University of Alberta

Evolutionary quantitative genetics and genomics applied to the study of sexually dimorphic traits in wild bighorn sheep (*Ovis canadensis)*

by

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ABSTRACT

The independent evolution of the sexes may often be constrained if male and female homologous traits share a similar genetic architecture. Thus, cross-sex genetic covariance is assumed to play a key role in the evolution of sexual dimorphism (SD) with consequent impacts on sexual selection, population dynamics and the speciation process. I used quantitative genetics tools to assess the importance of sex-specific genetic variance in facilitating the evolution of body mass and horn size SD in wild bighorn sheep from Ram Mountain, Alberta. I also developed a bighorn sheep genetic linkage map composed of 247 microsatellite markers to gain insights about the genetic architecture of trait variation. Finally, I conducted systematic reviews and meta-analyses of published cross-sex genetic correlations (r_{MF}, a standardized estimate of cross-sex genetic covariance) to test basic hypotheses about the importance of sex-specific genetic variance in the evolution of SD and mechanisms responsible for generating such variance. My results demonstrated that sex-specific genetic variance was present in bighorn sheep and that it likely played an important role in alleviating intralocus sexual conflicts. The quantitative trait locus (QTL) mapping analysis resulted in the identification of numerous loci influencing body mass and horn dimensions, some of which had apparent sex-specific effects. An analysis of 553 r_{MF} estimates recovered from 114 published sources allowed demonstrating that 1) the evolution of SD was generally constrained by positive cross-sex genetic covariance, 2) levels of SD were often sub-optimal, and 3) sex-specific genetic variance was an important mechanism allowing the evolution of SD. In addition, I

confirmed the long-standing hypothesis of a general decline in r_{MF} with age. Sexual dimorphism is an important evolutionary phenomenon, but our understanding of its evolution is still limited. After decades of speculation, my research has provided clear empirical evidence for the importance of sex-specific genetic variance in allowing its evolution.

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General introduction

1.1 Introduction

Males and females typically differ for a suite of morphological, physiological and behavioural traits due to their differing roles in reproduction (Fairbairn *et al*. 2007). For example, men are on average taller and heavier than women. This pattern, termed sexual dimorphism (SD), is believed to be adaptive, reflecting differences in sex-specific phenotypic optima (Fairbairn *et al*. 2007). However, the presence of such extensive and often dramatic differences is paradoxical because males and females share most of their genomes (Fairbairn 2007). Indeed, when selection acts to change the means of male and female traits in opposite directions, selection on genetic variants in one sex is expected to be counteracted by opposing selection on the same alleles in the other sex, resulting in negative cross-sex genetic covariance for fitness and evolutionary stasis (Lande 1980; Fairbairn 2007). Such situations, termed 'intralocus sexual conflicts' (ISCs), are believed to be widespread but remain poorly studied (Arnqvist & Rowe 2005, Bonduriansky & Chenoweth 2009).

In principle, the evolution of SD depends on the magnitude of the genetic correlation between homologous male and female traits (cross-sex genetic correlation, r_{MF}, Lande 1980, Lynch & Walsh 1998). Studying the extent and causes of variability in r_{MF} is therefore key to our understanding of ISCs (Rice & Chippindale 2001, Fairbairn 2007). Increased knowledge about the importance of sex-specific genetic variance in the evolution of SD and the ease with which it can evolve would also help develop more realistic sexual selection models, better understand population dynamics and assess the importance of sexual dimorphism

in the speciation process. General knowledge about the genetic basis and evolutionary dynamics of SD is also highly pertinent to plant and animal breeding as well as human medical genetics.

Most quantitative genetics and genomics studies related to ISCs have focussed on laboratory populations of model organisms such as Drosophila (e.g. Chippindale *et al.* 2001, Delcourt *et al.* 2009, Innocenti & Morrow 2010). Such experiments present obvious advantages including short generation times, large sample sizes, controlled environmental conditions, and the possibility to perform various breeding designs and fitness assays. However, they usually fail at portraying genetic variation and complex selection regimes present in wild populations. A complete understanding of ISCs therefore ultimately depends on our ability to apply evolutionary quantitative genetics and genomic tools to the study of free-living populations.

Studies of ISCs in wild populations are challenging because they require extensive pedigree information as well as fitness estimates. Nevertheless, the feasibility of such investigations was demonstrated by pioneering work in red deer, *Cervus elaphus*, and collared flycatchers, *Ficedula albicollis*, in which negative r_{MF} for fitness were documented (Foerster *et al.* 2007, Brommer *et al.* 2007). In addition, a few studies reported quantitative genetic parameters and sex-specific selection coefficients for traits other than fitness itself (e.g. Coltman *et al.* 2005, Jensen *et al.* 2008). However, such studies remain extremely rare and we still know very little about variability in fitness r_{MF} and the contribution of individual traits and genes to such covariance.

An organism offering great opportunities for the study of SD and ISCs in the wild is the bighorn sheep, *Ovis canadensis* (Valdez & Krausman 1999). This mountain ungulate from western North America exhibits important SD for body mass and horn size due to sexual selection through male-male combat during which males fiercely clash their horns (Geist 1971, Coltman *et al.* 2002). While both traits are obvious candidates for studies of ISCs, the study of horn size is particularly appealing because the horns of females have no clear fitness benefit. Horn size in this species could therefore provide an example of long-lasting ISC described by Lande (1980). Studies on the evolution and genetic basis of trait variation in bighorn sheep are made possible by the availability of DNA samples and large phenotypic datasets from multiple long-term field studies of individually recognizable individuals (e.g., Ram Mountain, Alberta, Coltman *et al.* 2005, National Bison Range, Montana, Hogg *et al.* 2006). Topics that have been addressed using these datasets are diverse and include the consequences of selective harvesting (Coltman *et al.* 2003), maternal effects (Wilson *et al.* 2005), genetic rescue (Hogg *et al.* 2006) and animal personality (Réale *et al.* 2009). The availability of large pedigrees combined with the possibility to transfer genomic resources from the closely related domestic sheep (*Ovis aries*, Maddox *et al.* 2001) also make bighorn sheep an obvious candidate for studies on the genetic architecture of fitness-related traits in the wild (Slate *et al.* 2010).

1.2 Description of thesis objectives and data chapters

For my doctoral research, I tested for the presence of ISC over body mass and horn size in wild bighorn sheep and assessed the importance of cross-sex genetic covariance in constraining the evolution of SD. I also developed genomic resources and mapped quantitative trait loci for these sexually dimorphic traits. Finally, I used meta-analysis techniques to empirically assess the importance of sex-specific genetic variance in facilitating the evolution of SD and test for the presence of mechanisms involved in generating such variance.

The thesis is divided in six data chapters, four of which describe research performed in bighorn sheep (2, 5, 6 and 7) while two describe meta-analyses of published cross-sex genetic correlations (3 and 4).

In **chapter 2**, I outline the results of a test for the presence of ISC over horn volume and body mass in bighorn sheep from Ram Mountain. I first assessed the importance of genetic constraints in limiting the evolution of sexual dimorphism by estimating sex-specific additive genetic variances and intersexual genetic correlations (r_{MF}). I then quantified sex-specific selection using field estimates of longevity and lifetime reproductive success.

In **chapter 3**, I present the first systematic review and meta-analysis of published cross-sex genetic correlations and assess the extent to which the evolution of SD is typically constrained and test several specific hypotheses. First, I tested if r_{MF} differ among trait types and especially between fitness components and other traits. I also tested the theoretical prediction of a negative relationship between r_{MF} and SD based on the expectation that increases in SD should be

facilitated by sex-specific genetic variance (Bonduriansky & Rowe 2005, Fairbairn & Roff 2006, Fairbairn 2007).

In **chapter 4**, I present a systematic review and meta-analysis of ontogenetic variability in r_{MF}. I tested if r_{MF} tended to decrease with age as originally hypothesized by Fisher (1958).

In **chapter 5**, I describe the development of genomic resources for North American mountain ungulates. I tested for the cross-amplification of microsatellite loci located throughout the domestic sheep genome in bighorn sheep and mountain goats (*Oreamnos americanus*) using 648 and 576 primer pairs, respectively.

In **Chapter 6,** I present a first-generation bighorn sheep linkage map constructed using DNA samples from two long-term field studies of pedigreed individuals (Ram Mountain and National Bison Range) and compare genome organization as well as recombination patterns between bighorn sheep and the closely related domestic sheep, *Ovis aries*.

In **Chapter 7**, I present a quantitative trait locus (QTL) mapping analysis for body mass and horn dimensions (length, base circumference, and volume) in wild bighorn sheep from Ram Mountain. Because these traits are sexually dimorphic, I estimated both sex-specific and cross-sex quantitative genetic parameters and tested for QTLs influencing either a single sex or both sexes similarly.

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Chapter 2

Quantitative genetics and sex-specific selection on sexually dimorphic traits in bighorn sheep

A version of this chapter has been published. Poissant J, Wilson AJ, Festa-Bianchet M, Hogg JT, Coltman DW (2008) *Proceedings of the Royal Society B* 275, 623-628.

2.1 Introduction

The widespread occurrence of sexual dimorphism suggests that optimal trait values often differ between the sexes (Fairbairn 2007). Because traits shared by the sexes are typically influenced by the same genes (Roff 1997), sexual conflicts at loci influencing shared traits (intralocus sexual conflicts, Arnqvist & Rowe 2005) may be common. While negative cross-sex genetic correlations for fitness in many laboratory and wild populations (Chippindale *et al*. 2001; Foerster *et al*. 2007; Brommer *et al*. 2007) suggest that such sexual conflicts may be common (Arnqvist & Rowe 2005), they have very rarely been studied in nature (Arnqvist & Rowe 2005; Rowe & Day 2006).

Since Darwin's (1871) suggestion that certain conspicuous male traits may have evolved through male-male combat, the massive sexually selected horns of male bighorn sheep (*Ovis canadensis*, Fig.2.1) have attracted much attention from evolutionary biologists (Geist 1966; Fitzsimmons *et al*. 1995; Coltman *et al*. 2002, 2003, 2005; Festa-Bianchet *et al*. 2004). On the other hand, the smaller horns of females have almost never been studied and have no clearly known fitness benefit. The presence of horns in females could result from a genetic correlation with male horns. Alternatively, horns may be useful to both sexes but differ in size if they have different functions. For example female horns may play an important role in defense against predators and intra-specific competition (Packer 1983; Roberts 1996).

The aim of this study was to test for the presence of sexual conflict at loci influencing horn size and body weight in a pedigreed population of wild bighorn

sheep studied extensively for over 35 years (Coltman *et al*. 2005). Because a sexual conflict at the genetic level requires heritable traits, I first quantified additive genetic variance in both sexes. I then assessed the importance of genetic constraints on the evolution of sexual dimorphism by estimating intersexual genetic correlations (r_G). Finally, I quantified sex-specific selection using field estimates of longevity and reproductive success. Significant heritability in both sexes for a shared trait could lead to sexual conflict at the genetic level if it was combined with sexually antagonistic selection and an intersexual $r_G > 0$. Conflict would also be present when selection is in the same direction in both sexes but where $r_G < 0$. I included body mass in my analyses to control for the influence of body size on horn size, but also to contrast quantitative genetic parameters and selection at traits varying in their degree of sexual dimorphism (horn size being much more dimorphic than body mass). This study represents a rare test of sexual conflict at loci influencing shared traits (Arnqvist & Rowe 2005; Rowe & Day 2006) and provides much needed information on the importance of genetic constraints on the evolution of sexual dimorphism in nature (Rice & Chippindale 2001; Fairbairn 2007).

2.2 Methods

2.2.1 Study site and data collection

The study population inhabits Ram Mountain, Alberta, Canada (52°N, 115°W, elevation 1080 to 2170 m). Techniques used to capture, mark, measure and monitor individuals are described in detail elsewhere (Jorgenson *et al*. 1993).

Data presented here were collected from 1970 to 2006. Briefly, animals were captured in a corral trap baited with salt from late May to September or early October each year. Almost all animals were marked as lambs or yearlings, so their exact age was known. Individuals first captured as adults were aged by counting horn growth rings. Marked sheep were monitored throughout their lifetime.

Ewes and young rams are usually captured multiple times each year, while rams 3 years and older are typically caught only 1-3 times per season, usually in June or July. At each capture, sheep are weighed to the nearest 250 g with a Detecto spring scale. Horn length along the outside curvature and horn base circumference are measured to the nearest mm for both horns using tape. Horn volume (cm^3) was calculated assuming a conical shape using the average horn base circumference of both horns and the length of the longest horn to reduce the influence of horn breakage.

2.2.2 Pedigree information

The pedigree used in this study includes 764 maternal and 435 paternal links. It differs from the one in Coltman *et al*. (2005) by the addition of individuals born between 2003 and 2006. Maternity was accurately determined from field observations of suckling behavior. Paternity was determined using paternity test and half-sib reconstruction based on genotypes at ~30 microsatellite loci for samples collected from 1988 to 2006. Laboratory and statistical methods are detailed in Coltman *et al*. (2005).

2.2.3 Quantitative genetic analysis

Phenotypic variance in horn volume and body mass was partitioned into additive genetic and other components using an animal model and restricted maximum likelihood (REML) with the program ASReml 2.0 (Gilmour *et al.* 2006). The animal model is a form of mixed model incorporating pedigree information where the phenotype of each individual is modeled as the sum of its additive genetic value and other random and fixed effects. This method is particularly useful for the study of natural populations because it optimizes the use of information from complex and incomplete pedigrees when estimating quantitative genetic parameters (Kruuk 2004).

Prior to analysis each trait for each age/sex class was standardized to a standard deviation of unity. Phenotypic variance left after taking into account fixed effects was then partitioned into five components: additive genetic (V_A), permanent environmental (V_{PE}), year (V_Y), year of birth (V_{YOB}) and residual (V_R). Attempts to include a maternal effect component often caused convergence problems for bivariate models. Since the influence of maternal effects for body size is known to be negligible by age 2 in the study population (Wilson *et al.* 2005), maternal effects were not fitted and analyses were restricted adult sheep (2 years old and older). Animals older than 5 were excluded because the distribution of phenotypes in older males is biased by trophy hunting (Coltman *et al.* 2003; Festa-Bianchet *et al.* 2004) and most rams become vulnerable to hunting at 5-7 years of age depending on their rate of horn growth. Year and year of birth were fitted to account for the influence of environmental variation (Postma 2006;

Kruuk & Hadfield 2007). Day of capture was included as a fixed effect (continuous, 2nd order polynomial, with May 24 as day 0) because different individuals were sampled at different points within sampling seasons. Since growth patterns differ between age classes, age (factor) and the age*date interaction were also fitted. Bivariate models were used to estimate covariances and correlations within and between the sexes. The significance of (co)variance components was assessed using likelihood ratio tests. Narrow sense heritability (h^2) and other ratios were calculated by dividing the appropriate variance component by V_P (e.g. V_A / V_P for h^2) where $V_P = V_A + V_{PE} + V_Y + V_{YOB} + V_R$. The significance of ratios and correlations was not explicitly tested but was instead inferred from the significance of their associated (co)variance components. Since a main objective of this study was to assess the importance of genetic constraints, likelihood ratio tests were used to verify whether genetic correlations were smaller than unity. Number of individuals and measurements included in the animal models are presented in table 2-1.

2.2.4 Selection analysis

Selection analyses were based on estimates of lifetime reproductive success (LRS, number of lambs produced that survived to weaning), longevity (in years) and mean reproductive success (MRS = LRS*longevity^{-1}). Separate analyses were performed for males and females. These analyses only included animals that were born before 1996 so that every individual had the opportunity to reach 10 years of age. For LRS and MRS, analyses only included genotyped

males that have been DNA sampled and therefore included in paternity analyses. Females that had received contraceptive implants and individuals removed for translocations or research purposes were excluded from the analysis. Year of birth was fitted in all models to account for changes in density and environmental conditions. Cohorts comprising a single informative individual were therefore omitted (1968 and 1994 for male longevity, 1980 and 1994 for male reproductive success and 1974 for female longevity and reproductive success).

Sex-specific standardized linear and quadratic selection differentials and gradients were estimated using linear regression (Lande & Arnold 1983). Body mass and horn volume at age 4 corrected to June 5 were used as phenotypic values. These corrected values were obtained using individual linear regressions for individuals sampled multiple times and using mean population growth rate for individuals sampled only once. The significance of coefficients was tested using generalized linear models with negative binomial error for LRS and Poisson error for longevity. For MRS, a linear model with a square root transformation was used. Neither quadratic nor interaction terms were statistically significant and are therefore not shown. These analyses were performed using SPLUS 7.0 (Insightful).

2.3 Results

2.3.1 Quantitative genetic parameters

Body mass and horn volume showed significant additive genetic variance in both sexes (Table 2-2). The proportion of phenotypic variance explained by

additive genetic effects after accounting for fixed effects ranged from 0.11 +/- 0.05 for FBM to 0.32 +/- 0.12 for MBM and MHV (Table 2-3). Year and year of birth were also significant for all traits and combined they explained 33% to 58% of the variation (Tables 2-2 and 2-3). Finally, permanent environmental effects which include non-additive genetic variance were also significant for all traits and accounted for 14 % to 27 % of the variation (Table 2-2 and 2-3).

Genetic correlation estimates were relatively large and significantly positive for three pairs of traits (Table 2-2). These included r_G for pairs of traits within each sex (body mass vs. horn volume) and between male and female body mass. On the other hand, intersexual r_G involving horn volume were all relatively small and significantly smaller than unity (Table 2-2).

With the exception of covariance between MHV and female traits, year and year of birth appeared to affect pairs of traits similarly (Table 2-2). In particular year and year of birth correlations were close to unity for pairs of traits within each sex. The within-sex correlation for permanent environmental effects was close to unity in males (0.75 +/- 0.20) and negligible in females (0.06 +/- 0.04, Table 2-2).

2.3.2 Selection analysis

Selection coefficients were relatively small in both sexes (Table 2-4). In males none of the selection coefficients for body mass and horn volume were significant. However, male horn volume showed a non-significant trend for a negative association with longevity after accounting for selection on body mass (-

0.11 +/- 0.06, p = 0.13, Table 2-4). In females selection differentials and gradients for body mass were all positive and significant. There was no evidence for directional selection on female horn volume.

2.4 Discussion

2.4.1 Quantitative genetic parameters

Body mass and horn volume showed significant additive genetic variance in both sexes. Quantitative genetic parameters had previously been estimated for female body mass and male traits (Réale *et al.* 1999; Coltman *et al.* 2003; Coltman *et al.* 2005; Pelletier *et al.* 2007) but not for female horn size. Heritability of horn volume in females was comparable with the male estimate (h^2 = 0.24 +/- 0.09 vs. 0.32 +/- 0.12, respectively).

The genetic correlation between horn size and body mass in females was significantly smaller then unity. This is important because it suggests that horn volume can evolve relative to body size in that sex. In contrast, the same genetic correlation was not significantly smaller then unity in males (0.74 +/- 0.15, p = 0.11). This is consistent with the results of Coltman *et al.* (2003, 2005) and suggests that the evolution of horn size relative to body mass may be more constrained in males.

A main goal was to evaluate the importance of genetic constraints on the evolution of sexual dimorphism in bighorn sheep. As previously shown (Coltman *et al.* 2003, 2005), the evolution of body size sexual dimorphism was found to be constrained by a large intersexual r_G (0.63 +/- 0.30). On the other hand, r_G were

smaller than unity for many other pairs of traits which suggests that horn volume should be able to evolve partly independently in each sex and that sex-specific optima could be reached more readily (Lande 1980). In particular the intersexual r_G for horn volume was quite small (0.24 +/- 0.28) and similar to estimates reported for other highly sexually dimorphic traits in other species (e.g. fat deposition in humans, Comuzzie *et al.* 1993; antenna length in the fly *Prochyliza xanthostoma*, Bonduriansky & Rowe 2005). This is consistent with the prediction that sexual dimorphism and intersexual r_G should be negatively correlated in response to sexually divergent selection (Bonduriansky & Rowe 2005; Fairbairn & Roff 2006).

2.4.2 Selection analysis

None of the selection coefficients differed significantly from zero in males. However, rams with fast growing horns are artificially selected against by trophy hunters in the study population (Coltman *et al.* 2003; Festa-Bianchet *et al.* 2004). Each year about 40% of rams with horns that satisfy the legal definition of a harvestable ram are shot. The trend towards a negative association between horn volume and longevity after controlling for selection on body mass (-0.11 +/- 0.06, $p = 0.13$) likely results from hunting pressure. A similar negative relationship between horn volume and longevity was documented in Soay sheep where it likely results from the cost of growing and carrying large horns (Robinson *et al.* 2006). In the Ram Mountain population, any natural selection against large horns is unlikely to be expressed as mortality of large-horned rams through trophy

hunters is quite strong (Coltman *et al*. 2003, Festa-Bianchet *et al*. 2004). It may also be that artificial selection more effectively targets total horn length or morphology rather than horn volume in bighorn sheep. For example, harvest restrictions are based on horn length and shape, not horn volume. Similarly, horn length is a good correlate of mating success in rams after accounting for age (Coltman et al. 2002). Horn volume may reflect the metabolic costs of growing and carrying horns, however, total horn length may be more relevant in terms of artificial and sexual selection.

Selection differentials and gradients for body mass were all significantly positive in females. Coltman *et al*. (2005) and Pelletier *et al*. (2007) also observed positive relationships between June body mass and female fitness. On the other hand, horn volume does not appear to be under directional selection in females. This contrasts with the negative association observed between horn size and LRS in female Soay sheep (Robinson *et al*. 2006). It may be that female horns in bighorn sheep are so small relative to body size that they do not incur an easily detectable fitness cost.

In summary, I tested for intralocus sexual conflict in a wild population of bighorn sheep by estimated quantitative genetic parameters and selection coefficients for two sexually dimorphic traits. Because all traits showed significant additive genetic variance and all genetic correlations were positive, sexual conflicts at the genetic level are possible in the presence of sexually antagonistic selection. However the absence of detectable sexually antagonistic selection suggests that there are currently no such conflicts.

Table 2-1. Phenotypic data for body mass (kg) and horn volume (cm^3) in bighorn sheep. Number of individuals and observations included in the animal models are indicated as well as age-specific trait means and variation (s.d.). Each sex/age class was standardized (s.d. of unity) prior to analysis.

	Males				Females			
Trait\Age	2	3	4	5	2	3	4	5
body mass								
individuals	203	169	142	119	235	222	199	177
observations	502	340	237	184	703	695	609	544
mean	56.6	69.1	77.3	83.5	48.6	56.3	60	62.5
s.d.	10.5	10.1	10.4	10.6	7.9	7.4	7.1	7.2
horn volume								
individuals	201	169	145	121	225	210	189	164
observations	498	339	240	186	620	596	526	457
mean	486.8	1133.7	1877.8	2412.6	70.6	103.2	120	124.9
s.d.	237.4	431.1	597.6	592.2	24.2	25.7	27.2	25.6

Table 2-2. Additive genetic, year, year of birth and permanent environmental (co)variance components and correlations within and between the sexes for body mass and horn volume in adult bighorn sheep. Variance components are on the diagonal while covariance components are below the diagonal and correlations are above. Variance components were obtained with sex-specific univariate animal models whereas covariances where obtained from bivariate models. Significance of (co)variance components was tested with likelihood ratio tests. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significance of genetic correlations (in italics when different from zero) was inferred from the significance of associated covariance components. †Identifies genetic correlations significantly smaller than unity ($^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$). Standard errors generated by ASReml are also presented. MBM, male body mass; MHV, male horn volume; FBM, female body mass and FHV, female horn volume.

