

## Demographic drivers of age-dependent sexual selection

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### Abstract

Sexual selection has a critical role in evolution, and it is fundamental to identify what ecological factors drive its variation. Disentangling the ecological correlates of sexual selection over the long term, however, is challenging and has rarely been done in nature. We sought to assess how demographic changes influenced the intensity, direction and form of sexual selection and whether selective pressures varied with age. We tested whether breeder sex ratio, number of competitors and age structure influenced selection differentials on horn length of wild bighorn rams (*Ovis canadensis*) of different age classes on Ram Mountain, Alberta. We used 21 years of data including a detailed pedigree, demographic parameters and repeated morphological measurements. Sexual selection on horn length of males of all ages was directional and positive. Selection intensity increased with the number of competitors, reflecting male–male encounter rate during the rut, but was independent of breeder sex ratio or age structure. This result can also be linked to changes in population size because the number of competitors was highly correlated to total number of sheep. This demographic effect likely arises from age-dependent mating tactics. Males aged 2–4 years are weakly competitive and experienced stronger sexual selection as they accounted for a greater proportion of all males. Selection experienced by mature males appeared independent of demography. Our study provides a rare description of the demographic determinants of sexual selection in nature.

### Introduction

Selection plays a key role in evolution (Rieseberg *et al.*, 2002) and is central to adaptation in nature (Endler, 1986; Schluter, 2009). Quantifying selection on phenotypes and identifying ecological drivers of selection have been cornerstones of modern evolutionary ecology since the seminal work of Lande & Arnold (1983). Variations in strength, direction and form are important features of selective pressures (Kingsolver *et al.*, 2001; Siepielski *et al.*, 2009; Bell, 2010). Fluctuations in selective pressure are driven by environmental conditions (Funk *et al.*, 2006; Siepielski *et al.*, 2009; Cornwallis & Uller, 2010) across spatial and temporal scales (Gosden & Svensson, 2008). The very few studies that identified

ecological drivers of selection mainly involved meta-analyses (but see Robinson *et al.*, 2008; Gosden & Svensson, 2008). Thus, we know little about the factors affecting variation in strength, direction and form of selection in nature (Morrissey & Hadfield, 2012). Our understanding of the link between selection and its ecological drivers is currently based upon a handful of studies, most of which relate changes in survival selection to demographic processes or climate (Calsbeek & Cox, 2010). Natural selection on beak size of Darwin's finches (*Geospiza* spp.) in response to drought is a seminal example (Grant & Grant, 2002). Only recently, sexual selection was shown to vary with ecological factors (Gosden & Svensson, 2008; Robinson *et al.*, 2008; Punzalan *et al.*, 2010) such as environmental quality or demographic parameters.

Very few studies have explored temporal variation in sexual selection and < 1% of studies linked these fluctuations to ecological variables (Cornwallis & Uller, 2010). Both inter- and intrasexual selection hinge on

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competition for access to mates (Andersson, 1994), whose intensity and outcome depend on interactions among individuals of different sex-age classes (West-Eberhard, 1983). As a consequence, sexual selection may vary with demography. The relative numbers of females and males ready to mate at a given time and place determine the operational sex ratio (OSR), which is thought to represent the 'degree of monopolizability of mates' (Emlen & Oring, 1977). The intensity of competition among males is expected to increase as OSR becomes more male-biased (Kvarnemo & Ahnesjö, 1996). As mate monopolization directly affects mating variance, a necessary condition for sexual selection, OSR was considered a primary determinant of sexual selection (Jones *et al.*, 2002; Mills *et al.*, 2007). An increase in the number of females leading to higher mating success for the most competitive males, is a textbook example of the direct effect of OSR on the potential for sexual selection (Andersson, 1994). Yet, the relationship between OSR and sexual selection may not be generally valid (Klug *et al.*, 2010). For instance, in a longitudinal study of ambush bugs (*Phymata americana*), intrasexual selection intensity on colour patterns decreased with OSR, rather than increasing as expected (Punzalan *et al.*, 2010); and in an experimental study on guppies (*Poecilia reticulata*), intersexual selection intensity on mating behaviour was not associated with OSR (Head *et al.*, 2007). Evolutionary models indicate that OSR may play a weaker role in sexual selection than the cost of breeding and sex-specific mortality (Kokko & Monaghan, 2001).

Despite its weaknesses in directly predicting sexual selection, OSR may be important in combination with other demographic parameters. For example, the OSR may remain stable but the number of females and males may change. For a given OSR, the density of competitors can influence competition by affecting the encounter rate among competitors (Hubbell & Johnson, 1987; Crowley *et al.*, 1991; Kokko & Rankin, 2006), especially where alternative mating tactics coexist. Alternative mating tactics may have evolved to overcome differences in mating competitiveness (Gross, 1996). As the number of competitors increases, less competitive individuals may experience a reduction in mating success. Consequently, the number of competitors could increase sexual selection intensity through its effects on mating competition. Although supported by a handful of studies (e.g. Jirotkul, 1999; Punzalan *et al.*, 2010), this relationship is not always evident (Head *et al.*, 2007; Aronsen *et al.*, 2013). Variance in competitiveness among males may also modulate mating monopolization (Klug *et al.*, 2010), regardless of OSR. In polygynous species, age is often associated with the development of morphological (Bonenfant *et al.*, 2009; Festa-Bianchet, 2012) and behavioural (Pelletier *et al.*, 2003; Martin *et al.*, 2013) sexually selected characters in males that increase mating success. Male age

structure is therefore another likely determinant of the strength of sexual selection.

Reproduction is age-dependent in many taxa (Forsslund & Pärt, 1995; Gaillard *et al.*, 1998). In males of species under sexual selection, it typically increases until individuals reach prime age, then declines with senescence (Festa-Bianchet, 2012). The seminal theoretical work of Fisher (1930), Medawar (1952) and Hamilton (1966) established that selection pressures on fitness-related genes should decrease with age after maturity (Charlesworth, 2000). Yet, sexual selection analyses usually disregard such heterogeneity in age or life-history stages and estimate selection differentials or gradients on all adults. We know little about age dependence in sexual selection pressures, as few individual-based studies have examined age-specific sexual selection (Coltman *et al.*, 2002). It is thus unclear if sexual selection documented in nature results from the combined effects of different age-specific selective pressures, or simply from differences in reproductive success between male age classes. For example, although, in most ungulates, prime-aged males have much higher reproductive success than younger males (Festa-Bianchet, 2012), we do not know how sexual selection differs between ages or life stages.

