Multilocus heterozygosity, parental relatedness and individual fitness components in a wild mountain goat, *Oreamnos americanus* population

**JULIEN MAINGUY,** * STEEVE D. CÔTÉ* and **DAVID W. COLTMAN†**

*Département de biologie et Centre d’études nordiques, Université Laval, Québec, QC, Canada G1V 0A6, †Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada T6G 2E9*

**Abstract**

Matings between relatives lead to a decrease in offspring genetic diversity which can reduce fitness, a phenomenon known as inbreeding depression. Because alpine ungulates generally live in small structured populations and often exhibit a polygynous mating system, they are susceptible to inbreeding. Here, we used marker-based measures of pairwise genetic relatedness and inbreeding to investigate the fitness consequences of matings between relatives in a long-term study population of mountain goats (*Oreamnos americanus*) at Caw Ridge, Alberta, Canada. We first assessed whether individuals avoided mating with kin by comparing actual and random mating pairs according to their estimated genetic relatedness, which was derived from 25 unlinked polymorphic microsatellite markers and reflected pedigree relatedness. We then examined whether individual multilocus heterozygosity $H$, used as a measure of inbreeding, was predicted by parental relatedness and associated with yearling survival and the annual probability of giving birth to a kid in adult females. Breeding pairs identified by genetic parentage analyses of offspring that survived to 1 year of age were less genetically related than expected under random matings. Parental relatedness was negatively correlated with offspring $H$, and more heterozygous yearlings had higher survival to 2 years of age. The probability of giving birth was not affected by $H$ in adult females. Because kids that survived to yearling age were mainly produced by less genetically related parents, our results suggest that some individuals experienced inbreeding depression in early life. Future research will be required to quantify the levels of gene flow between different herds, and evaluate their effects on population genetic diversity and dynamics.

**Keywords**: heterozygosity–fitness correlations, inbreeding, mate choice, pairwise genetic relatedness, ungulates, yearling survival

**Received 23 August 2008; revision received 14 March 2009; accepted 17 March 2009**

**Introduction**

Matings between relatives can lead to a decline in offspring fitness through the increased expression of homozygous deleterious recessive alleles inherited from a common ancestor, a phenomenon known as inbreeding depression (Charlesworth & Charlesworth 1987). Inbred offspring may exhibit lowered fitness as a result of both the fixation of partially recessive alleles in inbred lines (partial dominance hypothesis) and a heterozygote advantage at fitness-linked loci favouring outbred offspring (overdominance hypothesis; Charlesworth & Charlesworth 1999). The detrimental effects of inbreeding on fitness have been studied for over a century, and reported in many captive species and an increasing number of wild populations (Hedrick & Kalinowski 2000; Keller & Waller 2002). Thus, inbreeding depression is now regarded as a widespread phenomenon that can negatively impact individual fitness and the demography of populations (Spielman *et al*. 2004).

The avoidance of inbreeding is expected to occur through different mechanisms within a population (Pusey & Wolf 1996), such as dispersal (Rosenfield & Bielefeldt 1992; Bollinger *et al*. 1993) or kin recognition (Gerlach & Lysiak...
2006; Hoffman et al. 2007). For instance, kin recognition may occur when dispersal does not completely eliminate the risk of mating with relatives (Archie et al. 2007). In some wild populations, however, individuals do not appear to avoid inbreeding (Duarte et al. 2003; Lane et al. 2007) despite sometimes apparent signs of inbreeding depression in offspring (Keller & Arcese 1998; Hansson et al. 2007). This pattern could possibly be the result of a limited ability to discriminate between more or less related partners, or because the costs of inbreeding are potentially outweighed by other benefits, such as increased fitness through the spread of the same alleles (Kokko & Ots 2006).

