold, is a genomic measure of individual autozygosity (McQuillan *et al.*, 2008). The F_{ROH} inbreeding estimator is calculated from molecular information and, unlike F_{PED} (which is based on probability), F_{ROH} is based on observed homozygosity (Eusebi *et al.*, 2020). McQuillan *et al.* (2008) described the methodology used to calculate F_{ROH} by dividing the sum of an individual's ROH lengths by the total length of the genome that has SNP coverage. Due to this, F_{ROH} estimates are commonly higher than F_{SNP} measures because they cannot be

negative values. Pemberton *et al.* (2012) characterized ROH lengths and related the length to the time of inbreeding. Therefore, deriving inbreeding coefficients (F_{ROH}) from ROH not only provides information on the degree of inbreeding but also the “age” thereof (Ferenčaković, 2015). An advantage of ROH is the ability to distinguish between identical by descent (IBD) and identical by state (IBS), therefore showing which haplotypes are inherited and which ones are common haplotypes (Pryce *et al.*, 2012). Previous studies confirmed that F_{ROH} estimates are more accurate than pedigree-based estimates, which is advantageous because it accounts for the stochastic nature of inheritance on an individual marker level as well as a chromosome marker level (Sölkner *et al.* 2010; Ferenčaković *et al.*, 2011; Purfield *et al.*, 2012). The F_{ROH} inbreeding coefficient can be calculated as described by McQuillan *et al.* (2008);

$$F_{ROH} = \frac{\sum F_{ROH}}{\sum L_{AUTO}}$$

where: $\sum F_{ROH}$ = the length of ROH in one individual

$\sum L_{AUTO}$ = the length of the genome covered by SNPs

Aspects that affect the accuracy of ROH estimates include the varying parameters set to define an ROH between studies, which vary in both amount and distribution through the genome. The minimum length, minimum SNP density, minimum number of SNPs, and maximum distance between SNPs have the greatest effect on F_{ROH} values (Rodríguez-Ramilo *et al.*, 2020). The density of SNP panels is important because an increase in shorter lengths (<2Mb) may result in a higher rate of false positives (Zhang *et al.*, 2015), i.e. low SNP panel density results in genomic regions where it is impossible to detect short length ROHs (Meyermans *et al.*, 2020). Another factor that influences the accuracy of ROH is its association with effective population size (N_e). Populations with low N_e have more ROH present whereas the opposite is true for populations with larger N_e (Abied *et al.*, 2020). Due to the lack of uniformity of recombination rates across the genome, ROH with identical lengths located in genomic regions with differing recombination rates exposes a different inbreeding history. However, these ROHs will be considered to have equal F_{ROH} estimates (Eusebi *et al.*, 2020).

2.7 Within-population selection signatures

The genome-wide distribution and patterns of ROH are not random but may be shared amongst individuals and could, therefore, provide information on past selection events (Zhang *et al.*, 2015). Therefore, by using the conserved ROH approach, selection signatures can be identified (Kim *et al.*, 2013). Within-breed selection signatures (or selection sweeps) are characterized by genomic regions with reduced genetic variability (i.e., increased favourable allele frequencies within the genome) (Purfield *et al.*, 2017).

Selection signatures can be identified using different methods including the SNP-in-run approach and the conserved ROH approach. The SNP-in-run approach identifies genomic regions that are most associated with ROH by counting the proportion of times an SNP appears in an ROH and dividing it by the number of animals (Biscarini *et al.*, 2020). The conserved ROH approach identifies regions of reduced nucleotide diversity that have been subject to selection, otherwise known as ROH islands (Szmatola *et al.*, 2016; Purfield *et al.*, 2017). This study will focus on detecting conserved ROH regions that indicate genetic differentiation.

Almeida *et al.* (2019) demonstrated that functional analysis of identified genes within conserved ROH regions indicates the biological mechanisms that may be involved with performance and adaptation traits. A limitation of using the conserved ROH approach is the difficulty in determining which proportion of ROH is due to genetic drift and this may result in false positives (Almeida *et al.*, 2019). However, this can be overcome by detecting selection signatures that are overlapping in regions with QTL and using ROH regions in common with at least two animals (Ruiz-Larrañaga *et al.*, 2018).

2.8 Previous autozygosity studies on sheep

Studies on the inbreeding and autozygosity levels of SA sheep breeds are scarce (van Marle-Köster *et al.*, 2021). The SA Merino breed is the only South African sheep breed with sufficient genotypes to implement genomic selection (GS), thus there is a comprehensive data set of

sheep with SNP-genotypic information available to study the genomic inbreeding and autozygosity levels in this breed. It is difficult to compare results to previously published studies on a global level, due to the different SNP panels used, the varying ROH defining criteria, and the different sample sizes between studies (Mastrangelo *et al.*, 2018). Table 2.3 shows a summary of some recent autozygosity studies in global sheep populations.

Table 2.3 A non-comprehensive summary of recent autozygosity studies on sheep

| Breed ^a | Sample size | SNP density | Parameters ^b | References |
|------------------------------------------------|-------------|-------------|---------------------------------------------------|---------------------------------------|
| CM | 635 | 54241 | r^2 , Ne | Liu <i>et al.</i> , 2017 |
| CM | 635 | 54241 | F_{ROH} | He <i>et al.</i> , 2020 |
| NA, N, BP, SAM, DM, AM, CM, S, RA, S, MM | 400 | 36975 | MAF, F_{ST} , r^2 , H_O , H_E , F_{IS} | Dzomba <i>et al.</i> , 2020 |
| SAMM, DM, MM, BP, NA, SAM, S, D, A, N | 400 | 43556 | F_{ROH} | Dzomba <i>et al.</i> , 2021 |
| European sheep breeds (incl. MacArthur Merino) | 2536 | 60301 | F_{ROH} , H_O , H_E , F_{GRM} , F_{UNI} | Nosrati <i>et al.</i> , 2021 |
| BP, DAR, NA, P, SAM, DM, D | 319 | 54241 | MAF, F_{ROH} , r^2 , F_{ST} | van Marle-Köster <i>et al.</i> , 2021 |
| SPM | 403 | 56520 | H_O , H_E , F_{ROH} | Granero <i>et al.</i> , 2022 |
| AM, DM, D, SAMM, BL, CW, C, PD, WS, SAM, DR | 3509 | 47789 | F_{ST} , r^2 , H_O , H_E , Ne | Nel <i>et al.</i> , 2022 |

^a SAMM= South African Mutton Merino, DM= Dohne Merino, MM= Meat-master, BP= Blackhead Persian, NA= Namaqua Afrikaner, S= Swakara, SAM= South African Merino, D= Dorper, A= Afrino, N= non-descript Nguni sheep, AM= Australian Merino, BL= Border Leicester, CW= Coopworth, C= Corriedale, PD= Poll Dorset, WS= White Suffolk, DR= Dormer, CM= Chinese Merino, RA= Ronderib Afrikaner, RP= Redhead Persian, K= Karakual, DAR= Damara, P= Pedi, SW= Swazi, SAL= South African Landsheep, SPM= Spanish Merino

^b H_O = observed heterozygosity, H_E = expected heterozygosity, MAF= Minor allele frequency, F_{ROH} = F-statistic Runs of homozygosity, F_{GRM} = F-statistic Genomic relationship matrix, F_{UNI} = F-statistic Uniting gametes, F_{ST} = F-statistic Genetic differentiation, r^2 = linkage disequilibrium, Ne= effective population size

Among the afore-mentioned ROH studies, there was a consensus that ROH size fragments in sheep populations are mostly short and indicative of ancient inbreeding (He *et al.*, 2020; Dzomba *et al.*, 2021, Nosrati *et al.*, 2021; van Marle-Köster *et al.*, 2021). The level of genomic inbreeding in the studies was generally low, with all studies reporting F_{ROH} values below 0.037 (He *et al.*, 2020; Nosrati *et al.*, 2021; van Marle-Köster *et al.*, 2021; Granero *et al.*, 2022). A

common observation in these studies was the sufficiency of genetic diversity levels in sheep populations, with H_E ranges between 0.318 and 0.374 (van Marle-Köster *et al.*, 2021; Granero *et al.*, 2022), H_O ranging between 0.327 and 0.380 (van Marle-Köster *et al.*, 2021; Dzomba *et al.*, 2021), and a general tendency toward only small losses in genetic diversity (i.e., $H_O > H_E$). Furthermore, relatively low genome- and chromosome-wide LD (e.g., r^2 -value range: 0.09-0.25) were mostly reported, indicating high recombination rates (Liu *et al.*, 2017; Dzomba *et al.*, 2021; Nel *et al.*, 2022).

Chapter 3: Materials and Methods

3.1 Introduction

This study aimed to investigate the autozygosity and inbreeding levels of the South African Merino sheep breed using single nucleotide polymorphism (SNP) genotypic data. A total of 1 738 animals (1 567 females and 171 males) were available for analyses. The genotypes were first analyzed to quantify proxy-indicator estimators of genomic diversity, including heterozygosity levels, minor allele frequency (MAF), and linkage disequilibrium (LD). Autozygosity was then investigated by characterizing the genome-wide runs of homozygosity (ROH) and heterozygosity-rich region (HRR) profiles, as well as estimating several inbreeding statistics. Lastly, within-population selection signatures were identified using the conserved ROH approach. All genotypic data was used with the consent of the SA Merino Breeders' Association and provided by SA Stud Book. Ethics approval (NAS126/2023) for the use of all data in this study was obtained from the Ethics Committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria.

3.2 Materials

A total of 1 738 animals (1 567 females and 171 males), genotyped using different versions of the Illumina® Ovine 50K SNP panel, were included in the study. A descriptive summary of the panels used, as well as the number of animals genotyped with each, is given in Table 3.1.

The SNP-calling was done according to the standard genotyping protocols of each individual service provider. The resulting genotype input files were converted into PLINK-compatible input files (i.e., .map, and .ped) using a plug-in of Illumina® Genome Studio software v2.0 (Illumina, San Diego, California 92122 U.S.A).

Table 3.1 Summary of single nucleotide polymorphism (SNP) genotyping panels and the number of animals genotyped with each panel.

| Array Type | SNPs available per array ^a | Number of Females | Number of Males | Total Number of animals |
|--------------------------|---------------------------------------|-------------------|-----------------|-------------------------|
| Illumina [®] v1 | 52452 | 258 | 31 | 289 |
| Illumina [®] v2 | 51719 | 1170 | 49 | 1219 |
| Illumina [®] v3 | 50199 | 139 | 62 | 201 |
| Unistel v3 | 61954 | 0 | 29 | 29 |
| Total | | 1567 | 171 | 1738 |

^a SNP distributed over 27 autosomes and two sex chromosomes

3.3 Methods

For standardization, the autosomal SNPs in common between the panels were identified and extracted from each data set in PLINK using the *--extract* command. The data from the four panels were then merged to form one dataset using the *--merge-list* command. Thereafter, duplicate SNPs were removed from the merged dataset using the *--list-duplicate-vars ids-only suppress-first* and the *--exclude* commands. A subset of 50 169 SNPs was then subjected to quality control.