	MBM	MHV	FBM	FHV
additive genetic				
MBM	0.19 (0.07)**	*0.74 (0.15)*	*0.63 (0.30)*	0.27 (0.30)†
MVH	0.15 (0.07)*	0.22 (0.09)**	0.02 (0.29)††	0.24 (0.28)†
FBM	0.08 (0.04)*	0.00 (0.05)	0.10 (0.04)**	*0.63 (0.20)*†
FHV	0.06 (0.06)	0.06 (0.07)	0.10 (0.05)*	0.25 (0.10)**
year				
MBM	0.08 (0.02)***	*0.70 (0.11)*	*0.51 (0.16)*	*0.53 (0.15)*
MVH	0.06 (0.02)***	0.10 (0.03)***	*-0.56 (0.14)*	-0.23 (0.20)
FBM	0.07 (0.03)*	-0.11 (0.04)*	0.28 (0.08)***	*0.90 (0.04)*
FHV	0.05 (0.02)*	-0.02 (0.02)	0.19 (0.06)***	0.11 (0.03)***
year of birth				

MBM	0.12 (0.05)***	*0.96 (0.04)*	0.10 (0.26)	0.30 (0.25)
MVH	0.15 (0.06)***	0.18 (0.06)***	-0.10 (0.23)	0.37 (0.22)
FBM	0.02 (0.04)	-0.03 (0.06)	0.26 (0.08)***	*0.93 (0.04)*
FHV	0.05 (0.04)	0.08 (0.06)	0.29 (0.09)***	0.26 (0.09)***
permanent environment				
MBM	0.12 (0.06)**	*0.75 (0.20)*	-	-
MHV	0.10 (0.06)*	0.14 (0.08)*	-	-
FBM	-	-	0.13 (0.04)***	0.32 (0.17)
FHV	-	-	0.06 (0.04)	0.28 (0.08)***

Table 2-3. Sex-specific proportions of phenotypic variance explained by additive genetic (h^2), year, year of birth and permanent environmental effects. Standard errors generated by ASReml are also presented. MBM, male body mass; MHV, male horn volume; FBM, female body mass and FHV, female horn volume.

Trait	h^2	year	year of birth	perm. env.
MBM	0.32 (0.12)	0.13 (0.04)	0.20 (0.07)	0.21 (0.11)
MHV	0.32 (0.12)	0.14 (0.04)	0.25 (0.07)	0.20 (0.11)
FBM	0.11 (0.05)	0.30 (0.06)	0.28 (0.07)	0.14 (0.04)
FHV	0.24 (0.09)	0.11 (0.03)	0.25 (0.07)	0.27 (0.08)

Table 2-4. Sex-specific standardized directional selection differentials (S_i') and gradients (β_i') for body mass and horn volume in bighorn sheep. Male and female data were analyzed separately. Analyses were based on phenotypic values on June 5 at four years old. Fitness was defined as LRS (number of lambs produced that survived to weaning over an individual's lifetime), longevity (in years) and mean reproductive success (MRS, LRS*longevity^{-1}). Significant coefficients ($p < 0.05$) are italicized.

trait	fitness metric	n	S_i'	p	β_i'	p
male body mass	LRS	72	-0.09 (0.25)	0.68	-0.12 (0.36)	0.87
	longevity	129	-0.02 (0.04)	0.72	0.04 (0.05)	0.49
	MRS	72	0.03 (0.21)	0.99	-0.02 (0.29)	0.91
male horn volume	LRS	72	-0.05 (0.26)	0.50	0.03 (0.38)	0.58
	longevity	128	-0.08 (0.05)	0.15	-0.11 (0.06)	0.13
	MRS	72	0.06 (0.21)	0.89	0.07 (0.31)	0.86
female body mass	LRS	137	*0.13 (0.06)*	*< 0.05*	*0.16 (0.07)*	*< 0.01*
	longevity	137	*0.09 (0.04)*	*< 0.05*	*0.11 (0.04)*	*< 0.05*
	MRS	137	*0.08 (0.04)*	*< 0.05*	*0.10 (0.05)*	*< 0.05*
female horn volume	LRS	133	0.06 (0.05)	0.29	0.01 (0.06)	0.97
	longevity	133	0.03 (0.03)	0.39	-0.01 (0.04)	0.87
	MRS	133	0.01 (0.04)	0.73	-0.02 (0.04)	0.22

Figure 2-1. Adult male (left) and female (right) bighorn sheep from Ram Mountain, Alberta, Canada. Photos by Julien Martin.

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Chapter 3

Sex-specific genetic variance and the evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations

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3.1 Introduction

The phenotypic means of many traits differ between sexes in dioecious species. This pattern, termed sexual dimorphism (SD), is generally believed to be adaptive, reflecting differences in sex-specific phenotypic optima (Fairbairn *et al*. 2007). The independent occurrence of SD across a wide range of organisms suggests that, to some extent, divergence towards sex-specific phenotypic optima is often possible (Arnqvist & Rowe 2005; Fairbairn *et al*. 2007). Nevertheless, these optima may not always be reached as suggested by the commonly reported presence of sexually antagonistic selection for homologous male and female traits (i.e. situations where sex-specific selection coefficients are opposite in sign; Cox & Calsbeek 2009). In such cases, the independent evolution of the sexes may be constrained by shared genetic architecture manifested as a strong cross-sex genetic correlation (r_{MF}, Lande 1980, Roff 1997). Variability in sexual genetic constraints could influence the outcome of sexual selection models (Arnold 1985; Mead & Arnold 2004) but may also have implications for population dynamics and even speciation. For example, SD has been shown to correlate with population fitness (Kokko & Brooks 2003; Rankin & Arnqvist 2008), and may be involved in facilitating processes of ecomorphological diversification (Butler *et al*. 2007).

In principle, the short and long term evolution of SD mainly depends on the magnitude of r_{MF} between homologous traits and the nature of selection on each sex (Lande 1980). Since the evolution of SD is genetically constrained when selection acts to change the means of male and female characters against the sign

of their genetic correlation, the evolution of SD should be facilitated by the accumulation of alleles having sex-specific effects (Fisher 1958; Lande 1980, 1987; Rice 1984). However, the role of such sex-specific genetic variance in the evolution of SD is debated because SD can also arise from sex-specific environmental or maternal effects (Badyev 2002).

If sex-specific genetic variance is contributing to the resolution of sexual conflicts, an inverse relationship between SD and r_{MF} should be observed (Bonduriansky & Rowe 2005a; Fairbairn & Roff 2006; Fairbairn 2007b). This correlation could also be reinforced by the fact that SD can more readily increase when r_{MF} is small (Fairbairn 2007b). Thus far, a few population-specific empirical studies have added support to the hypothesis that sex-specific genetic variance is important in resolving sexual genetic conflicts. McDaniel (2005), Bonduriansky & Rowe (2005a), and Fairbairn (2007b) have all documented population-specific negative correlations between r_{MF} and SD. However, such a relationship was not observed in the largest population-specific dataset currently available (Cowley *et al.* 1986; Cowley & Atchley 1988) as noted by Fairbairn & Roff (2006). A lack of general correlation between r_{MF} and SD could be due to the fact that SD can still evolve when r_{MF} =1 if the sex-specific genetic variances are unequal (Lynch & Walsh 1998). Cross-sex genetic correlations may also generally tend to evolve toward unity if selection promotes the fixation (or loss) of mutations having sex-limited beneficial (or detrimental) effects.

The importance of genetic constraints in the evolution of SD remains largely unresolved, partly because little is known about the extant and causes of

variability in r_{MF} (Rice & Chippindale 2001; Fairbairn 2007a). It is especially unknown whether sexual genetic constraints differ in magnitude across types of traits, populations and environments. In this study, I compile r_{MF} estimates for dioecious plants and animals to provide the first empirical synthesis of r_{MF}. I also compare r_{MF} between different types of traits (morphological, developmental, behavioral, physiological and fitness components) and test the prediction that if positive genetic covariance constrains the evolution of SD, then r_{MF} for fitness components should on average be lower than for other trait types. I also briefly review variation in r_{MF} across populations and environments to assess whether evolutionary inferences from r_{MF} estimates in one system can be generalised more widely. Finally, I test the theoretical prediction of a negative relationship between r_{MF} and SD in order to evaluate whether sex-specific variance is an important contributor to the resolution of sexual genetic conflicts over shared traits.

3.2 Methods

3.2.1 Data collection

Scientific articles and book chapters containing r_{MF} estimates for homologous traits in dioecious species excluding humans were found using Google and PubMed. Searches included various combinations of the keywords including genetic correlation, r_G, intersex, inter-sex, intersexual, cross-sex, across sex, between sex, and gender. Additional studies were subsequently obtained from reference lists. Only studies that explicitly provided r_{MF} estimates were considered. This included estimates obtained with various experimental designs

and estimation methods. These estimates are only strictly comparable under the (untested) assumption that cross-sex correlations for additive genetic and other effects (maternal, dominance, epistasis) are similar. Another bias may arise from the exclusion of sex-linked effects in half-sib estimates. When both full-sib and half-sib estimates were provided, the half-sib estimate was used. When both maternal and paternal half-sib estimates were provided, the paternal half-sib estimate was used. Estimates from left and right side for symmetric traits (del Castillo 2005) were averaged. When estimates were published multiple times for the same population (e.g. Jensen *et al*. 2003, 2008), the most recent ones were used. An exception was made for the studies of waltzing flies (*Prochyliza xanthostoma*) by Bonduriansky and Rowe (2005a, 2005b) where the earliest estimates based on absolute rather than relative trait sizes were used to make results comparable with other studies. Estimates from studies where males and females were submitted to different treatments were excluded (Bisset *et al*. 1994, Hansen *et al*. 2003).

Absolute SD was quantified using phenotypic data made available in texts, tables or figures using the size dimorphism index (SDI) of Lovich & Gibbons (1992). This index is obtained by subtracting one from the ratio of the larger sex to the smaller sex. The SDI is considered the best estimator of SD because it is linear, intuitive, symmetrical and directional (Fairbairn 2007a). However, in the present case, absolute values of SDI were used so the index of SD is not entirely equivalent to the conventional SDI. It is therefore simply refered to as SD throughout the rest of this text. In a few instances, SD was calculated from

phenotypic means published in companion papers (Simons & Roff 1994, Merks 1988a, Singh *et al*. 1989, Havenstein *et al*. 1988a, 1988b)

Traits were categorized following Mousseau & Roff (1987) but with the difference that their "life history" category was separated into "fitness components" and "development" categories. This was done because the sign and magnitude of the cross-sex genetic correlation for fitness will depend on the signs of r_{MF} and sex-specific selection for traits contributing to fitness while it is not so for development traits. For example, the nature of sex-specific selection for growth rate is not expected to influence the sign of the r_{MF} for that trait, at least in the short term. On the other hand, the nature of selection for growth rate combined with the sign of the r_{MF} for that trait will influence the sign of the resulting cross-sex genetic covariance for fitness. "Fitness component" traits included estimates of survival, longevity, and reproductive success while development traits included traits such as growth rate and development time. Other categories were behavioural (e.g. activity level, food intake), physiological (e.g. hormone level, resistance to starvation and heat stress, conception rate in farmed animals) and morphological (e.g. body size, bristle number, bill color) traits. It must be acknowledged that classification was somewhat arbitrary for some traits. For example, life span under normal condition was classified as a fitness component but life span following heat shock or starvation were classified as physiological traits. Some traits classified as morphological such as flower number could also arguably have been included as fitness components. However, such cases were few and on the whole unlikely to alter conclusions

3.2.2 Statistical analyses

Variability in r_{MF} was first analysed for traits other than fitness components with a linear mixed effect model to test for a general relationship between r_{MF} and SD and quantify differences in r_{MF} among trait types. Fitness components r_{MF} were excluded from this analysis because they are expected to vary in sign depending on local sex-specific selection pressures while it is not so for other trait types. Weights were not used because it is often unclear how sample sizes should be determined and compared among and sometimes even within r_{MF} estimation methods. Relying on standard errors (SE) would also be unsatisfactory because SE estimation for r_{MF} is often based on different and sometimes problematic methods (Roff 1997). Sexual dimorphism (SD) and TRAIT TYPE were included as explanatory fixed effects in a model of r_{MF}. TRAIT (estimates for a trait obtained multiple times across ontogeny or in different environments for a single population were assigned to the same trait), POPULATION and SPECIES were fitted as random effects (intercepts) in an attempt to control for non-independence of traits due to shared genetic architectures. Differences between fitness components and other trait types were tested for using the same model except that TRAIT TYPE was collapsed into a two level factor (i.e., fitness components, all others). The significance of fixed effects was determined with conditional Wald F-tests using ASReml (Gilmour *et al*. 2006). These analyses assume that residual errors are normally distributed. However, while a strong central tendency was apparent in the distribution of residuals, there was also evidence of some negative skew and p-values presented

here should therefore be treated with slight caution. Nevertheless, analysis of r_{MF} after applying various normalising data transformation confirmed that all conclusions drawn here are statistically robust. In the interests of brevity and ease of interpreting effects sizes, only the models of the untransformed data are presented.

The relationship between r_{MF} and SD for traits other than fitness components was formally assessed using more traditional meta-analytic techniques based on population-specific effect sizes (Lipsey & Wilson 2001). Specifically, the distribution of population-specific effect sizes *r* (Spearman's correlation between r_{MF} and SD) was assessed. This necessarily required the exclusion of a substantial part of the data set since only results from studies in which r_{MF} was estimated for multiple traits in a single population could be included. *r* estimates obtained for the same population in multiple environments were averaged to limit the effect of non-independence in the estimation of the mean effect size. The analysis was based on untransformed effect sizes rather than Fisher transformed effect sizes as suggested in Hunter & Schmidt (2004). A sign test was used to test if effect sizes were more often negative than positive. Mean effect size was estimated by weighting each estimates by the inverse variance of Spearman's *r* (variance = $(n - 1)^{-1}$, where n is the number of traits). Mean effect size, 95% confidence intervals and heterogeneity of effect sizes (Q-statistic) were estimated with a random-effects model using the MiMa function developed by Viechtbauer (2006) in S-Plus 8.0 (Insightful).

3.3 Results

114 sources containing a total of 553 r_{MF} estimates were found (Table 3-1, see Supplementary File 1 at http://onlinelibrary.wiley.com/doi/10.1111/j.1558-5646.2009.00793.x/suppinfo). These studies included 3 plant, 3 fish, 9 mammal, 13 bird, and 19 invertebrate species. About half of the studies (58) were published in or after the year 2000. 488 r_{MF} estimates were left (Figures 3-1 and 3-2) after discarding duplicate studies and estimates obtained when males and females were submitted to different treatments, averaging left and right sides for symmetric traits, and excluding 2 two outliers from the study of Meagher (1984) since these were both highly inaccurate and very imprecise (inflorescence dry weight, $r_{MF} = -4.2 \pm 5.3$; inflorescence % of total dry weight, $r_{MF} = -32.2 \pm 29.4$). The vast majority of estimates were large and positive (Figure 3-1). Most of the rare negative estimates were for fitness components (Figure 3-2).

Mean r_{MF} and the influence of SD and trait type were approximated using a linear mixed model including trait, population and species as random effects. A total of 395 r_{MF} accompanied by estimates of SD were included in this analysis. These estimates were for 310 different traits (including 14 fitness components) measured in 101 populations from 42 species. Mean r_{MF} (predicted at SD = 0, i.e. no sexual dimorphism) was highest for morphological traits (0.80 ± 0.03) followed by behavioural (0.77 ± 0.09), developmental (0.73 ± 0.05), and physiological (0.62 ± 0.07) traits. Variation among trait types was significant (F-test, $p = 0.03$), a result attributable to the small mean r_{MF} for physiological traits (as compared to morphological traits). The relationship between r_{MF} and SD

estimated from this model was negative (slope = -0.07 ± 0.02, F-test, $p < 0.001$). Results were essentially the same when excluding putative outliers (n = 8) identified by ASReml.

Fitness components and other traits were contrasted using the mixed model methodology described above with the difference that trait types only consisted of two levels: fitness components and others. Cross-sex genetic correlations (predicted at SD = 0) appeared to be on average lower for fitness components than for other trait types (0.40 ± 0.07 vs. 0.76 ± 0.03, respectively; $p < 0.001$). Results were similar when excluding ten putative outliers (0.38 ± 0.07 vs. 0.77 ± 0.03, $p < 0.001$).

A test for a negative relationship between SD and r_{MF} at the population level was perfomed using 66 populations for which Spearman's *r* could be estimated (Figure 3-3). Effect sizes were marginally significantly more often negative than positive (38 vs. 24, sign test, one-tail $p = 0.049$). Effect sizes were not heterogeneous (Q-statistic, $p = 0.39$). The estimated mean effect size *r* (± 1 SE) was significantly negative (-0.12 ± 0.07, 95% CI: -0.26, 0.01, $z = -1.75$, one-tail test $p = 0.04$) and therefore consistent with the linear model analysis and the crude overall relationship between r_{MF} and SD (Figure 3-4).

3.4 Discussion

This study represents the first exhaustive synthesis of published estimates of cross-sex genetic correlations. Genetic correlations between homologous traits expressed in males and females were found to be predominantly large and

positive. This result confirms the conclusions drawn by others with regard to morphological traits (Roff 1997; Lynch & Walsh 1998), and shows that this pattern is fairly general across other trait types. Consequently, the rapid evolution of SD is, in general, genetically constrained. The evolution of sexual dimorphism is nonetheless often facilitated by cross-sex genetic correlations smaller than unity and in a few instances r_{MF} for non-fitness traits were very low or even negative. Negative correlations are not expected to limit the evolution of SD, but may nonetheless serve as evolutionary constraints in some contexts (e.g., when sex-specific selection coefficients are similar in sign).

Apart from fitness components (discussed further below), there was little evidence for differences in r_{MF} among most trait categories. The only exception being that physiological traits appeared to be characterized by smaller r_{MF} than morphological traits. This suggests that the evolution of SD may be generally less constrained for physiological traits. Trait categorization was admittedly subjective, particularly since traits assigned to different categories may often be closely related (e.g. daily food intake [behavioural], feed efficiency [physiological], growth rate [developmental], body weight [morphological]). Nonetheless such classification does allow for the detection of broad patterns (Mousseau & Roff 1987) and overall there appeared to be little evidence for clear differences in mean r_{MF} among most trait types after accounting for differences in SD.

Cross-sex genetic correlations for fitness components were on average smaller than for other trait types. This conclusion is in agreement with the results

of Kruuk *et al*. (2008) who focussed on quantitative genetic parameters obtained in wild populations only. The lower r_{MF} for fitness components compared to other trait types is also consistent with the common finding of sexually antagonistic selection on homologous male and female traits (Cox & Calsbeek 2009). The fact that r_{MF} for traits other than fitness components are typically positive combined with the frequent observation of negative r_{MF} for fitness (Chippindale *et al*. 2001; Foerster *et al*. 2007; Brommer *et al*. 2007) also support this hypothesis.

Cross-sex genetic correlations are population-specific due to differences in allele frequencies (Falconer 1989) but relatively few studies have estimated r_{MF} in different populations of the same species (e.g. Leips & Mackay 2000, Reverter *et al*. 2000, Stålhammar & Philipsson 1997, Nasholm 2004, McKay & Rahnefeld 1986, McDaniel 2005). For traits other than fitness, differences between populations varied from effectively non-existent (e.g., rib eye muscle area in Angus and Hereford cattle, r_{MF} = 0.89 and 0.9, Meyer & Graser 1999) to large (e.g., fat depth in Hereford and Santa Gertrudis cattle, r_{MF} = 0.87 and 0.35, Meyer & Graser 1999). For fitness component traits, the few estimates obtained in different populations grown under similar conditions were all substantially different (e.g., at least a 0.29 difference in r_{MF} between populations of fruit fly and seed beetle for life span; Leips & Mackay 2000, Fox *et al*. 2004). While such comparisons are rather arbitrary since SE for r_{MF} are typically either large, problematic, or simply not reported as in Meyer and Graser (1999), genetic constraints on the evolution of SD nonetheless appear to have the potential to vary considerably among populations.

In addition to being population-specific, r_{MF} estimates are expected to be environment specific due to genotype × environment interactions (Falconer 1989, Lyons *et al*. 1994, Simons & Roff 1996). Intuitively, this may be particularly true for fitness traits, the expression of which will be heavily influenced by environmentally determined selection regimes. The estimation of r_{MF} in different environments is uncommon (but see e.g., Lyons *et al*. 1994, Simons & Roff 1996, Vieira *et al*. 2000, Leips & Mackay 2000, Fox *et al*. 2004). Cross-sex genetic correlations sometimes varied greatly among environments for both fitness components and other trait types (e.g, r_{MF} = 0.23 to 1.23 for longevity raised at different temperatures in *D. melanogaster*, Vieira *et al*. 2000; r_{MF} = -0.68 and 0.94 for branch number under low and high density in white campion, Lyons *et al*. 1994). In a rare test of environmental heterogeneity, Lyons *et al*. 1994 detected significant variation in r_{MF} for two traits out of six (branch number and plant height). In the field cricket, r_{MF} were generally larger under field conditions compared to laboratory conditions (Simons & Roff 1996). These results suggest that r_{MF} estimates obtained in a single environment or averaged across environments may be insufficient to fully comprehend the evolutionary dynamic of sexual dimorphism when environmental conditions are heterogeneous over space and/or time.

Analyses have confirmed the prediction of a general negative correlation between r_{MF} and SD. This result supports the hypothesis that sex-limited genetic variance is important in the evolution of SD and implies that, in most cases, sex-specific genetic variance is not entirely depleted by sexually antagonistic

selection. The mean effect size estimate (-0.12 ± 0.07) was notably smaller than all previously published population-specific estimates (r = -0.89 in Fairbairn 2007; r = -0.74 in Bonduriansky & Rowe 2005a; r = -0.83 and -0.48 in McDaniel 2005) which may imply a publication bias toward large negative effect sizes. However, the sign of the relationships in these studies was nonetheless concordant with the results of the current meta-analysis. The mean effect size was also arguably somewhat diminished by a single positive effect size based on an uncommonly large number of traits (r = 0.08, n = 28, Cowley *et al*. 1986, Cowley & Atchley 1988) but the effect was marginal (mean r = -0.14 ± 0.07 when excluding that study). In fact, small effect sizes are not that surprising considering that r_{MF} estimates are usually highly imprecise and that many studies included suites of traits that were highly genetically correlated and/or varied little in their level of sexual dimorphism. It should also be noted that despite the presence of an overall trend toward a negative relationship between r_{MF} and SD, positive population-specific relationships appear possible and may in fact be relatively common.

Individual studies suggested that the relationship between r_{MF} and SD may be influenced by environmental conditions. For example, in white campion the mean effect size was highly negative (r = -0.71) when plants were watered daily at high density, yet highly positive (r = 0.66) when plants were watered every other day at low density (n = 6, Lyons *et al*. 1994). Conversely, r did not differ between laboratory and field conditions in field crickets (both r = -0.26, n = 6, Simons & Roff 1996). Difference in effect sizes among environmental conditions

could result from sex × genotype × environment interactions. Understanding how and why the relationship between r_{MF} and SD may vary with environmental conditions should be challenging since both variables could vary in unpredictable fashions as environments fluctuate.

Knowledge about the extant and causes of variability in r_{MF} is key to our understanding of the evolutionary dynamics of SD and of sexual conflicts. In particular, quantitative genetic models including SD which are often based on the assumption of stable quantitative genetic parameters (Mead & Arnold 2004) may benefit from incorporating potential short and long term variation in r_{MF}. Variability in sexual genetic constraints and the ease with which SD might evolve may also contribute to population dynamic heterogeneity and speciation rates (see Kokko & Brooks 2003, Butler *et al*. 2007, and Rankin & Arnqvist 2008 for examples of how SD may influence population dynamic and speciation). Despite the relatively large number of published r_{MF} estimates, there is still a clear need for more data relating to non-morphological traits, and to fitness components in particular. More studies that jointly estimate both genetic (co)variance structures and sex-specific selection regimes would also greatly improve our understanding of the evolutionary dynamics of intralocus sexual conflicts.

Table 3-1. Overview of compiled r_{MF} estimates for dioecious species.