Studying inbreeding in the wild is not trivial, as it requires detailed pedigree information over at least a few generations to determine inbreeding levels within individuals through the calculation of inbreeding coefficients $F$ (Keller 1998; Overall et al. 2005). When a pedigree is lacking or incomplete, an alternative is to use marker-based measures of genetic diversity such as multilocus heterozygosity $H$ and other derived metrics to infer inbreeding (Coulson et al. 1998; Amos et al. 2001). Depending on the demographic history and mating system of a population, however, the correlation between $H$ and $F$ can vary, but is generally weak (Balloux et al. 2004; Markert et al. 2004; Slate et al. 2004). Our capacity to detect the detrimental effects of inbreeding through marker-based methods can thus be limited and, when significant effects are found, they are also generally weak (reviewed in Colman & Slate 2003). Despite these limitations, new marker-based methods are still being developed to estimate levels of inbreeding (e.g. Aparicio et al. 2006), as homozygosity at a set of neutral markers partly reflects genome-wide homozygosity and, therefore, represents a surrogate of inbreeding levels (Aparicio et al. 2007). In addition, as inbreeding depression is often more severe in the wild than in captivity (Crnokrak & Roff 1999), generally due to the harsher environmental conditions occurring in nature (Jiménez et al. 1994; Halverson et al. 2006), studies of wild populations can enhance our capacity to detect inbreeding depression with $H$ (Jensen et al. 2007).

Detection of heterozygosity-fitness correlations (HFC) using ‘neutral’ loci such as microsatellites does not necessarily imply that inbreeding depression is the sole underlying mechanism originating from a ‘genome-wide’ effect (reviewed by Hansson & Westerberg 2002). This is because HFC may also be generated by linkage disequilibrium between some of the neutral markers and functional genes (‘local’ effect; Hansson & Westerberg 2002). Evidence for this second mechanism has been reported in some wild populations (Hansson et al. 2004; von Hardenberg et al. 2007), but some HFC are still best explained by genome-wide rather than local effects (Hoffman et al. 2004; Charpentier et al. 2005; Lesbarrès et al. 2005). Thus, there is likely a spectrum of explanations for HFC in nature (Balloux et al. 2004), with genome-wide effects having nonetheless a local cause, that is, functional genes of varying positive and negative effects (Lieutnant-Gosselin & Bernatchez 2006). In populations subjected to inbreeding, heterozygosity at coding loci is expected to be positively correlated to that found at neutral markers (Aparicio et al. 2007).

The mountain goat (Oreamnos americanus) is an alpine ungulate generally found in small populations with apparently limited but unknown levels of gene flow between them (Côté & Festa-Bianchet 2003). Therefore, mountain goats are at risk of inbreeding because they are thought to exhibit strong population structure such as in other alpine ungulates (e.g. Amills et al. 2004; Worley et al. 2006). The polygynous mating system of mountain goats (Mainguy et al. 2008) may also exacerbate the risks of inbreeding, as only a few males achieve high reproductive success each year (Mainguy 2008). This may explain why mountain goats exhibit low levels of genetic diversity at neutral (Mainguy et al. 2005) and even some functional genes (Mainguy et al. 2007). Altogether, the characteristics of this alpine ungulate suggest that genetic diversity at a set of neutral markers should correlate with $F$ (Balloux et al. 2004). Additionally, the occasional genetic contribution of immigrant males (Mainguy et al., unpublished data) combined with close inbreeding may increase the variance in inbreeding and favour genetic diversity levels that can promote the detection of HFC (Da Silva et al. 2006).

Here we examined whether actual mating pairs determined from genetic parentage analyses in a long-term study of mountain goats were less genetically related than what would be expected under random matings. Among mating pairs, we further tested whether age and social rank of females played a role in the degree of relatedness to their mating partner, expecting more experienced and higher-ranking females to be less related to their mate than younger and subordinate females. To assess the effect of inbred matings, we examined the relationship between parental marker-based relatedness and offspring genetic diversity ($H$), and the effect of individual genetic diversity on two life-history traits: yearling survival and the annual probability of giving birth in adult (≥3 years old) females. We predicted that most matings should occur between less-related individuals because of the generally negative effects of inbreeding (Keller & Waller 2002). Finally, assuming that $H$ would partially reflect $F$ in our study population, we predicted that yearling survival and the probability of giving birth to a kid in females should decrease with decreasing $H$ under a genome-wide effect, while also testing for potential local effects.

