The impact of SNP density on quantitative genetic analyses of body size traits in a wild population of Soay sheep

Caelinn James[1*], Josephine M. Pemberton[1], Pau Navarro[2], Sara Knott[1]

[1] Institute of Ecology and Evolution, School of Biological Sciences, The University of Edinburgh, Edinburgh

[2] MRC Institute of Genetics and Cancer, The University of Edinburgh, Edinburgh

[*] Corresponding author

Corresponding author mailing address: Caelinn James, Office 133, Ashworth Laboratories, Kings Buildings, Charlotte Auerbach Road, Edinburgh EH9 3FL

Corresponding author email address: c.james-5@sms.ed.ac.uk

Key words: GWAS, heritability, wild population, SNP density, animal model

Abstract

Understanding the genetic architecture underpinning quantitative traits in wild populations is pivotal to understanding the processes behind trait evolution. The 'animal model' is a popular method for estimating quantitative genetic parameters such as heritability and genetic correlation and involves fitting an estimate of relatedness between individuals in the study population. Genotypes at genome-wide markers can be used to estimate relatedness; however, relatedness estimates vary with marker density, potentially affecting results. Increasing density of markers is also expected to increase the power to detect quantitative trait loci (QTL). We estimated heritability and performed genome-wide association studies (GWAS) on five body size traits in an unmanaged population of Soay sheep using two different SNP densities: a dataset of 37,037 genotyped SNPs, and an imputed dataset of 417,373 SNPs. Heritability estimates did not differ between the two SNP densities, but the high-density imputed SNP dataset revealed five new SNP-trait associations that were not found with the lower density dataset. Conditional GWAS analyses after fitting the most significant SNPs revealed two more novel SNP-trait associations.

Introduction

Investigating the genetic architecture behind heritable traits is key to understanding the biological diversity of wild populations. If we know the number of loci influencing a trait and their effect size, we can better understand the evolutionary processes that underpin traits, improve inferences about trait evolution (Barton and Keightley 2002), and understand micro-evolutionary dynamics that occur due to environmental change. Most quantitative genetic research in animals is carried out in artificial populations; either domestic, agricultural or laboratory. Such populations experience controlled environmental conditions which make it easier to account for environmental factors when studying the effect of genetic variants on phenotypic variation. However, given that environmental factors can influence the phenotype of a quantitative trait (Charmantier et al. 2014), and that the presence of genotype-by-environment interactions can cause additive genetic variance to differ between environmental conditions, studies on artificial populations arguably cannot be fully extrapolated to wild populations (Kruuk et al. 2008). Therefore, it is important to also study quantitative traits in wild populations in their natural habitats. There is a wealth of quantitative genetics research in human populations (for examples, see Manolio et al. 2009; Kang et al. 2010; Yang et al. 2010; Zaitlen et al. 2013; Locke et al. 2015; Xia et al. 2016; Xia et al. 2021), but humans also arguably experience a more buffered environment than wild populations.

A popular method to decompose phenotypic variation in wild populations into genetic variance and environmental variance is the 'animal model', originally developed by animal breeders (Henderson 1984; Kruuk 2004; Wilson et al. 2010). As part of the model, genetic relatedness is fitted, which is often derived from a pedigree. Pedigrees can be constructed using field observations, assigning parentage using genetic markers, or a mixture of both (Pemberton 2008). However, wild pedigrees are often short, incomplete, and contain errors: observational data may be inaccurate due to incorrect parent-offspring assumptions, and if the genetic markers chosen are not sufficiently discriminatory they may result in misassigned parentage. Erroneous pedigree links can bias results of analyses using animal models; for example, misidentification of sires in cattle resulted in decreased heritability estimates for milk yield, fat yield, and milk-fat ratio (Van Vleck 1970).

In place of a pedigree, genotypes at multiple polymorphic loci can be used to estimate relatedness for use in an animal model. This has the advantage of not relying on recovering a pedigree and thus is not affected by incomplete or incorrect familial links (though knowledge of pedigree relationships is still valuable, for example so that maternal effects can be fitted). Relatedness estimated using genotype data is also potentially more precise than that from pedigrees – for example, with a pedigree it is presumed that full-sibs have a relatedness of 0.5, however the exact relationship varies depending on which DNA segments each sib has inherited (Visscher et al. 2006). Despite this greater accuracy, genotype-based relatedness estimates can still vary depending on which variants in the population have been genotyped, and the density of the genotyped variants. Increasing the density of genotyped polymorphisms means they are more likely to be in linkage disequilibrium (LD) with causal variants for the trait of interest, either by being physically closer to the causal variants or by matching the allele frequency of the causal variants more accurately. Thus, in species such as humans where genotyping is commonly of unrelated individuals and LD is generally low, the estimated heritability of a trait increases with SNP density due to an increase in the number of causal variants being in LD with genotyped SNPs (for instance, heritability of human height was estimated to be 0.45 when using 294,831 SNPs (Yang et

al. 2010) and 0.56 when using ~17 million imputed SNPs (Yang et al. 2015)). However, increasing the number of genotyped markers means larger, denser genotyping arrays with prices increasing with density. For commonly studied species, high-density arrays are more affordable due to high demand, but for more niche species, including wild populations, large genotyping arrays are often unaffordable. Genotyping-by-sequencing, e.g. ddRAD (Peterson et al. 2012) is a potentially useful alternative for upscaling SNP density, though the combination of bioinformatics and samples sizes required in quantitative genetic research means that this approach is not yet in widespread use.

As an alternative to expensive high-density genotyping, genotype imputation can be used to increase the number of variants analysed (Burdick et al. 2006). Imputation involves predicting genotypes at untyped SNPs in a 'target' population using a subset of the study population – or more generally a "reference" population – genotyped at a higher density, either through a high density SNP array or by genotyping-by sequencing. The genotypes at these untyped SNPs for individuals in the target population are inferred using their genotypes at typed markers and taking advantage of existing linkage disequilibrium (LD) between SNPs. Pedigree information can also be used to increase the accuracy of the imputation by identifying haplotype blocks that are identical by descent (Burdick et al. 2006).

The Soay sheep *(Ovis aries)* of St Kilda are a primitive, unmanaged breed of sheep that have been the focus of a longitudinal, individual-based study since 1985 (Clutton-Brock and Pemberton 2003). As part of the study, life history and environmental data is collected, DNA samples are collected, and a pedigree has been constructed using observation and genetic parentage inference. 7630 sheep have been genotyped on the Ovine SNP50 Illumina Beadchip, on which 37,037 SNPs are autosomal and polymorphic in this population.

To date, quantitative genetic analyses of the Soays have been performed using either the pedigree or the 50K SNP data. Bérénos et al. (2014) investigated the difference in quantitative genetic parameter estimates when using the pedigree or a genomic relationship matrix (GRM) constructed from the 50K SNP data. The authors estimated heritability, maternal genetic effects and genetic correlations for body size traits (weight, foreleg length, hindleg length, metacarpal length and jaw length) across four age groups. The additive genetic variance and the heritability estimates using the GRM were lower than when using the pedigree to estimate relatedness, with the SNPs explaining 84% of the additive genetic variance of the pedigree on average, though for the majority of the traits the standard errors of the pedigree-based and SNP-based heritability estimates were within one standard error of each other. Genetic correlations were found to differ little between analyses using the different relatedness estimates. A SNP rarefaction analysis of the heritability estimates was conducted on the adult traits, and it was found that heritability estimates asymptoted when 50% of the SNPs were used to estimate relatedness.

Linkage disequilibrium is high in Soay sheep (Bérénos et al. 2014), which may explain the results of the rarefaction analysis: the same causal variations can be represented by multiple genotyped SNPs, and so many genotyped SNPs are not providing unique information towards the additive genetic variance. However, given that heritability estimates when using SNP-based estimates of relatedness were lower than when using pedigree-based estimates, it is also possible that there are variants that contribute to variation in these traits that aren't in perfect LD with the genotyped SNPs. This may be depressing estimates of additive genetic variance in comparison to pedigree-based estimates. Increasing the

density of genotyped SNPs may therefore increase the SNP-based heritability estimates of these traits to closer match the pedigree-based estimates.