Standard quality control (QC) procedures were performed on the merged dataset using PLINK software (Purcell *et al.*, 2007). Sample-based as well as animal-based pruning was performed, to remove both non-informative SNPs and individuals from the dataset. Individual animals were removed if their genotyping call rate was less than 95% (using *--mind 0.05*), while SNPs were removed if their call rate was below 98% (using *--geno 0.02*). As suggested by Meyermans (2020) for ROH detection, neither minor allele frequency (MAF) nor linkage disequilibrium (LD) filtering was applied. This is because pruning for LD before ROH analysis results in a negative reduction of <70% of SNP detection, underestimated F_{ROH} estimates and a reduction in the number of ROH detected (Meyermans *et al.*, 2020). Furthermore, MAF pruning during quality control results in decreased ROH detection especially in high homozygous regions (Meyermans *et al.*, 2020). The MAF was, however, used as a QC

parameter for HRR detection. Table 3.2 summarizes the available number of SNPs and animals after quality control was applied.

Table 3.2 A summary of animal- and marker-based quality control, indicating the number of animals and SNPs removed for each parameter.

| Quality Control | PLINK (Purcell <i>et al.</i> , 2007) | Number of SNPs after QC | Number of animals after QC |
|--------------------------|--------------------------------------|-------------------------|----------------------------|
| Common SNPs ^a | --extract | 50169 | 1738 |
| Marker-based QC | --geno 0.02 ^b | 46952 | - |
| Animal-based QC | --mind 0.05 ^c | - | 258 |

^a unique SNPs i.e. after duplicate SNPs were removed from the dataset.

^b SNPs with 98% genotyping rate (2% missingness) were included.

^c Individuals with more than 5% missing genotypes were excluded.

3.3.1 Genetic diversity

Once QC procedures were applied, marker-based summary statistics including the proxy-indicators for genetic diversity (namely H_O & H_E , MAF, LD, N_e) were estimated using PLINK (Purcell *et al.*, 2007) as follows:

3.3.1.1 Observed and expected heterozygosity (H_O , and H_E)

The mean observed and expected heterozygosity estimates were produced using the --het command. The resulting .het file was then imported into Microsoft Excel version 16.59 (Microsoft Corporation, 2018) where H_O and H_E were calculated using the following formulas:

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

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

where: $N(NM)$ = the number of non-missing genotypes; $O(Hom)$ = the observed number of homozygous genotypes; $E(Hom)$ = the expected number of homozygous genotypes.

3.3.1.2 Minor allele frequency (MAF)

The MAF values were calculated using the `--freq` command. The resulting `.frq` file was imported into Microsoft Excel version 16.59 (Microsoft Corporation, 2018) where the mean MAF was calculated. The proportion of MAF per loci was visualized in a bar graph using the same software.

3.3.1.3 Linkage disequilibrium (LD)

The level of LD was calculated by adjacent pairwise analysis (measured as r^2) using the PLINK command `--r2`. To allow for all possible pairwise SNP comparisons per autosome, no minimum r^2 and inter-SNP distance restrictions were applied (i.e., `--ld-window-r2 0`, and `--ld-window-kb 99999` were applied). The resulting `.ld` file was imported into Microsoft Excel version 16.59 (Microsoft Corporation, 2018) where the mean r^2 estimate, mean inter-SNP distance, and minimum and maximum inter-SNP distance were calculated for all autosomes.

3.3.1.4 Effective population size (Ne)

The effective population size (N_e) was calculated using the SNeP v1.1 software tool (Barbato *et al.*, 2015). Microsoft Excel version 16.59 (Microsoft Corporation, 2018) was used to construct a line graph to visualize the N_e of the population over generations. The SNeP software predicts N_e using the following equation described by Corbin *et al.* (2012).

$$N_{T(t)} = (4f(c_t))^{-1} (E[r^2_{adj} | c_t]^{-1} - \alpha)$$

Where: $N_{T(t)}$ = the effective population size t generations ago calculated as $t = (2f(c_t))^{-1}$ (Hayes *et al.*, 2003), c_t is the recombination rate for a specific physical distance between SNPs

estimated using Sved and Feldman approximation (Sved & Feldman, 1973), r^2_{adj} is the LD value adjusted for sample size and α is a correction factor for the occurrence of mutations.

3.3.2 Runs of homozygosity (ROH) and heterozygosity-rich areas (HRR)

Both runs of homozygosity (ROH) and heterozygosity-rich areas (HRR) were detected using the *detectRUNS* R package (Biscarini *et al.*, 2018) through the execution of both the sliding window and consecutive runs approaches (SW, CR; Marras *et al.*, 2015). For a more comprehensive report on ROH profiles, both approaches were tested in this study, however, only the ROH detected through the SW approach were included in the calculation of the F_{ROH} inbreeding coefficient. The ROH and HRR defining parameters used in the SW and CR approaches are specified in Table 3.3 and Table 3.4, respectively. The ROH segments were assigned to five length categories, namely $<4\text{Mb}$, $4\leq\text{ROH}<8\text{Mb}$, $8\leq\text{ROH}<16\text{Mb}$, $16\text{Mb}\leq\text{ROH}<32\text{Mb}$, or $\geq 32\text{Mb}$. The HRRs were classified into three length categories, namely 0.5-1Mb, 1-2Mb, and $>2\text{Mb}$. The number and chromosome distribution of the ROH and HRR were counted.

Table 3.3. ROH and HRR defining parameters and code for the SW approach

| Parameter | Code | ROH value used | HRR value used |
|----------------------------------------------------------------------------------------------|---------------|---------------------|---------------------|
| Size of the sliding window (number of SNPs). | windowSize | 50 | 10 |
| The threshold of overlapping windows in the same state to call a SNP in a run. | Threshold | 0.05 | 0.05 |
| Minimum number of SNPs in a run. | minSNP | 49 | 20 |
| Maximum number of opposing genotypes in the sliding window. | maxOppWindow | 0 | 2 |
| Maximum number of missing SNP in the sliding window. | maxMissWindow | 2 | 1 |
| Maximum distance between consecutive SNP in a window to be still considered a potential run. | maxGap | 10 ⁶ bps | 10 ⁶ bps |
| Minimum length of a run in bps. | minLengthBps | 1000000 | 10000 |
| Minimum number of SNP per kbps. | minDensity | 0.001 | 0.000001 |
| Maximum number of opposite genotype SNPs in the run. | maxOppRun | NULL | NULL |
| Maximum number of missing SNPs in the run. | maxMissRun | NULL | NULL |

Table 3.4. ROH and HRR defining parameters and code for the CR approach.

| Parameter | Code | ROH value used | HRR value used |
|----------------------------------------------------------------------------------------------|--------------|---------------------|---------------------|
| Minimum number of SNPs in a run. | minSNP | 49 | 20 |
| Maximum distance between consecutive SNP in a window to be still considered a potential run. | maxGap | 10 ⁶ bps | 10 ⁶ bps |
| Minimum length of a run in bps. | minLengthBps | 1000000 | 10000 |
| Maximum number of opposite genotype SNPs in the run. | maxOppRun | 1 | 2 |
| Maximum number of missing SNPs in the run. | maxMissRun | 1 | 1 |

To reduce the number of SNPs that occur in a run due to chance, the following formula was used to calculate the minimum number of SNPs in a run (Purfield *et al.*, 2012). Using the formula below the minimum number of SNPs allowed in a run was set to 49 for ROH detection and 20 for HRR.

$$l = \frac{\log_e \frac{\alpha}{n_s \cdot n_i}}{\log_e (1 - h\bar{e}t)}$$

where: n_s is the number of SNPs per individual, n_i is the number of individuals, α is the percentage of false positive ROH (set to 0.05 in this study) and $h\bar{e}t$ is the mean SNP heterozygosity across all SNPs. For HRR, $(1-h\bar{e}t)$ was replaced with $h\bar{e}t$ in the formula.

3.3.3 Inbreeding estimation

Two methods were utilized to estimate inbreeding, namely 1) F_{SNP} and 2) F_{ROH} . Box plots were generated for each inbreeding coefficient in R using the function `boxplot` (Chambers *et al.*, 2018). Additionally, R was also used to calculate Pearson correlations between coefficients using the function `cor.test` (Best & Roberts, 1975).

3.3.3.1 SNP-based inbreeding coefficient (F_{SNP})

The SNP-by-SNP based inbreeding statistic (F_{SNP}) was calculated using the following formula defined by Keller *et al.* (2011):

$$F_{SNP} = \frac{O - E}{N - E}$$

where O is the observed number of homozygous SNPs per individual, E is the expected number of homozygous SNPs in Hardy-Weinberg equilibrium (HWE), calculated based on the estimated allele frequencies of the sample (Keller *et al.*, 2011), N is the total number of SNPs. The F_{SNP} inbreeding estimator can be negative when the observed homozygosity is lower than the expected homozygosity or be positive when the opposite is true (Ghoreishifar *et al.*, 2023).

3.3.3.2 ROH-based inbreeding coefficient (F_{ROH})

The ROH-based inbreeding coefficient (F_{ROH}) was estimated per individual as per the following formula defined by McQuillan *et al.* (2008);

$$F_{ROH} = \frac{\Sigma F_{ROH}}{\Sigma L_{AUTO}}$$

where ΣF_{ROH} represented the length of ROH in one individual, and ΣL_{AUTO} represented the length of the genome covered by SNPs.

The inbreeding coefficients relevant to each length category (<4Mb, $4 \leq ROH < 8$ Mb, $8 \leq ROH < 16$ Mb, $16 \text{Mb} \leq ROH < 32$ Mb, or ≥ 32 Mb) were calculated in R using the *detectRUNS* package (Biscarini *et al.*, 2018). The SW approach in the literature is most used for ROH detection across livestock species and hence a resource for F_{ROH} detection (Peripolli *et al.*, 2017), hence for comparability with other studies, the SW approach was used for F_{ROH} .

3.3.4 With-in population selection signatures

The *detectRUNS* (Biscarini *et al.*, 2018) package was used to identify population-wide overlaps in ROH as well as the candidate genes harboured within these overlapping regions. The ROH detected in the 99.7th percentile (threshold of 16.4%) were considered potential ROH islands, as suggested in previous studies (Baazaoui *et al.*, 2021; Ceccobelli *et al.*, 2023). In addition, to include more candidate genes, a threshold calculated from the mean percentage of SNPs in ROH plus three standard deviations was considered (Grilz-Seeger *et al.*, 2019).

The chromosome-wide proportion of times each SNP resided within a detected ROH was generated using Manhattan plots (Wickham, 2009). The *Ensembl* BioMart online tool (Cunningham *et al.*, 2022) was used to investigate candidate genes located within the overlapping ROH and HRR segments. Gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were carried out using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) version 6.8 software tool (Huang *et al.*, 2009). The DAVID software tool calculates a significance *p-value* for annotated genes, which uses a Fisher's Exact test that is adopted to measure the level of gene enrichment in annotation terms. Its biological interpretation is that the smaller the *p-value*, the more enriched the genes detected are for those ontology terms.

