

No inbreeding avoidance in an isolated population of bighorn sheep

Elise Rioux-Paquette^{a,b,*}, Marco Festa-Bianchet^{a,b}, David W. Coltman^{c,1}

^a Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec, Canada

^b Centre d'Études Nordiques, Université Laval, Québec City, Québec, Canada

^c Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

ARTICLE INFO

Article history:

Received 9 April 2010

Initial acceptance 7 June 2010

Final acceptance 9 August 2010

Available online 17 September 2010

MS. number: A10-00242

Keywords:

inbreeding
inbreeding avoidance
kin discrimination
mate choice
Ovis canadensis
relatedness
selective pressures

Inbreeding avoidance mechanisms such as mate choice should be selected for when inbreeding produces fitness costs. Several studies, however, suggest that animals tolerate inbreeding despite its costs. We studied inbreeding avoidance in bighorn sheep, *Ovis canadensis*, on Ram Mountain, Alberta, Canada, a population with limited dispersal. We used a randomization procedure to simulate a mean inbreeding coefficient of lambs if mating was random every year, and compared these random mating scenarios with known pairings from 1996 to 2007. We considered three sets of candidate males: all males aged 2 years or more, only males known to reproduce, and all males but accounting for age differences in reproductive success. In all cases, mean cohort inbreeding coefficients did not differ from those expected under random mating. We found no evidence of avoidance of mating between close relatives (half-cousins and higher degrees of relatedness). Mate choice was possibly constrained by the generally high level of relatedness among individuals in the population. Selective pressures for inbreeding avoidance, however, may also be weak for this species because of sex-differential costs of inbreeding, limited opportunities of meeting close relatives and breeding migrations of males. The apparent lack of inbreeding avoidance has important implications for the conservation of small and isolated populations of bighorn sheep, where high levels of inbreeding should be expected.

© 2010 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Inbreeding occurs when close relatives mate. It can reduce fitness and lead to inbreeding depression, a decline in the value of a trait (Keller & Waller 2002; Snustad & Simmons 2003). When inbreeding depression is substantial, theory predicts the evolution of inbreeding avoidance mechanisms (Pusey & Wolf 1996). Animals may reduce the risk of inbreeding through dispersal, decreasing the chance of meeting relatives where mating occurs (Blouin & Blouin 1988; Pusey & Wolf 1996). Alternatively, philopatric animals may avoid mating with close kin by suppressing offspring reproduction, delaying maturation, or through various kin-recognition mechanisms (Blouin & Blouin 1988). For example, female lions, *Panthera leo*, conceive at an earlier age if their father is evicted from their social group (Hanby & Bygott 1987).

Kin recognition can lead to active mate choice. Although females are generally the choosier sex, there is evidence that males can also be selective (Amundsen & Forsgren 2001; Doutrelant et al. 2008). Many mechanisms of kin recognition have been suggested.

Hansson et al. (2007) proposed that birds avoid mating with conspecifics to which they have been exposed during early development. Mate choice could also be based on smell associated with the major histocompatibility complex (MHC), and therefore, may depend on genotype (Brown & Eklund 1994). This hypothesis has been supported in studies of fish (Landry et al. 2001) and mammals (Piertney & Oliver 2006). Finally, copulations by females with multiple males (Pusey & Wolf 1996) can allow postmating kin recognition, again based on genotype. Chemoreceptors on spermatozoa, eggs, oviduct and ovarian liquid may enable post-copulatory or 'cryptic' choice by females (Eberhard 1996).

Several recent studies failed to detect inbreeding avoidance in natural populations (van Noordwijk et al. 1985; Keller & Arcese 1998; Hansson et al. 2007; Holand et al. 2007; Jamieson et al. 2009; Szulkin et al. 2009). Most of these studies were on birds, except for that of Holand et al. (2007) on reindeer, *Rangifer tarandus*. Inbreeding avoidance, however, has been reported in numerous other studies (reviewed in Pusey & Wolf 1996) on a wide variety of species including ants (Keller & Passera 1993), birds (Blomqvist et al. 2002; but see Griffith & Montgomerie 2003; Eimes et al. 2005) and mammals (Pusey 1990).

Inbreeding avoidance should be selected if its cost is lower than that of inbreeding (Waser et al. 1986). Possible costs of inbreeding avoidance include the loss of breeding opportunities, or higher risk

* Correspondence: E. Rioux-Paquette, Département de Biologie, Université de Sherbrooke, 2500 boul. de l'Université, Sherbrooke, Québec J1K 2R1, Canada.

E-mail address: elise.rioux-paquette@usherbrooke.ca (E. Rioux-Paquette).

¹ D. W. Coltman is at the Department of Biological Sciences, University of Alberta, 11455 Saskatchewan Drive, Edmonton, Alberta T6G 2E9, Canada.

of mortality while searching for an unrelated mate (Kokko & Ots 2006). Smith (1979) suggested that inbreeding depression would have to be substantial for inbreeding avoidance to be selected. Recently, Kokko & Ots (2006) tried to estimate inbreeding costs with a model examining changes in the opportunity to select between a related and an unrelated mate, the mate encounter rate and the number of mating opportunities. They estimated that inbreeding avoidance should evolve only if the cost of inbreeding was substantial, and they predicted that inbreeding tolerance should be frequent (Kokko & Ots 2006).

