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No evidence of inbreeding avoidance despite demonstrated survival costs in a polygynous rodent

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Abstract

Individuals are generally predicted to avoid inbreeding because of detrimental fitness effects. However, several recent studies have shown that limited inbreeding is tolerated by some vertebrate species. Here, we examine the costs and benefits of inbreeding in a largely polygynous rodent, the yellow-bellied marmot (*Marmota flaviventris*). We use a pedigree constructed from 8 years of genetic data to determine the relatedness of all marmots in our study population and examine offspring survival, annual male reproductive success, relatedness between breeding pairs and the effects of group composition on likelihood of male reproduction to assess inbreeding in this species. We found decreased survival in inbred offspring, but equal net reproductive success among males that inbred and those that avoided it. Relatedness between breeding pairs was greater than that expected by chance, indicating that marmots do not appear to avoid breeding with relatives. Further, male marmots do not avoid inbreeding: males mate with equal frequency in groups composed of both related and unrelated females and in groups composed of only female relatives. Our results demonstrate that inbreeding can be tolerated in a polygynous species if the reproductive costs of inbreeding are low and individuals that mate indiscriminately do not suffer decreased reproductive success.

Keywords: inbreeding, inbreeding avoidance, lethal equivalents, relatedness, reproductive success, yellow-bellied marmots

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Introduction

The extent to which an individual should inbreed depends on the costs of inbreeding relative to the costs of inbreeding avoidance (Waser *et al.* 1986; Kokko & Ots 2006). In a majority of studies, inbreeding has been shown to be prohibitively costly and thus avoided (Charlesworth & Charlesworth 1987; Pusey & Wolf 1996; Coltman *et al.* 1998; Keller & Waller 2002). Costs of inbreeding include lowered offspring birth weight (Coltman *et al.* 1998), decreased resistance to parasites (Acevedo-Whitehouse *et al.* 2003) and decreased reproductive success when offspring reach adulthood (Slate *et al.* 2000). Despite the costs, however, some species do not appear to discriminate against inbreeding (Bateson 1982; Part 1996; Keller & Arcese 1998; Hansson *et al.* 2007; Szulkin *et al.* 2009; Rioux-Paquette *et al.* 2010), and others may even seek inbred pairings (Schjørring & Jäger 2007; Thünken *et al.* 2007).

Inbreeding may be tolerated when some costs are low, such as a lack of deleterious fitness consequences with respect to offspring production or survival, as has been demonstrated in North American red squirrels (*Tamiasciurus hudsonicus*; Lane *et al.* 2007) and dwarf mongooses (*Helogale parvula*; Keane *et al.* 1996). Additionally, the inclusive fitness benefits gained in an inbred pairing are predicted to offset the costs of inbreeding in certain circumstances (Kokko & Ots 2006). For instance, an individual that accepts an inbred pairing receives benefits through direct fitness in the form of offspring, as well as indirect fitness from increasing the reproductive success of a relative (Kokko & Ots 2006). High costs of avoidance may also lead to inbreeding tolerance. Individuals that reject all inbred pairings may be forced to delay or forego reproduction entirely if no unrelated mates are

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available, especially in small or isolated populations (Pusey & Wolf 1996; Lehmann & Perrin 2003). High dispersal costs, either through increased mortality or decreased reproductive opportunities, may also favour inbreeding (Bengtsson 1978; Waser *et al.* 1986).

The prevention of excessive outbreeding, in which unrelated individuals from different populations reproduce, can also benefit individuals who inbreed. Outbreeding may cause locally adapted gene complexes, which can make an organism more suited to its immediate environment, to be disrupted (Lynch 1991). A further possible cost of outbreeding may be found in the representation of parental genes in the next generation, such that individuals that breed with relatives will contribute a comparatively greater amount of their own genes to the next generation than individuals that outbreed (Bateson 1983). Several studies have postulated a level of optimal inbreeding, in which individuals avoid outbreeding or close inbreeding, and prefer to mate with partners of intermediate relatedness (Bateson 1982; Hoogland 1992; Peacock & Smith 1997).