Common name	Latin name	Estimates	References
Baboon	*Papio hamadryas*	2	1-2
Barn owl	*Tyto alba*	2	3
Barn swallow	*Hirundo rustica*	1	4
Bighorn sheep	*Ovis canadensis*	3	5-6
Black field cricket	*Teleogryllus commodus*	4	7
Black tiger prawn	*Penaeus monodon*	7	8
Cattle	*Bos taurus*	47	9-16
Cellar spider	*Pholcus phalangioides*	6	17
Chicken	*Gallus gallus*	28	18-27
Collared flycatcher	*Ficedula albicollis*	3	28-30
Dark-eyed juncos	*Junco hyemalis*	3	31
Darwin's medium ground finch	*Geospiza fortis*	4	32
Devil's bit	*Chammaelirium luteum*	$10^{(8)*}$	33
Domestic sheep	*Ovis aries*	18	34-38
Dung fly	*Sepsis cynipsea*	11	39-40
Field cricket	*Gryllus pennsylvanicus*	12	41
Fly	*Zaprionus indianus*	4	42
Fruit fly	*Drosophila melanogaster*	100	43-65
Fruit fly	*Drosophila serrata*	16	66-67
House cricket	*Acheta domesticus*	$5^{(3)*}$	68
House sparrows	*Passer domesticus*	11	69-70
Mealworm beetle	*Tenebrio molitor*	5	71
Mice	*Mus musculus*	9	72-74
Moss	*Ceratodon purpureus*	10	75
Muskovy duck	*Cairina moschata*	4	22, 76

Nile tilapia	*Oreochromis niloticus*	5	77
Oriental ching bug	*Cavelerius saccharivorus*	2	78
Pig	*Sus scrofa domestica*	70	79-86
Pigeon	*Columba livia*	3	87
Quail	*Coturnix coturnix*	1	18
Rabbit	*Oryctolagus cuniculus*	2	88
Rainbow trout	*Oncorhynchus mykiss*	4	89
Red deer	*Cervus elaphus*	4	90
Red flour beetle	*Tribolium castaneum*	1	91
Red junglefowl	*Gallus gallus*	12	92-93
Sand cricket	*Gryllus firmus*	1	94
Seed beetle	*Callosobruchus maculatus*	8	95
Stalk-eye fly	*Cyrtodiopsis dalmanni*	2	96
Striped ground cricket	*Allonemobius socius*	2	97
Tilapia	*Oreochromis shiranus*	1	98
Turkey	*Meleagris gallopavo*	15	18, 99-100
Waltzing fly	*Prochyliza xanthostoma*	16	101-102
Water strider	*Aquarius remigis*	13	103-104
White Campion	*Silene latifolia*	58	105-110
Yellow dung fly	*Scathophaga stercoraria*	3	111-112
Zebra finch	*Taeniopygia guttata*	2	113-114

* Two highly inaccurate estimates were ignored (inflorescence dry weight, r_{MF} = -4.2 ± 5.3; inflorescence % of total dry weight, r_{MF} = -32.2 ± 29.4).

** Estimates from left and right sides were averaged.

(1) Towne *et al.* 1992, (2) Havill *et al.* 2004, (3) Roulin *et al.* 2001, (4) Moller 1993, (5) Coltman *et al.* 2005, (6) Poissant *et al.* 2008, (7) Zajitschek *et al.* 2007, (8) Kenway *et al.* 2008, (9) Van Vleck & Cundiff 1998, (10) Crews Jr *et al.* 2003,

(11) Crews Jr & Kemp 2001, (12) Reverter *et al.* 2000, (13) Meyer & Graser 1999, (14) Hansen *et al.* 2003, (15) Taylor *et al.* 1985, (16) Stålhammar & Philipsson 1997, (17) Ulh *et al.* 2004, (18) Becker *et al.* 1964, (19) Mignon-Grasteau *et al.* 1999, (20) Le Bihan-Duval *et al.* 1998, (21) Hagger 1994, (22) Mignon-Grasteau *et al.* 1998, (23) Tixier-Bouchard *et al.* 1995, (24) Buvanendran 1969, (25) Kinney & Shoffner 1965, (26) Singh *et al.* 1979, (27) Comstock 1956, (28) Merilä *et al.* 1998, (29) Brommer *et al.* 2007, (30) Qvarnström *et al.* 2006, (31) McGlothlin *et al.* 2005, (32) Price 1984, (33) Meagher 1984, (34) Coltman *et al.* 2001, (35) Nasholm 2004, (36) Mousa *et al.* 1999, (37) Bisset *et al.* 1994, (38) Lax & Jackson 1987, (39) Muhlhauser & Blanckenhorn 2004, (40) Reusch & Blanckenhorn 1998, (41) Simons & Roff 1996, (42) Araripe *et al.* 2008, (43) Kopp *et al.* 2003, (44) Nuzhdin *et al.* 1997, (45) Reeve & Fairbairn 1996, (46) Chippindale *et al.* 2001, (47) Mackay *et al.* 1996, (48) Cowley *et al.* 1986, (49) Cowley & Atchley 1988, (50) Frankham 1968, (51) Sheridan *et al.* 1968, (52) Long & Rice 2007, (53) Wang *et al.* 2007, (54) Vieira *et al.* 2000, (55) Jordan *et al.* 2007, (56) Morgan & Mackay 2006, (57) Wayne *et al.* 2001, (58) Sambandan *et al.* 2006, (59) Gurganus *et al.* 1998, (60) Harbison & Sehgal 2008, (61) Harbison *et al.* 2004, (62) Karan *et al.* 2000, (63) Leips & Mackay 2000, (64) Mignon-Grasteau *et al.* 2004, (65) Karan *et al.* 1999, (66) Cheneweth & Blows 2003, (67) Cheneweth *et al.* 2008, (68) del Castillo 2005, (69) Jensen *et al.* 2003, (70) Jensen *et al.* 2008, (71) Rolff *et al.* 2005, (72) Eisen & Legates 1966, (73) Hanrahan & Eisen 1973, (74) Cheverud *et al.* 2001, (75) McDaniel 2005, (76) Hu *et al.* 1999, (77) Rutten *et al.* 2005, (78) Fujisaki 1993, (79) McKay & Rahnefeld

1986, (80) Smith & Ross 1965, (81) Hicks *et al.* 1999, (82) Merks 1986, (83) Crump *et al.* 1997, (84) Merks 1988b, (85) Zhang *et al.* 2000, (86) Varona & Noguera 2001, (87) Mignon-Grasteau *et al.* 2000, (88) Piles *et al.* 2005, (89) Kause *et al.* 2003, (90) Foerster *et al.* 2007, (91) Enfield *et al.* 1966, (92) Parker & Garant 2004, (93) Parker & Garant 2005, (94) Roff & Fairbairn 1993, (95) Fox *et al.* 2004, (96) Wilkinson 1993, (97) Fedorka *et al.* 2007, (98) Maluwa *et al.* 2006, (99) Chapuis *et al.* 1996, (100) Toelle *et al.* 1990, (101) Bonduriansky & Rowe 2005a, (102) Bonduriansky & Rowe 2005b, (103) Preziosi & Roff 1998, (104) Fairbairn 2007b, (105) Meagher 1992, (106) Meagher 1994, (107) Steven *et al.* 2007, (108) Delph *et al.* 2004, (109) Meagher 1999, (110) Lyons *et al.* 1994, (111) Simmons & Ward 1991, (112) Blanckenhorn 2002, (113) Price & Burley 1993, (114) Price 1996.

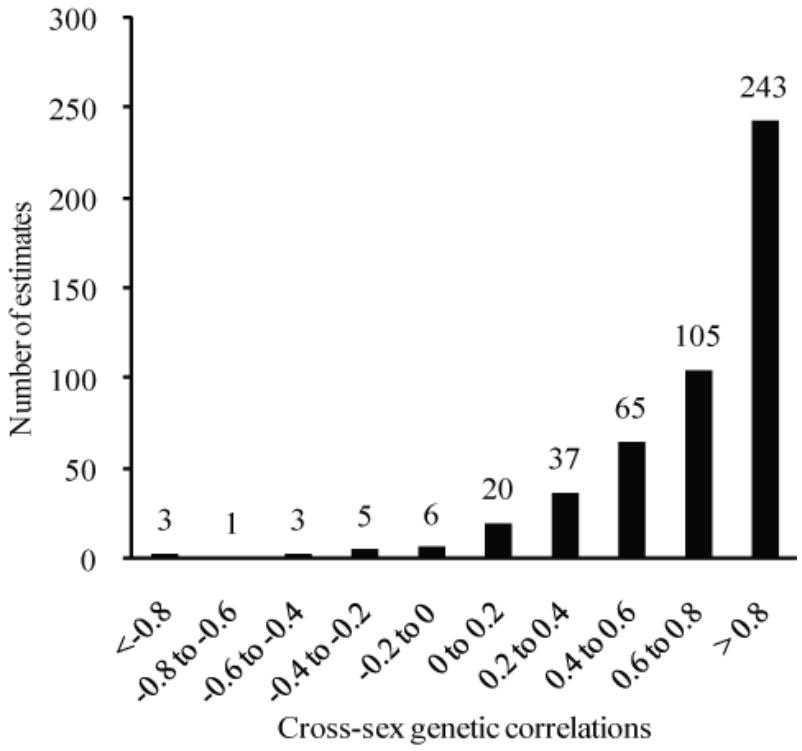

Figure 3-1. Distribution of compiled cross-sex genetic correlation estimates.

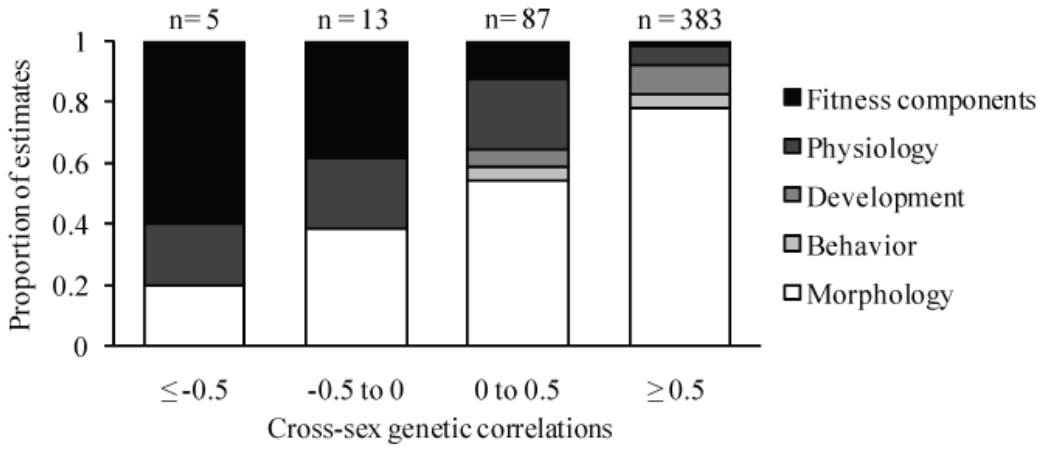

Figure 3-2. Proportional distribution of 488 cross-sex genetic correlations estimates according to trait type. (Categories on a black to white scale are fitness components, physiological, developmental, behavioural and morphological traits.)

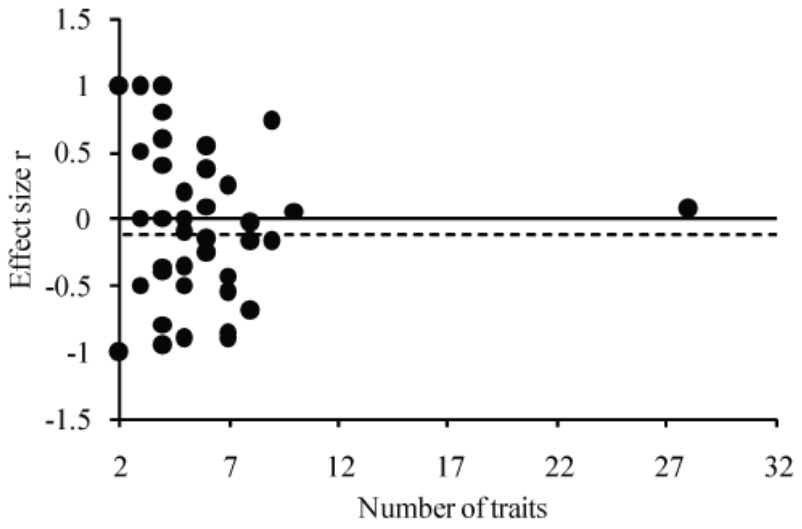

Figure 3-3. Variation in population-specific effect sizes (Spearman's r) as a function of sample size (number of traits) for the relationship between cross-sex genetic correlations and sexual dimorphism. (The dashed line depicts the mean weighted effect size (n = 66, r = -0.12 ± 0.07, 95% CI: -0.26, 0.01).)

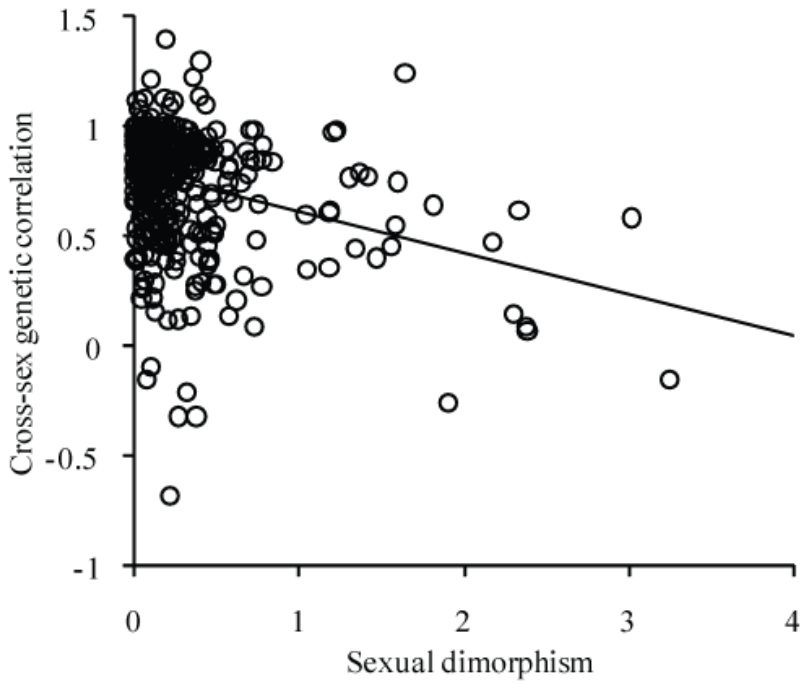

Figure 3-4. Crude relationship between cross-sex genetic correlations and sexual dimorphism based on 375 estimates (excluding fitness components; horn volume in bighorn sheep, SD = 12.2, was omitted to ease visualization).

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Chapter 4

The ontogeny of cross-sex genetic correlations: an analysis of patterns

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4.1 Introduction

Genetic values for male and female homologous traits usually covary (Poissant *et al*. 2010). This cross-sex genetic covariance acts as a constraint on the evolution of sexual dimorphism by limiting the independent responses of the sexes to sex-specific selection (Lande 1980). Cross-sex genetic correlations (r_{MF}, a standardized measure of cross-sex genetic covariance; Lynch & Walsh 1998) have been estimated for a variety of traits in a wide range of taxa (Poissant *et al*. 2010) but we still know little about their evolution. In particular, the ontogenetic dynamics of r_{MF} remains poorly understood and it therefore remains unknown if estimates obtained at a specific age, and the associated evolutionary inference, can generally be extrapolated to other ontogenetic stages.

Cross-sex genetic correlations are typically assumed to be stable with age (estimates are usually either obtained for a single time point, reviewed in Poissant *et al*. 2010, or averaged across ontogeny, e.g. Coltman *et al*. 2005, Poissant *et al*. 2008) despite evidence for frequent ontogenetic variation in quantitative genetic parameters (Wilson & Réale 2006, Bergen *et al*. 2007). In fact, there are many reasons to expect r_{MF} to vary with age. For example, r_{MF} could initially decrease with age followed by an increase if there are sex differences in the timing of gene action (Hanrahan & Eisen 1973). Alternatively, r_{MF} could increase or decrease with age for traits characterized by low levels of ontogenetic memory (sensu Atchley 1984) depending on the structure of genetic (co)variance matrix. For example, when survival of juveniles and adults is mainly determined by different pathogens, r_{MF} for survival in juveniles and adults will in part mirror r_{MF} for

resistance to these distinct pathogens and thus potentially change with age if resistance to the different pathogens is not perfectly genetically correlated. Similarly, r_{MF} could change late in ontogeny for some traits due to the accumulation of genetic (co)variance expected from the mutation accumulation theory of ageing (Medawar 1952, Kirkwood & Austad 2000). The behaviour of r_{MF} in this scenario will depend on how the accumulating sex-specific genetic effects tend to covary relative to the genetic effects present at earlier ontogenetic stages.

While both increases and decreases are possible at various ontogenetic stages, r_{MF} most likely typically declines with age as suggested by Fisher (1958) who hypothesized that most sex-specific genetic variance should start being expressed at sexual maturity if sex-limited mutations mainly act in conjuncture with the secretion of sexual glands. Downward trends in r_{MF} would also be consistent with what is typically observed between traits measured at increasing age intervals in the same sex (Atchley 1984) and concordant with the prediction of increasing sex-biased gene expression with age (Ellegren & Parsch 2007) as demonstrated in chicken (*Gallus gallus*; Mank & Ellengren 2008, 2009). A common decline in r_{MF} also seems more likely because sex-specific genetic variance tends to increase in the presence of sexually antagonistic selection (Bonduriansky & Rowe 2005, Fairbairn 2007, Poissant *et al*. 2010) and because such selection likely mainly operates in adults (Badyaev 2002). For example, selection favoured similar genotypes in juvenile fruit flies *Drosophila*

melanogaster yet was sexually antagonistic at the adult stage (Chippindale *et al*. 2001).

Here, I present the first compilation of r_{MF} for traits estimated at multiple time points in ontogeny and use meta-analysis techniques to test for the presence of a downward ontogenetic trend as originally hypothesised by Fisher (1958). Increased knowledge about ontogenetic variation in r_{MF} should in particular improve our understanding of the genetic basis of the development of sexual dimorphism and the evolutionary dynamics of sexual conflicts.

4.2 Methods

Studies containing r_{MF} estimates obtained at multiple points across ontogeny were identified from the database assembled by Poissant et al. (2010). Additional studies were obtained from the human research literature. Estimates from different populations were considered independent since r_{MF} is population-specific due to differences in allele frequencies (Falconer 1989).

Trait-specific relationships between r_{MF} and age were estimated using Spearman's *r*. A sign test was used to test if effect sizes were more often negative than positive. A mean effect size was obtained by weighting each estimate by the inverse variance of Spearman's *r* (variance = $(n - 1)^{-1}$, where n is the number of time points). Mean effect size, 95% confidence intervals and heterogeneity of effect sizes (Q-statistic) were estimated with a random-effects model using the MiMa function developed by Viechtbauer (2006) in S-Plus 8.0 (Insightful).

4.3 Results

22 studies reporting r_{MF} at multiple time points were found. However, the data from Eisen & Legates (1966) was excluded because the same population was also analysed by Hanrahan & Eisen (1973). The data of Svedberg *et al.* (2001) was also excluded because r_{MF} estimates were highly imprecise and/or bound to zero for most traits. Finally, the study of Nes *et al.* (2007) was excluded because trends in r_{MF} for the same trait inferred from different models were conflicting. In total, the analyses thus included 28 traits from 19 studies representing 11 species (Table 4-1).

Across all traits, the relationship between r_{MF} and age was significantly more often negative than positive (21 vs. 7, respectively, Table 4-1; sign test, $p < 0.01$). The mean weighted effect size (Spearman's r ± SE) was large and significantly negative (-0.49 ± 0.15, 95 % CI = -0.78 to -0.19, z = -3.19, one-tail $p < 0.001$, Figure 4-1). There was no evidence of heterogeneity (Q-Statistic = 23.06, $p = 0.68$).

4.4 Discussion

The analysis demonstrates that r_{MF} can vary across ontogeny and that such change usually involves a downward trend. The common decline in r_{MF} with age indicates that sexual dimorphism may evolve more readily for phenotypes expressed later in ontogeny and is consistent with the idea that selection for sexual dimorphism usually operates in adults (Badyaev 2002). The accumulation of age and sex-specific genetic variance may facilitate the resolution of

ontogenetic conflicts where selection favours differing patterns of SD in juveniles and adults (Badyaev 2002).

The exact proximate mechanisms leading to ontogenetic changes in r_{MF} remain largely unknown. In vertebrates, such changes likely arise from ontogenetic variation in levels of sex steroids as originally hypothesized by Fisher (1958). Interaction between sex steroids and loci distributed throughout the genome is readily recognized as an important source of sex-specific genetic variance (Rhen & Crews 2008) and many sex steroids are known to vary with age in sex-specific manners (Ober *et al*. 2008). Apart from well known sex differences occurring at puberty and persisting in adults, sex differences in sex steroids have also been documented in neonates (Corbier 1992) and ageing individuals (Chahal & Drake 2007), offering plenty of opportunity for the expression of age- and sex-specific genetic variance. While loci influencing males and females differently are known to be common (Mackay & Anholt 2006, Ober *et al*. 2008), few studies have investigated their relative importance in relation to age (but see Nuzhdin *et al*. 1997, 2005) and ontogenetic variation in r_{MF}. It should also be noted that genomic imprinting (Day & Bonduriansky 2004, Bonduriansky & Rowe 2005) could also play a role in ontogenetic variation in r_{MF} since it has been shown to have age-specific effects (Wolf *et al*. 2008).

Based on the studies compiled here, declines in r_{MF} with age appear to be the norm. However, additional research will be necessary to assess the prevalence of alternate patterns. In particular, there is a clear lack of data to verify if r_{MF} tends to increase following an initial decrease as a result of sex differences in the

timing of gene action and on the behaviour of r_{MF} during senescence. Most of the studies performed to date were also limited to vertebrates and it remains to be seen if the observed pattern can be generalized to other kinds of organisms such as invertebrates or plants.

The estimation of age-specific genetic parameters is notoriously difficult because it requires the collection of a large amount of phenotypic information. While longitudinal studies would generally be preferable, wild long-lived species with overlapping generations may also be well suited to such investigation since phenotypic data on related individuals of differing age can usually be obtained simultaneously. When small sample sizes lead to significant inaccuracy and imprecision, more data-efficient analytical strategies could include the use a sliding window where overlapping groups of age classes would be analysed sequentially or multivariate random regression (e.g. Robinson *et al*. 2009) where r_{MF} would be allowed to covary with age.

Results demonstrate that the magnitude and dynamics of genetic constraints with regard to the evolution of SD can vary with age. Care should thus be taken to relate evolutionary inferences to the ontogenetic stage at which r_{MF} was estimated. For example, it appears that r_{MF} estimates obtained in juveniles would usually tend to overestimate genetic constraints in the evolution of adult SD. The presence of variation with age also suggests that r_{MF} estimates obtained in different populations may not be comparable when obtained at different ontogenetic stages. Additional studies on the causes, mechanisms and consequences of ontogenetic variability in r_{MF} would greatly improve our

understanding of evolutionary patterns related to SD and the resolution of intralocus sexual conflicts.

Table 4-1. Compiled traits for which r_{MF} was estimated at multiple time points throughout ontogeny.