Inbreeding tolerance is expected to be higher in polygynous species than in monogamous species (Waser et al. 1986), based on the argument that mating with kin increases the parent's inclusive fitness (Bengtsson 1978). A female that mates with her brother may help a relative to spread genes that they share, provided that inbreeding has a weak effect or no negative effect on fitness. If a female mates with a close relative without reducing mating opportunities for the male, the female will have a direct benefit from this mating through her n offspring, and an indirect benefit by increasing the mating success of her male relative (m). The female's total inclusive fitness will be $(1 + r)n$ while it would have been only n for an unrelated mating (Fisher 1941). In this case there is an advantage for both the female and the male of mating with relatives. In monogamous species, however, mating with relatives does not increase the proportion of genes transmitted to progeny, because the male cannot mate with other females.

We took advantage of a detailed pedigree to investigate inbreeding avoidance in an isolated population of bighorn sheep, *Ovis canadensis*. Previous research on this population revealed a substantial negative effect of inbreeding on the survival of female lambs, suggesting a selective pressure for inbreeding avoidance. Inbred female lambs ($f > 0$) had to weigh about 60% more than non-inbred ones ($f = 0$) to have a similar probability of overwinter survival (Rioux-Paquette 2010). No inbreeding depression for overwinter survival was detected for male lambs, whose survival was much lower than that of female lambs.

The study population experiences almost no immigration and most breeders are philopatric. However, because bighorn sheep are highly polygynous (Coltman et al. 2002), they could be tolerant to inbreeding. We used a randomization method to compare yearly mean inbreeding coefficients with those expected from random matings. We used three sets of candidate males to represent different mating scenarios: all males that were present during the rut, only males that reproduced each year, and all males present during the rut but accounting for age differences in reproductive success. We also examined whether individuals avoided mating with close relatives.

METHODS

Study Area

We studied bighorn sheep on Ram Mountain, Alberta, 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. The population is isolated and philopatric. Since 1988, only three immigrant males have been known to contribute to reproduction. Bighorn sheep on Ram Mountain have been monitored from late May to late September since 1972. Sheep are captured several times each summer in a corral trap baited with salt. Males are marked with ear tags and females with collars. All research protocols were approved by the Université de Sherbrooke Animal Care Committee, affiliated with the Canadian Council for Animal Care (Certificate MFB2008-3). During 40 years of research and about 10 400 captures of over 1000 individuals, capture

operation led to only three sheep deaths and two broken bones. Since 1975, over 98% of sheep 1 year of age and older have been marked. We know precisely which individuals were present each year as the resighting rate is over 99% for females and 96% for males (Jorgenson et al. 1997). Between 1975 and 1981, the population was maintained at approximately 30 adult females by yearly removal of females (Jorgenson et al. 1997). When removals stopped, the population increased, peaking at 103 adult females in 1992. The population then declined to a minimum of 15 resident adult females in 2007 primarily because of density-dependent recruitment (Festa-Bianchet et al. 1995; Portier et al. 1998) and subsequent high cougar, *Puma concolor*, predation in 1998–2002 (Festa-Bianchet et al. 2006). Despite the cessation of high cougar predation, the population included only 21 locally born adult females in 2009. Introductions of sheep from another population in 2004 and in 2007 have so far had a minor impact on the population genetic structure.

Bighorn sheep have a polygynous mating system (Hogg 1987) with older, heavy males with large horns obtaining most paternities (Coltman et al. 2002). Age and body size are also positively correlated with male dominance status (Pelletier & Festa-Bianchet 2006). Yet, subordinate males sire up to 40% of lambs through alternative mating strategies (Hogg & Forbes 1997). So far, no variable associated with mating success of subordinate males has been identified.

Pedigree Building and Candidate Males

Maternal links were established by field observations of associations between marked females and lambs. Paternal links were based on genetic data from microsatellites. Sampling for DNA analyses began in 1988. Until 1993, blood samples were taken from all captured individuals. Tissue collection resumed in 1997 when hairs were collected from captured sheep. Since 1998, a small piece of ear tissue has been collected from each sheep using a biopsy punch. Polymerase chain reaction (PCR) amplification was executed at 32 ungulate-derived loci that showed no evidence of linkage disequilibrium (Coltman et al. 2005). Paternities were assigned using CER-VUS version 3.0 (Kalinowski et al. 2007) with a 95% confidence interval. We used COLONY version 2.0 (Wang 2004) to identify paternal half-siblings among unassigned offspring. A provisional dummy identity was given to the shared, unsampled father. Paternity was assigned to 350 of 524 lambs born since 1988. The pedigree included 1017 individuals and extended up to seven generations for some lambs born in 2007 and 2008. We calculated the inbreeding coefficient f , which gives the probability that two alleles at a given locus are identical by descent (Crow & Kimura 1970), using Pedigree Viewer version 5.5 (<http://www-personal.une.edu.au/~bkinghor/pedigree.htm>). We did not calculate relatedness between mating partners as it is equivalent to twice the inbreeding coefficient. We assumed that parents were related when their offspring's inbreeding coefficient f was different from 0. To assess the expected yearly level of inbreeding in the population if sheep mated at random, we used the pedigree to estimate the inbreeding coefficient value for dummy offspring that would have resulted from potential matings between each male and each female. We analysed these new pedigrees with Pedigree Viewer and calculated the inbreeding coefficient for each of these matings. This manipulation generated an estimated inbreeding coefficient for each possible male–female combination each year in the study population.