Materials and methods

Study area

Our study was conducted at Caw Ridge (54°N, 119°W), west-central Alberta, Canada, in the front range of the
Rocky Mountains. The native mountain goat population of Caw Ridge uses about 28 km² of alpine grassy slopes, short cliffs, and open subalpine forest that range from 1750 to 2170 m in altitude. The population, studied intensively since 1989, has ranged from 81 to 159 individuals, with ≥98% of individuals aged ≥1 year old marked since 1993. The Caw Ridge population is one of the largest in Alberta and is also geographically isolated from other mountain goat populations, the closest large herd being located about 20 km to the southeast on Mount Hammel (Hamed et al. 2006). A few immigrant males are however observed each year on Caw Ridge, and successful emigration to other nearby populations by males born at Caw Ridge has also been documented (Festa-Bianchet & Côté 2008). Further description of the study area can be found in Festa-Bianchet & Côté (2008).

Captures, measurements and genetic sampling
Goats were captured from late May to mid-September 1986–2007 (n = 756 captures of 398 individuals) in remotely controlled box traps and self-tripping nylon mesh Clover traps baited with salt (Côté et al. 1998a). All individuals were marked with plastic ear tags, visual or radiocollars. Since 1994, an ear tissue sample was also taken for DNA analyses using a punch during marking. Ear tissues were kept in 1.5 mL tubes filled a solution of 20% dimethyl sulfoxide saturated with sodium chloride at −20 °C. Goats that were adults (≥3 years old) at their first capture were aged according to the number of horn annuli (Côté et al. 1998b). Goats were weighed to the nearest 0.5 kg with a spring scale. Many goats were also weighed without handling (n = 1331 masses from 172 individuals aged ≥1 year during 2001–2007) using one to three electronic platform scales baited with salt (Bassano et al. 2003). All body masses were adjusted to 15 July according to the sex-specific growth rate of three age classes (yearlings, 2-year-olds and adults; Côté et al. 1998a; Mainguy & Côté 2008). Because the capture of a female with a kid at heel increases the risk of kid abandonment (Côté et al. 1998a), we did not capture kids starting in 1998 and therefore few data were available for the study of neonatal fitness traits in relation with genetic diversity. Côté et al. (1998a, b) provide further details of capture procedures.

Field observations
We used spotting scopes (15–45×) to sample goat behaviour at distances generally ranging from 200 to 700 m. Observations were conducted almost daily from May to September. In each year, we searched the study area intensively from mid-May to early June and attempted to find as many adult females as possible each day to determine which ones had given birth (Côté & Festa-Bianchet 2001a). Females produce only one kid annually generally starting at 4 or 5 years of age, with very few females primiparous at age 3 (Côté & Festa-Bianchet 2001b). The proportion of adult females giving birth at Caw Ridge generally increases rapidly until 6 years of age, peaks at 8 to 12 years, then declines slightly for females ≥13 years, yielding an average reproductive lifespan of about 9 to 12 years (Festa-Bianchet & Côté 2008). During summer, we noted the identity of individuals present and determined which marked females were nursing a kid until weaning in mid-September (Côté & Festa-Bianchet 2001a). We measured overwinter survival by determining which individuals survived to 1 June the following year. From 1994 to 2007, we also used all-occurrences sampling and focal observations to record agonistic encounters between adult females to determine social rank (Côté 2000), as dominance status influences kid production in females, especially at a young age (Côté & Festa-Bianchet 2001b). Individual females were ordered in annual dominance hierarchies using the methodology described in Côté (2000). Because annual matrix size varied from 38 to 61 adult females, we transformed social ranks according to the formula 1 – rank/N, where N is the number of adult females during year i (Côté 2000). Standardized social ranks therefore varied from 0 (subordinate) to 1 (dominant). As social rank is strongly correlated with age in adult females (r > 0.9), the residuals of standardized rank on age were used as a measure of social rank (Côté 2000).

Microsatellite genotyping
A total of 296 individuals, or 74% of the individuals marked and monitored since the beginning of the study, were sampled for genetic analyses. Genomic DNA was extracted from ear tissues with QIAGEN DNeasy extraction kits and then polymerase chain reaction-amplified using a set of 28 polymorphic microsatellite loci that were in Hardy–Weinberg equilibrium: ARO28, BL6, BM1225, BM1818, BM4025, BM4513, BM4630, BM6444, BR3510, HEL10, HUJ616, HUJ1177, ILSTS058, MAF36, MAF64, McM64, McM152, McM527, OarCP26, OarHH35, OarHH62, OarJMP29, OarJMP58, RT9, RT27, TGLA10, TGLA122, and URB038. We followed methods described in Mainguy et al. (2005) and obtained an overall genotyping success of 99.9%. These 28 loci were used for parentage analyses, whereas only a subsample of 25 loci were used for the estimation of molecular measures of relatedness and inbreeding, as some pairs of loci were in linkage disequilibrium (i.e. ARO28, MAF64 and OarHH62 were removed, see Mainguy et al. 2005).