Chapter 4: Results

4.1 Introduction

Genome-wide single nucleotide polymorphism (SNP) genotypic data of 1 738 SA Merino sheep was analyzed to evaluate their degree of genomic diversity, as well as uniformity in the form of inbreeding and autozygosity levels. Several within-breed population parameters relating to the population's diversity and inbreeding were estimated, followed by detailed analyses of both runs of homozygosity (ROH) and heterozygosity-rich areas (HRR). The identified ROH was further interrogated to identify conserved segments that may harbour candidate genes. Lastly, the identified genes were annotated, and their functional role was investigated.

4.2 Quality control

Before quality control (QC) was performed, a subset of 50 169 common autosomal SNPs was extracted from the genomic data generated and available through four variable SNP genotyping panels and merged into a single genotyping dataset. After QC, 46 952 SNPs and 1 738 animals were available for downstream analyses.

4.3 Genetic diversity parameters

The mean minor allele frequency (MAF) across all autosomes was 0.275 ± 0.14 with a median value of 0.287. The OAR 11 and OAR 23 autosomes had the lowest (0.245) and highest (0.284) chromosome-wide mean MAF values, respectively. The chromosome-wide mean MAF is presented in Figure 4.1. The highest percentage of monomorphic SNPs (MAF=0%) was observed on OAR 1 (11.25%) and the lowest on OAR 25 (1.23%). The highest percentage of SNPs had an MAF in the range of 0-0.19 (99.97%), with the lowest percentage of SNPs having an MAF of 0.5 (0.04%).

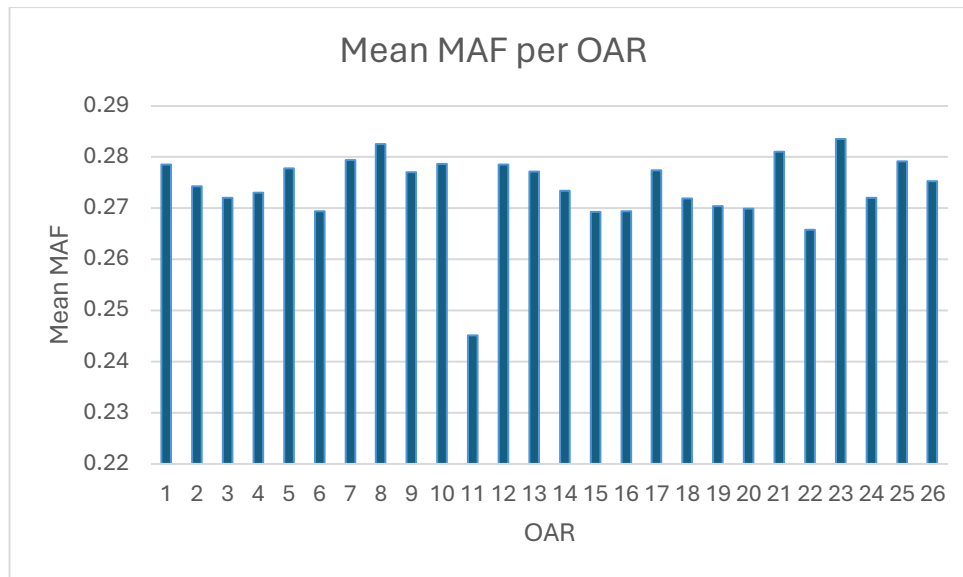


Figure 4.1 A graphic representation of the mean MAF per chromosome in SA Merino sheep.

The observed (H_o) and expected heterozygosity (H_E) levels were estimated for the population to identify a possible gain or loss in genetic diversity. The results indicated a loss in genetic diversity with a mean \pm standard deviation H_o of 0.351 ± 0.018 compared to a mean \pm standard deviation H_E of 0.365 ± 0.0001 . The H_o values ranged from a minimum of 0.236 to a maximum of 0.390, whilst the H_E values had a range of 0.362 to 0.366.

To calculate the chromosome-wide mean LD between all pairwise SNP comparisons per autosome, no restrictions were set on the minimum r^2 (default: $r^2=0.2$) and inter-SNP distance (default=1000kb) allowed for LD estimation. The mean \pm standard deviation r^2 -value obtained across all autosomes was 0.365 ± 0.180 . Table 4.2 shows the mean r^2 value per chromosome for different length windows namely 0-50kb, 0-100kb, and 0-1Mb. The average inter-SNP distance across all chromosomes was calculated to be 188.72kb, while the minimum and maximum inter-SNP distance across all chromosomes was 5.29kb and 998.07kb, respectively.

Table 4.1 Summary of the mean r^2 values and inter-SNP distance per chromosome for different length windows.

| OAR | Mean r^2 (50kb) | Mean r^2 (100kb) | Mean r^2 (1Mb) | Mean inter-SNP distance |
|-----|----------------------|-----------------------|---------------------|-------------------------|
| 1 | 0.472 | 0.437 | 0.364 | 185.584 |
| 2 | 0.484 | 0.454 | 0.377 | 178.812 |
| 3 | 0.460 | 0.435 | 0.367 | 191.859 |
| 4 | 0.477 | 0.449 | 0.370 | 188.339 |
| 5 | 0.485 | 0.440 | 0.361 | 178.443 |
| 6 | 0.437 | 0.419 | 0.355 | 206.076 |
| 7 | 0.458 | 0.425 | 0.358 | 170.726 |
| 8 | 0.467 | 0.428 | 0.359 | 174.648 |
| 9 | 0.491 | 0.433 | 0.368 | 181.837 |
| 10 | 0.498 | 0.456 | 0.394 | 199.474 |
| 11 | 0.468 | 0.431 | 0.361 | 233.932 |
| 12 | 0.455 | 0.419 | 0.355 | 176.988 |
| 13 | 0.484 | 0.457 | 0.378 | 181.823 |
| 14 | 0.457 | 0.426 | 0.357 | 192.856 |
| 15 | 0.468 | 0.425 | 0.355 | 191.300 |
| 16 | 0.453 | 0.416 | 0.344 | 195.062 |
| 17 | 0.435 | 0.417 | 0.356 | 209.292 |
| 18 | 0.485 | 0.439 | 0.369 | 190.476 |
| 19 | 0.512 | 0.469 | 0.377 | 202.203 |
| 20 | 0.484 | 0.443 | 0.358 | 190.830 |
| 21 | 0.468 | 0.435 | 0.355 | 181.334 |
| 22 | 0.502 | 0.457 | 0.371 | 175.042 |
| 23 | 0.424 | 0.389 | 0.333 | 201.111 |
| 24 | 0.456 | 0.427 | 0.344 | 225.198 |
| 25 | 0.508 | 0.456 | 0.376 | 163.959 |
| 26 | 0.423 | 0.402 | 0.356 | 178.976 |

4.4 Runs of homozygosity and heterozygosity-rich regions

4.4.1 Identified runs of homozygosity (ROH)

Both the sliding window (SW) and consecutive runs (CR) methods were employed to estimate the ROH profile of the SA Merino population. The descriptive statistics of the identified ROH per method are summarised in Table 4.2. The CR detection method identified more ($n=50291$ ROH) homozygous runs compared to the SW approach ($n=43147$ ROH). The mean (\pm standard deviation) ROH length per chromosome was $6.53\text{Mb} \pm 0.47\text{Mb}$ using the SW approach, compared to $6.36\text{Mb} \pm 0.44\text{Mb}$ for the CR approach. The mean ROH length was the longest for OAR 1 in both the SW and CR approaches (7.24Mb and 6.99Mb). The shortest mean ROH length was found on OAR 19 (5.52Mb) in the SW approach and OAR 13 for the CR approach (5.49Mb), respectively.

Table 4.2 Summary statistics of runs of homozygosity (ROH) identified for the SA Merino population using two ROH detection methods.

| | Sliding window | Consecutive runs |
|--------------------------------------|----------------|------------------|
| Number of ROH | 43147 | 50291 |
| Mean number of ROH / OAR | 1659.5 | 1934.269 |
| Mean ROH length / OAR (Mb) | 6.529 | 6.361 |
| Median ROH length / OAR (Mb) | 6.598 | 6.455 |
| Minimum ROH length / OAR (Mb) | 5.523 | 5.49 |
| Maximum ROH length /OAR (Mb) | 7.237 | 6.99 |
| Mean number of ROH / animal | 29.153 | 33.98 |
| Minimum ROH number / animal | 5 | 5 |
| Maximum ROH number / animal | 79 | 106 |

The highest number of ROH was detected on OAR 3 using both the SW and CR methods (4565 and 5205 , respectively), whilst both methods identified OAR 24 as having the lowest number of ROH (611 and 723 , respectively). Table 4.3 shows the proportion of detected ROH within different segment size categories for each method.

Table 4.3 Summary of the percentage of ROH detected per class size in the SA Merino population.

| | Sliding window | Consecutive runs |
|-------------------|-----------------------|-------------------------|
| Class (Mb) | Percentage (%) | Percentage (%) |
| <4 | 36.38 | 38.17 |
| 4≤ROH<8 | 40.55 | 40.37 |
| 8≤ROH<16 | 17.10 | 16.03 |
| 16≤ROH<32 | 5.15 | 4.65 |
| ≥32 | 0.81 | 0.78 |

The size category with the highest percentage of ROH was 4≤ROH<8Mb (40.55%, and 40.37%, respectively) for both methods. The ROH coverage (%) decreased with decreasing autosome length (i.e. roughly from OAR 1 to OAR 26). The peak percentage of ROH coverage (sum of ROH per chromosome divided by each chromosome length) was found on OAR 3 (10.5%) for the SW approach and OAR 2 (10.3%) for the CR approach. The lowest overall percentage of autosomal ROH coverage was recorded on OAR 24 (1.41% and 1.43%) for both the SW and CR approaches.

The number of ROH per animal was additionally identified for each method and the results are represented in Figure 4.2. The SW method identified less ROH per individual than the CR approach. The mean number of ROH per individual was 29.15 runs for the SW approach and 33.98 runs for the CR approach.