A major obstacle to an assessment of inbreeding avoidance in nature is identifying which males are possible mates. The study population is highly isolated, so we are confident that males alive in late September were present during the rut 2 months later. However, we do not know whether some males from the nearby

population on Shunda Mountain, across the North Saskatchewan River, may have migrated to Ram Mountain during the rut. Between 1988 and 2008, five nonresident males, seen irregularly on Ram Mountain and not locally born, were captured and sampled for their DNA. At least two of these five fathered lambs in the study population. For simulations of possible male choice, we considered resident and nonresident males alive at the end of September, plus any nonresident males that were not seen but were assigned a paternity during a given rut.

Statistical Analyses

We used a randomization procedure to test whether yearly mean inbreeding coefficients for the population were less than expected under random mating. We excluded the first 6 years (1987–1992) to eliminate possible inaccuracies due to lack of information in the pedigree. We decided to start analyses after years when no DNA samples were taken (ruts of 1993–1995). For the breeding seasons of 1996–2007, we calculated the mean observed inbreeding coefficient f of all lambs with two known parents and at least one known grandparent, following Kruuk et al. (2002) and Szulkin et al. (2007). Given an incomplete pedigree, the method only includes individuals for which it would be possible to calculate a minimal inbreeding coefficient of 0.125 (Marshall et al. 2002). Sample size available to calculate yearly mean inbreeding coefficients in 1996–2007 was 115 lambs.

For each year's cohort of lambs, we calculated a simulated mean inbreeding coefficient by randomly generating the same number of male–female pairings as the number of lambs for which both parents were known. We used the program Perm 1.0 (Duchesne et al. 2006), which paired one male to each female with a known mate. This procedure was repeated 10 000 times, and we then calculated the yearly mean inbreeding coefficient. We considered that evidence of inbreeding avoidance required that the observed mean inbreeding coefficient was less than the lowest 5% of the 10 000 simulated values.

We used three data sets to perform randomizations. The first set contained all males aged 2 years and older that were alive in September before each rut. The second set contained only males that were known to reproduce each year. To better represent the variability in reproductive success among males, we then considered two age classes: 5 years and younger and 6 years and older. In bighorn males, reproductive success increases with age (Coltman et al. 2002). Males younger than 6 years are unlikely to be able to adopt the highly successful tending tactic of defending individual oestrous females (Hogg & Forbes 1997). From 1988 to 2008, males 6 years and older sired four times more lambs than did younger males. Therefore, in the third simulation each male aged 6 years or older was entered four times as a possible partner, while younger males were entered only once. During the 1997 and 2000 ruts, one unsampled male each year (identified by COLONY) reproduced and was treated as an unknown father. He was removed from the data set, as was the female that he mated with.

To examine whether females avoided mating with closely related males, we considered mating matrices from all data sets and compared the observed and predicted numbers of lambs with an inbreeding coefficient of 0.03125 (half-cousins mating) or higher. We used a Fisher's exact test because of small expected frequencies, repeating the test for each year and for each class of inbreeding coefficients (≥ 0.0625 , ≥ 0.125 and ≥ 0.25).

RESULTS

Between 1996 and 2007, the data set including all males present in September produced 2157 possible matings. When we restricted

our analysis to males that reproduced, there were 690 possible matings. The data set including all males but accounting for age-specific male reproductive success led to 5013 possible matings. Inbreeding coefficients of lambs produced by all possible matings ranged from 0 to 0.314. In all three cases, 75% of pairings were among unrelated individuals ($f = 0$) (Fig. 1).

Overall, there were zero female-years with no related potential mates for the data set with all males, 15 for the data set with only males that reproduced and none when accounting for age-specific male reproductive success. Table 1 shows the distribution of potential inbreeding coefficients for the data set with all males accounting for the effect of age on reproductive success. All results presented below are for the age-specific data set. Other sets led to similar conclusions. The proportion of inbred lambs generated from all potential matings increased over time ($t_{10} = 5.714$, $r = 0.875$, $P < 0.001$), as did the observed proportion of inbred lambs ($t_{10} = 11.792$, $r = 0.966$, $P < 0.001$; Table 1).

Between 1996 and 2007, of 118 lambs for which we knew both parents and at least one grandparent, 54 (45%) were inbred (Fig. 1d). Simulated coefficients were similar for all data sets. Overall, there was no evidence of inbreeding avoidance, and mate choice appeared random with respect to kin (Fig. 2). For the nine ruts from 1999 to 2007, observed mean inbreeding coefficients were lower in four ruts and higher in five ruts than in the simulated ones. The first 3 years were removed from analyses because we lacked statistical power to detect inbreeding avoidance. During those years, a high proportion of candidate males were unrelated to females whose lambs were sampled (Table 1). In 1996 and 1997, 12.4% and 21.0% of simulated mean inbreeding coefficients equalled 0. In 1998, 3.9% of simulated values equalled 0. For all other years we had sufficient statistical power to detect inbreeding avoidance and we found none. A Kolmogorov–Smirnov test confirmed that the distributions of potential and observed inbreeding coefficients of lambs (Fig. 1) did not differ ($D = 0.062$, $N_{\text{simulated}} = 10\,000$, $N_{\text{observed}} = 115$, $P = 0.764$).

Because in most years we did not detect inbreeding avoidance, we assessed whether or not females avoided mating with closely related males. In data sets accounting for the effect of age on male reproductive success, there were some potential full sib matings (Fig. 1, Table 1), but no lamb had such a high inbreeding coefficient. Considering a level of inbreeding of 0.0625 or higher and accounting for age-specific reproductive success, expected and observed frequencies did not differ for any year (Table 2). We obtained similar results for all other categories of inbreeding coefficient tested ($f \geq 0.03125$, $f \geq 0.125$, $f \geq 0.25$) and for all three types of simulated matings (data not shown).