The costs and benefits of inbreeding are difficult to demonstrate in natural mammalian populations due to the difficulty of obtaining accurate pedigree information on wild populations. Many studies on inbreeding in the wild rely solely on measures of genetic heterozygosity as a proxy for inbreeding coefficients (Coltman *et al.* 1999; Amos *et al.* 2001). Several studies, however, have shown that the correlation between heterozygosity and inbreeding as determined by pedigrees is usually weak (Coltman & Slate 2003; Pemberton 2004; Slate *et al.* 2004). Pedigrees constructed from measures of relatedness obtained from microsatellite analysis are therefore generally more reliable (e.g. vonHoldt *et al.* 2008). Here, we use pedigree reconstruction supplemented with genetic pairwise relatedness estimates to examine the costs and benefits of male inbreeding in a wild population of yellow-bellied marmots in which inbreeding appears to be widespread.

Yellow-bellied marmots are semi-social rodents and live in geographically stable groups that vary in size from a male/female pair to several males and many females, as well as yearlings and juveniles (Allaine 2000; Olson & Blumstein 2010). Approximately 95% of male marmots and 50% of females disperse at 1 or 2 years of age (Armitage & Downhower 1974; Armitage & Schwartz 2000). Marmots of both sexes are capable of reproduction in their second year; however, fewer 2-year olds than expected mate successfully, possibly due to reproductive suppression (Armitage 2003). Marmots do not show evidence of paternal care; thus, males should have a greater tolerance for inbred pairings than females because of fewer lost reproductive opportunities when inbreeding (Kokko & Ots 2006). Previous research on this population of marmots, based solely on behavioural observations, has suggested both males and females achieve greater reproductive success when choosing to inbreed than forgoing reproduction (Armitage 2004).

Here, we investigate the costs and benefits that marmots accrue through breeding with relatives. We determine the reproductive costs of inbreeding to marmots through variation in offspring survival after weaning. We examine the relatedness of breeding pairs with respect to random expectations to determine whether marmots avoid mating with relatives. We then quantify the reproductive benefits accrued by inbreeding males using genetic parentage data and assess the likelihood of reproduction for males in social groups of varying relatedness to determine whether males forego reproduction to avoid inbreeding.

Methods

Pedigree construction

Our study site was located at the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA (38°57′N, 106°59′W). We collected hair and blood samples from marmots for DNA analysis from 2001 to 2008. DNA was extracted from hair using Qiagen™ DNA minikits following the protocol included in the kit (Qiagen Inc., Valencia, CA). We used 12 microsatellite primers developed for use in other sciurid species (May *et al.* 1997; Stevens *et al.* 1997; Goossens *et al.* 1998; Hanslik & Kruckenhauser 2000; da Silva *et al.* 2003; Kyle *et al.* 2004), multiplexed to include two primers per reaction. Multiplexed primers and DNA were amplified using polymerase chain reaction on a Peltier thermal cycler (MJ Research PTC-200) and genotyped on an ABI 3730 DNA capillary sequencer (Applied Biosystems). Alleles were analyzed using GeneMapper 3.0 software (Applied Biosystems). We found no significant deviations from Hardy–Weinberg equilibrium, and only a small percentage of geographic social groups displayed linkage disequilibrium (average 7% of locus pairs in a given year, range 2–13%), most likely due to population structure.

We genotyped a total of 997 individuals in 14 distinct geographic social groups, 819 of which were juveniles during the study period and for which both parents were identified. Average allelic diversity ranged from 9 to 22 alleles per locus (mean = 12.9), and average observed heterozygosity was 0.783 (range: 0.711–0.878). The combined non-exclusion probability, which represents the probability of two individuals sharing the same genotype by chance, was 1.15×10^{-16} (Kalinowski *et al.* 2007). We used the program Cervus 3.0 (Kalinowski *et al.* 2007) to assign parentage, which was then used to construct the pedigree. Cervus considers all possible mother/father pairs for a given offspring and calculates a likelihood score for each trio. Parentage is then assigned at a given confidence level by comparing this likelihood score to a critical value generated by simulation. We analyzed each year separately to generate accurate simulations of allelic diversity. Potential mothers were selected based on geographic social group residence and an index of nipple size (a measurement which reflects pregnancy and nursing). Candidate fathers were considered based on group residence. In addition, because male marmots are more likely to move among groups (Armitage 1974), fathers were divided into two distinct geographic groups, 'down valley' and 'up valley', separated by approximately 5 km, which represents a natural break in the marmot population. All offspring from each geographic group were given the same list of potential fathers. The majority of parent assignments were made at the 95% confidence level; five offspring were assigned parentage with 80% confidence. For 17 offspring, genetic samples were not available and parentage was assigned based solely on behavioural observations. Further details on pedigree construction are reported in Blumstein *et al.* (2010).

