

Male mating competitiveness and age-dependent relationship between testosterone and social rank in bighorn sheep

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Abstract In males, the acquisition and development of behavioral and morphological secondary sexual traits typically depends on testosterone and correlates with mating success. Testosterone level could affect competition for mates and thus be a target of sexual selection. We sought to relate testosterone levels to male mating competitiveness, by teasing apart the relationships between testosterone, behavior, and growth before the mating period. We monitored 24 adult bighorn rams (*Ovis canadensis*) at Ram Mountain, Alberta, from 2008 to 2011. Using linear mixed models, we tested the relationships between testosterone metabolites in feces, social rank, and both growth and size of two sexually selected traits: horns and body mass. The correlation between testosterone and social rank varied with age. Testosterone and rank were weakly and negatively correlated for young rams, positively correlated for prime-aged rams, and negatively correlated for older rams. Although testosterone had an increasingly positive effect on total horn length until 8 years of age, we could not detect any effects on annual growth rate of horns or body mass. Testosterone may be related to male’s ability to compete for mates through its relationship with behaviors determining social rank, rather than by influencing the development of morphological traits. Differences in

testosterone levels among competitors may be a proximate cause of variance in fitness.

Keywords Androgen · Ontogeny · *Ovis canadensis* · Secondary sexual traits · Sexual selection · Social hierarchy

Introduction

In polygynous species, some male armaments and ornaments such as horns, antlers, and colors are under sexual selection as they determine access to mates and affect reproductive success (Le Boeuf 1974; Andersson 1994; Coltman et al. 2002; Mainguy et al. 2009). Expression of these traits is often associated with the androgen testosterone (Becker and Breedlove 2002). For example, in birds testosterone correlates with the length of ornamental tail feathers (McGlothlin et al. 2008), and affects plumage patterns (but see Kimball and Ligon 1999; Gonzalez et al. 2001; Strasser and Schwabl 2004; Garamszegi et al. 2005). In mammals, testosterone increases muscle volume and body mass (Kousteni et al. 2001, 2002; Hartgens and Kuipers 2004). Inter-individual differences in testosterone levels during the growing season could therefore influence the development of sexually selected traits, and be under sexual selection.

Testosterone also influences sexually selected behaviors (Becker and Breedlove 2002), by promoting aggression and territoriality (McGlothlin et al. 2007; Hau and Beebe 2011; Pasch et al. 2011), courtship (Pinxten et al. 2003), dominance displays, and mate searching (Mills et al. 2009). The relationship between testosterone levels and mating behaviors, however, may not be universal. For instance, no correlation was found between testosterone and social rank in Himalayan tahr males (*Hemitragus jemlahicus*, Lovari et al. 2009). Alternatively, the “challenge hypothesis” (Wingfield et al. 1990) predicts that testosterone levels can rise in

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response to competition for mates. The meta-analysis of Hirschenhauser and Oliveira (2006), however, provided no clear evidence of an effect of male–male agonistic interactions on variation in testosterone (see also Apfelbeck and Goymann 2011).

High testosterone levels can promote competitiveness despite their potential cost (Folstad and Karter 1992). In several vertebrates, testosterone was associated with immunodeficiency (Zuk 1996). For instance, immunocompetence and resistance to protozoan infection were associated with increased testosterone and aggressive behaviors in mice (*Mus musculus*, Barnard et al. 1996; Smith et al. 1996). Skarstein et al. (2001) showed that reproductively active male arctic charr (*Salvelinus alpinus*) had higher intensities of macroparasite infections than non-reproductive males, apparently because of immune suppression related to the development of ornaments and to spermatogenesis. Males therefore appear to face trade-offs between physiological and morphological traits that may affect their fitness. A physiological strategy involving elevated testosterone only during the mating season may reduce the cost of testosterone-dependent immunosuppression.

Testosterone, however, can also enhance growth of body mass (Hartgens and Kuipers 2004) and armaments (Bartoš et al. 2009). Low levels outside the rut could thus reduce growth of sexually selected traits. To understand its importance in mediating life history trade-offs, it is thus important to monitor testosterone outside the breeding season and to evaluate its effects on age-specific growth of sexually selected traits. Age must be taken into account because the growth of these traits is strongly affected by age (Bonenfant et al. 2009; Festa-Bianchet 2012).

Although numerous studies investigated the links between morphology, behavior, and testosterone, our understanding of the importance of this hormone in shaping life history trade-offs in wild animals remains limited. That is partly because no study obtained repeated measurements of testosterone, morphological and behavioral traits of known-age wild animals. Here, we sought to quantify the relationships between fecal testosterone levels, the establishment of social rank, horn growth, and mass gain in wild bighorn rams (*Ovis canadensis*).

