Genomic analysis of morphometric traits in bighorn sheep using the Ovine Infinium® HD SNP BeadChip

Joshua M. Miller[1,3], Marco Festa-Bianchet[2] and David W. Coltman[1]

[1] Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada
[2] Département de biologie, Université de Sherbrooke, Sherbrooke, Québec, Canada
[3] Current affiliation: Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

ABSTRACT

Elucidating the genetic basis of fitness-related traits is a major goal of molecular ecology. Traits subject to sexual selection are particularly interesting, as non-random mate choice should deplete genetic variation and thereby their evolutionary benefits. We examined the genetic basis of three sexually selected morphometric traits in bighorn sheep (*Ovis canadensis*): horn length, horn base circumference, and body mass. These traits are of specific concern in bighorn sheep as artificial selection through trophy hunting opposes sexual selection. Specifically, horn size determines trophy status and, in most North American jurisdictions, if an individual can be legally harvested. Using between 7,994–9,552 phenotypic measures from the long-term individual-based study at Ram Mountain (Alberta, Canada), we first showed that all three traits are heritable ($h^2 = 0.15$–0.23). We then conducted a genome-wide association study (GWAS) utilizing a set of 3,777 SNPs typed in 76 individuals using the Ovine Infinium® HD SNP BeadChip. We found suggestive association for body mass at a single locus (OAR9_91647990). The absence of strong associations with SNPs suggests that the traits are likely polygenic. These results represent a step forward for characterizing the genetic architecture of fitness related traits in sexually dimorphic ungulates.

Subjects Conservation Biology, Evolutionary Studies, Genomics
Keywords GWAS, SNP, Animal model, *Ovis canadensis*

INTRODUCTION

A goal of molecular ecology is to identify the genetic architecture underlying traits of ecological relevance (*Ellegren & Sheldon, 2008*; *Slate et al., 2009*). There is a particular interest in finding regions associated with variation in fitness-related traits, as such traits and the loci underlying them are expected to be subject to strong selection. Under strong directional selection, the genetic variability underlying fitness-related traits should rapidly go to fixation, and yet much phenotypic variation in such traits is observed in the wild (*Kruuk, Slate & Wilson, 2008*; *Chenoweth & McGuigan, 2010*). Elucidating the genetic basis of fitness-related traits might help clarify how phenotypic variation is maintained, for example by overdominance or epistatic interactions (e.g., *Lappalainen et al., 2011*; *Johnston et al., 2013*).

Sexual selection poses particularly interesting scenarios for fitness-related traits, leading to the so called lek paradox (*Borgia, 1979*). Under sexual selection, mate choice is either non-random, based on an ornamental trait that may confer benefits to the offspring, or members of one sex compete for access to mates using dimorphic secondary sex characteristics. Over time, under both scenarios evolution should deplete the genetic variation underlying the selected trait, and thereby diminishing offspring benefits. Paradoxically, however, in many systems choice for such traits continues. A classic example of a secondary sexual characteristic often subject to sexual selection is horn size in bovids. Horns are characterized by a keratin sheath around a bony projection from the skull that grows continuously throughout life (*Davis, Brakora & Lee, 2011*). Across a number of species, horn size in males determines social status and mating access to females (*Bro-Jørgensen, 2007*). Thus, there is likely selection for variants that confer the ability of individuals to grow large horns, and yet variation remains.

In domestic sheep (*Ovis aries*) some breeds have horns while others are polled (lacking horns entirely), and from an agronomic production standpoint there is interest in removing horns from certain breeds (*Kijas et al., 2012*). Soay sheep, a primitive breed now living feral on the islands of St. Kilda, Scotland, have an additional horn "morph". In females, there are three morphs: normal, scurred (deformed horns composed only of keratin sheaths), and polled. In males there are only two morphs: normal horns and scurs (*Johnston et al., 2010*; *Johnston et al., 2011*).

The genetic basis of these differences in horn morphology and development in domestic sheep has been investigated. A single genomic region on chromosome 10 is associated with the presence and absence of horns in domestic breeds (*Kijas et al., 2012*) including Soay sheep (*Johnston et al., 2010*; *Johnston et al., 2011*), and is linked to quantitative differences in horn length of normal-horned male Soay sheep (*Johnston et al., 2011*). This region contains a single gene, relaxin/insulin-like family peptide receptor 2 (RXFP2). RXFP2 affects osteoporosis and testicular descent in mice and humans (*Ferlin et al., 2008*; *Feng et al., 2009*; *Yuan et al., 2010*); thus, its association with both bone development and secondary sexual characteristics make it an interesting candidate for influencing horn morphology. Furthermore, different genotypes at this locus in male Soay sheep are associated with trade-offs between reproductive success and longevity, which may maintain the different horn morphs through heterozygote overdominance (*Johnston et al., 2013*). Finally, though not the major QTL underlying horn phenotype, RXFP2 has been implicated in horn development in several association studies in cattle (*Bos taurus*) (*Gautier & Naves, 2011*; *Allais-Bonnet et al., 2013*; *Wiedemar & Drögemüller, 2015*), indicating that it may have similar function across species.

Bighorn sheep (*Ovis canadensis*) are iconic North American wild sheep named for their large horns. All individuals have normal horns, though males have much larger horns than females. Previous research has shown that horn size and body mass are important for intrasexual competition among males for reproductive access to females (*Coltman et al., 2002*; *Martin et al., 2013*). However, for female bighorn sheep horn length was found to be unrelated to social rank or other life history characteristics, which were more determined by body mass and age (*Favre, Martin & Festa-Bianchet, 2008*). In addition, horn size

determines the trophy status of a male and, in many jurisdictions, whether it can be legally harvested. Such regulations directly influence longevity, as males with fast-growing horns are removed at a younger age (*Festa-Bianchet et al., 2008*; *Festa-Bianchet et al., 2014*; *Bonenfant et al., 2009*; *Hengeveld & Festa-Bianchet, 2011*; *Pigeon et al., 2016*).

Previous studies of bighorn sheep have shown that both horn size and body mass are heritable (*Coltman, 2005*; *Coltman et al., 2005*; *Poissant et al., 2008*; *Poissant et al., 2012*) and quantitative trait locus (QTL) mapping with microsatellite loci highlighted several suggestive regions for different aspects of horn morphology (e.g., volume and base circumference) as well as body mass (*Poissant et al., 2012*). These regions appear on several chromosomes, but notably included suggestive QTLs for horn volume and base circumference in males on chromosome 10 that spans the region predicted to contain RXFP2. This same region was highlighted by *Kardos et al. (2015)* who used pooled genome sequencing to search for signatures of selective sweeps in bighorn sheep. Analyses from pooled samples representing 58 bighorn sheep from three populations across Montana (USA) found 21 regions showing signs of selective sweeps. The strongest signal from these regions contained RXFP2, and a comparison to haplotypes in Soay sheep (*Johnston et al., 2013*) suggested that the selected alleles are associated with large horn size (*Kardos et al., 2015*). However, there has yet to be an individual-based study of the genomics of horn size, as *Kardos et al. (2015)* was based on pooled sequencing, and no assessments have been done of other fitness related traits.

In this study, we build on these results using individual phenotypic data from a long-term study of marked sheep followed throughout their lives at Ram Mountain, Alberta Canada. Though the costs of genome sequencing continue to decline, large-scale resequencing projects are still out of reach for many study systems. Therefore, we capitalize on the close evolutionary relationship between domestic and bighorn sheep (*Bunch et al., 2006*) to efficiently genotype many SNPs in many individual bighorn sheep using a genomic technology developed for domestic sheep. By investigating a suite of morphological characters, we aim to examine the genetic architecture underlying complex quantitative traits in wild sheep, and with respect to horn size, assess if the architecture is similar to its domestic relative.

