

1 Genomic analysis reveals a polygenic architecture of 2 antler morphology in wild red deer (*Cervus elaphus*).

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9 Abstract

10 Sexually-selected traits show large variation and rapid evolution across the animal
11 kingdom, yet genetic variation often persists within populations despite apparent
12 directional selection. A key step in solving this long-standing paradox is to determine the
13 genetic architecture of sexually-selected traits to understand evolutionary drivers and
14 constraints at the genomic level. Antlers are a form of sexual weaponry in male red deer.
15 On the island of Rum, Scotland, males with larger antlers have increased breeding
16 success, yet there has been no response to selection observed at the genetic level. To
17 better understand the underlying mechanisms of this observation, we investigate the
18 genetic architecture of ten antler traits and their principle components using genomic data
19 from >38,000 SNPs. We estimate the heritabilities and genetic correlations of the antler
20 traits using a genomic relatedness approach. We then use genome-wide association and
21 haplotype-based regional heritability to identify regions of the genome underlying antler
22 morphology, and an Empirical Bayes approach to estimate the underlying distributions of

allele effect sizes. We show that antler morphology is heritable with a polygenic architecture, highly repeatable over an individual's lifetime, and that almost all aspects are positively genetically correlated with some loci identified as having pleiotropic effects. Our findings suggest that a large mutational target and pleiotropy with traits sharing similar complex polygenic architectures are likely to contribute to the maintenance of genetic variation in antler morphology in this population.

29 Introduction

Sexually-selected traits show a great variety and complexity across the animal kingdom, ranging from traits and behaviours that increase attractiveness, to those which increase intra-sexual competitiveness for access to mates (Andersson, 1994). Sexual traits are typically under strong selection (Kingsolver *et al.*, 2001), with phenotypic differences between related species suggesting that they can evolve rapidly, with downstream consequences for other phenomena such as adaptation, speciation and extinction probability (Lorch *et al.*, 2003; Ritchie, 2007; Servedio & Bürger, 2014; Wilkinson *et al.*, 2015; Martínez-Ruiz & Knell, 2017). Theory predicts that such strong sexual selection will reduce genetic variation within populations, yet empirical studies often show that sexual traits have substantial underlying genetic variation despite evidence of selection (Pomiankowski & Moller, 1995; Kotiaho *et al.*, 2001; Kruuk *et al.*, 2002; Svensson & Gosden, 2007). This contradiction presents an evolutionary paradox, for which several explanations have been proposed. These include differences in selection between the sexes, developmental stages or environmental conditions (Bourret *et al.*, 2017; Barson *et al.*, 2015), phenotypic plasticity (Charmantier & Gienapp, 2014), condition dependence (Dugand *et al.*, 2019) and trade-offs with survival (Johnston *et al.*, 2013). In addition, these observations could be due to genetic correlations with traits under opposing selection gradients and linkage disequilibrium between causal loci and deleterious alleles (Lande, 1982; Lande & Arnold, 1983; Connallon & Hall, 2018). Quantitative genetic studies have provided some insight into these different explanations, through estimating the relative contributions of additive genetic (i.e. the heritability, h^2) and environmental effects to phenotypic variance, as well as examining phenotypic and genetic correlations

52 with other traits, including fitness (Emlen, 1994; Griffith *et al.*, 1999; Kruuk *et al.*, 2002;
53 Robinson *et al.*, 2006). However, a key limitation of most studies to date is that the genetic
54 architecture of sexual traits is generally unknown - that is, the underlying loci, their
55 number, distribution and relative effect sizes, and the extent of pleiotropy, epistasis and
56 other interactions (Timpson *et al.*, 2018; Chenoweth & McGuigan, 2010). By identifying
57 the genetic architecture of sexual traits, we can better understand the underlying
58 molecular mechanisms and evolutionary processes that drive their variation (Dobzhansky,
59 1971; Lewontin *et al.*, 1974; Kuijper *et al.*, 2012; Wilkinson *et al.*, 2015).

60 Recent genomic advances in natural populations have led to a number of studies
61 characterising genetic architectures using genome-wide association studies (GWAS,
62 reviewed in Santure & Garant 2018). Yet, relatively few studies exist for sexually selected
63 traits, with much of the focus on discrete traits with Mendelian or relatively simple genetic
64 architectures (Johnston *et al.*, 2011; Barson *et al.*, 2015; Hendrickx *et al.*, 2021). In these
65 rare cases, mapping specific genomic variants associated with sexual trait variation can
66 allow investigation of sex, age and environment-specific effects at individual loci. As such,
67 they have revealed compelling cases of heterozygote advantage due to trade-offs
68 between reproductive success and survival (Johnston *et al.*, 2013), or due to differences
69 in optimal trait expression between the sexes (Barson *et al.*, 2015). However in most
70 cases, sexual traits are likely to have oligogenic or polygenic architectures (i.e. moderate
71 to large numbers of underlying loci), particularly in cases where they are condition
72 dependent (Rowe & Houle, 1996). One issue is that as the number of loci increase and
73 their relative effect sizes decrease, it becomes more difficult to implicate individual loci in
74 trait variation; for example, in heights of people of European ancestry, only a fraction of
75 the loci underpinning variation has been identified (Yengo *et al.*, 2018). On the other
76 hand, being able to determine that a trait has a polygenic architecture can still shed light
77 on how sexual traits evolve for the following reasons. First, polygenic traits present a large
78 mutational target, contributing to the maintenance of genetic variance via the introduction
79 of new variants (Rowe & Houle, 1996). Second, selection induced allele frequency
80 changes at a great number of loci is expected to result in a rapid change in trait mean,
81 and thus trait evolution, which is sustained by the aforementioned large mutational input,
82 leaving the distribution of genetic effects unperturbed (Barton *et al.*, 2017; Sella & Barton,

2019). Third, pleiotropy and/or linkage between loci could maintain variation through conflicts between traits sharing a similar or linked polygenic architecture (Ruzicka *et al.*, 2019). Therefore, studies of sexual traits should aim to identify specific genetic variants with large effects on phenotype, and should also aim also to determine the distribution of polygenic effect sizes and the degree to which these underlying loci are shared between traits. This will not only shed light on potential evolutionary processes and mechanisms in empirical studies, but will also inform the mechanistic details of theoretical models to allow better assumptions to account for the complexities of natural populations (McNamara & Houston, 2009; Wilkinson *et al.*, 2015).

Antlers are a form of sexual weaponry in deer (Cervidae) that are generally only present in males and are shed and regrown annually (Davis *et al.*, 2011). They are used as weaponry in intra-male competition for access to females, with larger antlers often associated with increased reproductive success (Kruuk *et al.*, 2002; Malo *et al.*, 2005), yet antler weights and dimensions are often moderately heritable in both wild and captive populations (Lukefahr & Jacobson, 1998; Williams *et al.*, 1994; Wang *et al.*, 1999; Van Den Berg & Garrick, 1997; Kruuk *et al.*, 2002, 2014; Jamieson *et al.*, 2020). In male red deer (*Cervus elaphus*) on the island of Rum, Scotland, there is directional selection for increased antler weight and number of points (known as “form”), yet both are substantially heritable ($h^2 = 0.38$ & 0.24 , respectively) with no phenotypic response to selection observed over a 30 year study period (Kruuk *et al.*, 2002, 2014). Whilst both antler weight and form are positively genetically correlated, the selection gradients on the genetic components were estimated as zero and negative, respectively (Kruuk *et al.*, 2002, 2014). This suggested that selection for antler weight is environmentally driven, whereas negative selection on antler form is constrained by genetic associations with a trait that is genetically unresponsive to selection, meaning that genetic constraints may contribute to the maintenance of genetic variance. Alternatively, the association with breeding success may be driven by indirect correlations with environmental variables (Kruuk *et al.*, 2014). To better understand the mechanisms constraining selection, a logical next step is to determine the genetic architecture of antler morphology. As antlers present a multi-dimensional phenotype, adding more information from different measures may contribute to understanding potential evolutionary conflict and constraints within the antler

114 and to characterise the specific genetic variants underpinning heritable variation
115 (Chenoweth & McGuigan, 2010).