Bérénos et al. (2015) focused on the same traits as Bérénos et al. (2014) in adults only, and partitioned the genetic variance for each trait, first by chromosome and then by 150 SNP windows, and performed GWAS. For the three leg length phenotypes, a disproportionately high amount of variance was explained by two SNPs on chromosome 16 (s23172.1) and chromosome 19 (s74894.1). For the remaining traits, the proportion of additive genetic variance explained by a chromosome was proportional to its length, suggesting that variation in these traits is influenced by many genetic variants of small effect, and no SNP-trait associations were discovered. The authors performed a two-step GWAS analysis; first they modelled the traits by fitting fixed and random effects, and then they extracted the residuals from the models and tested for association between SNPs and the residuals. Three traits (weight, foreleg length and hindleg length) are measured in the same individual across multiple years – for these traits, the authors analysed the mean residual values.

Since the studies from 2014 and 2015, 1895 more individuals have been both phenotyped and genotyped on the Ovine SNP50 Illumina Beadchip. In addition, 188 individuals were genotyped on the Ovine Infinium High Density chip with 600K attempted SNPs, which has enabled the genotypes of the remaining individuals to be accurately imputed to that higher density using LD and pedigree data (Stoffel et al. 2021).

In this study we performed a direct comparison of heritability estimates and GWAS associations between the lower density SNP data and the imputed high density SNP data in the Soay population using an increased sample size and compared the results to the previous studies (Bérénos et al. 2014; Bérénos et al. 2015). We focused on the same five traits as the previous studies in neonates, lambs and adults. Unlike the 2015 study (Bérénos et al. 2015), we performed GWAS by fitting fixed and random effects in the same step as testing for SNP-trait associations. This has the advantage of correctly propagating error throughout the analysis, reducing the chance of false positive results. We also carried out a two-step GWAS approach similar to that of Bérénos et al. 2015, focusing on the adult traits using the 50K SNP data, to investigate whether any SNP-trait associations identified using our approach were due to the increased population size or due to the different methodology (single-step vs. two-step GWAS).

Our aims were as follows:

1) To determine whether the increased density of SNPs changes the heritability estimates of the traits using the same individuals for both the low SNP density and the high SNP density analysis. Given the previous rarefaction analysis (Bérénos et al. 2014), our prediction was that there would be no change.

2) To determine whether the imputed SNP data enables the identification of new SNP-trait associations via GWAS. We predicted we would find more associations, either due to increased power to detect small effect size SNPs through increased LD with the imputed SNPs or due to tagging of new causal variants.

3) To examine how a single-step GWAS methodology compares with the two-step approach previously used on the Soay population, to investigate whether any novel SNP-trait associations

identified with the 50K SNP data were due to increased population size or due to the difference in methods.

METHODS

Phenotypic data

We focused on five body size traits in three age groups: neonates, lambs, and adults. Of the five traits, three (weight, foreleg length and hindleg length) are live measures, recorded in April for neonates and in August for lambs and adults. The remaining two traits (metacarpal length and jaw length) are *post mortem* measures taken from skeletal material. The sheep are ear-tagged when they are first captured which allows for reidentification for life. Both birth and August weight are measured to the nearest 0.1kg, whilst the remaining traits are all measured to the nearest mm. A detailed description of trait measurements can be found in Beraldi et al. (2007).

We defined neonates as individuals who were caught and weighed between two and ten days after birth – birth weight was the only trait recorded for this age group. Lambs were classed as individuals who had phenotypic data recorded in the August of their birth year for the live traits, and as individuals who died before 14 months of age for the *post mortem* measures. Individuals were classed as adults if they had August phenotypic data recorded at least two years after birth, or if they died after 26 months of age for *post mortem* measures. Unlike Bérénos et al. (2014), we chose not to analyse yearling data due to the small sample sizes in comparison to the other age classes, which is due to high first winter mortality.

Genetic data

Most of the sheep in our study population have been genotyped using the Ovine SNP50 Illumina BeadChip, which targets 54,241 SNPs across the sheep genome. After removing SNPs which failed quality control standards (minor allele frequency (MAF) > 0.001, call rate > 0.99, deviation from Hardy-Weinberg Equilibrium P > 1e-05), 39,368 polymorphic variants remained for 7630 individuals (3643 female, 3987 male). See Bérénos et al. (2014) for information on genetic sampling protocol and marker characteristics).

Of these 7630 individuals, 188 have also been genotyped using the Ovine Infinium HD SNP BeadChip which types 606,066 SNPs. This has allowed for the low density genotypes to be imputed to the higher density using AlphaImpute, which combines shared haplotype and pedigree information for phasing and genotype imputation (Hickey et al. 2012) (see Stoffel et al. (2021) for information on imputation). We used imputed genotype "hard" calls (rather than genotype probabilities) in downstream analyses. After filtering SNPs that failed quality control standards, 419,281 autosomal SNPs remained for 7621 individuals (3639 females, 3982 males).

Both the 50K SNP data and the imputed SNP data are mapped to the OAR_v3.1 genome assembly.

Narrow sense heritability estimation

We used animal models to partition the phenotypic variance for each trait in each age class into genetic and non-genetic variance components. Fixed and random effects were fitted for all models, with the effects differing between traits and age classes (Table 1). We implemented these analyses in DISSECT (Canela-Xandri et al. 2015) using the following model:

$$y = X\beta + \sum_r Z_r u_r + Wg + \varepsilon$$

where y is the vector of phenotypic values; X is a design matrix linking individual records with the vector of fixed effects β, Z_r is an incidence matrix that relates a random effect to the individual records; u_r is the associated vector of non-genetic random effects; g is the vector of additive genetic random effects with W the incidence matrix; and ε is the vector of residuals. It is assumed that g ~ $MVN(0, M\sigma_g^2)$, where σ_g^2 is the additive genetic variance and M is the genomic relationship matrix (GRM). For each trait in each age class, we ran this model twice: first with M being a GRM calculated from the 50K genotype data, and second with M being a GRM calculated from the imputed SNP genotypes. The GRMs (VanRaden 2008) were computed using DISSECT (Canela-Xandri et al. 2015), and the genetic relationship between individuals i and j is computed as:

$$A_{ij} = \frac{1}{N} \sum_{k=1}^{N} \frac{(s_{ik} - 2p_k)(s_{jk} - 2p_k)}{2p_k(1 - p_k)}$$

where s_{ik} is the number of copies of the reference allele for SNP k of the individual i, p_k is the frequency of the reference allele for the SNP k, and N is the number of SNPs.

The narrow sense heritability was estimated by dividing the additive genetic variance (the variance explained by the GRM) by the total estimated phenotypic variance (the sum of the variance explained by the GRM and other fitted random effects after fitting fixed effects).

In adults, there are multiple records for August weight, foreleg length and hindleg length for the same individual due to individuals being caught across multiple years. For these traits we used a repeatability model in order that uncertainty was correctly propagated through all estimations (Mrode 2014). To implement a repeatability model in DISSECT, we edited the input files so that each measurement had its own row in the genotype and covariate files. Individual ID was replaced with a unique capture reference number, and individual permanent environment was fitted as a random effect (see Supplementary Methods for a more detailed explanation).

Sample sizes and total number of phenotypic measurements for all traits are shown in Table 1, with effects fitted in all models.

Genome wide association analysis

We also conducted genome-wide association analyses using DISSECT (Canela-Xandri et al. 2015). We fitted the same fixed and random effects for each trait and age class as for the heritability estimation (Table 1). To account for population structure, when testing SNPs on a given chromosome for association with the phenotype, a GRM calculated from the remaining autosomes (referred to as Leave One Chromosome Out GRM (Yang et al. 2014)) was fitted. Input files for repeated-measure traits were reformatted as above. Our significance threshold was corrected for multiple testing using the SimpleM

method (Gao et al. 2008), which accounts for linkage disequilibrium between markers in order to calculate the effective number of independent tests.

We estimated the variance explained by SNPs that passed the significance threshold using the equation

$$V(SNP) = 2pq\alpha^2$$

where p and q are the major and minor allele frequencies of the SNP, and α is the estimated SNP effect. We then calculated the proportion of additive genetic variance explained by each SNP by dividing by the total additive genetic variance estimated for that trait.