METHODS AND MATERIALS

Population history and phenotypic data collection

Bighorn sheep at Ram Mountain (RM) are native, isolated and philopatric. Individual-based monitoring of the population began in 1972 (*Jorgenson et al., 1993*; *Jorgenson et al., 1997*). Most individuals are marked with unique tags as lambs, so over 95% are of known age. Individuals are followed throughout their lives. Every spring and summer, sheep are drawn into a corral trap baited with salt where several phenotypic measurements are taken, including body mass and horn measures (*Jorgenson et al., 1993*). Genetic sampling of the population began in 1988, and was used to build a genetic pedigree (*Coltman et al., 2002*; *Poissant et al., 2008*). In some cases, full or half siblings were inferred from unsampled males using the program COLONY (*Wang, 2013*). By 2013, the pedigree contained 864 maternal and 528 paternal links involving 1,134 sheep.

Phenotypic measures

This study uses data from animals captured under research protocols that were approved by the University of Alberta Animal Use and Care Committee, affiliated with the Canadian Council for Animal Care (Certificate 610901). We considered three morphological characteristics: average horn length, average horn base circumference, and body mass. Specifically, sheep were weighed to the nearest 250 g with a Detecto spring scale, while horn length (measured along the outside curvature) and base circumference were measured to the nearest millimeter with tape. Each trait measurement was standardized to a sex and age specific standard deviation of one (value divided by the SD for that sex in that age class). We only considered individuals aged 1 or greater to avoid maternal effects (*Wilson, Kruuk & Coltman, 2005*; *Poissant et al., 2012*), and pooled males aged ≥9 years and females aged ≥14 years to increase sample sizes in those age classes. Fewer than 10% of either sex lives to these ages (*Loison et al., 1999*).

Quantitative genetic analyses for morphological characteristics

Quantitative genetic variation in our morphological characteristics was estimated using a series of 'animal models'. Animal models are linear mixed effects models that incorporate pedigree information along with phenotypic measures to partition phenotypic variation (V_p) into that due to additive genetic variation (V_a), permanent environmental effects (V_{pe}), and residual variation (V_r) (*Kruuk, 2004*; *Wilson et al., 2010*). For our analyses, fixed effects included sex, age (as a factor), date of measurement (as a continuous, second-order polynomial), as well as all interactions between the three variables. Random effects were individual identity to account for permanent environmental effects associated with having repeated measures of individuals (V_{pe}), as well as year of birth (V_{yb}) and year of measurement (V_y) to account for environmental effects. Thus, phenotypic variation was broken into five components $V_p = V_a + V_{pe} + V_y + V_{yb} + V_r$.

The three morphological traits were modeled independently using univariate animal models run in ASReml version 3.0 (*Gilmour et al., 2009*), based on measurements taken between 1972 and 2012. To maximize statistical power, we considered both sexes simultaneously. Combining the sexes into a single model is justified as cross-sex genetic correlations were either large and positive (1.00 for horn length and 0.76 for body mass) or positive but not significant (0.03 for horn base circumference) indicating that we should capture the same genetic variation in both sexes (*Poissant et al., 2012*). The effect size of each random effect was calculated as the proportion of V_p explained by the random effect, and its significance tested by comparing a model with the term removed to the full model using a likelihood ratio test with one degree of freedom. From these models, we calculated heritability (h^2) of each trait as the ratio of V_a/V_p. We also recorded estimates of individual breeding values (V_a), calculated using best linear unbiased predictors (BLUPs), for use in selecting individuals for the association analyses (see below).

SNP genotyping

We chose 95 individuals for genotyping based on their breeding value for horn length. Specifically, we attempted to maximize our chances of detecting an association by choosing

an approximately equal number of individuals of each sex with the highest and lowest breeding values with respect to horn length (*Li et al., 2011*; *Barnett, Lee & Lin, 2013*). The range of high values was 0.18 to 0.65 for males and 0.16 to 0.44 for females, while the range of low values was −0.41 to −0.81 for males and −0.35 to −0.61 for females. The selected individuals were typed on the Ovine Infinium® HD SNP BeadChip an array originally developed for domestic sheep that contains 606,006 loci distributed throughout the domestic sheep genome (*Kijas et al., 2014*). Initial assessment of genotype quality was performed using Genome Studio version 2011.1 (Genotyping Module 1.9; Illumina, San Diego, CA, USA). We used cluster information based on 288 domestic sheep samples representing a diversity of breeds (provided by the International Sheep Genomics Consortium) and discarded all loci with GenCall scores less than 0.6 and GenTrain scores less than 0.8. GenCall and GenTrain scores are calculated in Genome Studio as quality measures of individual genotypes and locus clustering, respectively. Genotypes were then exported to PLINK version 1.07 (*Purcell et al., 2007*) for additional filtering. Specifically, we considered only those loci which mapped to the autosomes in domestic sheep, had a minor allele frequency >5%, and were in Hardy-Weinberg Equilibrium (adjusted $p > 1.28 \times 10^{-5}$) in our sample set ($N = 3{,}777$ remaining). Finally, we used VIPER (*Paterson et al., 2012*) to check for evidence of pedigree inconsistencies in our dataset. Specifically, this program implements an inheritance-checking algorithm based on a provided pedigree.

Genome-wide association study (GWAS) analyses

Traditional GWAS methods are not designed for repeated measure data (*Rönnegård et al., 2016*). Alternatives have included fitting individual average values or using breeding values as the phenotypic measure (*Johnston et al., 2011*; *Santure et al., 2013*). However, both methods produce undesirable results including inflated association statistics (*Hadfield et al., 2010*; *Ekine et al., 2014*). Therefore, we used an alternative method designed for repeated measure data that simultaneously considers phenotypic and SNP data. Specifically, we used the R package *RepeatABEL* version 1.8-0 (*Rönnegård et al., 2016*) an extension of the *GenABLE* package (*Aulchenko et al., 2007*; *Karssen, Van Duijn & Aulchenko, 2016*).

RepeatABEL solves the issue of using repeated measures in GWAS by conducting analyses in two steps. First, a base linear-mixed effect model is fit without SNP effects but including a genome-wide relationship matrix (GRM) to account for polygenic effects and individual ID as a random effect to account for repeated measures. In our analyses, the base model had the same structure as that used in the ASREML analyses above. Second, the estimated (co)variance matrix from the first step is used when individual SNPs are tested for association with the phenotype. Specifically, associations are assessed using a linear model and p-values are calculated with a Wald statistic. We fit separate models for horn length, horn base circumference, and body mass.

To correct for multiple testing we used *Keff* (*Moskvina & Schmidt, 2008*) to determine significance thresholds genome-wide, and for each chromosome individually assuming an alpha value of 0.05. Association results were then visualized with Manhattan plots created using the *ggplot2* package version 1.0.0 (*Wickham, 2009*). All analyses were conducted in R version 3.2.4 (*R Core Team , 2015*).

