

1 Evaluating regional heritability mapping methods for identifying QTLs
2 in a wild population of Soay sheep

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18 **Abstract**

19 Regional heritability mapping (RHM) is a method that estimates the heritability of genomic segments
20 that may contain both common and rare variants affecting a complex trait. We compared three RHM
21 methods: SNP-RHM, which uses genomic relationship matrices (GRMs) based on SNP genotypes;
22 Hap-RHM, which uses GRMs based on haplotypes; and SNHap-RHM, which uses both SNP-based and
23 haplotype-based GRMs jointly. We applied these methods to data from a wild population of sheep,
24 analysed eleven polygenic morphometric traits and compared the results with previous genome
25 wide association analyses (GWAS). We found that whilst the inclusion of the regional matrix did not
26 explain significant variation for all regions that were associated with trait variation using GWAS, it
27 did for several regions that were not previously associated with trait variation.

28 **Introduction**

29 Genome-wide association studies (GWAS) are commonly used to identify genotyped SNPs in linkage
30 disequilibrium (LD) with causal loci. The regions around the SNPs associated with the focal trait can
31 then be examined as these SNPs serve as markers for the causal loci. For example, the function of
32 nearby genes can be investigated to see if they are involved in biological pathways related to the
33 trait, or fine-mapping can be performed to narrow down the relevant region and pinpoint the causal
34 variant. However, GWAS has some limitations and challenges that prevent it from finding all the
35 genetic factors that contribute to complex traits. One of these limitations is the power of GWAS,
36 which is the ability to detect true associations. The power of GWAS depends on several factors, such
37 as the sample size, the variant effect size, whether genotyped SNPs are in LD with causal SNPs, and
38 the allele frequency of the causal variant.

39 To overcome the limitations of GWAS, especially when a trait is influenced by multiple independent
40 effects and/or rare variants in a region, regional heritability mapping (RHM) methods have been
41 developed (Nagamine et al. 2012; Shirali et al. 2018; Oppong et al. 2021). RHM is a technique that
42 estimates the heritability of a trait that is explained by a specific region of the genome. To estimate
43 the heritability of a region, RHM uses a genomic relationship matrix (GRM), which is a matrix that
44 captures the genetic similarity between individuals based on their SNP genotypes in that region.
45 RHM also corrects for the genetic similarity across the whole genome by fitting another GRM that
46 includes all the SNPs in the genome (or a leave-one-chromosome-out (LOCO) GRM that excludes the
47 chromosome where the region of interest is located). By comparing the model fit with and without
48 the regional GRM (rGRM), RHM can identify regions that contain causal variants for the trait, and by
49 using the variance estimate for the rGRM, RHM can estimate how much heritability that region
50 contributes.

51 RHM can be performed using different types of rGRMs and region sizes, depending on the
52 assumptions and goals of the analysis. There are three main types of RHM that have been proposed.
53 The first type is SNP-RHM, which uses rGRMs that are based on the sharing of SNP alleles across a
54 region. The regions are usually defined as windows that contain a fixed number of SNPs (Nagamine
55 et al. 2012). SNP-RHM aims to identify regions with multiple SNPs that are in LD with the multiple
56 causal variants that have too small an effect on the trait individually to be detected by GWAS.
57 However, SNP-RHM only captures effects associated with genotyped SNPs. The second type is Hap-
58 RHM, which uses rGRMs that are based on the sharing of haplotype alleles across a region. The
59 regions are defined as haplotype blocks (Shirali et al. 2018). Hap-RHM aims to identify regions where
60 the causal variant is in LD with the haplotype allele, but not necessarily with any specific genotyped
61 SNPs, which allows for detection of variance that is not captured by genotyped SNPs. This method
62 can capture the effect of rare causal variants due to rare haplotype alleles being more likely to be in
63 LD with rare variants than individual, genotyped SNPs. In addition, haplotype effects may reflect the
64 interaction effects of closely linked causal variants. The third method, SNHap-RHM, simultaneously
65 fits two rGRMs: one SNP-based and one haplotype-based, and defines regions as haplotype blocks
66 (Oppong et al. 2021). This combines the advantages of both SNP-RHM and Hap-RHM to increase
67 power to detect regions containing variants influencing the phenotype. On occasions where SNP-
68 RHM and Hap-RHM can detect genetic variance in the same haplotype block, SNHap-RHM can also
69 be used to give more insight into the underlying genetic architecture.

70 Here, we evaluate the three RHM methods for their ability to identify regions containing potentially
71 causal loci in a sample of wild Soay sheep. In this study, we analysed 11 polygenic morphometric
72 traits in the Soay sheep population using RHM. These traits include the same traits measured at
73 different ages, as they are affected by different non-genetic factors (and potentially different genetic

74 factors) and vary in heritability across different stages of life. Despite using various methods to
75 search for the genetic variants that affect these traits, such as GWAS (Bérénois et al. 2015; James et
76 al. 2022), genomic prediction (Ashraf et al. 2021) and chromosome partitioning (Bérénois et al.
77 2015), most of the genetic variation in these traits remains unexplained by the genotyped and
78 imputed SNPs. Moreover, for some of these traits, there are no SNPs that show significant
79 association with the trait variation to date.

80 Our aims were as follows:

- 81 1) To determine the suitability of RHM methods for the Soay sheep data given the smaller
82 sample sizes, lower density SNP data and more potential for missing data in comparison to
83 the human data for which these methods were developed.
- 84 2) To compare the results of RHM with those of GWAS to determine the extent to which RHM
85 recovers known associations and identifies new associations.
- 86 3) To investigate regions for which including regional matrices in the RHM framework improves
87 model fit, to better characterise the underlying genetic architecture of the focal traits and
88 identify potential causal genes based on known functional data.

89

90 **Methods**

91 Phenotypic data

92 The Soay sheep (*Ovis aries*) is a primitive breed of sheep that lives on the St. Kilda archipelago. Since
93 1985, a long-term, individual-based study has been conducted on the population residing on the
94 island of Hirta (Clutton-Brock and Pemberton 2003). Each individual is sampled for DNA analysis and
95 ear-tagged when it is first captured (usually within ten days of birth) so that it can be re-identified
96 later. The study involves regular recaptures to measure various traits throughout an individual's life,
97 and collection and measurement of skeletal remains after death.