For any trait for which several SNPs in the same region were associated with variation in the trait and thus had strong support for at least one QTL in the region, we carried out conditional analysis to understand if the region could harbour potentially several independent QTL, or if further QTL could be uncovered elsewhere in the genome. To that aim, the genotypes of the SNP with the smallest association p value from each associated region (hereafter called the "top SNP") were added to the GWAS model as a fixed covariate and removed from the GRMs and genotype data. The GWAS analysis was re-run accounting for those associations to try and reveal novel peaks either in the same regions or elsewhere in the genome.

Genes in QTL regions

For each trait x SNP association, we investigated the genes within a 0.5Mb window either side of the top SNP to identify any genes which could be contributing to trait variation. We extracted a list of genes for each trait using the biomaRt package in R (Durinck et al. 2005; Durinck et al. 2009) from the OAR_v3.1 genome assembly and reviewed each gene against the NCBI Gene (Bethesda (MD): National Library of Medicine (US) 2004 - 2022), Animal QTLdb (Hu et al. 2022), and Ensembl (Howe et al. 2020) databases to examine function and expression annotations. When possible, we also compared with human and mouse orthologues due to the high level of annotation data available for these two species.

Two-step GWAS analysis

To investigate whether any novel SNP associations identified (since Bérénos et al. 2015) by performing GWAS on the adult traits using the 50K SNP data were due to the increased population sample or due to the change in methodology, we also performed a two-step GWAS, focusing on adults only and using the 50K SNP data. We performed mixed model analyses using ASReml-R (Butler et al. 2017) for each trait fitting the same fixed and random effects as in our single-step analyses, including whole-genome relatedness (in the form of a GRM) and, for repeated-measure traits, permanent environment. We then extracted the residuals from the mixed models and performed GWAS with the residuals as the trait phenotypes using DISSECT (Canela-Xandri et al. 2015). For repeated measure traits, we used the mean residual value for each individual. We used the Bonferroni correction calculated in Bérénos et al. 2015 to determine the significance threshold for our two-step GWAS in order to best compare with previous GWAS performed on the Soay sheep.

RESULTS

Heritability estimation

Neonates

In neonates, the heritability of birth weight was 0.051 (S.E. 0.020) both when using the 50K SNPs to calculate relatedness, and when using the imputed SNPs (Figure 1, Supplementary Table 1). Given that both estimates are identical to 3 decimal places, there is no difference between the estimates.

Lambs

In lambs, the heritability estimates for the live August measures were lower than those for the *post mortem* measures (Figure 1, Supplementary Table 1). Across all the traits, heritability estimates were similar when using the 50K SNP data and the imputed SNP data, with the biggest difference being 0.024 for metacarpal length. For all traits, estimates were within one standard error of each other, indicating that the small differences in heritability estimates between the two SNP densities were not significant.

Adults

As observed in lambs, heritability estimates for live measures in adults were lower than those of the *post mortem* measures. Across all traits, heritability estimates were higher in adults than in lambs. Estimates obtained using the 50K SNPs and using the imputed SNPs were similar and were within one standard error of each other (Figure 1, Supplementary Table 1), meaning that the imputed SNPs provided no additional information to partition the variation into genetic and environmental variance.

Estimates for all variance components are listed in Supplementary Table 1.

GWAS

50K SNP data

To correct for multiple testing, we calculated the effective number of tests to be 20082 using the SimpleM method (Gao et al. 2008), giving a genome-wide significance threshold of $2.49e^{-06}$ for the 50K SNP data.

For weight in neonates (birth weight), and lambs (August weight), no SNPs were found to have an association p value smaller than this threshold, suggesting that any variants that influence weight variation are either of small effect or were not tagged by SNPs in the 50K SNP data (Figure 2A, Supplementary Figure 1B and 1G). For adult August weight, three SNPs had a p value lower than the genome-wide significance threshold; one SNP on chromosome 6 and two SNPs on chromosome 9.

For all three leg length measures in lambs, we found associations with the same region on chromosome 16. SNP s23172.1 was the SNP with the lowest p value for lamb foreleg and hindleg, explaining 0.52% and 0.69% of the genetic variance for each trait respectively (Supplementary Table 2, Supplementary Figure 1C and 1D). For lamb metacarpal, SNP 22142.1 in the same chromosome 16 region had the lowest p value and explained 0.97% of the genetic variance. There was also a single SNP on chromosome 3 (OAR3_100483326.1) and a cluster of SNPs on chromosome 19 that had p values smaller than the genome-wide significance threshold and were associated with variation in lamb metacarpal length, with

the SNP with the lowest p value from each region explaining 2.08% and 2.40% of the genetic variance respectively (Supplementary Table 2, Supplementary Figure 1E).

The two regions on chromosomes 16 and 19 that were associated with lamb metacarpal length variation were also significantly associated with all three leg length measures in adults, with SNP s22142.1 on chromosome 16 and SNP s74894.1 on chromosome 19 respectively explaining 0.80% and 2.04% of the genetic variation in adult foreleg, 0.88% and 1.32% of the genetic variation in adult hindleg, and 0.55% and 2.02% of the genetic variation in adult metacarpal length. There were other regions of the genome also associated with variation in the adult leg length traits; a region on chromosome 11 was significant across all three adult leg length traits, with the most significant SNP explaining 2.35%, 2.25% and 1.13% of the genetic variance in adult foreleg, hindleg and metacarpal respectively (Figure 2B, Supplementary Table 2, Supplementary Figure 1H and 1J). For adult foreleg, a SNP on chromosome 7 and two on chromosome 9 were also associated, with the most significant SNPs in each region explaining 1.31% and 2.99% of the genetic variance respectively for this trait (Supplementary Table 2, Supplementary Figure 1H).

In lambs, there were no associations with jaw length found (Supplementary Figure 1F). In adults, a SNP on chromosome 20 was associated with jaw length variation, explaining 2.05% of the genetic variance for this trait (Supplementary Table 2, Supplementary Figure 1K).

In total, we identified 85 SNP-trait associations with 39 unique SNPs.

Imputed data

Using the SimpleM method (Gao et al. 2008), we calculated the number of effective tests to be 48635, giving a genome-wide significance threshold of 1.03e-06.

When performing GWAS using the imputed SNP data, we were able to recover significant SNPs in the same locations for all traits as those we found using the 50K SNP data. Of the 85 SNP-trait associations that we identified with the 50K SNP data, 81 were significant using the imputed SNP data – the remaining four SNPs were no longer significant due to the increased multiple testing burden (which leads to a more stringent significance threshold) between the 50K SNP data and the imputed SNP data ($2.49e^{-06}$ and $1.03e^{-06}$ respectively).

We also identified 795 new SNP-trait associations using the imputed SNP data with 425 unique SNPs (Supplementary Table 2). The majority of new associations were in the same regions as the SNPs identified using the 50K SNP data, but we also found new associations: four SNPs on chromosome 1 and three SNP on chromosome 7 was associated with birth weight (Figure 2A, Supplementary Table 2), one SNP on chromosome 3 was associated with adult August weight (Figure 2B, Supplementary Table 2), and one SNP on chromosome 17 was associated with adult metacarpal length (Figure 2C, Supplementary Table 2).

Manhattan and QQ plots for all traits can be found in Supplementary Figure 1.

Conditional analysis

For any trait that had at least two SNPs on the same region associated with variation in that trait, we fitted the genotype of the SNP with the lowest p value in each region in the GWAS model and removed the SNP from the genotype file. For traits that had multiple SNP associations on more than one

chromosome, we fitted the genotypes of the SNP with the lowest p value from each associated chromosome simultaneously. We performed conditional analysis on all three leg length traits in both lambs and adults, as well as on birth weight, adult August weight adult jaw length (See Supplementary Table 2 for all SNPs that were fitted for each trait). For all of these traits we performed the conditional analysis using both the 50K SNP data and the imputed SNP data, with the exception of birth weight, which did not have any significant SNP associations using the 50K data.

Six of the nine traits we performed conditional analysis on had significant SNPs after fitting the SNPs with the lowest p value, however for four of these traits (lamb metacarpal length, adult August weight, foreleg length and hindleg length), these were SNPs that were also significant in our original GWAS analysis but were not fitted in the conditional analysis due to being the only SNP that was significantly associated with the trait in that region (Supplementary Table 3). The remaining two traits (birth weight and adult jaw length) both had a new association, both of which were on chromosome 2. For birth weight, nine SNPs had p values lower than the genome-wide significance threshold, all around ~81Mb (Figure 3A, Supplementary Table 3). For adult jaw length, only one SNP had a lower p value than the genome-wide significance threshold, at position 137,162,126 (Figure 3B, Supplementary Table 3).