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Table 1 Proportion of phenotypic variance after having accounted for fixed effects in the full datasets. Variance components of morphometric traits after having accounted for fixed effects in the full datasets; standard errors generated by the statistical software package ASReml version 3.0 (*Gilmour et al., 2009*) are shown in parentheses unless otherwise noted.

| Trait	Ind[a]	Obs[b]	Mean (s.d.)	Transformed data mean (s.d)	V_p	h^2	V_y	V_{yb}	V_{pe}
Horn length (mm)	652	8,011	27.40 (16.98)	6.62 (2.46)	0.85 (0.04)	0.15 (0.05)[**]	0.07 (0.02)[**]	0.10 (0.03)[**]	0.42 (0.05)[**]
Horn base circumference (mm)	637	7,994	17.33 (8.33)	12.00 (4.49)	0.84 (0.04)	0.23 (0.05)[**]	0.08 (0.02)[**]	0.11 (0.03)[**]	0.27 (0.04)[**]
Body mass (kg)	677	9,552	58.69 (15.85)	7.39 (2.00)	0.58 (0.03)	0.20 (0.04)[**]	0.16 (0.03)[**]	0.07 (0.02)[**]	0.24 (0.04)[**]

Notes.
[a] Numer of individuals.
[b] Number of phenotypic measurements.
[**] $P < 0.00001$.

We examined gene annotations in the domestic sheep genome near suggestive loci (see 'Results'). To determine the genomic window within which to search, we estimated the 'half-length' of linkage disequilibrium (LD) for our marker set, i.e., the inter-marker distance at which LD decreased to half its maximal value (*Reich et al., 2001*). This value is thought to reflect the extent to which an association between genotypes at a given locus and a QTL can be detected. For this analysis we used PLINK version 1.90b2l (*Chang et al., 2015*) to calculate pairwise values of r^2 between syntenic markers on all chromosomes ($n = 370,568$ pairwise comparisons). These estimates were then compared to inter-marker physical distance based on map positions from the domestic sheep genome, and half-length was calculated using a custom script which calculated LD decay rate as in Appendix 2 of *Hill & Weir (1988)*.

RESULTS

Average horn length, horn base circumference, and body mass all showed positive phenotypic correlations, with the magnitude much stronger in males than females (Table S1). All three morphological traits also exhibited significant additive genetic variation, with values on par with other studies of this population (Table 1). In total, 95 individuals were genotyped on the SNP chip and used to filter loci based on GenTrain and GenCall scores. One individual was subsequently removed from further analyses after significant (>5%) pedigree inconsistencies were found. Of the original 606,006 loci on the chip, 474,277 returned genotypes in bighorn sheep. Subsequent filtering removed 8,528 loci based on their levels of missing data, 469,822 based on our minor allele threshold, and 127 loci based on HWE equilibrium. The final dataset contained 3,777 loci, with at least 60 markers on each autosome (average $\pm$ SD $= 145.3 \pm 88.6$; Table S2). Such reductions in the number of polymorphic loci are expected in cross-species application of SNP chips (*Miller et al., 2012*). Of the 94 originally genotyped individuals, 76 had morphological measures and were used in subsequent analyses.
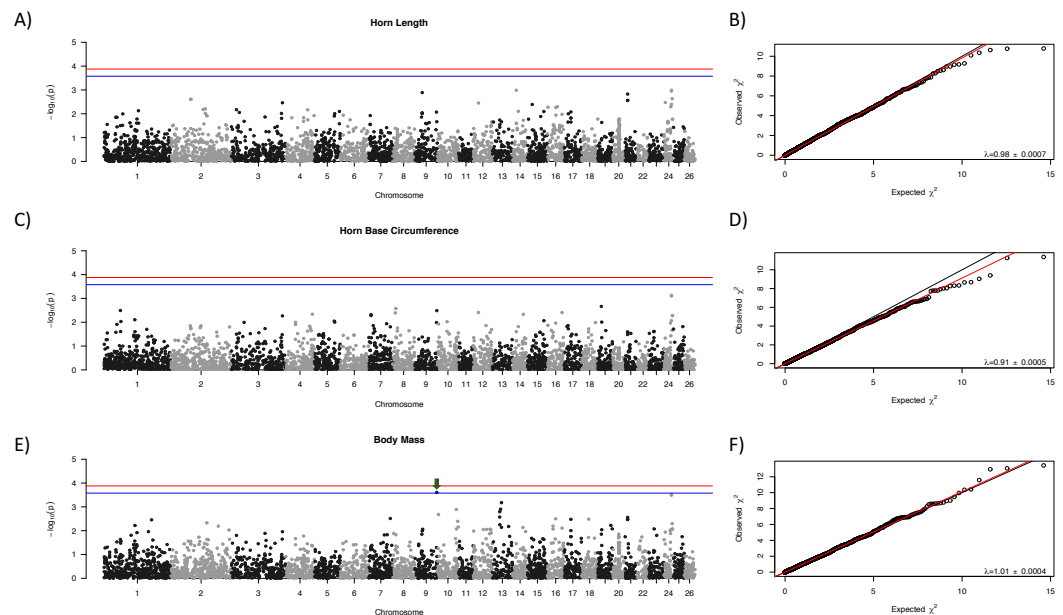
Figure 1 Manhattan plots for morphological characteristics. Horn length (A), horn base circumference (C), and body mass (E). The blue line represents the genome-wide significance threshold; the red line represents the threshold for suggestive association. Positions are relative to the domestic sheep genome assembly (version 3.1; *Jiang et al., 2014*). The green arrow indicates the suggestive locus for body mass. Next to each Manhattan plot is the corresponding QQ-plot (B, D, and F), with the genomic inflation factor (λ) and standard error indicated in the bottom right of each plot. The black line shows a 1:1 correspondence while the red line is a regression through the observed data.

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Manhattan plots for each trait are shown in Fig. 1 with corresponding QQ-plots. In all cases genomic inflation (λ) was ≤1, indicating that there was no underlying population structure or other factors which could lead to false positive associations (*Freedman et al., 2004*; *François et al., 2016*). No loci were associated at the genome-wide significance level to any of the morphological traits examined. One locus, OAR9_91647990, showed suggestive association with body mass (Fig. 1, indicated with a green arrow).

As expected, there was a general decrease in LD with increasing inter-marker distance, and half-length was estimated to be 412,834 bp (Fig. 2). Based on this half-length estimate we extracted gene names from the *Ovis aries* gene set (Oar v3.1, genebuild Mar 2015) within a 413,000 bp window on either side of the candidate marker using BioMart (*Kinsella et al., 2011*) and Ensembl version 89 (*Flicek et al., 2014*). This returned two genes: U6 spliceosomal RNA, and ENSOARG00000026555, a long intergenic non-coding RNA. No gene ontology (GO) terms were available for either of these genes, and we do not see an immediate connection with body mass.