Bighorn sheep are highly sexually dimorphic (Leblanc et al. 2001), polygynous, and rams can live up to 14 years. Horn length and body mass are correlated with breeding success for rams aged 6 years and older (Coltman et al. 2002). Social rank increases with both age and mass, which are strongly correlated, especially for young adults (Pelletier and Festa-Bianchet 2006). Horns grow from spring to autumn (Leblanc et al. 2001), while rams establish a social hierarchy (Pelletier and Festa-Bianchet 2006). During the rut in late November and early December, rams use rank-specific reproductive tactics. Dominant males use a mate-guarding tactic called tending (Hogg 1984). Tending leads to many paternities for rams in the top 1–4 ranks of the hierarchy, but up to 40 % of lambs are

fathered by subordinates (Coltman et al. 2002) that use alternative tactics (Hogg 1984). Little is known about the determinants of breeding success for subordinate rams (Coltman et al. 2002). In the Sheep River population, testosterone was positively correlated with social rank during the rut (Pelletier et al. 2003).

Given the importance of horns, body mass, and social rank for mating success of bighorn rams, we predicted that testosterone levels should be positively related to social rank outside the breeding season. We also expected that testosterone would be positively correlated with horn growth and body mass gain. As morphological and behavioral sexually selected traits vary with age, we hypothesized an age-dependent relationship between testosterone and mating competitiveness. Because growth rates decrease with age (Festa-Bianchet et al. 1996; Bonenfant et al. 2009), we predicted that the positive relationship between testosterone and horn growth, body mass gain, and social rank should be stronger in young than in old males.

Material and methods

Study population

Bighorn sheep on Ram Mountain (52°N, 115°W, elevation 1,080–2,170 m), Alberta, Canada, have been monitored since 1971. Sheep are captured in a corral trap baited with salt between mid-May and September (Jorgenson et al. 1993). All rams included in this study were marked during their first year, and their exact ages were known. The resighting rate of rams exceeds 95 % (Bonenfant et al. 2009), providing a very accurate measure of survival. We only considered rams aged 2 years and older, as yearling rams appear unable to obtain paternities (Coltman et al. 2002).

Morphological parameters

We measured horns (millimeters), mass (kilograms), and testis (millimeters) at each capture. We measured horn base circumference (hereafter referred to as horn base) and horn length along the outer curvature. We standardized horn measurements to spring (June 5th) and autumn (September 15th, see “Statistical analysis” section for details) before analysis. Horns stop growing in winter and form a distinct annulus, under which the new horn growth becomes evident in the spring. We focused on the growth of the newly grown horn increment. We defined horn growth as the yearly increment grown from June 5th to September 15th, calculated as the difference in horn length between spring and autumn. We applied the same definition to quantify the growth of horn base. We obtained testis size by measuring the diameter of one testis using a caliper (± 0.5 mm). All

scrotal measurements were taken at the largest section of the scrotum for one testicle. This measure was taken four times at each capture to assess repeatability. The more frequent measure within 1 mm was recorded.

Social rank

Daily observations were conducted from June to September 2009 to 2011. Groups of males were observed until all rams disappeared from sight. All agonistic interactions were noted. We observed rams at a minimum distance of 400 m from 10 min to 6 h. We recorded six dyadic behaviors: eye rubbing, front kick, displacement, butt, homosexual mount, and clash (Geist 1971; Hogg 1987). Agonistic interactions were used to rank each ram according to de Vries (1995), a method previously used for bighorn rams (Pelletier and Festa-Bianchet 2006). We first tested the linearity of the hierarchy using the h' index (de Vries 1995), which ranges from 0 for no linearity to 1 for perfect linearity, corrected for the number of unknown dyadic relationships. As the linearity of social hierarchies was highly significant each year (Table 1), we then used an iterative procedure of 1,000 randomizations to find the optimal near-linear order of individuals (de Vries 1998). This procedure organizes the social hierarchy by minimizing first the number and then the strength of inconsistencies in dyadic matrices. An inconsistency occurs when individual j dominates i , but j is ranked below i . The strength of an inconsistency is the absolute difference between the ranks of the individuals involved. Predictability in the outcome of encounters between two individuals is measured by the directional consistency index (Van Hoof and Wensing 1987). This index ranges from 0 for dyads where each member wins half of the interactions to 1 when one individual wins all interactions. We standardized social ranks by the yearly number of rams as $1 - (\text{rank}/N_x)$ where N_x is the number of rams in year x (Pelletier and Festa-Bianchet 2006). All calculations relative to social hierarchy were performed with Matman 1.0 (de Vries et al. 1993).

Sample collection

Fecal samples were collected at each capture. We collected 174 fecal samples from 2008 to 2011 ($N=39, 50, 49, 36,$

respectively). On average, we sampled each individual 7.2 ± 3.8 times. We recorded the date and time of collection, kept samples at -15°C for about 10 days, then dried them at 70°C (see Montiglio et al. 2012) and re-froze them to minimize bacterial degradation during storage.