Genes in QTL regions

Given that all of the region-trait associations that were found to be significant with the 50K SNP data were also significant with the imputed SNP data, we chose to focus on top SNPs in the imputed dataset (See Supplementary Table 2 for the list of SNPs, and Supplementary Table 4 for the list of genes).

We found a total of 179 genes in the regions around the SNPs associated with our traits. 56 of these genes were unannotated in the current sheep genome build, and of those that were annotated, three did not have a listed mouse homologue and a further six had neither a mouse nor a human homologue. Of the genes that did have annotation and homologue data, we found nine that are associated with similar traits to our focal traits in humans and mice, suggesting that they may be contributing to the genetic variation of our traits (Table 2). However, without intimate knowledge of the genes surrounding the focal SNPs, it is likely that there are other genes that are also contributing. It is also worth noting that the causal variant may not be in any of the genes in proximity to the SNPs we identified as being associated with our traits, but instead in upstream regulatory sequences that effect expression of either these or other genes.

We also compared our GWAS results with QTL from Animal QTLdb (Hu et al. 2022). We found that the region on chromosome 6 that we found to be associated with adult August weight overlaps with a region previously found to be associated with carcass weight and final body weight in an (Awassi x Merino) x Merino backcross population (Cavanagh et al. 2010) and is ~0.5Mb upstream of a 2.5Mb region that has also previously been associated with body weight in a population of Australian Merino sheep (Al-Mamun et al. 2015). In addition, the region on chromosome 9 we found to be associated with adult August weight is 1Mb upstream of a region previously found to be associated with live weight in a population of Chinese Merino sheep, however this trait was studied in yearlings rather than adults (Zhao et al. 2021).

Chromosome 6 has previously been associated with adult body weight in a smaller population of Soays (Beraldi et al. 2007), however the markers flanking the associated region are not located close to the region we identified.

Two-step GWAS

To compare our results using the 50K SNP data to previous GWAS of adult traits in the Soays, we also performed a two-step GWAS. For our two-step analysis, we used the significance threshold previously calculated in Bérénos et al. (2015) ($1.35e^{-6}$).

Across all 5 traits, we recovered the SNP-trait associations identified by Bérénos et al. (2015). However, we were unable to recover any of the novel SNP-trait associations we had found when performing our single-step GWAS on the 50K SNP data, with the exception of the association between chromosome 16 and adult foreleg (though the authors noted that SNPs in this region approached significance in their analysis). Despite the genome-wide significance threshold used by Bérénos et al. (2015) being more stringent than the significance threshold we calculated using the SimpleM method for the 50K SNP data, no additional associations are recovered when using our less stringent threshold

Our QQ plots using the two-step method also matched the QQ plots of Bérénos et al. (2015). In both, the observed p values were higher than the expected p values, causing the majority of points in the plots to fall below the x=y line (Supplementary Figure 3).

DISCUSSION

Heritability

Our results corroborate previous findings that all five body size traits we studied in Soay sheep are influenced by genetic variation in the population (Bérénos et al. 2014), that *post mortem* measures (metacarpal length and jaw length) have higher heritability estimates than live measures (weight, foreleg length and hindleg length), that leg measures have higher heritability than weight (Wilson et al. 2006; Beraldi et al. 2007; Bérénos et al. 2014), and that heritability estimates increase with age (Wilson et al. 2006; Bérénos et al. 2014).

The heritability estimates for the 50K data were very similar to those estimated using a GRM based on the 50K data in a smaller sample of the same population of sheep by Bérénos et al. (2014), with estimates for the same trait falling within one standard error of each other. The biggest difference was in adult metacarpal length with a heritability difference of 0.05 (estimates were 0.644 (0.047) and 0.594 (0.047) for our and Bérénos et al.'s results respectively). Given that we used the same models as Bérénos et al., it is likely that the small differences between heritability estimates for each trait is due to our increased sample sizes.

Comparing the heritabilities estimated using the imputed SNP data against the estimates using the 50K SNP data, we found little difference between the two SNP densities in any traits in any age class. The additional genotypes at the imputed SNPs do not give any additional information on additive genetic variation for these traits. This result is not surprising given the previous rarefaction analysis showing that the heritability of these body size traits in adults asymptoted when about half the 50K SNP data was used (Bérénos et al. 2014). There is high LD between nearby SNPs in the Soay sheep genome, which suggests that most, if not all, of the causal variants tagged by the imputed SNP data may have already been tagged by the 50K SNPs. The high LD was reflected when calculating GWAS significance thresholds – whilst the number of SNPs between the 50K SNP data and the imputed SNP data increased by a factor of ten, the number of effective tests only doubled (39K SNPs, 20082 effective tests and 401K SNPs and 48635 effective tests respectively).

For some of the traits we have analysed there is still a difference in heritability estimated using SNP data versus heritability estimated using pedigree – for example, the highest SNP-based heritability estimate for lamb metacarpal length (the estimate using the imputed SNP data) gave an estimate 59% of Bérénos et al.'s pedigree-based estimate (Bérénos et al. 2014). Given that our SNP-based heritability estimates were similar when using the 50K SNP data as when using the imputed SNP data, and the results of Bérénos et al.'s rarefaction analysis (Bérénos et al. 2014), we believe it is unlikely that increasing the density of genotyped SNPs that are common in the population will increase heritability estimates of these traits. It is possible instead that the difference in heritability estimates obtained from pedigree and genomic data is due to rare familial variants that do not segregate widely in the population, as well as due to dominance and epistasis.

GWAS

Body size traits have been the focus of many kinds of analyses in Soay sheep (Beraldi et al. 2007; Ozgul et al. 2009; Bérénos et al. 2014; Bérénos et al. 2015; Pemberton et al. 2017; Regan et al. 2017; Ashraf et

al. 2021), and several SNPs have already been identified as being associated with variation in these traits. A 2015 study aiming to find SNP-trait associations for these body size traits in adults identified QTL for leg length measures on chromosomes 16 and 19 (s23172.1 and s74894.1 respectively) (Bérénos et al. 2015). A more recent study comparing genomic prediction methods in Soays using the 50K SNP data identified s48811.1 on chromosome 7 and s50107.1 on chromosome 9 as having a probability higher than 0.9 of having a non-zero effect on adult foreleg length in addition to the previously discovered regions on chromosomes 16 and 19 (Ashraf et al. 2021). We were able to identify all four of these associations in our GWAS, alongside associations that have not previously been identified in this population. Use of the imputed SNP data allowed us to discover two more associations with loci that were not genotyped in the 50K SNP data, suggesting that future identification of polymorphisms influencing trait variation in the Soay sheep may benefit from using the imputed data.

Performing a two-step analysis confirmed that the novel SNP-trait associations we were able to identify using the 50K SNP data were due to being able to fit the fixed and random effects for each trait whilst performing GWAS all in a single step, rather than the increased population sample. Given the increase in SNP-trait associations when using the single-step methodology, we suggest that a two-step GWAS is redundant with the availability of software like DISSECT which is able to fit fixed and random effects whilst performing GWAS. As we have shown, although DISSECT does not currently have the option to automatically run a repeated measures GWAS, it is possible to modify input files to allow for repeated measures.

The imputed SNP data revealed SNP-trait associations in four regions of the genome that were not discovered using the 50K SNP data; a region on chromosome 1 and a region on chromosome 7 and birth weight, a region on chromosome 3 and adult August weight, and a region on chromosome 17 and adult metacarpal length. (Supplementary Table 2). When examining the Manhattan plot for the 50K data for each trait (Figure 2A, 2B and 2C, Supplementary Table 2) it is clear that, with the exception of the region on chromosome 1 associated with birth weight, there was a small cluster of SNPs just under the significance threshold in the 50K analyses. The additional (imputed) SNPs may have matched the allele frequency of the underlying causal variants more accurately, resulting in a smaller association p value.