The final reconstructed pedigree consisted of 149 reproductive pairs and their 819 offspring (1–29 offspring per pair), with a maximum depth of five generations and an average depth of three generations per offspring. When individuals of unknown origin immigrated into a social group, their relatedness to others was assessed using pairwise relatedness calculated by KINGROUP 2.0, a program that uses methods developed by Queller & Goodnight (1989) to calculate the level of genetic similarity between two individuals (Konovalov *et al.* 2004). To verify that the relatedness values generated by KINGROUP were comparable to those produced by the pedigree, we calculated the relatedness coefficient (using KINGROUP) for individuals whose relationships were known from the pedigree and compared these values for 100 randomly chosen pairs of parent/offspring, full sibling and half-sibling dyads. Relatedness values generated with this method for parent/offspring pairs averaged 0.48 (SD = 0.13), full siblings averaged 0.48 (SD = 0.16) and half-siblings averaged 0.29 (SD = 0.19).

To determine the overall level of inbreeding that occurred in the population, we counted each offspring for which the required relatives to determine both close and moderate inbreeding events were known, as described in Marshall *et al.* (2002). Close inbreeding (inbreeding coefficient $f = 0.25$) includes parent/offspring and full sibling pairs, while moderate inbreeding events ($f = 0.125$) are those between grandparent/grandchild, aunt/nephew, uncle/niece and half-siblings. We then counted the number of actual pairings observed for each type of inbreeding event and from this calculated their frequency of occurrence. To control for immigration of unknown individuals into the study area, we used the equation provided by Marshall *et al.* (2002), which discounts the rates of each type of inbreeding event by the proportion of unknown reproductive males in the population. To compute frequencies of total, close and moderate inbreeding, we again used an equation provided by Marshall *et al.* (2002), which assumes that rates of inbreeding are independent of each other.

Offspring survival

To determine whether costs associated with inbreeding depression were likely to influence an individual's decision to inbreed, we examined the lifetime survival after weaning of inbred and outbred individuals. Survival prior to weaning could not be determined because juvenile marmots do not leave the natal burrow until weaned (Frase & Hoffmann 1980). Marmots disperse during June or July at 1 or 2 years of age (Van Vuren 1990). We began live-trapping by mid to late May in all years; thus, we were able to census the yearly survival of 1- or 2-year olds prior to dispersal. Because the fate of dispersing individuals was not followed, we examine local survival, i.e., the probability of staying in the group and surviving. We used observation and live-trapping data (methods detailed in Armitage & Johns (1982) and Olson & Blumstein (2010)) from field work performed in May–August 2001–2002, April–August 2003 and April–September 2004–2008.

We used a proportional odds discrete-time survival model because marmot survival was censused once per year (Allison 1982) when the animal was first live-trapped. Discrete-time survival analysis splits each individual by the number of time periods the individual was present in the analysis, with the dependent variable a binary indicator of whether or not the subject died during the study. We used a logistic regression and included seven dummy variables indicating whether or not an individual had died in a given time period (no individuals occurred in the data set for more than 7 years). We clustered the analysis on the individual, which adjusts the estimates of standard errors to account for non-independence between individuals. We performed univariate analyses to test the significance of possible covariates before including them in the multivariate model; possible covariates included inbreeding coefficient, colony, sex and year of birth. Statistical analysis for this and all other analyses were performed using STATA 10 (StataCorp 2007). Tests were deemed significant when $P < 0.05$.

To further quantify the effects of inbreeding on survival, we calculated the number of lethal gene equivalents in the genome resulting from inbreeding. Lethal equivalents estimate the number of recessive alleles in the genome that, when homozygous, will cause death (Templeton & Read 1984; Keller *et al.* 2002). To determine the number of lethal recessive gene equivalents (*B*) (Morton *et al.* 1956), we divided individuals into six inbreeding classes based on inbreeding coefficient as determined by pedigree and calculated the probability of survival for offspring in each class by dividing the number of surviving inbred or outbred offspring by the total number known to be born (Keller 1998). We performed a weighted least squares regression equation to explain variation in the natural log of offspring survival from both zero to 1 and zero to 2 years as a function of an offspring's inbreeding coefficient. The slope of this line indicates the number of lethal equivalents in the gamete (Morton *et al.* 1956). The significance of the *B* value was tested using methods outlined in Templeton & Read (1984).