Parentage analyses
A total of 194 goats had a mother identified through behavioural associations in the field (Gendreau et al. 2005). We tested 111 of these relationships by comparing microsatellite
genotypes and verified all but one relationship based on simple genetic exclusion using the parentage-assignment software Cervus 3.0 (Kalinowski et al. 2007). Because not all individuals were assigned to a mother, however, we attempted to determine genetic maternity using the likelihood-based approach implemented in Cervus. Briefly, this programme calculates a logarithm-of-the-odds (LOD) score for each candidate mother based on simulations of offspring genotypes, and then assigns each offspring to the most likely mother at 95% (strict) or 80% (relaxed) statistical confidence. Simulations were carried out for each year separately to estimate the critical difference in LOD scores for assignments based on the number of candidate mothers and the proportion of them sampled in that year. Input parameters common to all years included simulation of 100 000 offspring genotypes based on allele frequencies of the whole population, a typing error of 0.2% according to the mean observed error rate across loci of known mother–offspring pairs, and the overall proportion of loci typed (99.9%). Candidate mothers considered must have been observed with a kid at heel in the year of birth of the offspring tested, and their kid must have survived to weaning in September. Information about kid sex was also used in assigning the mother, as sex determination from field observations was highly accurate (Côté & Festa-Bianchet 2001c). Then, to maximize the confidence level of paternity assignments, only kids whose mothers had been genotyped and confirmed with Cervus were tested (n = 191). Based on field observations conducted during the rut (Mainguy & Côté 2008; Mainguy et al. 2008), only males aged ≥ 3 years that were observed at least once during the year preceding the kid birth were considered as candidate fathers. Because a few (three to five) adult immigrant males were also observed to rut on Caw Ridge (Mainguy et al. 2008), we added five unsampled males in the simulations for paternal assignments in each year to provide us with a conservative proportion of sampled males. Only paternities assigned under strict statistical confidence in Cervus and with no more than one mismatch in the trio ‘kid-mother-father’ entered the pedigree. Because some individuals (mainly males) were not sampled for DNA in the early years of the study, the pedigree was incomplete and our ability to calculate inbreeding coefficients restricted. The pedigree of 399 individuals included 278 maternal and 100 paternal links (see Mainguy 2008).

Molecular measures of pairwise relatedness

Because the accuracy of relatedness estimators generally varies between different data sets (Van De Casteele et al. 2001), we tested two commonly used estimators of pairwise genetic relatedness (Queller & Goodnight 1989; Lynch & Ritland 1999) and a more recent one for structured populations (Oliehoek et al. 2006) to estimate true genetic relatedness in our study population. The first two estimators were computed using SPAGeDi 1.2 (Hardy & Vekemans 2002) and can theoretically vary between −1 and 1, with negative values suggesting non-kin and positive values kin. The most recent estimator (Oliehoek et al. 2006) was computed using rea 0.2 available at www.geneticdiversity.net/estimators.html and which can vary on a scale from 0 to 1 (none to highly related). We determined the ‘minimal’ true genetic relatedness among all possible pairs of individuals in the Caw Ridge pedigree (R Pedro ) using cfc 1.0 (by M. Sarolzaei, H. Iwaisaki, & J.-J. Colleau; available at www.agr.niigata-u.ac.jp/~iwsk/cfc.html). Then, we compared the three estimators of pairwise genetic relatedness to R Pedro once individuals with values of 0 were excluded (range of R Pedro = 0.016 to 0.625; n = 2431 pairwise comparisons). Based on these comparisons (Table 1), we chose the estimator of Lynch & Ritland (1999), as it explained the most variance in known relatedness. This is consistent with findings of Csilléry et al. (2006) who reported that this estimator performed generally better than others in five different wild populations, including one alpine ungulate.