We performed conditional analysis on all three leg length traits in both lambs and adults, as well as on birth weight (only using the imputed SNP data), adult August weight and adult jaw length. For each trait, we simultaneously fitted the genotype for the SNP with the lowest p value for any chromosome that had at least two SNPs found to be associated with the trait (see Supplementary Table 2 for a list of SNPs fitted for each trait). We found that all of the SNPs that were significant in the GWAS analysis were no longer significant in the conditional analysis when a significant SNP on the same chromosome was fitted (Figure 3, Supplementary Table 3). We suggest that any future work looking to pinpoint the exact location of the genetic variants affecting body size traits in Soay sheep primarily focus on the regions around the SNPs listed in Supplementary Table 2.

We identified 179 genes within 0.5Mb of the top SNPs for each trait (Supplementary Table 4), and of these genes, we found nine that are potential candidate genes for further analyses due to their association with similar traits in other species. However, we stress that it is possible that these nine genes may not be totally responsible for the associations we identified via GWAS – given that we do not have intimate knowledge of genes we identified, we believe that any analyses seeking to confirm gene-trait associations should not just focus on the nine genes listed in Table 2.

Across all traits for all age classes, the QQ plots showed deviation from the expected distribution of test statistics under the null hypothesis (x=y line) for a wide range of test statistics, including low values, indicative of underlying population structure not accounted for by the GRMs. The first 20 genomic principal components accounted for 10.68% of the variance in the genetic data, and repeating the GWAS analysis fitting these first 20 genomic principal components in addition to the GRM did not change the p values of the SNPs nor the QQ plots. This shows that the principal components in this case were not useful in adjusting for population structure in the presence of the GRM.

In order to have sufficient power to detect associations between markers and a trait of interest, GWAS primarily requires two factors: i) a very high density of genotyped SNPs, and ii) a large number of individuals that have been genotyped and phenotyped (Santure and Garant 2018). For intensively studied organisms, both are achievable; such populations tend to have more individuals accessible to collect data from, high density genotyping can be done at a lower cost due to higher demand, and, as in humans, data from different populations can be combined to create larger sample sizes. GWA studies of humans are the most obvious example of this; studies often have study populations made up of hundreds of thousands of individuals and human SNP chips commonly genotype hundreds of thousands of variants (for example, see Wood et al. 2014; Ishigaki et al. 2020; Wu et al. 2021). In comparison, wild study population samples are much smaller – often struggling to reach one thousand individuals – and the number of SNPs genotyped is much lower (for example, see Silva et al. 2017; Malenfant et al. 2018; Perrier et al. 2018). Analyses of wild populations therefore generally lack the power of more intensively studied study organisms. Here, we have increased power by increasing the number of genotyped markers via imputation. Despite high LD in the Soay sheep population, use of imputed data has allowed us to identify four new SNP-trait associations, including an association with birth weight, which had yet to be associated with any QTL in the Soay population. We have therefore shown that for a given sample size, more information can be obtained by increasing the density of markers for those individuals have been phenotyped. We suggest that, where possible, analyses of wild populations impute SNP data in order to increase power and obtain results that may otherwise remain undiscovered.

Although we have discovered new SNP-trait associations, it is likely that there are still causative variants that remain undetected. GWAS lacks power to detect rare causative variants and variants with very small effect sizes (Yang et al. 2010). Also, GWAS power drops when the same amount of phenotypic variation is a consequence of multiple variants in the same region as opposed to a single variant (Nagamine et al. 2012). Regional mapping methods have been developed that partition trait variance into regions by simultaneously fitting a whole genome and a regional GRM, with the regions either being defined as fixed SNP windows (Nagamine et al. 2012) or haplotype blocks (Shirali et al. 2018). Such methodologies have the potential to identify regions of the genome that contain variants associated with a trait that are unable to be identified by GWAS either due to being rare, or individually having small effects on trait variation. Genomic prediction, which simultaneously estimates all marker effects drawn from multiple distributions, can also be used to study the genetic architecture of traits by estimating the posterior inclusion probability of a SNP having a non-zero effect on a trait. Genomic prediction has already been used on adult body size traits in Soays, and has identified several of the SNPs we identified through our GWAS approach (Ashraf et al. 2021). Ultimately, we believe that it is important to use a variety of methodologies when studying the genetic architecture of complex traits, as different analyses have different strengths and may be able to identify different QTL.

523 References

524

525 Al-Mamun HA, Kwan P, Clark SA, Ferdosi MH, Tellam R, Gondro C. 2015. Genome-wide association study
526 of body weight in australian merino sheep reveals an orthologous region on oar6 to human and
527 bovine genomic regions affecting height and weight. Genetics, selection, evolution : GSE.
528 47(1):66.
529 Arts HH, Bongers EM, Mans DA, van Beersum SE, Oud MM, Bolat E, Spruijt L, Cornelissen EA, Schuurs-
530 Hoeijmakers JH, de Leeuw N et al. 2011. C14orf179 encoding ift43 is mutated in sensenbrenner
531 syndrome. Journal of medical genetics. 48(6):390-395.
532 Ashraf B, Hunter DC, Bérénos C, Ellis PA, Johnston SE, Pilkington JG, Pemberton JM, Slate J. 2021.
533 Genomic prediction in the wild: A case study in soay sheep. Molecular Ecology. 1– 15.
534 Barton NH, Keightley PD. 2002. Understanding quantitative genetic variation. Nature reviews Genetics.
535 3(1):11-21.
536 Beraldi D, McRae AF, Gratten J, Slate J, Visscher PM, Pemberton JM. 2007. Mapping quantitative trait
537 loci underlying fitness-related traits in a free-living sheep population. 61(6):1403-1416.
538 Bérénos C, Ellis PA, Pilkington JG, Lee SH, Gratten J, Pemberton JM. 2015. Heterogeneity of genetic
539 architecture of body size traits in a free-living population. 24(8):1810-1830.
540 Bérénos C, Ellis PA, Pilkington JG, Pemberton JM. 2014. Estimating quantitative genetic parameters in
541 wild populations: A comparison of pedigree and genomic approaches. 23(14):3434-3451.
542 Ncbi gene. 2004 - 2022. [accessed 11/02/2022]. https://www.ncbi.nlm.nih.gov/gene/.
543 Burdick JT, Chen W-M, Abecasis GR, Cheung VG. 2006. In silico method for inferring genotypes in
544 pedigrees. Nature genetics. 38(9):1002-1004.
545 Butler DG, Cullis BR, Gilmour AR, G. GB, R. T. 2017. Asreml-r reference manual version 4. Hemel
546 Hempstead, HP1 1ES, UK.: VSN International Ltd.
547 Campos-Xavier B, Rogers RC, Niel-Bütschi F, Ferreira C, Unger S, Spranger J, Superti-Furga A. 2018.
548 Confirmation of spondylo-epi-metaphyseal dysplasia with joint laxity, exoc6b type.
549 176(12):2934-2935.
550 Canela-Xandri O, Law A, Gray A, Woolliams JA, Tenesa A. 2015. A new tool called dissect for analysing
551 large genomic data sets using a big data approach. Nature Communications. 6(1):10162.
552 Cavanagh CR, Jonas E, Hobbs M, Thomson PC, Tammen I, Raadsma HW. 2010. Mapping quantitative trait
553 loci (qtl) in sheep. Iii. Qtl for carcass composition traits derived from ct scans and aligned with a
554 meta-assembly for sheep and cattle carcass qtl. Genetics, selection, evolution : GSE. 42(1):36.
555 Charmantier A, Garant D, Kruuk LEB. 2014. Quantitative genetics in the wild. Oxford University Press.
556 Clutton-Brock TH, Pemberton JM. 2003. Soay sheep: Dynamics and selection in an island population.
557 Clutton-Brock TH, Pemberton JM, editors. Cambridge: Cambridge University Press.
558 Cornish J, Naot D. 2010. Lactoferrin as an effector molecule in the skeleton. BioMetals. 23(3):425-430.
559 Duchatelet S, Ostergaard E, Cortes D, Lemainque A, Julier C. 2005. Recessive mutations in pthr1 cause
560 contrasting skeletal dysplasias in eiken and blomstrand syndromes. Human molecular genetics.
561 14(1):1-5.
562 Durinck S, Moreau Y, Kasprzyk A, Davis S, De Moor B, Brazma A, Huber W. 2005. Biomart and
563 bioconductor: A powerful link between biological databases and microarray data analysis.
564 Bioinformatics (Oxford, England). 21(16):3439-3440.
565 Durinck S, Spellman PT, Birney E, Huber W. 2009. Mapping identifiers for the integration of genomic
566 datasets with the r/bioconductor package biomart. Nature protocols. 4(8):1184-1191.
567 Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S,
568 Danielsson A, Edlund K et al. 2014. Analysis of the human tissue-specific expression by genome-