Here, we seek to disentangle the effects of demographic factors on intrasexual selection in nature. We first test for annual fluctuations in strength, form, and direction of intrasexual selection on males. We then explore how intrasexual selection is affected by the breeder sex ratio, the number of competitors and the age structure of adult males. We take advantage of long-term monitoring of wild bighorn sheep (*Ovis canadensis*) on Ram Mountain, including repeated individual measures of horn length and body mass, a deep pedigree, and detailed demographic information. Bighorn sheep are polygynous ungulates where male–male competition for reproduction leads to sexual selection on horn length and body mass (Coltman *et al.*, 2002), with little evidence of female choice (Hogg, 1987). Depending on their social rank and age, rams use different mating tactics (Hogg, 1987; Hogg & Forbes, 1997), leading to a skewed distribution of paternities. Coltman *et al.* (2002) showed that large horns increased mating success for rams aged 7 years and older. The mating success of young rams, however, appeared to be random in respect to morphological characters (Coltman *et al.*, 2002). We used data collected over twenty-one breeding seasons to investigate three questions that provide insights on how sexual selection varies according to demography: (i) Does sexual selection on horn length fluctuate over time in strength and direction? (ii) Are secondary sexual traits of young rams, which use alternative mating tactics, under sexual selection? (iii) Does population demography (including sex ratio, number of competitors and ram age structure) drive sexual selection on horn length, possibly in an age-dependent manner?

## Materials and methods

### Study population and mating system

We used data collected from 1988 to 2011 from marked known-age bighorn sheep on Ram Mountain (52°N, 115°W, elevation 1080–2170 m), Alberta, Canada. Sheep have been captured in a corral trap baited with salt since 1971 (Jorgenson *et al.*, 1993) and marked with unique combinations of ear tags and collars. All individuals included in this study were marked during their first or second summer and their exact age was known. Annual resighting probabilities exceeded 95% and 99% for males and females, respectively (Bonenfant *et al.*, 2009), providing accurate information on demographic parameters (Table S1).

Variability in male breeding success arises partly from the use of alternative mating tactics (Hogg, 1984, 1987). Rams establish a linear dominance hierarchy where rank increases with age, horn size and body mass (Pelletier & Festa-Bianchet, 2006; Martin *et al.*, 2013). The rut usually starts in late November and over 80% of conceptions typically occur over two weeks (Pelletier & Festa-Bianchet, 2006). Males in the top 1–4 ranks of the social hierarchy use a mate guarding tactic called ‘tending’ which leads to high mating success (Hogg, 1984). Alternative tactics used by subordinates include courting (trying to mate with a female during courting chases after bypassing the tending male) and blocking (sequestration of a female away from dominant males until she enters oestrus). Copulation rate of courting rams increases with age (Hogg & Forbes, 1997) but previous studies detected no effect of phenotype on mating success of young rams (Coltman *et al.*, 2002).

### Demographic parameters

#### *Number of competitors*

The number of males aged two or older alive in late September.

#### *Male age structure*

Only males five years and older adopt the highly successful tending tactic (Pelletier & Festa-Bianchet, 2006). Male age structure was the ratio of males aged two to four years over the total number of males two years and older.

#### *Breeder sex ratio*

We approximated the operational sex ratio by the breeder sex ratio because the study site is not accessible during the rut. The breeder sex ratio was the ratio of parturient females the following spring over the number of rams two years and older about six weeks before the rut. We identified parturient females as those with colostrum or milk at first capture in late May or early June.

### Morphological measurements

We measured total horn length from the base to the tip, along the outer curvature. To account for growth during summer, we adjusted individual horn length and body mass to September 15th, consistent with previous studies (Festa-Bianchet *et al.*, 2000). We estimated individual growth rates by fitting linear mixed models on repeated individual measurements each summer (Martin & Pelletier, 2011). Models included male identity (intercept) and the square root of capture date (slope) as random effects and age and the square root of date as fixed effects. The square-root transformation of capture date linearizes growth over the summer (Festa-Bianchet *et al.*, 1996; Martin & Pelletier, 2011).

### Paternity assignments and fitness

Tissue sampling began in 1987. Samples were genotyped at 26 microsatellite loci (Coltman *et al.*, 2005). We assigned 242 paternities to 65 fathers from a pool of 136 candidate males from 1988 to 2011 with 95% confidence in CERVUS (Marshall *et al.*, 1998). Tissue samples were not collected in 1994 to 1996. Consequently, we excluded from analyses ruts from 1993 to 1995 because paternities for lambs conceived during these three ruts could only be established if they survived to 1997. The 242 paternities thus excluded those from these three ruts. Paternities of lambs that died before capture were unknown. We measured fitness as breeding success, the number of offspring assigned to each male during a rut.

### Statistical analyses

We investigated how age, horn length and body mass affected breeding success by fitting generalized linear mixed models. We used a log link to account for the Poisson error structure of breeding success. Effects of horn length and body mass were modelled separately because their strong correlation over the entire range of ram ages ( $r = 0.93$ ,  $P < 0.001$ ) led to strong collinearity (variance inflation factor = 6.25). We refer to these variables as ‘morphological characters’. Models included the linear and quadratic terms of age and of morphological characters, interactions between the linear age term and the linear and quadratic terms of morphological characters and interactions between the quadratic term of age and linear terms of morphological characters as explanatory variables. We included male identity as a random variable, after verifying its significance with a log-likelihood ratio test (Bolker *et al.*, 2009), which follows a  $\chi^2$  distribution. The distribution of paternities was overdispersed. It is not straightforward, however, to fit a quasi-Poisson or a negative binomial linear model in a mixed effect model to handle

overdispersion. Following Atkins *et al.* (2012), we extended the Poisson mixed models by including an overdispersion factor as an additional random term, which is a per-observation error term that captures overdispersion. Because this per-observation error term is similar to a residual error term, we tested its significance with a log-likelihood ratio test. Analyses on body mass are reported in the Appendix S1.