Considering all males, only 23 of 2157 possible mating pairs led to inbreeding coefficients equal to or higher than 0.25. Of these, 17 were mother–son pairs. The mean inbreeding coefficient of lambs potentially sired by each male within a given year decreased with increasing male age ($t_{186} = -2.980$, $r = -0.213$, $P = 0.003$).

DISCUSSION

Observed matings over 9 years in a small population of bighorn sheep provided no evidence of inbreeding avoidance. Mean inbreeding coefficients were not different from those predicted under random mating and females did not avoid mating with close relatives (Table 2). Lack of evidence of inbreeding avoidance, even when inbred matings generate fitness costs, may suggest a constraint on female choice (Kokko & Ots 2006). The average relatedness between potential mating partners was most likely higher during the study years (ruts of 1999–2007) than during earlier years at Ram Mountain. A severe population decline began

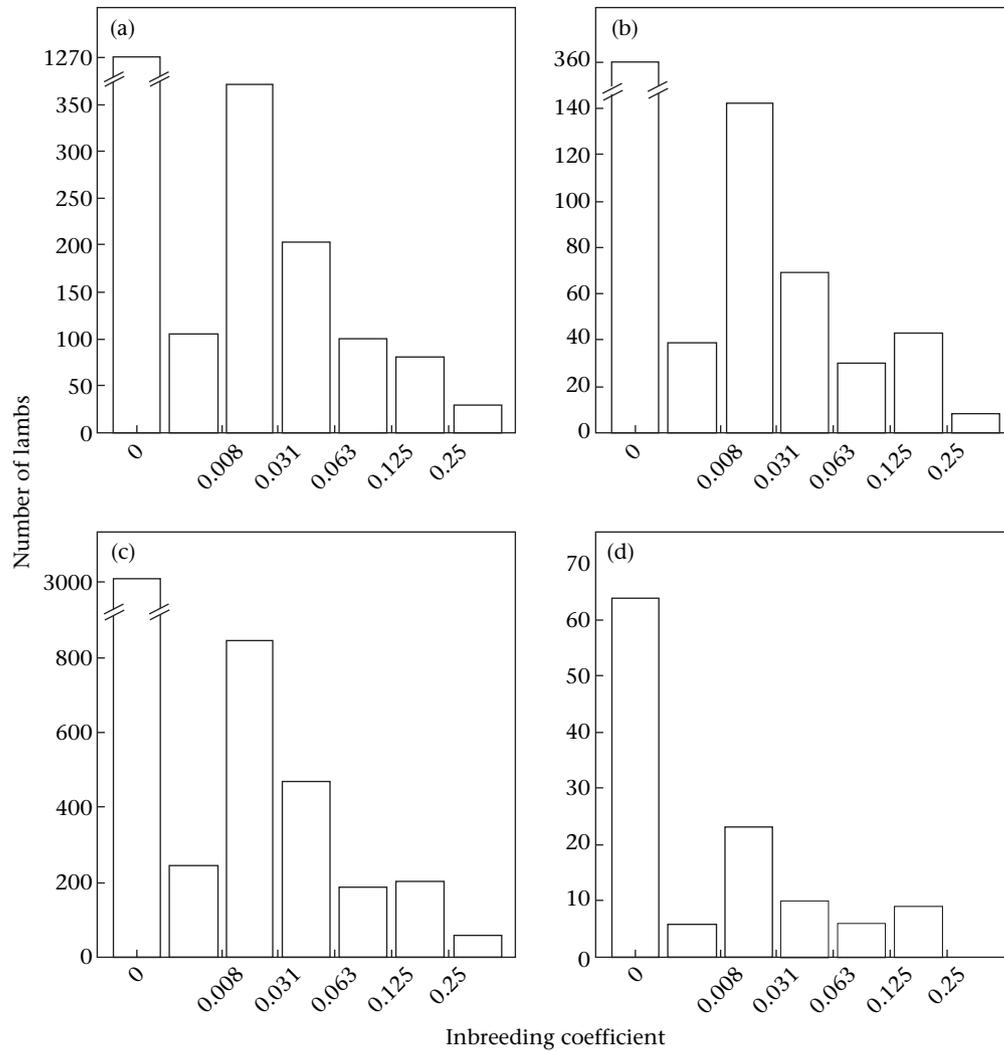


Figure 1. Distributions (a–c) of potential inbreeding coefficients f for lambs on Ram Mountain, Alberta, during 1996–2007, obtained by pairing each female to each candidate male using (a) all males aged 2 years and older that were alive in September before the rut, (b) only males that reproduced at each rut and (c) all males aged 2 years and older but accounting for expected age-related differences in reproductive success (see text). The observed distribution of inbreeding coefficients is shown in (d). Only females that reproduced and whose mate was known are included. Maximum potential value of inbreeding coefficient is 0.314.

in 1992, and as the population decreased, mean inbreeding coefficients of lambs increased (Fig. 2; Rioux-Paquette 2010).