569 wide integration of transcriptomics and antibody-based proteomics. Molecular & cellular
570 proteomics : MCP. 13(2):397-406.
571 Fathzadeh M, Li J, Rao A, Cook N, Chennamsetty I, Seldin M, Zhou X, Sangwung P, Gloudemans MJ, Keller
572 M et al. 2020. Fam13a affects body fat distribution and adipocyte function. Nature
573 communications. 11(1):1465.
574 Gao X, Starmer J, Martin ER. 2008. A multiple testing correction method for genetic association studies
575 using correlated single nucleotide polymorphisms. 32(4):361-369.
576 . Applications of linear models in animal breeding. 1984.
577 Hickey JM, Kinghorn BP, Tier B, van der Werf JH, Cleveland MA. 2012. A phasing and imputation method
578 for pedigreed populations that results in a single-stage genomic evaluation. Genetics, selection,
579 evolution : GSE. 44(1):9.
580 Howe KL, Achuthan P, Allen J, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, Azov AG, Bennett R,
581 Bhai J et al. 2020. Ensembl 2021. Nucleic Acids Research. 49(D1):D884-D891.
582 Hu Z-L, Park CA, Reecy JM. 2022. Bringing the animal qtldb and corrdb into the future: Meeting new
583 challenges and providing updated services. Nucleic Acids Research. 50(D1):D956-D961.
584 Ishigaki K, Akiyama M, Kanai M, Takahashi A, Kawakami E, Sugishita H, Sakaue S, Matoba N, Low S-K,
585 Okada Y et al. 2020. Large-scale genome-wide association study in a japanese population
586 identifies novel susceptibility loci across different diseases. Nature genetics. 52(7):669-679.
587 Kang HM, Sul JH, Service SK, Zaitlen NA, Kong S-y, Freimer NB, Sabatti C, Eskin E. 2010. Variance
588 component model to account for sample structure in genome-wide association studies. Nature
589 genetics. 42(4):348-354.
590 Kruuk LEB. 2004. Estimating genetic parameters in natural populations using the "animal model".
591 Philosophical transactions of the Royal Society of London Series B, Biological sciences.
592 359(1446):873-890.
593 Kruuk LEB, Slate J, Wilson AJ. 2008. New answers for old questions: The evolutionary quantitative
594 genetics of wild animal populations. 39(1):525-548.
595 Laue K, Pogoda H-M, Daniel PB, van Haeringen A, Alanay Y, von Ameln S, Rachwalski M, Morgan T, Gray
596 MJ, Breuning MH et al. 2011. Craniosynostosis and multiple skeletal anomalies in humans and
597 zebrafish result from a defect in the localized degradation of retinoic acid. Am J Hum Genet.
598 89(5):595-606.
599 Li Q, Zhao J, Hu W, Wang J, Yu T, Dai Y, Li N. 2018. Effects of recombinant human lactoferrin on
600 osteoblast growth and bone status in piglets. Animal Biotechnology. 29(2):90-99.
601 Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J
602 et al. 2015. Genetic studies of body mass index yield new insights for obesity biology. Nature.
603 518(7538):197-206.
604 Malenfant RM, Davis CS, Richardson ES, Lunn NJ, Coltman DW. 2018. Heritability of body size in the
605 polar bears of western hudson bay. Molecular Ecology Resources. 18(4):854-866.
606 Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon
607 LR, Chakravarti A et al. 2009. Finding the missing heritability of complex diseases. Nature.
608 461(7265):747-753.
609 Mendez IA, Ostlund SB, Maidment NT, Murphy NP. 2015. Involvement of endogenous enkephalins and
610 β-endorphin in feeding and diet-induced obesity. Neuropsychopharmacology : official
611 publication of the American College of Neuropsychopharmacology. 40(9):2103-2112.
612 Mrode RA. 2014. Linear models for the prediction of animal breeding values.
613 Nagamine Y, Pong-Wong R, Navarro P, Vitart V, Hayward C, Rudan I, Campbell H, Wilson J, Wild S, Hicks
614 AA et al. 2012. Localising loci underlying complex trait variation using regional genomic
615 relationship mapping. PloS one. 7(10):e46501.

616 Oralová V, Chlastáková I, Radlanski RJ, Matalová E. 2014. Distribution of bmp6 in the alveolar bone
617 during mouse mandibular molar eruption. Connective tissue research. 55(5-6):357-366.
618 Ozgul A, Tuljapurkar S, Benton TG, Pemberton JM, Clutton-Brock TH, Coulson T. 2009. The dynamics of
619 phenotypic change and the shrinking sheep of st. Kilda. Science (New York, NY). 325(5939):464-
620 467.
621 Pemberton JM. 2008. Wild pedigrees: The way forward. Proceedings Biological sciences. 275(1635):613-
622 621.
623 Pemberton JM, Ellis PE, Pilkington JG, Bérénos C. 2017. Inbreeding depression by environment
624 interactions in a free-living mammal population. Heredity (Edinb). 118(1):64-77.
625 Perrier C, Delahaie B, Charmantier A. 2018. Heritability estimates from genomewide relatedness
626 matrices in wild populations: Application to a passerine, using a small sample size. Molecular
627 Ecology Resources. 18(4):838-853.
628 Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double digest radseq: An inexpensive
629 method for de novo snp discovery and genotyping in model and non-model species. PloS one.
630 7(5):e37135.
631 Qiu T, Xian L, Crane J, Wen C, Hilton M, Lu W, Newman P, Cao X. 2015. Pth receptor signaling in
632 osteoblasts regulates endochondral vascularization in maintenance of postnatal growth plate. J
633 Bone Miner Res. 30(2):309-317.
634 Regan CE, Pilkington JG, Bérénos C, Pemberton JM, Smiseth PT, Wilson AJ. 2017. Accounting for female
635 space sharing in st. Kilda soay sheep (ovis aries) results in little change in heritability estimates.
636 Journal of Evolutionary Biology. 30(1):96-111.
637 Santure AW, Garant D. 2018. Wild gwas—association mapping in natural populations. Molecular Ecology
638 Resources. 18(4):729-738.
639 Schipani E, Provot S. 2003. Pthrp, pth, and the pth/pthrp receptor in endochondral bone development.
640 Birth defects research Part C, Embryo today : reviews. 69(4):352-362.
641 Shirali M, Knott SA, Pong-Wong R, Navarro P, Haley CS. 2018. Haplotype heritability mapping method
642 uncovers missing heritability of complex traits. Scientific Reports. 8(1):4982.
643 Silva CNS, McFarlane SE, Hagen IJ, Rönnegård L, Billing AM, Kvalnes T, Kemppainen P, Rønning B, Ringsby
644 TH, Sæther BE et al. 2017. Insights into the genetic architecture of morphological traits in two
645 passerine bird species. Heredity (Edinb). 119(3):197-205.
646 Stoffel MA, Johnston SE, Pilkington JG, Pemberton JM. 2021. Genetic architecture and lifetime dynamics
647 of inbreeding depression in a wild mammal. Nature communications. 12(1):2972.
648 Tang J, Zhou H, Sahay K, Xu W, Yang J, Zhang W, Chen W. 2019. Obesity-associated family with sequence
649 similarity 13, member a (fam13a) is dispensable for adipose development and insulin sensitivity.
650 International Journal of Obesity. 43(6):1269-1280.
651 Van Vleck LD. 1970. Misidentification in estimating the paternal sib correlation. Journal of Dairy Science.
652 53(10):1469-1474.
653 VanRaden PM. 2008. Efficient methods to compute genomic predictions. J Dairy Sci. 91(11):4414-4423.
654 Visscher PM, Medland SE, Ferreira MAR, Morley KI, Zhu G, Cornes BK, Montgomery GW, Martin NG.
655 2006. Assumption-free estimation of heritability from genome-wide identity-by-descent sharing
656 between full siblings. PLOS Genetics. 2(3):e41.
657 Wardhana DA, Ikeda K, Barinda AJ, Nugroho DB, Qurania KR, Yagi K, Miyata K, Oike Y, Hirata KI, Emoto N.
658 2018. Family with sequence similarity 13, member a modulates adipocyte insulin signaling and
659 preserves systemic metabolic homeostasis. Proceedings of the National Academy of Sciences of
660 the United States of America. 115(7):1529-1534.
661 Warrington NM, Beaumont RN, Horikoshi M, Day FR, Helgeland Ø, Laurin C, Bacelis J, Peng S, Hao K,
662 Feenstra B et al. 2019. Maternal and fetal genetic effects on birth weight and their relevance to
663 cardio-metabolic risk factors. Nature genetics. 51(5):804-814.