98 We focused on 11 age-specific morphometric traits which have been repeatedly analysed by
99 different approaches and are known to be polygenic (Bérénos et al. 2015; Ashraf et al. 2021; Hunter
100 et al. 2022; James et al. 2022). We analysed these traits separately by age class (neonate, lamb and
101 adult). Birth weight was the only trait analysed in neonates, defined as individuals who were caught
102 and weighed between two and ten days after birth. In August, lambs (aged approximately 4 months)
103 and adults were caught and measured for weight, foreleg length and hindleg length. Due to adults
104 being recaptured across multiple years, the adult live traits included repeated measurements.
105 Metacarpal length and jaw length were measured from the skeletons after death. We classified
106 "lambs" as individuals who had live trait data recorded in the August of their birth year, or who died
107 before 14 months of age for post mortem measures. We classified "adults" as individuals who had
108 live trait data recorded at least two years after birth, or who died after 26 months of age for post
109 mortem measures. Birth and August weights are recorded to the nearest 0.1kg, whilst the length
110 traits are measured to the nearest mm (Beraldi et al. 2007). We did not analyse yearlings due to low
111 sample size.

112 See Table 1 for the number of individuals and records per trait.

113 Genetic data

114 8557 sheep have been genotyped on the Ovine SNP50 Illumina BeadChip, of which 38,130 SNPs are
115 autosomal and polymorphic in the population. 188 individuals have additionally been genotyped on
116 the Ovine Infinium HD SNP BeadChip which genotypes 600K SNPs – this allowed for imputation of
117 the remaining genotyped individuals to this higher density. AlphaImpute v1.98 (Hickey et al. 2012)
118 was used for the imputation as it combines shared haplotype and pedigree information to increase
119 imputation accuracy (see Stoffel et al. 2021 for details on our imputation). Genotypes with a
120 probability of < 0.99 were excluded, resulting in 419,281 autosomal SNPs remaining for 8557
121 individuals (4035 females, 4452 males). Imputed genotype "hard" calls were used instead of
122 genotype probabilities in the analyses detailed in this manuscript. Locus positions for both sets of
123 genetic data were based on the OAR_v3.1 genome assembly.

124 Regional heritability mapping

125 Prior to regional heritability mapping, the traits were pre-corrected to account for genome-wide
126 genetic diversity by fitting a LOCO GRM, constructed from all autosomes with the exception of the
127 chromosome containing the focal region. We also fitted fixed and non-genetic random effects during
128 pre-correction (see Table 1 for a full list of fixed and non-genetic random effects fitted). Pre-
129 correction for the non-repeated measures traits was performed in DISSECT (Canela-Xandri et al.
130 2015) using the following model:

$$y = X\beta + \sum_r Z_r u_r + Wg_{LOCO} + \varepsilon$$

131 where y is the vector of phenotypic values; X is a design matrix linking individual records with the
132 vector of fixed effects β , Z_r is an incidence matrix that relates a random effect to the individual
133 records; u_r is the associated vector of non-genetic random effects; g_{LOCO} is the vector of additive
134 genetic random effects from all autosomes except for that containing the focal region with W the
135 incidence matrix linking individual phenotypes with the genetic effect; and ε is the vector of
136 residuals. It is assumed that $g_{\text{LOCO}} \sim MVN(0, M\sigma_{g_{\text{LOCO}}}^2)$, where $\sigma_{g_{\text{LOCO}}}^2$ is the additive genetic variance
137 explained by all autosomes except the excluded one, and M is the LOCO GRM.

138 The GRMs (VanRaden 2008) were computed using DISSECT, and the genetic relationship between
139 individuals i and j is computed as:

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

140

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

143 The residual for each individual was then taken as the phenotype for RHM. Pre-correction for the
144 three repeated measures traits (adult August weight, adult foreleg length and adult hindleg length)
145 was performed using ASReml-R (version 4.1, Butler et al. 2017) using the same model as given
146 above, and the mean of the residuals summed with the permanent environment effect for each
147 individual was taken as the phenotype for RHM.

148 For all three regional heritability methods, we used the same regions to allow for direct comparisons
149 between the methods. Due to Hap-RHM and SNHap-RHM requiring regions to be defined as
150 haplotype blocks, we used haplotype blocks for all three methods. Haplotype blocks were estimated
151 with Plink v1.90 using the `--blocks`, `--blocks-max-kb 500` (which allows pairs of variants within 500kb
152 of each other to be considered within the same block) and `--blocks-min-maf 0.01` options (which
153 instructs Plink to include all SNPs with a MAF higher than 0.01 when estimating the haplotype blocks
154 (Purcell et al. 2007; Purcell 2014)). Using a higher max kb threshold or lower MAF threshold did not
155 alter the haplotype block boundaries estimated. No haplotype block was allowed to have only one
156 SNP, due to the SNP-based GRM and haplotype-based GRM being identical for such blocks. Any
157 block containing only one SNP was therefore omitted from the analysis. Blocks were determined
158 using all 8557 individuals with imputed genotypes to ensure consistency across phenotypes.

159 Phased data is required for Hap-RHM and SNHap-RHM; genotypes were phased using SHAPEIT v4.2
160 (Delaneau et al. 2019).

161 Regional heritability mapping was performed using the following models:

$$y_{\text{pre-corrected}} = Wr_{\text{SNP}} + \varepsilon$$

$$y_{\text{pre-corrected}} = Wr_{\text{Hap}} + \varepsilon$$

$$y_{\text{pre-corrected}} = Wr_{\text{SNP}} + Wr_{\text{Hap}} + \varepsilon$$

162 for SNP-RHM, Hap-RHM and SNHap-RHM respectively, where $y_{\text{pre-corrected}}$ is the vector of pre-
163 corrected phenotypic values, r_{SNP} is the vector of individual additive genetic random effects from all