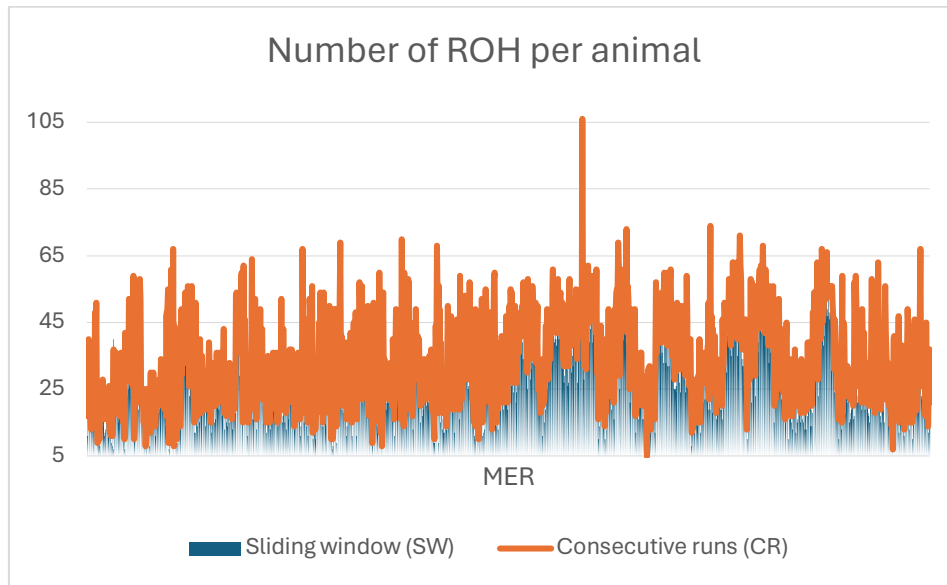


Figure 4.2 A graph representing the number of ROH detected per individual SA Merino sheep for both methods.

An increasing trend was observed regarding the length of ROH segments (in megabase pairs; Mb) and the number of ROH per individual, as shown in Figure 4.3. The figure shows that most individuals have detected ROHs that are short i.e. the sheep with a higher total ROH coverage had a higher number of short ROHs detected.

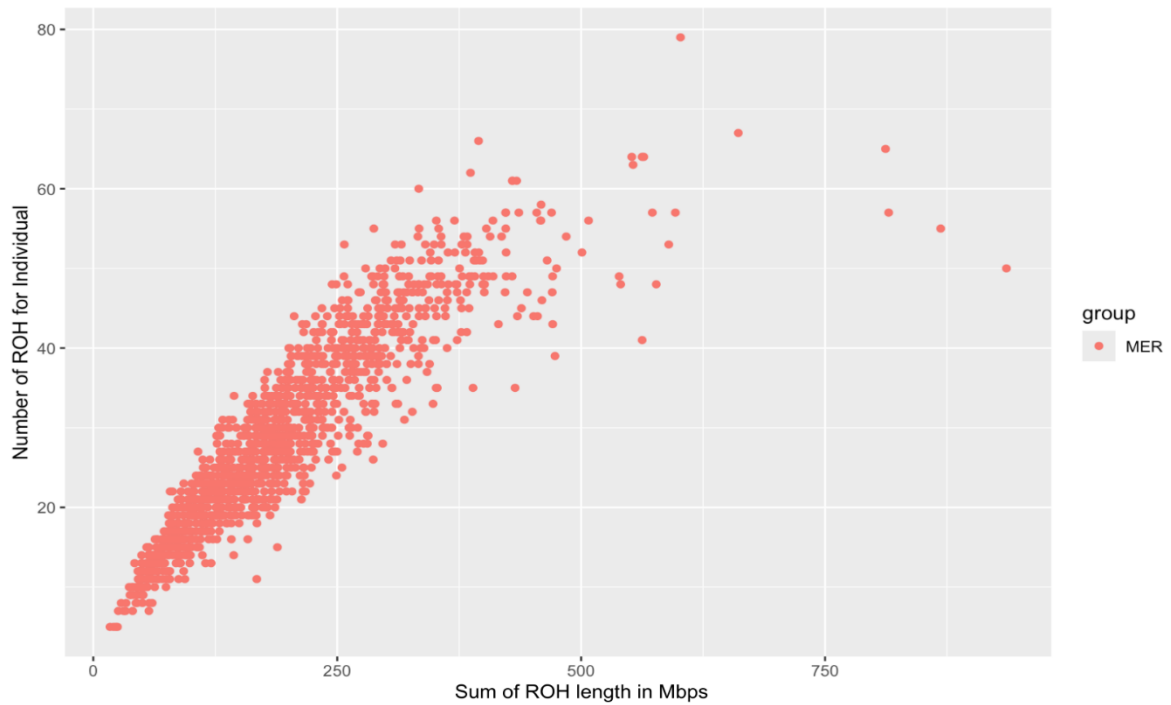


Figure 4.3. A plot showing the sum of ROH lengths (in Mb) against the number of ROH per individual.

4.4.2 Identified heterozygosity-rich regions (HRR)

Similar to ROH, HRR was identified using both the SW and CR methods. The SW detection method identified more heterozygous runs compared to the CR approach. The mean (\pm standard deviation) HRR length per chromosome was $1.3\text{Mb} \pm 0.14\text{Mb}$ for the SW approach and $1.1\text{Mb} \pm 0.17\text{Mb}$ for the CR approach. The statistics of the identified HRR per method are summarised in Table 4.4.

Table 4.4. Summary statistics of heterozygosity-rich areas identified for the SA Merino population using two HRR detection methods.

| | Sliding window | Consecutive runs |
|--------------------------------------|-----------------------|-------------------------|
| Number of HRR | 11744 | 911 |
| Mean number of HRR / OAR | 451.692 | 39.609 |
| Mean HRR length / OAR (Mb) | 1.3 | 1.129 |
| Median HRR length / OAR (Mb) | 1.269 | 1.142 |
| Minimum HRR length / OAR (Mb) | 1.095 | 0.818 |
| Maximum HRR length / OAR (Mb) | 1.653 | 1.695 |
| Mean number of HRR / animal | 7.93 | 1.33 |
| Minimum HRR number / animal | 1 | 1 |
| Maximum HRR number / animal | 21 | 4 |

The OAR 2 autosome had the longest mean length of HRR in both the SW and CR approaches (1.65Mb and 1.69Mb, respectively). Additionally, the highest HRR count was also detected on OAR 2 in both methods (n=1647, and n=164, respectively). The shortest mean length of HRR was found on OAR 4 (1.09Mb) in the SW approach and OAR 10 for the CR approach (0.81Mb), whilst the lowest HRR count was found on OAR 11 for both the SW and CR methods (n=76, and n=3, respectively). Table 4.5 shows the percentage of HRR detected per class size for both methods.

Table 4.5 Summary of the percentage of HRR detected per class size in the SA Merino population.

| | Sliding window | Consecutive runs |
|-------------------|-----------------------|-------------------------|
| Class (Mb) | Percentage (%) | Percentage (%) |
| 0.5-1 | 22.98 | 32.49 |
| >1-2 | 71.65 | 63.67 |
| >2 | 5.37 | 3.84 |

The size class with the highest percentage of HRR detected was 1-2Mb (71% and 63% for the SW and CR methods, respectively). The number of HRR detected per class size showed a

general decreasing trend from OAR 1 to 26 for the SW method, whereas the CR method showed a decreasing trend from OAR 1 to 25 with peaks on OAR 10 and OAR 13. The highest HRR coverage was detected on OAR 2 for both the SW and CR approaches (14.02% and 18%), respectively. The lowest overall HRR coverage was recorded on OAR 11 (0.64% and 0.32%) for the SW and CR approaches, respectively.

The number of HRR per animal was additionally identified for each method and the results are represented in Figure 4.4. A higher number of HRRs were identified per individual in the SW approach than in the CR approach. The mean number of HRRs per individual was 7.93 runs for the SW approach and 1.33 runs for the CR approach.

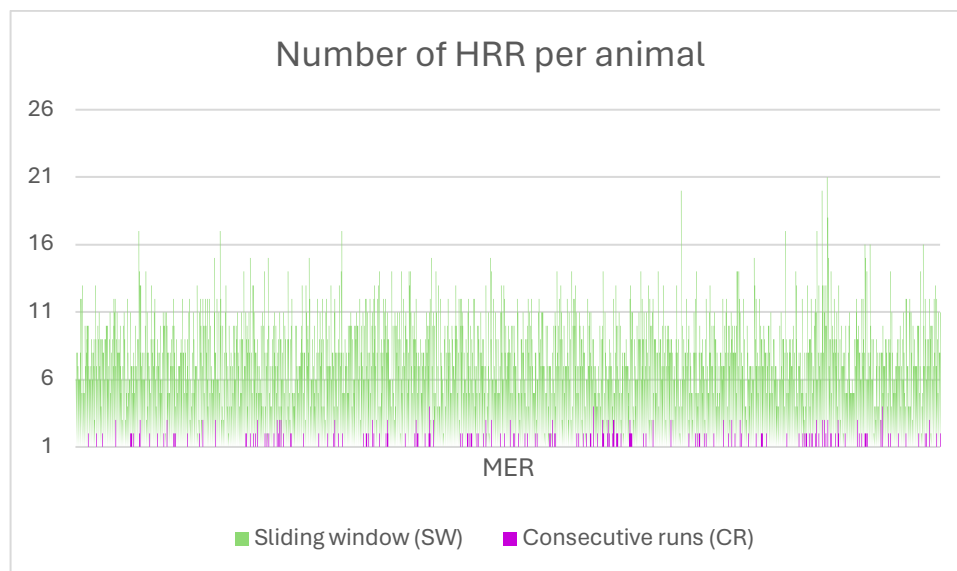


Figure 4.4 The number of HRR detected per individual SA Merino sheep for both methods.

The average length of HRR segments (Mb) showed a positive relationship with the average number of HRR per individual as shown in Figure 4.5. The midsection of the figure indicates that most individuals had HRR lengths of moderate size compared to the average number of runs per individual i.e. few sheep had extremely short and extremely long lengths of HRRs.

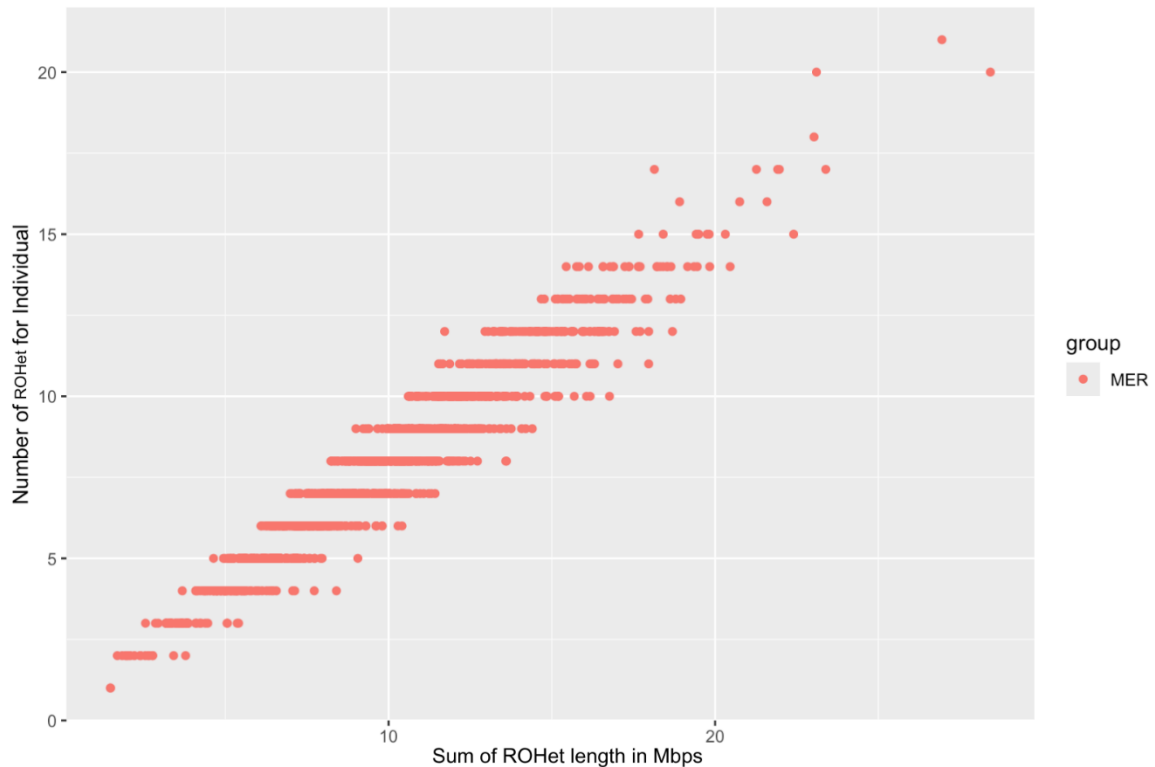


Figure 4.5. A scatter plot indicating the relationship between the sum of HRR lengths (in Mb) and the number of HRR per individual.