The standardized selection differential of each selection event was estimated as the covariance between character value, annually standardized to zero mean and unit variance, and relative fitness, calculated annually by dividing individual breeding success by mean breeding success. For each estimation on each character, the strength of linear selection was obtained by fitting a model with the linear term of the character, and the strength of the nonlinear selection was obtained by fitting a model with both linear and quadratic terms of the character (Lande & Arnold, 1983). Annual linear standardized selection differentials ( $i$ ) were estimated by separate annual regressions of standardized horn length and body mass against relative breeding success (Lande & Arnold, 1983). Annual quadratic standardized selection differentials ( $j$ ) were estimated by separate annual regressions of both linear and quadratic terms of each standardized phenotypic character against relative breeding success. The standardized quadratic selection differentials ( $j$ ) are twice the estimates of the quadratic terms of phenotypic characters. Statistical significance for each selection differential was estimated with generalized quasi-Poisson linear models where unstandardized characters were regressed against breeding success.

To study variation in sexual selection on horn size and body mass, we tested first whether selection differentials varied according to age and year, and second whether selection differentials varied according to age and demographic parameters through time. To do so, we first ran a model including as fixed effects phenotype, age and year with their two- and three-way interactions. This model controlled for pseudoreplication by including individual identity as a random intercept. We used a log-likelihood ratio test and a stepwise simplification to assess the significance of the random and fixed effects, respectively. We then used a sequential model building approach (Chenoweth & Blows, 2005) to test the effects of demographic parameters in a model in which year was included as a random intercept and phenotype (horn length or body mass) was fitted as a random slope. Age, phenotype and demographic parameters were included as fixed effects along with their two- and three-way interactions. We used sequential log-likelihood ratio tests to assess the significance of random and fixed effects by comparing a full model with one in which each of these variables was removed one at a time. This test assessed the effect of each interaction independently of the others.

The difference in log-likelihood between two models follows a chi-squared distribution with one degree of freedom. We first assessed the significance of random effects using the maximum-likelihood (ML) method and then reported the estimates of variance components and the significance of fixed effects using the restricted maximum-likelihood (REML) method. Significance of three-way interactions was tested before that of two-way interactions.

To estimate global linear and nonlinear selection differentials over all years, we added individual identity as a random effect in generalized quasi-Poisson linear mixed models. To investigate age-dependent fluctuation of selection, we estimated selection differentials for each age. Models included standardized character values and relative reproductive success at the scale of the entire population as previously explained. We pooled males ten years and older ( $n = 18$  ram-years) to estimate age-specific selection differentials. We used Z-tests to compare estimates of linear selection differentials between age classes and between phenotypic characters. Z-scores were calculated as  $Z = \frac{\beta_i - \beta_j}{\sqrt{SE_i^2 + SE_j^2}}$ , where  $\beta$  and SE are, respectively, the selection differential and associated standard errors. A Z-score  $> 1.96$  indicates a significant difference between two selection differentials.

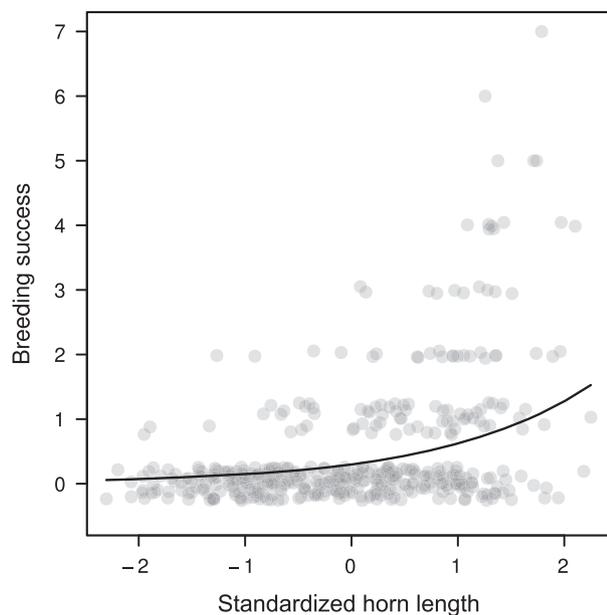
To further investigate whether demographic parameters could explain annual changes in the strength of sexual selection, we fitted linear models including the number of competitors, breeder sex ratio and male age structure as explanatory variables. We did not include interactions to avoid overparameterization because we had twenty-one data points (years). In these models, the number of competitors, used as an explanatory variable, is also involved in the calculation of mean fitness as the denominator of annual fitness. To test whether this mathematical dependence influences the conclusions of the models, we modelled the relationship between the above-mentioned set of demographic parameters and both standardized selection differentials and the covariance between each standardized character and absolute fitness. To account for differences in the accuracy of estimation of selection differentials with differing sample sizes, we weighted our regression models by  $1/SE^2$  where SE is the standard error in models quantifying the significance of selection differentials. To test whether selection events were autocorrelated, we used a log-likelihood ratio test for the significance of a first-order autocorrelation structure. We fitted linear models to investigate how the proportion of males aged two to four years and five years and older influenced the proportion of offspring produced by males of each age class. We simplified each full model through backward stepwise deletion (Zuur *et al.*, 2009). We accompanied each deletion step with a log-likelihood ratio test to ensure that model fit was not reduced.

All independent variables were standardized to zero mean and unit variance. All statistical analyses were performed using R version 2.14.1 (R Development Core Team, 2011). We used the 'GLS' function from the 'NLME' library (Pinheiro *et al.*, 2011) to test for autocorrelation. Other linear models and generalized linear mixed models were fitted using the 'lm' and 'GLMER' functions of the 'LME4' library (Bates *et al.*, 2011).