Wilson AJ, Pemberton JM, Pilkington JG, Clutton-Brock TH, Coltman DW, Kruuk LEB. 2006. Quantitative genetics of growth and cryptic evolution of body size in an island population. Evolutionary Ecology. 21(3):337.

Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LE, Nussey DH. 2010. An ecologist's guide to the animal model. The Journal of animal ecology. 79(1):13-26.

Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S, Chu AY, Estrada K, Luan Ja, Kutalik Z et al. 2014. Defining the role of common variation in the genomic and biological architecture of adult human height. Nature genetics. 46(11):1173-1186.

Wu Y, Murray GK, Byrne EM, Sidorenko J, Visscher PM, Wray NR. 2021. Gwas of peptic ulcer disease implicates helicobacter pylori infection, other gastrointestinal disorders and depression. Nature communications. 12(1):1146-1146.

Xia C, Amador C, Huffman J, Trochet H, Campbell A, Porteous D, Hastie ND, Hayward C, Vitart V, Navarro P et al. 2016. Pedigree- and snp-associated genetics and recent environment are the major contributors to anthropometric and cardiometabolic trait variation. PLoS Genet. 12(2):e1005804.

Xia C, Canela-Xandri O, Rawlik K, Tenesa A. 2021. Evidence of horizontal indirect genetic effects in humans. Nature human behaviour. 5(3):399-406.

Yang J, Bakshi A, Zhu Z, Hemani G, Vinkhuyzen AA, Lee SH, Robinson MR, Perry JR, Nolte IM, van Vliet-Ostaptchouk JV et al. 2015. Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. Nature genetics. 47(10):1114-1120.

Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, Madden PA, Heath AC, Martin NG, Montgomery GW et al. 2010. Common snps explain a large proportion of the heritability for human height. Nature genetics. 42(7):565-569.

Yang J, Zaitlen NA, Goddard ME, Visscher PM, Price AL. 2014. Advantages and pitfalls in the application of mixed-model association methods. Nature genetics. 46(2):100-106.

Yashiro K, Zhao X, Uehara M, Yamashita K, Nishijima M, Nishino J, Saijoh Y, Sakai Y, Hamada H. 2004. Regulation of retinoic acid distribution is required for proximodistal patterning and outgrowth of the developing mouse limb. Developmental cell. 6(3):411-422.

Zaitlen N, Kraft P, Patterson N, Pasaniuc B, Bhatia G, Pollack S, Price AL. 2013. Using extended genealogy to estimate components of heritability for 23 quantitative and dichotomous traits. PLOS Genetics. 9(5):e1003520.

Zhao B, Luo H, Huang X, Wei C, Di J, Tian Y, Fu X, Li B, Liu GE, Fang L et al. 2021. Integration of a single-step genome-wide association study with a multi-tissue transcriptome analysis provides novel insights into the genetic basis of wool and weight traits in sheep. Genetics, selection, evolution : GSE. 53(1):56.

Age	Trait	No. individuals	No. records	Fixed effects	Random effects
Neonate	Birth weight	2678	2678	Sex	Year of birth
				Litter size	Mother ID
				Population size year before birth	
				Age of mother (quadratic)	
				Ordinal date of birth	
				Age (days)	
Lamb	Weight	2228	2228	Sex	Year of birth
				Litter size	Mother ID
				Population size	Permanent environment
				Age (days)	
	Foreleg	2284	2284	Sex	Year of birth
				Litter size	Mother ID
				Population size	Permanent environment
				Age (days)	
	Hindleg	2349	2349	Sex	Year of birth
				Litter size	Mother ID
				Population size	Permanent environment
				Age (days)	
	Metacarpal	2059	2059	Sex	Year of birth
				Litter size	Mother ID
				Age at death (months)	
	Jaw	2113	2113	Sex	Year of birth
				Litter size	Mother ID
				Age at death (months)	
Adult	Weight	1152	3553	Sex	Year of capture
				Population size	Permanent environment
				Age (years)	
	Foreleg	1121	3331	Sex	Year of capture
				Population size	Permanent environment
				Age (years)	
	Hindleg	1135	3444	Sex	Year of capture
				Population size	Permanent environment
				Age (years)	
	Metacarpal	945	945	Sex	Year of birth
				Age at death (years)	
	Jaw	991	991	Sex	Year of birth
				Age at death (years)	

700 **Table 1** Number of individuals and records, fixed and random effects fitted in each trait x age class
701 model in addition to the GRM. The same individuals and records were used for both heritability
702 estimates and for GWAS.

703

Gene Name	Ensembl Gene ID	Chr	Associated trait	Effects in other species
Cytochrome P450 26B1	ENSOARG00000011582	3	Lamb metacarpal length	Associated with skeletal abnormalities in humans and zebrafish (Laue et al. 2011), knockouts produce reduced limbs in mice (Yashiro et al. 2004).
EXOC6B	ENSOARG00000011607	3	Lamb metacarpal length	Associated with spondyloepimetaphyseal dysplasia (resulting in short stature) in humans (Campos-Xavier et al. 2018).
FAM13A	ENSOARG00000018727	6	Adult August weight	Modulates body fat distribution and adipocyte function in humans and mice (Fathzadeh et al. 2020) as well as adipose insulin signalling in mice (Wardhana et al. 2018), also linked with obesity in mice (Tang et al. 2019)
ONECUT1	ENSOARG00000020928	7	Birth weight	Associated with birth weight in humans (Warrington et al. 2019).
IFT43	ENSOARG00000002065	7	Adult foreleg length	Associated with Sensenbrenner syndrome (resulting in growth retardation and dwarfism due to femoral and humeral limb shortening) in humans (Arts et al. 2011).
PENK	ENSOARG00000020184	9	Adult August weight	PENK knock-out mice found to have diminished food motivation, lower baseline body weight and attenuated weight gain (Mendez et al. 2015)
PTH1R	ENSOARG00000006638	19	Lamb metacarpal length, adult foreleg length, adult hindleg length, adult metacarpal length	Involved in osteoblast development in mice (Qiu et al. 2015), associated with skeletal disorders such as JMC (Schipani and Provot 2003), EKNS (Duchatelet et al. 2005) and BLC (Schipani and Provot 2003) in humans.
LTF	ENSOARG00000008620	19	Lamb metacarpal length, adult	Human LTF associated with increased bone growth when

			metacarpal length	injected into piglets (Li et al. 2018), found to stimulate osteoblast proliferation (Cornish and Naot 2010). High expression levels in human bone marrow (Fagerberg et al. 2014).
BMP6	ENSOARG00000017264	20	Adult jaw length	Involved in bone development and expressed in the jaw bone in mice (Oralová et al. 2014).

704 **Table 2** Potential candidate genes for future analyses. From left to right: gene name, Ensembl gene ID,
705 chromosome, associated trait, and evidence for association in other species.

706

Figure 1 Estimates of VA/VP for body size traits in neonates, lambs, and adult Soay sheep when using a GRM calculated from the 50K SNP data (blue) compared with using a GRM calculated from the imputed SNP data (yellow). Error bars represent standard error estimates.

Figure 2 Manhattan plots for A) birth weight GWAS using 50K SNP data (left) and imputed SNP data (right); B) adult August weight GWAS using 50K SNP data (left) and imputed SNP data (right); and C) adult metacarpal length GWAS using 50K SNP data (left) and imputed SNP data (right). The red line represents the significance threshold (2.49e-06 for the 50K SNP data and 1.03e-06 for the imputed SNP data) – any SNPs above this threshold are considered to be significantly associated with variation in their respective traits.