116 In this study, we used an extensive antler morphology data set with 948 to 3972
117 observations in 336 to 891 unique males and genomic data from 38,000 polymorphic
118 SNPs to estimate the heritability and genetic correlations of ten antler traits using
119 genomic relatedness matrices. We then use genome-wide association and
120 haplotype-based regional heritability to identify regions of the genome underlying antler
121 morphology, and an Empirical Bayes approach to estimate the underlying distributions of
122 allele effect sizes. We show that antler morphology is heritable with a polygenic
123 architecture, and that almost all aspects are positively genetically correlated with some
124 loci identified as having pleiotropic effects. Our findings suggest that genetic variation in
125 antler morphology is maintained via a large mutational target and pleiotropy with traits
126 sharing similar complex polygenic architectures in the red deer population.

127 **Methods**

128 **Study system**

129 The red deer study population is situated in the North Block of the Isle of Rum, Scotland
 130 (57°02'N, 6°20'W) and has been subject to individual monitoring since 1971
 131 (Clutton-Brock *et al.*, 1982). Deer calves are marked with ear tags shortly after birth to
 132 enable recording of detailed life histories of individuals. DNA is routinely extracted from
 133 neonatal ear punches, post-mortem tissue and/or cast antlers (see Huisman *et al.* 2016).
 134 A pedigree of 4,515 individuals is available for the population, and was previously
 135 constructed using single nucleotide polymorphism (SNP) data in the R package Sequoia
 136 (Huisman, 2017). Research was conducted following approval of the University of
 137 Edinburgh's Animal Welfare and Ethical Review Body and under appropriate UK Home
 138 Office licenses.

139 **Antler measures**

140 Male red deer cast and regrow their antlers every year from the age of one or two (Kruuk
 141 *et al.*, 2002). Ten antler measures are routinely taken from cast antlers and antlers from
 142 deceased individuals between 1971 and 2017 (see Figure 1 and Table 1 for full details
 143 and sample sizes of each measure). All measures of length were taken following the curve
 144 of the antler, and measures of circumference were taken at the narrowest point between
 145 antler tines (points). Total antler length was defined as the distance from the coronet (base)
 146 to the furthest point of the antler. All length and circumference measures were taken in
 147 centimetres. Antler weight was measured as the total dry mass of the antler in grams.
 148 Antler form was defined as the number of tines, and as this trait can be determined visually
 149 on living deer, was determined from observations in the field and therefore has the greatest
 150 sample size (3972 observations from 891 stags). Where measurements from both the
 151 left and right antlers were available, the mean was taken. Individual measurements were
 152 excluded if the antler part was broken and antler weight was discarded if any part of the
 153 antler was broken. Only antlers from stags aged 3 years or older were considered, as cast

154 antlers recovered in the field can be reliably assigned to known individuals by their shape
 155 from this age onwards (Kruuk *et al.*, 2002, 2014).

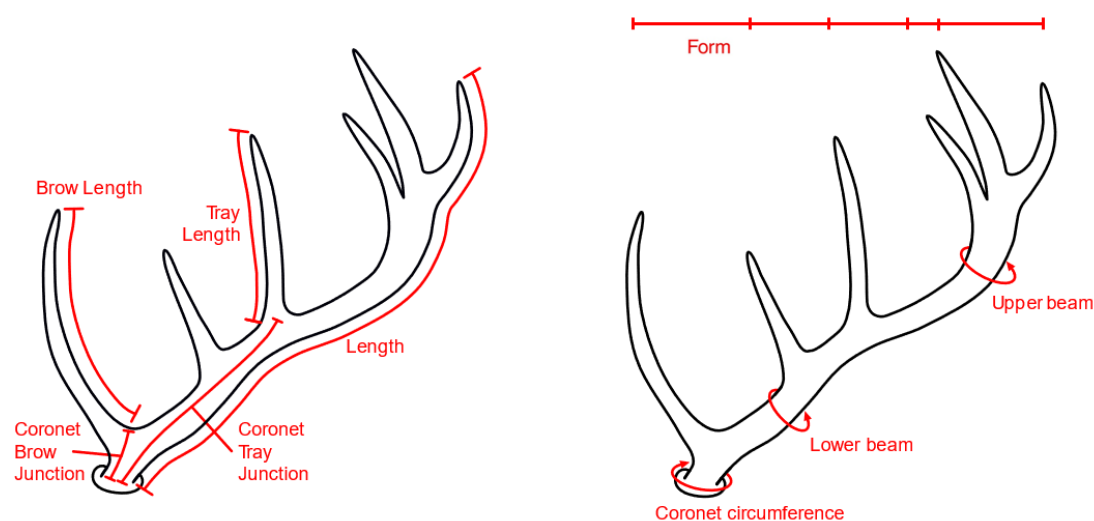


Figure 1: A schematic of the antler measures used in this study (measured in *cm*). Antler weight was also measured in *g*. Further details for each measurement are given in the main text and in Table 1.

156

Table 1: Summary statistics of all 10 antler measures as shown in Figure 1 in SNP genotyped males. *N Obs* is the number of observations, *N IDs* is the number of unique individuals for each measure. All lengths and circumferences were measured in *cm*, weight was measured in *g*. *SD* denotes the standard deviation.

Trait	N Obs	N IDs	Mean	SD
Antler Length	1060	444	65.168	12.029
Coronet Circumference	1224	471	15.626	2.013
Lower Beam Circumference	1204	460	10.504	1.533
Upper Beam Circumference	1123	436	9.446	1.358
Coronet-Brow Junction	1162	466	5.720	1.025
Coronet-Tray Junction	1184	455	27.313	5.398
Brow Length	1139	457	20.568	5.461
Tray Length	948	388	14.484	4.177
Antler Weight	1003	336	649.858	243.098
Form (Total Number of Points)	3972	891	4.498	1.072

157 Genomic data-set

158 DNA samples from 2,870 individuals have been genotyped at 51,248 SNP markers
159 (Huisman *et al.*, 2016) on the Cervine Illumina BeadChip (Brauning *et al.*, 2015) using an
160 Illumina genotyping platform and Illumina GenomeStudio software (Illumina Inc., San
161 Diego, CA, USA). All SNPs on the Cervine Illumina BeadChip are named based on their
162 synteny with the cattle genome BTA vUMD 3.0 (e.g. SNP ID *cela1_red_15_1479373* is
163 orthologous to position 1479373 on cattle chromosome 15). In addition, a linkage map
164 specific to the Rum population is available, with 38,083 SNPs assigned to linkage groups
165 corresponding to the 33 deer autosomes and X chromosome (Johnston *et al.*, 2017).
166 Quality control was carried out in PLINK v1.9 (Chang *et al.*, 2015) with the following
167 thresholds: SNP genotyping success >0.99, minor allele frequency >0.01 and individual
168 genotyping success >0.99. Further quality control of mapped SNPs and X-linked markers
169 (i.e heterozygous state in males) was conducted using the *check.marker* function with

170 default thresholds in the R library GenABEL v1.80 in R v3.4.2 (Aulchenko *et al.*, 2007).
 171 The final SNP dataset consisted of 2,138 individuals and 38,006 markers. Genome-wide
 172 linkage disequilibrium (LD) was calculated between all SNPs within 1Mb of each other
 173 using Spearman's Rank correlation (ρ). Based on a linear regression of ρ on the log base
 174 pair distance between SNPs, LD decayed at a relatively low rate of 0.031 ρ per Mb (SE =
 175 5.56×10^{-4} , Figure S1).

