

Sex-differential effects of inbreeding on overwinter survival, birth date and mass of bighorn lambs

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Keywords:

inbreeding depression;
 lambs growth;
 lambs survival;
Ovis canadensis;
 selection;
 sex-differential effect.

Abstract

Although it is generally expected that inbreeding would lower fitness, few studies have directly quantified the effects of inbreeding in wild mammals. We investigated the effects of inbreeding using long-term data from bighorn sheep on Ram Mountain, Alberta, Canada, over 20 years. This population underwent a drastic decline from 1992 to 2002 and has since failed to recover. We used a pedigree to calculate inbreeding coefficients and examined their impact on lamb growth, birth date and survival. Inbreeding had a substantial effect on female survival: for a given mass in September, the probability of overwinter survival for inbred female lambs was about 40% lower than that of noninbred ones. Contrary to our expectations, inbred female lambs were born earlier than noninbred ones. Earlier birth led to inbred female lambs being heavier by mid-September than noninbred ones. There was a nonsignificant trend for inbred female yearlings to weigh more than noninbred ones. A stronger mass-dependent viability selection for inbred compared to noninbred female lambs may explain why surviving inbred females were heavier than noninbred ones. Survival of male lambs was not affected by inbreeding. Sex-differential effects of inbreeding may be a general pattern in sexually dimorphic mammals, because of sex-biased maternal care or sexual differences in early development strategies.

Introduction

Inbreeding occurs when an individual's parents are related. Compared to the population average, inbred individuals have a greater chance of inheriting identical alleles at a given locus, increasing their homozygosity (Keller & Waller, 2002). Inbreeding can reduce a population's genetic variability and an individual's fitness (Frankham *et al.*, 1999). Decreases in values of fitness-related traits are called inbreeding depression (Keller & Waller, 2002; Snustad & Simmons, 2003). In natural populations, the occurrence of inbreeding is related to population size: when populations decline, the probability of mating with relatives increases. Therefore, inbreeding is a concern for endangered species (Frankham *et al.*, 2002).

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Many studies of captive and domestic mammals found negative effects of inbreeding on traits ranging from juvenile survival and adult fertility (Sausman, 1984; Hass, 1989; Alados & Escòs, 1991) to mass at birth (Cassinello & Alados, 1996), and even to behaviour (Mariette *et al.*, 2006). In domestic sheep, inbreeding reduces conception rate at the first oestrus, litter weight, litter size, lamb survival and ewe survival (Ercanbrack & Knight, 1991; Wiener *et al.*, 1992a,b,c). Numerous studies found that inbreeding reduced birthweight of sheep (Boujenene & Chami, 1997; Analla *et al.*, 1999; Rzewuska *et al.*, 2005). In cows, it decreases survival, milk production and reproductive performance (Hodges *et al.*, 1979; Hudson & Van Vleck, 1984; Hermas *et al.*, 1987), but it has little effect on morphological traits (Nelson & Lush, 1950; Thomson & Freeman, 1967; Hudson & Van Vleck, 1984).

Joron & Brakefield (2003), however, pointed out that the fitness effects of inbreeding can be underestimated in captivity because animals are constrained in their behaviour, interactions with the environment are rare,

resources are not limited, and mortality is reduced compared to natural situations. Accordingly, Crnokrak & Roff (1999) found that reported effects of inbreeding were generally higher in wild populations than in captivity. To quantify the role of inbreeding in evolution and in population dynamics, it is therefore important to study wild populations, where environmental factors may interact with the expression of deleterious recessive alleles (Szulkin & Sheldon, 2007). Studies of inbreeding in the wild are rare, however, because they require detailed pedigrees and data on fitness-related traits for most of the study population. Even with a relatively complete pedigree, the detection of inbreeding depression in the wild remains difficult (Keller & Waller, 2002) because kin-recognition mechanisms can reduce the frequency of inbreeding events and so lower the sample size of inbred individuals. Furthermore, although drastic reductions in population size can result in increasing levels of inbreeding, they can also purge recessive deleterious alleles from the population (Frankham *et al.*, 2002).

Inbreeding depression has been documented in the wild (Keller & Waller, 2002). For example, in song sparrows (*Melospiza melodia*), survival was reduced by inbreeding in years when environmental stress was severe (Keller *et al.*, 1994). Amos *et al.* (2001) used data on grey seals (*Halichoerus grypus*), long-finned pilot whales (*Globicephala melas*) and three species of albatross (*Diomedea exulans*, *Thalassarche chrysostoma* and *Thalassarche melanophris*) to show that individuals born from more genetically distant parents had higher reproductive success. Coltman *et al.* (1999) showed that Soay sheep (*Ovis aries*) that were more homozygous at microsatellite loci had more parasites and reduced winter survival. Inbreeding can also affect development early in life: heterozygous harbour seal (*Phoca vitulina*) pups are heavier at birth (Coltman *et al.*, 1998). A few studies reported severe inbreeding depression in the wild (Kruuk *et al.*, 2002; Szulkin *et al.*, 2007) and even extinction because of inbreeding (Saccheri *et al.*, 1998). The loss of genetic variability that should accompany high levels of inbreeding is of concern for the conservation of biodiversity (Frankham *et al.*, 2002) and it is important to understand how inbreeding may affect population dynamics.