Dispersal can be effective in avoiding inbreeding in birds (Szulkin & Sheldon 2008), and in some ungulates such as feral horses (Linklater & Cameron 2009). In our study population,

however, there is almost no dispersal of either sex and almost all lambs are sired by males born in the population. When dispersal is rare, the ability of individuals to recognize relatives may increase (Jamieson et al. 2009). Mammals may avoid inbreeding through active kin recognition because they have a highly developed sense

Table 1

Distribution of potential inbreeding coefficients for all possible male–female pairs of bighorn sheep on Ram Mountain, Alberta, Canada

Year	$f=0$	$0 < f < 0.03125$	$0.03125 \leq f \leq 0.0625$	$0.0625 < f \leq 0.125$	$f > 0.125$	Total
1996	753 (12)	83 (0)	87 (0)	20 (0)	14 (0)	957 (12)
1997	668 (8)	81 (0)	79 (0)	42 (0)	10 (0)	880 (8)
1998	601 (10)	106 (1)	90 (0)	28 (0)	11 (0)	836 (11)
1999	232 (9)	52 (1)	72 (2)	18 (1)	0 (0)	374 (13)
2000	214 (6)	79 (2)	42 (3)	22 (1)	3 (0)	360 (12)
2001	70 (3)	65 (1)	30 (1)	9 (0)	1 (0)	175 (5)
2002	36 (4)	47 (2)	39 (2)	4 (0)	10 (0)	136 (8)
2003	107 (3)	98 (4)	32 (1)	8 (1)	15 (1)	260 (10)
2004	30 (3)	47 (1)	15 (1)	13 (1)	7 (1)	112 (7)
2005	68 (2)	96 (5)	18 (0)	9 (0)	7 (2)	198 (9)
2006	99 (3)	193 (6)	47 (4)	20 (0)	33 (1)	392 (14)
2007	133 (1)	141 (6)	32 (1)	6 (1)	21 (0)	333 (9)

Inbreeding coefficients of lambs were calculated by pairing each female to each candidate male, including all males aged 2 years and older that were present in late September before each rut and accounting for age-related differences in reproductive success (see text). Numbers in parentheses indicate the observed number of lambs for each level of inbreeding in each year.

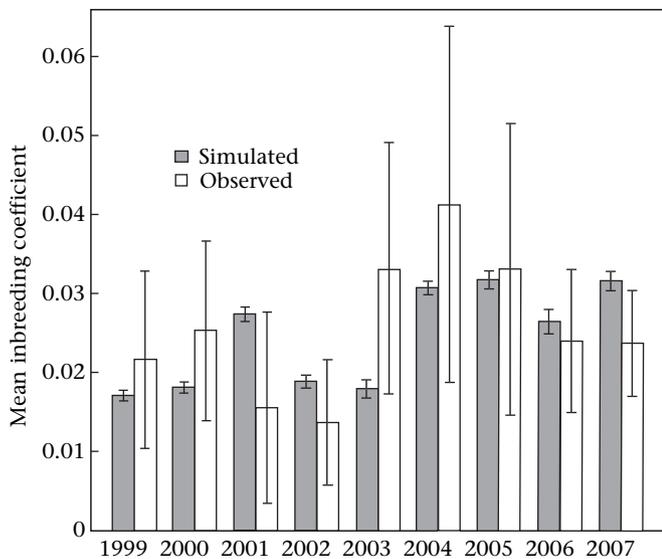


Figure 2. Simulated and observed mean inbreeding coefficient $f \pm$ SE of bighorn lambs with two known parents and at least one known grandparent on Ram Mountain, Alberta, during 1999–2007. The simulated mean coefficient was calculated from a data set including all males that were present at the end of September preceding the rut and accounting for age-related differences in reproductive success (see text).

of smell (Pusey & Wolf 1996). Genetic variability in the MHC complex can be used to select dissimilar mates (Potts et al. 1991; Ziegler et al. 2005). In feral sheep, however, MHC diversity did not explain mating preferences (Paterson & Pemberton 1997). Bighorn sheep have a polygynous social mating system and a few males sire most lambs each year (Coltman et al. 2002). Oestrous females often copulate with several males (Hogg 1988), which could allow for postcopulatory mate choice (Brown 1997; Zeh & Zeh 1997). Our results, however, do not suggest mate choice based on relatedness. We found no evidence for inbreeding avoidance, similarly to several studies of wild birds (van Noordwijk et al. 1985; Keller & Arcese 1998; Hansson et al. 2007; Jamieson et al. 2009; Szulkin et al. 2009). Holand et al. (2007) found no inbreeding avoidance in polygynous reindeer, where the cost of searching unrelated males and delaying conception may override the fitness costs of parental relatedness.

In polygynous species, mate choice theory predicts that females should be more selective than males (Trivers 1972; Andersson

1994). Apparent mate selection has been observed in lekking species (Clutton-Brock et al. 1989; Gosling & Petrie 1990; Balmford et al. 1992; Bro-Jørgensen 2002). Evidence for female choice in ungulates is limited (bighorn sheep: Hogg 1987; pronghorn, *Antilocapra americana*: Byers et al. 1994). We found no evidence for female choice against related males.