Hormone assay

We used methanol-based extraction for fecal testosterone (Brown et al. 2004). Samples were dried for 7 days at 50°C then ground to $\approx 5 \pm 0.5$ mm. We weighed feces in clean culture glass tubes (16×100 mm) to obtain 100 ± 10 mg per sample, then added 5 ml of 90 % methanol to each sample. Tubes were vortexed at 250 rpm at room temperature for 4 h and then at 400 rpm for 30 min. We filtered samples with a $0.45\text{-}\mu\text{m}$ non-sterile filter fixed on a syringe and stored at -20°C until we measured testosterone concentration.

We performed competitive enzyme-linked immunosorbent assay in triplicate for each extract using a 96-well microtiter plate (Nunc-Immuno, Maxisorp Surface; Fisher Scientific, Pittsburgh, PA, USA). The testosterone polyclonal antibody (R156/7, University of California, Davis, CA, USA) was diluted 1:10,000 in a commercial coating buffer (CB1, ImmunoChemistry Technologies, Bloomington, MN, USA), and 50 μl was added to each well, except the blank, and incubated for 18 h. The next morning, we blocked plates and rinsed them with wash buffer (distilled water with 0.2 % of Tween-20). To perform the assay, 50 μl of standards and samples was added to wells with the horseradish peroxidase conjugate (tracer; 1:150,000) provided with the kit and incubated for 1 h at room temperature on an orbital shaker. Standards (testosterone solution; Sigma-Aldrich, St. Louis, MO) were assayed in duplicates in a range of 78.125 to 10,000 pg/ml^{-1} in assay buffer (NaH_2PO_4 0.65 M, Na_2HPO_4 1.03 M, NaCl 0.15 M, pH 7.0). We washed plates five times, then 100 μl of fresh substrate buffer (40 mMABTS[2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] diammonium salt, 1.6 mM H_2O_2 , 0.05 M citric acid pH 4.0) was added to each well and incubated on an orbital shaker for 45 min. Absorbance was read at 405 nm with a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Cross-reactivity of the testosterone antibody with each steroid is testosterone 100.0 %, 5α -dihydrotestosterone, 7.4 %; androstenedione, 0.27 %; and androsterone, dehydroepiandrosterone, cholesterol, $17\text{-}\alpha$ estradiol, progesterone, and pregnenolone <0.05 % (Muir et al. 2001). Sensitivity of the testosterone polyclonal antibody is $0.1 \text{ pg/wells}^{-1}$ (Munro and Lasley 1988). Sample testosterone concentrations were calculated and expressed as a function of dry fecal mass (picograms per milligram). The assay was repeated for any sample in which triplicates differed by 20 % or greater.

Table 1 Descriptive statistics of dominance matrices of bighorn rams 2 years and older, from 2009 to 2011, Ram Mountain, Alberta, Canada

Year	Number of males	Number of interactions	% of dyads observed	h'	P	DC
2009	16	76	63.33	0.45	0.002	0.88
2010	13	37	47.44	0.47	0.017	0.89
2011	16	120	65.80	0.6	<0.001	0.92

h' Landau's corrected linearity index, DC directional consistency index

Assay validations

To validate assays (Buchanan and Goldsmith 2004), pooled pellets from five fecal samples were weighted and extracted together to test for parallelism with the standard curve. We also used this extract (diluted 1:25) as an inter-assay standard. The intra-assay coefficient of variation (CV) was calculated based on triplicates for each sample ($N=217$). To calculate repeatability, we measured testosterone for four individuals with nine independent replicates per individual. To estimate the efficacy of our extraction method, we repeated four serial extractions on the same samples for four individuals. The pool was serially diluted 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 to confirm parallelism with the standard curve. Mean intra- and inter-assay CV were 10.18 ± 1.55 and 7.68 ± 5.95 %, respectively. The efficiency of extraction was 77 ± 12 % for the first extraction. Repeatability was 94.51 % [confidence interval (CI), 0.826, 0.995; nine replicates per individual].

Statistical analysis

We adjusted horn size and body mass for capture date to account for growth during the summer. We first estimated individual daily growth rates by fitting a linear mixed effects model on repeated individual measurements taken throughout the sampling seasons (Martin and Pelletier 2011). Models included ram identity (intercept) and the square root of date (slope) as random effects, and age and square root of date as fixed effects to correct for the mean effects of age and date. The square-root transformation of date linearizes phenotypic changes over the summer (Festa-Bianchet et al. 1996). We included linear and quadratic terms of age to account for the asymptotic pattern of growth with age. Horn length, body mass, and testis diameters for all individuals were adjusted to the beginning (June 5th) and the end of the growing period (September 15th). We used these two dates, referred to as spring and autumn, for consistency with previous studies (Festa-Bianchet et al. 2000). The length of horn annual increments was the difference between autumn and spring horn lengths.