Recently, an increasing number of studies reported a sex-differential effect of inbreeding in a variety of taxa: insects (Tran & Credland, 1995; Saccheri *et al.*, 2005; Fox *et al.*, 2006), birds (Reid *et al.*, 2007) and mammals (Sausman, 1984; Coulson *et al.*, 1999 but see Slate & Pemberton (2002); Charpentier *et al.*, 2006). In all studies except for Saccheri *et al.* (2005), females were more likely than males to be affected by inbreeding. Reid *et al.* (2007) found that the sex-differential effect was inconsistent for different traits associated with immune response. Many mechanisms have been proposed to explain why inbreeding can affect one sex more than the other: nonrandom mortality early in life (Fox *et al.*,

2006), sex-linked deleterious alleles with gender-specific expression (Fox *et al.*, 2006), sex-differential trade-offs in allocation of resources (Fox *et al.*, 2006; Reid *et al.*, 2007), gender-dependent maternal investment (Charpentier *et al.*, 2006), gender differences in food acquisition (Charpentier *et al.*, 2006) or sex-differential early growth strategies (Coulson *et al.*, 1999). Our understanding of sex-differential effects of inbreeding remains limited and more studies are required.

Pedigrees can be used to calculate an index of inbreeding based on mating between individuals that share common ancestors (Keller & Waller, 2002). Most studies of inbreeding in wild mammals, however, used proxies based on multilocus heterozygosity and information on allele size or frequency (Coulson *et al.*, 1999; Balloux *et al.*, 2004; Pemberton, 2004; Slate *et al.*, 2004). Correlations between inbreeding measured from pedigrees and these indices, however, tend to be very low regardless of how many loci are used (Balloux *et al.*, 2004; Slate *et al.*, 2004).

Our study population of bighorn sheep offers a unique opportunity to evaluate the effects of inbreeding in the wild using a pedigree. Coefficients calculated from pedigrees remain the best estimator for detecting inbreeding depression (Pemberton, 2004). This small population is isolated and has a polygynous breeding system (Hogg, 1987), likely increasing the strength of any effect of inbreeding and therefore our ability to detect them (Komers & Curman, 2000). The population underwent a severe decline, partly because of high predation (Festa-Bianchet *et al.*, 2006) and in 2002–2006, it included on average only 18 females of breeding age. Despite many years at low density, the population continued to show very low recruitment. Inbreeding may have contributed to its poor performance. Here, we first compared the rate of inbreeding with population size, then tested for inbreeding effects on lamb birth date. We finally tested the hypothesis that inbred lambs would show reduced mass and survival compared to noninbred individuals. We chose to investigate the effect of inbreeding on these particular traits because they have an important implication for the dynamic of this population. Birth date influences mass of lambs (Feder *et al.*, 2008), and heavy lambs have high survival overwinter (Festa-Bianchet *et al.*, 1997). If inbreeding affects these traits, it could possibly prevent the expected increase in lamb survival at low population density (Portier *et al.*, 1998).

Material and methods

Study area and bighorn sheep population

Ram Mountain, Alberta, is a mountainous complex (elevation 1080–2170 m) 30 km east of the Canadian Rockies (52°N, 115°W), with 38 km² of alpine and subalpine habitat used by sheep. The population is isolated: between 1988 and 2008, only three known

immigrant males contributed to reproduction. The population has been monitored from late May to late September each year since 1972. Sheep are repeatedly captured in a corral trap baited with salt. More than 98% of the population is marked, so that its exact size and sex-age structure are known each year. Since 1975, the only unmarked animals have been immigrant males and a few lambs not captured in the year of birth. We suspect that immigrants are from the nearby population on Shunda Mountain, across the North Saskatchewan River. From 1972 until 1981, the population was maintained at about 30 adult females by yearly removals of ewes (Jorgenson *et al.*, 1997). When removals stopped, the population increased, peaking at 103 females in 1992. A combination of density-dependent effects on recruitment (Festa-Bianchet *et al.*, 1995; Portier *et al.*, 1998) and high cougar (*Puma concolor*) predation in 1997–2001 (Festa-Bianchet *et al.*, 2006) led to a decline. The population was reduced to between 15 and 20 ewes in 2002–2008 and failed to recover despite the cessation of high predation. Introductions of sheep from another population in 2004 and 2007 have so far had only a minor impact on the population genetic structure. By July 2009, there were 53 residents, eight introduced sheep and only two that were born to one introduced ewe.

Pedigree building and inbreeding estimation

Maternal links were established from observations of marked lambs suckling from marked ewes. Paternal links were determined using microsatellites. Tissue collection for DNA analyses began in 1988, with blood sampling of each individual captured until 1993. Sampling resumed in 1997 with the collection of hairs. From 1998 onwards, a tissue sample from the ear was obtained for all captured sheep. Polymerase chain reaction amplification was performed at 32 ungulate-derived loci (Coltman *et al.*, 2005). No evidence of linkage disequilibrium at these loci was found (Coltman *et al.*, 2005). Paternities were assigned using CERVUS version 3.0 (Marshall *et al.*, 1998) at $a > 95\%$ confidence level (Coltman *et al.*, 2002). Some individuals were identified as paternal half-siblings using COLONY version 2.0 (Wang, 2004) even though their father was not sampled because it died before we began tissue collections. Of 524 lambs born since 1988, paternity was known for 350 (67%). Most lambs of unknown paternity died soon after birth and were not captured. The available pedigree in 2008 contained 1017 individuals and extended up to seven generations. We calculated the inbreeding coefficient f (Wright, 1922; Keller & Waller, 2002), the probability that two alleles at a given locus in an individual are identical by descent (Crow & Kimura, 1970), using PEDIGREE VIEWER version 5.5 (<http://www-personal.une.edu.au/~bkinghor/pedigree.htm>). To ensure that for every individual we could calculate a minimum inbreeding coefficient f of 0.125 or higher (Marshall *et al.*,