Reproductive benefits

To determine whether inbreeding tolerance conveys reproductive benefits, we examined the annual reproductive success of all adult males in the population that produced offspring ($N = 44$). We determined the reproductive benefits of inbreeding tolerance by quantifying the net number of offspring for males that did not inbreed vs. those males that did inbreed or performed a mixed strategy. To determine the net number of offspring produced, we multiplied inbred and outbred offspring by their respective probabilities of survival, as calculated earlier (1 year survival: 0.625 outbred and 0.323 inbred; 2 year survival: 0.594 outbred and 0.282 inbred). We calculated this value for survival to both 1 and 2 years of age. Reproductive success between males that mated with relatives and those that did not was compared using a non-parametric Mann–Whitney *U* test. Variance in male reproductive success was calculated by quantifying the total number of offspring each male produced during the study period, and the mode was used to determine the number of offspring per male most frequently represented in the population.

Inbreeding avoidance

To evaluate whether male marmots forego reproduction in order to avoid inbreeding, we examined the reproductive decisions of males in three different types of social groups. There were 104 unique males in the data set. However, individuals were present multiple years and in multiple groups per year. Thus, we defined the unit of analysis as the group-year, which is a given social group in a given year. With respect to a specific male, groups consisted of only related females ($N = 48$ group-years), only unrelated females ($N = 54$ group-years) or both related and unrelated females ($N = 145$ group-years). Group membership was inferred if a male was seen in a social group during the breeding season, or if a male was determined to have parented offspring there based on genetic analysis ($N = 22$ group-years).

We fitted a logistic regression clustered by group-year with breeding (reproduce or not) as the dependent variable and group type (only relatives, no relatives, mixed) as a covariate. Because group type is a categorical variable, we converted two of the categories (mixed and no relatives) into dummy variables and compared them to the third group type (only relatives), which we used as a reference category. We also included the male-to-female sex ratio of the group, the group size and the proportion of females a male was related to in the group as covariates.

To determine whether females avoided mating with relatives, we compared the observed relatedness of actual reproductive pairs to simulated relatedness values generated by pairing a given reproductive female with all potential reproductive males. Potential mates were determined based on geographic proximity, using the same two geographically distinct groups, 'down valley' and 'up valley', as previously mentioned. We performed a randomization procedure to determine whether the average relatedness of reproductive pairs differed from that expected by random mating. We used data from only 2003 to 2008 for this analysis, because the pedigree for 2001 and 2002 was fairly sparse compared to other years. For each year, we randomly drew pairs with replacement from among all possible combinations of females and males in each geographic group and calculated the mean relatedness of all chosen reproductive pairs. Because 2-year-old males may be less reproductively competitive than older males, we performed separate analyses on two data sets: one with all males 2 years and older and one with males 3 years and older.

Randomizations were performed in MATLAB v.7.12 (2007, The MathWorks, Natick, MA, USA) using the built-in 'randsample' function. We performed 10 000 iterations for each year, with the number of pairs selected at each iteration determined by the number of offspring produced in each area. The mean relatedness of potential parent pairs was calculated at each iteration, and we considered females in a group to have avoided inbreeding when the observed mean pair relatedness was below the lowest 5% of the 10 000 simulated mean relatedness values (Rioux-Paquette *et al.* 2010).

The proportion of breeding males that were immigrants during the study was 0.17 in the 'down valley' sites and 0.26 in the 'up valley' sites. The relatedness of these immigrants to other reproductive-age individuals could not be determined by the pedigree. Therefore, to insure consistent relatedness values between adults of known and unknown ancestry for this analysis, we used the program Kingroup 2.0 to determine relatedness values between all male/female dyads in each social group.

Results

The total frequency of inbreeding, as measured by Marshall *et al.* (2002), for all social groups combined was 13.9%, with close inbreeding events (father/daughter, mother/son and full siblings) making up 7.2% (Table 1). Moderate inbreeding events included paternal half-siblings, maternal grandmother/grandson, and maternal aunt/nephew and also occurred at a frequency of 7.2%. When the proportion of immigrant males in the population was taken into account, the adjusted overall frequency of inbreeding was estimated more conservatively at 10.9% (Table 1). The adjusted frequencies may be excessively conservative however, because it is not possible to say with certainty whether the immigrant individuals were completely unrelated to all individuals in the social group (Marshall *et al.* 2002). The most frequent type of inbreeding event was father/daughter, occurring in 25 pairings (6.6%), with paternal half-siblings the second most frequent, with 15 occurrences. There were many more known opportunities for half-sibling pairings however, so that the frequency of half-sibling inbreeding was only 2.2% (Table 1).