176 Principal components of antler measures

177 A principal component analysis (PCA) was conducted to create a second dataset
 178 combining information from the different antler measures, while also increasing the
 179 differentiation among the different principal components (PCs). As PCA does not allow for
 180 missing data, we imputed missing antler measures using the Bayesian *bpca* algorithm in
 181 the packages *pcaMethods* v1.7 in R v3.4.2 (Stacklies & Redestig, 2018; Oba *et al.*, 2003).
 182 We used the default settings which assumes a flat prior distribution for imputation, and the
 183 most appropriate number of PCs was determined using the *kEstimate* function. To
 184 improve imputation accuracy, the form was split into the number of tines on the lower
 185 beam and upper beam, respectively, resulting in 11 antler measures. Imputation accuracy
 186 was quantified by calculating the error of prediction (E) from a complete subset of the
 187 data with no missing values and the same subset with randomly missing data at a similar
 188 level to the whole data set ($\sim 9\%$). The error of prediction was calculated as follows:

$$E = \sum (V - I)^2 / \sum (V)^2 \quad (1)$$

189 where I refers to the imputed values of the data subset with missing values and V to
 190 their counterpart in the complete data subset (Stacklies & Redestig, 2018). Our analysis
 191 found that the imputation of missing values using a Bayesian PCA approach achieved high
 192 accuracy ($E = 0.015$). To account for variation among antlers due to age structure, antler
 193 measure values were modelled using a linear model approach (following Pallares *et al.*
 194 2014). All models had the same structure:

$$y = Age + Age^2 + e \quad (2)$$

195 where y is a vector of the antler measure and e is a residual error term. Age effects were
 196 fitted as a fixed quadratic term to account for the non-linear change in antler measures
 197 with age (see also Nussey *et al.* 2009 and Kruuk *et al.* 2002). All antler measures were
 198 modelled using a Gaussian distribution. All models were fitted using the *lm* function in R
 199 v3.4.2. The residuals of these models were then used in a standard PCA using the *prcomp*
 200 function in R and the scores of the PCs used as trait values in the downstream analysis.

201 **Estimating heritability using the animal model**

202 All antler measures and PCs were modelled using a restricted maximum-likelihood
 203 (REML) approach within the mixed ‘animal model’ framework (Henderson, 1975) in
 204 ASREML-R v4.0 (Butler *et al.*, 2009) in R v3.4.2. The animal model estimates the effect
 205 sizes of fixed effects and partitions phenotypic variance (V_P) into several random effects,
 206 including the variance attributed to additive genetic effects (V_A). Previous studies have
 207 estimated V_A using a pedigree relatedness matrix (Kruuk *et al.*, 2002, 2014); here, we
 208 wished to compare estimates of V_A using both pedigree and genomic relatedness
 209 information. Therefore, all models were carried out estimating V_A in one of two ways: 1)
 210 using a numerator relationship matrix A based on the pedigree, using the *ainv* function in
 211 ASReml-R; and 2) using a genomic relatedness matrix (GRM) calculated using
 212 autosomal SNPs ($N = 37,271$) in the *--make-grm* function in GCTA v1.24.3 (Yang *et al.*,
 213 2011a). The GRM was adjusted to assume similar frequency spectra of genotyped and
 214 causal loci with the argument *--grm-adj 0*.

215 All 10 antler measures and 11 PCs were modelled in univariate animal models with the
 216 following structure:

$$y = X\beta + Z_1a + Z_ru_r + e \quad (3)$$

217 where y is a vector of the antler measure or PC, X is an incidence matrix relating
 218 individual measures to the vector of fixed effects β ; Z_1 and Z_r are incidence matrices
 219 relating individual measures to additive genetic and other random effects respectively; a is
 220 a vector of relatedness matrix A or GRM; u_r is a vector of additional random effects; and e
 221 is a vector of residual effects. Fixed effects included age in years as both a linear and

quadratic term for the antler measures and an intercept only for the PC models (as age structure was accounted for prior to PC estimation). Random effects included: the additive genetic effect; permanent environment (i.e. individual identity) to account for pseudoreplication due to repeated measures in the same individual; and birth year and year of antler growth to account for common environmental effects between individuals. The narrow sense heritability (h^2) was calculated as V_A/V_P , where V_P was defined as the sum of the variance attributed to all random effects, including the residual variance (Falconer & Mackay, 1996). The significance of fixed effects was calculated with a Wald test, while the significance of the random effects was tested using a likelihood ratio test (LRT) between models with and without random effect of interest (i.e. $2 \times$ the difference between the model log-likelihoods, assuming a χ^2 distribution with 1 degree of freedom).

Bivariate models were run to determine genetic correlations between the 10 antler measures, with the following structure:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} a_{a1} \\ a_{a2} \end{bmatrix} + \begin{bmatrix} Z_{r1} & 0 \\ 0 & Z_{r2} \end{bmatrix} \begin{bmatrix} u_{r1} \\ u_{r2} \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix} \quad (4)$$

All variables are as defined in equation (3), with subscripts referring to antler traits 1 and 2, respectively. The GRM was used to model the additive genetic covariance. The genetic correlation r^2 can be obtained from the genetic covariance, as $r_a = cov_a(1, 2)/\sigma_1\sigma_2$, where $cov_a(1, 2)$ stands for the covariance between trait 1 and 2, and σ represents the respective standard deviations for traits 1 and 2. The significance of r_a was determined using an LRT as above, by comparing the model to another where r_a was constrained to either zero or one.

Genome-wide association studies

Genome-wide association studies (GWAS) were conducted in RepeatABEL v1.1 (Rönnegård *et al.*, 2016) implemented in R v3.4.2. First, the *prefitModel* function was used to fit a linear mixed model (without fixed SNP effects), specifying the same fixed and random effect structure as Equation 3. The resulting covariance matrix of the random

effects was then input to the *rGLS* function, which fits each SNP genotype as an additive linear covariate. This approach accounts for population structure by fitting the GRM as a random effect and allows for repeated measures per individual. The significance of each SNP was determined using Wald tests, distributed as χ^2 with 1 degree of freedom. These statistics were corrected for potential inflation due to population structure not captured by the GRM by dividing them by the genomic inflation factor λ , defined as the observed median χ^2 statistic divided by the null expectation median χ^2 statistic (Devlin & Roeder, 1999). This was done separately for each antler measure. After correction, the genome-wide significance threshold was set to a p-value of 1.42×10^{-6} , equivalent to $\alpha = 0.05$ after correcting for multiple-testing and accounting for non-independence due to LD between SNP markers (see Johnston *et al.* 2018).

Regional heritability analysis

In addition to GWAS, we used a regional heritability approach to identify regions of the genome associated with antler trait variation. This method uses information from multiple loci to determine the proportion of V_P explained by defined genomic regions, and has increased power to detect variants of small effect sizes and low minor allele frequencies (Yang *et al.*, 2011b; Nagamine *et al.*, 2012). Regions were defined using a 'sliding window' approach with SNPs of known position on the Rum deer linkage map (Johnston *et al.*, 2017). Each window was 20 SNPs wide and overlapped the preceding window by 10 SNPs. If the last window in the linkage group contained less than 20 SNPs, the last 20 SNPs of that linkage group were taken instead. SNPs in linkage group 34, which corresponding to the X chromosome, were excluded from this analysis, as models using X-linked markers did not converge. This resulted in a total of 3,608 genomic windows. The contribution of each genomic region to V_A and V_P for each antler measure and PC was modelled as follows:

$$y = X\beta + Z_1(a - v_i) + Z_2v_i + Z_ru_r + e \quad (5)$$

with variables defined as in Equation (3), but with the additive genetic components split into two terms: $Z_1(a - v_i)$ and Z_2v_i , where Z_1 is an incidence matrix of the GRM

constructed based on all autosomal SNPs excluding those in window i , $(a - v_i)$ is the additive genetic effect excluding the window i ; Z_2 is an incidence matrix of the GRM constructed with only the SNPs in window i and v_i is the additive genetic effect of the window i . The significance of an association between a window i and an antler trait was determined using a LRT comparing models containing and omitting the term Z_2v_i . The distribution of χ^2 statistics from the LRTs across all windows was corrected using the genomic control parameter λ , calculated using the same approach as above (Devlin & Roeder, 1999). A genome-wide significance threshold was calculated using a Bonferroni correction, where the α significance level (here 0.05) was divided by the number of effective tests. For this, we divided the number of windows by two to account for the overlap of half the number of total SNPs within each window, resulting in a significance threshold of $P = 2.77 \times 10^{-5}$.