We tested the repeatability of our measurements for testis size and testosterone levels for the same individual using the package “ICC” (Wolak et al. 2012) with R version 2.14.1 (R Development Core Team 2011). To evaluate factors affecting individual testosterone levels, we fitted a model including date, age, a quadratic term for age, testis size, year, and the interactions between date and the two age terms, using log-normal models. We used a log-normal linear model because ram identity as random variable was not significant, with a Gaussian family and a log link to maximize homogeneity of residuals. We included year as a categorical fixed variable in all models because there were only four levels (Bolker et al. 2009).

We also fitted linear models to determine the factors affecting social rank, and growth in horn length and base circumference. Ram identity as random variable for these models was not significant according to a log-likelihood ratio test (Pinheiro and Bates 2000). We modeled total horn length with linear mixed models as ram identity was significant according to LRT. All models of morphological parameters included as explanatory variable age and age², mean testosterone level, year, and the interactions between mean summer level of testosterone and the two age terms. Models of social ranks included as explanatory variables age and age², mean testosterone level, year, and the interactions between mean summer testosterone and the two age terms. As ranks were standardized by the annual number of males, we did not include year effects in these models. We simplified each full model through backward stepwise deletion based on the significance of the variables (Zuur et al. 2008). We accompanied each deletion step with a log-likelihood ratio test to ensure that model fit was not reduced. All estimates of continuous variables were calculated on standardized variables, with zero means and unit standard deviations. All analyses were performed using R version 2.14.1 (R Development Core Team 2011). Linear mixed models were fitted using the “nlme” package (Pinheiro et al. 2012).

Results

Temporal variation in testosterone levels

Individual testosterone levels ranged from 6.63 to 229.87 pg/mg. Average level decreased over the summer (Table 2, Fig. 1) and was not associated with testis size or age (Table 2). Interactions between date and either age or age² were not significant (Table 2).

Effect of testosterone on social rank

Social rank was influenced by the interaction between testosterone and age (Table 3, Fig. 2). Testosterone had a positive association with rank for prime-aged rams, between about 4 and 7 years of age, while it had a weaker and possibly negative effect for both younger and older rams. Horn length had a positive effect on social rank.

Effect of testosterone on morphology

Repeatability of testis size was 0.99 (CI, 0.985, 0.994). After controlling for age, there was no effect of testosterone on horn growth (Table 4). Total horn length in autumn was affected by an interaction between testosterone and age: testosterone had increasingly positive effects on length as

Table 2 Factors affecting individual testosterone levels from June 5th to September 15th in bighorn sheep rams, Ram Mountain, Alberta, Canada

Variable		Estimate	SE	<i>t</i>	<i>P</i>
Intercept		4.541	0.048	95.051	<0.001
Date		-0.102	0.026	-3.973	<0.001
Years					
2009		-0.112	0.066	-1.706	0.090
2010		-0.418	0.077	-5.455	<0.001
2011		0.092	0.064	1.429	0.155
Age	(5)	-0.041	0.026	-1.592	0.113
Age ²	(4)	0.047	0.115	0.410	0.682
Testis size	(3)	0.138	0.071	1.926	0.056
Date×age	(2)	-0.008	0.025	-0.331	0.741
Date×age ²	(1)	0.095	0.113	0.846	0.399

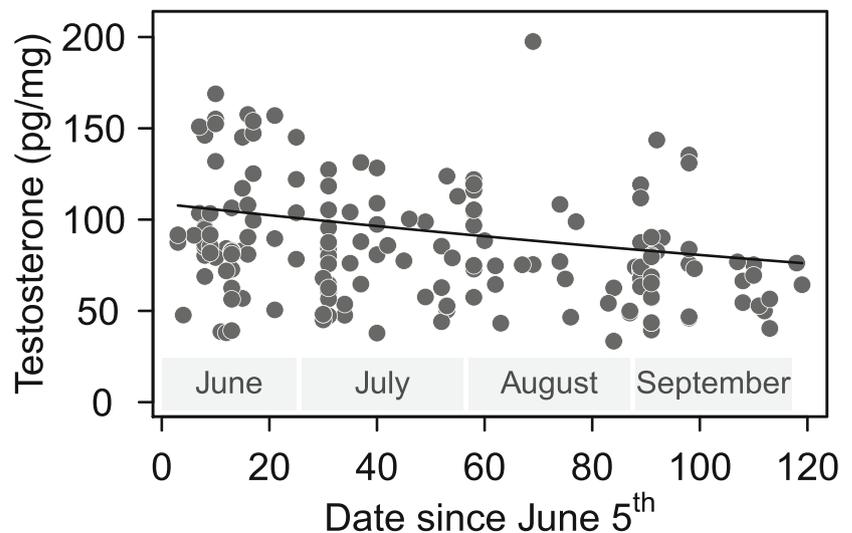
Estimates are from loglinear models including 169 observations of 23 rams in 2008–2011. Year 2008 was considered as the reference year. Ram identity as a random effect was excluded after testing its significance by log-likelihood ratio test (log-likelihood ratio=0.008, $P=0.928$). Variables in bold were retained in the final model. The order of deletion by backward simplification is in parenthesis. The final model explained 33.62 % of the deviance

individuals aged (Table 4, Fig. 3). Body mass appeared independent of testosterone levels. Because results for mass were similar to those for horn size, they are reported in (Table S1) the [Electronic supplemental material](#).