164 SNPs contained within the focal haplotype block and r_{Hap} is the vector of individual additive genetic
165 random effects from the haplotype alleles for the focal haplotype block. It is assumed that $r_{\text{SNP}} \sim$
166 $MVN(0, M\sigma_{r_{\text{SNP}}}^2)$ and $r_{\text{Hap}} \sim MVN(0, M\sigma_{r_{\text{Hap}}}^2)$, where $\sigma_{r_{\text{SNP}}}^2$ is the additive genetic variance from all
167 SNPs in the haplotype block, $\sigma_{r_{\text{Hap}}}^2$ is the additive genetic variance from the haplotype alleles and M
168 is the respective GRM. The GRMs were computed using DISSECT (Canela-Xandri et al. 2015). The
169 SNP-based GRMs were calculated using the same method as the LOCO GRMs, except they were
170 constructed from the SNPs located in the focal haplotype block. For the haplotype-based GRMs, the
171 genetic relationship for individuals i and j is calculated as follows

$$H_{ij} = \frac{1}{h} \sum_{k=1}^h \frac{(d_{ik} - 2p_k)(d_{jk} - 2p_k)}{2p_k(1 - p_k)}$$

172 where d_{ik} is the diplotype code (coded as the number of copies of haplotype k) for individual i and
173 takes the values 0, 1, and 2, p_k is the frequency of haplotype k and h is the number of haplotypes in
174 the region (see Oppong et al. 2021 for further information and examples).

175 To test whether the regional heritability models explained significant variation for each region, we
176 compared them against the null model:

$$y_{\text{precorrected}} = \varepsilon$$

177 using loglikelihood ratio testing (LRT). We performed five comparisons; SNP-RHM, Hap-RHM and
178 SNHap-RHM were all compared with the null model, and SNHap-RHM was additionally compared to
179 each of SNP-RHM and Hap-RHM individually. LRTs were performed with 1 degree of freedom, with
180 the exception of the comparison of SNHap-RHM to the null model, which was performed with 2
181 degrees of freedom. P values were calculated as $0.5 \times$ the p-value of a chi-squared distribution with
182 one degree of freedom for the 1 degree of freedom tests. For the 2 degrees of freedom tests, the p
183 values were calculated as $0.25 \times$ the p-value of a chi-squared distribution with two degrees of
184 freedom plus $0.5 \times$ the p-value of a chi-squared distribution with one degree of freedom (Self and
185 Liang 1987). Model fit was considered to be significantly improved if the resulting p value was less
186 than $1.04e^{-06}$ (0.05 divided by $48,125$, the total number of haplotype blocks).

187 Comparison with GWAS

188 To determine how well the different RHM methods detected previously discovered loci, we
189 identified which haplotype blocks contained the top SNP from each peak significantly associated
190 with phenotypic variation for each trait when performing GWAS. GWAS and conditional GWAS
191 analysis has recently been performed using the high density genotype data (James et al. 2022), so
192 we used the results from that analysis. The significance threshold used in the GWAS analysis was
193 $1.03e^{-06}$ ($0.05/48635$), which accounted for multiple testing using the SimpleM method (Gao et al.
194 2008). This method accounts for linkage disequilibrium between markers in order to calculate the
195 effective number of independent tests.

196 Identification of candidate genes

197 We extracted a list of genes overlapping any haplotype block for which model fit was improved by at
198 least one RHM model, using the R biomaRt package (Durinck et al. 2005; Durinck et al. 2009) from
199 the OAR_v3.1 genome assembly. Each gene was then reviewed against the Ensembl (Howe et al.
200 2020) and NCBI Gene (Bethesda (MD): National Library of Medicine (US) 2004 - 2023) databases to

201 examine expression and functional annotations. Human and mouse orthologues were also used to
202 characterise gene function due to the high level of genetic annotation in these two species.

203 **Results**

204 Soay sheep haplotype blocks

205 Setting the maximum kb between any two variants within the same haplotype block to 500Kb and
206 the minimum minor allele frequency (MAF) for variants to be considered to 0.01 resulted in 48,125
207 haplotype blocks being estimated across the 26 Soay sheep autosomes. The maximum number of
208 SNPs in a given haplotype block was 111, the minimum was 2 (as blocks with one SNP were omitted),
209 and the average number of SNPs per haplotype was 8.19. 75% of haplotype blocks contained 10 or
210 less SNPs, and 99% of blocks contained 50 or less. Block statistics for each chromosome are shown in
211 Table 2.

212 Across the whole sample of genotyped individuals, haplotype allele frequency ranged from
213 0.00005843 to 0.9895992, with 0.00005843 being the most commonly observed haplotype
214 frequency (28.95% of haplotype alleles). A frequency of 0.00005843 equates to a haplotype allele
215 being present on one chromosome in the entire sample. 75% of haplotype alleles had a frequency
216 lower than 0.072 (present on less than 1232 chromosomes in the entire sample), and 90% had a
217 frequency lower than 0.316 (present on less than 5408 chromosomes in the entire sample) (Figure