4.5 Inbreeding estimation

4.5.1 F-statistics

The mean±standard deviation genome-wide F_{ROH} was estimated as 0.074 ± 0.04 . The chromosome-wide F_{ROH} mean values ranged between 0.076 (OAR 1) and 0.352 (OAR 21). The mean F_{ROH} values per chromosome are reported in Table A1. The mean F_{ROH} values per ROH length category (in Mb) were $F_{ROH<4}=0.074$, $F_{\leq ROH<8}=0.061$, $F_{8\leq ROH<16}=0.037$, $F_{16\leq ROH<32}=0.023$, and $F_{ROH\geq 32}=0.022$, respectively. The mean±standard deviation F_{SNP} across the genome was marginally lower at 0.037 ± 0.05 . The mean F_{SNP} values estimated had a minimum value of -0.075 and a maximum value of 0.353. There was a strong, positive relationship between the genomics-based F-statistics (i.e., F_{SNP} and F_{ROH}), with a Pearson correlation of 0.95. Figure 4.6 shows boxplots, illustrating the mean, median, and outliers for the F_{SNP} and F_{ROH} estimators. Characteristically, the F_{SNP} coefficient showed more variation than the F_{ROH} coefficient.

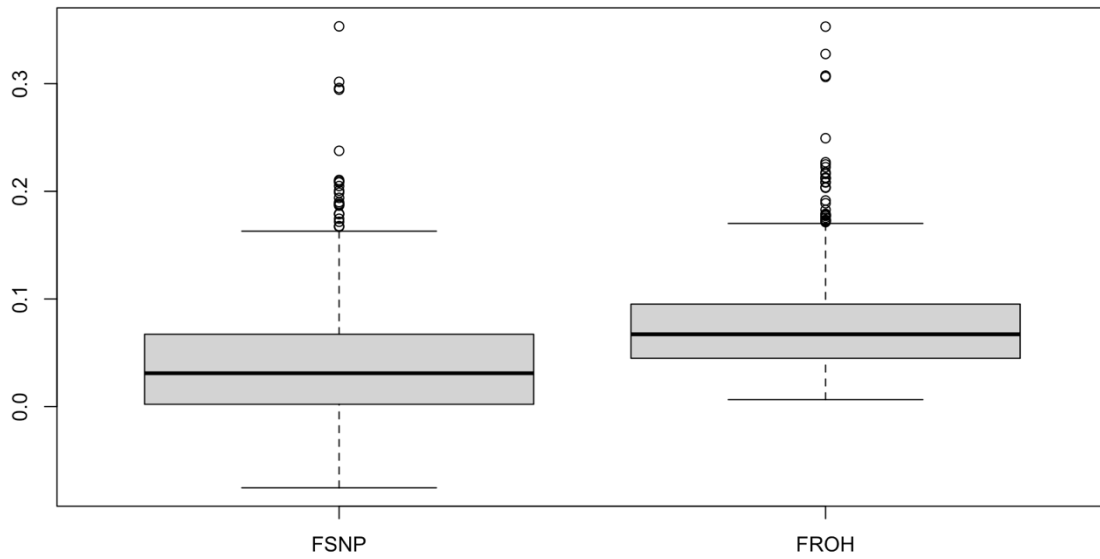


Figure 4.6 Boxplots for the F_{SNP} and F_{ROH} inbreeding estimators showing the mean, median, maximum, minimum, and outliers of each.

4.5.2 Effective population size (N_e)

The SNP-based N_e estimates were computed from 912 generations ago until 12 generations ago. Due to limitations in the panel density, the most recent estimate that the software could calculate was 12 generations ago, and this relates to ~38 and ~2900 years ago based on an estimated generation interval of 3.2 years (Cloete *et al.*, 2009). The N_e of the SA Merino breed decreased over time, as represented in Figure 4.7. The N_e declined from 3062 for the oldest generation (912 generations ago) to 305 for the most recent generation (12 generations ago).

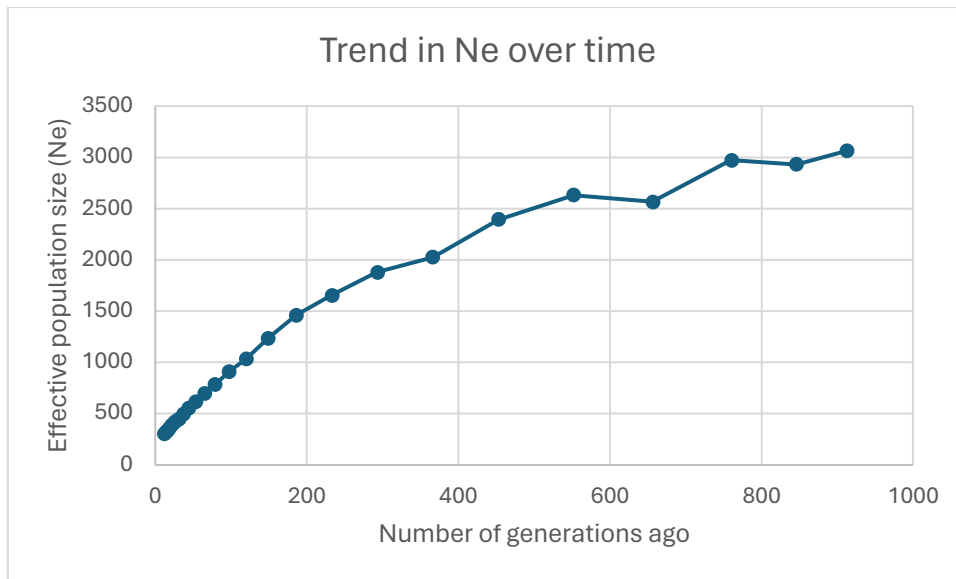


Figure 4.7 The decline in N_e from generation 912 to generation 12 of the SA Merino sheep population.

4.6 Within-population selection signatures

The chromosome-wide proportion of times an SNP resided within a detected ROH using the SW method is summarised in Table 4.6. A total of 275 genes (within a threshold of 16.4%) and 374 SNPs across four autosomes were detected.

Table 4.6 A summary of the number of genes located within each ROH region detected using a threshold of 16.4%.

| OAR | n SNP ^a | Position (from) | Position (to) | Length (Mb) | n Genes ^b |
|--------------|--------------------|--------------------|------------------|----------------|----------------------|
| 2 | 60 | 13707962 | 16493535 | 2785573 | 15 |
| 2 | 41 | 17960258 | 20898157 | 2937899 | 12 |
| 3 | 1 | 47595933 | 47595933 | - | 1 |
| 3 | 5 | 47755179 | 48428503 | 673324 | 2 |
| 3 | 18 | 48950632 | 50074939 | 1124307 | 3 |
| 3 | 22 | 50229700 | 51353599 | 1123899 | 1 |
| 3 | 7 | 51607921 | 51922042 | 314121 | 1 |
| 3 | 1 | 63916843 | 63916843 | - | 0 |
| 6 | 57 | 39124095 | 43178901 | 4054806 | 6 |
| 6 | 6 | 53238259 | 53470583 | 232324 | 0 |
| 6 | 14 | 54029176 | 54762520 | 733344 | 0 |
| 11 | 142 | 23318858 | 33174792 | 9855934 | 234 |
| Total | 374 | - | - | - | 275 |

^a Number of SNPs ^b Number of genes detected

A Manhattan plot of the proportion of times each SNP resides within detected ROH per chromosome is shown in Figure 4.8. The highest occurring consensus ROH haplotype was on OAR 11 (base pair position: 23318858-33174792), and this region contained 234 protein-coding genes. In descending order, the highest occurring consensus ROH regions were followed by OAR 2 with 27 protein-coding genes, OAR 3 with 8 protein-coding genes, and OAR 6 with 6 protein-coding genes.

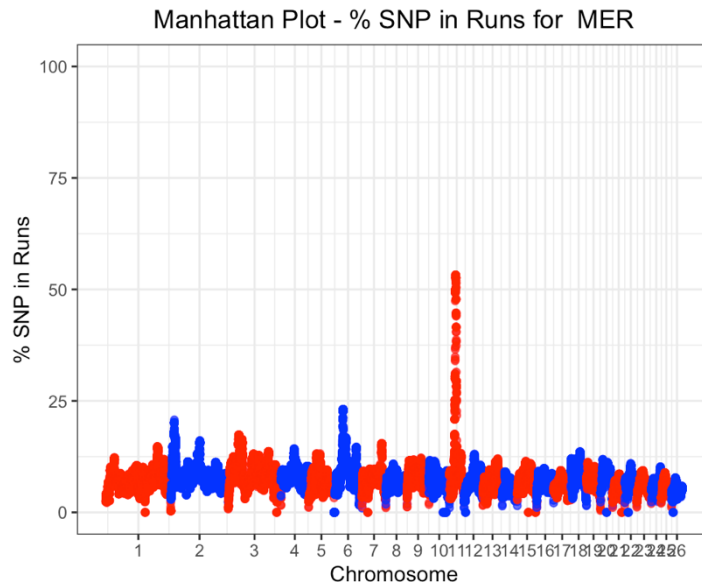


Figure 4.8 The chromosome-wide proportion of times each SNP resides within a detected ROH using the SW method.

Table 4.7 summarises the associated functions of each protein-coding gene located within the detected ROH regions. A more comprehensive version of the table is included in the addendum as Table A2. The protein-coding genes in ROH that were the most enriched (i.e., p -value of $1.70E-07$) were associated with the lipoxygenase pathway and linoleic acid metabolic processes.