Offspring survival

We found that as inbreeding coefficient increased, offspring survival decreased (Coeff = 2.33, $z = 3.67$, $P < 0.001$) (Table 2). The social group a marmot was in, as well as the year of its birth, did not have significant effects on its survival; however, sex was a significant indicator, with females surviving longer than males (Coeff = 0.39, $z = 3.47$, $P < 0.001$).

In the weighted least squares regression of the natural log of marmot survival on inbreeding coefficients, we found the equivalent of 3.09 lethal recessive genes (SE = 0.68, $r^2 = 0.84$) in the haploid genome of inbred marmots that survived from zero to 1 year. We found 4.21 lethal recessives (SE = 1.54, $r^2 = 0.71$) in inbred marmots that survived from zero to 2 years. Both of these estimates were significantly greater than the case of no inbreeding depression (i.e. 0 lethal equivalents) (1 year: $N = 6$, $t = 3.74$, $P = 0.02$; 2 years: $N = 5$, $t = 3.46$, $P = 0.04$).

Reproductive benefits

Males that mated with relatives achieved annual reproductive success similar to males that did not (Table 3).

Table 1 Frequency of the occurrence of each type of inbreeding event out of the total times a given event could have been detected from the marmot pedigree data, 2001–2008

	Up valley			Down valley			All groups		
	Frequency	Raw (%)	Adjusted (%)	Frequency	Raw (%)	Adjusted (%)	Frequency	Raw (%)	Adjusted (%)
Father/daughter	12/181	6.6	4.9	13/196	6.6	5.5	25/377	6.6	5.1
Mother/son	0/242	0	0	1/214	0.5	0.4	1/456	0.2	0.2
Full siblings	1/404	0.2	0.2	2/404	0.5	0.4	3/808	0.4	0.3
Paternal half-siblings	4/390	1.0	0.8	11/341	3.2	2.7	15/731	2.1	1.6
Maternal half-siblings	0/243	0	0	0/332	0	0	0/575	0	0
Paternal grandmother/grandson	0/61	0	0	0/29	0	0	0/90	0	0
Maternal grandmother/grandson	4/170	2.4	1.7	1/133	0.8	0.6	5/303	1.7	1.3
Paternal grandfather/granddaughter	0/38	0	0	0/22	0	0	0/60	0	0
Maternal grandfather/granddaughter	0/119	0	0	0/144	0	0	0/263	0	0
Paternal uncle/niece	0/40	0	0	0/22	0	0	0/62	0	0
Maternal uncle/niece	0/90	0	0	0/110	0	0	0/200	0	0
Paternal aunt/nephew	0/37	0	0	0/23	0	0	0/60	0	0
Maternal aunt/nephew	9/111	8.1	6.0	0/132	0	0	9/243	3.7	2.9
Double first cousin	0/57	0	0	0/34	0	0	0/91	0	0
Total measured inbreeding		17.3	12.9		11.2	9.3		13.9	10.9
Close inbreeding		6.9	5.0		7.5	6.2		7.2	5.6
Moderate inbreeding		10.3	7.6		4.0	3.3		7.2	5.6

Frequencies were calculated for the entire population (All Groups), only the 'Up Valley' groups, and only the 'Down Valley' groups. Adjusted values represent inbreeding frequencies after controlling for immigrants of unknown ancestry.

Table 2 Probability of survival for outbred ($f = 0$) to increasingly inbred ($f = 0.375$) marmot offspring, from 1 to 7 years of age

Inbreeding coefficient	*N* offspring	1	2	3	4	5	6	7
0	474	0.625	0.323	0.208	0.170	0.144	0.117	0.088
0.063	93	0.594	0.282	0.169	0.133	0.109	0.085	0.059
0.125	74	0.520	0.208	0.109	0.080	0.062	0.045	0.028
0.188	32	0.445	0.148	0.066	0.044	0.032	0.021	0.012
0.250	134	0.373	0.100	0.038	0.023	0.015	0.009	0.004
0.375	3	0.246	0.041	0.010	0.005	0.002	0.001	0.000

Number of offspring produced at each level of inbreeding is also shown.