Molecular measures of inbreeding

In addition to H, which varied from 0.24 to 0.80 in the Caw Ridge population (mean ± SD = 0.50 ± 0.09), we computed two molecular metrics of inbreeding: internal relatedness IR (Amos et al. 2001) and homozygosity by loci H L (Aparicio et al. 2006). Pairwise comparisons of these three metrics revealed, however, that H measured the same extent of genetic diversity as IR and H L, (all r’s > 0.96). Thus, we only present the results obtained with H.

Table 1 Comparisons of the performance of three estimators of pairwise genetic relatedness in reflecting genetic relatedness determined from the pedigree (R Pedro ) in a population of mountain goats (n = 296 genotyped individuals out of 399) at Caw Ridge, Alberta, Canada. Because the pedigree was incomplete, R Pedro sometimes represented a minimal value of true genetic relatedness. All correlations are significant at P < 0.0001

<table>
<thead>
<tr>
<th>Estimators of pairwise genetic relatedness</th>
<th>Variance explained (r²) in R Pedro</th>
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<tbody>
<tr>
<td>Queller &amp; Goodnight (1989)</td>
<td>0.35</td>
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<tr>
<td>Lynch &amp; Ritland (1999)</td>
<td>0.42</td>
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<tr>
<td>Oliehoek et al. (2006)*</td>
<td>0.30</td>
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*The weighted equal drift similarity (WEDS) estimator was applied with a β2 correction (see Oliehoek et al. 2006).

Data analyses

We examined whether mating pairs that produced a kid surviving to 1 year of age (i.e. age at first capture) were less...
genetically related than random mating pairs following methods described in Holand et al. (2007). Briefly, we compared the estimated pairwise genetic relatedness of actual mating pairs to that of all potential nonmating pairs using a permutation test based on 10,000 randomizations. We considered all individuals that were adults during rut 1994 to 2005 in the simulations to compare them to the actual mating pairs that produced a kid caught at 1 year of age in 1996–2007. In addition, we used a Cochran-Armitage permutation test (Agresti 1990) with 10,000 randomizations to test whether actual mating pairs had more often than expected negative (non-kin) rather than positive (kin) pairwise genetic relatedness compared to all potential nonmating pairs. Because we expected individuals to avoid inbreeding due to its detrimental effects, we used one-tailed tests for these analyses. We also examined the effect of females’ characteristics on the degree of pairwise genetic relatedness due to its detrimental effects, we used one nonmating pairs. Because we expected individuals to avoid pairwise genetic relatednesses compared to all potential expected negative (non-kin) rather than positive (kin) relatedness with their mating partner using a linear mixed model (LMM) with ‘female identity’ and ‘year’ fitted as random terms. The estimator of genetic relatedness, which varied between –0.27 and 0.50, was normalized before the analysis by performing a log-transformation \[\log_e(x + 1)\]. As both random terms did not explain variance in the degree of genetic relatedness between mating partners (covariance parameter estimate of ‘female identity’ was \[\pm 0.004, Z = 0.93, P = 0.18\], whereas estimate for ‘year’ was 0), we present the results of a generalized linear model (GLM).

Before testing for HFC, we evaluated whether pairwise genetic relatedness between individuals of actual mating pairs predicted \(H\) in their offspring using a GLM. We then explored non-genetic sources of variation in yearling survival, defined as the probability of surviving from 1 to 2 years of age, and kid production in adult females, expressed as the annual probability of giving birth to a kid, by fitting these dependent variables as binary responses in logistic regressions. Explanatory variables considered to examine variance in yearling survival were yearling sex and mass because Festa-Bianchet & Côté (2008) reported a nearly significant 11% difference in survival from 1 to 2 years of age favouring females, and kid mass was known to affect positively survival to 1 year (Côté & Festa-Bianchet 2001a). Because the ability to provide maternal care increases with age in female mountain goats (Côté & Festa-Bianchet 2001b, c) and this could have influenced survival later, we fitted maternal age at birth as a third explanatory variable. For kid production, we considered female age, age\(^2\), social rank, and an interaction between age and social rank, as these factors have all been previously reported to influence the annual probability of giving birth (Côté & Festa-Bianchet 2001b). For both yearling survival and kid production, population density was also added as an explanatory variable expressed as the number of adult females on 1 June, because density nearly doubled since the beginning of the study (Festa-Bianchet & Côté 2008) and recent analyses have revealed density-dependent effects, such as increased costs of reproduction in females at high density (Hamel 2008). For both analyses, we used generalized estimating equations (GEE; Liang & Zeger 1986) with ‘year’ fitted as a repeated term to account for variation in fitness components that could be attributable to between-year differences.