## Results

Demographic parameters were not significantly correlated (Fig. S1, number of competitors – breeder sex ratio:  $r = -0.006$ ,  $P = 0.77$ ; number of competitors – age structure:  $r = 0.28$ ,  $P = 0.22$ ; breeder sex ratio – age structure:  $r = -0.14$ ,  $P = 0.53$ ), allowing us to include them together in the same model for subsequent analyses. Controlling for age, horn length had a linear positive effect on breeding success ( $\beta = 0.720 \pm 0.163$ ,  $z = 4.432$ ,  $P < 0.001$ , Table S2a, Fig. 1). Mass had a quadratic positive effect on breeding success that increased with age (Fig. S2, Table S2b): it was weak for rams aged two to six years and increasingly stronger afterwards.

Global linear selection differentials on horn length were positive and significant, for all ages or by age class (Table 1, Table S3 for body mass). The global linear selection differential for horn length was 9.5% stronger than that for body mass ( $Z = 7.620$ ,  $P < 0.001$ ). Nonlinear selection differentials were not significant (Table S4).



**Fig. 1** Relationship between annually standardized horn length and breeding success in bighorn rams. Selection events in 1988–1992 and 1996–2011, Ram Mountain, Alberta, Canada.

All annual linear selection differentials for all ages were positive except for 2001, when the differential was negative but not significantly different from zero (Table S5). Selection on horn length estimated for all ages annually did not vary in direction, and its variation in strength was unclear as the three-way interaction horn length  $\times$  age  $\times$  year was nearly significant (Table 2; Table S6 for body mass; Fig. S3 for both horn length and body mass). The coefficient of variation and variance for annual linear selection differentials on horn length for all ages were  $CV = 0.55$ ,  $\sigma^2 = 0.44$ . We found no evidence of temporal autocorrelation, as the inclusion of an autoregressive process did not reduce model deviance (LRT = 0.853,  $P = 0.35$ ). For horn length, coefficients of variation and variances calculated for selection differentials were  $CV = 1.25$ ,  $\sigma^2 = 0.38$  for males aged two to four years and  $CV = 2.51$ ,  $\sigma^2 = 3.11$  for older males (Fig. S3). The global selection differential on horn length was stronger for mature than for young males ( $Z = 58.98$ ,  $P < 0.001$ ). Annual selection differentials for the two age classes were not correlated ( $r = -0.35$ , d.f. = 18,  $P = 0.12$ ). Horn length was under sexual selection that increased with age (Fig. S4,  $R^2 = 0.71$ ,  $\beta = 0.638 \pm 0.154$ ,  $t = 4.138$ ,  $P = 0.004$ ).

Age influenced linear selection differentials on horn length (Table 3) and body mass (Table S7) in interaction with demographic parameters. Annual selection differentials calculated for all ages on horn length increased with the number of competitors (Table 4a: adjusted  $R^2 = 0.19$ ,  $\beta = 0.252 \pm 0.076$ ,  $t = 3.337$ ,  $P = 0.003$ ). Breeder sex ratio and age structure did not affect annual selection differentials on horn length ( $P > 0.15$ , Table 4). Annual selection differentials on horn length for males aged two to four increased as these young males made up a greater proportion of all males (Table 4b, adjusted  $R^2 = 0.30$ ,  $\beta = 0.221 \pm 0.071$ ,  $t = 3.130$ ,  $P = 0.006$ , Fig. 2) but were independent of breeder sex ratio or the number of males. The proportion of paternities assigned to males two to four years increased with their proportion among males (adjusted  $R^2 = 0.23$ ,  $\beta = 0.098 \pm 0.037$ ,  $t = 2.647$ ,  $P = 0.016$ , Table S1). For males five years and older, selection on horn length was independent of demographic parameters (Table 4c). The effects of demographic parameters

**Table 1** Linear sexual selection differentials on horn length estimated for bighorn rams of all ages, young (2 to 4 years) and mature (5 years and older) in 1988–1992 and 1996–2011, Ram Mountain, Canada.

Age class	Horn length		
	$i \pm SE$	$t$	$P$
All ages	$0.934 \pm 0.007$	9.675	< 0.001
2 to 4 years old	$0.298 \pm 0.020$	3.481	0.001
5 years and older	$1.738 \pm 0.014$	4.531	< 0.001

**Table 2** Test of annual variation of the standardized selection differential  $i$  on horn length of bighorn rams for 1988–1992 and 1996–2011, Ram Mountain, Canada. Significance of ram identity was confirmed by a log-likelihood ratio test (LRT:  $P < 0.0001$ , variance explained = 19.6%).

	Estimate $\pm$ SE	$t$	$P$
Intercept	0.327 $\pm$ 0.337	0.971	0.332
Horn length	−0.433 $\pm$ 0.225	1.926	0.055
Year	0.002 $\pm$ 0.016	0.095	0.924
Age	0.033 $\pm$ 0.083	0.395	0.693
Horn length $\times$ year	0.006 $\pm$ 0.026	0.211	0.833
Horn length $\times$ age	0.279 $\pm$ 0.055	5.074	< 0.001
Age $\times$ year	−0.013 $\pm$ 0.007	1.938	0.053
Horn length $\times$ age $\times$ year	−0.016 $\pm$ 0.009	1.760	0.079

**Table 3** Effects of demographic parameters and age tested by a sequential model building approach on selection differentials on horn length of bighorn rams in 1988–1992 and 1996–2011, Ram Mountain, Canada. Horn length was standardized to zero mean and unit variance. Irrespective of their nonsignificance, random effects were kept in the model when testing for fixed effects to account for the nonindependence of observations across individuals and years.

	d.f.	log-L	$\chi^2$	$P$
Random effects				
Identity	17	−878	0	1
Year (HL year)	18	−878	0	1
Fixed effects				
HL $\times$ age $\times$ male age structure	20	−880	3.425	0.064
HL $\times$ age $\times$ breeder sex ratio	20	−879	1.962	0.161
HL $\times$ age $\times$ $N$ competitors	20	−881	6.253	0.012

HL, horn length; log-L, log-likelihood.

on selection differentials on mass were similar to those on horn length (Table S8). The links between sexual selection differentials and demographic parameters were not driven by the potential mathematical dependency between the dependent and independent variables because models included only the covariance between the trait and absolute breeding success, therefore not scaled by mean breeding success, led to the same conclusions (Tables 4 and S8).