Figure 3 Miami plots for A) birth weight using imputed SNP data (top) and birth weight conditional analysis using imputed SNP data (bottom); and B) adult jaw length GWAS using 50K SNP data (top left) and imputed SNP data (top right), adult jaw length conditional analysis using 50K SNP data (bottom left) and imputed SNP data (bottom right). The red line represents the significance threshold (2.49e-06 for the 50K SNP data and 1.03e-06 for the imputed SNP data) – any SNPs above this threshold are considered to be significantly associated with variation in their respective traits.

Data Availability Statement

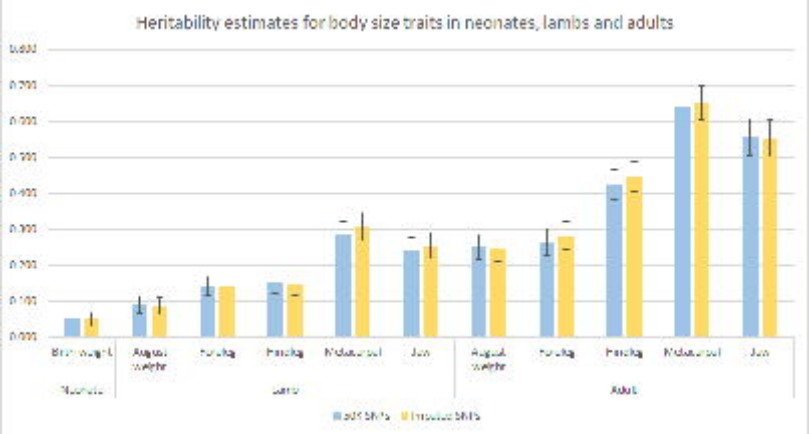
All scripts and data can be found at
https://github.com/CaelinnJames/Impact_of_SNPDensity_on_Soay_Sheep

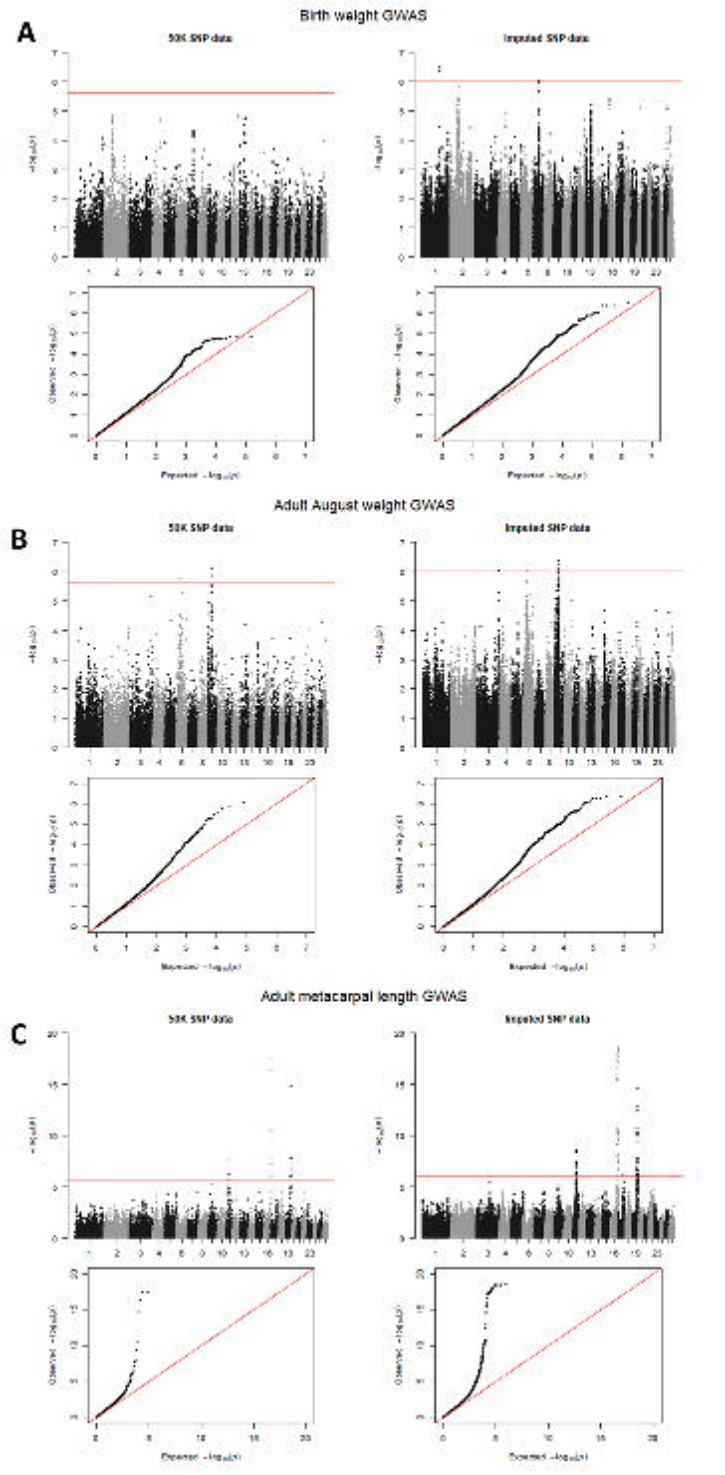
Acknowledgments

We thank the National Trust for Scotland and Scottish Natural Heritage for permission to work on St Kilda and QinetiQ and Eurest for logistics and other support on the island. We also thank all those who have been involved in the long-term project, including those who helped with field work on the island. We thank the Wellcome Trust Clinical Research Facility Genetics Core in Edinburgh for SNP genotyping.

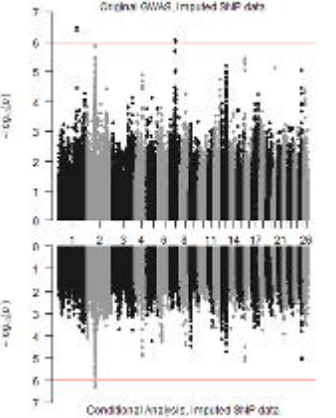
Funding

This work was supported by a NERC Doctoral Training Partnership grant (NE/S007407/1). The long-term field project on St Kilda has been largely funded by the UK Natural Environment Research Council. The SNP genotyping was funded by a European Research Council Advanced Grant.

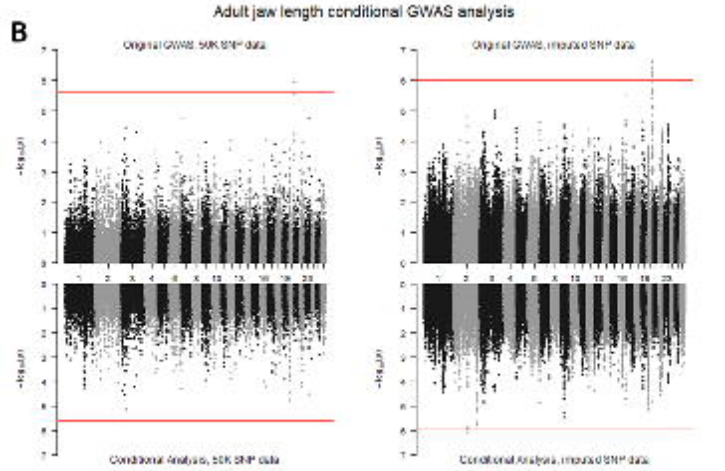

Heritability estimates for body size traits in neonates, lambs and adults
Birth weight
August weight
February
March
Midautumn
June
August weight
February
March
Midautumn
June
Neonate
Lamb
Adult


A
Birth weight GWAS
50K SNP data
Imputed SNP data
B
Adult August weight GWAS
50K SNP data
Imputed SNP data
C
Adult metacarpal length GWAS
50K SNP data
Imputed SNP data

A


Birth weight conditional GWAS analysis
Original GWAS, imputed SNP data
-log10(p)
Conditional Analysis, imputed SNP data

B


Adult jaw length conditional GWAS analysis
Original GWAS, 50K SNP data
Original GWAS, imputed SNP data
-log10(p)
Conditional Analysis, 50K SNP data
Conditional Analysis, imputed SNP data