286 Estimation of SNP effect size distribution

We investigated the distribution of allele effect sizes and false discovery rates for all antler measures and PCs using the *ash* function in the R package *ashR* v2.2-32 (Stephens, 2016). This uses “adaptive shrinkage”, an Empirical Bayes method that uses the slopes and standard errors of the additive SNP effects from the GWAS models above to compute a posterior distribution of SNP effect sizes across all loci. This approach estimates the local false discovery rate (*lfdr*), which is the probability that the SNP effect is zero. The significance of a SNP effect was then determined by a local false sign rate (*lfsr*), defined as the probability of making an error when assigning a sign (positive or negative) to an effect, with a cut-off at $\alpha = 0.05$. The prior distribution was specified to be any symmetric unimodal distribution when applying the *lfdr* estimation.

We then re-estimated the effect sizes of SNPs with the highest non-zero effects using adaptive shrinkage using the animal model framework (Equation 3) in ASREML-R v4.0 (Butler *et al.*, 2009). A maximum number of 10 SNPs per trait were taken. SNP genotype was fit as a two or three level factor and significance was tested using a Wald test. Fitting SNP genotype as a factor allowed the quantification of both the dominance deviation and

the variance co-variance structure of the genotypes, which are needed to estimate the variance attributed to each SNP, calculated as follows (Falconer & Mackay, 1996):

$$V_{SNP} = 2pq(a + d(q - p))^2 \quad (6)$$

where p and q are the allele frequencies of alleles A and B , respectively; a is the additive genetic effect defined as the mid-point between the effect sizes of the genotypes AA and BB ; and d is the dominance deviation defined as the difference between a and the effect size of the heterozygote AB . The proportion of V_A attributed to a SNP was calculated as the ratio of V_{SNP} to the sum of V_{SNP} and the V_A obtained from an animal model where the SNP effect was omitted. Standard errors of V_{SNP} were estimated using the *deltamethod* function in the R library *msm* v1.6.7 (Jackson, 2011) in R v3.4.2.

Data Accessibility Statement

Data for this study will be archived in a public repository upon manuscript acceptance. All results and data underlying the figures in this manuscript are provided as Supplementary Material. All scripts for the analysis are provided at https://github.com/Lucy-Peters/Red_deer_antler_genetic_architecture.

316 Results

317 Principal component analysis of antler measures

318 A principal component analysis (PCA) of 11 antler measures resulted in the maximum
 319 number of principal components (i.e. 11 PCs). The composition of the PCs showed that
 320 PC1, which explained around 41% of the variance, combined approximately equal amounts
 321 of information from all 11 measures, while PCs 2 to 11 explained increasingly less variance,
 322 mostly representing one or two antler measures (Figure 2).

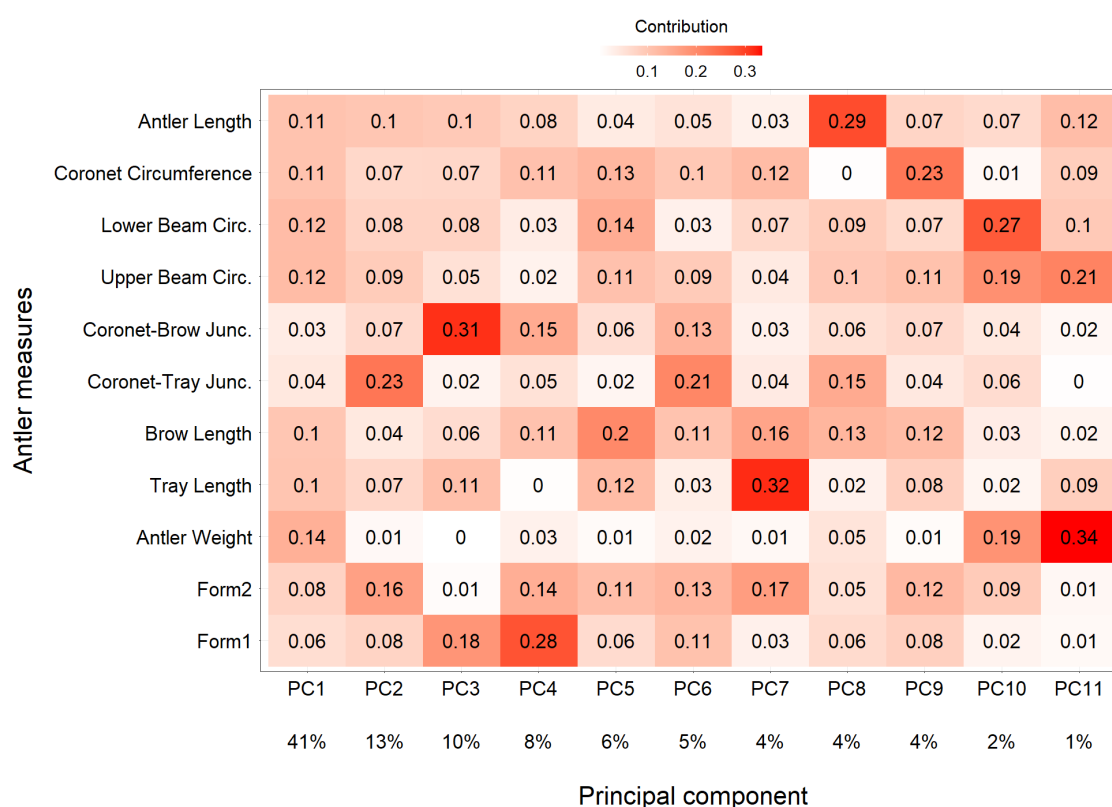


Figure 2: Heat-map showing the contribution (as a proportion) of each antler measurement to each of the 11 principal components.

323 **Animal models of antler measures.**

324 Antler measures were significantly heritable, with estimates ranging from $h^2 = 0.211$ to
 325 0.436 for the pedigree estimates, and $h^2 = 0.229$ to 0.414 for the genomic estimates
 326 (Figure 3; Tables 2 & S1). Heritability estimates for antler weight, antler length, coronet
 327 circumference, brow length and form were generally consistent with previous findings by
 328 Kruuk *et al.* (2014, Figure 3). All antler PCs were also significantly heritable, although
 329 estimates decreased substantially for higher order PCs, which explained relatively small
 330 proportions of variance in antler morphology (Figure S2, Table S2). For all antler
 331 measures and PCs, confidence intervals between pedigree and genomic relatedness
 332 estimates were highly similar; there was no trend when comparing estimates of h^2 for the
 333 same trait, suggesting that both the pedigree and genomic relatedness matrices capture
 334 the additive genetic variance to a similar degree in this population. Therefore, all results
 335 described from this point onwards are from models fitting a GRM, unless otherwise
 336 stated.

337 The permanent environmental effect (which includes dominance and epistatic effects)
 338 was generally significant for all antler measures, explaining up to 28.0% (upper beam
 339 circumference) of the phenotypic variance (Table 2). Year of antler growth explained a
 340 significant proportion of phenotypic variance for most antler measures and PCs (Tables 2
 341 and S2, respectively). Conversely, birth year was not significant for any antler measure or
 342 PC; nevertheless, we retained this random effect in all models to account for potential
 343 cohort effects (Tables 2 & S2). Trait repeatabilities, calculated as the sum of contributions
 344 from the additive genetic, the permanent environment and birth year components, was
 345 high for all antler measures, ranging from 38.5% (antler length) to 66.1% (tray length;
 346 Table 2). Age as both linear and quadratic fixed effect terms was significantly associated
 347 with all antler measures (Table S3).