DISCUSSION

We examined the genetic bases of three fitness-related characteristics in bighorn sheep. To do so, we utilized a new genomic technology originally designed for domestic sheep to rapidly genotype markers in a wild species, then combined these data with phenotypic
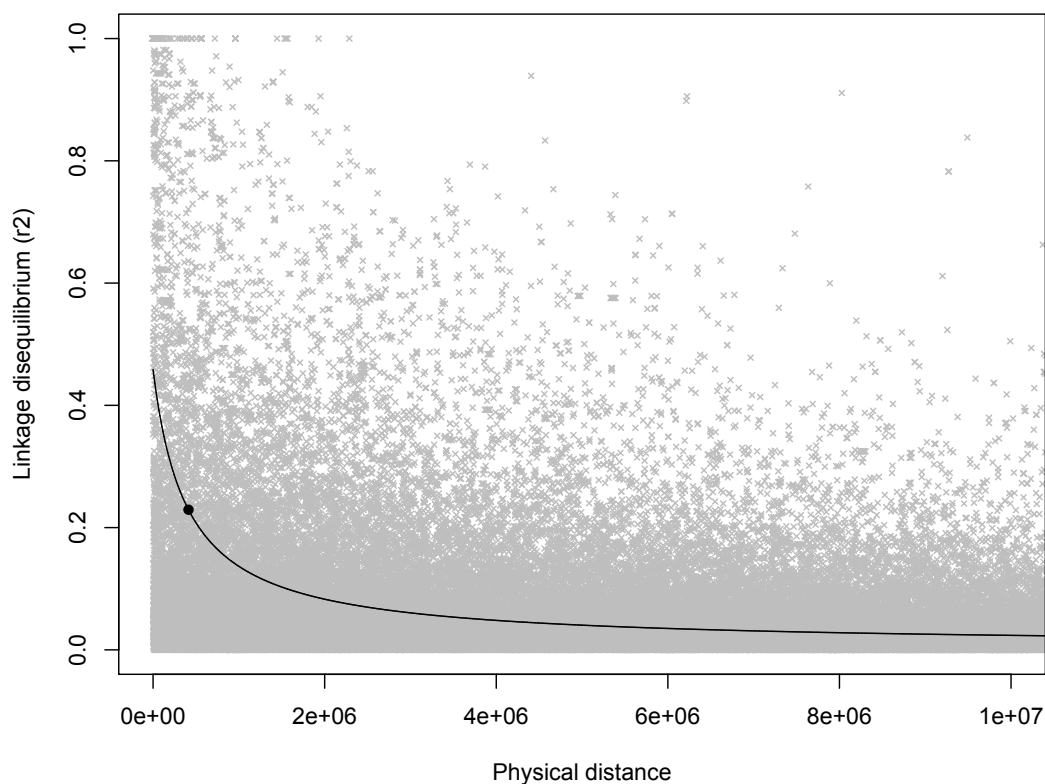
Figure 2 Scatterplot of LD estimates versus inter-markers distance. A non-linear least squares regression line is shown, with the round point indicating the half-length estimate.

measures from a long-term individual-based study. We found one locus with suggestive associations to body mass (Fig. 1). Previous QTL mapping with microsatellite loci for these same traits in the RM population highlighted several candidate regions (*Poissant et al., 2012*); however, our suggestive locus is not near any of the QTLs described in *Poissant et al. (2012)*. In addition, we found no overlap in location between the locus found here and morphological traits in the domestic sheep QTL database (*Hu, Fritz & Reecy, 2007*; *Hu et al., 2013*). While it is possible that the sample sizes used in the *Poissant et al. (2012)* led to an overestimation of effect sizes due to the Beavis Effect (*Slate, 2013*) we note that the methods underlying QTL mapping and GWAS analyses are different (*Slate et al., 2010*). Specifically, QTL mapping relies on informative meioses within a pedigree of related individuals, while GWAS uses linkage disequilibrium between loci. In addition, the sample sizes differed, with fewer individuals included in the work presented here. These differences could influence the associations detected.

It is somewhat surprising that we did not see even a suggestive association between horn morphology and the region surrounding RXFP2 on chromosome 10 given the very strong links seen in both domestic sheep and cattle (*Gautier & Naves, 2011*; *Johnston et al., 2011*; *Johnston et al., 2013*; *Kijas et al., 2012*; *Wiedemar et al., 2014*) as well as the suggestive QTL for horn volume in bighorn sheep in this same region (*Poissant et al., 2012*). However,

based on the estimate of half-length (412,834 bp) it appears as if we did not have sufficient marker coverage to adequately test for associations in the horns region. Within our set of loci the closest marker to RXFP2 was 698,861 bp away.

It is interesting that the extent of LD reported here (~400,000 bp) is an order of magnitude less than found in a previous assessment of LD in bighorn sheep from RM (~4,000,000 by *Miller et al., 2011*) using an order of magnitude fewer markers (308 vs. 3,777 loci). Analogous decreases in LD with the addition of markers have been seen in other species including cattle (*McKay et al., 2007*; *Porto-Neto, Kijas & Reverter, 2014*), domestic sheep (*García-Gámez et al., 2012*; *Kijas et al., 2014*), and flycatchers (*Ficedula albicollis*; *Backström et al., 2006*; *Kawakami et al., 2014*).

In light of our failure to detect genome-wide significant associations, we more formally quantified the expected power of a marker to detect a hypothetical causal QTL given the average minor allele frequency and genome wide critical *p*-value for the loci in this study. To do so we used an R script developed by *Minikel (2012)* which implements the QTL association feature of the Genetic Power Calculator (*Sham et al., 2000*; *Purcell, Cherny & Sham, 2003*). Specifically, this script estimates the expected power to detect an association given an estimate of the QTL effect size, the number of samples genotyped, and the average level of linkage disequilibrium among markers. For our analyses we varied effect sizes from 0–1.0, sample sizes between 50–500 individuals, and three levels of linkage disequilibrium (0.75, 0.50, and 0.25). This exploration showed that even at extreme effect sizes for the QTL and levels of LD well above what was seen at the half-length estimate (~0.23; Fig. 2), the number of samples used in our GWAS analyses was likely not enough to have the power to detect all associations (Fig. 3). Note that these simulations assume that unrelated individuals were used in the GWAS, so the presence of related individuals in our test set will boost power slightly. In general, the simulations indicate that our marker coverage likely increased the chance of Type II errors (missing true associations). Similar results were found with simulations and whole genome sequences of collared flycatchers (*Kardos et al., 2016*). However, we do not believe this diminishes the association observed, as it has no effect on Type I errors (detecting false associations).

The power of our association analyses was likely also weakened by the cross-species application of a SNP chip originally derived for domestic sheep. While the two species are closely related (*Bunch et al., 2006*), and have a highly syntenic karyotype (*Poissant et al., 2010*), loci were selected for inclusion on the chip based on variability in domestic sheep, leading to ascertainment bias when applied to bighorn sheep (*Lachance & Tishkoff, 2013*). This bias would also increase the chance of Type II errors, as we are unable to assess bighorn sheep specific variants.

Recent research has suggested that a selective sweep occurred around the RXFP2 region in bighorn sheep (*Kardos et al., 2015*). In this scenario, multiple generations of sexual selection for large horns led to the fixation of genetic variation in the RXFP2 regions. If true, that fixation would preclude detection of associations in the current study. The region described by *Kardos et al. (2015)* spans ~350,000 bp and while the Ovine Infinium® HD SNP BeadChip contains 57 SNPs in this region, none of these loci were polymorphic in our sample of sheep from RM.