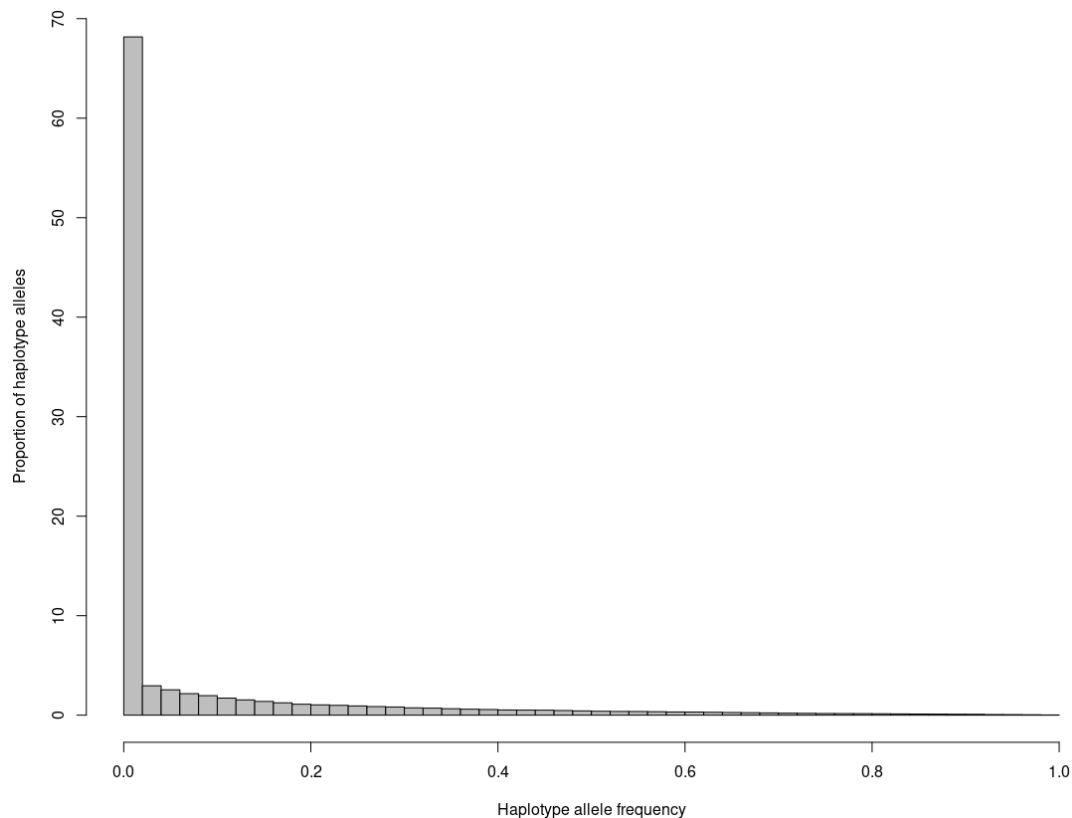


Figure 1: Proportion of haplotype alleles at each haplotype frequency over all regions.

218 1).

219

220 Comparison of RHM

221 A summary of results for the RHM analyses are shown in Table 3, whilst detailed results are shown in
222 Supplementary Tables 1 – 10.

223 There were two traits for which none of the RHM models significantly improved model fit for any
224 haplotype blocks: birth weight and lamb August weight, meaning that no regions of the genome
225 were found to significantly explain additional genetic variance not accounted for during pre-
226 correction.

227 For lamb foreleg length and lamb hindleg length, Hap-RHM was the only model which significantly
228 improved model fit in comparison to the null model. Hap-RHM improved model fit for one haplotype
229 block on chromosome 1 (haplotype number 1717) and one on chromosome 11 (726) for lamb
230 foreleg length, and one on chromosome 2 (4160) and chromosome 3 (3432) for lamb hindleg length
231 (Supplementary Tables 1 – 3).

232 For lamb metacarpal length, SNP-RHM significantly improved model fit for 16 haplotype blocks on
233 chromosome 16. Model fit was improved for 14 of these blocks using Hap-RHM, with the other two
234 being non-significant. 10 of the blocks for which model fit was improved by both SNP-RHM and Hap-
235 RHM were also improved by SNHap-RHM when compared to the null model, however no blocks
236 showed improved model fit when using SNHap-RHM when compared to either single-GRM model.
237 For the same trait, SNP-RHM also improved model fit for 23 haplotype blocks on chromosome 19,
238 whilst Hap-RHM improved model fit for 11 of the same blocks. When compared to the null model,
239 SNHap-RHM improved model fit for 20 of these haplotype blocks on chromosome 19, and four
240 haplotype blocks on chromosome 19 when compared to Hap-RHM. SNHap-RHM did not improve
241 model fit for any blocks when compared to SNP-RHM (Supplementary Tables 1 and 4).

242 For lamb jaw length, model fit was only significantly improved by Hap-RHM and SNHap-RHM when
243 compared to SNP-RHM. Hap-RHM improved model fit for one haplotype block on chromosome 3
244 (270), two blocks on chromosome 13 (1025 and 1041), one block on chromosome 14 (762) and one
245 on chromosome 17 (923). When compared to SNP-RHM, SNHap-RHM significantly improved model
246 fit for the same two blocks on chromosome 13 for which model fit was improved by Hap-RHM
247 (Supplementary Tables 1 and 5).

248 For adult August weight, Hap-RHM significantly improved model fit for 83 haplotype blocks over 22
249 chromosomes. In comparison to the null model, SNHap-RHM improved model fit for 35 haplotype
250 blocks over 21 chromosomes, whilst in comparison to SNP-RHM, model fit was improved for 56
251 haplotype blocks over 17 chromosomes. The blocks for which model fit was significantly improved
252 by SNHap-RHM in comparison to either the null model or SNP-RHM were all ones that were also
253 significantly improved by Hap-RHM, with the exception of one on chromosome 9 (1898), for which
254 model fit was only significantly improved by SNHap-RHM in comparison to the null model. SNHap-
255 RHM did not improve model fit for any haplotype blocks in comparison to Hap-RHM, nor did SNP-
256 RHM when compared to the null model (Supplementary Tables 1 and 6).

257 For adult foreleg length, Hap-RHM significantly improved model fit for six haplotype blocks; one on
258 chromosome 1 (1284), one on chromosome 6 (1064), one on chromosome 11 (113), one on
259 chromosome 12 (23), one on chromosome 23 (1020) and one on chromosome 26 (259). Model fit
260 was also improved for these same six haplotype blocks when comparing SNHap-RHM to the model
261 and against SNP-RHM. SNHap-RHM did not improve model fit for any haplotype blocks in
262 comparison to Hap-RHM, nor did SNP-RHM when compared to the null model (Supplementary
263 Tables 1 and 7).

264 For adult hindleg length, SNP-RHM significantly improved model fit for four haplotype blocks on
265 chromosome 16. Hap-RHM improved model fit for 25 haplotype blocks over 15 chromosomes,
266 including two of the blocks for which model fit was significantly improved by SNP-RHM. SNHap-RHM
267 improved model fit for 14 haplotype blocks over 11 chromosomes when compared to the null
268 model, and 17 blocks over 12 chromosomes when compared to SNP-RHM. SNHap-RHM did not
269 significantly improve model fit for any blocks when compared to Hap-RHM (Supplementary Tables 1
270 and 8).

271 For adult metacarpal length, SNP-RHM significantly improved model fit for 15 haplotype blocks on
272 chromosome 16. Hap-RHM significantly improved model fit for 12 of these blocks. When compared
273 to the null model, SNHap-RHM improved model fit for 12 haplotype blocks on chromosome 16 – all
274 of which were blocks that experienced significant improvement in model fit by SNP-RHM. In
275 addition, SNP-RHM and SNHap-RHM improved model fit for the same four blocks on chromosome
276 19 when compared to the null model. When compared to the single GRM RHM models, SNHap-RHM
277 did not improve model fit for any haplotype blocks (Supplementary Tables 1 and 9).