## Discussion

Twenty-one years of detailed morphological and reproductive fitness measurements revealed that sexual selection on horn length and body mass of bighorn rams varied according to demography. Sexual selection on horn length and body mass was directional, positive, and varied in strength but not in direction. Sexual selection also affected young rams that likely used alternative mating tactics. More importantly, we found that sexual selection was affected by the number of

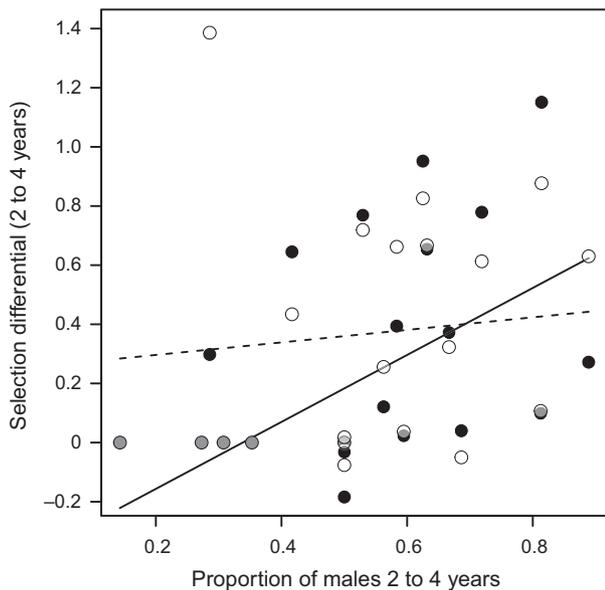
**Table 4** Demographic effects on the intensity of sexual selection on horn length of bighorn rams of (a) all ages, (b) 2 to 4 years and (c) 5 years and older. Covariance refers to the annual linear selection differentials estimated on absolute reproductive success and standardized values of a character. Selection events in 1988–1992 and 1996–2011, Ram Mountain, Alberta, Canada. Variables in bold were retained in the final models.

Variable	Estimate $\pm$ SE	$t$	$P$
(a) All ages			
Selection differential			
Intercept	<b>0.854 <math>\pm</math> 0.076</b>	<b>11.201</b>	<b>&lt; 0.001</b>
$N$ competitors	<b>0.252 <math>\pm</math> 0.076</b>	<b>3.337</b>	<b>0.003</b>
Male age structure	0.146 $\pm$ 0.099	1.476	0.157
Breeder sex ratio	0.058 $\pm$ 0.082	0.712	0.486
Covariance			
Intercept	<b>1.038 <math>\pm</math> 0.112</b>	<b>9.263</b>	<b>&lt; 0.001</b>
$N$ competitors	<b>0.327 <math>\pm</math> 0.111</b>	<b>2.940</b>	<b>0.008</b>
Male age structure	−0.017 $\pm$ 0.155	0.108	0.916
Breeder sex ratio	0.111 $\pm$ 0.123	0.909	0.376
(b) 2 to 4 years			
Selection differential			
Intercept	<b>0.231 <math>\pm</math> 0.062</b>	<b>3.753</b>	<b>0.001</b>
$N$ competitors	0.057 $\pm$ 0.072	0.793	0.439
Male age structure	<b>0.221 <math>\pm</math> 0.071</b>	<b>3.130</b>	<b>0.006</b>
Breeder sex ratio	0.073 $\pm$ 0.073	0.995	0.333
Covariance			
Intercept	<b>0.730 <math>\pm</math> 0.245</b>	<b>2.976</b>	<b>0.008</b>
$N$ competitors	0.360 $\pm$ 0.277	1.299	0.210
Male age structure	<b>0.769 <math>\pm</math> 0.281</b>	<b>2.733</b>	<b>0.013</b>
Breeder sex ratio	0.252 $\pm$ 0.290	0.869	0.397
(c) 5 years and older			
Selection differential			
Intercept	0.860 $\pm$ 0.679	1.267	0.221
$N$ competitors	1.233 $\pm$ 0.648	1.903	0.074
Male age structure	−0.206 $\pm$ 0.634	0.324	0.750
Breeder sex ratio	1.187 $\pm$ 0.640	1.856	0.080
Covariance			
Intercept	0.779 $\pm$ 0.308	2.530	0.021
$N$ competitors	0.259 $\pm$ 0.318	0.815	0.426
Male age structure	−0.294 $\pm$ 0.303	0.971	0.346
Breeder sex ratio	0.423 $\pm$ 0.290	1.456	0.163

competitors, but not by the breeder sex ratio or age structure.

## Variation in strength of selection

Of the standardized selection differentials for horn length and body mass estimated on all ages, 76% were higher than 0.5 and 62% were higher than 0.75, indicating very strong selection (Kingsolver *et al.*, 2001). The median selection differentials on horn length and body mass were 0.84 and 0.89, or 4 and 4.2 times stronger than the absolute median directional selection differential of 0.21 for fecundity selection estimated on the number of offspring produced in Hereford *et al.*



**Fig. 2** Positive relationship between annual selection differentials on horn length (black dots) and body mass (clear dots) for bighorn sheep rams aged two to four years and their proportion among all adult rams. Grey dots indicate overlapping black and clear dots. Solid and dashed lines are the predictions of the models presented in the text and Table S8, respectively, for horn length and body mass. Selection events in 1988–1992 and 1996–2011, Ram Mountain, Alberta, Canada.

(2004). Changes in direction of selection can influence evolution over the long term. Fluctuations in direction may maintain diversity by sequentially selecting for different phenotypic optima. These fluctuations, typical of survival selection (Siepielski *et al.*, 2011), may weaken or prevent evolutionary responses (Siepielski *et al.*, 2009; Kingsolver & Diamond, 2011). In bighorn sheep, however, we found consistent sexual selection favouring long-horned and heavy rams. There was no clear evidence of variation in the intensity of sexual selection over 21 years.