Table 4.7 A summary of the associated function of each protein-coding gene located within the defined ROH regions.

| Associated function | No of genes | Significance level (p-value) |
|---------------------------------------|--------------------|-------------------------------------|
| Arachidonic acid metabolic process | 6 | 9.00E-08 |
| Arachidonate 12-lipoxygenase activity | 4 | 4.70E-06 |
| Actin filament binding | 9 | 4.70E-05 |
| Arachidonate 15-lipoxygenase activity | 3 | 3.30E-04 |
| Myofibril | 4 | 9.40E-04 |
| Myosin complex | 5 | 0.001 |
| Olfactory receptor activity | 15 | 0.019 |

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were constructed for genes within the detected ROH regions. The KEGG pathways for a) genes associated with Arachidonic acid metabolism, b) genes associated with motor proteins, and c) genes associated with olfactory transduction are indicated in Figure 4.9.

Figure 4.9a shows the genes that play a role in arachidonic acid metabolism are also associated with linolenic acid metabolism, namely, *ALOX12B*, *ALOX12* (Arachidonate 12-lipoxygenase, OAR 11), *ALOX15* (Arachidonate 15-lipoxygenase, OAR 11) and *ALOXE3* (Arachidonate lipoxygenase 3, OAR 11).

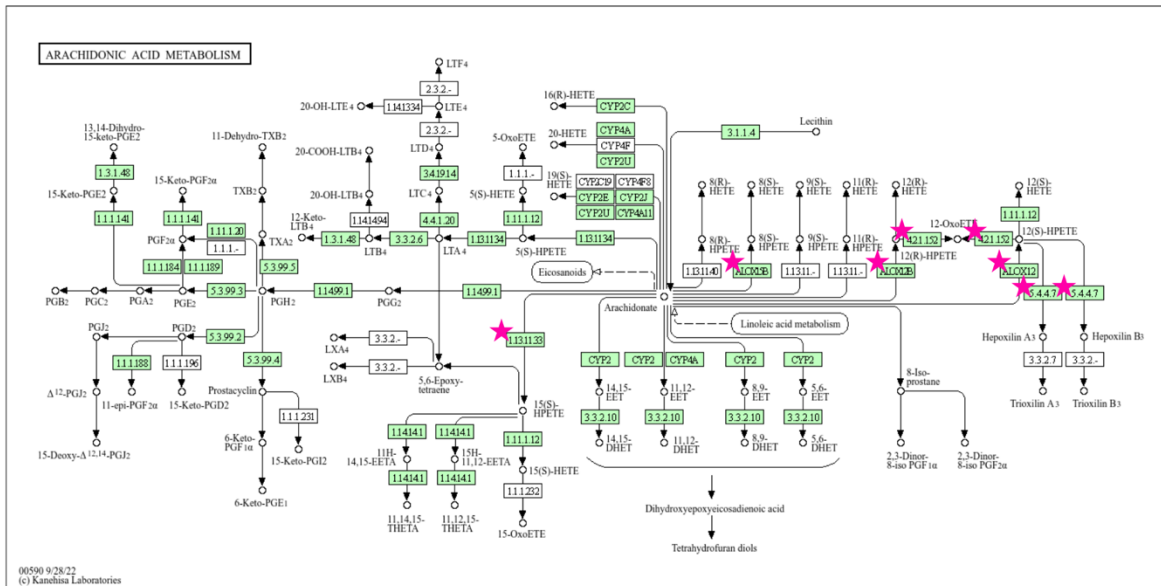


Figure 4.9a. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for genes associated with Arachidonic acid metabolism in sheep.

Figure 4.9b shows genes that are associated with motor proteins and play a role in myofibril formation, namely, Genes *DNAH2* (dynein axonemal heavy chain 2, OAR 11), *DNAH9* (dynein axonemal heavy chain 9, OAR 11), *KIF1C* (kinesin 3, OAR 11), *MYH1*, *MYH2* *MYH8* and *MYH10* (OAR 11).

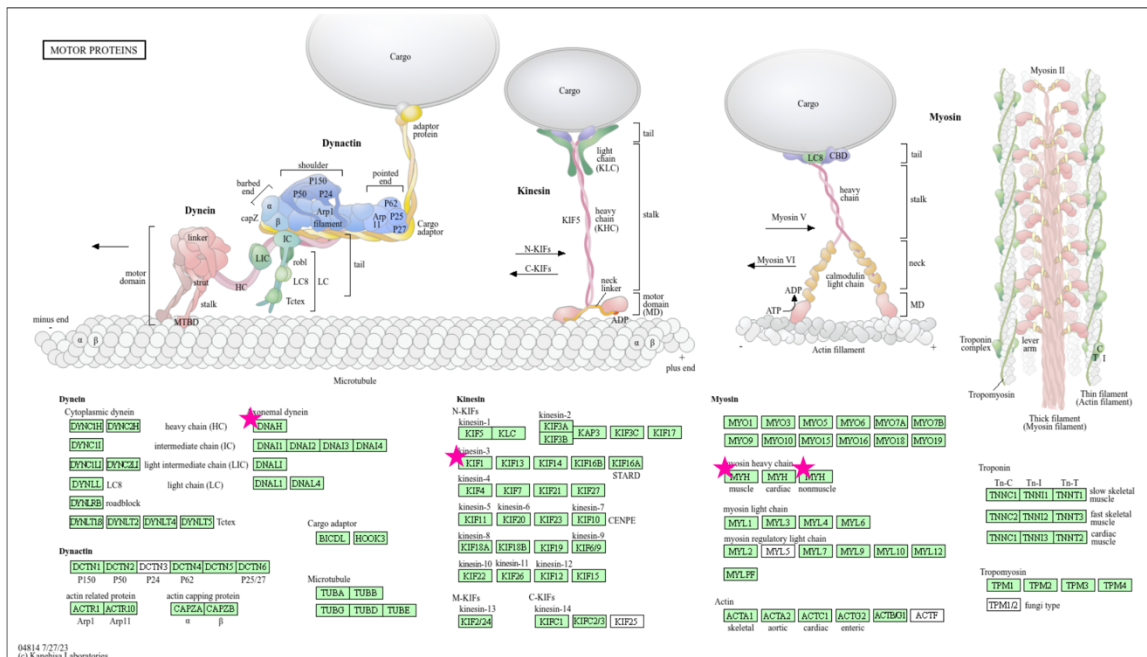


Figure 4.9b. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for genes associated with functioning of motor proteins in sheep.

Figure 4.9c shows genes that are associated with olfactory transduction, namely, *ARRB2* (Arrestin beta 2, OAR 11), *GUCY2D* (Guanylate cyclase 2D retinal, OAR 11) and *DTMT* (Olfactory receptor-like protein, OAR 11).

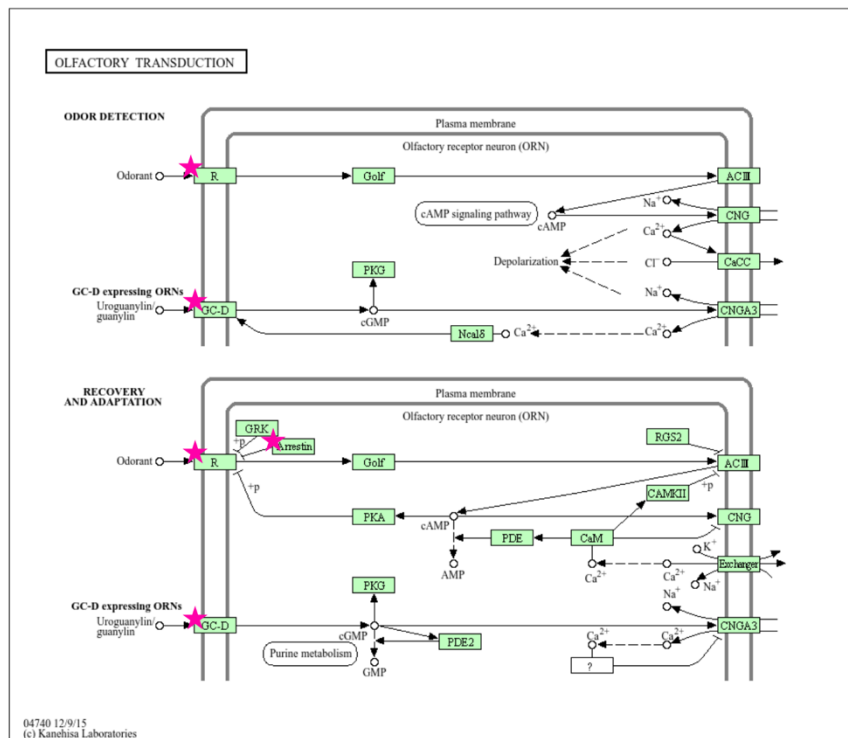


Figure 4.9c. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway for genes associated with olfactory transduction in sheep.

Figure 4.9 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for genes associated with a. Arachidonic acid metabolism, b. functioning of motor proteins, and c. olfactory transduction within enriched ROH regions.

Chapter 5: Discussion

5.1 Introduction

Merino and Merino-derived breeds are dispersed globally and are arguably the most abundant sheep breed and primary producer of the world's wool supply (Ciani *et al.*, 2015). Along the evolutionary timeline from the domestication of sheep to the development of commercial flocks, several factors (e.g. intense directional selection, genetic drift, related mating, population bottlenecks, and natural selection) have influenced the autozygosity levels of sheep flocks (Ferenčaković, 2015; Xu *et al.*, 2019).

This study aimed to perform an SNP-based investigation of the current autozygosity levels in the South African Merino sheep breed. Several population parameters that serve as proxy indicators of genetic diversity and/or inbreeding, including observed and expected heterozygosity- rates and effective population size (N_e) were used to quantify the genetic variability present within the SA Merino breed. Additionally, the genome-wide ROH profiles and associated F-statistics were used to reflect the autozygosity levels in the SA Merino sheep breed. Although previous local and global studies have explored within and across-population autozygosity levels in numerous sheep breeds, the present investigation provides a the first comprehensive overview of autozygosity levels in the SA Merino breed, specifically.

5.2 Genetic Diversity

Mean expected heterozygosity (H_E) and observed heterozygosity (H_O) are both measures of genetic diversity that are estimated from molecular information and the relationship between the two statistics reveals a gain or loss of genetic diversity (Eusebi *et al.*, 2020). The mean observed heterozygosity of the SA Merino, estimated in the current study, was moderate ($H_O=0.35$) and consistent with previously reported heterozygosity levels for the breed. Previous studies, of notably smaller sample sizes (e.g. 10 and 33 SA Merino sheep, respectively), reported similar observed heterozygosity values of 0.37 (e.g. Ceccobelli *et al.*,

2023; Dzomba *et al.* 2023). For a larger sample size (n=697 SA Merino sheep), Nel *et al.* (2022), for example, obtained an average observed heterozygosity value of 0.36 for SA Merino sheep. In the current study, including a larger and more representative sample of the breed, the expected ($H_E=0.365$) heterozygosity was slightly higher than the observed heterozygosity level and this agreed with previous studies where $H_E= 0.378$ (Ceccobelli *et al.*, 2023), 0.39 (Dzomba *et al.*, 2023), and 0.37 (Nel *et al.*, 2022) were reported for SA Merino sheep.