278 For adult jaw length, Hap-RHM improved model fit for six haplotype blocks; one on chromosome 1
279 (3843), one on chromosome 3 (2046), one on chromosome 11 (1027), one on chromosome 18 (665)
280 and two on chromosome 23 (342 and 434). Model fit was improved for the same haplotype blocks
281 on chromosome 1 and 3 when comparing SNHap-RHM against both the null model and against SNP-
282 RHM, with the haplotype block on chromosome 23 (434) also showing improved model fit when
283 compared against SNP-RHM. SNHap-RHM did not improve model fit for any haplotype blocks in
284 comparison to Hap-RHM, nor did SNP-RHM (Supplementary Tables 1 and 10).

285 Comparison with GWAS results

286 Of the 11 traits, lamb August weight and lamb jaw length were the only two to have no previously
287 associated genetic loci (Béréños et al. 2014; James et al. 2022). Of the traits for which GWAS has
288 previously identified SNP-trait associations, RHM only significantly improved model fit for blocks
289 containing top SNPs associated with lamb metacarpal length, adult hindleg length, and adult
290 metacarpal length on chromosomes 16 and 19.

291 The underlying causal variant on chromosome 16 influencing lamb metacarpal length is presumed to
292 be the same variant influencing adult hindleg length and adult metacarpal length – the top GWAS
293 SNP on chromosome 16 for adult hindleg length and lamb metacarpal length is the same (James et
294 al. 2022), adult hindleg and metacarpal length have been shown to have a genetic correlation of
295 0.827 (S.E. 0.232) (Béréños et al. 2014), and SNP-leg trait associations in this region have been
296 shown to be dependent on each other; when a SNP genotype from this region is fitted during
297 conditional analyses, no new SNP associations appear in this region. We can therefore combine the
298 RHM results for these three traits to characterise the architecture of genetic variance in this region.
299 Whilst SNP-RHM significantly improved model fit for blocks on chromosome 16 that Hap-RHM did
300 not, there were no blocks on chromosome 16 for which Hap-RHM improved model fit but SNP-RHM
301 did not (Supplementary Tables 1, 4, 8 and 9). In fact, in the case of adult hindleg length, Hap-RHM
302 did not improve model fit for any blocks on chromosome 16 (Supplementary Table 8). This suggests
303 that the additive genetic variance being attributed to the regional GRMs is due to individual SNP
304 genotypes, rather than due to a specific haplotype allele. Block 1363 (which contains s22142.1, the
305 top GWAS SNP for lamb metacarpal length and adult hindleg length) contains 17 SNPs and has 18
306 haplotype alleles in the population. The minor allele for s22142.1 appears in 3 haplotype alleles, with
307 two of these haplotype alleles being relatively rare (each appearing on 17 chromosomes in the
308 genotype population).

309 Again, the underlying causal variant on chromosome 19 influencing lamb metacarpal length is
310 presumed to be the same variant influencing adult metacarpal length – whilst the top GWAS SNPs
311 are different for these two traits, they still fall in the same haplotype block (Supplementary Table
312 11), and when the genotype of the top SNP is fitted during conditional analysis, no new SNP-trait
313 associations appear (James et al. 2022). Again, we can combine the RHM results for both lamb
314 metacarpal length and adult metacarpal length to characterise the underlying architecture. For both
315 traits, model fit for the block containing the top GWAS SNPs was only significantly improved by SNP-
316 RHM and SNHap-RHM when compared to the null model (and SNHap-RHM compared to Hap-RHM
317 in the case of lamb metacarpal length). This suggests that this association is being driven by the SNP
318 alleles in this region, rather than the haplotype alleles. Block 952, which contains both top GWAS
319 SNPs, has 37 SNPs and 52 haplotype alleles in the genotyped population. The minor alleles for each
320 of the top SNPs each appear in two haplotype alleles, with one haplotype allele containing both
321 minor SNP alleles. The haplotype alleles each containing one of the minor SNP alleles for the top
322 GWAS SNPs were both rare in the population (appearing on one and 50 chromosomes in the
323 population).

324 Novel associations

325 Novel block-trait associations were identified using at least one RHM method for all but four traits –
326 birth weight, lamb August weight, lamb metacarpal length and adult metacarpal length. In the case
327 of the former two traits, RHM did not improve model fit for any haplotype blocks in comparison to
328 the null model, whilst in the case of the latter two, RHM only significantly improved model fit in the
329 same regions as previously identified QTL for these traits.

330 Across the lamb traits, at least one RHM method significantly improved model fit for a total of nine
331 haplotype blocks; two blocks for lamb foreleg length (on chromosomes 1 and 11), two blocks for
332 lamb foreleg length (on chromosomes 2 and 3), and five blocks for lamb jaw length (one on
333 chromosome 3, two on chromosome 13, one on chromosome 14 and one on chromosome 17). For
334 all of these trait-block associations, Hap-RHM was the only model to significantly improve model fit –
335 with the exception of the two blocks on chromosome 13 for lamb jaw length, for which SNHap-RHM
336 also improved model fit when compared to SNP-RHM.

337 At least one RHM method significantly improved model fit for adult August weight for 85 haplotype
338 blocks that did not contain previously identified QTL across a total of 22 chromosomes. For the adult
339 leg traits, at least one RHM method significantly improved model fit for multiple blocks that had not
340 previously been identified via GWAS; six blocks across six chromosomes for adult foreleg length, and
341 23 blocks across 14 chromosomes for adult hindleg length respectively. For adult jaw length, model
342 fit was significantly improved by at least one RHM method for six blocks across five chromosomes
343 that had not previously been identified by GWAS. For all of these blocks, model fit was improved by
344 a mixture of Hap-RHM, and SNHap-RHM when compared to either the null model or SNP-RHM.