All statistical analyses were conducted in sas 9.1 (SAS Institute 2003). Nonsignificant variables were removed from the full models of non-genetic terms based on their \(P\) values (i.e. by removing the least significant term) using a backward stepwise procedure with statistical significance set at \(\alpha = 0.05\). We then added \(H\) or single-locus heterozygosity to the reduced models to examine if they explained significant additional variance. Inspection of residuals and collinearity diagnostics indicated no violation of the assumptions of normality and homoscedasticity when required, or of multicollinearity among explanatory variables. ‘Dispersion parameter’ values were close to 1 in the modelling of binary response variables, suggesting no problems of under- or overdispersion. Probabilities were corrected using the sequential Bonferroni method for single-locus analyses to account for multiple tests (Rice 1989). All means are presented ± SE.

**Results**

*Pairwise genetic relatedness of actual vs. random mating pairs*

There were 95 mating pairs that produced a kid surviving to 1 year of age and in which both parents were adults during the rut. The relatedness of these mating pairs was compared to that of 8222 possible nonmating pairs. Mean pairwise genetic relatedness of actual mating pairs (–0.03 ± 0.01) was more than three times lower than that of random mating pairs (–0.009 ± 0.002), but the difference was not statistically significant (\(P = 0.09\); Fig. 1). The values of pairwise genetic relatedness of actual mating pairs, however, were significantly more often negative than positive compared to that of all possible nonmating pairs (68% vs. 59%, respectively) under a Cochran–Armitage permutation test (\(P = 0.04\); Fig. 1). The pairwise genetic relatedness of mating pairs was not affected by female age (\(F = 1.25\), d.f. = 1, 71, \(P = 0.27\)) nor social rank (\(F = 1.87\), d.f. = 1, 72, \(P = 0.07\)). As expected, offspring \(H\) was negatively related to the pairwise genetic relatedness of their parents (\(F_{1,98} = 19.4\), d.f. = 1, 98, \(P < 0.0001\), \(r^2 = 0.16\)).

**Yearling survival**

Based on a sample of 155 yearlings monitored over 15 years, yearling sex, mass, and linear effects of maternal age did
not affect the probability of survival from age 1 to 2, whereas population density had a positive effect on survival (Table 2). After accounting for this non-genetic effect, yearling $H$ was associated with survival (Table 2), as more heterozygous individuals had higher survival probabilities than less heterozygous ones (Fig. 2). When considered alone, $H$ still marginally affected yearling survival positively ($\beta = 3.68 \pm 1.79$, $\chi^2 = 3.61$, d.f. = 1, 153, $P = 0.057$). When comparing the first (most homozygous) and last (most heterozygous) quartiles, the difference in yearling survival was 10% (82% vs. 92%, respectively). Because more than half of the mothers (59%, $n = 64$) contributed more than 1 yearling in this analysis (range = 2–5; 18 of the 155 yearlings used in this analysis were not assigned to a mother) and some of these mothers could have been of higher ‘quality’, this could have also affected yearling survival positively. To test for such a possible confounding effect of ‘mother identity’ on yearling survival, we repeated the GEE analysis on a reduced data set by randomly selecting a single yearling from each mother (see Hoffman et al. 2006 for details). We then repeated this analysis on four other randomly selected subsets of yearlings. The $P$ values of the five data sets varied by less than 0.03, arguing against a confounding influence of ‘mother identity’ on the relationship between yearling survival and $H$. No single locus had a significant effect on yearling survival after sequential Bonferroni correction (critical $P$ value = 0.05/25 loci = 0.002) when accounting for population density. However, as Bonferroni correction can sometimes be regarded as too stringent (Nakagawa 2004), we verified whether removing one of the two loci with $0.002 < P$ values $< 0.05$ (HEL10 and McM152, both loci with a positive effect of heterozygosity on yearling survival) in the calculation of $H$ changed the relationship. The removal of HEL10 reduced the positive association between $H$ and yearling survival to a trend ($P = 0.06$), whereas the removal of McM152 did not change the results ($P = 0.03$). When we removed both loci in the calculation of $H$, the positive relationship was no longer significant ($P = 0.16$).