Discussion

Our analyses suggest that testosterone may be related to a male's ability to compete for mates through behaviors that determine social rank, rather than through development of morphological traits. As expected, we also found that the

Fig. 1 Decrease in testosterone levels over spring–summer (day 0 is May 25th) for bighorn rams on Ram Mountain, Alberta, Canada. The fitted line controls for year effects presented in Table 2

**Table 3** Effects of age, age², fecal testosterone levels, their interactions, and horn length in autumn on social rank from 2009 to 2011 in bighorn rams, Ram Mountain, Alberta, Canada

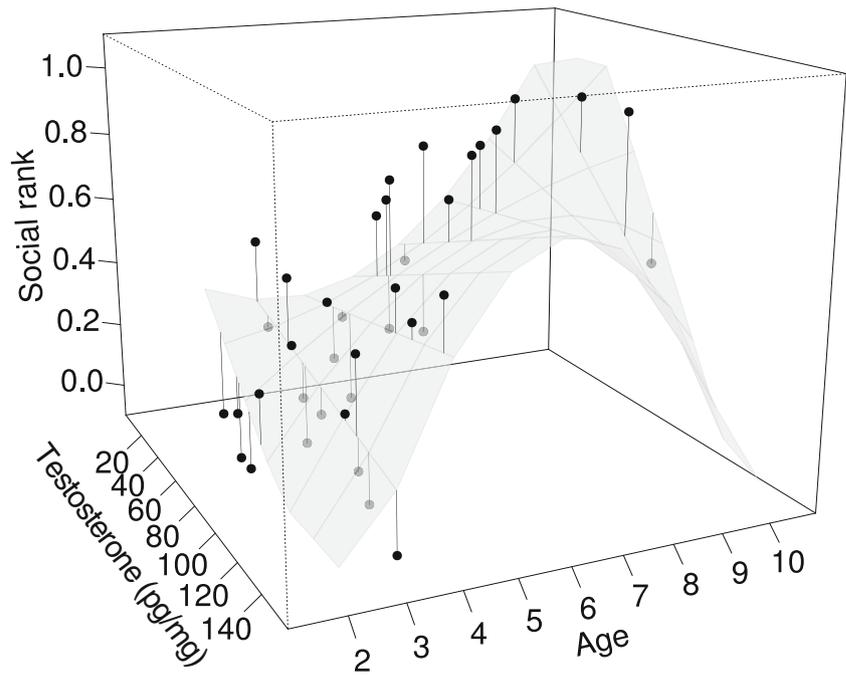
Fixed effects	Estimate	SE	<i>t</i>	<i>P</i>
Intercept	0.463	0.038	12.025	<0.001
Mean (<i>T</i>)	-0.036	0.034	-1.050	0.302
Age	0.273	0.226	1.211	0.235
Age ²	-0.175	0.167	-1.043	0.305
Horn length	0.214	0.080	2.667	0.012
Years				
2010	-0.158	0.060	-2.636	0.013
2011	-0.106	0.063	-1.690	0.101
Mean (<i>T</i>)×age	0.575	0.201	2.854	0.008
Mean (<i>T</i>)×age ²	-0.528	0.200	-2.636	0.013

Estimates are from a linear model including 39 observations of 20 rams in 2009–2011. Ram identity as a random effect was excluded after testing its significance by log-likelihood ratio tests (log-likelihood ratio=0.441, $P=0.506$). Adjusted $R^2=0.776$. Mean [*T*] is the mean fecal testosterone level. Year 2009 was considered as reference

relationship between testosterone and social rank varied with ram age. Testosterone had a positive correlation with rank for rams aged 4 to 7 years, but not for those younger or older. We could not detect any relationship between testosterone and horn growth, horn base circumference, or body mass. Testosterone, however, had an age-dependent correlation with horn length. By integrating hormonal, behavioral, and morphological measurements of marked known-age individuals over 3 years, our research underlines the complex roles of behavioral endocrinology in the development of fitness-related traits in wild long-lived mammals.