There was no difference in gross number of offspring (offspring surviving to weaning) or net number of offspring (number of offspring discounted by survival to 1 or 2 years of age) between males whose offspring included either all inbred or a mix of inbred and outbred offspring vs. males who avoided inbreeding (Mann–Whitney *U*: total offspring: $N = 42$, $U = 0.30$, $P = 0.76$; 1 year survival: $N = 42$, $U = 1.15$, $P = 0.25$; 2 year survival: $N = 42$, $U = 1.36$, $P = 0.17$). We also found considerable variance in annual reproductive success among adult males, such that the modal number of offspring per adult male when all adult males in the population were considered was 0 (range: 0–115, $N = 104$), and only 42% of adult males in the population ever reproduced.

Inbreeding avoidance

During the 8-year study period, we found that inbreeding between kin at the level of first cousins or greater ($f > 0.0625$) occurred at least once during the breeding tenure of 59% of adult males that bred in the study population (26 out of 44 males). The type of social group a male was in (relatives, no relatives or mixed) was not a significant predictor of a male's breeding behaviour with respect to kin (Table 4). There was no difference in a male's breeding likelihood as a function of the number of related females in his group, but group sex ratio and group size were both significant predictors of breeding (Table 4). Males were less likely to breed when there were more males per female and when group sizes were large.

Table 3 Mean and range of number of offspring that survived to weaning, 1, and 2 years produced by male marmots that inbred or performed a mixed strategy ($N = 26$) and males that outbred only ($N = 18$)

	Inbred/mixed strategy			Outbred only		
	Mean	Range	95% CI	Mean	Range	95% CI
Weaning	19.8	1–115	8.92–30.85	12	2–24	8.21–15.79
1 Year	12.0	0.6–68	4.02–13.34	7.5	1.3–15	5.13–9.87
2 Year	5.9	0.3–32	3.65–12.22	3.9	0.6–7.8	4.88–9.38

Based on the results from the randomization procedure, female marmots do not appear to avoid breeding with close relatives. The average number of males 2 years and older present in a given year in the 'down valley' group was 11 (range: 6–14), and the average number of females 2 years and older was 23 (range: 11–33). Thus, the number of potential random pairings ranged from 66 to 462. In the 'up valley' group, average number of males per year was 20 (range: 13–32) and the average number of females per year was 37 (range: 20–47). The number of potential pairings for this group ranged from 260 to 1504. Table 5 shows the distribution of simulated parent–pair relatedness values compared to the relatedness between reproductive pairs actually observed in the population each year (Rioux-Paquette *et al.* 2010).

The observed mean pair relatedness values for the 'up valley' group were higher than the top 95% of mean simulation values for 5 of the 6 years examined, indicating that reproductive pairs were more related

Table 4 Effects of group type (mixed and no relatives compared to only relatives as the reference group), male-to-female group sex ratio, proportion of females related to a male out of all females in the group and group size on a male marmot's breeding likelihood

Variable	Regression coefficient	Z-score	*P*-value	95% confidence interval
No relatives	0.21	0.34	0.73	−0.98 to 1.39
Mixed	0.23	0.53	0.59	−0.62 to 1.09
Sex ratio	−1.15	−4.19	**0.001**	−1.69 to −0.61
Proportion related	−0.63	−1.61	0.11	−1.40 to 0.14
Group size	−0.06	−4.39	**0.001**	−0.08 to −0.03
Intercept	1.48	2.51	0.01	0.33 to 2.64

Significant variables indicated in bold.

Table 5 Per cent distribution of relatedness values for all potential parent pairs of yellow-bellied marmots

Year	$r = 0$	$0 > r < 0.125$	$0.125 \le r < 0.25$	$0.25 \le r < 0.375$	$0.375 \le r < 0.5$	$0.5 \le r < 1$
2003	54.6 (53.8)	21.1 (22.1)	13.1 (5.8)	5.0 (6.7)	3.9 (10.6)	2.4 (1.0)
2004	54.2 (38.1)	22.2 (20.6)	9. 8 (15.3)	5.2 (10.3)	5.8 (10.7)	2.8 (5.0)
2005	45.9 (41.3)	24.6 (22.8)	15.8 (12.4)	5.8 (7.7)	4.7 (7.7)	3.2 (8.1)
2006	46.8 (41.4)	25.2 (20.1)	16.4 (13.7)	6.1 (8.2)	2.7 (9.2)	2.9 (7.4)
2007	39.2 (48.1)	23.9 (19.6)	17.9 (12.2)	7.4 (8.0)	6.2 (5.3)	5.5 (6.8)
2008	49.7 (31.6)	22.8 (26.5)	12.3 (16.2)	7.1 (11.6)	4.2 (8.3)	3.8 (5.7)