Although we had over 20 years of data on a population where all individuals are monitored from birth to death, in some years we had a limited sample size. Sampling error from small sample sizes may artificially inflate variation in selection estimates (Kingsolver *et al.*, 2001; Kingsolver & Diamond, 2011; Siepielski *et al.*, 2011; Morrissey & Hadfield, 2012). This highlights the burden of stochasticity on selection analyses for studies which cannot monitor entire populations, and suggests that small sample size could be negatively associated with selection intensity. In our study, all animals were marked and the sample size directly reflects the number of competitors in the population. Its relationship with selection intensity was positive. In the last years of monitoring, some standardized selection differentials on horn length and body mass for males five years and

older were negative (Table S5, Fig. S3). In 2008 and 2010, two rams aged five years achieved the highest reproductive success despite being relatively small-horned and light: each obtained three paternities for a relative reproductive success of 5 and 6.3. These exceptional events may have been partly attributable to the small population size in the last decade of study (Table S1).

### Demographic effects on selection and age dependence

By measuring sexual selection over twenty-one mating seasons, we tested whether its strength was affected by demographic factors likely to influence mating competition: mean mating opportunities per ram approximated by the breeding sex ratio, encounter rate among competitors approximated by their number and age structure approximating changes in the relative competitiveness of males. The importance of the number of competitors is controversial as it has been found to increase (Tomkins & Brown, 2004; Mobley & Jones, 2007), decrease (Jirotkul, 1999; Pomfret & Knell, 2008) or have no effect on sexual selection (Head *et al.*, 2007; Wacker *et al.*, 2013). We provide empirical evidence in nature for adult males of all ages that intrasexual selection intensity on horn length increases with the number of competitors: differences in phenotypic characters played a stronger role in mating success as the number of competitors increased (consistently with Tomkins & Brown, 2004; and Punzalan *et al.*, 2010; Mobley & Jones, 2007). Bighorn rams use alternative mating tactics that have different siring probabilities (Hogg & Forbes, 1997). The number of top-ranked rams that can use the tending tactic is constrained by the number of ewes that are in oestrus at the same time. In most days during the rut, there are only 1–3 ewes in oestrus, so that all but the top 1–3 rams in the hierarchy are forced to use alternative mating tactics. Our results suggest that an increase in the number of competitors makes breeding competition more difficult to sustain for small rams with short horns than for large rams with long horns. Horn size and body mass are associated with social rank (Pelletier & Festa-Bianchet, 2006; Martin *et al.*, 2013). An increase in the number of rams mostly reflects an increase in the number of subordinates that resort to alternative tactics, likely reducing access to oestrous females for weakly competitive rams. The sex-specific reproductive biology of bighorn sheep, with male reproductive success mainly affected by competition over access to oestrous ewes, justifies our test of the relationship between the number of competitors and selection differentials experienced by rams. Population size, however, might also correlate with the intensity of sexual selection. A comparison of models revealed that the number of competitors and population size were equivalent predictors of variation in

selection intensity (see results in Appendix S1). Thus, although we suggest that the number of competitors is more biologically meaningful in our study system, we cannot exclude that the effects we found might be partially driven by fluctuation in population size, these two variables being closely correlated (number of competitors—population size:  $r = 0.95$ ,  $P < 0.001$ ).

Most studies in nature have disregarded how age influenced sexual selection experienced by individuals. As a consequence, determinants of breeding success or drivers of sexual selection for young males remained poorly known, and are often attributed to random consequences of sperm competition (Hogg & Forbes, 1997). For instance, sexual selection was only detected for rams seven years and older in the study population in 1994–1999 (Coltman *et al.*, 2002), a restricted period which reduced the power to detect sexual selection in young rams. Our study of the demography and age dependence of selection revealed the conditions under which young males may also experience weak but significant sexual selection on horn length and body mass. The coursing tactic leads to scramble competition in which the number of mates shared by males two to four years depends upon the effectiveness of mate defence by older and more competitive males. Facing a decreasing proportion of older competitors, males up to age four obtained an increasing proportion of mates, mirrored by increasing selection differentials for young males. The lightest young rams with the smallest horns were less competitive as the number of coursing rams increased, when young rams with relatively longer horns and heavier mass achieved high relative reproductive success. For rams 5 years and older, however, no demographic parameter influenced selection intensity on horn length or body mass. This result suggests that mating competition among tending rams is independent of demographic parameters.

### Selection by hunting relative to intrasexual selection

Our results help to understand how selective harvesting may perturb phenotypic evolution. The strong and consistent positive linear selection on horn length, with an estimated heritability  $h^2$  of  $0.39 \pm 0.13$  (Coltman *et al.*, 2005), is expected to lead to an increase of horn length, or counterbalance potential negative selection on genetically correlated characters (Lande & Arnold, 1983). Horn length and its breeding values, however, decreased while our study population was under unrestricted harvesting of rams with large horns (Pigeon *et al.*, 2016). In addition to a direct negative effect on the evolution of secondary sexual characters, unrestricted selective harvesting may decrease the number of competitors and hence indirectly diminish sexual selection on horn length and body mass. The number of males involved in mating competition was

also a key factor affecting population dynamics in Milner *et al.* (2007)'s model of how selective harvesting may affect population growth rate, highlighting that the number of males is a demographic parameter with major importance in both ecological and evolutionary processes.

### Conclusion

This study sought to identify the ecological drivers of sexual selection to explain its temporal variation and to further our knowledge of how it operates in nature. We explored how demographic parameters influenced the dynamics of sexual selection on horn length and body mass in a polygynous and dimorphic species in natural conditions. We provide evidence that an increasing number of competitors intensify sexual selection on horn length, by making competition less sustainable for smaller males. Finally, we show that young males may experience sexual selection and their reproductive success is not just a random consequence of sperm competition. Our study is one of the few documenting how ecological variables affect sexual selection. It emphasizes the value of detailed long-term monitoring to couple ecological and evolutionary processes to obtain insights into phenotypic evolution in nature. Understanding the importance of ecological drivers in the dynamics of selective processes is an important but challenging step towards understanding how ecological drivers shape phenotypic evolution in nature.

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### Conflict of interest

The authors declared that they have no conflict of interest.

### References

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.