Species	Latin name	Trait	Age range	Time points	r_{MF} trend	reference
Black tiger prawn	*Penaeus monodon*	Growth rate	16-30 to 16-54 weeks	3	-	Kenway et al. 2006
Black tiger prawn	*Penaeus monodon*	Weight	16 - 54 weeks	4	-	Kenway et al. 2006
Cattle	*Bos taurus*	Weight	0 - 1 year	3	+	Van Vleck & Cundiff 1998
Cattle	*Bos taurus*	Backfat	12 vs. 14 months	2	-	Crews Jr. & Kemp 2001
Cattle	*Bos taurus*	Longissimus muscle area	12 vs. 14 months	2	+	Crews Jr. & Kemp 2001
Cattle	*Bos taurus*	Mortality[(1)]	1-14 vs. 61-180 days	2	-	Hansen et al. 2003
Cattle	*Bos taurus*	Weight gain[(2)]	Birth to weaning vs. post weaning	2	-	Stålhammar & Philipsson 1997
Cattle	*Bos taurus*	Weight gain[(3)]	Birth to weaning vs. post weaning	2	-	Stålhammar & Philipsson 1997
Cattle	*Bos taurus*	Weight gain[(4)]	Birth to weaning vs. post weaning	2	-	Stålhammar & Philipsson 1997
Cattle	*Bos taurus*	Weight gain[(5)]	Birth to weaning vs. post weaning	2	+	Stålhammar & Philipsson 1997
Cattle	*Bos taurus*	Weight gain[(6)]	Birth to weaning vs. post weaning	2	-	Stålhammar & Philipsson 1997
Chicken	*Gallus gallus*	Weight	8 vs. 36 weeks	2	-	Mignon-Grasteau et al. 1999
Chicken	*Gallus gallus*	Weight	0 - 8 weeks	3	-	Singh et al. 1979
Human	*Homo sapiens*	Liability to major depression	Younger vs. older adults	2	-	Kendler & Prescott 1999
Human	*Homo sapiens*	Anxiety and depression	8-9 to 19-20 years	4	-	Kendler et al. 2008
Human	*Homo sapiens*	Subjective well-being	Younger vs. older	2	-	Nes et al. 2006
Human	*Homo sapiens*	Fat patterning	7-11 vs. 13-17 years	2	-	Towne 1995

Mice	*Mus musculus*	Weight	3 - 8 weeks	3	+	Hanrahan & Eisen 1973
Muskovy duck	*Cairina moschata*	Weight	10 vs. 18 weeks	2	-	Hu et al. 1999
Nile tilapia	*Oreochromis niloticus*	Weight	122 - 293 days	5	-	Rutten et al. 2005
Pig	*Sus scrofa domestica*	Feed per day	50 - 200 lbs	3	-	Smith & Ross 1965
Pig	*Sus scrofa domestica*	Weight	4 - 22 weeks	3	-	Zhang et al. 2000
Rainbow trout	*Oncorhynchus mykiss*	Weight	2 vs. 3 years	2	-	Kause et al. 2003
Red junglefowl	*Gallus gallus*	Tarsus length	0 - 18 weeks	3	+	Parker & Garant 2005
Red junglefowl	*Gallus gallus*	Weight	0 - 26 weeks	3	+	Parker & Garant 2005
Turkey	*Meleagris gallopavo*	Weight[(7)]	12 vs. 16 weeks	2	-	Chapuis et al. 1996
Turkey	*Meleagris gallopavo*	Weight[(8)]	12 vs. 16 weeks	2	+	Chapuis et al. 1996
Turkey	*Meleagris gallopavo*	Weight[(9)]	12 vs. 16 weeks	2	-	Chapuis et al. 1996

[(1)] Non-transferred males only, [(2)] Aberdeen Angus, [(3)] Charolais, [(4)] Hereford, [(5)] Limousin, [(6)] Simmental, [(7)] Strain A, [(8)] Strain B, [(9)] Strain C.

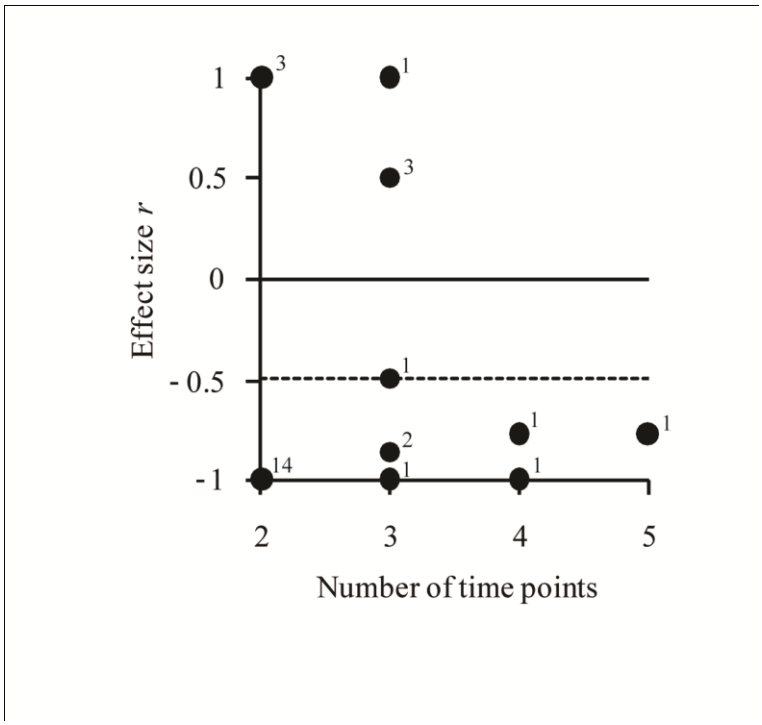

Figure 4-1. Variation in trait-specific effect sizes (Spearman's r) as a function of sample size (number of time points) for the relationship between cross-sex genetic correlations and age. (Numbers represents the amount of trait-specific effect sizes estimated. The mean weighted effect size (n = 28, r = -0.49 ± 0.15) is depicted by a dashed line.)

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Chapter 5

Genome-wide cross-amplification of domestic sheep microsatellites in bighorn sheep and mountain goats

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5.1 Introduction

Population genomics (Luikart *et al*. 2003) and linkage mapping studies in the wild (Slate 2005) are key to our understanding of the molecular genetic basis of phenotypic variation and adaptation (Stinchcombe & Hoekstra 2007). However, there are only a few wild species for which sets of highly polymorphic molecular markers offering genome-wide coverage are available. Currently 80 microsatellite loci are available for bighorn sheep (*Ovis canadensis*, Boyce *et al*. 1997; Wilson *et al*. 1997; Maudet *et al*. 2004; Whittaker *et al*. 2004; Coltman *et al*. 2005; Hogg *et al*. 2006) and 31 for mountain goats (*Oreamnos americanus*; Wilson & Strobeck 1999; Mainguy *et al*. 2005). Given that microsatellite primer pairs used in domestic sheep often amplify polymorphic loci in wild sheep and goats (Maudet *et al*. 2004; Mainguy *et al*. 2005), I took advantage of the relatively dense domestic sheep linkage map (Maddox & Cockett 2007) to characterize a large number of polymorphic microsatellite loci putatively distributed throughout the genomes of my focal species.

5.2 Methods

This study focussed on two populations of bighorn sheep (Ram Mountain, RM, Alberta, Canada, Poissant *et al*. 2008, and National Bison Range, NBR, Montana, USA, Hogg *et al*. 2006) and one population of mountain goats (Caw Ridge, CR, Alberta, Canada, Festa-Bianchet & Côté 2008) where long term studies of marked individuals have allowed pedigrees to be developed. Marker amplification was initially tested for using 648 microsatellite primer pairs in 7

bighorn sheep from RM and 576 primer pairs in 7 mountain goats. Primer pairs amplifying products in RM were subsequently tested in 8 individuals from NBR. Primer pairs consistently amplifying polymorphic (≥ 3 alleles in one population) products in sheep were finally genotyped on 22 additional individuals per population. For mountain goats, 12 additional individuals were genotyped for all loci that had ≥ 2 alleles in the first round of screening. About half of the individuals were males (NBR = 16, RM = 16, CR = 11). All primer sequences were obtained from Dr. J. Maddox's sheep genetic resources web site: http://rubens.its.unimelb.edu.au/~jillm/jill.htm. Primer information originated from a large number of studies. In cases where primer sequences differed between the original publication and Dr. Maddox's web site, the information from the web site was used.

Genomic DNA was extracted from tissue samples using Qiagen DNeasy tissue kits. Microsatellites were amplified using a reverse primer, a forward primer with an M13(-21) tail (TGTAAAACGACGGCCAGT) and an M13(-21) labelled (FAM, PET, NED or VIC) primer (Schuelke 2000). Each 15 ul PCR reaction contained 1X PCR buffer (1 mM Tris pH 8.8, 0.01% Triton X-100, 5 mM KCl, 0.016 mg/ml BSA), 1.9 mM $MgCl_2$, 0.2 mM dNTP, forward (0.04 uM), reverse (0.16 uM) and M13(-21) (0.16 uM) primer, 0.5 U of Taq polymerase isolated as in Engelke *et al.* (1990), 25 ng of DNA and water. PCR reactions were performed in an epgradient mastercycler (Eppendorf) under the following conditions: 94°C for 1 minute, 3 cycles of 30 seconds (sec) at 94°C, 20 sec at 52°C and 5 sec at 72°C, 30 cycles of 15 sec at 94°C, 20 sec at 52°C and 2 sec at

72°C, followed by a 15 minute extension at 72°C. Annealing temperatures were only optimized for a few loci in bighorn sheep (AGLA269, BM226, BM4006, BM6466, BMS381, BP33, CSRD81, CSSM19, MNS101A, MNS89A, URB058, Appendix 1) using two additional animals from the RM population (1 male and 1 female). Products were separated on a 3730 DNA Analyser (Applied Biosystems, AB) and sized using the Genescan LIZ500 size standard (AB). Genotypes were compiled using GeneMapper v4.0 (AB).

Descriptive statistics (number of alleles, observed and expected heterozygosity) were obtained using Microsatellite Analyser (MSA) 4.05 (Dieringer & Schlotterer 2003). Allelic sizes were not directly comparable between sheep and goats because different dye labelled M13 primers were sometimes used in the 2 species. Departure from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) was tested using GENEPOP 4.07 (Rousset 2008). Tests for the presence of null alleles were performed using MICRO-CHECKER (Van Oosterhout *et al*. 2004).

5.3 Results and Discussion

247 and 149 new polymorphic markers were characterized in bighorn sheep and mountain goats, respectively (see supplementary file online at http://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2009.02575.x/suppinfo). The greater discovery rate in bighorn sheep compared to mountain goats may be due to the closer evolutionary relationship between domestic sheep and bighorn sheep (Fernández & Vrba 2005). However, differences in polymorphism could

also be due to the demographic history of species and/or populations. The mean number of chromosomes genotyped, number of alleles per locus, observed and expected heterozygosity are presented in Table 5-1. 17, 10 and 8 markers were found to be out of HWE ($p < 0.05$) and 2, 4 and 2 loci showed signs of null alleles in the NBR, RM and CR populations, respectively. No marker was out of HWE or showing signs of null alleles following Bonferroni correction. Sheep and goats were not directly comparable because the number of genotyped individuals as well as marker selection criteria differed between species (i.e., ≥ 3 alleles in sheep vs. ≥ 2 alleles in goats). In sheep, genetic diversity and LD were higher in the NBR than in the RM population (Table 5-1). This probably results from the recent translocation of animals to the NBR population (Hogg *et al*. 2006).

Several primer pairs amplified 2 loci in bighorn sheep, as previously found in domestic sheep (Maddox *et al*. 2001). Seven of these primer pairs amplified non-overlapping, polymorphic products that resembled independently segregating loci (BMS2466, BM3212, HBB2, MCMA54, MNS97A, TGLA176, TGLA377). TGLA176/i and TGLA176/ii as well as BM3212/i and BM3212/ii were in LD (both $p < 0.0001$) while the remaining pairs of markers were not.

Putative locations of the amplified fragments in the domestic sheep genome based on the Australian Sheep Gene Mapping project sex averaged linkage map (http://rubens.its.unimelb.edu.au/~jillm/jill.htm) are presented in a supplementary file available online at http://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2009.02575.x/suppinfo. One pair of primers amplifying an autosomal marker in domestic sheep (FCB19,

linkage group 15) appeared to amplify an X-linked marker in bighorn sheep based on the absence of heterozygous males (0 /32 heterozygous males vs. 20/27 heterozygous females). This marker was uninformative in mountain goats. The average distance between all known polymorphic bighorn sheep and mountain goat markers inferred from the Australian domestic sheep linkage map (mean ± 1 SD) was 11.1 ± 9.2 and 15.8 ± 13.8 centimorgans (cM), respectively. Overall, markers appeared to cover most of the domestic sheep genome except for 2 and 5 intervals > 50 cM in bighorn sheep and mountain goats, respectively (Figure 5-1). Markers were also absent from a few telomeric regions, especially in mountain goats. Ultimately, linkage analysis will be necessary to map these markers in the genomes of focal species. The recently acquired domestic sheep genome sequence (http://www.sheephapmap.org/isgc_genseq.htm) could subsequently be used to develop markers for regions with low coverage.

These markers will be useful for improving parentage analyses, marker-based relatedness estimates and landscape genetic studies. They will also be used to perform genome scans to detect loci influenced by selection, develop linkage maps and locate quantitative trait loci (QTL) in wild populations. This will allow for investigations into the molecular genetic basis of local adaptation, harvesting-induced evolution (Coltman *et al.* 2003), development of sexual dimorphism (Poissant *et al.* 2008), genetic rescue (Hogg *et al.* 2006) and animal personality (Réale *et al.* 2000).

Table 5-1. Mean number of chromosomes genotyped, number of alleles, observed and expected heterozygosity (H) ± 1 standard deviation, as well as proportion of marker pairs in linkage disequilibrium at the 0.01, 0.001 and 0.0001 significance levels in bighorn sheep (Ram Mountain, RM, National Bison Range, NBR) and mountain goat (Caw Ridge, CR) populations.

	RM	NBR	CR
Number of chromosomes	56.1 +/- 5.0	58.0 +/- 4.4	37.0 +/- 2.4
Number of alleles	3.9 +/- 1.6	4.7 +/- 1.6	2.9 +/- 1.0
Observed H	0.56 +/- 0.19	0.63 +/- 0.14	0.47 +/- 0.19
Expected H	0.56 +/- 0.17	0.64 +/- 0.14	0.48 +/- 0.17
LD, $p < 0.01$	2.0%	3.7%	1.0%
LD, $p < 0.001$	0.4%	0.8%	0.1%
LD, $p < 0.0001$	0.1%	0.3%	0.01%

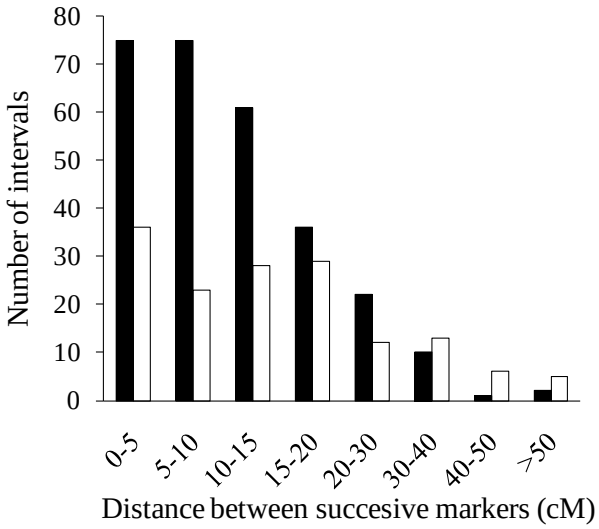

Figure 5-1. Putative distance (in centimorgans, cM) between successive polymorphic bighorn sheep (black bars) and mountain goat (white bars) markers based on the Australian Sheep Gene Mapping Project sex averaged linkage map.

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Chapter 6

Genetic linkage map of a wild genome: genomic structure, recombination and sexual dimorphism in bighorn sheep

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6.1 Introduction

The construction of genetic linkage maps in model organisms and domesticated species enables studies of the genetic architecture of trait variation and genome evolution. However, such resources for free-living populations of non-model species are still rare because it is difficult to acquire large enough pedigrees and associated sets of molecular markers (Slate 2005, 2008). The utility of genetic linkage maps developed using pedigreed wild populations has been demonstrated by pioneering studies on the genetic architecture of trait variation (Slate *et al*. 2002, Beraldi *et al*. 2006, 2007a, 2007b, Gratten *et al*. 2007, Johnston *et al*. 2010), genetic constraints (Gratten *et al*. 2008) and patterns of linkage disequilibrium (Backström *et al*. 2006, Slate & Pemberton 2007) under semi-natural settings. Yet, we still know very little about these specific topics and the potential to address a variety of additional subjects remains largely unexploited (Slate *et al*. 2010). The development of linkage maps for additional natural populations is therefore clearly desirable.

The bighorn sheep (*Ovis canadensis*), a mountain ungulate inhabiting western North America (Valdez and Krausman 1999), is one species for which linkage map construction using free-living individuals is possible. DNA samples from intensively studied pedigreed populations have been collected over many decades by field biologists (e.g. Hogg *et al*. 2006, Poissant *et al*. 2008) and a large set of polymorphic microsatellite markers was recently derived from domestic sheep genomic resources (Poissant *et al*. 2009). A bighorn sheep linkage map would enable one to dissect the molecular genetic basis of fitness-related traits,

study the molecular basis of inbreeding depression and genetic rescue (Hogg *et al*. 2006), and potentially reveal the molecular genetic basis of human-influenced evolution (Coltman 2008).

In addition to generating species-specific research opportunities, a bighorn sheep map would shed light on the levels of genomic re-organization between bighorn and domestic sheep. While few differences are expected between these species due to their recent divergence (~3 million years, Bunch *et al*. 2006), shared karyotype (Bunch *et al*. 1999) and ability to produce fertile hybrids (Delgadillo *et al*. 2003), enough time has elapsed for rearrangements to accumulate (Pontius *et al*. 2007). For example, numerous small-scale rearrangements have been documented between domestic sheep and the slightly more genetically distant domestic goat, *Capra hircus* (Maddox & Cockett 2007), which can also interbreed with domestic sheep (Tucker *et al*. 1989]. Reorganization has also been observed among domestic sheep breeds (Beraldi *et al*. 2006, McRae & Beraldi 2006). A bighorn sheep linkage map could therefore be used to detect recent chromosomal rearrangements in sheep species and would help with inferring ancestral marker order for regions showing intra-specific variation.

While genome structure is anticipated to be similar between closely related sheep species, expectations for sex-averaged and sex-specific recombination rates are less clear. This is because domestication may have led to an increase in recombination rates and unusual male-biased heterochiasmy in domestic sheep (Burt & Bell 1987, Coop & Przeworski 2007). However, the role

of domestication in the evolution of mammalian recombination rates remains unclear due to the absence of data on wild relatives (Ross-Ibarra 2004, Dumont & Payseur 2008). A bighorn sheep linkage map would enable such a comparison and help to determine if domestication played a role in the evolution of the atypical recombination patterns seen in domestic sheep.

In this article, I report on the development of a first-generation bighorn sheep genetic linkage map based on the genotyping of 252 polymorphic microsatellites in 498 animals from two pedigreed wild populations: National Bison Range (NBR), Montana, USA (Hogg *et al*. 2006), and Ram Mountain (RM), Alberta, Canada (Poissant *et al*. 2008). The availability of multiple mapping populations permitted a comparison of intra-specific variability in map characteristics as well as the construction of a more contiguous map that should in principle be more representative of the species as a whole. Marker synteny and order were then compared between bighorn sheep and domestic sheep to test for recent chromosomal rearrangements. Finally, I contrasted intervals between species in terms of sex-averaged length and sexual dimorphism to gain insights into the impacts of domestication on the evolution of mammalian recombination rates.

6.2 Methods

6.2.1 Study populations

6.2.1.1 National Bison Range

The NBR population was established by transplanting four rams and eight potentially pregnant ewes from Banff National Park (Alberta, Canada) in 1922 (Hogg *et al.* 2006). The population remained isolated until the introduction of five rams in 1985 and 10 sheep (three rams and seven ewes) from 1990 to 1994. Fourteen of these more recently introduced animals were derived from a native Montana population (Sun River) while one ewe was from a native Wyoming herd (Whisky Basin). Individuals from these latter introductions were highly successful (Hogg *et al.* 2006), resulting in relatively high levels genetic diversity and linkage disequilibrium (Poissant *et al.* 2009). All sheep were individually recognizable through physical characteristics from 1979 onward and collection of blood/tissue samples for genetic analysis began in 1988. Analyses included a combination of descendants from the original introduction, recent immigrants and admixed individuals.

6.2.1.2 Ram Mountain

The RM population is native to a small isolated mountain range located about 50 km east of the Canadian Rockies in Alberta, Canada (Poissant *et al.* 2008). Immigration and emigration is highly restricted and mainly limited to exchanges with a smaller unmonitored herd located on the same mountain range. Animals were captured in a corral trap baited with salt and marked with unique tags as lambs or yearlings. Population monitoring began in the early 1970s and collection of hair/blood/skin samples for genetic analysis began in 1988.

6.2.3 Mapping pedigrees

In both populations, parentage was originally determined with ~30 microsatellite loci using the 95% confidence threshold in Cervus (Marshall *et al.* 1998). For the RM population, the markers used are presented in Poissant *et al.* (2008) and references therein. For the NBR population, the markers included the ones listed in (Hogg *et al.* 2006) as well as BL25, BM1225, BM1818, BM4505, BM4630, BM848, BMC1222, MAF92, OarJMP29, TGLA126, TGLA387, EPCDV21, MCMA54/i and MCMA54/ii. Laboratory methods are detailed in Hogg *et al.* (2006), Poissant *et al.* (2008) and references therein. References for primer sequences are available in Additional file 1 at http://www.biomedcentral.com/1471-2164/11/524. Reconstructed pedigrees were used to identify animals expected to contribute the most information for linkage mapping purposes (e.g. large sibships and their parents) and these animals were then genotyped at more than 200 microsatellite loci (details below). Once genome-wide genotypes were obtained, animals for which genotyping success was low (< 65%) were discarded and the pedigrees were updated based on new parentage analyses. Following these steps, no more than 2-3 mismatches were observed between parent-offspring pairs. The software Pedcheck (O'Connell & Weeks 1998) was then used to identify Mendelian inconsistencies which were corrected when possible or otherwise eliminated by deleting the genotypes of the individuals involved. The resulting NBR and RM mapping pedigrees spanned seven and six generations and included 212 and 286 related individuals, respectively (Figures 6-1 and 6-2). The NBR pedigree contained 184 paternal

links (42 sires, mean ± 1 SD of 4.4 ± 3.5 offspring per sire) and 173 maternal links (51 dams, 3.4 ± 2.1 offspring per dam). The RM pedigree consisted of 168 paternal links (43 sires, 3.9 ± 3.3 offspring per sire) and 172 maternal links (71 dams, 2.4 ± 1.3 offspring per dam).

6.2.4 Microsatellite selection and genotyping

In addition to markers used for the initial pedigree reconstruction, microsatellites putatively distributed throughout the bighorn sheep genome were identified using the domestic sheep IMF map version 4.7 (Poissant *et al*. 2009, http://rubens.its.unimelb.edu.au~jillm/hill.htm). Markers were selected based on their predicted genomic location and level of polymorphism (assessed in ~30 individuals/population) with the aim of optimising genomic coverage and meiotic information. Most but not all markers were typed in both populations. Eleven markers were only genotyped in the NBR population while 17 were only typed in the RM population (see Additional file 1 at http://www.biomedcentral.com/1471-2164/11/524). Laboratory methods are available in Poissant *et al*. (2009) and references for primer sequences (Johnston *et al*. 2010, Poissant *et al*. 2009, Maudet *et al*. 2004, Georges & Massey 1992, Ede *et al*. 1995, Stone *et al*. 1995, http://rubens.its.unimelb.edu.au~jillm/hill.htm) are presented in Additional file 1 at http://www.biomedcentral.com/1471-2164/11/524. In total, 252 markers, amplified using 244 pairs of primers (8 primer pairs amplified two markers: BM3212, BMS2466, HBB2, MCMA54, MNS97A, MNS101A, TGLA176, TGLA377), were included in the linkage analysis. Descriptive statistics (typing

success, number of alleles and observed heterozygosity) were obtained using MSA 4.05 (Dieringer & Schlötterer 2003).