A) **LD=0.75**

B) **LD=0.50**

C) **LD=0.25**

D)
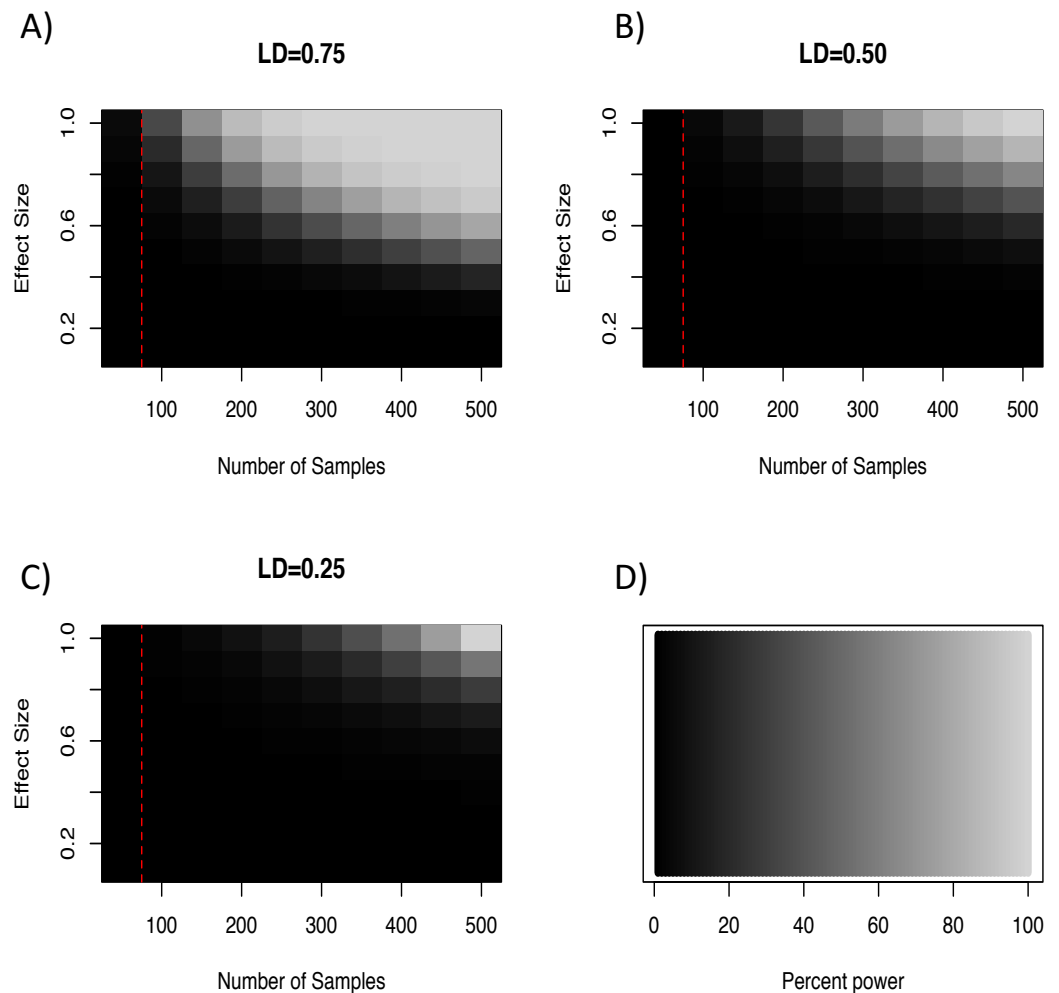
Figure 3 Heat maps of expected percent power of a GWAS as a function of sample size and effect size for linkage disequilibrium (LD) estimates of 0.75 (A), 0.50 (B), and 0.25 (C). Light colors indicate higher power to detect associations (D). Dotted red lines correspond to the number of samples used in this study ($N = 76$).

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Finally, the lack of strong associations could be due to the fact that in this species these complex phenotypes are not single-locus traits. Instead, there may be many loci of small effect that jointly contribute to the phenotype, similar to the "missing heritability" phenomenon seen in many quantitative traits (*Manolio et al., 2009*; *Yang et al., 2010*). Other studies of the genetic architecture of complex phenotypes in wild populations have also found that they tend not to be controlled by single loci of large effect, but rather are polygenic (*Husby et al., 2015*; *Bérénos et al., 2015*; *Kardos et al., 2016*; *Silva et al., 2017*). New methods, such as chromosome partitioning, can now investigate this possibility (*Yang et al., 2011*; *Robinson et al., 2013*; *Santure et al., 2013*). Unfortunately, we cannot utilize chromosome partitioning at this time due to the small number of individuals typed on the 700k SNP chip. Attempts to use this method with our data produced unstable estimates

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of per-chromosome heritability (results not shown). More broadly, if these traits are truly polygenic it helps to explain how their variation is maintained despite strong directional selection (*Rowe & Houle, 1996*).

CONCLUSION

The lack of associations found here highlights the challenges of identifying genes underlying traits in non-model systems. While cross-species application of this SNP chip provided a rapid and affordable way to genotype many loci across a large number of individuals, as high-throughput sequencing costs continue to decline we expect this method to be superseded by those that allow for simultaneous marker discovery and genotyping in the species of interest (e.g., *Andrews et al., 2016*). Future studies could build on our findings by using high-throughput sequencing to increase the number of loci, individuals, and populations used. Improved genomic resources for bighorn sheep (*Coltman, Hogg & Miller, 2013*; *Kardos et al., 2015*; *Miller et al., 2015*) including whole genome sequence will enable fine mapping of associations, as well as detection of novel associations. Consideration of additional populations will allow for assessing the consistency of associations observed. In addition, haplotype-based analyses (*Browning & Browning, 2011*) or chromosome partitioning methods (*Yang et al., 2011*; *Robinson et al., 2013*; *Santure et al., 2013*) can detect novel associations and highlight if the traits fit a polygenic framework.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

David W Coltman is an Academic Editor for PeerJ.

Author Contributions

- Joshua M. Miller conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Marco Festa-Bianchet contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
- David W. Coltman conceived and designed the experiments, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

This study uses data from animals captured under research protocols that were approved by the University of Alberta Animal Use and Care Committee, affiliated with the Canadian Council for Animal Care.

Data Availability

The following information was supplied regarding data availability:

SNP genotypes and morphological measurements are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.c0p090f.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.4364#supplemental-information.

REFERENCES

Allais-Bonnet A, Grohs C, Medugorac I, Krebs S, Djari A, Graf A, Fritz S, Seichter D, Baur A, Russ I, Bouet S, Rothammer S, Wahlberg P, Esquerre D, Hoze C, Boussaha M, Weiss B, Thepot D, Fouilloux MN, Rossignol MN, Van Marle-Koster

E, Hreidarsdottir GE, Barbey S, Dozias D, Cobo E, Reverse P, Catros O, Marchand JL, Soulas P, Roy P, Marquant-Leguienne B, Le Bourhis D, Clement L, Salas-Cortes L, Venot E, Pannetier M, Phocas F, Klopp C, Rocha D, Fouchet M, Journaux L, Bernard-Capel C, Ponsart C, Eggen A, Blum H, Gallard Y, Boichard D, Pailhoux E, Capitan A. 2013. Novel insights into the bovine polled phenotype and horn ontogenesis in bovidae. *PLOS ONE* **8**(5):e63512 DOI 10.1371/journal.pone.0063512.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* **17**:81–92 DOI 10.1038/nrg.2015.28.

Aulchenko YS, Ripke S, Isaacs A, Van Duijn CM. 2007. GenABEL: an R library for genome-wide association analysis. *Bioinformatics* **23**:1294–1296 DOI 10.1093/bioinformatics/btm108.

Backström N, Qvarnström A, Gustafsson L, Ellegren H. 2006. Levels of linkage disequilibrium in a wild bird population. *Biology Letters* **2**:435–438 DOI 10.1098/rsbl.2006.0507.

Barnett IJ, Lee S, Lin XH. 2013. Detecting rare variant effects using extreme phenotype sampling in sequencing association studies. *Genetic Epidemiology* **37**:142–151 DOI 10.1002/gepi.21699.

Bérénos C, Ellis PA, Pilkington JG, Lee SH, Gratten J, Pemberton JM. 2015. Heterogeneity of genetic architecture of body size traits in a free-living population. *Molecular Ecology* **24**:1810–1830 DOI 10.1111/mec.13146.

Bonenfant C, Pelletier F, Garel M, Bergeron P. 2009. Age-dependent relationship between horn growth and survival in wild sheep. *Journal of Animal Ecology* **78**:161–171 DOI 10.1111/j.1365-2656.2008.01477.x.

Borgia G. 1979. Sexual section and the evolution of mating system. In: Blum MS, Blum NA, eds. *Sexual selection and reproductive competition in insects*. New York: Academic Press, 19–80.