- Aronsen, T., Berglund, A., Mobley, K.B., Ratikainen, I.I. & Rosenqvist, G. 2013. Sex ratio and density affect sexual selection in a sex-role reversed fish. *Evolution* **67**: 3243–3257.
- Atkins, D.C., Baldwin, S., Zheng, C., Gallop, R.J. & Neighbors, C. 2012. A tutorial on count regression and zero-altered count models for longitudinal substance use data. *Psychol. Addict. Behav.* **27**: 166–177.
- Bates, D., Maechler, M. & Bolker, B. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. <http://CRAN.R-project.org/package=lme4>.
- Bell, G. 2010. Fluctuating selection: the perpetual renewal of adaptation in variable environments. *Phil. Trans. R. Soc. B* **365**: 87–97.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. *et al.* 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* **24**: 127–135.
- Bonenfant, C., Pelletier, F., Garel, M. & Bergeron, P. 2009. Age-dependent relationship between horn growth and survival in wild sheep. *J. Anim. Ecol.* **78**: 161–171.
- Calsbeek, R. & Cox, R.M. 2010. Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* **465**: 613–616.
- Charlesworth, B. 2000. Fisher, Medawar, Hamilton and the evolution of aging. *Genetics* **156**: 927–931.
- Chenoweth, S.F. & Blows, M.W. 2005. Contrasting mutual sexual selection on homologous signal traits in *Drosophila serrata*. *Am. Nat.* **165**: 281–289.
- Coltman, D.W., Festa-Bianchet, M., Jorgenson, J.T. & Strobeck, C. 2002. Age-dependent sexual selection in bighorn rams. *Proc. R. Soc. B* **269**: 165–172.
- Coltman, D.W., O'Donoghue, P., Hogg, J.T. & Festa-Bianchet, M. 2005. Selection and genetic (co)variance in bighorn sheep. *Evolution* **59**: 1372–1382.
- Cornwallis, C.K. & Uller, T. 2010. Towards an evolutionary ecology of sexual traits. *Trends Ecol. Evol.* **25**: 145–152.
- Crowley, P.H., Travers, S.E., Linton, M.C., Cohn, S.L., Sih, A. & Sargent, R.C. 1991. Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. *Am. Nat.* **137**: 567–596.
- Emlen, S.T. & Oring, L.W. 1977. Ecology, sexual selection, and the evolution of mating systems. *Science* **197**: 215–223.
- Endler, J.A. 1986. *Natural Selection in the Wild*. Princeton University Press, New Jersey.
- Festa-Bianchet, M. 2012. The cost of trying: weak interspecific correlations among life-history components in male ungulates. *Can. J. Zool.* **90**: 1072–1085.
- Festa-Bianchet, M., King, W.J., Jorgenson, J.T., Smith, K.G. & Wishart, W.D. 1996. The development of sexual dimorphism: seasonal and lifetime mass changes in bighorn sheep. *Can. J. Zool.* **74**: 330–342.
- Festa-Bianchet, M., Jorgenson, J.T. & Réale, D. 2000. Early development, adult mass, and reproductive success in bighorn sheep. *Behav. Ecol.* **11**: 633–639.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford, UK.
- Forslund, P. & Pärt, T. 1995. Age and reproduction in birds - hypotheses and tests. *Trends Ecol. Evol.* **10**: 374–378.
- Funk, D.J., Nosil, P. & Etges, W.J. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proc. Natl. Acad. Sci. USA* **103**: 3209–3213.
- Gaillard, J.-M., Festa-bianchet, M. & Yoccoz, N.G. 1998. Population dynamics of large herbivores: variable recruitment with constant adult survival. *Trends Ecol. Evol.* **13**: 58–63.
- Gosden, T.P. & Svensson, E.I. 2008. Spatial and temporal dynamics in a sexual selection mosaic. *Evolution* **62**: 845–856.
- Grant, P.R. & Grant, B.R. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* **296**: 707–711.
- Gross, M. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* **11**: 92–98.
- Hamilton, W.D. 1966. The moulding of senescence by natural selection. *J. Theor. Biol.* **12**: 12–45.
- Head, M.L., Lindholm, A.K. & Brooks, R. 2007. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. *Evolution* **62**: 135–144.
- Hereford, J., Hansen, T.F. & Houle, D. 2004. Comparing strengths of directional selection: how strong is strong? *Evolution* **58**: 2133–2143.
- Hogg, J.T. 1984. Mating in bighorn sheep: multiple creative male strategies. *Science* **225**: 526–529.
- Hogg, J.T. 1987. Intrasexual competition and mate choice in Rocky Mountain bighorn sheep. *Ethology* **75**: 119–144.
- Hogg, J.T. & Forbes, S.H. 1997. Mating in bighorn sheep: frequent male reproduction via a high-risk “unconventional” tactic. *Behav. Ecol. Sociobiol.* **41**: 33–48.
- Hubbell, S.P. & Johnson, L.K. 1987. Environmental variance in lifetime mating success, mate choice, and sexual selection. *Am. Nat.* **130**: 91–112.
- Jirotkul, M. 1999. Population density influences male-male competition in guppies. *Anim. Behav.* **58**: 1169–1175.
- Jones, A.G., Arguello, J.R. & Arnold, S.J. 2002. Validation of Bateman's principles: a genetic study of sexual selection and mating patterns in the rough-skinned newt. *Proc. R. Soc. B* **269**: 2533–2539.
- Jorgenson, J.T., Festa-Bianchet, M. & Wishart, W.D. 1993. Harvesting bighorn ewes: consequences for population-size and trophy ram production. *J. Wildl. Manag.* **57**: 429–435.
- Kingsolver, J.G. & Diamond, S.E. 2011. Phenotypic selection in natural populations: what limits directional selection? *Am. Nat.* **177**: 346–357.
- Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hoang, A. *et al.* 2001. The strength of phenotypic selection in natural population. *Am. Nat.* **157**: 245–261.
- Klug, H., Heuschele, J., Jennions, M.D. & Kokko, H. 2010. The mismeasurement of sexual selection. *J. Evol. Biol.* **23**: 447–462.
- Kokko, H. & Monaghan, P. 2001. Predicting the direction of sexual selection. *Ecol. Lett.* **4**: 159–165.
- Kokko, H. & Rankin, D.J. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Phil. Trans. R. Soc. B* **361**: 319–334.
- Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* **11**: 404–408.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Marshall, T.C., Slate, J., Kruuk, L.E.B. & Pemberton, J.M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* **7**: 639–655.
- Martin, J.G.A. & Pelletier, F. 2011. Measuring growth patterns in the field: Effects of sampling regime and methods on standardized estimates. *Can. J. Zool.* **89**: 529–537.