2002), we followed Kruuk *et al.* (2002) and Szulkin *et al.* (2007) and included in our analyses only lambs for which we knew both parents and at least one grandparent. Immigrant males were assumed to be noninbred.

To analyse the effects of inbreeding on lamb mass, survival and birth date, we coded individuals as 'noninbred' when $f = 0$ and as inbred when $f > 0$ because of the nonlinear effect of inbreeding and to maximize statistical power. There were 6 (10%) lambs with an inbreeding coefficient inferior to 0.008 among the inbred ones. To avoid the erroneous classification as noninbred of lambs with only 1–3 known grandparents, analyses included as 'noninbred' only those for which all four grandparents were known. Despite this additional criterion, we had more noninbred ($n = 94$) than inbred lambs ($n = 60$), which included lambs with both known parents and at least one known grandparent to maximize sample size. Although these data-selection criteria mean that the level of inbreeding may have been underestimated for some inbred lambs, of 60 lambs with $f > 0$, none had only 1 known grandparent, only one had two known grandparents, 12 had three known grandparents and all four grandparents were known for 47. We excluded from these analyses sheep that were first sampled when aged 1 year or older, to avoid possible biases resulting from differential survival over the first year according to level of inbreeding.

Definition and collection of variables

Mass

Seventy-five per cent of lambs were weighed at least twice between June and September. Almost all ewes were weighed 2–7 times each summer. Lamb mass was adjusted to June 15 and to September 15 using a linear mixed model (Pelletier *et al.*, 2007). Female mass was adjusted to June 5 and September 15 using the same procedure. Lamb mass was not adjusted to June 5 because some lambs were not yet born by that date, leading to very low or negative adjusted mass on June 5 (Festa-Bianchet *et al.*, 1996). Absolute summer mass gain was the difference between estimated mass in September and in June.

Birth date

Estimated by field observations of ewes with newborn lambs with a precision of ± 5 days (Feder *et al.*, 2008).

Lamb survival

Only lambs that survived to September were included in analyses, because almost none of those that died earlier were captured and therefore we did not know their fathers. We measured lamb survival from September to the following June. Resighting probability is over 99% for ewes and yearlings (Jorgenson *et al.*, 1997), and no lamb thought to have died was ever sighted in a subsequent year.

Neonatal mortality

When ewes are captured in late May or early June, their lactation status is determined through udder examination. Lactating ewes never seen with a lamb were assumed to have lost their lamb at birth. Sex and paternity of these lambs were unknown. We calculated the proportion of neonatal mortality among all lambs born each year. This measure was used to take into account differences in environmental factors that could explain variation in yearly mass and survival.

Previous reproductive status

Previous reproductive status was a binomial variable coded 0 if the ewe did not wean a lamb the previous year and 1 if her lamb survived to September 15 (Feder *et al.*, 2008). Mothers that did not wean a lamb the previous year may have had lower energy expenditure and this may affect the condition of their lambs the year after (Martin & Festa-Bianchet, in press).

Population size

The number of ewes aged 2 years and older in June each year.

Faecal crude protein

A measure of summer forage quality (Blanchard *et al.*, 2003) also used in other ungulates studies (Leslie & Starkey, 1985; Côté & Festa-Bianchet, 2001) that may influence mass and survival of lambs. Higher faecal crude protein indicates better forage quality. We estimated faecal crude protein in summer as the area under a smoothed cubic spline relating the natural logarithm of faecal crude protein to date, derived from faecal samples collected from late May to late September (Blanchard *et al.*, 2003).

Weather

We used weather records from the Environment Canada station in Nordegg (elevation 1320 m, about 20 km west of Ram Mountain). 'Summer' mean temperature and total precipitation were from May 15 to June 15 and 'winter' mean temperature and total snowfall were from December 1 to March 31, following Portier *et al.* (1998). Feder *et al.* (2008) previously reported an effect of weather on birth date. When snowfall was heavier during the rut, lambs were born later the following spring.

Statistical analyses

We used generalized linear mixed models to test the effects of inbreeding on lamb mass in June and September, summer mass gain and birth date using a Gaussian error structure. To test for the effects of inbreeding on survival, we used a binomial error structure. Because some mothers produced lambs in multiple years, we included mother identity and year as random factors. The

Table 1 Variable abbreviations used in models shown in Tables 2 and 3.