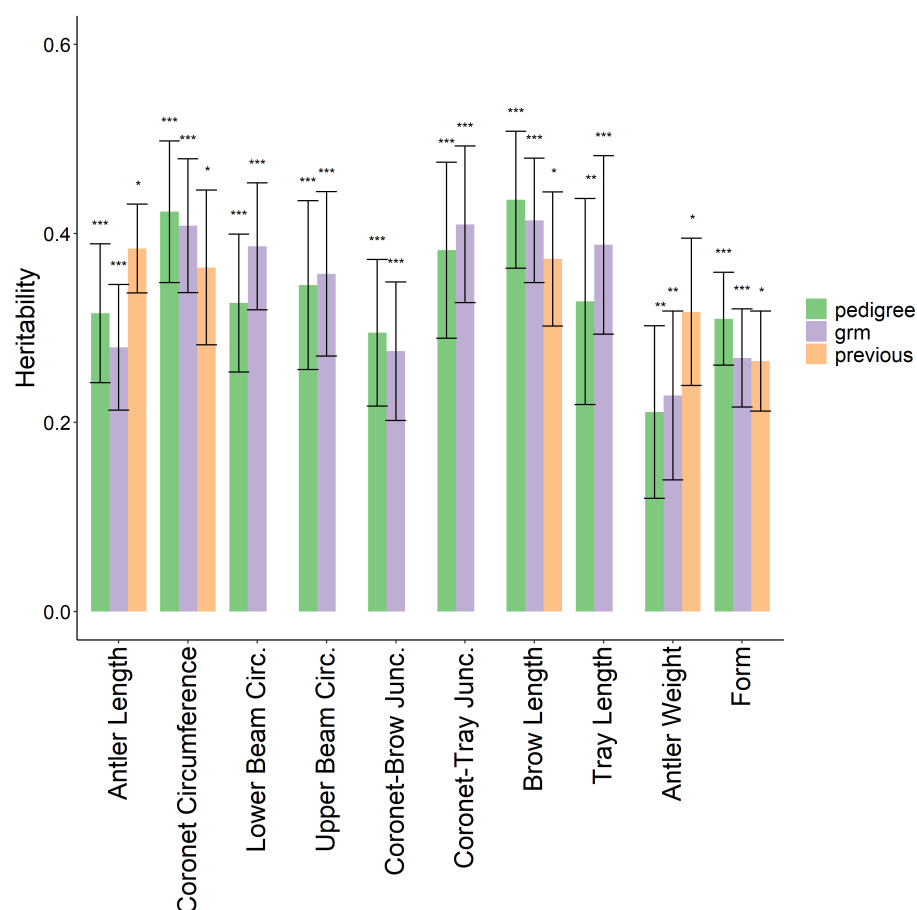


Figure 3: Heritability estimates for all 10 antler measures. Both estimates from the pedigree and the GRM models are shown as well as results from a previous study by Kruuk *et al.* (2014) for antler weight and form. * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$. Underlying data is provided in Table S1

Table 2: Proportions of phenotypic variance (V_P) explained by random effects in animal models of the ten antler measures. The additive genetic effect was estimated using the GRM. Standard errors are given in brackets. Information on sample sizes and mean measures is provided in Table 1.

Trait	V_P	Additive Genetic	Permanent Environment	Birth Year	Growth Year	Residual	Repeatability
Antler Length	61.155	0.279 (0.066)	0.105 (0.058)	1.04E-08 (0.014)	0.222 (0.047)	0.393 (0.035)	0.385
Coronet Circ.	3.047	0.408 (0.071)	0.128 (0.056)	0.018 (0.018)	0.243 (0.047)	0.203 (0.020)	0.554
Lower Beam Circ.	1.323	0.386 (0.067)	0.096 (0.054)	2.22E-07 (1.64E-08)	0.211 (0.042)	0.307 (0.027)	0.483
Upper Beam Circ.	1.084	0.357 (0.087)	0.280 (0.081)	5.16E-08 (0.017)	0.164 (0.035)	0.199 (0.018)	0.637
Coronet-Brow Junc.	0.872	0.275 (0.073)	0.254 (0.068)	0.026 (0.022)	0.152 (0.037)	0.292 (0.025)	0.555
Coronet-Tray Junc.	20.930	0.410 (0.083)	0.148 (0.072)	0.001 (0.016)	0.019 (0.009)	0.422 (0.032)	0.559
Brow Length	21.555	0.414 (0.066)	0.068 (0.050)	6.33E-09 (0.013)	0.269 (0.050)	0.250 (0.025)	0.481
Tray Length	13.809	0.388 (0.094)	0.273 (0.088)	7.65E-08 (5.34E-09)	0.028 (0.012)	0.311 (0.028)	0.661
Antler Weight	30727.710	0.229 (0.089)	0.263 (0.086)	0.023 (0.027)	0.252 (0.050)	0.233 (0.025)	0.514
Form	0.907	0.268 (0.052)	0.249 (0.046)	0.024 (0.015)	0.044 (0.011)	0.416 (0.019)	0.541

348 Genetic correlations between antler measures

349 Most antler measures were positively genetically correlated, suggesting some degree of
 350 shared genetic architecture (Table 3). This was also reflected in the disproportionate
 351 contribution of all antler measures to PC1 (Figure 2). Antler weight was significantly
 352 positively correlated with almost all other measures (with the exception of coronet-tray
 353 junction), ranging from $r^2 = 0.40$ for coronet-brow junction to $r^2 = 0.94$ for upper beam
 354 circumference. The only significant negative genetic correlations were observed between
 355 coronet-tray junction and tray length, form and lower beam circumference (Table 3).

356 Genome-wide and regional heritability studies

357 No genomic regions were significantly associated with any antler measure or PC using
 358 GWAS (Figures 4 and S3, and Tables S4 and S5, respectively). The regional heritability
 359 analysis found no regions of the genome significantly associated with any antler measure
 360 or PC (Figures 5 and S4, and tables S6 and S7, respectively), with the exception of PC9,
 361 which was significantly associated with three overlapping windows (corresponding to a
 362 ~ 4.4 Mb region) on CEL linkage group 21. The most significantly associated window
 363 explained 16.8% (SE = 8.0%) of the phenotypic variance and 66.3% (SE = 22.0%) of the
 364 additive genetic variance. However, PC9 accounts for only 4% of overall phenotypic
 365 variance among all antler measures (see Figure 2). Homology with the cattle genome
 366 (version ARS-UCD1.2) suggested that there are a total of 19 coding regions within the
 367 region covered by all three significant windows, Details of SNPs within this region can be
 368 found in Table S8 and associated GO terms can be found in Table S9.

Table 3: Genetic correlations among all 10 antler measurements. Standard errors are given in brackets, $*P \leq 0.05$, $**P \leq 0.01$ and $***P \leq 0.001$. AL = Antler Length, CC = Coronet Circumference, LBC = Lower Beam Circumference, UBC = Upper Beam Circumference, CBJ = Coronet-Beam Junction, CTJ = Coronet-Tray Junction, BL = Brow Length, TL = Tray Length, AW = Antler Weight, F = Form.