When single-locus heterozygosity was fitted as a unique explanatory variable, we found 14 positive and 11 negative associations with yearling survival, a ratio not different from unity (Fig. 3a, $\chi^2 = 0.18$, d.f. = 1, $P = 0.67$).

An annual probability of giving birth in adult females

As in Côté & Festa-Bianchet (2001b), the annual probability of giving birth to a kid was influenced by age, age$^2$, social
rank, and an interaction between age and social rank (Table 3) in a sample of 120 individual females monitored over 12 years, whereas population density did not affect the probability of producing a kid (Table 3). Fitting $H$ in a reduced model of the non-genetic terms did not explain additional variance for this trait (Table 3). Using only one randomly selected observation from each female led to similar results. When tested alone, no single locus had a significant effect on the probability of giving birth to a kid after sequential Bonferroni correction. Although there were almost twice more positive ($n = 16$) than negative ($n = 9$) associations between single-locus heterozygosity and the probability of giving birth in adult females (Fig. 3b), the difference was not statistically significant ($\chi^2 = 1.00$, d.f. = 1, $P = 0.32$).

**Discussion**

In the Caw Ridge mountain goat population, actual mating pairs had more often negative than positive values of pairwise genetic relatedness compared to all possible nonmating pairs. The degree of genetic relatedness of females with their mate was independent of maternal age and dominance status. Estimated parental genetic relatedness was negatively correlated with offspring genetic diversity, which was in turn positively related to survival from 1 to 2 years of age. As opposed to yearling survival, the annual probability of giving birth in adult females was not affected by individual genetic diversity. Our study provides evidence that inbreeding depression occurs at an early life stage in mountain goats from the Caw Ridge population.

The results of the analysis of mate choice based on estimated pairwise genetic relatedness are likely to originate from inbreeding depression rather than indicating inbreeding avoidance, or could possibly be a combination of these two processes. This is because we studied mating pairs that produced a kid that survived to 1 year of age and that inbreeding often affects survival and other fitness traits more strongly during early life stages than later in life (Pujolar et al. 2006; Fessehaye et al. 2007; Rijks et al. 2008, but see Szulkin et al. 2007). As a consequence, it is possible that kids that died before 1 year old were more inbred than those that survived, potentially decreasing our estimate of parental genetic relatedness. In other words, mating pairs that produced an inbred offspring which did not survive to yearling age would be considered as nonmating pairs in the analyses. Our results support this hypothesis since $H$ was positively related with survival from 1 to 2 years of age after accounting for a positive effect of population density.
on survival, which was somewhat puzzling, but could have originated from a benefit of gaining higher probabilities of detecting predators in larger than in smaller groups, similarly to what was reported in a gregarious bird (Watson et al. 2007). This is possible as predation is a common cause of mortality in kids and yearlings (Côté & Beaudoin 1997; Côté et al. 1997; Festa-Bianchet & Côté 2008). Although speculative, it is also possible that population density was partly correlated with another environmental factor (e.g. the length of the vegetation growth period), that was also positively correlated with yearling survival. Altogether, our results suggest that matings between relatives occur in our study population, but may be more difficult to detect if inbred offspring die before being sampled at 1 year of age (Marshall et al. 2002). Nevertheless, our results may still be indicative of inbreeding avoidance. For instance, we did not find close inbreeding (F = 0.25) in the pedigree. Therefore, mountain goats may be able to partly discriminate among more or less related kins for matings, unless all highly inbred offspring are being removed from the population by natural selection. On the other hand, low to moderate inbreeding, which can account for most of the overall inbreeding in a population (Marshall et al. 2002), may be responsible for the signs of inbreeding depression that we found.