The small difference in H_O and H_E values indicates a negligible loss of within-population genetic diversity (Eusebi *et al.*, 2020), which could be due to maintaining breed purity or breed standards by the breed society. The moderate heterozygosity levels reported here were expected as the SA Merino is a well-managed commercial sheep breed with moderate to high population sizes and well-defined breeding programs (Dzomba *et al.*, 2021).

Minor allele frequency (MAF) relates to genetic diversity and indicates the level of informative and segregating markers within a population (Keller *et al.*, 2011). Highly polymorphic markers are associated with high MAF values which correspond to higher levels of H_E and consequently indicate a greater genetic diversity (Engelsma *et al.*, 2014). On the contrary, low MAF indicates possible fixation of one allele in the population and, hence, lower genetic diversity (Engelsma *et al.*, 2014). The high MAF values (highly informative SNPs) obtained for the Illumina® OvineSNP50 Beadchip genotyping panel are expected as this genotyping panel was specifically designed to include SNPs validated in the most economically- and globally-important *Ovis aries* breeds, including 127 samples from Merino-type breeds (Kijas *et al.*, 2009).

The mean MAF (0.275 ± 0.14) reported in this study is similar to Ovine 50K-based values previously reported for smaller sample sizes of the SA Merino breed; these MAF values ranged from 0.26 to 0.27 (Dzomba, 2021; Sandenbergh, 2015; Sandenbergh *et al.*, 2016). The mean MAF values estimated are also comparable to previous Ovine 50K-based estimates for global Merino populations that ranged from 0.29 to 0.3 (Al-Mamun *et al.*, 2015; Nosrati *et al.*, 2021).

Linkage disequilibrium (LD) is a genetic diversity parameter that shows the extent of non-random association between two alleles located at different loci and is a direct reflection of the recombination rate within a population (Zavattari, 2000; Kijas *et al.*, 2014). The level of LD in a population reflects how closely SNPs are related to one another within the genome (Qanbari *et al.*, 2010). In this study, LD was estimated using r^2 as proposed by Bohmanova *et al.* (2010) because of the inverse relationship between r^2 and the sample size required to detect associations between SNPs (Bohmanova *et al.*, 2010). High levels of LD and, hence, more relatedness result in higher levels of specific allelic combinations inherited over generations than can be expected by chance (Eusebi *et al.*, 2020).

The average r^2 -value calculated in this study was 0.365, which is consistent with other studies on similar sample sizes of Merino sheep. Liu *et al.* (2017); Dzomba *et al.* (2021), and Nel *et al.* (2022) reported r^2 -values in a wide range of 0.14 to 0.31.

The effective population size (N_e) of an existing population can be defined as the ideal population size that will result in the same amount of genetic diversity as that which is present in the current existing population (Wright, 1974). The N_e size directly influences a population's inbreeding level (Gholizadeh & Ghafouri-Kesbi, 2016). In concordance with the low estimates of inbreeding reported here, the most recent estimate of N_e falls within the recommended range of 50 (Frankham *et al.*, 2002) and 500 (Holt *et al.*, 2005; Meuwissen, 2009), proposed for the survival of a breed by the FAO.

In this study, the most recent genomics-based N_e estimate achievable (considering the SNP density and, hence, capturable LD level) was for 12 generations ago. There was a decline in the SA Merino's estimated N_e ; over the span of ~2800 years (based on a generation interval of 3.2 years), the N_e decreased from 3 062 animals for the oldest generation (912th generation) to 305 animals for the most recent generations ago (i.e., 12). This decreasing trend observed for N_e over generations is in concordance with previous studies on SA Merino sheep (Dzomba, 2021; van Marle-Köster *et al.*, 2021; Nel *et al.*, 2022; Ceccobelli *et al.*, 2023).

There were however discrepancies between previously reported N_e estimates, and those reported here. For example, Dzomba (2021) reported a decline from 2809 (ancestral) to 167

animals (most recent). Furthermore, van Marle-Köster *et al.* (2021), Nel *et al.* (2022) and Ceccobelli *et al.* (2023) reported a decline in N_e population sizes from 697 (ancestral) to 33 (most recent) for smaller sample sizes of SA Merino sheep. The fairly large discrepancies may, however, be attributable to the sample size and composition. Higher relatedness within a sample could overestimate various of the proxy-indicator parameters studied here, for example, the inbreeding coefficients and the degree of LD. Furthermore, the density of the SNP genotyping panel can be a limiting factor in achievable estimation of N_e in more recent generations, as it determines the chromosome-wide LD that can be captured (Goddard & Hayes, 2007; Visser *et al.*, 2023).

5.3 Runs of homozygosity and Heterozygosity-rich regions

Runs of homozygosity (ROH) are long stretches of homozygous regions throughout the genome that reflect autozygosity due to consanguineous mating (Ferenčaković, 2015). The lengths of ROH reflect recombination events, with longer segments referring to recent inbreeding and shorter segments referring to ancestral inbreeding (Pemberton *et al.*, 2012).

For ease of comparison, only the descriptive statistics of the ROH identified (and corresponding F_{ROH} calculations) by means of the SW approach will be referred to in this discussion. In this study, a total of 43 147 ROH with a mean per chromosome length of 6.53Mb were identified. The OAR 1 and OAR 19 autosomes had the longest and shortest mean ROH length, respectively. The number of genome-wide ROH present and identifiable depends on the population dynamics; for instance, Nosrati *et al.* (2021) identified an average number of 16.8 ROH per individual ROH from a sample size of 356 animals from Chinese Merino sheep populations. On the contrary, Dzomba *et al.* (2021) identified a total number of 5931 ROH from a sample size of 56 SA Merino sheep and total number of 4071 ROH from a sample size of 50 Australian Merino sheep.

The majority of ROH detected in this study, were classified into the second shortest length category (i.e. 4-8Mb), reflecting the predominance of distant inbreeding effects. In support of this observation, a positive relationship (or trend) was observed between the number of ROH

per individual and its summed ROH length (i.e. the sheep with higher total ROH coverage), implying that SA Merino animals generally had more ROH per individual albeit smaller ROH in size.

Due to discrepancies in ROH defining criteria across studies, the comparison of reported ROH descriptive statistics to previously published ROH results is limited, however, general trends can be deduced. Two previous studies have investigated ROH in the SA Merino; in van Marle-Köster *et al.* (2021), for example, the majority (69%) of identified ROH were classified into the shortest length category (ROH<4Mb). Ceccobelli *et al.* (2023) found a similar mean per-individual ROH length (6.93Mb) to this study, with most (45.51%) of the identified ROH falling within a 5-10Mb length category. In international Merino studies, similar trends in ROH descriptive statistics were reported. Al-Mamun *et al.* (2015) and Wanjala *et al.* (2023), for example, observed the majority of ROH in a 1-10Mb length category. All the afore-mentioned studies reported a general decreasing trend of the number of ROH per increasing length category, hence, reiterating the suggestion of less recent inbreeding in local and global Merino populations.

Heterozygosity-rich regions (HRR) are consecutive stretches of heterozygous SNP genotypes (Williams *et al.*, 2016), which provide information regarding a population's evolutionary history and diversity (Samuels *et al.*, 2016). The HRR can be used to identify specific genomic areas where it would be beneficial to maintain a higher level of genetic diversity in specific areas (Biscarini *et al.*, 2020), typically associated with adaptive traits (Mc Parland *et al.*, 2009; Selli *et al.*, 2021).

In this study, a total of 11 744 HRR were detected, most (71%) of which were classified as short in length (1-2Mb); the mean HRR length per chromosome was 1.3Mb. These findings suggest that HRRs are small and frequent within the SA Merino sheep genome. Studies investigating HRR in livestock species are scarce in literature, thus making it difficult to find comparable results. In a study by Selli *et al.* (2021), however, a mean of 139.59 HRR (with a mean length of 459.894kb) per animal was identified across populations. Higher density SNP genotyping panels are expected to contribute more knowledge to the HRR profiles in populations.

In HRR-focused studies on other livestock species, the average number of HRR regions detected per animal was lower than the average number of ROH regions detected per animal (HRR_{TURKEY}=57.80, ROH=126.21, Marras *et al.*, 2018; HRR_{CATTLE}=9.87, ROH=22.27, Biscarini *et al.*, 2020; HRR_{HORSE}=52.17, ROH= 75.63, Bizarria dos Santos *et al.*, 2021), which is a similar trend observed in this study. In contrast, Selli *et al.* (2021) investigated HRR for international Merino sheep and detected a higher total average number of HRR (165 558) than the total average number of ROH (80 639). This could be due to differences in defining parameters when calculating HRR.

5.4 Inbreeding estimation

The F_{SNP} inbreeding coefficient estimates the excess in homozygosity (Rodríguez-Ramilo *et al.*, 2020). The genomic inbreeding coefficients, i.e. F_{SNP} and F_{ROH} , are highly correlated, with a strengthening correlation as the effective population size decreases, and this is because F_{ROH} defined by short ROH (ancient inbreeding) correlates poorly with homozygous mutation load when N_e is small (Keller *et al.*, 2011). Dzomba (2021) recommends a safe threshold of $F_{SNP} < 0.1$ as sufficient to allow population diversity.

In this study, the mean F_{SNP} (0.037) was comparable with mean values reported in other studies on SA Merino sheep. Sandenbergh *et al.* (2015) and Dzomba *et al.* (2023), for example, both reported mean F_{SNP} values closest in comparison to this study (i.e., $F_{SNP}=0.060$ and 0.040, respectively). van Marle-Köster *et al.* (2021) reported an F_{SNP} value remarkably lower than that of other studies ($F_{SNP}=0.009$). Overall, the tendency towards small F_{SNP} values suggests that there is sufficient genetic diversity within the breed to avoid the effects of inbreeding depression.

The F_{ROH} coefficient is a genomic measure of individual autozygosity (McQuillan *et al.*, 2008). Due to the nature of the formula to calculate F_{ROH} , estimates are commonly higher than those of F_{SNP} measures because they cannot include negative values. The added benefit of deriving inbreeding coefficients from ROH (F_{ROH}) is that it provides additional information to the

degree of inbreeding; the length characteristics also provides an indication of the “age” of inbreeding effects (Ferenčaković, 2015).

In this study, the mean genome-wide F_{ROH} was estimated at 0.074. The average F_{ROH} values per ROH length category (in Mb) were furthermore produced. The highest F_{ROH} value (0.074) was found within the short length category (1-4Mb) and showed an inverse relationship with ROH length (i.e. the F_{ROH} values decreased for increasing ROH length). These results are in close agreement with previous results, with reported F_{ROH} ranges between 0.055 and 0.14 for smaller sample sizes of SA Merino (Dzomba, 2021; van Marle-Köster *et al.*, 2021; Ceccobelli *et al.*, 2023). As expected, the F_{ROH} values were all higher than F_{SNP} values, conveying sufficient autozygosity at the individual level to avoid negative inbreeding effects.