	CC	LBC	UBC	CBJ	CTJ	BL	TL	AW	F
AL	0.678 (0.068) ***	0.640 (0.077) ***	0.664 (0.093) ***	0.674 (0.103) ***	0.665 (0.096) ***	0.549 (0.075) ***	0.447 (0.109) ***	0.854 (0.063) ***	0.406 (0.108) ***
CC	—	0.800 (0.054) ***	0.745 (0.084) ***	0.168 (0.109) ***	0.369 (0.089) ***	0.650 (0.067) ***	0.603 (0.088) ***	0.913 (0.065) ***	0.565 (0.095) ***
LBC	—	—	0.940 (0.024) ***	0.442 (0.102) ***	-0.322 (0.091) ***	0.592 (0.068) ***	0.810 (0.059) ***	0.928 (0.059) ***	0.780 (0.064) ***
UBC	—	—	—	0.266 (0.132) *	-0.179 (0.112) ***	0.541 (0.073) ***	0.733 (0.069) ***	0.943 (0.059) ***	0.902 (0.041) ***
CBJ	—	—	—	—	0.492 (0.108) ***	-0.079 (0.109) ***	-0.006 (0.137) ***	0.396 (0.185) *	-0.163 (0.126) ***
CTJ	—	—	—	—	—	0.055 (0.065) ***	-0.713 (0.073) ***	0.188 (0.146) ***	-0.521 (0.099) ***
BL	—	—	—	—	—	—	0.739 (0.070) ***	0.710 (0.073) ***	0.608 (0.065) ***
TL	—	—	—	—	—	—	—	0.811 (0.082) ***	0.822 (0.064) ***
AW	—	—	—	—	—	—	—	—	0.890 (0.061) ***

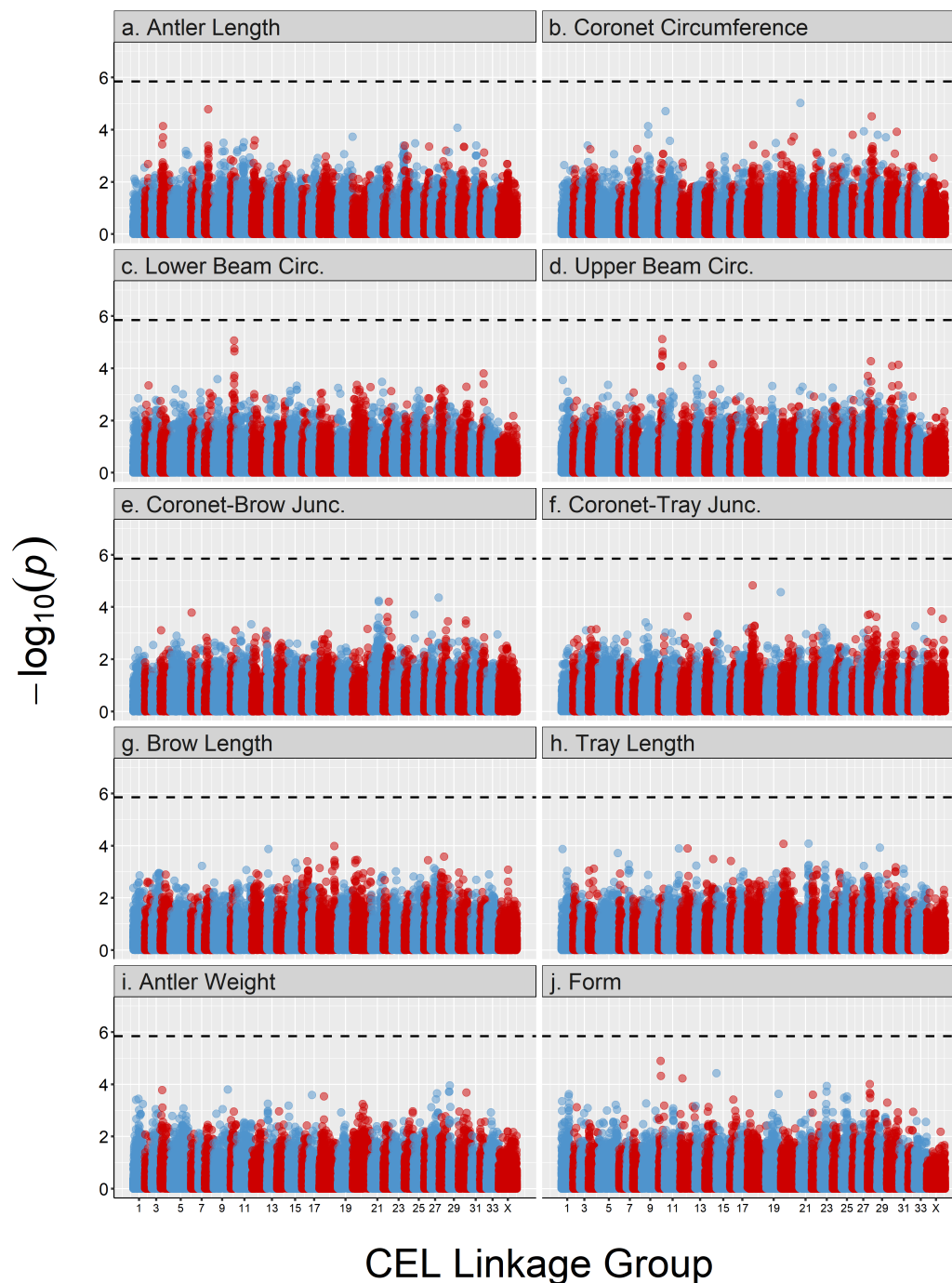


Figure 4: Genome-wide association study for antler measures. The dashed line indicates the significance threshold equivalent to $\alpha = 0.05$. Points are colour-coded by chromosome. Underlying data is provided in Table S4.