We interpreted the decrease in yearling survival with increasing levels of homozygosity as evidence of inbreeding depression under a genome-wide effect (Hansson & Westerberg 2002). We advocate that this is likely for three main reasons. First, we found no significant single-locus effects (as in Hoffman et al. 2004; Charpentier et al. 2005; Ortego et al. 2007), although heterozygosity at some of the loci examined had stronger positive effects on juvenile survival than others. This result is not surprising, however, given that we used a fairly large number of loci of which none had a significant effect on survival after correcting for multiple tests. Second, our estimates of pairwise genetic relatedness were correlated with pedigree relatedness and parental estimated relatedness was positively correlated with offspring homozygosity, as expected for inbred matings. Third, our study population met the conditions in which H should be correlated with F, as mountain goats are found in small structured populations and exhibit a polygynous mating system (Balloux et al. 2004). At Caw Ridge, five males out of 57 sampled sired more than half of the yearlings genetically assigned over 12 years (Mainguy 2008), suggesting that many individuals could be related paternally. Thus, inbred matings stemming from a common paternal ancestor are likely, whereas father–daughter matings are much less likely. This is because most males start to reproduce intensely at 7–9 years of age only (Mainguy 2008), but rarely survive past 10 years (Festa-Bianchet & Côté 2008), whereas females are generally primiparous at 4–5 years of age (sometimes as late as 6 or 7 years), precluding them from mating with their fathers who are unlikely to be alive by that age. On the other hand, females are more likely to reproduce with a paternally related half-sib, as observed in our pedigree, due to the numerous offspring sired by a few mature dominant males (Mainguy 2008). Because of this polygynous mating system and likely limited genetic exchanges among populations, F is thus expected to correlate moderately to strongly with H in this alpine ungulate. Continued monitoring of the individuals of the Caw Ridge population will allow us to assess the extent of this relationship, as well as increasing our statistical power to study inbreeding depression.

To conclude, we used marker-based measures of relatedness and inbreeding to show an apparent lack or weak evidences for the presence of inbreeding avoidance, and subsequent inbreeding depression on yearling survival in a long-term study population of mountain goats. These findings suggest that despite generations of matings between relatives, mountain goats are still showing signs of inbreeding depression. This may thus represent an evolutionary stable state for this alpine ungulate, similarly to that found in insular metapopulations of birds which also exhibit signs of inbreeding depression partly generated by strong population structure (e.g. Jensen et al. 2007). A future and important area of research will be to quantify the genetic exchanges among different mountain goat populations, and assess their effects on population genetic diversity and dynamics.

Acknowledgements

Many people helped with fieldwork at Caw Ridge over the years and we thank all of them. M. Festa-Bianchet at the Université de Sherbrooke and K. G. Smith from the Alberta Natural Resources Service (ANRS) were pivotal in the inception of the Caw Ridge study and provided valuable logistical help, as did M. Ewald and D. Hobson from ANRS. We also thank A. S. Llewellyn and K. Worley for genotyping mountain goats captured in 1994–2003. We are grateful to C. Barrette, L. Bernatchez, L. E. B. Kruuk, and J. M. Pemberton for valuable discussions, and L. Bernatchez, M. Festa-Bianchet, D. Fortin, J.-M. Gaillard, A. G. McElligott, J. Poissant and an anonymous reviewer for comments that improved the quality of the manuscript. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Natural Environment Research Council (UK), ANRS, the Alberta Conservation Association (ACA), and Alberta Ingenuity. J. Mainguy was supported by NSERC, Fonds québécois de la recherche sur la nature et les technologies, Fonds Richard-Bernard, Fondation J.-Arthur-Vincent, and ACA grants in biodiversity scholarships.

References


J.M. conducted his PhD on mountain goats at Université Laval (Québec) with S.C. and D.C. He is now working as a wildlife biologist for the Québec Ministry of Natural Resources and Wildlife. His research interests include behavioural and molecular ecology. S.C. is a professor in the Biology Department of Université Laval and senior scientist at the Centre for Northern Studies. His research interests include behavioural ecology of large herbivores, evolution of life-history strategies, wildlife management, conservation biology and population genetics. D.C. is a professor in the Department of Biological Sciences of University of Alberta. His research centers on conservation genetics and ecological genomics of handsome ungulates and other wildlife.