The cost of avoiding inbreeding may at times be greater than the cost of tolerating it (Pärt 1996) because of lost breeding opportunities, especially when mates are difficult to locate. Bighorn sheep, however, form mixed-sex groups in open habitat during the rut and oestrous females are typically pursued by several males (Hogg 1987). Recently, Kokko & Ots (2006) argued that inbreeding should be tolerated if the increased inclusive fitness from inbred matings outweighs its cost. Therefore, even if there is a cost to inbreeding, inbreeding avoidance mechanisms do not always evolve (Frère et al. 2010). In our study population, inbreeding depressed the survival of female lambs but did not appear to affect the survival of male lambs (Rioux-Paquette 2010). A sex-specific effect of inbreeding would lower its overall cost, potentially weakening selection for inbreeding avoidance. In polygynous species, reproductive success varies substantially among males (Trivers 1972), and in bighorn sheep, male reproductive success increases with age, mass and horn length (Coltman et al. 2002). A female could benefit by mating with a related dominant male if she conceives a son that may inherit his father's phenotypic traits. Horn size and body mass have a strong genetic component in bighorn sheep (Coltman et al. 2005).

Inbreeding avoidance mechanisms should not be strongly selected if the chance of mating with a relative is low (Kokko & Ots 2006). None of the sampled lambs had an inbreeding coefficient of 0.25 or higher. There were a few potential pairings that could have produced an inbreeding coefficient $f \geq 0.25$ in the population, mostly from mother–son matings. In bighorn sheep, father–daughter matings should be rare because most lambs are sired by males aged 6 years and older (Coltman et al. 2002) and the survival of mature males is low (Gaillard et al. 2000), so that most die before their daughters are sexually mature 2–4 years later. Accordingly, we found that relatedness with potential mates was higher for younger males.

In other bighorn sheep populations, many males leave their natal population to rut elsewhere (Hogg 2000), reducing the selective pressure for inbreeding avoidance mechanisms. In the Ram Mountain population, however, almost all lambs are fathered by philopatric males. In addition, most bighorn sheep populations are larger than the Ram Mountain population during our study, and large population size would reduce the opportunity for inbred matings. Both demography and male behaviour suggest that for most bighorn sheep populations inbreeding depression may not be an important selective pressure. The apparent absence of inbreeding avoidance in our study population may simply be due to low opportunities for inbred matings during the evolution of our study species. Given the similarity in demography and the frequency of male dispersal for most large herbivores (Gaillard et al. 2000), selective pressures favouring the evolution of inbreeding avoidance may also be low in many other ungulate species.

Mate choice in our study population appeared to be random with respect to relatedness. Lack of mechanisms for inbreeding avoidance, however, can have a substantial negative demographic impact on small populations, as in the case of the Ram Mountain population over the last few years (Rioux-Paquette 2010). Populations of species that have not evolved mechanisms to avoid inbreeding may be particularly vulnerable to declines caused by overexploitation, habitat fragmentations or barriers to genetic exchange (Epps et al. 2005). Our results underline the need to carefully examine both the costs and the opportunities of

Table 2

Observed and expected proportions of lambs with an inbreeding coefficient equal to or greater than 0.0625 for Ram Mountain bighorn sheep

Year	Observed proportion	Predicted proportion	Odds ratio	95% CI	<i>P</i>
1996	0	0.051	0	0–6.89	1
1997	0	0.082	0	0–6.71	1
1998	0	0.086	0	0–4.33	0.61
1999	0.154	0.107	1.52	0.16–7.33	0.64
2000	0.250	0.100	2.99	0.50–12.70	0.12
2001	0.200	0.103	2.17	0.04–23.54	0.43
2002	0.125	0.110	1.15	0.02–10.04	1
2003	0.200	0.069	3.33	0.33–17.99	0.16
2004	0.286	0.179	1.83	0.16–12.19	0.61
2005	0.222	0.086	3.02	0.29–17.65	0.20
2006	0.071	0.138	0.48	0.01–3.34	0.70
2007	0.111	0.084	1.36	0.03–10.79	0.55

Proportions were generated including all males aged 2 years and older that were present at the end of September preceding each rut and accounting for age-related differences in reproductive success (see text). *P* values were obtained with a Fisher's exact test. CI = confidence interval.

inbreeding in wild animals to understand why some species may not avoid inbreeding despite its fitness costs.

Acknowledgments

We are grateful for funding provided by the Natural Sciences and Engineering Research Council of Canada, the Ministère de l'Éducation, du Loisir et du Sport du Québec and the Université de Sherbrooke. Jon Jorgenson, Chiarastella Feder and Anne Hubbs, biologists with Alberta Fish & Wildlife, provided logistic support. Dany Garant provided constructive criticism of an earlier draft of the manuscript. This long-term study would not have been possible without the help of numerous students and field assistants. We particularly wish to thank Julien Martin, Julien Hénault-Richard, Alexandre Martin, Hubert Désilets and Alice Brambilla for their help in 2008–2009.