Bro-Jørgensen J. 2007. The intensity of sexual selection predicts weapon size in male bovids. *Evolution* **61**:1316–1326 DOI 10.1111/j.1558-5646.2007.00111.x.

Browning SR, Browning BL. 2011. Haplotype phasing: existing methods and new developments. *Nature Reviews Genetics* **12**:703–714.

Bunch TD, Wu C, Zhang YP, Wang S. 2006. Phylogenetic analysis of snow sheep (Ovis nivicola) and closely related taxa. *Journal of Heredity* **97**:21–30 DOI 10.1093/jhered/esi127.

Chang C, Chow C, Tellier L, Vattikuti S, Purcell S, Lee J. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* **4**:7 DOI 10.1186/s13742-015-0047-8.

Chenoweth SF, McGuigan K. 2010. The genetic basis of sexually selected variation. *Annual Review of Ecology, Evolution, and Systematics* **41**:81–101 DOI 10.1146/annurev-ecolsys-102209-144657.

Coltman DW. 2005. Testing marker-based estimates of heritability in the wild. *Molecular Ecology* **14**:2593–2599 DOI 10.1111/j.1365-294X.2005.02600.x.

Coltman DW, Festa-Bianchet M, Jorgenson JT, Strobeck C. 2002. Age-dependent sexual selection in bighorn rams. *Proceedings of the Royal Society B: Biological Sciences* **269**:165–172 DOI 10.1098/rspb.2001.1851.

Coltman DW, Hogg JT, Miller JM. 2013. Genomic resources notes accepted 1 April 2013–31 May 2013. *Molecular Ecology Resources* **13**:965–965 DOI 10.1111/1755-0998.12142.

Coltman DW, O'Donoghue P, Hogg JT, Festa-Bianchet M. 2005. Selection and genetic (CO)variance in bighorn sheep. *Evolution* **59**:1372–1382 DOI 10.1111/j.0014-3820.2005.tb01786.x.

Davis EB, Brakora KA, Lee AH. 2011. Evolution of ruminant headgear: a review. *Proceedings of the Royal Society B: Biological Sciences* **278**:2857–2865 DOI 10.1098/rspb.2011.0938.

Ekine CC, Rowe SJ, Bishop SC, De Koning D-J. 2014. Why Breeding Values Estimated Using Familial Data Should Not Be Used for Genome-Wide Association Studies. *G3: Genes|Genomes|Genetics* **4**:341–347 DOI 10.1534/g3.113.008706.

Ellegren H, Sheldon BC. 2008. Genetic basis of fitness differences in natural populations. *Nature* **452**:169–175 DOI 10.1038/nature06737.

Favre M, Martin JGA, Festa-Bianchet M. 2008. Determinants and life-history consequences of social dominance in bighorn ewes. *Animal Behaviour* **76**:1373–1380 DOI 10.1016/j.anbehav.2008.07.003.

Feng S, Ferlin A, Truong A, Bathgate R, Wade JD, Corbett S, Han S, Tannour-Louet M, Lamb DJ, Foresta C, Agoulnik AI. 2009. INSL3/RXFP2 signaling in testicular descent. *Annals of the New York Academy of Sciences* **1160**:197–204 DOI 10.1111/j.1749-6632.2009.03841.x.

Ferlin A, Pepe A, Gianesello L, Garolla A, Feng S, Giannini S, Zaccolo M, Facciolli A, Morello R, Agoulnik AI, Foresta C. 2008. Mutations in the insulin-like factor 3 receptor are associated with osteoporosis. *Journal of Bone and Mineral Research* **23**:683–693 DOI 10.1359/jbmr.080204.

Festa-Bianchet M, Coltman DW, Hogg JT, Jorgenson JT. 2008. Age-related horn growth, mating tactics, and vulnerability to harvest: why horn curl limits may select for small horns in bighorn sheep. *Biennial Symposium of the Northern Wild Sheep and Goat Council* **15**:42–49.

Festa-Bianchet M, Pelletier F, Jorgenson JT, Feder C, Hubbs A. 2014. Decrease in horn size and increase in age of trophy sheep in Alberta over 37 years. *The Journal of Wildlife Management* **78**:133–141 DOI 10.1002/jwmg.644.

**Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, Gil L, Giron CG, Gordon L, Hourlier T, Hunt S, Johnson N, Juettemann T, Kahari AK, Keenan S, Kulesha E, Martin FJ, Maurel T, McLaren WM, Murphy DN, Nag R, Overduin B, Pignatelli M, Pritchard B, Pritchard E, Riat HS, Ruffier M, Sheppard D, Taylor K, Thormann A, Trevanion SJ, Vullo A, Wilder SP, Wilson M, Zadissa A, Aken BL, Birney E, Cunningham F, Harrow J, Herrero J, Hubbard TJP, Kinsella R, Muffato M, Parker A, Spudich G, Yates A, Zerbino

DR, Searle SMJ. 2014. Ensembl 2014. *Nucleic Acids Research* **42**:D749–D755 DOI 10.1093/nar/gkt1196.

François O, Martins H, Caye K, Schoville SD. 2016. Controlling false discoveries in genome scans for selection. *Molecular Ecology* **25**:454–469 DOI 10.1111/mec.13513.

Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D. 2004. Assessing the impact of population stratification on genetic association studies. *Nature Genetics* **36**:388–393 DOI 10.1038/ng1333.

García-Gámez E, Sahana G, Gutiérrez-Gil B, Arranz J-J. 2012. Linkage disequilibrium and inbreeding estimation in Spanish Churra sheep. *BMC Genetics* **13**:43 DOI 10.1186/1471-2156-13-43.

Gautier M, Naves M. 2011. Footprints of selection in the ancestral admixture of a New World Creole cattle breed. *Molecular Ecology* **20**:3128–3143 DOI 10.1111/j.1365-294X.2011.05163.x.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R. 2009. *ASReml user guide. Release 3.0.* Hemel Hempstead: VSN International Ltd.

Hadfield JD, Wilson AJ, Garant D, Sheldon BC, Kruuk LEB. 2010. The misuse of BLUP in ecology and evolution. *American Naturalist* **175**:116–125 DOI 10.1086/648604.

Hengeveld PE, Festa-Bianchet M. 2011. Harvest regulations and artificial selection on horn size in male bighorn sheep. *The Journal of Wildlife Management* **75**:189–197 DOI 10.1002/jwmg.14.

Hill WG, Weir BS. 1988. Variances and covariances of squared linkage disequilibria in finite populations. *Theoretical Population Biology* **33**:54–78 DOI 10.1016/0040-5809(88)90004-4.

Hu ZL, Fritz ER, Reecy JM. 2007. AnimalQTLdb: a livestock QTL database tool set for positional QTL information mining and beyond. *Nucleic Acids Research* **35**:D604–D609 DOI 10.1093/nar/gkl946.

Hu ZL, Park CA, Wu XL, Reecy JM. 2013. Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era. *Nucleic Acids Research* **41**:D871–D879 DOI 10.1093/nar/gks1150.

Husby A, Kawakami T, Rönnegård L, Smeds L, Ellegren H, Qvarnström A. 2015. Genome-wide association mapping in a wild avian population identifies a link between genetic and phenotypic variation in a life-history trait. *Proceedings of the Royal Society B: Biological Sciences* **282**:20150156 DOI 10.1098/rspb.2015.0156.