Abbreviation	Description
bdate	Lamb birth date
Sex	Lamb sex
<i>F</i>	Lamb inbreeding coefficient
mlj	Lamb mass in June
mls	Lamb mass in September
mmps	Maternal mass the previous September
mmj	Maternal mass in June
gmm	Maternal mass gain during summer
mage	Maternal age
PRS	Previous reproductive status
snowP	Total amount of snowfall previous winter
prec	Total precipitation during summer
TPw	Mean temperature previous winter
Ts	Mean summer temperature
Tw	Mean temperature the following winter
FCPPs	Faecal crude protein previous summer
FCPs	Faecal crude protein during summer
popsize	Number of adult ewes in June
neo	Yearly neonatal mortality

significance of random terms was tested with a likelihood ratio test of models including and excluding random factors and testing the change in deviance against a χ^2 distribution with one degree of freedom (Pinheiro & Bates, 2000). In all models, we included the inbreeding coefficient and kept it in the final model selected to assess its effect (see Table 1 for a description of variables used in models). All analyses were conducted in R 2.8.1 (R Development Core Team 2008). Models were fitted using the lmer function and the maximum-likelihood (ML) estimation procedure developed in the lme4 and matrix libraries. ML was used for the selection of final models. Parameter estimates for final models were obtained with restricted maximum-likelihood following Hox (2002). We used a stepwise method to select the final model following McCullagh & Nelder (1989). Model selection using Akaike information criterion (AIC), a numerical value representing the maximum log-likelihood and penalized by two times the number of parameters included in the model (Burnham & Anderson, 2002), led to similar results. Inspection of residuals for models with a normal error distribution was used to check assumptions of normality and homoscedasticity. Birth date was log-transformed to respect those criteria. Sample sizes varied between models because of gaps in the data.

Results

Occurrence of inbreeding vs. population size

Of 331 lambs for which we could calculate an inbreeding coefficient because they had at least one known grandparent, 60 (18.1%) had an $f > 0$. Only 3.6% (12) had a coefficient of 0.125 or more. Coefficients > 0 ranged from

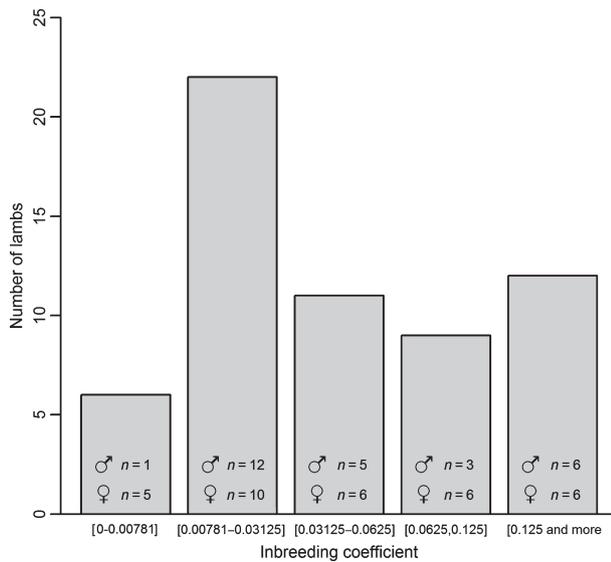


Fig. 1 Distribution of inbreeding coefficients > 0 for bighorn lambs with at least one known grandparent on Ram Mountain, Alberta, 1988–2008.

0.00195 to 0.13184 (Fig. 1). The mean inbreeding coefficient of lambs with four known grandparents varied among cohorts ($F_{1,17} = 2.187$, $P = 0.007$; Fig. 2a). To account for a possible increase in inbreeding with time because of the increasing depth of the pedigree, we fitted a linear regression of the mean inbreeding coefficient with year ($t_{16} = 2.315$, $r = 0.501$, $P = 0.034$). We then assessed the Spearman rank correlation of the residuals of this regression with either the number of males aged 5 years and more or the number of breeding-age ewes, because residuals were not normally distributed. The numbers of breeding-age ewes and of rams aged 5 years and more were correlated ($t_{16} = 4.808$, $r = 0.769$, $P < 0.001$). Males aged 5 years and more obtained more than 80% of known paternities since 1988. The adjusted mean inbreeding coefficient of lambs, obtained by the regression explained previously, was negatively correlated with the number of males aged 5 years and older during the previous rut ($S = 1553$, $r_s = -0.604$, $P = 0.008$, Fig. 2b) but not with the number of breeding-age ewes ($S = 1252$, $r_s = -0.292$, $P = 0.239$). This result held when using the mean inbreeding coefficient of lambs with at least one known grandparent.

Inbreeding effects on birth date and lamb mass

For birth date, the selected model included population size and an interaction between lamb sex and inbreeding coefficient (Table 2), with year (variance = 0.048, variance ratio = variance divided by the sum of residual variance and variance associated with random factors) = 37.5%, $\chi^2 = 20.331$, $P < 0.001$) as a random