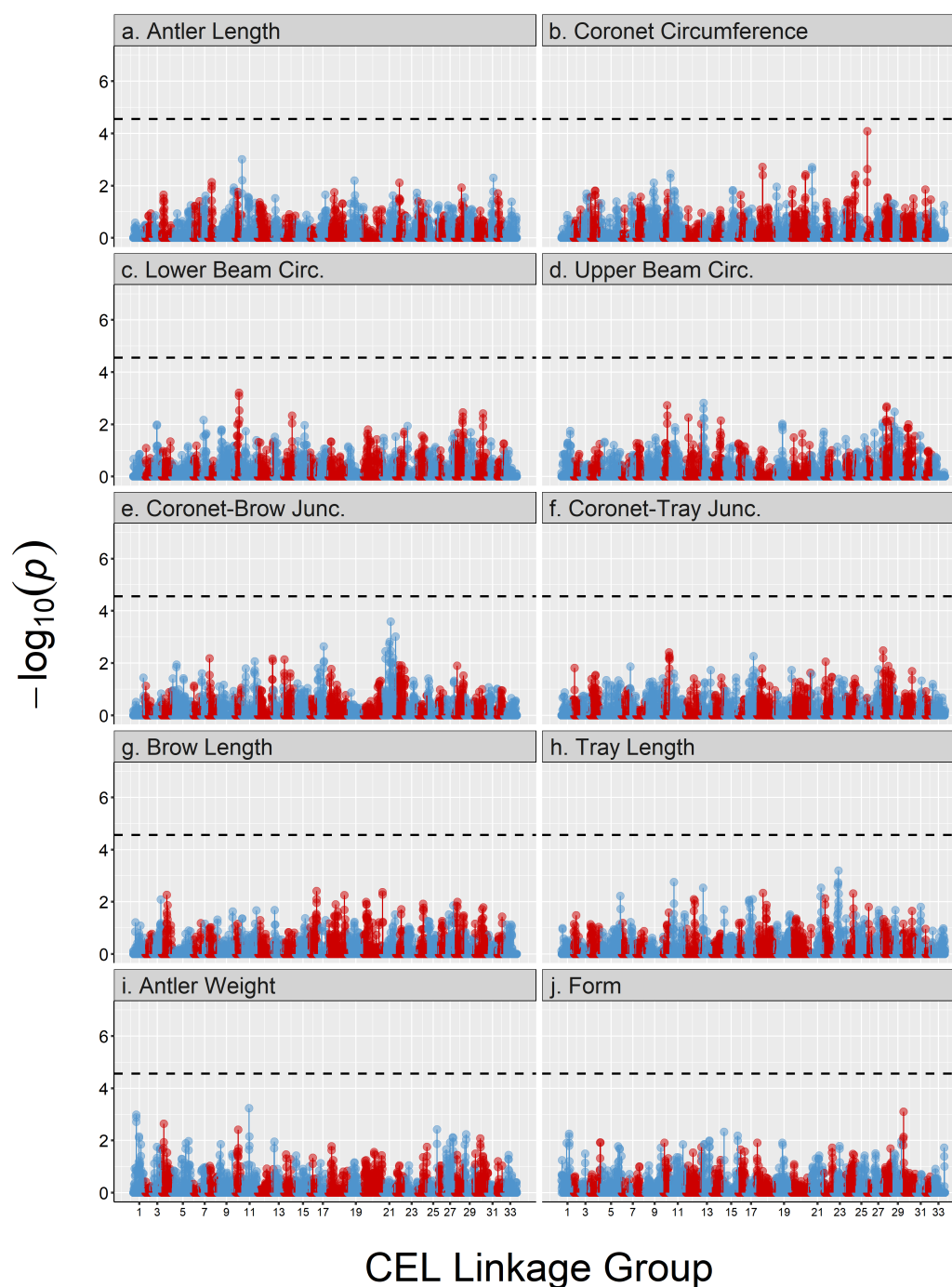


Figure 5: Regional heritability analysis for antler measures. The dashed line indicates the significance threshold equivalent to $\alpha = 0.05$. Points are colour-coded by chromosome. Underlying data is provided in Table S6

369 Distribution and quantification of SNP effect sizes

370 A total of 897 unique SNPs had significant non-zero effects across the ten antler
 371 measures (Table 4; full results are provided in Table S11). The number of significant
 372 non-zero SNPs ranged from 15 SNPs (antler length) to 279 SNPs (lower beam
 373 circumference; Table 4), although antler weight and upper beam circumference had no
 374 significant non-zero effect SNPs. Several SNPs showed pleiotropic effects i.e. they were
 375 associated with more than one measure (Table S11), with the underlying proportion
 376 ranging from 6% (coronet-tray junction) to 68% (tray length). Lower beam circumference
 377 and antler form showed distributions that included more extreme SNPs with large effects
 378 on phenotype outside the 5% to 95% quantile boundaries, whereas most other measures
 379 had more uniformly distributed effect sizes (Table 4). For the 11 antler PCs, only 159
 380 unique SNPs had non-zero effects (Table S10; full results in Table S12). No pleiotropic
 381 SNPs were observed, most likely due to the independence of each PC. PC4 and PC9 had
 382 no non-zero effect SNP associations. Only about 25% of the 159 SNPs were in common
 383 with the 897 SNPs in the antler measure analysis. This was mainly due to the higher
 384 order PCs (PC6 to PC11) sharing no SNP associations with any antler measures, while
 385 other PCs had similar or more numbers of shared and unique SNPs (PC1 and PC2).

386 Some of the non-zero effect SNPs with large effect size estimates after FDR explained
 387 large proportions of overall genetic and phenotypic variance for their respective traits. The
 388 SNP *cela1_red_2_101997097* explained about 17% of the overall additive genetic and
 389 4% of phenotypic variance in antler length (SNP variance = 2.609, SE = 1.094), while for
 390 PC8 (which is strongly representative of antler length; Figure 2) the marker
 391 *cela1_red_4_41756873* explained 39% of the additive genetic and 4% of the phenotypic
 392 variance (SNP variance = 0.019, SE = 0.008). Despite these seemingly large effect
 393 marker associations the standard errors for the SNP variance estimates were generally
 394 large (see Tables S13 and S14 for summaries of variances, and Tables S15 and S16 for
 395 full results for the antler measures and PCs, respectively). Overall, as the uncertainty
 396 around the importance of the SNP effects is large and none of these marker achieved
 397 genome-wide significance in any of the GWAS, the influence of specific SNP loci on
 398 variation in antler measures and PCs must be interpreted with caution.

Table 4: Summary of SNPs with non-zero effects on antler measures. N SNPs is the number of SNPs with non-zero effects. Proportion pleiotropic is the proportion of SNPs with non-zero effects on other antler measures. Maximum and minimum effects are given relative to the data scale (units = cm for lengths, g for weight). Lower and upper quantiles refer to the 5% and 95% boundaries of the effect size distribution respectively.

Antler Measure	N SNPs	Proportion Pleiotropic	Maximum Effect	Minimum Effect	Lower Quantile	Upper Quantile
Antler Length	15	0.4001	0.668	-0.622	-0.619	0.653
Coronet Circ.	56	0.214	0.151	-0.159	-0.152	0.146
Lower Beam Circ.	279	0.136	0.251	-0.151	-0.120	0.144
Upper Beam Circ.	0	-	-	-	-	-
Coronet-Brow Junc.	29	0.138	0.092	-0.084	-0.084	0.091
Coronet-Tray Junc.	110	0.055	0.460	-0.490	-0.435	0.441
Brow Length	272	0.081	0.538	-0.532	-0.484	-0.477
Tray Length	6	0.667	0.357	-0.357	-0.355	0.354
Antler Weight	0	-	-	-	-	-
Form	193	0.124	1.018	-0.097	-0.091	0.096

399 Discussion

400 In this study, we have used a genomic approach to determine the genetic architecture of
 401 antler morphology in the red deer of Rum. We have shown that antler morphology is
 402 heritable and repeatable over an individual's lifetime, and that it is likely to be highly
 403 polygenic with a moderate degree of shared genetic architecture across different antler
 404 traits. Genome-wide association and regional heritability studies failed to identify any
 405 genomic regions associated with antler measures and their principal components, with
 406 the exception of a single region on linkage group 21 associated with variation in PC9.
 407 Most traits were underpinned by SNPs with uniformly small effect sizes, whereas others,
 408 such as coronet circumference and antler form, showed distributions that included some
 409 SNPs with larger effects on phenotype. Our findings suggest that antler morphology has a
 410 highly polygenic architecture with many loci of small effect. Here, we discuss how our
 411 findings build on previous quantitative genetic studies in the Rum red deer system, and
 412 how they inform the broader question of the distribution of genetic architectures of
 413 sexually selected male weaponry and the consequences for its evolution.

414 Heritability and repeatability of antler morphology.

415 All 10 antler measures were significantly heritable (ranging from 0.229 to 0.414, Table 2)
 416 and were similar to previous heritability estimates for antler weight and form in the same
 417 population (Kruuk *et al.*, 2014). The strong agreement of estimates from both the pedigree
 418 and GRM approaches indicate that the SNPs present on the red deer SNP chip are in
 419 sufficiently high LD with causative loci to allow accurate estimation of trait heritabilities
 420 in this population (Yang *et al.* 2010; Figure 3). Indeed, LD is maintained at a relatively
 421 constant level over a distance of up to 1Mb, after which it starts to slowly decay (Figure
 422 S1). All antler traits were also highly repeatable, with between 38.5% and 66.1% of the
 423 phenotypic variance explained by additive genetic, permanent environment and birth year
 424 effects. This indicates that antler morphology is temporally stable over an individual's adult
 425 life, despite the annual shedding and regrowth of antlers. Similar findings were found for

the 11 principal components, with lower order PCs generally exhibiting higher heritabilities and repeatabilities (Table S2); we discuss why we think this is the case in the section on genetic correlations and constraints below. It should be noted that the heritabilities may be slightly over-estimated due to any common environments on a fine spatio-temporal scale that cannot be captured by the animal models (Stopher *et al.*, 2012). These findings support previous work showing that traits under sexual selection can have substantial underlying genetic variation in wild populations (Kruuk *et al.*, 2008; Merila *et al.*, 2001; Pomiankowski & Moller, 1995), where morphological traits often show heritabilities of a similar magnitude even when associated with fitness (Johnston *et al.*, 2013; Bérénos *et al.*, 2014; Bourret *et al.*, 2017; Malenfant *et al.*, 2018).