- Martin, A.M., Presseault-Gauvin, H., Festa-Bianchet, M. & Pelletier, F. 2013. Male mating competitiveness and age-dependent relationship between testosterone and social rank in bighorn sheep. *Behav. Ecol. Sociobiol.* **67**: 919–928.
- Medawar, P.B. 1952. *Unsolved Problem of Biology*. H. K. Lewis, London, UK.
- Mills, S.C., Grapputo, A., Koskela, E. & Mappes, T. 2007. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proc. R. Soc. B* **274**: 143–150.
- Milner, J.M., Nilsen, E.B. & Andreassen, H.P. 2007. Demographic side effects of selective hunting in ungulates and carnivores. *Conserv. Biol.* **21**: 36–47.
- Mobley, K.B. & Jones, A.G. 2007. Geographical variation in the mating system of the dusky pipefish (*Syngnathus floridae*). *Mol. Ecol.* **16**: 2596–2606.
- Morrissey, M.B. & Hadfield, J.D. 2012. Directional selection in temporally replicated studies is remarkably consistent. *Evolution* **66**: 435–442.
- Pelletier, F. & Festa-Bianchet, M. 2006. Sexual selection and social rank in bighorn rams. *Anim. Behav.* **71**: 649–655.
- Pelletier, F., Bauman, J. & Festa-Bianchet, M. 2003. Fecal testosterone in bighorn sheep (*Ovis canadensis*): behavioural and endocrine correlates. *Can. J. Zool.* **81**: 1678–1684.
- Pigeon, G., Festa-Bianchet, M., Coltman, D.W. & Pelletier, F. 2016. Intense selective hunting leads to artificial evolution in horn size. *Evol. Appl.* **9**: 521–530.
- Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. & the R Development Core Team 2011. *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-102.
- Pomfret, J.C. & Knell, R.J. 2008. Crowding, sex ratio and horn evolution in a South African beetle community. *Proc. R. Soc. B* **275**: 315–321.
- Punzalan, D., Rodd, F.H. & Rowe, L. 2010. Temporally variable multivariate sexual selection on sexually dimorphic traits in a wild insect population. *Am. Nat.* **175**: 401–414.
- R Development Core Team 2011. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL <http://www.R-project.org/>.
- Rieseberg, L.H., Widmer, A., Armtz, A.M. & Burke, J.M. 2002. Directional selection is the primary cause of phenotypic diversification. *Proc. Natl. Acad. Sci. USA* **99**: 12242–12245.
- Robinson, M.R., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. & Kruuk, L.E.B. 2008. Environmental heterogeneity generates fluctuating selection on a secondary sexual trait. *Curr. Biol.* **18**: 751–757.
- Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science* **323**: 737–741.
- Siepielski, A.M., Dibattista, J.D. & Carlson, S.M. 2009. It's about time: the temporal dynamics of phenotypic selection in the wild. *Ecol. Lett.* **12**: 1261–1276.
- Siepielski, A.M., DiBattista, J.D., Evans, J.A. & Carlson, S.M. 2011. Differences in the temporal dynamics of phenotypic selection among fitness components in the wild. *Proc. R. Soc. B* **278**: 1572–1580.
- Tomkins, J.L. & Brown, G.S. 2004. Population density drives the local evolution of a threshold dimorphism Joseph. *Nature* **431**: 1099–1103.
- Wacker, S., Mobley, K., Forsgren, E., Myhre, L.C., de Jong, K. & Amundsen, T. 2013. Operational sex ratio but not density affects sexual selection in a fish. *Evolution* **67**: 1937–1949.
- West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.* **58**: 155–183.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

## Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

### Appendix S1 Results

**Table S1** Demography of bighorn sheep at Ram Mountain, Alberta, Canada, during breeding seasons in 1988–1992 and 1996–2011.

**Table S2** Effect of (a) horn length and (b) body mass on reproductive success of bighorn rams for 1988–1992 and 1996–2011, Ram Mountain, Canada.

**Table S3** Linear sexual selection differentials on body mass estimated for bighorn rams of all ages, young (2 to 4 years) and mature (5 years and older) in 1988–1992 and 1996–2011, Ram Mountain, Canada.

**Table S4** Linear (*i*) and non-linear (*j*) selection differentials on horn length and body mass of bighorn rams for 1988–1992 and 1996–2011, Ram Mountain, Canada.

**Table S5** Annual linear selection differentials *i* on horn length and body mass of bighorn rams of all ages, 2 to 4 years, and 5 years and older in 1988–1992 and 1996–2011, Ram Mountain, Canada.

**Table S6** Test of annual variation of the standardized selection differential *i* on body mass for 1988–1992 and 1996–2011, Ram Mountain, Canada.

**Table S7** Effects of demographic parameters and age tested by a sequential model building approach on selection differentials on body mass of bighorn rams in 1988–1992 and 1996–2011, Ram Mountain, Canada.

**Table S8** Demographic effects on the intensity of sexual selection on body mass of bighorn rams of (a) all ages, (b) 2 to 4 years, and (c) 5 years and older.

**Figure S1** Number of competing rams, male age structure, and breeder sex ratio over twenty-one years for bighorn sheep at Ram Mountain, Alberta.

**Figure S2** Age-dependence of annually standardized body mass and breeding success for bighorn rams, 1988–1992 and 1996–2011, Ram Mountain, Alberta, Canada.

**Figure S3** Annual linear selection differentials *i* on a) horn length and b) body mass of bighorn rams of all ages (dark grey), 2 to 4 years (light grey), and 5 years and older (black).

**Figure S4** Age-specific linear selection differentials on horn length and body mass of bighorn rams at Ram Mountain, Canada, increase with age.

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