5.5 Within-population selection signatures

Selection signatures are characterized by genomic regions with reduced genetic variability (i.e., increased allele frequencies within the genome) (Purfield *et al.*, 2017). Studying the distribution of ROH patterns across the genome allows the detection of selection signatures (Zhang *et al.*, 2015), which most likely reflect selection events (Kim *et al.*, 2013). Therefore, the functional analysis of identified genes within selection signatures may indicate traits under selection pressure (Almeida *et al.*, 2019).

In this study, a total of 275 genes had an incidence of more than 16.4% in SNP-in-ROH regions. Ceccobelli *et al.* (2023) similarly identified a total of 166 genes which had an incidence above a 99.9th percentile threshold. Biscarini *et al.* (2020) identified 58 genes using a 99.9 quantile of the SNP-in-ROH frequency distribution (corresponds to a frequency threshold of 0.41). The highest occurring consensus ROH haplotype was on OAR 11 which covered many base pairs (9855934bp) and this region contained 234 protein-coding genes. This indicates that genes within this region of OAR 11 have been under extensive selection pressure. The protein-coding genes found within the selection signatures were associated with the arachidonic acid metabolic process, motor proteins, and olfactory transduction.

Arachidonic acid metabolic process (which includes lipoxygenase pathways and linoleic acid metabolism) are important in sheep as they play an important role in inflammatory responses (Selli *et al.*, 2021) as well as wool production (Wang *et al.*, 2020). The *ALOX12B* and *ALOX12* (Arachidonate 12-lipoxygenase, OAR 11) genes are associated with body size and feed efficiency in sheep (Kominakis *et al.*, 2017). The *ALOX15* (Arachidonate 15-lipoxygenase, OAR 11) gene plays a role in *H. contortus* resistance in sheep (Guo *et al.*, 2016). The *ALOXE3* (Arachidonate lipoxygenase 3, OAR 11) gene encodes the enzyme arachidonate lipoxygenase 3 (Dzomba *et al.*, 2021).

Detected genes were found to play a role in the functioning of motor proteins (i.e. myosin complex) and involved in myofibril formation, which is important in fibre formation (Megdiche *et al.*, 2019). The *DNAH2* (dynein axonemal heavy chain 2, OAR 11) and *DNAH9* (dynein axonemal heavy chain 9, OAR 11) genes are involved in MAP2K4-metabolic functioning in the liver (Dzomba *et al.*, 2023). The *KIF1C* (kinesin 3, OAR 11) gene is involved in the functioning of motor proteins (Megdiche *et al.*, 2019). The *MYH1*, *MYH10* and *MYH8* genes (all located on OAR 11) collectively facilitate diverse roles in energy metabolism, including muscular contraction, cytokinesis, and phagocytosis (Miyagi *et al.*, 1999). The *MYH2* gene (OAR 11) plays a role in motor activity, actin cytoskeleton, actin binding, contractile fibre and calmodulin binding (Selli *et al.*, 2021).

Several genes associated with olfactory transduction were in selection signatures, mostly located on OAR 11. The *ARRB2* (Arrestin beta 2, OAR 11) gene is involved in regulating anti-inflammatory responses in the lungs (Walker *et al.*, 2003). The *GUCY2D* (Guanylate cyclase 2D retinal, OAR 11) gene encodes a transmembrane GC receptor protein expressed in the murine olfactory epithelia (Yuge, 2021). The *DTMT* (Olfactory receptor-like protein, OAR 11) gene is involved in the binding of odour molecules for olfaction (Muñoz *et al.*, 2019).

The notable genes within ROH regions associated with metabolic and immune response pathways (*DNAH2*, *DNAH9*, *MYH1*, *MYH10*, *MYH8* and *ARRB2*) relate to selection pressure for traits necessary for survival in harsh, arid environments of South Africa (Mdladla *et al.*, 2018).

5.6 Conclusion

It is important to characterize and monitor autozygosity levels within a population to conserve population genetic diversity and ensure the sustainability of future breeding programs. In this study, genome-wide SNP data of 1 738 SA Merino sheep were utilized to characterize their autozygosity and inbreeding levels.

Observed and expected heterozygosity as well as MAF, LD, and N_e estimates were quantified as proxy-indicators of the genetic variability present within the SA Merino breed. The reported H_o and H_E were indicative of a gain in within-population genetic diversity, and conveys that mating programs and intense selection are well managed within the breeding objectives of SA Merino sheep. Additionally, high MAF values were reported along with low levels of LD within the SA Merino population. The relationship between MAF and LD presents opportunities to further develop genomic applications such as imputation. The Food and Agriculture Organization (FAO) (1998) proposed a threshold value of $50 < N_e > 500$ animals per herd to prevent the loss of genetic diversity. The values reported in this study were within the recommendations provided by the FAO for a healthy genetic diversity status, and indicate that genetic diversity is maintained at a reasonable level for the SA Merino.

Runs of homozygosity (ROH) and genomic estimators of inbreeding, i.e. genomic F-statistics, were used to reflect the autozygosity and inbreeding levels in the SA Merino sheep breed. The ROH length profiles were indicative of more ancient inbreeding and may reflect historic population bottlenecks. Protein-coding genes found within the selection signatures were associated with various functions in sheep involving reproduction, fibre formation, inflammatory responses, wool production, lactate metabolism, DNA, lipid, and carbohydrate synthesis.

The sufficient levels of genetic diversity retained in the current study population indicate that the SA Merino sheep breed has the potential for future sustainable production. Accounting for the genetic diversity within the SA Merino sheep breed allows for more widespread exploration of genomic applications such as genomic selection (GS) within the breed, and if

successfully applied, can pave the way for (and be transferable to) GS in other SA sheep breeds.

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Addendum

Table A1. Average F_{ROH} values per chromosome.

| OAR | Average F_{ROH} |
|-----|-------------------|
| 1 | 0.0762 |
| 2 | 0.0885 |
| 3 | 0.0930 |
| 4 | 0.1096 |
| 5 | 0.1012 |
| 6 | 0.1117 |
| 7 | 0.1066 |
| 8 | 0.1145 |
| 9 | 0.1251 |
| 10 | 0.1128 |
| 11 | 0.1672 |
| 12 | 0.1139 |
| 13 | 0.1120 |
| 14 | 0.1262 |
| 15 | 0.1151 |
| 16 | 0.1214 |
| 17 | 0.1186 |
| 18 | 0.1355 |
| 19 | 0.1381 |
| 20 | 0.1502 |
| 21 | 0.1635 |
| 22 | 0.1497 |
| 23 | 0.1355 |
| 24 | 0.1797 |
| 25 | 0.1550 |
| 26 | 0.1438 |

Tables A2. Comprehensive summary of the associated functions of each protein-coding gene located within the detected ROH regions.

| Associated function | No of genes | Significance level (p-value) | Gene names |
|-------------------------------------------------------------------------------------------------------------------------------|-------------|------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Arachidonic acid metabolic process & Actin filament binding | 6 | 9.00E-08 | ALOX12B, ALOX12, LOC101121185, ALOX15, ALOXE3 |
| Inoleic acid metabolic process | 9 | 4.70E-05 | |
| Lipoxygenase pathway | 5 | 1.70E-07 | |
| Linoleate 13S-lipoxygenase activity | 5 | 1.70E-07 | |
| Lipoxin A4 biosynthetic process | 4 | 4.70E-06 | |
| Cytoplasm | 3 | 3.30E-04 | |
| Myofibril & Myosin complex (Motor Proteins) | 37 | 6.90E-04 | |
| Response to heat | 4 | 9.40E-04 | DNAH2, DNAH9, KIF1C, MYH1, MYH10, MYH2, MYH8, LOC101113252, LOC101111980 |
| Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen | 5 | 0.001 | |
| Microtubule associated complex | 3 | 1.50E-03 | |
| Histone deacetylase binding | 3 | 0.003 | |
| Microfilament motor activity | 3 | 4.10E-03 | |
| ATP binding | 5 | 9.50E-03 | |
| Negative regulation of transcription from RNA polymerase II promoter | 3 | 0.011 | |
| ATP metabolic process | 25 | 0.012 | |
| Olfactory receptor activity | 10 | 0.014 | |
| | 3 | 0.018 | |
| | 15 | 0.019 | ARRB2, GUCY2D, LOC101119277, LOC101106784, LOC101107296, LOC115611253, LOC114117013, LOC101120175, LOC101119916, LOC101120421, LOC101120929, LOC101106536, LOC101108065, LOC101120480, DTMT, LOC101108585 |

| | | |
|--------------------------------------------------------------------------------------|----|-------|
| Flagellated sperm motility | 4 | 0.02 |
| Peptide secretion | 2 | 0.021 |
| Cellular response to interleukin-13 | 2 | 0.021 |
| Linoleate 9S-lipoxygenase activity | 2 | 0.021 |
| Establishment of skin barrier | 3 | 0.024 |
| Positive regulation of telomerase activity | 3 | 0.024 |
| Iron ion binding | 6 | 0.025 |
| Ceramide biosynthetic process | 3 | 0.026 |
| Adult walking behaviour | 3 | 0.028 |
| Phosphatidylinositol 3-kinase complex, class IB | 2 | 0.033 |
| MLL1 complex | 3 | 0.035 |
| Calcium | 11 | 0.039 |
| 1-phosphatidylinositol-3-kinase regulator activity | 2 | 0.042 |
| Cytoskeleton | 7 | 0.043 |
| G-protein coupled receptor activity | 16 | 0.045 |
| Sulfotransferase activity | 3 | 0.048 |
| ATPase activity | 7 | 0.048 |
| Motor activity | 3 | 0.05 |
| Positive regulation of cardiac muscle cell differentiation | 2 | 0.052 |
| Positive regulation of peroxisome proliferator activated receptor signalling pathway | 2 | 0.062 |
| Third ventricle development | 2 | 0.062 |
| Elongator holoenzyme complex | 2 | 0.064 |
| Protein self-association | 3 | 0.064 |

| | | |
|---------------------------------------------------------------------------------|----|-------|
| Transcriptional repressor complex | 3 | 0.065 |
| Ubiquitin protein ligase binding | 6 | 0.067 |
| Protein lipidation | 2 | 0.071 |
| Extracellular ATP-gated cation channel activity | 2 | 0.072 |
| Leading edge membrane | 2 | 0.074 |
| Lateral plasma membrane | 3 | 0.074 |
| Cytosol | 33 | 0.075 |
| Neuromuscular junction | 3 | 0.081 |
| Bone marrow development | 2 | 0.081 |
| Regulation of calcium ion transport | 2 | 0.081 |
| Nuclear migration | 2 | 0.081 |
| Purinergic nucleotide receptor activity | 2 | 0.082 |
| Dendritic spine | 3 | 0.084 |
| External side of plasma membrane | 5 | 0.085 |
| Protein desumoylation | 2 | 0.091 |
| Positive regulation of production of MIRNAS involved in gene silencing by MIRNA | 2 | 0.091 |
| Negative regulation of production of MIRNAS involved in gene silencing by MIRNA | 2 | 0.091 |
| Behavioural response to pain | 2 | 0.091 |
| Transcription corepressor activity | 4 | 0.093 |