6.2.5 Linkage analysis

Population-specific linkage maps as well as an integrated map where populations were treated as independent families were constructed using CRI-MAP (Green *et al*. 1990). The same construction procedure was used for all maps. First, two-point linkage analyses were performed for all pairs of markers assuming equal recombination rates between the sexes using a modified version of CRI-MAP developed by Liu & Grosz (2006). The program AUTOGROUP (Liu & Grosz 2006) was then used to identify sets of markers likely residing in the same LG (pairwise LOD scores > 4). For markers unassigned to a LG following that analysis, two-point LOD scores were inspected and in cases where the most likely linkage was with a marker known to be adjacent in the domestic sheep IMF map, the marker was assumed to be part of the same LG in bighorn sheep. In cases where multiple bighorn sheep LGs were composed of markers known to be part of the same chromosome in domestic sheep, two-point LOD scores between markers residing at the end of each bighorn sheep LG were inspected and linkage was assumed when the LOD scores were among the highest for these respective markers. For each putative LG, the most likely marker order was recovered using the BUILD and FLIPSn options of a CRI-MAP version recently developed by Jill Maddox and Ian Evans (2.503) that more efficiently deals with large datasets. Specifically, LGs were first constructed using BUILD and a LOD > 3 threshold.

Markers were then successively added to these LGs using less stringent LOD thresholds of 2, 1, 0.5 and 0. The FLIPSn option was then used to compare the likelihood of alternate orders produced by shuffling up to five adjacent loci and markers were re-ordered when a more likely order was identified. Doubtful tight double recombinants were identified using the CHROMPIC option of CRI-MAP and responsible erroneous genotypes were corrected when present. Finally, sex-averaged and sex-specific recombination fractions for individual LGs were estimated using the FIXED option of CRI-MAP 2.503 and transformed to centimorgans (cM) using the Kosambi map function (Kosambi 1944). In cases where estimated sex-averaged intervals were greater than 50 cM, LGs were broken in two and separate analyses were performed for markers on each side of the interval.

6.2.6 Comparison of linkage maps

In order to assess intra- and inter-specific variability in genomic structure and recombination rates, the NBR and RM linkage maps as well as the bighorn sheep integrated map and domestic sheep IMF map version 4.7 were compared. Differences in marker synteny and order were identified by visual inspection. In cases where the most likely marker order differed between populations/species, support for alternate orders was determined by comparing $\log_{10}$ likelihoods. The relative sex-averaged length of different maps was compared by summing the length of intervals that were present in both maps (raw data available in Additional files 3 and 4 at http://www.biomedcentral.com/1471-2164/11/524).

To test for a genome-wide difference in sex-averaged recombination rate between population/species, two-tailed sign tests contrasting the number of shared intervals greater in length in one map than in the other were used. Reduced major axis regressions were also used to describe the relationship between interval-specific sex-averaged recombination rates between populations/species. The slopes, intercepts and their errors were obtained using the formula from Sokal & Rohlf (Sokal & Rohlf 1995) implemented in the software RMA version 1.17 (Bohonak 2004). Confidence intervals including the ones for correlation coefficients were obtained by performing 10000 bootstraps.

To assess variation in heterochiasmy across populations and species genomes, sexual dimorphism for individual intervals was quantified using the sexual dimorphism index (SDI) of Lovich and Gibbons (Lovich & Gibbons 1992). This index is considered the best estimator of sexual dimorphism because it is intuitive, linear, symmetrical, and directional (Fairbairn 2007). The SDI was obtained by subtracting 1 from the ratio of the largest sex-specific value to the smallest sex-specific value. Following convention, estimates were then made positive when the female value was largest and negative when the male value was largest. The presence of genome-wide bias in sexual dimorphism was tested for using sign tests. Reduced major axis regressions were used to compare interval-specific sexual dimorphism of different maps.

The validity of comparing intervals between maps constructed using different number of markers is questionable since the length of an interval partly depends on the subset of markers included in a linkage analysis (the domestic

sheep IMF map 4.7 contains about 1400 markers). Therefore, cross-species analyses were repeated using information derived from additional linkage maps based solely on markers mapped in both species. Results and conclusions were essentially the same as for previous analyses and are therefore not presented. These maps are available in Additional file 6 at http://www.biomedcentral.com/1471-2164/11/524.

6.3 Results

6.3.1 Genotyping success and marker polymorphism

Genotyping success was high (~95%) in both populations and is summarised in Table 6-1 with additional details available in Additional file 1 at http://www.biomedcentral.com/1471-2164/11/524. Marker diversity (number of alleles and observed heterozygosity) and the number of informative meioses tended to be greater in the NBR population despite a smaller number of genotyped individuals.

6.3.2 Population-specific maps

Linkage analysis for population-specific datasets yielded very similar outcomes. For this reason, only salient features of these maps are presented here while specific details are made available in Additional file 1 at http://www.biomedcentral.com/1471-2164/11/524. In brief, all markers assigned to a linkage group (LG) appeared to be part of the same chromosome in both populations (Figure 6-3). Map contiguity was slightly greater in the NBR map,

with 230 markers distributed along 29 LGs compared to 232 markers distributed along 34 LGs in the RM map. The NBR sex-averaged map spanned 2910 cM while the RM sex-averaged map spanned 2581.4 cM. For both populations, while the overall female autosomal map was longer than the equivalent male map (ratio of 1.13 in NBR and 1.06 in RM), two chromosomes (5 and 15) had longer male maps than female maps. In addition, NBR linkage groups 10, 21, 24 and 25 were longer in the male map while RM linkage groups 2a, 2b, 3c, 8a, 9, 11, 12, 13, 15, 16a, 18, and 19 were longer in the male map.

The most likely marker order differed between maps in only one instance. This involved two tightly linked markers on chromosome 1 (MCM137 and BM7145) for which order was reversed between maps. However, while support for the inferred order was moderate in the NBR map ($\log_{10}$ likelihood difference of 2.02, MCM137-BM7145, 0.47 cM), support for the alternate order in the RM map was weak ($\log_{10}$ likelihood difference of 0.34, BM7145-MCM137, 0.86 cM).

A comparison of the intervals present in both maps revealed that localized sex-averaged recombination rates were generally similar between populations (r^2 = 0.61, raw data available in Additional file 3 at http://www.biomedcentral.com/1471-2164/11/524). The sum of these intervals was accordingly similar (2382.9 cM in NBR vs. 2427.8 cM in RM). In both populations, intervals were more often longer in the female map than in the male map (102 vs. 61 in NBR and 92 vs. 72 in RM) but only significantly so in the NBR population (NBR, $p < 0.01$; RM, $p = 0.14$). Sexual dimorphism in interval length (sexual dimorphism index, SDI) was significantly more often in the same

direction than not (94 out of 160, $p < 0.05$). However, interval-specific SDI was only weakly correlated between populations ($r^2 = 0.03$, 95% CI = 0 - 0.14).

6.3.3 Integrated bighorn sheep map

Combining the two datasets in a single linkage analysis produced a highly contiguous map (Figure 6-4). In that analysis, 247 markers were assigned to 27 LGs representing all ovine autosomes and the X chromosome. Since 7 markers were perfectly linked to another marker, the map only truly depicted the locations of 240 unique mapped positions for an average of 8.9 ± 4.3 loci per chromosome. Sex-averaged intervals were on average 14.3 ± 9.1 cM long and usually shorter than 30 cM (Table 6-3). The sex-limited and pseudo-autosomal regions of chromosome X were separated by slightly more than 50 cM in the sex-averaged map due to an absence of linkage in the male map. However, the X chromosome LG was left intact due to evidence for tighter linkage (21.5 cM) in the female map. OarFCB11 was excluded from chromosome 2 because it was estimated to be more than 50 cM away from its closest neighbouring marker (INHA). BMS1247, BMS1948 and HBB2/ii could not be assigned to a chromosome while GHRHR was excluded due to having too few informative meioses. The length of the complete sex-averaged map was 3050.9 cM while the autosomal female and male maps were 3166.1 cM and 2832.2 cM long (1.12 ratio), respectively. Intervals were significantly more often longer in the female map than in the male map (119 vs. 87, $p < 0.05$), however four chromosomes (5, 15, 18 and 24) had longer male than female chromosome maps.

6.3.4 Comparison of bighorn sheep and domestic sheep maps

Synteny was highly similar between the bighorn sheep and the domestic sheep International Mapping Flock (IMF) maps with only three observed differences (Figure 6-4). First, FCB19 mapped to chromosome X in bighorn sheep but to chromosome 15 in domestic sheep. Second, BM4005 mapped to chromosome 2 in bighorn sheep but to chromosome 24 in domestic sheep. Finally, neither of the two markers amplified in bighorn sheep with the primer pair used for MCMA54 in domestic sheep mapped to the location of this marker predicted from the IMF map (chromosome 21). Instead, MCMA54/i and MCMA54/ii mapped to bighorn sheep chromosomes 1 and 9, respectively. For the three other primer pairs which amplified two unlinked markers in bighorn sheep (TGLA377, BMS2466, MNS97A), one of the markers mapped to its predicted position while the other mapped to a different chromosome (TGLA377/ii, MNS97A/ii and BMS2466/ii were assigned to chromosome 3, 5 and 10, respectively). One additional putative difference between species was observed on chromosome 10 for markers not mapped in the IMF but mapped in Soay sheep, a feral domestic sheep breed (Johnston *et al*. 2010). The most likely order for this region in bighorn sheep was OarSEJ10, OarSEJ11, AGLA226 and OarSEJ13 versus AGLA226, OarSEJ10, OarSEJ11 and OarSEJ13 in Soay sheep. The difference in $\log_{10}$ likelihood between marker orders in bighorn sheep was 3.01.

The length of orthologous intervals was highly correlated between species ($r^2 = 0.71$, $p < 0.01$, Figure 6-5) and their sum very similar (3044 cM in bighorn sheep vs. 3001 cM in domestic sheep; a difference of ~1.5 %). This excluded the

intervals located at the tip of bighorn sheep chromosomes 5 (MNS97A/ii to WNT3K13, 6 cM) and 10 (OarSEJ10 to AGLA226, 0.5 cM) that have no equivalent in the IMF map. Intervals did not tend to be larger in one species than the other (105 larger in domestic sheep vs. 98 larger in bighorn sheep, $p = 0.67$). Based on coverage of these intervals in the version 4.7 IMF map, the current genome coverage by the integrated map in bighorn sheep was estimated to correspond to ~ 84 % of the domestic sheep linkage map.

Mean SDI in length (± 1 SD) considering only orthologous intervals was 0.10 ± 1.27 in bighorn sheep and -0.34 ± 1.33 in domestic sheep. The positive mean SDI in bighorn sheep reflected a tendency for larger intervals in the female map (114 out of 197, $p < 0.05$) while the negative mean SDI in domestic sheep indicated a tendency for larger intervals in the male map (120 out of 197, $p < 0.01$). Interval-specific SDI was significantly correlated between species ($r^2 = 0.21$, $p < 0.01$, Figure 6-6). The intercept and slope were both significantly positive (intercept ± 1 SE: 0.42 ± 0.08, $p < 0.01$; slope: 0.95 ± 0.06, $p < 0.01$). In general, SDI values in bighorn sheep tended to be greater than in domestic sheep (124 times out of 189, $P < 0.001$).

6.4 Discussion

As expected, marker synteny and order were generally congruent between bighorn sheep maps. This suggests that datasets were mostly free of errors and justified combining individual maps. The NBR population was generally more informative than the RM population. This was likely a consequence of the more

complete NBR pedigree combined with greater marker variability resulting from recent admixture (Hogg *et al*. 2006, Poissant *et al*. 2009). Nonetheless, information provided by both populations was generally complementary and ultimately allowed construction of a highly contiguous map covering approximately 84 % of the species genome. This is greater coverage than for a similar map for free-ranging red deer (*Cervus elephus*, 39 %, Slate *et al*. 2002) and almost on a par with one for Soay sheep (*Ovis aries*, 90 %, Beraldi *et al*. 2006) for which virtually all genetic resources developed for domestic sheep can be used. The coverage of the presented map is therefore similar to a first-generation map for a domestic species and outstanding for a wild species.

Recombination fractions were very similar between bighorn sheep populations. Combining pedigrees into a single analysis therefore likely resulted in map distances that were generally representative of the species as a whole. While genuine intra-specific differences may exist in map distances, the integrated map is likely to more accurately depict recombination fractions of individual populations than the estimates derived from the population-specific maps. This is because interval estimates for population-specific maps were often based on relatively few informative meioses, especially in the RM population. Relying on distances from the integrated map in future downstream population-specific studies is therefore advisable.

As predicted, marker synteny and order were generally congruent between the *Ovis* species maps. This is in line with the expectation of 1 to 2 rearrangements per million years in most mammalian lineages (Delgadillo *et al*.

2003). However, it has to be acknowledged that marker coverage was generally too sparse to detect subtle rearrangements. Cross-species comparison was also made difficult by the fact that some primer pairs amplified two loci. For example, BM4005 mapped to different locations in each species but I am aware of a second locus for BM4005 in bighorn sheep that could not be reliably genotyped. Since primers for BM4005 are also known to amplify multiple sets of bands in domestic sheep (Maddox *et al.* 2001), the BM4005 loci mapped in the two species are probably not orthologous. Similarly, FCB19 mapped to chromosome X in bighorn sheep but to chromosome 15 in domestic sheep. This marker is definitely autosomal in domestic sheep given that a fraction of males are undoubtedly heterozygous (J. Maddox, unpublished data) so the discrepancy in map location is not spurious. However, FCB19 markers amplified in the two species might not be orthologous given that a single primer pair can amplify multiple markers. In contrast, convincing evidence for cross-species rearrangement came from the primers used to amplify MCMA54 in domestic sheep. In that case, neither of the two markers amplified using this primer pair in bighorn sheep mapped to the location of the MCMA54 locus in domestic sheep (the MCMA54 primers amplified two band sets that both mapped to chromosome 21 in domestic sheep vs. 1 and 9 in bighorn sheep). The comparison of the bighorn sheep map with the Soay sheep map (Johnston *et al.* 2010) also suggested the presence of a minor rearrangement on chromosome 10. While some of these cases may depict genuine rearrangements, it is clear from this study that the organization of the two species genomes is very similar.

Genomic analysis in a close relative of domestic sheep offered the opportunity to infer ancestral marker order for chromosomal regions showing variation among domestic sheep breeds (Beraldi *et al*. 2006, McRae & Beraldi 2006). For chromosome 1, the order of two loci located in the rearranged region (MCM137 and BM7145) was similar between the NBR and the IMF maps (McRae & Beraldi 2006). On the other hand, the most likely marker order in the RM map was similar to an alternate order documented in Soay sheep (McRae & Beraldi 2006). Inferred marker orders were arguably not significantly more likely than the alternate orders. Yet, it is worth noting that this chromosomal region was the only one for which the most likely marker order differed between bighorn sheep maps. If both orders are present in bighorn sheep, it would mean that this region is either prone to rearrangements or that polymorphism in marker order originated millions of years ago rather than recently as hypothesised by McRae and Beraldi (McRae & Beraldi 2006). For a second putatively varying region located on chromosome 12 (Beraldi *et al*. 2006), only one (BM4025) of the two markers used to infer rearrangement in domestic sheep (BM4025 and TGLA53) was amplified. However, marker order in bighorn sheep for that region appeared to be the same as in the IMF map based on a marker located only 2 cM away from TGLA53 in domestic sheep (CSAP01E). Therefore, the IMF appeared to portray the ancestral marker order.

The comparison of orthologous intervals suggested high similarity in localized sex-averaged recombination rates between the *Ovis* species. While the near perfect concordance in total map length (~1 %) may be coincidental, given

that variation in the order of 10% has been documented among domestic sheep breeds (Beraldi *et al*. 2006), it nonetheless strongly suggests little difference between species. Assuming that results were not unduly biased by missing and erroneous genotypes (which can be a concern when using CRI-MAP in complex pedigrees, Slate 2008, Cox *et al*. 2009), it appears that the elevated recombination rates observed in domestic sheep are a characteristic of *Ovis* species rather than a consequence of domestication. Alternatively, recombination rates may have increased rapidly in both species since their recent divergence as a consequence of domestication in domestic sheep and for a different reason in bighorn sheep. But, this later explanation seems unlikely since the evolution of mean recombination rates in mammals is generally slow and most likely governed by neutral processes (Dumont & Payseur 2008).

Contrary to what has been found for domestic sheep, recombination rates in bighorn sheep tended to be greater in females than in males. The unusual pattern observed in domestic sheep therefore appeared to be species-specific. This finding is not overly surprising given the low phylogenetic inertia of the trait (Lenormand & Dutheil 2005). The magnitude of heterochiasmy in sheep species is also arguably modest when compared with species such as the saltwater crocodile (*Crocodylus porosus*, ratio of 5.7:1, Miles *et al*. 2009) or the zebrafish (*Danio rerio*, ratio of 2.74:1, Singer *et al*. 2002). Yet, the presence of male-biased recombination in domestic sheep remains puzzling given that recombination in placental mammals is generally female-biased (Coop & Przeworski 2007). An intuitive explanation is that altered sex-specific recombination patterns in

domesticated mammals (cattle are also atypical, exhibiting no heterochiasmy, Ihara *et al*. 2004) might be an incidental result of strong artificial selection during the process of domestication. Alternatively, the unusual heterochiasmy pattern documented in the domestic IMF might simply be an artefact resulting from the facts that the population size was small, all sires descended from a single grand-sire and there were only three maternal grandsires compared to 13 granddams. Knowing that recombination rates can vary substantially among individuals, and that such differences can have a large genetic component (e.g. Dumont *et al*. 2009), it could be that the paternal grand-sire was characterised by an uncommonly high recombination rate breeding value and/or that some of the maternal grandsires were characterised by uncommonly small recombination rate breeding values (assuming that male and female recombination rates are positively genetically correlated, Poissant *et al*. 2010). A comparison of sex-specific recombination rates in additional domestic sheep pedigrees might answer this question.

As in other taxa (e.g. Paigen *et al*. 2008, Broman *et al*. 1998), great variability was observed in patterns of heterochiasmy across and along chromosomes. For example, recombination appeared to be male-biased for a few chromosomes despite the presence of a genome-wide tendency for greater recombination in females. However, no clear pattern emerged at the chromosomal level with the NBR and RM maps yielding mainly inconsistent results. At the interval scale, patterns of sexual dimorphism were conserved across populations and species. This means, for example, that genomic regions characterized by low

SDI values in one species were mirrored by similarly low SDI values in the other species. This could be due to conserved sex-specific and/or sex-biased recombination hot-spots. However, fine-scale analyses of recombination rates in other pairs of closely related species (e.g. human and chimpanzee, Winckler *et al*. 2005, Ptak *et al*. 2005) suggest that this is unlikely at the inter-specific level. Inter-specific congruence in localized recombination rate sexual dimorphism could also be due to the position of intervals along chromosomes relative to centromeres and telomeres, irrespective of the exact location of individual hot-spots. For example, in humans, recombination tends to be greater in females near centromeres but greater in males near telomeres (reviewed in Lynn *et al*. 2004). In domestic sheep, recombination in telomeric and centromeric regions is usually greater in males (J. Maddox, unpublished data). To verify if a similar pattern was also present in bighorn sheep, bighorn sheep interval-specific SDI was contrasted to the relative distance of these intervals from centromeres and telomeres inferred from the location of these intervals in the IMF map. A pattern similar to that seen in domestic sheep was observed, with recombination being greater in males near centromeres and telomeres while being greater in females in more central parts of chromosomes (Figure 6-7).

6.5 Conclusion

A first-generation bighorn sheep linkage map was constructed using DNA from two wild pedigreed population and genomic resources originally developed for domestic sheep. Since bighorn sheep and domestic sheep genomes are very

similar, future efforts to increase marker density in specific chromosomal regions should be relatively straightforward. This could be achieved using bighorn sheep single nucleotide polymorphism (SNP) markers recently discovered using the OvineSNP50 Beadchip (Miller *et al.* 2011), additional microsatellites already mapped in domestic sheep and/or by taking advantage of the recently acquired domestic sheep genome sequence (http://www.sheephapmap.org/) to develop novel markers. The high similarity between the genomes of the two species should also greatly facilitate future efforts to assemble a bighorn sheep genome sequence and to develop additional SNPs.

The main reason for developing genomic resources in bighorn sheep is to allow studies of complex trait genetic architecture and evolution under natural settings. In the NBR population, genomic resources will enable investigations into the genetic basis of fitness, inbreeding depression and genetic rescue (Hogg *et al.* 2006). In RM, it will be possible to study the genetic architecture of additional traits including body mass, horn size and animal personality (Poissant *et al.* 2008, Réale *et al.* 2009). Finally, genomic information could eventually be combined with population genetic approaches to study adaptive population differentiation (Stinchcombe & Hoekstra 2007), especially in the context of parasitism (Luikart *et al.* 2008) and selective harvesting (Coltman *et al.* 2003).

While resources developed for domestic sheep are obviously highly useful to bighorn sheep research, genomic research in bighorn sheep can also yield valuable information through comparisons with domestic sheep in return. For example, I have demonstrated how linkage mapping in bighorn sheep can be used

to infer ancestral marker order in domestic sheep. Also, by comparing the domestic sheep map with the map of a close wild relative, I was able to determine that the elevated recombination rates observed in domestic sheep were likely a characteristic of *Ovis* species while the unusual male-biased heterochiasmy might have been a consequence of domestication. Finally, I have demonstrated that interval-specific patterns of sexual dimorphism could be conserved among closely related species, possibly due to the position of these intervals relative to centromeres and telomeres.