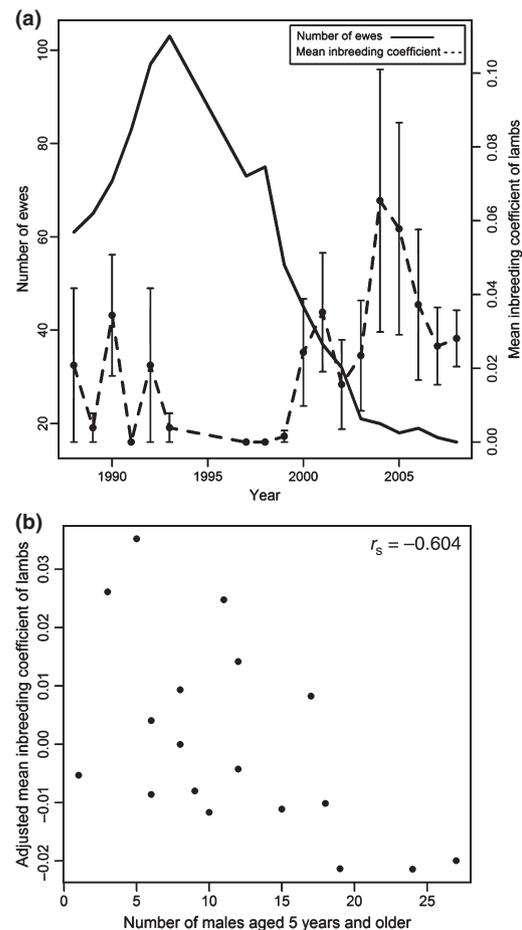


Fig. 2 (a) Number of adult bighorn ewes aged 2 years and older and mean inbreeding coefficient of lambs with four known grandparents on Ram Mountain, Alberta, 1988–2008. (b) Mean inbreeding coefficient, adjusted for yearly variability, of lambs with four known grandparents compared to the number of rams aged 5 years and older during the previous rut.

factor; mother identity (variance = 0.019, variance ratio = 15.3%, $\chi^2 = 1.378$, $P = 0.241$) was not significant. In years with many ewes, births were delayed and there was a strong tendency for female lambs to have later birth dates than male lambs (Table 2). Inbreeding had a sex-differential effect on birth date of lambs, but contrary to our expectations, inbred females were born on average 9 days earlier than noninbred ones (Fig. 3). The final model explained 11.3% of the variance.

Lamb mass in June increased with maternal mass the previous September and with earlier birth date (Table 2), with random effects of year (variance = 0.506, variance ratio = 31.8%, $\chi^2 = 10.162$, $P = 0.001$) and mother identity (variance = 0.412, variance ratio = 25.9%, $\chi^2 = 7.183$, $P = 0.007$). Fixed effects explained 61.4% of variance. Inbreeding did not explain any additional variance.

Table 2 Parameter estimates of fixed effects for the determinants of mass in June and September, summer mass gain and birth date of bighorn lambs on Ram Mountain, Alberta, 1988–2008.

	Estimates	95% CI	P-value
Birth date ($n = 91$)			
Full model: mmPs + F + sex \times F + mage + PRS + FCPPs + snowP + TPw			
Final model			
Intercept	0.754	0.474–1.035	< 0.001
Population size	0.006	0.001–0.012	0.013
Lamb sex (female)	0.210	0.005–0.410	0.055
Inbreeding ($f > 0$)	0.171	–0.025 to 0.387	0.124
Lamb sex (female): inbreeding ($f > 0$)	–0.292	–0.549 to –0.031	0.039
Lamb mass in June ($n = 91$)			
Full model: bdate + mmPs + F + sex \times F + sex + mage + PrevRS + snowP + TPw + FCPPs + popsize			
Final model			
Intercept	4.183	0.633–7.042	0.041
Birth date	–0.113	–0.134 to –0.092	< 0.001
Maternal mass previous fall	0.063	0.021–0.108	0.022
Inbreeding ($f > 0$)	–0.255	–0.809 to 0.333	0.312
Lamb mass in September ($n = 91$)			
Full model: bdate + sex + mmj + F + sex \times F + mage + PRS + prec + Ts + FCPs + popsize			
Final model			
Intercept	14.214	5.089–20.434	0.005
Birth date	–0.252	–0.303 to –0.207	< 0.001
Lamb sex (male)	2.908	1.899–4.183	< 0.001
Maternal mass in June	0.241	0.129–0.388	0.004
Inbreeding ($f > 0$)	–0.609	–1.766 to 0.878	0.497
Lamb mass gain ($n = 154$)			
Full model: sex + ml + popsize + Ts + F + sex \times F + mmPs + mage + PRS + bdate + FCPs + prec			
Final model			
Intercept	10.068	8.97–11.748	< 0.001
Lamb sex (male)	2.012	1.666–2.531	< 0.001
Lamb mass in June	1.169	1.162–1.258	< 0.001
Population size	–0.038	–0.058 to –0.024	< 0.001
Inbreeding ($f > 0$)	0.059	–0.614 to 0.525	0.497

The variable ‘inbreeding’ was kept in all models even if it was not significant to show its effect size. Year and mother identity were included as random effects for all models except that for birth date, where only year was included. Male lambs and an inbreeding coefficient f of 0 (noninbred lambs) were used as references. Analyses used restricted maximum-likelihood linear mixed models. CI, confidence interval.