345 Genes in QTL regions

346 Across all haplotype blocks for which model fit was improved by at least one RHM method for at
347 least one of the 11 focal traits, there were 351 genes overlapping these blocks. 91 of these genes are
348 completely uncharacterised in sheep and classed as “novel genes”, and a further 14 genes were RNA
349 genes. Of the 246 characterised protein coding genes, 13 genes had functional annotations that
350 related to the traits for which model fit was improved (Table 4). One of these genes was in a
351 haplotype block associated with lamb jaw length, four in haplotype blocks associated with adult
352 August weight, one associated with adult foreleg and adult hindleg length, one in haplotype blocks

353 associated only with adult hindleg length and eight in haplotype blocks associated with lamb
354 metacarpal length (one of which was also associated with adult metacarpal length). One of these
355 genes – *PTH1R* – was previously identified as a putative causal gene due to its functional data and
356 proximity to top GWAS SNPs for multiple Soay sheep leg length measures (James et al. 2022).

357 **Discussion**

358 *RHM overview*

359 In total, there were 169 haplotype blocks for which model fit was improved for at least one trait by
360 at least one RHM model. We found that Hap-RHM improved model fit more often than SNP-RHM,
361 which is due in part to the fact that Hap-RHM improved model fit for more traits than SNP-RHM.
362 Hap-RHM also improved model fit more often than SNHap-RHM when SNHap-RHM was compared
363 to either the null model or either of the single regional GRM models. Additionally, SNP-RHM only
364 improved model fit for haplotype blocks in regions surrounding QTL previously identified by GWAS.

365 We found that there were some trait associated regions identified via GWAS for which none of the
366 RHM methods improved model fit – for instance, the regions on chromosomes 1 and 7 associated
367 with birth weight, and the region on chromosome 16 associated with lamb foreleg length and
368 hindleg length. There are two main differences between GWAS and RHM that may be contributing
369 to the observed differences in results: firstly, SNP genotypes are fitted as fixed effects in GWAS,
370 which confers more power than random effects. Secondly, we performed pre-correction of fixed and
371 random effects prior to performing RHM but fitted them during the GWAS step.

372 We have previously shown that pre-correcting for fixed and random effects reduces power of GWAS
373 to detect variant-trait associations. When pre-correction is performed for the adult traits, the only
374 significant GWAS associations are those between SNPs on chromosome 16 and adult foreleg, hindleg
375 and metacarpal lengths, and SNPs on chromosome 19 and adult metacarpal length (James et al.
376 2022). This mirrors our RHM results; the only haplotype blocks for which model fit was improved by
377 the RHM methods were those two regions on chromosomes 16 and 19, with model fit for the latter
378 region only improving for metacarpal length. Pre-correction may therefore explain why we did not
379 see the RHM methods improving model fit for all of the haplotype blocks containing previously
380 identified variants. Currently pre-correction is a necessary step when performing RHM with DISSECT
381 due to DISSECT being unable to fit all of the necessary fixed and random effects during RHM. It
382 would be interesting to rerun this analysis when suitable software is developed for single-step RHM,
383 to determine whether single-step RHM improved model fit for all haplotype blocks containing
384 significant GWAS associations.

385 The significance threshold used during GWAS in James et al. (2022) was similar to the threshold used
386 during the RHM analyses ($1.03e^{-06}$ and $1.04e^{-06}$ respectively). This is because we used SimpleM (Gao
387 et al. 2008) to calculate the number of independent tests during GWAS – this was estimated to be
388 48,635, whilst the number of haplotype blocks estimated by Plink (Purcell et al. 2007; Purcell 2014)
389 was 48,125. This is therefore not a major difference and won't contribute to explaining why we
390 obtained different results from GWAS and RHM.

391 RHM did, however, improve model fit for some regions associated with trait variation using GWAS;
392 RHM improved model fit for haplotype blocks in regions previously found to be associated with lamb
393 metacarpal length, adult hindleg length and adult metacarpal length on chromosome 16, and lamb
394 metacarpal length and adult metacarpal length on chromosome 19.

395 SNP-RHM has previously been performed in a smaller sample of this same population, focusing on
396 only adult morphometric traits (Béréños et al. 2015). 37K autosomal SNPs were split into 150 SNP
397 windows with a 75 SNP overlap. When comparing the results of (Béréños et al. 2015) to our results
398 for the same traits, we find six regions for which SNP-RHM improved model fit for (Béréños et al.
399 2015) and at least one RHM method improved model fit in our own analyses; two regions on
400 chromosome 1 and one region on chromosome 6 were associated with adult August weight

401 (1:119,553,142 – 1:139,871,327, 1:163,370,112 – 1:173,759,083, 6:38,952,950 – 6:48762234), one
402 region on chromosome 6 was associated with adult hindleg length (6:32,615,209 – 6:43,798,415), a
403 region on chromosome 16 associated with adult hindleg and metacarpal length (16:64,064,879 –
404 16:71,555,691), and a region on chromosome 19 associated with adult metacarpal length
405 (19:41,742,622 – 19:58,334,807).

406 Genetic architecture of traits

407 As previously mentioned, RHM failed to significantly improve model fit for some haplotype blocks in
408 regions previously associated with our focal traits by GWAS. However, RHM also identified some
409 novel block-trait associations – for instance, at least one RHM method improved model fit for 85
410 haplotype blocks across a total of 22 chromosomes for adult August weight. None of these blocks
411 were within 1Mb of a previously identified GWAS association. Interestingly, neither SNP-RHM
412 compared to the null model nor SNHap-RHM when compared to Hap-RHM improved model fit for
413 any haplotype blocks for adult August weight. This suggests that the majority of genetic variance
414 contributing to variation in adult August weight is not due to small effect causal variants in LD with
415 genotyped SNPs, but instead due to rare SNPs in LD with rare haplotype alleles or due to multiple
416 SNPs in the same region interacting epistatically. We have previously shown that family-associated
417 non-additive genetic variance such as dominance and epistasis may be making up 37.1% of previous
418 narrow-sense heritability estimations for this trait (James et al. 2023). This finding would be
419 consistent with Hap-RHM detecting regions in which multiple variants are acting in an epistatic
420 manner. We found three genes with functional data suggesting an association with adult August
421 weight that overlapped with haplotype blocks for which model fit was significantly improved by at
422 least one RHM method: *LEPR*, *TBX15* and *EPHX2*. For the blocks overlapping all three genes, model
423 fit is significantly improved by the presence of the haplotype GRM; Hap-RHM significantly improved
424 model fit for all of the overlapping blocks, and SNHap-RHM significantly improved model fit when
425 compared to the null model and to SNP-RHM for the blocks overlapping *LEPR* and *EPHX2*. This may
426 explain why these regions were not identified as being associated with adult August weight when
427 performing GWAS (James et al. 2022), as the variance influencing adult August weight in those
428 regions is likely due to specific haplotype alleles, rather than individual SNP effects.