Actual per cent of parent–pair relatedness values for each category are given in parentheses.

than expected by random mating, and not different than expected in 1 year (Fig. 1). Actual reproductive pairs' mean relatedness for all years in the 'up valley' group was 0.09 (SD = 0.06), while the simulated expected relatedness was 0.02 (SD = 0.02). In the 'down valley' group, mean reproductive pair relatedness was higher than expected for 3 years and not different from random mating in 3 years. The mean actual relatedness for this group was 0.19 (SD = 0.06), while the simulated expected relatedness was 0.15 (SD = 0.03). The age of males included when considering potential reproductive pairs had no effect on the results.

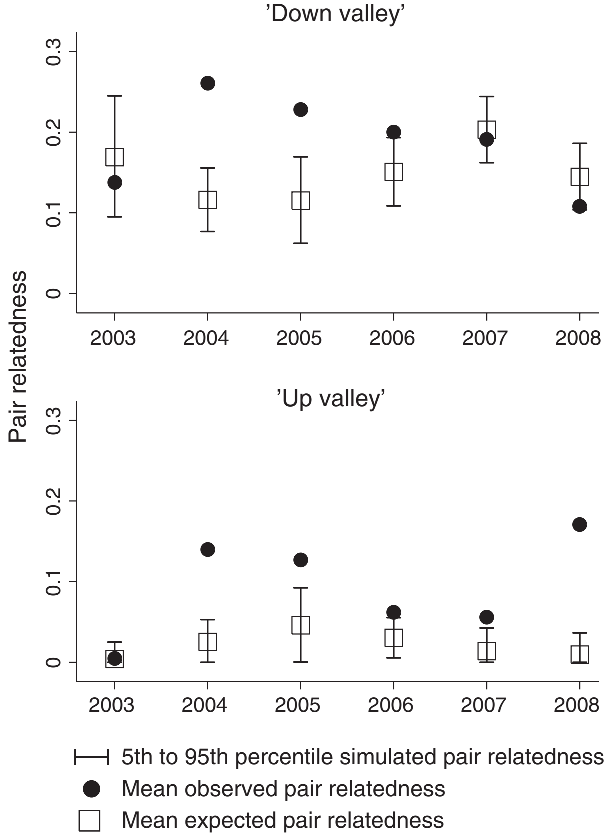

Fig. 1 Observed relatedness values between reproductive pairs of yellow-bellied marmots were higher than the 95th percentile of simulated expected relatedness values for eight of the 12 group-years studied.

Discussion

Yellow-bellied marmots in our study population do not appear to avoid inbreeding, and in fact may demonstrate a preference for inbred pairings, despite demonstrated survival costs to inbred offspring. Relatedness between reproductive pairs was higher than that expected from random mating during a majority of the years studied, indicating that female marmots do not routinely discriminate against inbred pairings. Additionally, individual reproductive output was similar for males that inbred and for those that did not (Table 3), despite the demonstrated decreased survival of inbred offspring (Table 2). Thus, the benefits of inbred pairings for marmots appear to outweigh, or at least equal, the costs.

Moderate inbreeding in a mammal can be a successful strategy to achieve reproductive success when it is not negated by costs such as decreased survival of offspring or lost reproductive opportunities. Male mammals especially are predicted to have a higher tolerance for inbreeding, because they invest less in each pairing and therefore stand to lose fewer reproductive opportunities when they accept an inbred pairing (Wheelwright *et al.* 2006). Alternatively, males may be more tolerant of inbreeding because of reproductive skew, in which few males achieve the majority of reproductive success, while most males achieve little or none (Johnstone 2000). In this case, a male may be more likely to accept an inbred pairing because the cost of inbreeding avoidance in missed reproductive opportunities would be great (Kokko & Ots 2006). In yellow-bellied marmots, the proximate factor favouring inbreeding in males appears to be the large variance in reproductive success, because most males in our study population achieved little to no reproduction. Therefore, males that mated with relatives did not suffer decreased annual reproductive success, even after accounting for decreased offspring survival due to inbreeding depression, relative to the majority of other males in the population.