Table 6-1. Marker variability in bighorn sheep mapping populations (range and mean ± 1 SD)

	National Bison Range	Ram Mountain
Marker typing success (%)	42.0 - 100	52.5 - 100
	(95.8 ± 7.8)	(94.7 ± 8.8)
Number of alleles	2 - 12	2 – 12
	(5.40 ± 1.89)	(4.65 ± 1.73)
Observed heterozygosity	0.06 - 0.90	0.14 - 0.84
	(0.66 ± 0.13)	(0.60 ± 0.15)
Total informative meiosis	16 - 310	42 - 285
	(225.9 ± 54.4)	(171.3 ± 54.1)
Female informative meiosis	15 - 146	20 - 142
	(106.3 ± 25.6)	(83.2 ± 26.6)
Male informative meiosis	1 - 181	18 - 154
	(118.1 ± 31.6)	(86.4 ± 28.9)

Table 6-2. Descriptive statistics for the integrated bighorn sheep map

			Map length (cM)			No. of intervals (Sex-averaged length)		
Linkage group	No. of markers	No. of intervals	Sex-averaged	Female	Male	0 - 15 cM	15 - 30 cM	> 30 cM
1	22	21	302.8	326.3	284.9	13	7	1
2	18	17	274.3*	290.9*	264*	11	3	3
3	16	15	272.7	303.5	250.1	7	6	2
4	10	8	142.7	167.6	124	3	4	1
5	10	8	132.8	125.2	148.1	3	5	0
6	13	11	138.9	148.3	135.4	8	3	0
7	9	8	125.3	136.8	116.8	4	4	0
8	9	8	127.9	155.6	122.9	6	1	1
9	12	10	115.5	122.5	109.9	7	3	0
10	10	8	64.2	65	64	8	0	0
11	6	5	108.2	118.3	99.2	2	1	2
12	9	8	102.9	107.3	99.5	4	4	0
13	9	8	120.6	122.5	119.7	5	2	1
14	9	7	82.5	92.2	75.3	6	1	0
15	11	10	112.8	110.5	118.8	9	1	0
16	5	4	67.6	77.4	62.3	2	2	0
17	9	7	97.3	100.5	97.3	3	4	0
18	10	9	96.9	94.4	97.4	6	3	0
19	6	5	75.5	75.5	74.8	3	2	0
20	6	5	71.5	77.9	66.3	4	1	0
21	3	2	16.3	16.6	16	2	0	0
22	5	4	51.9	60.9	45.8	3	1	0
23	8	7	71.9	82	63.9	6	1	0

24	3	2	44	41.3	47.9	1	0	1
25	4	3	83.3	89	80.8	0	1	2
26	6	5	51.3	58	46	4	1	0
X	9	8	99.2	170.6	1.3**	7	0	1
Total	247	213	3050.8	3336.6	2832.4	137	61	15

*excluding FCB11 which is more than 50 cM away

**Pseudo-autosomal region

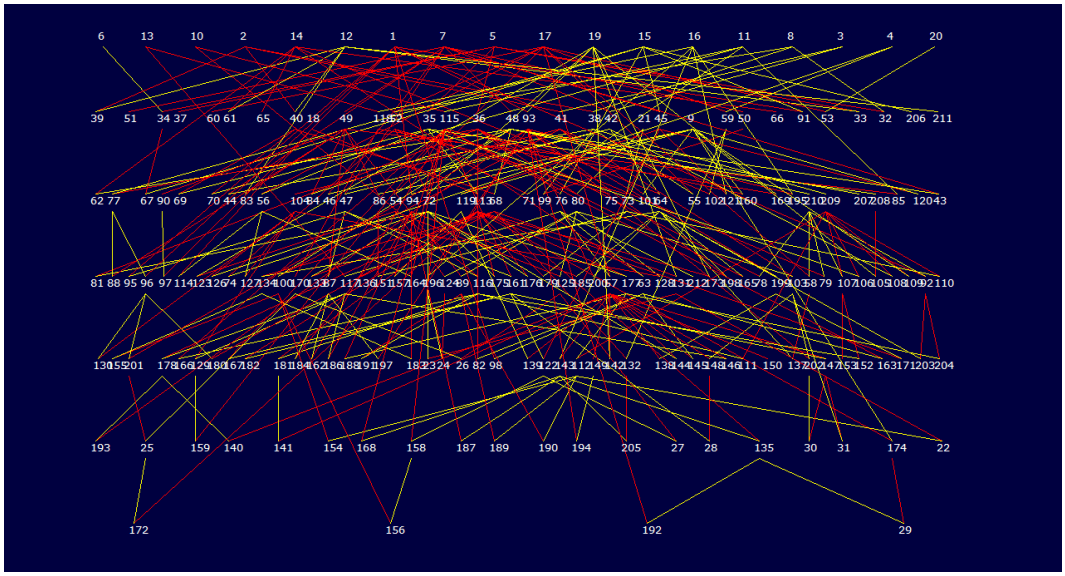


Figure 6-1. National Bison Range bighorn sheep mapping pedigree.

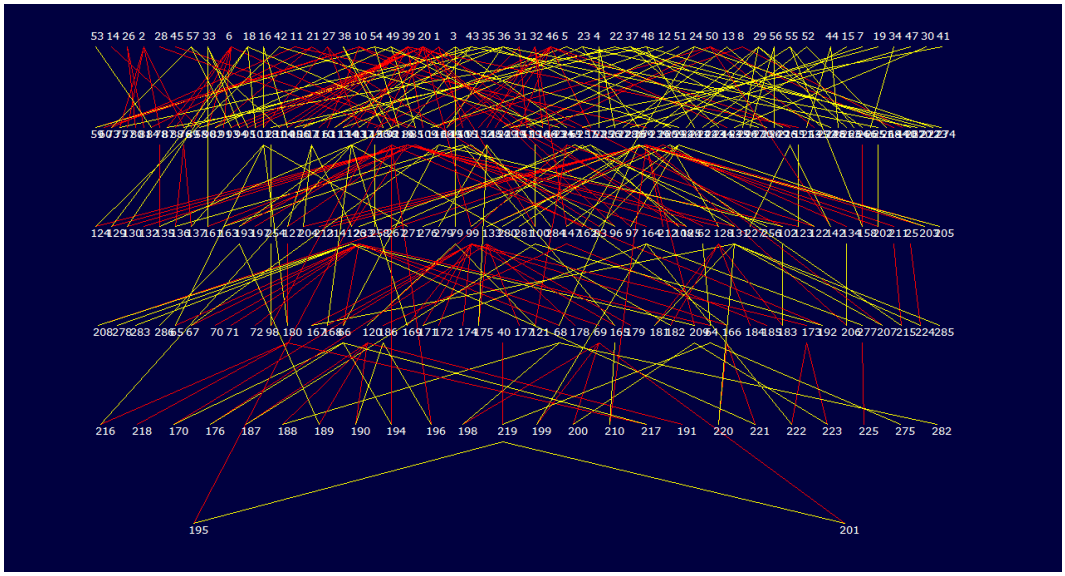
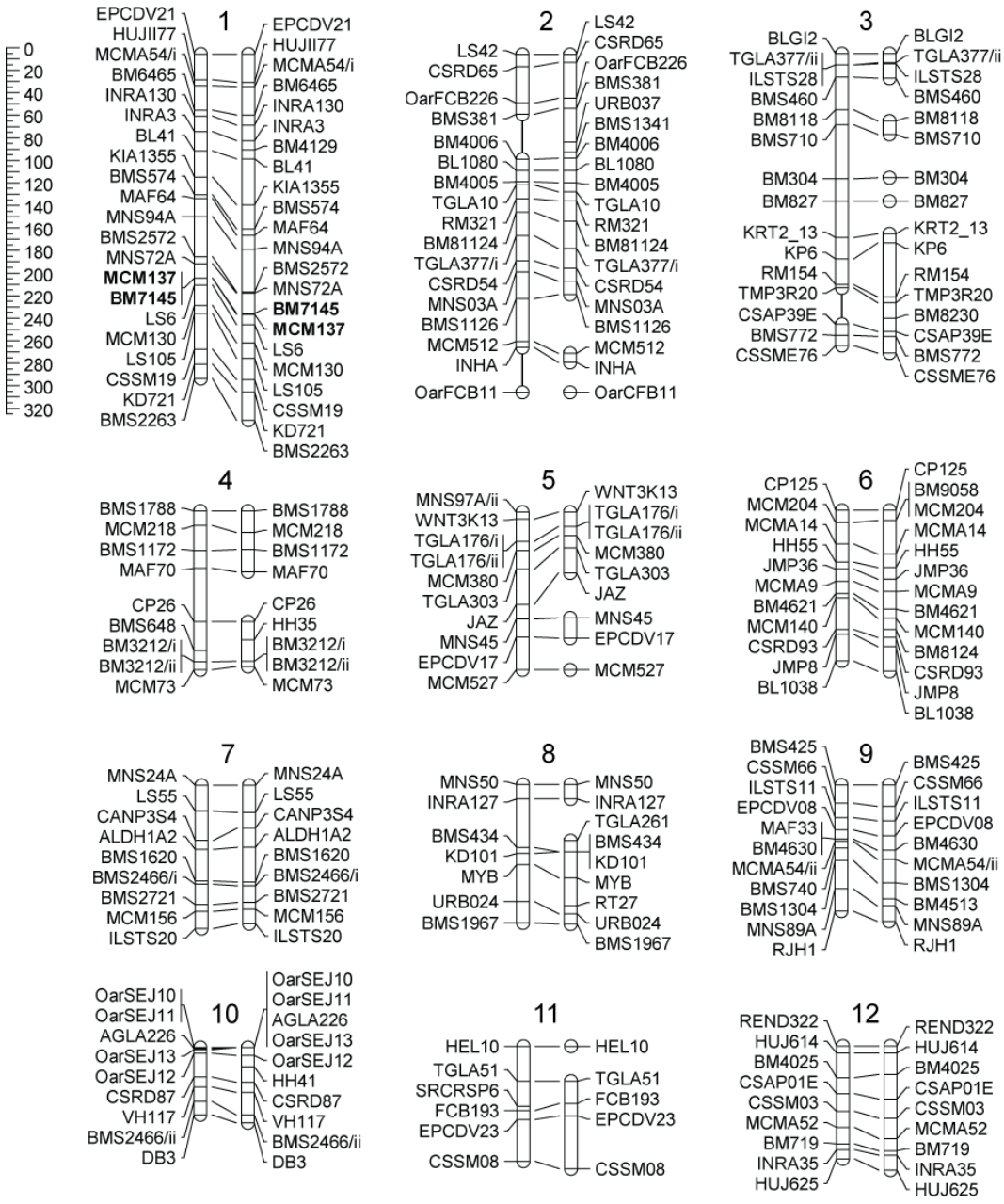


Figure 6-2. Ram Mountain bighorn sheep mapping pedigree.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HUJII77
MCMA54/i
BM6465
INRA130
INRA3
BL41
KIA1355
BMS574
MAF64
MNS94A
BMS2572
MNS72A
MCM137
BM7145
LS6
MCM130
LS105
CSSM19
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BMS2263
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CSRD65
OarFCB226
BMS381
BM4006
BL1080
BM4005
TGLA10
RM321
BM81124
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CSRD54
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BMS1126
MCM512
INHA
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OarCFB11
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BM827
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KP6
RM154
TMP3R20
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BM8230
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MCM218
BMS1172
MAF70
CP26
BMS648
BM3212/i
BM3212/ii
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TGLA176/ii
MCM380
TGLA303
JAZ
MNS45
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MCMA14
HH55
JMP36
MCMA9
BM4621
MCM140
CSRD93
JMP8
BL1038
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MCM156
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TGLA261
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MYB
RT27
URB024
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BM4513
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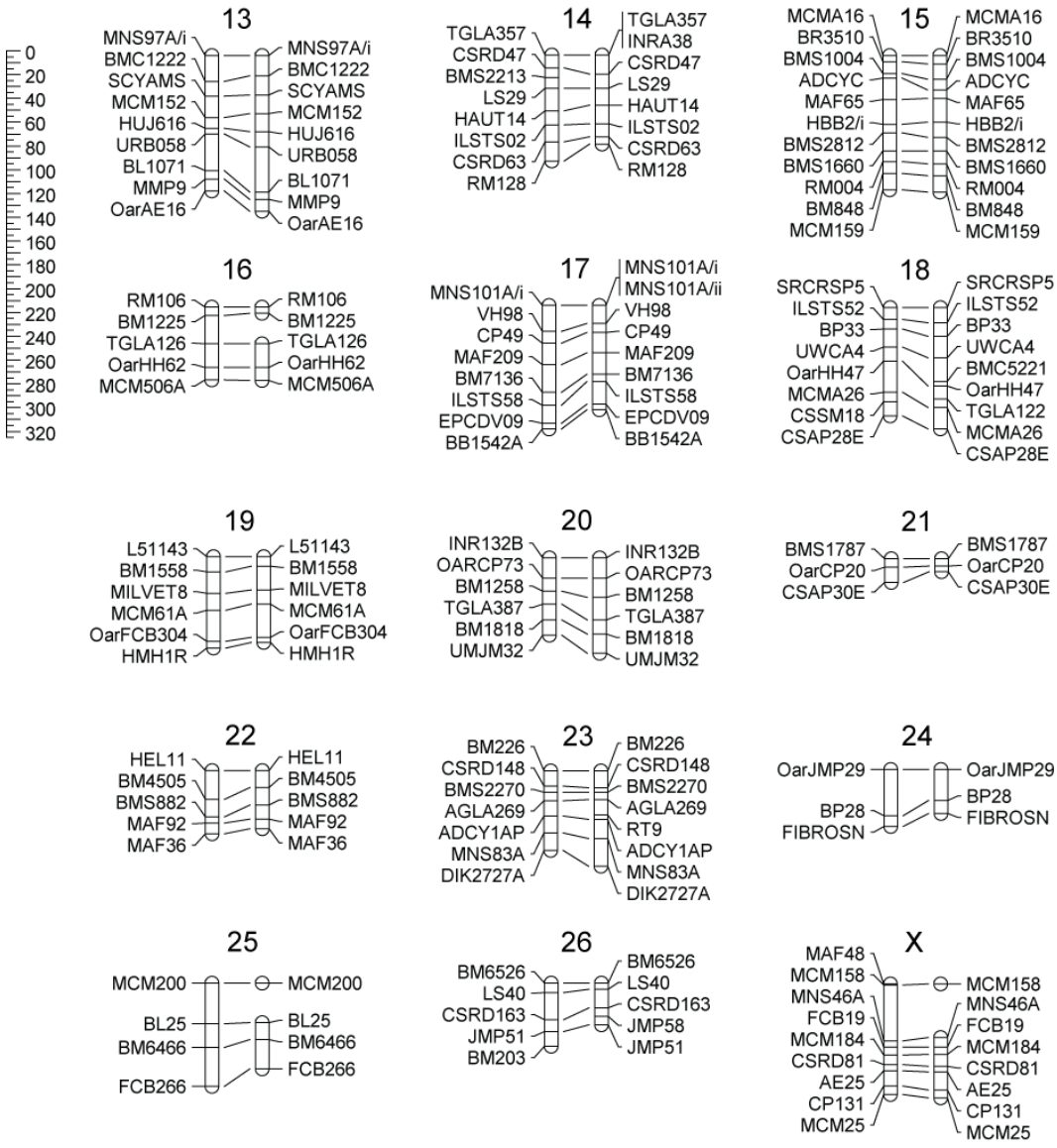
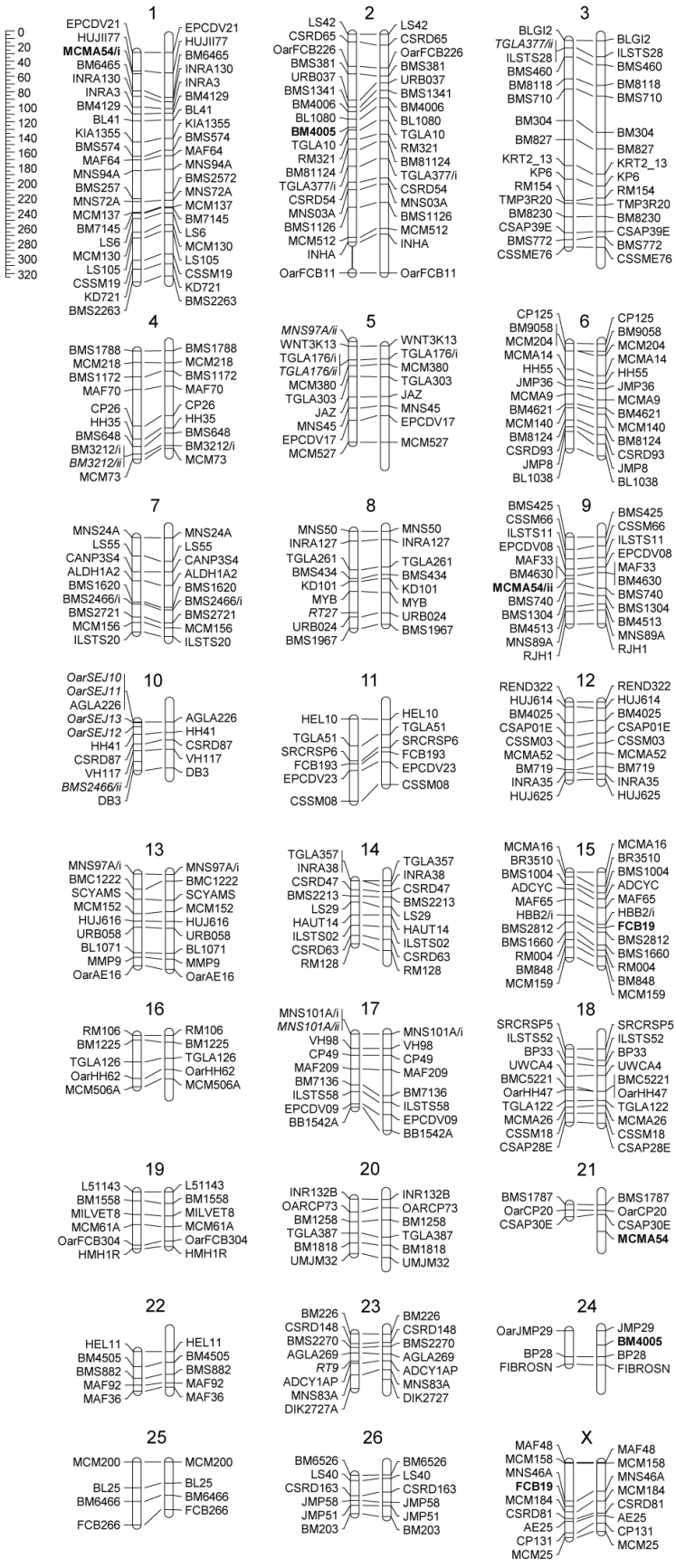


Figure 6-3. Comparison of population-specific bighorn sheep linkage maps. For each chromosome, the National Bison Range linkage groups are on the left while the Ram Mountain linkage groups are on the right. Lines connect homologous loci. Markers not mapping to the same location in the two populations are in bold. Thin vertical lines connecting OarFCB11 to chromosome 2 as well as some linkage groups indicate that linkage was inferred based on LOD scores but that separate analyses were ultimately performed for both side of the intervals because

these intervals were estimated to exceed 50 cM in length. These intervals were not included in the estimation of total map lengths and their length in the figure is arbitrary. The ruler at the top left corner represents a cM scale.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OarFCB226
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URB037
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BM4006
BL1080
BM4005
TGLA10
RM321
BM81124
TGLA377/i
CSRD54
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CSRD81
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CP131
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MCM184
CSRD81
AE25
CP131
MCM25

Figure 6-4. Bighorn sheep sex-average linkage map compared with the domestic sheep IMF map. (For each chromosome, the bighorn sheep linkage groups (LGs) are on the left while the domestic sheep LGs are on the right. Lines connect orthologous loci. Markers not mapping to the same location in the two species are in bold while markers only mapped in bighorn sheep are italicized. The thin vertical line connecting OarFCB11to chromosome 2 indicates that this marker was assigned to that chromosome but was excluded from the linkage analysis for being more than 50 centimorgans (cM) away from the closest neighbouring marker. That interval was not included in the total map length estimate and its length in the figure is arbitrary. The ruler at the top left corner represents a cM scale.)

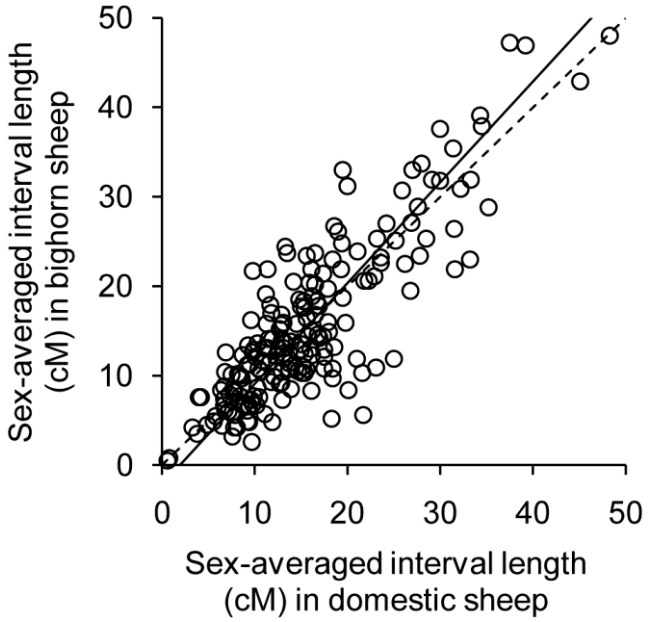

Figure 6-5. Comparison of sex-averaged interval length (cM) in bighorn sheep and domestic sheep for 203 pairs of adjacent markers. (The solid line depicts the relationship between bighorn sheep and domestic orthologous intervals (reduced major axis regression, $y = 1.14 x – 1.83$, $r^2 = 0.72$, 95 % CI = 0.60, 0.80) while the dashed line separates intervals larger in bighorn sheep (above line, n = 105) from intervals larger in domestic sheep (below line, n = 98).)

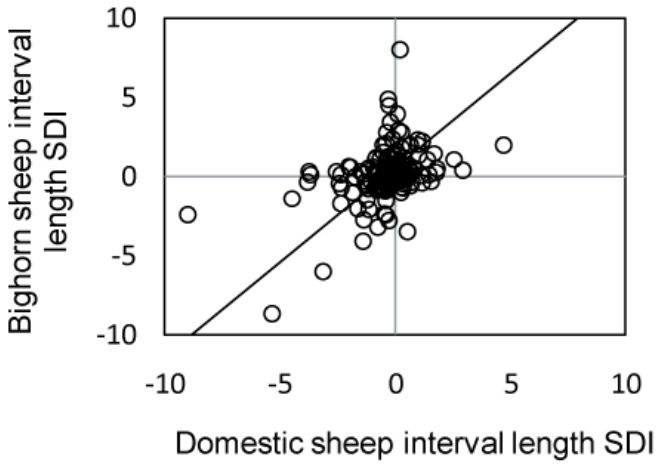

Figure 6-6. Comparison of sexual dimorphism (SDI) in interval length between orthologous bighorn sheep and domestic sheep intervals. (192 intervals between adjacent markers were compared (reduced major axis regression, $y = 0.95 x + 0.42$, $r^2 = 0.21$, 95% CI = 0.09, 0.37). SDI values are positive when intervals are larger in females and negative when intervals are larger in males.)

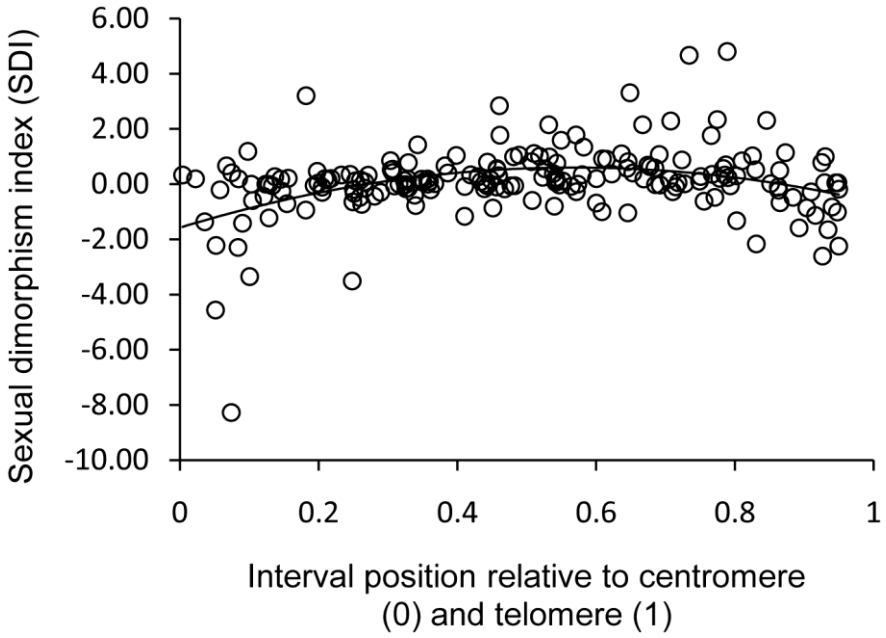

Figure 6-7. Relationship between interval length sexual dimorphism (SDI) and relative distance from centromeres and telomeres in bighorn sheep. **(**The location of each interval relative to centromeres (0) and telomeres (1) were inferred using the position of orthologous intervals in the domestic sheep IMF map version 4.7. The fitted curve is a second order polynomial ($r^2 = 0.16$, quadratic term fitted in a linear model, $p < 0.001$).)

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Chapter 7

QTL mapping in wild bighorn sheep reveals striking between-species similarity in the genetic architecture of sexually selected traits

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7.1 Introduction

Dissecting the genetic architecture of ecologically important traits is key to understanding the mechanisms and processes allowing the maintenance of adaptive genetic variation (Ellegren & Sheldon 2008). While a variety of approaches can identify relevant loci (Ellegren & Sheldon 2008), genomic studies performed using long-term field studies of individually recognizable individuals offer unparalleled opportunities to study topics requiring realistic fitness estimates (Kruuk & Hill 2008, Kruuk *et al.* 2008, Clutton-Brock & Sheldon 2010, Slate *et al.* 2010). These include the genetic basis and evolutionary dynamics of sexually selected traits (Chenoweth & McGuigan 2010) and sexually antagonistic genetic variation (Bonduriansky & Chenoweth 2009). However, genomic studies in free-living pedigreed populations remain extremely rare due to difficulties in maintaining multigenerational pedigrees and assembling adequate genotype-phenotype datasets (Slate 2005, Slate *et al.* 2010).