For lamb mass in September, fixed effects of the selected model explained 60.5% of the variance and included birth date, sex and maternal mass in June (Table 2), with year (variance = 3.228, variance ratio = 33.8%, $\chi^2 = 15.687$, $P < 0.001$) and mother identity (variance = 2.921, variance ratio = 30.6%, $\chi^2 = 7.129$, $P = 0.008$) as random variables. Lamb mass increased with earlier birth date and with larger maternal mass. Males were about 2.9 kg (or 10%) heavier than females, but there was no effect of inbreeding. There was no interaction between inbreeding and sex (estimate: –0.259, 95% CI: –2.329–2.376, $P = 0.875$). Birth date explained 50% and 46.1% of variance in lamb mass adjusted to June and September, respectively. Because inbreeding affected birth date of female lambs and birth date has an important effect on mass in September, we examined a possible indirect effect of inbreeding on mass. To do so, we excluded birth date from the final model

in Table 2. We obtained a significant interaction between sex and inbreeding status (estimate: 2.395, 95% CI: 0.060–5.195, $P = 0.041$, $n = 154$) suggesting an indirect positive effect of inbreeding on female lambs’ mass in September. Estimates for all other explicative variables were similar to those of the model including birth date. Inbred females were 1.9 kg (about 8%) heavier than noninbred ones ($t_{80} = -2.023$, $P = 0.046$; Fig. 4), and the result held after removing outliers ($t_{78} = -2.361$, $P = 0.021$; Fig. 4). Mass in September was strongly correlated with mass in mid-June ($t_{80} = 11.839$, $r = 0.798$, $P < 0.001$). Inbred female yearlings ($n = 17$) were 1.0 kg (about 4%) heavier than noninbred ones ($n = 29$) but the difference was not significant ($t_{44} = -1.149$, $P = 0.257$; Fig. 4).

For absolute lamb summer mass gain, fixed effects of the selected model explained 61.2% of the variance (Table 2), with year (variance = 2.027, variance

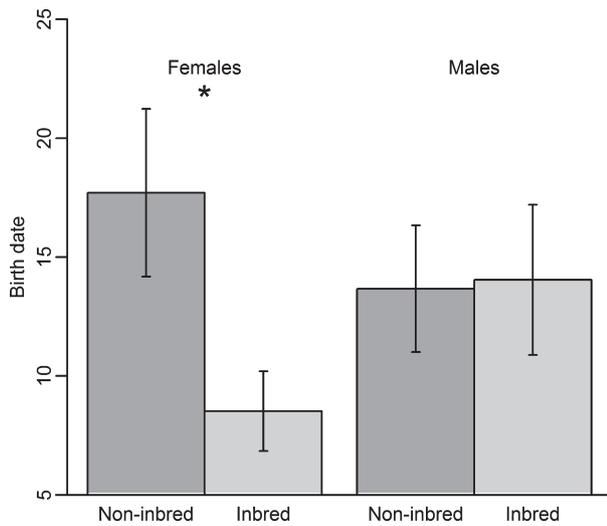


Fig. 3 Mean birth dates of bighorn lambs on Ram Mountain, Alberta, 1988–2008 according to sex and inbreeding status. Sample sizes for noninbred females, inbred females, and noninbred males and inbred males are respectively 15, 26, 24 and 26. Asterisk shows significant difference in mean birth dates between inbred and noninbred females.

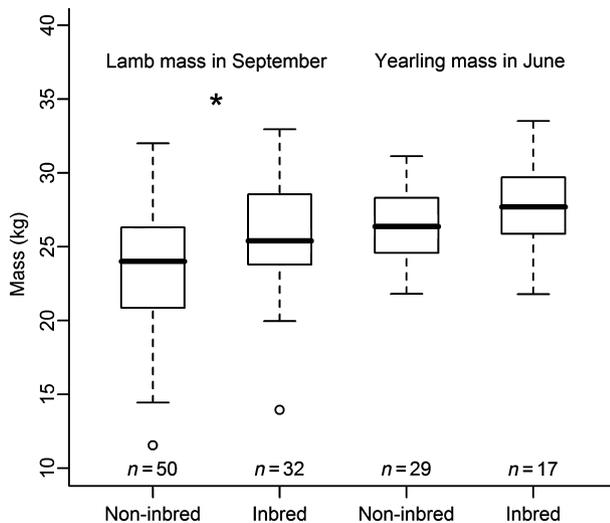


Fig. 4 Distribution of mass for female bighorn sheep on Ram Mountain, Alberta: noninbred and inbred lambs in September (1988–2008) and yearlings in June (1989–2009). Box shows 25th to 75th percentiles, and moustaches show the first and last 25 percentiles. Mean and median are similar in this boxplot. Empty circles are outliers. Asterisk shows significant difference in mass between inbred and noninbred females.

ratio = 54.8%, $\chi^2 = 80.590$, $P < 0.001$) and mother identity (variance = 1.142, variance ratio = 30.9%, $\chi^2 = 11.739$, $P < 0.001$) as random variables. Males gained about 2.0 kg (or 9%) more than females, and mass gain

Table 3 Parameter estimates for selected generalized linear models of the determinants of overwinter survival for bighorn lambs ($n = 154$) on Ram Mountain, Alberta, 1988–2008.