The positive association between testosterone and social rank for rams aged 4 to 7 years suggests that for prime-aged adults testosterone may be correlated with aggressiveness,

Fig. 2 Effects of age, testosterone, and their interaction on social rank of bighorn rams, 2009–2011, Ram Mountain, Alberta, Canada. The *grey surface* is the prediction of the model presented in Table 3. *Points* are raw data and are connected to their respective prediction on the surface



endurance, or other factors that improve ability to attain high rank. This is a key result because most of these rams are likely to use alternative reproductive tactics, which can account for as much as 40 % of paternities (Coltman et al. 2002). The age-dependent relationship between testosterone and social rank was 2.6 times stronger than that between testosterone and horn length. This result suggests that testosterone may mainly influence mating competitiveness through its relationship with social rank. That contention is supported by the correlation

between aggression rate and testosterone levels observed in rams from another population (Pelletier et al. 2003). The strong relationship between age, mass, and social rank found among young rams began to break down at about 6 years of age (Pelletier and Festa-Bianchet 2006), when age was no longer the predominant determinant of body mass. Consequently, some prime-aged individuals were able to outcompete some older conspecifics and increase their rank. The declining effect of testosterone on social ranks we observed beginning at about

Fig. 3 Effects of age, testosterone, and their interaction on horn length adjusted to September 15, 2008–2011, in bighorn rams, Ram Mountain, Alberta, Canada. The *grey surface* is the prediction of the final model presented in Table 4c. *Points* are raw data and are connected to their respective prediction on the surface

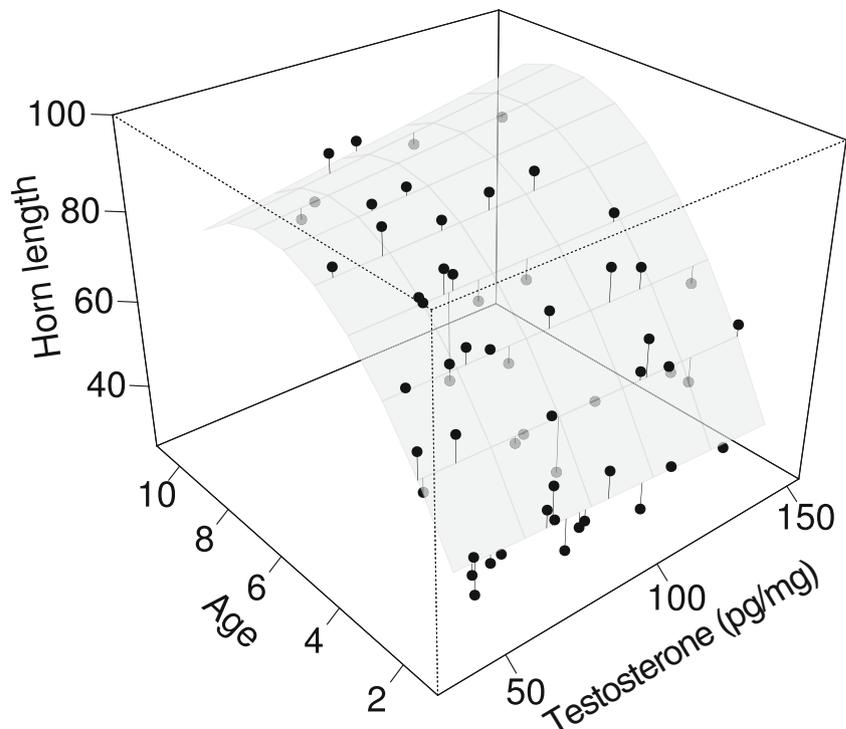


Table 4 Effects of year, fecal testosterone level, age, and their interaction on the growth of horn length and horn base from spring to autumn, and on annulus length and total horn length in bighorn rams, Ram Mountain, Alberta, Canada

Variable		Estimate	SE	<i>t</i>	<i>P</i>
(a) Horn length growth. Adjusted $R^2=0.618$ (LRT=2, $P=0.157$)					
Intercept		7.515	0.152	49.521	<0.001
Age		-1.492	0.153	-9.747	<0.001
Years					
2009	(5)	0.163	0.431	0.379	0.706
2010	(5)	0.115	0.418	0.275	0.785
2011	(5)	-0.823	0.420	-1.961	0.055
Mean (<i>T</i>)	(4)	-0.032	0.181	-0.177	0.860
Age ²	(3)	-0.065	0.713	-0.091	0.928
Mean (<i>T</i>)×age	(2)	0.120	0.198	0.609	0.545
Mean (<i>T</i>)×age ²	(1)	1.123	1.012	1.109	0.273
(b) Horn base growth. Adjusted $R^2=0.591$ (LRT=0.001, $P=0.999$)					
Intercept		3.965	0.053	75.092	<0.001
Age		-1.473	0.245	-6.013	<0.001
Age²		1.062	0.245	4.336	<0.001
Years					
2009	(4)	0.098	0.155	0.633	0.529
2010	(4)	-0.088	0.149	-0.588	0.559
2011	(4)	-0.211	0.154	-1.371	0.176
Mean (<i>T</i>)	(3)	-0.020	0.065	-0.313	0.755
Mean (<i>T</i>)×age	(2)	0.022	0.070	0.313	0.755
Mean (<i>T</i>)×age ²	(1)	0.291	0.361	0.804	0.425
(c) Horn length (LRT=33.235, $P<0.001$)					
Intercept		60.482	1.024	59.059	<0.001
Mean (<i>T</i>)		-0.077	0.331	-0.234	0.817
Age		46.137	1.777	25.968	<0.001
Age²		-30.527	1.884	-16.2	<0.001
Mean (<i>T</i>)×age		1.315	0.413	3.18	0.003
Years					
2009	(2)	0.061	1.045	0.058	0.954
2010	(2)	-0.058	1.294	-0.045	0.965
2011	(2)	-0.847	1.539	-0.551	0.586
Mean (<i>T</i>)×age ²	(1)	-3.197	2.333	-1.371	0.181