References

- Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences, U.S.A.*, **98**, 13155–13160.
- Andersson, M. B. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.
- Balmford, A., Albon, S. & Blakeman, S. 1992. Correlates of male mating success and female choice in a lek-breeding antelope. *Behavioral Ecology*, **3**, 112–123.
- Bengtsson, B. O. 1978. Avoiding inbreeding: at what cost? *Journal of Theoretical Biology*, **73**, 439–444.
- Blomqvist, D., Andersson, M., Küpper, C., Cuthill, I. C., Kis, J., Lanctot, R. B., Sandercock, B. K., Székely, T., Wallander, J. & Kempenaers, B. 2002. Genetic similarity between mates and extra-pair parentage in three species of shorebirds. *Nature*, **419**, 613–615.
- Blouin, S. F. & Blouin, M. 1988. Inbreeding avoidance behaviors. *Trends in Ecology & Evolution*, **3**, 230–233.
- Bro-Jørgensen, J. 2002. Overt female mate competition and preference for central males in a lekking antelope. *Proceedings of the National Academy of Sciences, U.S.A.*, **99**, 9290–9293.
- Brown, J. L. 1997. A theory of mate choice based on heterozygosity. *Behavioral Ecology*, **8**, 60–65.
- Brown, J. L. & Eklund, A. 1994. Kin recognition and the major histocompatibility complex: an integrative review. *American Naturalist*, **143**, 435–461.
- Byers, J. A., Moodie, J. D. & Hall, N. 1994. Pronghorn females choose vigorous mates. *Animal Behaviour*, **47**, 33–43.
- Clutton-Brock, T. H., Hiraiwa-Hasegawa, M. & Robertson, A. 1989. Mate choice on fallow deer leks. *Nature*, **340**, 463–465.
- Coltman, D. W., Festa-Bianchet, M., Jorgenson, J. T. & Stroebeck, C. 2002. Age-dependent sexual selection in bighorn rams. *Proceedings of the Royal Society 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.
- Crow, J. F. & Kimura, M. 1970. *An Introduction to Population Genetics Theory*. New York: Harper & Row.
- Doutrelant, C., Grégoire, A., Grnac, N., Gomez, D., Lambrechts, M. M. & Perret, P. 2008. Female coloration indicates female reproductive capacity in blue tits. *Journal of Evolutionary Biology*, **21**, 226–233.
- Duchesne, P., Étienne, C. & Bernatchez, L. 2006. PERM: a computer program to detect structuring factors in meaningful social units. *Molecular Ecology Notes*, **6**, 965–976.
- Eberhard, W. J. 1996. *Female Control Sexual Selection by Cryptic Female Choice*. Princeton, New Jersey: Princeton University Press.
- Eimes, J. A., Parker, P. G., Brown, J. L. & Brown, E. R. 2005. Extrapair fertilization and genetic similarity of social mates in the Mexican jay. *Behavioral Ecology*, **16**, 456–460.
- Epps, C. W., Palsbøll, P. J., Wehausen, J. D., Roderick, G. K., Ramey, B. R. II & McCullough, D. R. 2005. Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecology Letters*, **8**, 1029–1038.
- Festa-Bianchet, M., Jorgenson, J. T., Lucherini, M. & Wishart, W. D. 1995. Life history consequences of variation in age of primiparity in bighorn ewes. *Ecology*, **76**, 871–881.
- Festa-Bianchet, M., Coulson, T., Gaillard, J.-M., Hogg, J. T. & Pelletier, F. 2006. Stochastic predation events and population persistence in bighorn sheep. *Proceedings of the Royal Society B*, **273**, 1537–1543.
- Fisher, R. A. 1941. Average excess and average effect of a gene substitution. *Annals of Eugenics*, **11**, 53–63.
- Frère, C. H., Krütsen, M., Kopps, A. M., Ward, P., Mann, J. & Sherwin, W. B. 2010. Inbreeding tolerance and fitness costs in wild bottlenose dolphins. *Proceedings of the Royal Society B*, **277**, 2667–2673.
- Gaillard, J. M., Festa-Bianchet, M., Yoccoz, N. G., Loison, A. & Toigo, C. 2000. Temporal variation in fitness components and population dynamics of large herbivores. *Annual Review of Ecology and Systematics*, **31**, 367–393.
- Gosling, L. M. & Petrie, M. A. 1990. Lekking in topi: a consequence of satellite behaviour by small males at hotspots. *Animal Behaviour*, **40**, 272–287.
- Griffith, S. C. & Montgomerie, R. 2003. Why do birds engage in extra-pair copulation? *Nature*, **422**, 833.
- Hanby, J. P. & Bygott, J. D. 1987. Emigration of subadult lions. *Animal Behaviour*, **35**, 161–169.
- Hansson, B., Jack, L., Christians, J. K., Pemberton, J. M., Akesson, M., Westerdahl, H., Bensch, S. & Hasselquist, D. 2007. No evidence for inbreeding avoidance in a great reed warbler population. *Behavioral Ecology*, **18**, 157–164.
- Hogg, J. T. 1987. Intrasexual competition and mate choice in Rocky Mountain bighorn sheep. *Ethology*, **75**, 119–144.
- Hogg, J. T. 1988. Copulatory tactics in relation to sperm competition in Rocky Mountain bighorn sheep. *Behavioral Ecology and Sociobiology*, **22**, 49–59.
- Hogg, J. T. 2000. Mating systems and conservation at large spatial scales. In: *Vertebrate Mating Systems* (Ed. by M. Apollonio, M. Festa-Bianchet & D. Mainardi), pp. 214–252. Singapore: World Scientific.
- Hogg, J. T. & Forbes, S. H. 1997. Mating in bighorn sheep: frequent male reproduction via a high-risk 'unconventional' tactic. *Behavioral Ecology and Sociobiology*, **41**, 33–48.