Full model				
mls + sex × popsize + sex × neo + sex × F + mls × F + bdate × mjs + gmm + bdate + mage + snow + Tw + FCPs				
Minimal model	Estimates	95% CI	<i>P</i> -value	
Intercept	-1.783	-4.773 to 1.159	0.233	
Lamb mass in September	0.169	0.080–0.268	< 0.001	
Lamb sex (male)	-2.562	-5.659 to 0.400	0.095	
Population size	-0.029	-0.056 to -0.007	0.013	
Inbreeding ($f > 0$)	-1.745	-3.294 to -0.372	0.018	
Neonatal mortality	2.528	-2.813 to 8.211	0.364	
Sex (male): population size	0.042	0.010–0.075	0.011	
Sex (male): inbreeding ($f > 0$)	2.257	0.235–4.407	0.033	
Sex (male): neonatal mortality	-7.395	-14.472 to -0.757	0.033	

Male lambs and an inbreeding coefficient f of 0 (noninbred lambs) were used as references. CI, confidence interval.

was positively correlated with mass in June. Summer mass gain decreased with population size and, as previously reported (Feder *et al.*, 2008), was independent of birth date. Inbreeding coefficient did not explain additional variance in mass gain. Similar results were observed when using the relative mass gain of lambs.

Inbreeding effect on lamb overwinter survival

Although males appeared to have lower survival (50%, $n = 72$) than females (60%, $n = 82$), the difference was not significant ($\chi^2 = 1.107$, d.f. = 1, $P = 0.293$). Neither year (variance = 0.126, variance ratio = 11.2%, $\chi^2 = 0.299$, $P = 0.585$) nor mother's identity (variance = 1.399×10^{-15} , variance ratio = 0%, $\chi^2 = 0$, $P = 1$) were significant as random effects; therefore, we used generalized linear models (GLM's) to analyse lamb survival.

The selected model included three interactions because the effect of many variables differed according to sex. For both sexes, greater mass in September improved overwinter survival (Table 3). Yearly neonatal mortality had an important negative effect on survival of males but not of females (Table 3). For females, overwinter survival showed negative density dependence (Table 3). Inbreeding substantially decreased the survival of females, but did not affect that of males (Table 3). An inbred female weighing 24.3 kg in mid-September (the mean adjusted mass of female lambs) had an overwinter survival probability 40% lower than that of a noninbred one of the same mass (Fig. 5). Males had a mass-specific probability of surviving the winter intermediate between those of noninbred and inbred females (Fig. 5). The selected model for overwinter survival of lambs explained 18.1% of the variance.

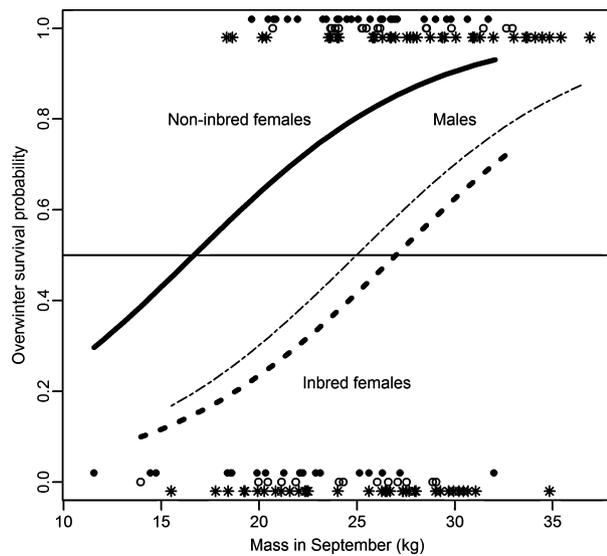


Fig. 5 Overwinter survival of bighorn lambs on Ram Mountain, Alberta, 1988–2008 as a function of mass in September, sex and, for females, inbreeding status, adjusted to mean population size and neonatal mortality for males. Filled points represent noninbred females ($f = 0$), open points represent inbred females ($f > 0$) and asterisks represent males. The horizontal line shows the mass in September associated with a 50% survival probability. Sample sizes were 50 noninbred females, 32 inbred females and 72 males.

Discussion

Our study revealed that inbreeding occurs in the study population, but close inbreeding is rare. Inbreeding reduced substantially the survival of female lambs and increased in both frequency and intensity as the number of mature males declined. We found an unexpected and probably indirect favourable effect of inbreeding on birth date and mass of female lambs.

The increase in the mean inbreeding coefficient of lambs with decreasing number of mature males was to be expected considering the polygynous mating system of bighorn sheep, where dominant, older males obtain most paternities (Hogg & Forbes, 1997; Coltman *et al.*, 2002). As the number of mature males declines, those remaining may obtain more paternities. Population dynamics models often include only females and assume that male availability does not affect reproduction, sex ratio is even and the life history of both sexes is similar (Caswell & Weeks, 1986). Our study supports the use of two-sex models as suggested by Rankin & Kokko (2007) because males may play a role in population dynamics by affecting lamb survival.

The effect of inbreeding on birth date was unexpected. Inbred female lambs were born earlier: 40.0% (6/15) of noninbred female lambs were born during the last quartile of the lambing season, compared to only 19.2% (5/26) of inbred females. Lambs born earlier in

the spring are heavier (Table 2, Feder *et al.*, 2008) and this increases their overwinter survival (Festa-Bianchet *et al.*, 1997). Probably because inbred female lambs were born earlier, they appeared heavier than noninbred ones in September, another unexpected result. Rather than a direct positive effect of inbreeding, however, these two results suggest that more inbred lambs died neonatally, before we could capture them. We could only estimate birth dates for lambs that were seen and we could measure mass and inbreeding coefficient only for lambs that we captured. If late-born inbred lambs suffered higher neonatal mortality, we would have underestimated the effects of inbreeding on early survival. The greater mass in September for inbred female lambs when unadjusted for birth date may also imply that late-born inbred females were unlikely to survive long enough to be captured. Other studies reported that inbreeding had a strong effect early in life (Crnokrak & Roff, 1999). Results for male lambs did not suggest the same trend as for females, because 26.9% (7/26) of inbred males were born during the fourth quartile of the lambing season compared to 25.0% (6/24) for noninbred males. We found no effect of inbreeding on male mass in September (Table 2).