Jiang Y, Xie M, Chen W, Talbot R, Maddox JF, Faraut T, Wu C, Muzny DM, Li Y, Zhang W, Stanton J-A, Brauning R, Barris WC, Hourlier T, Aken BL, Searle SMJ, Adelson DL, Bian C, Cam GR, Chen Y, Cheng S, DeSilva U, Dixen K, Dong Y, Fan G, Franklin IR, Fu S, Fuentes-Utrilla P, Guan R, Highland MA, Holder ME, Huang G, Ingham AB, Jhangiani SN, Kalra D, Kovar CL, Lee SL, Liu W, Liu X, Lu C, Lv T, Mathew T, McWilliam S, Menzies M, Pan S, Robelin D, Servin B, Townley D, Wang W, Wei B, White SN, Yang X, Ye C, Yue Y, Zeng P, Zhou Q, Hansen JB, Kristiansen K, Gibbs RA, Flicek P, Warkup CC, Jones HE, Oddy VH, Nicholas FW,

McEwan JC, Kijas JW, Wang J, Worley KC, Archibald AL, Cockett N, Xu X, Wang W, Dalrymple BP. 2014. The sheep genome illuminates biology of the rumen and lipid metabolism. *Science* **344**:1168–1173 DOI 10.1126/science.1252806.

Johnston SE, Beraldi D, McRae AF, Pemberton JM, Slate J. 2010. Horn type and horn length genes map to the same chromosomal region in Soay sheep. *Heredity* **104**:196–205 DOI 10.1038/hdy.2009.109.

Johnston SE, Gratten J, Berenos C, Pilkington JG, Clutton-Brock TH, Pemberton JM, Slate J. 2013. Life history trade-offs at a single locus maintain sexually selected genetic variation. *Nature* **502**:93–95 DOI 10.1038/nature12489.

Johnston SE, McEwan JC, Pickering NK, Kijas JW, Beraldi D, Pilkington JG, Pemberton JM, Slate J. 2011. Genome-wide association mapping identifies the genetic basis of discrete and quantitative variation in sexual weaponry in a wild sheep population. *Molecular Ecology* **20**:2555–2566 DOI 10.1111/j.1365-294X.2011.05076.x.

Jorgenson JT, Festa-Bianchet M, Gaillard J-M, Wishart WD. 1997. Effects of age, sex, disease, and density on survival of bighorn sheep. *Ecology* **78**:1019–1032 DOI 10.1890/0012-9658(1997)078[1019:EOASDA]2.0.CO;2.

Jorgenson JT, Festa-Bianchet M, Lucherini M, Wishart WD. 1993. Effects of body size, population density, and maternal characteristics on age at first reproduction in bighorn ewes. *Canadian Journal of Zoology* **71**:2509–2517 DOI 10.1139/z93-344.

Kardos M, Husby A, McFarlane SE, Qvarnström A, Ellegren H. 2016. Whole-genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. *Molecular Ecology* **16**:727–741 DOI 10.1111/1755-0998.12498.

Kardos M, Luikart G, Bunch R, Dewey S, Edwards W, McWilliam S, Stephenson J, Allendorf FW, Hogg JT, Kijas J. 2015. Whole-genome resequencing uncovers molecular signatures of natural and sexual selection in wild bighorn sheep. *Molecular Ecology* **24**:5616–5632 DOI 10.1111/mec.13415.

Karssen LC, Van Duijn CM, Aulchenko YS. 2016. The GenABEL project for statistical genomics. *F1000Research* **5**:914 DOI 10.12688/f1000research.8733.1.

Kawakami T, Backström N, Burri R, Husby A, Olason P, Rice AM, Ålund M, Qvarnström A, Ellegren H. 2014. Estimation of linkage disequilibrium and interspecific gene flow in Ficedula flycatchers by a newly developed 50k SNP array. *Molecular Ecology Resources* **14**:1248–1260 DOI 10.1111/1755-0998.12270.

Kijas JW, Lenstra JA, Hayes B, Boitard S, Porto Neto LR, San Cristobal M, Servin B, McCulloch R, Whan V, Gietzen K, Paiva S, Barendse W, Ciani E, Raadsma H, McEwan J, Dalrymple B. 2012. Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLOS Biology* **10**:e1001258 DOI 10.1371/journal.pbio.1001258.

Kijas JW, Porto-Neto L, Dominik S, Reverter A, Bunch R, McCulloch R, Hayes BJ, Brauning R, McEwan J. 2014. Linkage disequilibrium over short physical distances measured in sheep using a high-density SNP chip. *Animal Genetics* **45**:754–757 DOI 10.1111/age.12197.

Kinsella RJ, Kahari A, Haider S, Zamora J, Proctor G, Spudich G, Almeida-King J, Staines D, Derwent P, Kerhornou A, Kersey P, Flicek P. 2011. Ensembl BioMarts: a hub for data retrieval across taxonomic space. *Database* **2011**:bar030 DOI 10.1093/database/bar030.

Kruuk LEB. 2004. Estimating genetic parameters in natural populations using the "animal model". *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**:873–890 DOI 10.1098/rstb.2003.1437.

Kruuk LEB, Slate J, Wilson AJ. 2008. New answers for old questions: the evolutionary quantitative genetics of wild animal populations. *Annual Review of Ecology Evolution and Systematics* **39**:525–548 DOI 10.1146/annurev.ecolsys.39.110707.173542.

Lachance J, Tishkoff SA. 2013. SNP ascertainment bias in population genetic analyses: why it is important, and how to correct it. *BioEssays* **35**:780–786 DOI 10.1002/bies.201300014.

Lappalainen T, Montgomery SB, Nica AC, Dermitzakis ET. 2011. Epistatic selection between coding and regulatory variation in human evolution and disease. *American Journal of Human Genetics* **89**:459–463 DOI 10.1016/j.ajhg.2011.08.004..

Li DL, Lewinger JP, Gauderman WJ, Murcray CE, Conti D. 2011. Using extreme phenotype sampling to identify the rare causal variants of quantitative traits in association studies. *Genetic Epidemiology* **35**:790–799 DOI 10.1002/gepi.20628.

Loison A, Festa-Bianchet M, Gaillard J-M, Jorgenson JT, Jullien J-M. 1999. Age-specific survival in five populations of ungulates: evidence of senescence. *Ecology* **80**:2539–2554 DOI 10.1890/0012-9658(1999)080[2539:ASSIFP]2.0.CO;2.

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TFC, McCarroll SA, Visscher PM. 2009. Finding the missing heritability of complex diseases. *Nature* **461**:747–753 DOI 10.1038/nature08494.

Martin AM, Presseault-Gauvin H, Festa-Bianchet M, Pelletier F. 2013. Male mating competitiveness and age-dependent relationship between testosterone and social rank in bighorn sheep. *Behavioral Ecology and Sociobiology* **67**:919–928 DOI 10.1007/s00265-013-1516-7.

McKay SD, Schnabel RD, Murdoch BM, Matukumalli LK, Aerts J, Coppieters W, Crews D, Dias E, Gill CA, Gao C, Mannen H, Stothard P, Wang ZQ, Van Tassell CP, Williams JL, Taylor JF, Moore SS. 2007. Whole genome linkage disequilibrium maps in cattle. *BMC Genetics* **8**:74 DOI 10.1186/1471-2156-8-74.

Miller JM, Kijas JW, Heaton MP, McEwan JC, Coltman DW. 2012. Consistent divergence times and allele sharing measured from cross-species application of SNP chips developed for three domestic species. *Molecular Ecology Resources* **12**:1145–1150 DOI 10.1111/1755-0998.12017.

Miller JM, Moore SS, Stothard P, Liao X, Coltman DW. 2015. Harnessing cross-species alignment to discover SNPs and generate a draft genome sequence of a bighorn sheep (Ovis canadensis). *BMC Genomics* **16**:397 DOI 10.1186/s12864-015-1618-x.