Females are predicted to have a lower tolerance for inbreeding owing to greater parental investment (Waser *et al.* 1986; Kokko & Ots 2006) and fewer lifetime reproductive opportunities than males, which results in selection for offspring to be as fit as possible (Trivers 1972; Kokko & Ots 2006). Female marmots in our study did not appear to discriminate against inbreeding, however, and actual relatedness between reproductive pairs was higher than expected from simulations for the majority of years examined. The prevalence of reproduction between individuals that are more related than expected by random chance may indicate a preference for inbred pairings in this marmot population. The mean relatedness of observed pairings in both 'up valley' and 'down valley' (r = 0.09 and 0.19, respectively) is consistent with low levels of inbreeding (Marshall *et al.* 2002). The relatedness of pairings expected by chance differed greatly between geographic groups, with 'up valley' expected to have fairly low (r = 0.02) relatedness and 'down valley' to have fairly high (r = 0.15). That both groups had observed pairings consistent with a fairly similar level of relatedness suggests that marmots may be optimizing their level of inbreeding, as has been reported for other species (Bateson 1982; Hoogland 1992; Peacock & Smith 1997).

The composition of the social group may also play a role in a marmot's likeliness to inbreed. For instance, groups with males with long reproductive tenures would be more likely to be composed of relatives, especially father/daughter pairs or grandfather/granddaughter pairs, because males are the primary dispersing sex (Van Vuren & Armitage 1994). Our analysis of the potential routes to inbreeding found that father/daughter inbreeding events occurred in 6.6% of all individuals for whom this pairing could have been detected. This was the highest frequency of any single inbreeding event. Interestingly, aunt/nephew pairings were also fairly frequent, at 3.7%. This pairing is likely to occur when juvenile males remain philopatric, which should be fairly rare among polygynous mammals (Dobson 1982). The relatively low frequencies of these inbreeding events reflect the fact that while few individuals reproduce in each group in a given year, many offspring are produced. Thus, while many known routes to inbreeding exist, the proportion of inbred pairings that actually occur is relatively small. The differences in the frequencies of inbreeding events may represent a conflict of interest between the sexes for acceptable levels of inbreeding. Theory predicts that a trait may persist that is harmful to one sex but beneficial to another (Waser *et al.* 1986; Parker 2006). In such situations, males are predicted to favour inbreeding, and because older animals are more likely to prevail in a conflict of interest, father/daughter inbreeding should be seen more often than mother/son (Waser *et al.* 1986). Our data support this prediction, with 25 instances of father/daughter mating out of 72 group-years with fathers and daughters in the same group, and one instance of mother/son mating in 62 group-years with mothers and sons in the same group.

Despite the lack of inbreeding avoidance demonstrated by yellow-bellied marmots, the lethal equivalents found in our study are greater than 80% of the estimates of lethal equivalents in a study on captive mammals (range: 0.68–15.16) (Ralls *et al.* 1988). Consequently, inbreeding appears to exact a significant cost in survival for offspring that is consistent with inbreeding depression. Yet, we did not find evidence to indicate that marmots adjust their frequency of reproduction to avoid inbreeding. When in social groups with only related females, male marmots do not forego mating opportunities, but instead mate at similar frequencies to males that have the opportunity to avoid inbreeding. This result is contrary to that found in many other species in which inbreeding has been examined (Wolff *et al.* 1988; Sillero-Zubiri *et al.* 1996). Our results support the theory, however, that inbreeding can be tolerated if the benefits gained by a lack of inbreeding avoidance outweigh the costs imposed by inbreeding depression and lost opportunities to outbreed (Waser *et al.* 1986; Kokko & Ots 2006). The absence of inbreeding avoidance in this marmot population suggests that the cost of inbreeding may be lower than the reproductive benefit individuals gain by tolerating it.

Inbreeding is common in plants and eusocial species (Jain 1976; Reeve *et al.* 1990; Keller & Waller 2002), but is rare in mobile vertebrates with small population sizes where inbreeding will rapidly result in substantial inbreeding depression (Pusey & Wolf 1996; vonHoldt *et al.* 2008). Our results, based on both observational and genetic data, indicate that the benefits marmots receive from inbred pairings outweigh the costs. Therefore, though we found that the survival costs of inbreeding were high, we conclude that they must be lower than some threshold that would make inbreeding intolerable. We suggest inbreeding in mammals should be modelled as phenomena with costs and benefits and may be a viable strategy for managing populations existing in small and fragmented habitats or in captivity. Given the theoretical expectation that inbreeding should be more common than reported (Kokko & Ots 2006), we expect that detailed analyses, like the one presented here, will find more evidence of inbreeding in natural populations.

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Data accessibility

Data deposited in the Dryad repository: doi:10.5061/dryad.fg4bc331.