The significance of ram identity as a random effect was tested by log-likelihood ratio tests (LRT). Models (a) and (b) are linear, while (c) is a linear mixed model. Horn length was adjusted to September 15th. In model (c; Fig. 3), the proportion of deviance explained by random effects is 0.817 and that explained by fixed effects is 0.057. The order of deletion by backward simplification is in parenthesis. Variables in bold constitute the final model. The models included 59 observations of 23 rams in 2008–2011. Mean (*T*) is the mean faecal testosterone level. 2008 was considered as the reference year

age 7 may result from an increasing importance of morphology relative to testosterone in the acquisition of rank as rams age. Given the limited sample size of older individuals, however,

the apparently negative relationship between rank and testosterone for older rams must be interpreted cautiously.

Testosterone levels may affect social ranks, or testosterone may vary in response to social challenges as proposed by the “challenge hypothesis” (Wingfield et al. 1990). Given the dramatic effects of hormone levels on life histories, we did not manipulate individual hormone levels in our wild study population. Although path analysis (Shiple 2009) may circumvent the difficulty of establishing the direction of causality, our sample sizes precluded us from using it. The combination of our results with those of Pelletier and Festa-Bianchet (2006) can help address the unclear direction of causality between behavior and testosterone. Testosterone levels decreased from 110 to 75 pg/mg of feces following the June congregation of rams (Festa-Bianchet 1986) until late September, and Pelletier et al. (2003) showed that testosterone levels decreased from 60 to 15 pg/mg of feces between mid-September and mid-December. This pattern follows a general decline of testosterone levels with time, and suggests that rams exhibit the highest testosterone levels in spring, a critical period in the establishment of male social rank (Geist 1971).

Our study contrasts with earlier reports of testosterone effects on social rank (Schwarzenberger 2007), mostly from experimental studies that did not account for the possible effects of age (but see Pelletier et al. 2003) and morphology. In many sexually dimorphic mammals, age has a strong effect on the development of male secondary sexual traits (Pelletier and Festa-Bianchet 2006; Bergeron et al. 2010) that may be affected by testosterone in an age-dependent manner. Consequently, reported effects of testosterone on social rank may have been confounded by age and by the size of sexually selected traits. For example, Pelletier et al. (2003) did not find a relationship between testosterone and social rank of bighorn rams after accounting for age, likely because testosterone and age were correlated. In our study population, testosterone was not correlated with age or horn length; therefore, we were able to assess their relationships with social rank.

Based on the extensive literature on testosterone dependence of secondary sexual traits in vertebrates (e.g., Garamszegi et al. 2005; McGlothlin et al. 2008; Roberts et al. 2009; Gaspar-López et al. 2010), we expected positive correlations between testosterone and both horn growth and mass gain. In line with this prediction, testosterone had a weak but increasingly positive effect on total horn length as individuals aged. We did not, however, detect any effect of testosterone on annual horn growth. These conflicting results suggest that the age-dependent effects of testosterone on total horn length likely emerged from the positive association between social rank on the one hand, and testosterone levels and horn length on the other hand. This interpretation is supported by the very weak age-dependent effects size of testosterone in a model where fixed effects explained less than 6 % of the deviance (Table 4) and the

independence of testosterone and body mass, while mass is strongly correlated with total horn length. Malo et al. (2009) also reported testosterone independence of antler length and number of points in red deer (*Cervus elaphus*), although the diameter of the antler burr decreased as testosterone level increased. In bovids, keratin is the main component of horns and is produced by keratinocytes controlled by the epidermal growth factor (Tomlinson et al. 2004). This growth factor is itself under the control of androgens, the lactogenic hormone prolactin, and the stress hormones glucocorticoids (Hendry et al. 1999). Keratinization thus integrates short- and long-term environmental stimuli, and testosterone may have a limited influence on the amount of keratin proteins synthesized.