429 When performing RHM on lamb foreleg and hindleg lengths, only two blocks showed significantly
430 improved model fit for each trait – none of which had previously been indicated to be associated
431 with leg lengths in the Soay population. For all four blocks, Hap-RHM was the only model that
432 significantly improved model fit, suggesting any variance being contributed by these blocks to their
433 respective traits is due to the haplotypes, rather than individual SNPs within the blocks.

434 RHM improved model fit for a block on each of six chromosomes for adult foreleg length and 27
435 blocks across 15 chromosomes for adult hindleg length – of these only the blocks on chromosome 16
436 were close to top GWAS SNPs from any previous leg length GWAS results (Béréanos et al. 2014; James
437 et al. 2022). Blocks on chromosome 16 overlapping top GWAS hits also showed improved model fit
438 for both lamb and adult metacarpal length. It is hard to separate whether the variance being
439 contributed by this region is due to individual SNP effects or an overall haplotype effect, as SNP-
440 RHM, Hap-RHM and SNHap-RHM all significantly improved model fit when compared to the null
441 model, but SNHap-RHM did not significantly improve model fit when compared to either of the
442 single-GRM models. This does suggest, however, that the variance is not due to independent SNP
443 and haplotype effects in the same block.

444 Blocks overlapping the top GWAS SNPs on chromosome 19 also showed significant improvement in
445 model fit for lamb and adult metacarpal lengths when performing SNP-RHM and when comparing

446 SNHap-RHM against the null model (and when compared to Hap-RHM for lamb metacarpal length).
447 This suggests that the variance contributing to these traits is solely due to SNP genotypes, rather
448 than haplotype alleles. Conditional analyses fitting the top GWAS SNP have shown that there are no
449 secondary SNP-trait associations on chromosome 19 independent of the top SNP (James et al. 2022),
450 implying that the variance is being contributed entirely by a single SNP.

451 RHM identified five block-trait associations for lamb jaw length. For all of these blocks, Hap-RHM
452 was the only RHM model that improved model fit, with the exception of two blocks on chromosome
453 13 for which SNHap-RHM also improved model fit when compared to SNP-RHM. Similarly, six trait-
454 block associations were identified for adult jaw length (none of which overlapped with lamb jaw
455 length). Hap-RHM improved model fit for all six blocks, and SNHap-RHM improved model fit for two
456 and three of these blocks when compared to the null model and SNP-RHM respectively. This
457 suggests that these regions either contain a rare variant that influences jaw length that is only in LD
458 with a low number of haplotype alleles, or they contain multiple variants interacting in an epistatic
459 manner. It also implies that variation in lamb and adult jaw length are influenced by different genetic
460 factors, which corroborates previous GWAS findings (James et al. 2022).

461 Concluding remarks

462 We have demonstrated that RHM methods are a useful tool for detecting regions that contribute
463 genetic variation to traits in a wild population and complement other analyses such as GWAS. We
464 found that Hap-RHM and SNHap-RHM improved model fit for more haplotype blocks than SNP-RHM,
465 but all three can be used together to better characterise the underlying genetic architecture within a
466 region. Using these methods, we detected multiple haplotype blocks that improved model fit with at
467 least one RHM method. From these regions, we characterised the genetic regions influencing trait
468 variation and identified 13 potential genes that influence trait variation that have not previously
469 been associated with variation in these traits in the Soay population.

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531

Age	Trait	Number of individuals	Number of records	Fixed effects	Random effects
Neonate	Birth weight	2975	2975	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	2424	2424	Sex	Year of birth
				Litter size	Mother ID
				Population size	
				Age (days)	
	Foreleg	2512	2512	Sex	Year of birth
				Litter size	Mother ID
				Population size	
				Age (days)	
	Hindleg	2577	2577	Sex	Year of birth
				Litter size	Mother ID
				Population size	
				Age (days)	
	Metacarpal	2117	2117	Sex	Year of birth
				Litter size	Mother ID
				Age at death (months)	
	Jaw	2172	2172	Sex	Year of birth
				Litter size	Mother ID
				Age at death (months)	
Adult	Weight	2092	3860	Sex	Year of capture
				Population size	Permanent environment
				Age (years)	
	Foreleg	1936	3594	Sex	Year of capture
				Population size	Permanent environment
				Age (years)	
	Hindleg	2027	3481	Sex	Year of capture
				Population size	Permanent environment
				Age (years)	
	Metacarpal	987	987	Sex	Year of birth
				Age at death (years)	
	Jaw	1057	1057	Sex	Year of birth
Age at death (years)					

Table 1 Number of individuals and records, fixed and random effects fitted in each trait and age class model during RHM pre-correction, alongside the LOCO GRM.

Chromosome	Number of SNPs	Number of haplotype blocks	Mean number of SNPs per haplotype block
1	47318	5466	8.66
2	41917	4426	9.47
3	38265	4081	9.38
4	20659	2259	9.15
5	18545	2132	8.70
6	19205	1908	10.07
7	17647	2267	7.78
8	15815	2029	7.79
9	16517	2177	7.59
10	15517	2223	6.98
11	11567	1371	8.44
12	13909	1665	8.35
13	13626	1427	9.55
14	10785	1205	8.95
15	13897	1537	9.04
16	11992	1400	8.57
17	12598	1694	7.44
18	11718	1357	8.64
19	10230	1058	9.67
20	9074	1115	8.14
21	7879	813	9.69
22	9352	1246	7.51
23	10004	1062	9.42
24	6362	492	12.93
25	7530	842	8.94
26	7353	873	8.42

Table 2 Number of SNPs per chromosome, number of haplotype blocks per chromosome, and average number of SNPs per haplotype block.