- Holand, O., Askim, K. R., Røed, K. H., Weladji, R. B., Gjøstein, H. & Nieminen, M. 2007. No evidence of inbreeding avoidance in a polygynous ungulate: the reindeer (*Rangifer tarandus*). *Biology Letters*, **3**, 36–39.
- Jamieson, I. G., Taylor, S. S., Tracy, L. N., Kokko, H. & Armstrong, D. 2009. Why some species of birds do not avoid inbreeding: insights from New Zealand robins and saddlebacks. *Behavioral Ecology*, **20**, 575–584.
- Jorgenson, J. T., Festa-Bianchet, M., Gaillard, J.-M. & Wishart, W. D. 1997. Effects of age, sex, disease, and density on survival of bighorn sheep. *Ecology*, **78**, 1019–1032.
- Kalinowski, S. T., Taper, M. L. & Marshall, T. C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.
- Keller, L. F. & Arcese, P. 1998. No evidence for inbreeding avoidance in a natural population of song sparrows (*Melospiza melodia*). *American Naturalist*, **152**, 380–392.
- Keller, L. F. & Passera, L. 1993. Incest avoidance, fluctuating asymmetry, and the consequences of inbreeding in *Iridomyrmex humilis*, an ant with multiple queen colonies. *Behavioral Ecology*, **33**, 191–199.
- Keller, L. F. & Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, **17**, 230–241.
- Kokko, H. & Ots, I. 2006. When not to avoid inbreeding. *Evolution*, **60**, 467–475.
- Kruuk, L. E. B., Sheldon, B. C. & Merilä, J. 2002. Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society B*, **269**, 1581–1589.
- Landry, C., Garant, D., Duchesne, P. & Bernatchez, L. 2001. 'Good genes as heterozygosity': the major histocompatibility complex and mate choice in Atlantic salmon (*Salmo salar*). *Proceedings of the Royal Society B*, **268**, 1279–1285.
- Linklater, W. L. & Cameron, E. Z. 2009. Social dispersal but with philopatry reveals incest avoidance in a polygynous ungulate. *Animal Behaviour*, **77**, 1085–1093.
- Marshall, T. C., Coltman, D. W., Pemberton, J. M., Slate, J., Spalton, J. A., Guinness, F. E., Smith, J. A., Pilkington, J. G. & Clutton-Brock, T. H. 2002. Estimating the prevalence of inbreeding from incomplete pedigrees. *Proceedings of the Royal Society B*, **269**, 1533–1539.
- van Noordwijk, A. J., van Tienderen, P. H., de Jong, G. & van Balen, J. H. 1985. Genealogical evidence for random mating in a natural population of the great tit (*Parus major* L.). *Naturwissenschaften*, **72**, 104–106.
- Pärt, T. 1996. Problems with testing inbreeding avoidance: the case of the collared flycatcher. *Evolution*, **50**, 1625–1630.
- Paterson, S. & Pemberton, J. M. 1997. No evidence for major histocompatibility complex dependent mating patterns in a free-living ruminant population. *Proceedings of the Royal Society B*, **264**, 1813–1819.
- Pelletier, F. & Festa-Bianchet, M. 2006. Sexual selection and social rank in bighorn rams. *Animal Behaviour*, **71**, 649–655.
- Piertney, S. B. & Oliver, M. K. 2006. The evolutionary ecology of the major histocompatibility complex. *Heredity*, **96**, 7–21.
- Portier, C., Festa-Bianchet, M., Gaillard, J.-M., Jorgenson, J. T. & Yoccoz, N. G. 1998. Effects of density and weather on survival of bighorn sheep lambs (*Ovis canadensis*). *Journal of Zoology*, **245**, 271–278.
- Potts, W. K., Manning, C. J. & Wakeland, E. K. 1991. Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature*, **352**, 619–621.
- Pusey, A. E. 1990. Mechanisms of inbreeding avoidance in non-human primates. In: *Biosocial Dimensions* (Ed. by J. R. Feirman), pp. 201–220. Berlin: Springer-Verlag.
- Pusey, A. & Wolf, M. 1996. Inbreeding avoidance in animals. *Trends in Ecology & Evolution*, **11**, 201–206.
- Rioux-Paquette, E. 2010. Effets de la consanguinité sur les traits d'histoire de vie, les caractéristiques morphologiques et le choix de partenaire du mouflon d'Amérique (*Ovis canadensis*). M.Sc. thesis, Université de Sherbrooke.
- Smith, R. H. 1979. On selecting for inbreeding in polygynous animals. *Heredity*, **43**, 205–211.
- Snustad, D. P. & Simmons, M. J. 2003. *Principles of Genetics*. 3rd edn. New York: J. Wiley.
- Szullkin, M. & Sheldon, B. C. 2008. Dispersal as a means of inbreeding avoidance in a wild bird population. *Proceedings of the Royal Society B*, **275**, 703–711.

- Szulkin, M., Garant, D., McCleery, R. H. & Sheldon, B. C.** 2007. Inbreeding depression along a life-history continuum in the great tit. *Journal of Evolutionary Biology*, **20**, 1531–1543.
- Szulkin, M., Zelazowski, P., Nicholson, G. & Sheldon, B. C.** 2009. Inbreeding avoidance under different null models of random mating in the great tit. *Journal of Animal Ecology*, **78**, 778–788.
- Trivers, R. L.** 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136–176. Chicago: Aldine.
- Wang, J. L.** 2004. Sibship reconstruction from genetic data with typing errors. *Genetics*, **166**, 1963–1979.
- Waser, P. M., Austad, S. N. & Keane, B.** 1986. When should animals tolerate inbreeding? *American Naturalist*, **128**, 529–537.
- Zeh, J. A. & Zeh, D. W.** 1997. The evolution of polyandry II: post copulatory defences against genetic incompatibility. *Proceedings of the Royal Society B*, **264**, 69–75.
- Ziegler, A., Kentenich, H. & Uchanska-Ziegler, B.** 2005. Female choice and the MHC. *Trends in Immunology*, **26**, 496–502.