The bighorn sheep (*Ovis canadensis*), a mountain ungulate endemic to Western North America, has been the focus of numerous ecological and evolutionary quantitative genetics investigations (e.g. Réale *et al.* 1999, 2009, Coltman *et al.* 2003, 2005, Poissant *et al.* 2008) and is emerging as a prime organism for studies of evolution in the wild. Recently, a first generation bighorn sheep genetic linkage map was developed to enable QTL mapping (Poissant *et al.* 2010a). Two traits of interest are horn size and body mass due to their importance to male mating success (Coltman *et al.* 2002). Both traits are sexually selected through male-male combat (Geist 1971). The genetic architecture of horn size and

body mass is also key to better understand the evolution of sexual dimorphism (Poissant *et al*. 2008, 2010b) and the evolutionary impacts of selective harvesting (Coltman *et al*. 2003, Coltman 2008).

Differentiating real QTL from false positives is a major challenge in any QTL mapping study (Lander & Krugliak 1995), especially for studies of wild populations in which sample sizes are typically small (Slate *et al*. 2010). Data interpretation in bighorn sheep could be facilitated by previous research in domestic sheep (*Ovis aries*, ~3 million years divergence, Bunch *et al*. 2006) if QTLs tend to be conserved between sheep species. A number of QTLs have already been identified for growth-related traits and horn size in domestic sheep (Cavanagh *et al*. 2010, Johnston *et al*. 2010) and their co-localization with similar QTL in bighorn sheep would confirm their presence in both species. QTLs conserved across species are not uncommon (e.g. Reid *et al*. 2005, Moghadam *et al*. 2007) but expectations for sexually selected traits are unclear (Chenoweth & McGuigan 2010). The absence of overlap between loci known to influence horn development in domestic sheep, goat and cattle (Georges *et al*. 1993, Vaiman *et al*. 1996, Montgomery *et al*. 1996, Asai *et al*. 2004) as well as little evidence for the presence of shared carcass traits QTLs between domestic sheep and cattle (Cavanagh *et al*. 2010) suggests that the genetic basis of horn size and body mass may differ substantially among bovid species.

I performed a genome-wide scan for body mass and horn dimension (length, base circumference and volume) QTLs in wild bighorn sheep from Ram Mountain, Alberta, Canada. Because adult bighorn sheep are highly sexually

dimorphic for body mass and horn size (Poissant *et al*. 2008), I estimated both sex-specific and cross-sex quantitative genetic parameters and searched for QTLs influencing either a single sex or both sexes similarly. Results were then compared with the location of QTLs for similar traits in domestic sheep. Horn size and body mass experience similar selective pressures in bighorn sheep and domestic sheep (Coltman *et al*. 2002, Preston *et al*. 2003). A comparison with domestic sheep would therefore also provide a rare opportunity to contrast the genetic architecture of sexually selected traits among closely related species and potentially lead to the identification of genes involved in similar micro-evolutionary processes across species. This research is an important step towards the integration of ecological, quantitative genetics and genomic approaches to deepen our understanding of sexual selection as an evolutionary process (Chenoweth & McGuigan 2010).

7.2 Methods

7.2.1 Study population

The Ram Mountain population is native to a small isolated mountain range located about 50 km east of the Canadian Rockies in Alberta, Canada (52° N, 115° W, elevation 1080-2170 m). The present study is based on data collected from 1970 to 2009. Techniques used to capture, mark, measure and monitor animals were described in detail by Jorgenson *et al*. (1993). Briefly, animals were captured in a corral trap baited with salt from late May to September or early October each year. Almost all animals were marked early in life, so their exact

age was known. Individuals captured for the first time as adults were aged by counting horn growth rings. Marked sheep were subsequently monitored throughout their lifetime.

7.2.2 Phenotypic data

Most females and young males were captured multiple (> 3) times each year, while males 3 years and older were typically caught one to three times per season, usually in June or July. At each capture, sheep were weighed and the size of their horns was measured. Horn measurements included length along the outside curvature and horn base circumference. As in Poissant *et al*. (2008), horn volume was subsequently calculated assuming a conical shape using the average horn base circumference of both horns and the length of the longest horn to reduce the influence of horn breakage. Horn length measurements of females with 2 severely broken horns were excluded. To reduce the potentially confounding influence of maternal effects (Wilson *et al*. 2005, Kruuk & Hadfield 2007) and age × QTL interactions (Poissant & Coltman 2009), analyses focused on phenotypes measured in adults aged 2 to 10.

7.2.3 Pedigree information

Over the entire study period, maternity was inferred in the field using suckling behavior. Genetic analyses (described below) showed that this technique is accurate in > 99% of cases. Since 1988, the collection of DNA samples permitted formal genetic parentage analyses. These were based on ~30

microsatellite loci (see Coltman *et al*. 2005 for details) and the 95% confidence threshold in Cervus (Marshall *et al*. 1998). In addition, the software Colony (Wang 2004) was used to infer sibships resulting from sires that were not DNA sampled (see Coltman *et al*. 2005 for details). The accuracy of parts of the pedigree was also recently re-assessed using > 200 microsatellite loci used for linkage map construction (details below). The current pedigree contains 803 maternal links resulting from 236 dams (mean number of offspring ± 1 SD = 3.40 ± 2.52) and 454 paternal links resulting from 70 sampled and 36 unsampled sires (mean number of offspring per sire = 4.28 ± 4.40).

Only parts of the full pedigree are informative for QTL mapping purposes because genome-wide genotypes have only been obtained for a subset of individuals. The QTL mapping analyses were therefore based on a restricted pedigree composed of 310 fully typed animals (172 females, 138 males). Animals that were either untyped (n = 18) or only typed at markers used for initial parentage analyses (n = 41) were also included if they helped to connect fully typed animals in the pedigree (i.e. parents). The QTL mapping pedigree included 201 females and 159 males connected by 301 maternal links (mean number of offspring per dam ± 1 SD = 2.59 ± 1.49) and 259 paternal links (mean number of offspring per sire = 4.05 ± 3.28).

7.2.4 Bighorn sheep linkage map

The bighorn sheep linkage map is based on information from two wild pedigreed populations (Ram Mountain, Alberta, Canada, and National Bison

Range, Montana, USA) and contains 247 microsatellites ordered along all 26 autosomes and the X chromosome (Poissant *et al.* 2010a). A total of 241 markers have been genotyped in the Ram Mountain population, and all but three (BMS1247, BMS1948, OarFCB11) are positioned in the species map. In this study, map distances from the integrated species map instead of the Ram Mountain population-specific map were used because they are likely more accurate (Poissant *et al.* 2010a). Details about markers, laboratory techniques, map construction and map characteristics are available in Poissant *et al.* (2009, 2010a).

7.2.5 Quantitative genetic analyses

Phenotypic variance was partitioned into additive genetic and other components using the animal model and restricted maximum likelihood implemented in the program ASReml 3.1 (Gilmour *et al.* 2009). The animal model is a form of mixed model incorporating pedigree information where the phenotype of each individual is modelled as the sum of its additive genetic value and other random and fixed effects. The method has a long history in animal breeding and is now commonly used for studies of free-living populations due to its ability to optimize the use of information in complex and incomplete pedigrees (Lynch & Walsh 1998, Kruuk 2004, Wilson *et al.* 2010).

In a typical animal model:

$$y = X\beta + Za + e$$

y is the vector of individual phenotypes, X and Z are incidence matrices relating fixed and random effects to each individual, $ß$ is a vector of fixed effects, a is a vector of polygenic (additive genetic) effects, and e is the vector of residual errors. In all models, permanent environmental (identity), year of capture and year of birth random effects were also fitted. The permanent environmental effect was included to account for inter-individual variation resulting from non-genetic causes (e.g. horn breakage) as well as dominance and epistasis. The year of capture and year of birth effects were fitted to account for common environmental conditions (Kruuk and Hadfield 2007). Phenotypic variance (V_p) was therefore partitioned into five components after having taken fixed effects into account (described below): additive genetic (V_a), permanent environmental (V_{pe}), year of capture (V_y), year of birth (V_{yob}), and residual (V_r). All components were retained in final models even when not significant to prevent biasing V_a upwardly (Wilson *et al.* 2010).

Some of the traits included in this study are known to be partly genetically independent in males and females (e.g. horn volume, Poissant *et al.* 2008) while others have not been studied from a quantitative genetics perspective in females (horn length and horn base circumference). Male and female traits were therefore treated separately. However, doing so considerably reduces the amount of

phenotypic information included in any given analysis and diminishes the probability of detecting QTLs influencing both sexes similarly. All analyses were therefore repeated treating male and female traits as a single trait. Each trait in each age/sex class was standardized to a standard deviation of unity prior to analysis because phenotypic variance differed between the sexes and increased with age, especially in males. In sex-specific analyses, fixed effects included age (factor), date of capture (continuous, second-order polynomial, with May 24 as day 0), and the age × date interaction. In analyses where male and female homologous traits were combined, fixed effects also included sex and all possible interactions. Bivariate models were used to estimate covariances and correlations. All analyses were performed using the full Ram Mountain pedigree as well as the more restricted QTL mapping pedigree for comparative purposes.

Heritability (h^2) and other ratios were obtained by dividing individual variance components by V_p where $V_p = V_a + V_{pe} + V_y + V_{yob} + V_r$. Significance of (co)variance components and ratios was tested using likelihood ratio tests contrasting models including and excluding individual random effects. To test if correlations were significantly smaller than unity, a similar approach was used where unconstrained models were contrasted to models in which correlations were constrained to unity.

7.2.6 QTL mapping

QTLs were mapped using a variance component approach (George *et al.* 2000, Slate *et al.* 2005). This was done by extending the animal model described

above with the addition of a QTL component estimated using pairwise estimates of identity-by-descent (IBD) for specific genomic locations. IBD matrices were estimated every 2 cM (Haldane map function) as well as for unassigned markers using pedigree information, genotypes and map distances with the software Loki (Heath 1997). Loki does not estimate proper IBD matrices for the sex chromosomes (Lange & Sobel 2006). As in Beraldi *et al*. (2007a,b), IBD matrices for the X chromosome werer therefore estimated with Loki by treating the Y chromosome as a non-variable X chromosome. After a burn-in period of 50 cycles, 1 million iterations were performed with statistics being stored every 2 iterations. Significance of QTL effects was determined using LOD scores calculated as

$$LOD = (L_{QTL} - L_{polygenic}) / \ln(10)$$

where L was the log likelihood of models with and without a QTL component. Because linkage maps of bighorn sheep and domestic sheep are very similar (Poissant *et al*. 2010a), the significance thresholds previously calculated for domestic sheep by Johnston *et al*. (2010) based on the formula from Lander & Kruglyak (1995) were used. QTL were therefore considered significant and suggestive when LOD scores were greater than 3.31 and 1.88, respectively. For locations where similar QTLs had already been mapped in domestic sheep, a nominal p value < 0.01 (LOD > 1.175) was considered sufficient for QTL confirmation (Lander & Krugkyak 1995). Following Lander & Botstein (1989),

95% confidence intervals for QTL positions were approximated using the one-LOD drop-off method.

7.3 Results

When analyzing the entire dataset for both sex-specific and sexes-combined analyses, all traits showed significant additive genetic, year, year of birth and permanent environmental variance after accounting for fixed effects (Table 7-1). Similar results were observed when analyzing the smaller QTL mapping dataset, except that year of birth and permanent environmental effects were not all significant (Table 7-2). The proportion of phenotypic variance explained by each component was similar between datasets, except that heritability tended to be higher in the QTL mapping dataset (0.18-0.38 vs. 0.21-0.50). In sex-specific analyses, year of capture and year of birth together explained ~20-40 % of the phenotypic variance while permanent environmental effects explained ~20-25 %. In the sexes-combined analyses, year of capture and year of birth explained ~20-25 % of the phenotypic variance while permanent environmental effects explained ~30-50 %.

Genetic correlation estimates were generally positive (31 of 34) but many were also significantly smaller than unity (24 of 34, Tables 7-3 and 7-4). The only (non-significant) negative estimates were between female horn base circumference and male traits. Of the four cross-sex genetic correlations involving homologous male and female traits, two were significantly smaller than unity and close to zero (horn volume and horn base circumference) while two were large

and not significantly different from unity (body mass and horn length). Estimates obtained using the full and the smaller QTL mapping dataset were similar (Tables 7-3 to 7-6).

The QTL analysis resulted in the identification of 5 suggestive loci at the genome-wide level (LOD > 1.88) and another 20 were significant at the nominal p < 0.01 value (Table 7-7, Figures 7-1 and 7-2). Three of the suggestive QTL were for sex-specific traits and located on chromosomes 1 (male horn length), 23 (male horn length), and 26 (female body mass) while the other two were for male and female horn volume and horn base circumference treated as single traits and co-localized on chromosome 18. Estimates of individual QTL effects were generally large and comprised most or all of the additive genetic variance (Table 7-7).

7.4 Discussion

Results from the variance component analyses were consistent with findings from previous quantitative genetic studies in the Ram Mountain population (Réale *et al.* 1999, Coltman *et al.* 2005, Poissant *et al.* 2008). The presence of additive genetic variance for all traits combined with varying levels of genetic covariance indicated that QTL detection was possible for all traits and that loci with and without pleiotropic effects were to be expected.

This study is the first to estimate sex-specific and cross-sex quantitative genetic parameters for female horn length and horn base circumference in bighorn sheep. Results suggest that the genetic decoupling of male and female horn volume in bighorn sheep reported by Poissant *et al.* (2008) may largely be due to

the evolution of sex-specific genetic variance for horn base circumference. The cross-sex genetic correlation for horn base circumference was one of the lowest ever estimated for a pair of homologous male and female traits (Poissant *et al.* 2010b). The reason for this is unclear but horn base circumference may have experienced greater sexually antagonistic selection than horn length. While most studies of sexual selection in sheep have focused on male horn length (e.g. Coltman *et al.* 2002, Preston *et al.* 2003), there is no obvious reason to expect sexual selection to act on horn length more than horn base circumference. Horn base circumference is likely more important than horn length for fighting because males clash their horns near the base. The observed pattern would also be consistent with the presence of sexually antagonistic selection on horn volume or horn mass rather than base circumference or length since a change in horn base circumference will have a greater influence on horn volume than a proportional change in horn length.

The genome scan for body mass QTL yielded one suggestive locus (chromosome 26) and five regions with nominal p values < 0.01 (chromosomes 2, 22, 23, 24, X). Such numbers (i.e. few QTLs with low LOD scores) are typical of QTL mapping studies in free-living wildlife populations (Slate *et al.* 2010) including feral domestic sheep (Beraldi *et al.* 2007a,b). The positions of putative bighorn sheep body mass QTLs were highly concordant with the locations of similar QTLs in domestic sheep. This includes QTLs for carcass weight, muscularity and scan weight on chromosome 2 (Laville *et al.* 2004, Walling *et al.* 2004), carcass weight, body mass, total fat and growth rate on chromosome 23

(Margawati *et al*. 2006, 2009, Raadsma *et al*. 2009), body weight, growth rate and muscle mass on chromosome 24 (Campbell *et al*. 2003, Raadsma *et al*. 2009), and growth rate on chromosome 26 (Raadsma *et al*. 2009). On the other hand, we are unaware of growth-related QTLs in domestic sheep for the regions of chromosome 22 and X identified here. While these results may be spurious, I also note that chromosomes 22 and X have rarely been included in domestic sheep QTL studies, making comparison difficult. Results for the X chromosome should also be interpreted with caution given difficulties in estimating IBD matrices for that chromosome (Lange & Sobel 2006). Testing whether overlapping QTLs are due to the same genes will be challenging (Ellegren & Sheldon 2008). Nonetheless, the rapid pace at which new sheep markers are being developed (e.g. Miller *et al*. 2011) and the presence of numerous candidate genes in the QTL regions (e.g. myostatin, beta-3-adrenergic receptor, melanocortin 4 receptor, erythropoietin, elastin, and fibrosin genes, Raadsma *et al*. 2009) are encouraging.

Four of the five QTLs reaching suggestive significance were for horn dimension traits. Two of them were for male horn length (chromosome 1 and 23) while the other two were for horn volume and horn base circumference when pooling male and female phenotypes (co-localized on chromosome 18). Results also confirmed the presence of a male horn size QTL on chromosome 10 in the region containing the domestic sheep horn locus (Montgomery *et al*. 1996; Johnston *et al*. 2010). However, as in domestic sheep (Johnston *et al*. 2010), none of the QTLs overlapped with loci known to influence horn morphology in other genera (Georges *et al*. 1993, Vaiman *et al*. 1996, Asai *et al*. 2004).

Most QTLs appeared to have sex-specific effects. For example, the horn size QTL on chromosome 10 was only detected in the male-specific analysis. On the other hand, pooling the sexes in a joint analysis revealed QTLs that remained undetected in sex-specific analyses. In particular, a suggestive QTLs was identified for horn dimensions on chromosome 18 for which LOD scores were relatively large but still below the genome-wide significance threshold in both sex-specific analyses. Sex-specific QTLs have been documented in a variety of organisms (e.g. Nuzhdin *et al.* 1997, Farber & Medrano 2007, Moghadam *et al.* 2007) including domestic sheep (Raadsma *et al.* 2009). Keeping in mind that sex-specific QTLs may often be artifacts of small sample sizes (Curtsinger 2002), their detection in bighorn sheep is nonetheless consistent with the presence of sexual dimorphism and weak cross-sex genetic correlations for horn traits (Poissant *et al.* 2010b).

As opposed to what was observed in cross-sex comparisons, QTLs appeared to influence multiple traits within each sex. This is consistent with the expectation for sexually selected genes to have important pleiotropic effects (Fitzpatrick 2004). For example, a region of chromosome 23 potentially contains QTLs influencing male horn development and body mass. Similarly, a region of chromosome 10 was found to influence both male horn volume and horn length. Such results were expected given that most studied traits are highly phenotypically and genetically correlated within each sex. QTLs influencing variation in both male horn size and body mass were also expected simply because the horns of rams make up a substantial proportion of their total mass

(Blood *et al.* 1970). While larger sample sizes will allow a finer dissection of each trait's genetic architecture, it is clear that a number of loci have similar influence on multiple morphological traits.

All QTLs appeared to explain all or most of the additive genetic variation. Such results are typical of QTL studies in free-living wildlife populations (Slate *et al.* 2010) and likely a consequence of small sample sizes combined with the well known upward bias occurring when QTL effects are estimated in the population in which they were discovered (Goring *et al.* 2001). It could be argued that effect sizes of QTLs already discovered in domestic sheep are less likely to be upwardly biased (Slate *et al.* 2010). However, even in these cases, the magnitude of locus-specific effect sizes was clearly incompatible with the polygenic genetic architecture suggested by the QTL analysis.

In this study, genomic resources originally developed for domestic sheep and a long-term phenotypic dataset from individually marked individuals were used to identified QTLs for sexually selected traits in wild bighorn sheep. Identifying genes of ecological importance in this species will allow to address a variety of topics including the molecular impacts of selective harvesting (Coltman *et al.* 2003) as well as the genetic basis of genetic rescue (Hogg *et al.* 2006) and adaptive differentiation (Luikart *et al.* 2003). A comparison between bighorn sheep and domestic sheep revealed that the same QTLs were likely responsible for variability in sexually selected traits in these two species despite ~3 million years of divergence. Horn development and body mass in sheep may therefore

provide excellent systems to study the link between individual loci and micro- and macro-evolutionary processes in nature.

Table 7-1. Proportion of phenotypic variance explained by additive genetic (h^2), year, year of birth, and permanent environmental effects in the Ram Mountain bighorn sheep population for horn volume (cm^3), horn length (cm), horn base circumference (cm) and body mass (kg). Number of individuals and observations included in the full dataset analysis as well as corresponding numbers for the QTL mapping dataset (in parentheses) are presented. Trait means and ratios (s.d. in parentheses) are from univariate analyses of the entire dataset. Significance of ratios was assessed using likelihood ratio tests ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

trait	individuals	observations	mean	h2	year	year of birth	perm. env
Male traits							
Horn volume	261 (86)	1711 (863)	1546 (1057)	0.27 (0.12)**	0.08 (0.02)***	0.31 (0.07)***	0.21 (0.11)**
Horn length	262 (86)	1718 (866)	52.19 (18.41)	0.26 (0.13)*	0.14 (0.03)***	0.26 (0.07)***	0.24 (0.12)**
Horn base circ.	261 (86)	1715 (865)	30.60 (6.91)	0.30 (0.11)***	0.06 (0.02)***	0.30 (0.07)***	0.19 (0.11)*
Body mass	262 (86)	1708 (868)	72.29 (16.84)	0.34 (0.12)**	0.14 (0.04)***	0.13 (0.05)***	0.22 (0.11)**
Female traits							
Horn volume	311 (154)	4028 (3121)	111.6 (32.0)	0.28 (0.10)***	0.06 (0.02)***	0.17 (0.05)***	0.33 (0.09)***
Horn length	313 (154)	4089 (3146)	22.54 (4.21)	0.22 (0.11)*	0.05 (0.01)***	0.15 (0.06)***	0.49 (0.11)***
Horn base circ.	313 (156)	4292 (3356)	13.55 (1.08)	0.38 (0.08)***	0.08 (0.02)***	0.12 (0.04)***	0.16 (0.06)***
Body mass	318 (156)	4791 (3741)	60.10 (9.37)	0.20 (0.07)***	0.16 (0.04)***	0.10 (0.04)***	0.22 (0.06)***
Sexes combined							
Horn volume	572 (240)	5739 (3984)	-	0.19 (0.06)***	0.05 (0.01)***	0.18 (0.05)***	0.41 (0.06)***
Horn length	575 (240)	5807 (4012)	-	0.18 (0.06)***	0.06 (0.02)***	0.17 (0.05)***	0.47 (0.07)***
Horn base circ.	574 (242)	6007 (4221)	-	0.23 (0.06)***	0.07 (0.02)***	0.15 (0.04)***	0.31 (0.05)***
Body mass	580 (242)	6499 (4609)	-	0.24 (0.06)***	0.13 (0.03)***	0.07 (0.03)***	0.28 (0.05)***

Table 7-2. Proportion of phenotypic variance explained by additive genetic (h^2), year, year of birth, and permanent environmental effects in the Ram Mountain bighorn sheep population for horn volume (cm^3), horn length (cm), horn base circumference (cm) and body mass (kg) based on the restricted QTL mapping dataset. Significance of ratios was assessed using likelihood ratio tests ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

trait	Ind.	Obs.	trait mean	h2	year	year of birth	perm. env
Male traits							
Horn volume	86	863	1538 (1036)	0.36 (0.20)*	0.11 (0.03)***	0.25 (0.11)**	0.16 (0.18)
Horn length	86	866	52.33 (18.37)	0.50 (0.21)*	0.14 (0.04)***	0.19 (0.10)**	0.07 (0.18)
Horn base circ.	86	865	30.58 (6.77)	0.42 (0.18)**	0.09 (0.03)***	0.25 (0.10)**	0.09 (0.15)
Body mass	86	868	72.72 (16.60)	0.38 (0.17)**	0.16 (0.05)***	0.06 (0.08)	0.23 (0.16)*
Female traits							
Horn volume	154	3121	114.2 (31.53)	0.34 (0.13)***	0.07 (0.02)***	0.16 (0.07)***	0.27 (0.11)**
Horn length	154	3146	22.91 (4.15)	0.32 (0.15)*	0.05 (0.02)***	0.17 (0.08)**	0.36 (0.14)**
Horn base circ.	156	3356	13.61 (1.05)	0.41 (0.10)***	0.10 (0.03)***	0.11 (0.05)***	0.13 (0.08)*
Body mass	156	3741	61.01 (9.15)	0.22 (0.09)**	0.16 (0.04)***	0.00 (0.00)	0.26 (0.08)***
Sexes combined							
Horn volume	240	3984	-	0.27 (0.09)***	0.06 (0.02)***	0.13 (0.05)***	0.37 (0.08)***
Horn length	240	4012	-	0.29 (0.10)***	0.06 (0.02)***	0.13 (0.06)***	0.40 (0.09)***
Horn base circ.	242	4221	-	0.29 (0.08)***	0.09 (0.02)***	0.11 (0.05)***	0.27 (0.07)***
Body mass	242	4609	-	0.21 (0.07)***	0.15 (0.03)***	0.04 (0.03)	0.29 (0.06)***

Table 7-3. Additive genetic (co)variances and correlations for sex-specific morphological traits in adult bighorn sheep. Variance components are on the diagonal while covariance components are below the diagonal and correlations are above the diagonal. Significance was assessed using likelihood ratio tests. * identifies (co)variances and correlations significantly different from zero ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) while † identifies correlations significantly smaller than unity ($^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$). Standard errors generated by ASREML are presented in parentheses. MHV, male horn volume; MHL, male horn length; MHB, male horn base circumference; MBM, male body mass; FHV, female horn volume; FHL, female horn length; FHB, female horn base circumference; FBM, female body mass. Estimates are based on the entire Ram Mountain dataset.