Miller JM, Poissant J, Kijas J, Coltman DW. 2011. A genome-wide set of SNPs detects population substructure and long range linkage disequilibrium in wild sheep. *Molecular Ecology Resources* **11**:314–322 DOI 10.1111/j.1755-0998.2010.02918.x.

Minikel E. 2012. Power for GWAS and extreme phenotype studies. CureFFI.org. *Available at* http://www.cureffi.org/2012/12/05/power-for-gwas-and-extreme-phenotype-studies/.

Moskvina V, Schmidt KM. 2008. On multiple-testing correction in genome-wide association studies. *Genetic Epidemiology* **32**:567–573 DOI 10.1002/gepi.20331.

Paterson T, Graham M, Kennedy J, Law A. 2012. VIPER: a visualisation tool for exploring inheritance inconsistencies in genotyped pedigrees. *BMC Bioinformatics* **13**:S5 DOI 10.1186/1471-2105-13-S8-S5.

Pigeon G, Festa-Bianchet M, Coltman DW, Pelletier F. 2016. Intense selective hunting leads to artificial evolution in horn size. *Evolutionary Applications* **9**:521–530 DOI 10.1111/eva.12358.

Poissant J, Davis CS, Malenfant RM, Hogg JT, Coltman DW. 2012. QTL mapping for sexually dimorphic fitness-related traits in wild bighorn sheep. *Heredity* **108**:256–263 DOI 10.1038/hdy.2011.69.

Poissant J, Hogg JT, Davis CS, Miller JM, Maddox JF, Coltman DW. 2010. Genetic linkage map of a wild genome: genomic structure, recombination and sexual dimorphism in bighorn sheep. *BMC Genomics* **11** DOI 10.1186/1471-2164-11-524.

Poissant J, Wilson AJ, Festa-Bianchet M, Hogg JT, Coltman DW. 2008. Quantitative genetics and sex-specific selection on sexually dimorphic traits in bighorn sheep. *Proceedings of the Royal Society B: Biological Sciences* **275**:623–628 DOI 10.1098/rspb.2007.1361.

Porto-Neto LR, Kijas JW, Reverter A. 2014. The extent of linkage disequilibrium in beef cattle breeds using high-density SNP genotypes. *Genetics Selection Evolution* **46**:22 DOI 10.1186/1297-9686-46-22.

Purcell S, Cherny SS, Sham PC. 2003. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19**:149–150 DOI 10.1093/bioinformatics/19.1.149.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ, Sham PC. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics* **81**:559–575 DOI 10.1086/519795.

R Core Team. 2015. R: a language and environment for statistical computing, reference index version 3.2.2. *Available at* https://www.r-project.org.

Reich DE, Cargill M, Bolk S, Ireland J, Sabeti PC, Richter DJ, Lavery T, Kouyoumjian R, Farhadian SF, Ward R, Lander ES. 2001. Linkage disequilibrium in the human genome. *Nature* **411**:199–204 DOI 10.1038/35075590.

Robinson MR, Santure AW, DeCauwer I, Sheldon BC, Slate J. 2013. Partitioning of genetic variation across the genome using multimarker methods in a wild bird population. *Molecular Ecology* **22**:3963–3980 DOI 10.1111/mec.12375.

Rönnegård L, McFarlane SE, Husby A, Kawakami T, Ellegren H, Qvarnström A. 2016. Increasing the power of genome wide association studies in natural populations using repeated measures—evaluation and implementation. *Methods in Ecology and Evolution* **7**:792–799 DOI 10.1111/2041-210X.12535.

Rowe L, Houle D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society B: Biological Sciences* **263**:1415–1421 DOI 10.1098/rspb.1996.0207.

Santure AW, De Cauwer I, Robinson MR, Poissant J, Sheldon BC, Slate J. 2013. Genomic dissection of variation in clutch size and egg mass in a wild great tit (Parus major) population. *Molecular Ecology* **22**:3949–3962 DOI 10.1111/mec.12376.

Sham PC, Cherny SS, Purcell S, Hewitt JK. 2000. Power of linkage versus association analysis of quantitative traits, by use of variance-components models, for sibship data. *American Journal of Human Genetics* **66**:1616–1630 DOI 10.1086/302891.

Silva CNS, McFarlane SE, Hagen IJ, Ronnegard L, Billing AM, Kvalnes T, Kemppainen P, Ronning B, Ringsby TH, Saether B-E, Qvarnstrom A, Ellegren H, Jensen H, Husby A. 2017. Insights into the genetic architecture of morphological traits in two passerine bird species. *Heredity* **119**:197–205 DOI 10.1038/hdy.2017.29.

Slate J. 2013. From beavis to beak colour: a simulation study to examine how much QTL mapping can reveal about the genetic architecture of quantitative traits. *Evolution* **67**:1251–1262 DOI 10.1111/evo.12060.

Slate J, Gratten J, Beraldi D, Stapley J, Hale M, Pemberton JM. 2009. Gene mapping in the wild with SNPs: guidelines and future directions. *Genetica* **136**:97–107 DOI 10.1007/s10709-008-9317-z.

Slate J, Santure AW, Feulner PGD, Brown EA, Ball AD, Johnston SE, Gratten J. 2010. Genome mapping in intensively studied wild vertebrate populations. *Trends in Genetics* **26**:275–284 DOI 10.1016/j.tig.2010.03.005.

Wang J. 2013. An improvement on the maximum likelihood reconstruction of pedigrees from marker data. *Heredity* **111**:165–174 DOI 10.1038/hdy.2013.34.

Wickham H. 2009. *ggplot2: elegant graphics for data analysis.* New York: Springer.

Wiedemar N, Drögemüller C. 2015. A 1.8-kb insertion in the 3′-UTR of RXFP2 is associated with polledness in sheep. *Animal Genetics* **48**:457–461 DOI 10.1111/age.12309.

Wiedemar N, Tetens J, Jagannathan V, Menoud A, Neuenschwander S, Bruggmann R, Thaller G, Drogemuller C. 2014. Independent polled mutations leading to complex gene expression differences in cattle. *PLOS ONE* **9(3)**:e93435 DOI 10.1371/journal.pone.0093435.

Wilson AJ, Kruuk LEB, Coltman DW. 2005. Ontogenetic patterns in heritable variation for body size: using random regression models in a wild ungulate population. *The American Naturalist* **166**:E177–E192 DOI 10.1086/497441.

Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2010. An ecologist's guide to the animal model. *Journal of Animal Ecology* **79**:13–26 DOI 10.1111/j.1365-2656.2009.01639.x.

Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, Madden PA, Heath AC, Martin NG, Montgomery GW, Goddard ME, Visscher PM. 2010.

Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics* **42**:565–569 DOI 10.1038/ng.608.

Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM, De Andrade M, Feenstra B, Feingold E, Hayes MG, Hill WG, Landi MT, Alonso A, Lettre G, Lin P, Ling H, Lowe W, Mathias RA, Melbye M, Pugh E, Cornelis MC, Weir BS, Goddard ME, Visscher PM. 2011. Genome partitioning of genetic variation for complex traits using common SNPs. *Nature Genetics* **43**:519–525 DOI 10.1038/ng.823.

Yuan FP, Li X, Lin J, Schwabe C, Büllesbach EE, Rao CV, Lei ZM. 2010. The role of RXFP2 in mediating androgen-induced inguinoscrotal testis descent in LH receptor knockout mice. *Reproduction* **139**:759–769 DOI 10.1530/REP-09-0518.