The trend for heavier mass in June for inbred yearling females was contrary to our expectation of a negative effect of inbreeding on physical development. Because of the strong mass-related selection on survival over the first winter, some of the heaviest yearling females were inbred and therefore likely more homozygous than the population average. Because very few studies of wild mammals have obtained accurate pedigrees, it is unknown whether similar survival selection may affect the distribution of phenotypes among adults in other species. Our results provide an explanation for why phenotypic measures such as mass do not necessarily correlate with genetic measures of quality (Moyes *et al.*, 2009). Because inbred lambs can only survive the winter if they accumulate more mass than noninbred ones, they require an environment with enough resources to support rapid mass gain. It therefore seems likely that inbreeding depression would be stronger in years with severe environmental stress (Keller *et al.*, 1994).

We were unable to detect any effects of inbreeding on males. There are few studies of inbreeding effects on free-ranging mammals and therefore little is known about sexual differences. Coltman *et al.* (1999) used microsatellite heterozygosity to determine that less heterozygous adult Soay sheep had more parasites, which reduced their survival. There was no interaction with sex, and this effect was not detected for lambs. However, other data suggest that inbreeding effects may be stronger for females than for males. Sausman (1984) examined pedigrees in captive bighorn sheep and found that inbreeding reduced lamb survival, but the effect was greater for females. Coulson *et al.* (1999) also found that the effect of inbreeding on red deer calf survival was

positive for males and negative for females. However, their index of inbreeding (d^2) and their results were later criticized (Slate & Pemberton, 2002). Similar results were found in the sexually dimorphic mandrill (*Mandrillus sphinx*): inbreeding had a negative effect on body size of females but not of males (Charpentier *et al.*, 2006). In many sexually dimorphic species, young males have lower survival than young females, especially when resources are scarce (Clutton-Brock *et al.*, 1985), as confirmed by the lower overwinter survival of males than of noninbred females (Fig. 5). Male lamb survival was reduced in years of high neonatal mortality (Table 3). A larger sample from Ram Mountain (not constrained by the requirement for data on ancestry) revealed that late birth had a stronger negative effect on the survival of male than of female lambs (Feder *et al.*, 2008). Mean inbreeding coefficient of males ($\mu = 0.018$, $n = 72$) was the same as that of females ($\mu = 0.018$, $n = 82$) and it is possible that in bighorn lambs, inbreeding has a stronger effect on females than males.

Coulson *et al.* (1999) suggested that the stronger effects of inbreeding in females may be related to different developmental strategies between sexes: young males grow more muscles, whereas females accumulate more fat. Sex hormones are also associated with the level of inbreeding (Kosowska & Zdrojewicz, 1996); when homozygosity increases, sex hormone levels decrease. Consequently, outbred males could have more androgens and higher muscle growth, possibly reducing survival overwinter. By reducing androgen levels, inbreeding may increase survival of young males. It is also possible that differential maternal investment may partly compensate inbreeding effects in males. Bighorn ewes provide greater maternal care to sons than to daughters (Bérubé *et al.*, 1996), potentially compensating for the negative effects of inbreeding for young males but not for young females.

Inbreeding had a substantial effect on the overwinter survival of female lambs. The lower survival of inbred female lamb likely decreased the rate of population growth during our study by lowering female recruitment. Lamb survival during our study, when the population was at very low density, was much lower than what we expected based on density-dependent lamb survival over the first 20 years of the Ram Mountain study (Portier *et al.*, 1998). Lower recruitment is a conservation concern because the frequency of inbred matings increased as the population declined. Our results are broadly relevant to understand the effects of inbreeding for animals that survive their first year of life. If inbred juveniles face strong survival selection, only those with well-developed fitness-related traits may survive. In our study, stronger selection against inbred than noninbred juveniles led to the paradoxical result that inbred young adult females appeared to be of greater phenotypic quality than noninbred ones. Survival selection may partly explain why studies often find that inbreeding depression is stronger among juveniles, first by reducing

sample sizes for the study of inbreeding on adults and second because inbred survivors possess fitness-related phenotypic traits. Further investigations are required to confirm whether inbreeding depression is more difficult to detect in males than in females.

Acknowledgments

This research was financially supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Ministère de l'Éducation, du Loisir et du Sport of Québec. Dany Garant and Fanie Pelletier provided constructive comments on an earlier draft of the manuscript. We are grateful to the many field assistants and students who contributed to data collection at Ram Mountain over the last 35 years, especially Julien Martin, Julien Hénault-Richard, Alexandre Martin, Hubert Désilets and Alice Brambilla for their help in 2008–2009. Our research at Ram Mountain depends upon the logistic support and collaboration of the Alberta Fish and Wildlife Division, particularly Jon Jorgenson, Anne Hubbs and Chiara Feder.

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Received 18 February 2010; revised 10 September 2010; accepted 14 September 2010