Trait	SNP-RHM	Hap-RHM	SNHap-RHM	SNHap-RHM vs Hap-RHM	SNHap-RHM vs SNP-RHM
Birth weight	0	0	0	0	0
Lamb August weight	0	0	0	0	0
Lamb foreleg length	0	2	0	0	0
Lamb hindleg length	0	2	0	0	0
Lamb metacarpal length	40	25	30	4	2
Lamb jaw length	0	5	0	0	2
Adult August weight	0	83	35	0	56
Adult foreleg length	0	6	6	0	6
Adult hindleg length	4	25	14	0	17
Adult metacarpal length	19	12	16	0	0
Adult jaw length	0	6	2	0	3

Table 3 Number of haplotype blocks for which inclusion of regional GRMs improved model fit. *SNP-RHM* column compares the SNP-RHM model against the null model to see if the inclusion of the regional SNP GRM improves model fit. *Hap-RHM* column compares the Hap-RHM model against the null model to see if the inclusion of the regional haplotype GRM improves model fit. *SNHap-RHM* column compares the SNHap-RHM model against the null model to see if the simultaneous inclusion of both the regional SNP GRM and the regional haplotype GRM improves model fit. *SNHap-RHM vs Hap-RHM* column compares the SNHap-RHM model against the Hap-RHM model to see if the additional inclusion of the regional SNP GRM improves model fit. *SNHap-RHM vs SNP-RHM* column compares the SNHap-RHM model against the SNP-RHM model to see if the additional inclusion of the regional haplotype GRM improves model fit.

Trait	Models with improved fit	Chromosome	Haplotype or SNP location(s) (bp)	Gene	Functional annotation
Adult August weight	Hap-RHM, SNHap-RHM vs null, SNHap-RHM vs SNP-RHM	1	40773086 – 40841455, 40847219 – 40872161	LEPR	Codes for the leptin receptor, an adipocyte-specific hormone that regulates body weight. Repeatedly associated with body weight and physiological factors contributing to body weight variation across multiple species (Chagnon et al. 1999; Yiannakouris et al. 2001; Israel and Chua 2010; Ros-Freixedes et al. 2016; Solé et al. 2021), including sheep (Macé et al. 2022).
Adult August weight	Hap-RHM	1	95596363 – 95600207	TBX15	Identified as a potential causal gene for birth weight in Barki sheep (Abousoliman et al. 2021). Found to be a master transcriptional regulator in human adipose tissue and has downstream effects on abdominal obesity (Pan et al. 2021), correlates with BMI and hip-to-waist ratio in humans (Heid et al. 2010) and TBX15 knock-out mice are shown to have increased body weight gain in comparison to control mice (Sun et al. 2019).
Adult August weight	Hap-RHM, SNHap-RHM vs null, SNHap-RHM vs SNP-RHM	2	38080434 – 38081124	EPHX2	Linked with obesity in humans (Khadir et al. 2020) and affects insulin sensitivity in both rodents and humans (Luther and Brown 2016).
Adult hindleg	Hap-RHM	6	103234182 – 103331382	EVC2	Humans: his gene encodes a protein that functions in bone formation and skeletal development. Mutations in this gene cause Ellis-van Creveld syndrome (characteristics include small stature and short limbs) (Baujat and Le Merrer 2007) and Weyers acrofacial dysostosis (characteristics include short limbs) (Ye et al. 2006). Deletion within EVC2 causes chondrodysplastic (short-legged) dwarfism in cattle (Murgiano et al. 2014).
Adult foreleg length Adult	Hap-RHM, SNHap-RHM vs null, SNHap-RHM vs SNP-RHM	12	1315546 – 1498521	PPP1R15B	Missense mutation in PPP1R15B causes a disease in humans resulting in short stature (Abdulkarim et al. 2015)

hindleg length					
Adult August weight	Hap-RHM, SNHap-RHM vs null, SNHap-RHM vs SNP-RHM	12	31886985 - 32003794	SDCCAG8	Mutations result in Bardet-Biedl Syndrome (characterisation includes obesity) in humans (Schaefer et al. 2011). Associated with obesity in human children and adolescents (Scherag et al. 2012)
Lamb jaw length	Hap-RHM, SNHap-RHM vs SNP-RHM	13	53300575 – 53763103	LOC101112800	Expressed in mouse lower jaw mesenchyme (Diez-Roux et al. 2011)
Lamb metacarpal length	SNP-RHM, SNHap-RHM vs null	19	48699277 – 48839372	POC1A	Mutations in this gene result in SOFT syndrome (characterisation includes short stature) in humans (Min Ko et al. 2016).
Lamb metacarpal length	SNP-RHM, Hap-RHM, SNHap-RHM vs null	19	50594654 – 50934237	TCTA	Induces osteoclastogenesis (Kotake et al. 2009)
Lamb metacarpal length	SNP-RHM, Hap-RHM, SNHap-RHM vs null	19	50594654 – 50934237	RHOA	Promotes osteoclastogenesis (Wang et al. 2023). Inhibition of RHOA induces chondrogenesis in chick limbs (Kim et al. 2012). Inhibits bone formation by suppressing IGF1 (Negishi-Koga et al. 2011).
Lamb metacarpal length	SNHap-RHM vs null, SNHap-RHM vs Hap-RHM, SNHap-RHM vs SNP-RHM	19	51192408 - 51582246	PLXNB1	Involved in negative regulation of osteoblast proliferation. Activates RHOA. (Negishi-Koga et al. 2011)
Lamb metacarpal length	SNHap-RHM vs null, SNHap-RHM vs Hap-RHM, SNHap-RHM vs SNP-RHM	19	52376602 – 52543759	PTH1R	Previously identified as potential causal gene for multiple leg length measures in Soay sheep via GWAS (James et al. 2022). Involved in osteoblast development in mice (Qiu et al. 2015), associated with skeletal disorders such as EKNS (Duchatelet et al. 2005), JMC, and BLC (Schipani and Provot 2003) in humans.
Adult metacarpal length	SNHap-RHM vs null				

Lamb metacarpal length	SNP-RHM	19	53372619 – 53548690	LIMD1	Influences osteoblast differentiation and function in mice (Luderer et al. 2008)
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Table 4 - Potential candidate genes for future analyses. From left to right: associated trait, method that resulted in the gene being identified, chromosome, haplotype block, gene name, and evidence for association in sheep and other species.