Testosterone levels fluctuate both seasonally and over the lifetime. A typical pattern involves seasonal covariation of sexual activity and testosterone (but see Lynch et al. 2002): testosterone rises before the breeding season, peaks during it, and then returns to its baseline level. This pattern arises from environmental cues including changes in photoperiod (Wingfield and Kenagy 1991; Bronson 2009), as shown for instance in lemurs (*Microcebus murinus*, Perret 1992), macaques (*Macaca mulatta*, Herndon et al. 1996), and feral sheep (*Ovis aries*, Lincoln and Ebling 1985). In several sheep breeds, Lincoln et al. (1990) found that testosterone levels increased with decreasing day length. In contrast, we found that testosterone in wild bighorn sheep decreased with decreasing day length after the summer solstice. Similar results were obtained for wild Alpine ibex (*Capra ibex*), where fecal testosterone decreased from 6.60 ± 3.55 pg/mg in late June to 3.18 ± 1.18 pg/mg in late August (Decristophoris et al. 2007). The seasonal decrease common to bighorn sheep and ibex may also explain the lack of correlation between rank and testosterone among wild male tahr sampled in late autumn (Lovari et al. 2009). The reason for the contrasting seasonal pattern between domestic and wild sheep remains to be investigated.

We explored the importance of age on testosterone effects on male social rank and on the development of sexually selected traits in a polygynous and highly sexually dimorphic species in natural conditions. We provided evidence that testosterone levels are related to mating competitiveness through sexual behaviors in an age-dependent manner, but not to growth of sexually selected traits. Our results therefore support the hypothesis that differences in testosterone among individuals could be a proximate cause of variance in reproductive success (Hillgarth et al. 1997), which is a necessary condition for selection (Arnold and Wade 1984). Our study emphasizes the need of long-term monitoring of wild populations and the importance of integrating endocrinology within a framework of evolutionary ecology to refine our understanding of sexual selection.

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Ethical standards This research project was approved by the Animal Care Committee of the Université de Sherbrooke (MFB2009-01 and FP2012-01), an affiliate of the Canadian Council on Animal Care.

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1 Table S1. Effects of testosterone levels, age and year on body mass of bighorn sheep
 2 rams, adjusted to spring (June 5th) and autumn (September 15th), Ram Mountain, Alberta,
 3 Canada. Estimates are from linear mixed models with ram identity as random effects. The
 4 order of deletion by backward simplification is indicated in parenthesis. Variables in bold
 5 were retained in the final model. The models included 59 observations of 23 rams during
 6 2008-2011. Mean [T] is the mean faecal testosterone metabolites level. Year 2008 was
 7 considered as reference.
 8

Variable		Estimate	SE	<i>t</i>	<i>P</i>
a) Spring body mass. Proportion of deviance explained by random effects is 0.845 (LRT=37.050, <i>P</i> <0.0001). Proportion of deviance explained by fixed effects is 0.148					
Intercept		67.821	1.485	45.680	<0.001
Age		41.851	2.300	18.192	<0.001
Age²		-28.285	2.467	-11.463	<0.001
Years : 2009	(4)	1.415	1.366	1.036	0.308
2010	(4)	1.779	1.662	1.07	0.293
2011	(4)	1.072	2.117	0.506	0.616
Mean [T]	(3)	0.105	0.542	0.194	0.848
Mean [T] × Age	(2)	1.013	0.572	1.771	0.087
Mean [T] × Age ²	(1)	-1.239	3.109	-0.399	0.693

b) Autumn body mass. Proportion of deviance explained by random effects is 0.884
(LRT=44.428, *P*<0.001). Proportion of deviance explained by fixed effects is 0.205

Intercept		90.540	1.838	49.248	<0.001
Age		41.892	2.455	17.061	<0.001
Age²		-27.482	2.651	-10.366	<0.001
Mean [T]	(4)	-0.083	0.449	-0.186	0.854
Years : 2009	(3)	1.314	1.528	0.860	0.397
2010	(3)	2.447	2.021	1.210	0.236
2011	(3)	1.256	2.555	0.492	0.627
Mean [T] × Age	(2)	0.798	0.616	1.294	0.206
Mean [T] × Age ²	(1)	-1.267	3.359	-0.377	0.709

c) **Mass gain.** Proportion of deviance explained by random effects is 0.537

(LRT=13.081, $P<0.001$). No deviance explained by fixed effects.

Intercept		22.715	0.429	52.928	0.000
Age	(6)	0.548	0.322	1.700	0.098
Mean [T]	(5)	0.113	0.213	0.531	0.599
Age ²	(4)	-0.282	1.199	-0.235	0.815
Years : 2009	(3)	0.093	0.675	0.138	0.891
2010	(3)	0.643	0.755	0.851	0.401
2011	(3)	0.086	0.808	0.107	0.916
Mean [T] × Age	(2)	-0.200	0.304	-0.658	0.516
Mean [T] × Age ²	(1)	0.497	1.628	0.305	0.763

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2