The polygenic architecture of antler morphology.

Genome-wide association studies and regional heritability analyses across all antler traits and PCs showed no significant associations, with the exception PC9 (discussed in the next section). Generally, GWAS can only detect loci with moderate to large effects on phenotype and which only partially explain the trait heritabilities, with the remainder termed the “missing heritability” (Manolio *et al.*, 2009; Golan *et al.*, 2014). For example, a meta-analysis of human GWAS found that the heritability attributed to all common SNP variants was significantly higher than that of the SNPs that achieved genome-wide significance. (Shi *et al.*, 2016), meaning that large numbers of “non-significant” SNPs will contribute to the additive genetic variation. To characterise the genetic architecture beyond GWAS alone, we employed two additional approaches. The first, regional heritability (Nagamine *et al.*, 2012), incorporated the haplotypic diversity within genomic regions. This approach detected a large contribution of defined genomic regions to a single PC, but not to any of the other antler measures or PCs. The second, the Empirical Bayes false discovery rate and effect size estimation, incorporated information from the GWAS effect size estimates and their error to show that a substantial number of SNPs had non-zero effects on antler morphology (Stephens, 2016). Taken together, these analyses provide compelling evidence that most aspects of antler morphology have a highly polygenic architecture (Fisher, 1930; Barton *et al.*, 2017). This is in line with findings

from other studies of wild organisms that have identified polygenic architectures for morphological and life history traits (Robinson *et al.*, 2013; Santure *et al.*, 2013; Pallares *et al.*, 2014; Berenos *et al.*, 2015; Husby *et al.*, 2015). In this study, we modelled antler morphology using both independent measures of antler morphology and a principal component framework to characterise different dimensions of shape variation. An advantage of using both approaches was that it allowed us to identify a greater number of potentially causal loci, which supports the usefulness of this approach when trying to characterise the genetic architecture of a complex morphological trait (such as in Pallares *et al.* 2014).

Genomic regions associated with antler morphology.

Three genomic windows within the CEL linkage group were associated with PC9 at the genome-wide level. The main contributing antler measure to PC9 is coronet circumference. The region covered by the most highly associated window explained ~66% of the additive genetic variance, representing a large part of the overall heritability estimated using the whole GRM (~19%). The region contains a number of candidate genes among which are gasdermin C (*GSDMC*); MYC proto-oncogene (*MYC*); ArfGAP with SH3 domain, ankyrin repeat and PH domain 1 (*ASAP1*) and cellular communication network factor 4 (*CCN4*). Whilst none have previously been implicated in antler morphology, they have associated functions that make them potential candidate genes. Both the enhancer protein *ASAP1* and *CCN4*, which is a type of connective tissue growth factor, are linked to bone ossification and bone cell differentiation in mice (The Jackson Laboratory, 2019; Schreiber *et al.*, 2019; Maeda *et al.*, 2015), processes which are likely to be vital to antler regeneration, rapid growth and the ability of antlers to withstand impact (Goss, 1983). Upregulation of *GSDMC* is implicated in carcinogenesis in mice, as a consequence of an interrupted growth factor signalling pathway (Miguchi *et al.*, 2016) and *MYC* is a potent oncogene that is implicated in many human cancers and promotes rapid tumor cell proliferation (Beroukhi *et al.*, 2010; Lin *et al.*, 2012); recent work suggests that rapid regeneration of bony antlers has evolved by upregulating cell proliferation pathways while suppressing tumorigenesis (Wang *et al.*, 2019).

484 Despite being linked to compelling candidate gene regions, with our current data it is
485 virtually impossible to determine exactly which genes in this region drive the association
486 with PC9. Furthermore, as PC9 only represents ~4% of the overall phenotypic variance
487 among all antler PCs, we expect effect sizes of causal loci to be very small. Validating the
488 findings for this association would be challenging, as replication of a similar PC in other
489 deer populations would have to consider its main contributing antler measures (e.g.
490 coronet circumference); additionally, the same variants may not be associated with this
491 trait in different populations. A more feasible approach may be to type a higher density of
492 SNP loci to characterise more variation at (or in tight linkage) with potential causal loci in
493 the Rum deer population.

494 **Genetic correlations and constraints on antler morphology.**

495 Almost all antler traits were positively genetically correlated, with the exception of the
496 coronet-tray junction, which was negatively genetically correlated with tray length, lower
497 beam circumference and antler form. These findings were reflected by the PC analysis,
498 where the PC explaining the most variance in antler morphology (PC1, ~41%) combined
499 equal information from all antler measures, whereas those explaining declining amounts
500 of variation represented one or two antler traits. The Empirical Bayes analysis identified
501 varying degrees of marker pleiotropy associated with the antler measures (Table 4) that
502 were consistent with the observed genetic correlations. Nevertheless, there were some
503 exceptions, such as for brow length and antler weight, which both showed strong positive
504 genetic correlations yet had small proportions of shared loci (brow length) or no
505 associated loci at all (antler length). This incongruity may be explained by the large
506 variation in the number of non-zero effect SNPs detected between antler measures, which
507 could be due to differences in the effect size distribution of markers.

508 The large heritabilities of individual antler traits suggest there is potential for response to
509 selection, but our results further add to previous findings that constraints at the genetic
510 level may affect how the population may respond to selection. Previous work showed that
511 a large part of genetic variance in antler weight is not available to selection due to the lack

of genetic covariance between weight and fitness (Kruuk *et al.*, 2002, 2014). In the current study, antler weight was highly correlated with most other antler measures on a genetic level, which may likely indicate that large parts of the genetic variance of these antler measures are also unavailable for selection, thus limiting the evolutionary potential of antler morphology despite large heritability estimates. In the PC analysis, higher order PCs explained much smaller amounts of variation and had very low additive genetic variation. It is possible that these PCs could represent morphologically stable aspects of the antlers, where lower heritabilities could indicate past strong stabilising selection on the combination of trait aspects represented by that PC. Other studies exploring multivariate sexual selection on a suite of traits found that despite large genetic variance in univariate analyses, there can be very little genetic variance available in the trait composition that is the target of selection (Hunt *et al.*, 2007; Van Homrigh *et al.*, 2007).

The discovery that the genetic architecture of antler morphology is highly polygenic also suggests that further evolutionary mechanisms may be partially responsible for the maintenance of genetic variation in this trait. As discussed in the introduction, traits with many genes of small effect can present a large mutational target for the introduction of novel genetic variation which can contribute to the genetic variation in a trait (Rowe & Houle, 1996). Consequently, although selection on polygenic traits can lead to rapid changes in trait mean, under the infinitesimal model the distribution of underlying genetic effects is expected to remain relatively constant, counteracting the loss of genetic variation. (Barton *et al.*, 2017; Sella & Barton, 2019). Finally, pleiotropic effects of loci that share a similar complex architecture with other traits and/or are in LD with loci associated with fitness could maintain genetic variation through conflicts and trade-offs (Lande, 1982).

Conclusions

In this study, we have shown that antler morphology is heritable, has a polygenic genetic architecture, and some degree of shared genetic architecture between different antler measures. A single region association is linked to candidate genes that could potentially

540 have an effect on antler morphology, but more work would be required to validate this
541 finding in this and other populations. Future work in this system will integrate knowledge
542 of the genomic architecture of antler morphology with fitness measures to further dissect
543 constraints on trait evolution within this population. Ultimately, our findings corroborate the
544 expectation for a quantitative trait such as multidimensional weaponry traits to conform to
545 a polygenic genetic architecture of many genes with small effects.

546 **Author Contributions**

547 L.P. and S.E.J. designed the study. J.M.P and L.K provided the data. L.P., J.H and S.E.J.
548 analysed the data. L.P. and S.E.J. wrote the first draft of the paper and all authors
549 contributed to revisions.

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