	MHV	MHL	MHB	MBM	FHV	FHL	FHB	FBM
MHV	0.21 (0.09)**	0.89 (0.07)*	0.93 (0.04)**†	0.73 (0.14)*†	0.37 (0.28)††	0.88 (0.37)**	-0.10 (0.25)††	0.24 (0.28)††
MHL	0.18 (0.09)*	0.20 (0.10)*	0.72 (0.15)*†	0.48 (0.20)†	0.40 (0.30)†	1.20 (0.41)**	-0.28 (0.26)††	0.43 (0.31)
MHB	0.20 (0.09)**	0.15 (0.09)*	0.23 (0.09)***	0.86 (0.11)**	0.42 (0.26)††	0.78 (0.34)*	0.03 (0.24)†††	0.22 (0.27)††
MBM	0.16 (0.07)*	0.11 (0.07)	0.18 (0.07)**	0.20 (0.08)**	0.44 (0.28)†	0.82 (0.35)*	-0.07 (0.25)††	0.76 (0.24)**
FHV	0.09 (0.07)	0.09 (0.07)	0.10 (0.07)	0.10 (0.06)	0.27 (0.10)***	0.75 (0.12)†	0.95 (0.07)***	0.71 (0.17)**†
FHL	0.17 (0.07)**	0.21 (0.07)**	0.16 (0.07)*	0.15 (0.06)*	0.17 (0.10)	0.22 (0.12)*	0.37 (0.21)†	0.60 (0.27)
FHB	-0.03 (0.07)	-0.07 (0.07)	0.01 (0.07)	-0.02 (0.07)	0.23 (0.08)***	0.10 (0.08)	0.34 (0.08)***	0.52 (0.16)**†††
FBM	0.04 (0.04)	0.06 (0.04)	0.03 (0.04)	0.11 (0.04)**	0.12 (0.05)**	0.08 (0.05)	0.10 (0.04)**	0.11 (0.04)***

Table 7-4. Additive genetic (co)variances and correlations for morphological traits in adult bighorn sheep. Variance components are on the diagonal while covariance components are below the diagonal and correlations are above the diagonal. Significance was assessed using likelihood ratio tests. * identifies (co)variances and correlations significantly different from zero ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) while † identifies correlations significantly smaller than unity ($^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$). Standard errors generated by ASREML are presented in parentheses. Estimates are based on the entire Ram Mountain dataset.

	Horn volume	Horn length	Horn base circ.	Body mass
Horn volume	0.17 (0.05)***	0.80 (0.08)**†††	0.94 (0.04)***†	0.74 (0.11)***†††
Horn length	0.13 (0.05)**	0.16 (0.06)***	0.51 (0.16)*†††	0.69 (0.14)**†††
Horn base circ.	0.16 (0.05)***	0.09 (0.05)*	0.20 (0.05)***	0.57 (0.12)**†††
Body mass	0.11 (0.04)***	0.10 (0.04)**	0.10 (0.04)**	0.14 (0.04)***

Table 7-5. Additive genetic (co)variances and correlations for sex-specific morphological traits in adult bighorn sheep based on the Ram Mountain QTL mapping dataset. Variance components are on the diagonal while covariance components are below the diagonal and correlations are above the diagonal. Standard errors generated by ASREML are presented in parentheses. MHV, male horn volume; MHL, male horn length; MHB, male horn base circumference; MBM, male body mass; FHV, female horn volume; FHL, female horn length; FHB, female horn base circumference; FBM, female body mass.

	MHV	MHL	MHB	MBM	FHV	FHL	FHB	FBM
MHV	0.30 (0.18)	0.94 (0.04)	0.96 (0.03)	0.98 (0.08)	0.79 (0.35)	0.93 (0.36)	0.32 (0.34)	0.52 (0.33)
MHL	0.40 (0.18)	0.42 (0.20)	0.80 (0.10)	0.73 (0.12)	1.01 (0.50)	1.30 (0.52)	-0.01 (0.28)	0.47 (0.30)
MHB	0.29 (0.17)	0.32 (0.17)	0.33 (0.16)	0.95 (0.08)	0.59 (0.30)	0.64 (0.32)	0.33 (0.29)	0.46 (0.29)
MBM	0.23 (0.12)	0.23 (0.08)	0.26 (0.12)	0.21 (0.10)	0.49 (0.35)	0.73 (0.36)	0.00 (0.30)	0.67 (0.30)
FHV	0.21 (0.10)	0.22 (0.09)	0.18 (0.10)	0.11 (0.08)	0.32 (0.13)	0.77 (0.11)	0.88 (0.08)	0.64 (0.20)
FHL	0.24 (0.10)	0.29 (0.10)	0.19 (0.10)	0.16 (0.08)	0.23 (0.13)	0.33 (0.16)	0.29 (0.25)	0.53 (0.25)
FHB	0.09 (0.10)	-0.01 (0.11)	0.11 (0.10)	0.00 (0.08)	0.26 (0.10)	0.09 (0.09)	0.37 (0.11)	0.47 (0.19)
FBM	0.10 (0.06)	0.10 (0.06)	0.09 (0.06)	0.10 (0.05)	0.12 (0.06)	0.10 (0.07)	0.09 (0.06)	0.11 (0.05)

Table 7-6. Additive genetic (co)variances and correlations for morphological traits in adult bighorn sheep based on the Ram Mountain QTL mapping dataset. Variance components are on the diagonal while covariance components are below the diagonal and correlations are above the diagonal. Standard errors generated by ASREML are presented in parentheses.

	Horn volume	Horn length	Horn base circ.	Body mass
Horn volume	0.24 (0.09)	0.80 (0.08)	0.86 (0.06)	0.68 (0.15)
Horn length	0.20 (0.09)	0.27 (0.10)	0.35 (0.20)	0.62 (0.17)
Horn base circ.	0.20 (0.07)	0.09 (0.07)	0.26 (0.08)	0.48 (0.17)
Body mass	0.11 (0.05)	0.11 (0.05)	0.09 (0.04)	0.12 (0.04)

Table 7-7. Putative QTL (LOD > 1.175) for horn size and body mass in the Ram Mountain bighorn sheep population and their estimated parameters (q^2, proportion of phenotypic variance explained by the QTL; h^2, residual heritability after having fitted the QTL effect). * denotes suggestive QTL (LOD > 1.88).

Trait	LOD	Chromosome	Position (cM)	Closest marker	1-LOD drop (cM)	q^2	h^2
Male traits							
Horn volume	1.59	1	361	BMS2263	-	0.56 (0.08)	0.00 (0.00)
	1.66	10	0	OarSEJ10, 11	-	0.39 (0.10)	0.00 (0.00)
	1.37	23	28	AGLA269	-	0.56 (0.13)	0.00 (0.00)
Horn length	1.91*	1	361	BMS2263	346 - 361	0.60 (0.08)	0.00 (0.00)
	1.31	18	12	ILSTS52	-	0.48 (0.15)	0.11 (0.13)
	2.82*	23	26	AGLA269	16 - 38	0.73 (0.06)	0.00 (0.00)
Horn base circ.	1.45	10	0	OarSEJ10, 11	-	0.37 (0.12)	0.06 (0.16)
Body mass	1.34	23	35	AGLA269	-	0.45 (0.13)	0.00 (0.00)
Female traits							
Horn volume	1.74	18	12	ILSTS52	-	0.38 (0.10)	0.00 (0.00)
	1.33	18	108	CSAP28E	-	0.41 (0.11)	0.00 (0.00)
Horn base circ.	1.3	5	55	TGLA303	-	0.44 (0.09)	0.00 (0.00)
	1.58	6	66	JMP36	-	0.43 (0.08)	0.00 (0.00)
	1.59	18	12	ILSTS52	-	0.29 (0.12)	0.12 (0.14)
	1.42	19	49	MCM61A	-	0.43 (0.09)	0.00 (0.00)
Body mass	1.32	2	190	BM81124	-	0.22 (0.07)	0.00 (0.00)
	1.44	24	44	BP28	-	0.24 (0.07)	0.00 (0.00)
	2.15*	26	40	JMP58	30 - 44	0.26 (0.07)	0.00 (0.00)
Sexes combined							
Horn volume	1.95*	18	9	ILSTS52	0 - 48	0.33 (0.08)	0.00 (0.00)
	1.21	18	108	CSAP28E	-	0.29 (0.08)	0.00 (0.00)
Horn length	1.29	22	50	MAF92	-	0.31 (0.08)	0.00 (0.00)

Horn base circ.	2.35*	18	1	SRCRSP5	0 - 30	0.33 (0.07)	0.00 (0.00)
	1.27	19	46	MCM61A	-	0.31 (0.07)	0.00 (0.00)
Body mass	1.47	2	190	BM81124	-	0.21 (0.06)	0.00 (0.00)
	1.25	22	10	HEL11	-	0.23 (0.06)	0.00 (0.00)
	1.18	X	124	MCM25	-	0.15 (0.07)	0.08 (0.08)

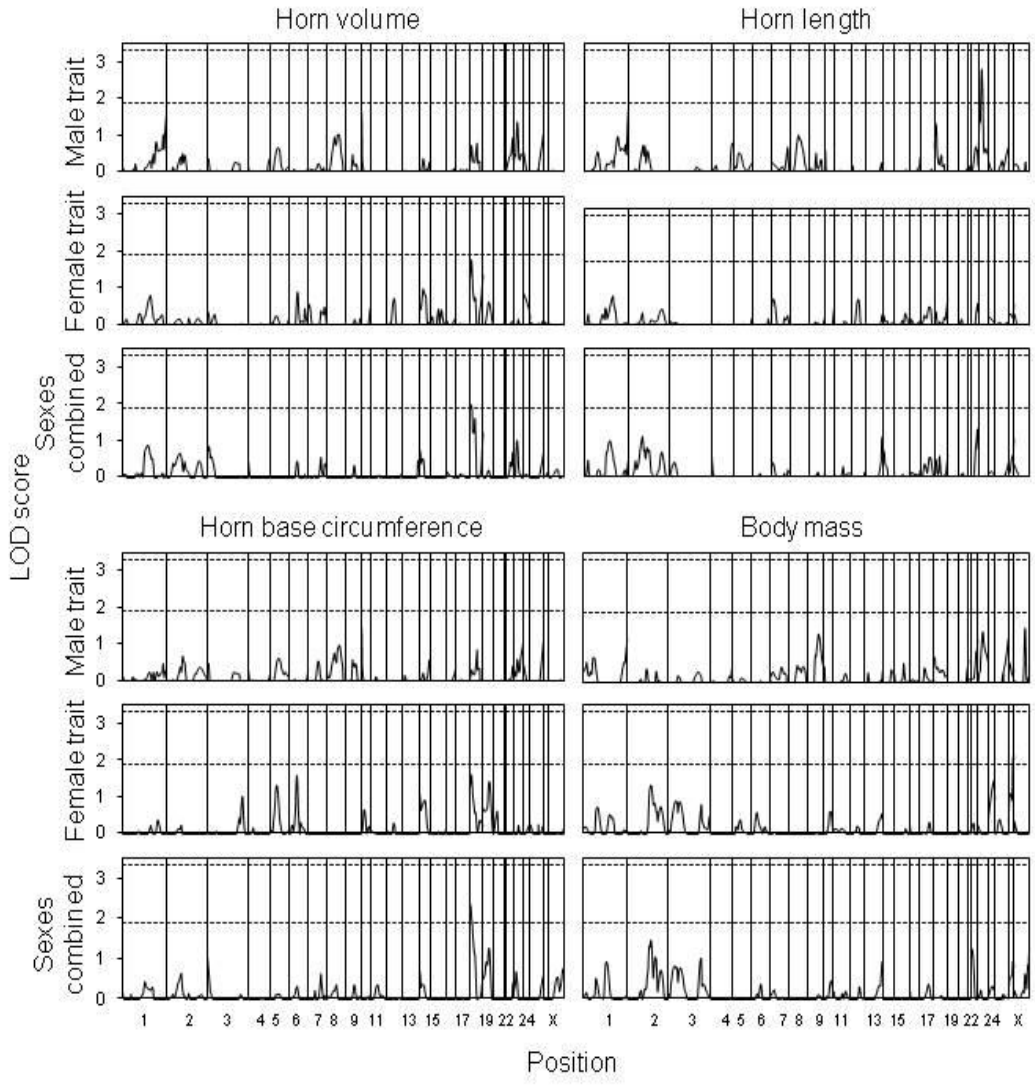

Figure 7-1. LOD scores along the 26 autosomes and the X chromosome for the presence of horn size and body mass QTL in the Ram Mountain bighorn sheep population. Dashed horizontal lines depict genome-wide thresholds used to identify suggestive (LOD > 1.88) and significant (LOD > 3.31) QTLs.

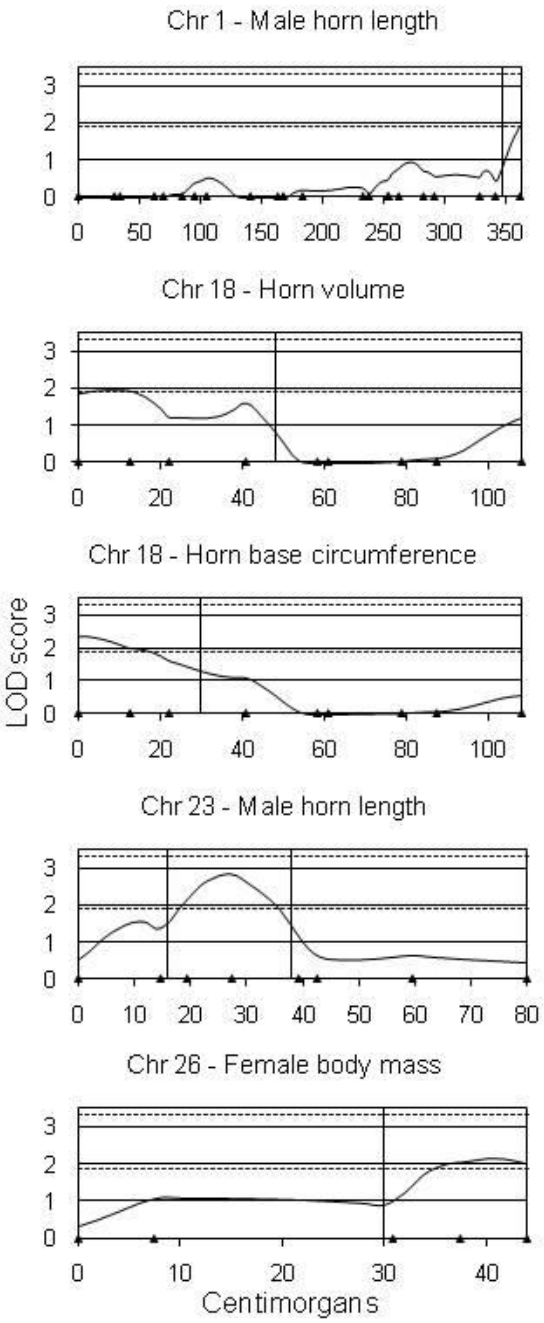

Figure 7-2. LOD scores along chromosomes for the 5 suggestive QTL (LOD > 1.88) in the Ram Mountain bighorn sheep population. Triangles on the X axis depict marker positions. Dashed horizontal lines depict genome-wide thresholds used to identify suggestive (LOD > 1.88) and significant (LOD > 3.31) QTLs. Vertical lines depict 1-LOD 95% confidence intervals.

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Chapter 8

General conclusion

8.1 Conclusion

My doctoral research was centered on the importance of sex-specific genetic variance in allowing the evolution of sexual dimorphism (SD). This included quantifying sex-specific and cross-sex quantitative genetic parameters as well as sex-specific selection for highly sexually dimorphic traits in free-living bighorn sheep (chapters 2 and 7). I also constructed a bighorn sheep genetic linkage map (chapters 5 and 6) and mapped quantitative traits loci influencing body mass and horn dimensions (chapter 7). Finally, I performed the first exhaustive review of published cross-sex genetic correlation (r_{MF}) estimates to test hypotheses about the importance of sex-specific genetic variance in allowing the evolution of SD and ontogenetic variability (chapters 3 and 4).

In **chapter 2**, I demonstrated that variation in body mass and horn volume in the Ram Mountain bighorn sheep population had an additive genetic basis in both sexes, and that male and female traits were positively genetically correlated. For horn volume, selection coefficients did not significantly differ from zero in either sex. For body weight, selection coefficients were positive in females but did not differ from zero in males. The absence of detectable sexually antagonistic selection suggested that intralocus sexual conflicts (ISCs) over body mass and horn size had been for the most part resolved through the evolution of SD. In hindsight, this was expected, considering that the most sexually dimorphic traits are typically the ones for which the evolution of SD is least constrained (chapter 3). Future research aimed at identifying the source of ISCs would therefore benefit from using a bottom-up approach. In particular, genome scans based on

large panels of single nucleotide polymorphism (SNP) markers could dissect the genetic architecture of ISCs in wild populations. Such research would allow us to answer a number of fundamental questions about the mechanisms underlying the maintenance of ISCs and genetic variation.

In **chapter 3**, I compiled 553 r_{MF} estimates from 114 sources. Results showed that r_{MF} tended to be large and positive but that they were typically smaller for fitness components. This demonstrated that the evolution of SD was typically genetically constrained and that sex-specific selection coefficients were often opposite in sign due to sub-optimal levels of SD. Finally, after decades of speculation, I empirically confirmed that sex-specific genetic variance was an important contributor to the evolution of SD by validating the prediction of a negative correlation between r_{MF} and SD.

The large number of r_{MF} estimates gathered from the literature defied the common perception that such estimates were rare (e.g. Rice & Chippindale 2001, Parker & Garant 2005, Fairbairn 2007). In particular, a relatively large number of estimates were amassed for morphological traits in animals. However, there was still a clear need for more data relating to non-morphological traits and fitness components in particular. More studies that jointly estimate genetic (co)variances, SD, and sex-specific regimes would also greatly improve our understanding of the evolutionary dynamics of ISCs. Finally, very little is known about interactions between r_{MF} and environmental conditions and future research on this topic in bighorn sheep and other organisms would be greatly informative.

In **chapter 4**, I assembled a database on ontogenetic variability in r_{MF} comprising estimates from 22 studies. Based on patterns observed for 28 traits from 11 species, I demonstrated that r_{MF} could vary across ontogeny and that such change usually involved a downward trend. This suggested that SD could generally evolve more readily for phenotypes expressed later in ontogeny and was consistent with the idea that selection for SD usually operates in adults. The accumulation of age- and sex-specific genetic variance may play an important role in the resolution of ontogenetic conflicts where selection favors differing patterns of SD in juveniles and adults. Despite the presence of general patterns, additional studies on ontogenetic variability in r_{MF} in bighorn sheep and other organisms are clearly needed.

In **chapter 5**, I reported on the discovery of 247 and 149 new polymorphic microsatellite markers in bighorn sheep and mountain goats, respectively. Most markers were in Hardy-Weinberg equilibrium and showed no sign of null alleles. The average distance between successive markers in the domestic sheep genome (mean ± 1 SD) was 11.9 ± 9.2 and 15.8 ± 13.8 centimorgans for bighorn sheep and mountain goat markers, respectively. These new markers will be useful for improving parentage analyses, marker-based relatedness estimates and landscape genetic studies. The development of genomic resources in these wildlife species also enabled studies of the genetic architecture of trait variation.

In **Chapter 6,** I presented a highly contiguous bighorn sheep genetic linkage map composed of 247 microsatellite markers distributed along all 26 autosomes and the X chromosome. The map covered about 84% of the species

genome. The 240 unique positions spanned a sex-averaged distance of 3051 cM with an average inter-marker distance of 14.3 cM. I found that marker synteny, order, sex-averaged interval lengths and sex-averaged total map lengths were all very similar between bighorn sheep and the closely related domestic sheep, *Ovis aries*. However, in contrast to domestic sheep, but consistent with the usual pattern for a placental mammal, recombination rates in bighorn sheep were significantly greater in females than in males. Finally, I found that interval-specific patterns of sexual dimorphism were preserved among closely related sheep species, possibly due to the conserved position of these intervals relative to the centromeres and telomeres. An exciting extension of this work would be to perform evolutionary quantitative genetics analyses of recombination rates in the study populations. In particular, I am unaware of r_{MF} estimates for recombination rates or studies on micro-evolutionary change and the extent of SD for that trait.

In **Chapter 7**, I estimated sex-specific and cross-sex genetic parameters for body mass and horn dimensions in the Ram Mountain population and performed a quantitative trait locus mapping analysis using 241 microsatellite loci typed in 310 pedigreed animals. Consistent with results presented in chapter 2, all traits showed significant additive genetic variance and genetic correlations tended to be positive. The QTL analysis resulted in the identification of one body mass and four horn dimension QTLs that reached genome-wide suggestive significance (LOD > 1.88) and 20 more loci with nominal p-values < 0.01. As expected for sexually dimorphic traits involved in male-male combat, most QTLs appeared to have sex-specific effects. A comparison with domestic sheep revealed striking

similarities in the location of QTLs between species. Fine-mapping of the QTLs presented in this thesis using a larger number of markers will allow for groundbreaking analyses of the evolutionary dynamics of QTL in natural environments. Another exciting extension of this work would be to test for the presence of conserved QTL in additional closely related species such as mountain goats.

When I started this doctoral research five years ago, little was known about the importance of genetic constraints in limiting the independent evolution of the sexes. Empirical studies explicitly focussing on ISCs were scarce and comments about the lack of r_{MF} estimates were frequent. This gap in the literature was made obvious in the 2005 book "Sexual conflict" by Arnqvist & Rowe in which less than 4 pages were devoted to ISCs. But things have changed. In this thesis, I provided new empirical insights into the evolution of ISCs and filled important gaps in our knowledge of r_{MF}. Articles on ISCs and sexual antagonism are now appearing on a regular basis in top evolution journals such as Evolution, Proceedings of the Royal Society B and the Journal of Evolutionary Biology and a number of reviews synthesising the field have recently been published (e.g. Rowe & Day 2006, Fairbairn *et al*. 2007, Cox & Calsbeek 2009, Bonduriansky & Chenoweth 2009, Chenoweth & McGuigan 2010). With the advent of new sequencing and genotyping technologies (Stapley *et al*. 2010) and the ever increasing number of long-term field studies of pedigreed individuals (Clutton-Brock & Sheldon 2010), the future of ISC research looks rosy.

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