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Temporal dynamics of genetic variability in a mountain goat (*Oreamnos americanus*) population

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Abstract

The association between population dynamics and genetic variability is of fundamental importance for both evolutionary and conservation biology. We combined long-term population monitoring and molecular genetic data from 123 offspring and their parents at 28 microsatellite loci to investigate changes in genetic diversity over 14 cohorts in a small and relatively isolated population of mountain goats (*Oreamnos americanus*) during a period of demographic increase. Offspring heterozygosity decreased while parental genetic similarity and inbreeding coefficients (F_{IS}) increased over the study period (1995–2008). Immigrants introduced three novel alleles into the population and matings between residents and immigrants produced more heterozygous offspring than local crosses, suggesting that immigration can increase population genetic variability. The population experienced genetic drift over the study period, reflected by a reduced allelic richness over time and an 'isolation-by-time' pattern of genetic structure. The temporal decline of individual genetic diversity despite increasing population size probably resulted from a combination of genetic drift due to small effective population size, inbreeding and insufficient counterbalancing by immigration. This study highlights the importance of long-term genetic monitoring to understand how demographic processes influence temporal changes of genetic diversity in long-lived organisms.

Keywords: genetic diversity, genetic drift, heterozygosity, inbreeding, relatedness, ungulate

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Introduction

In finite and closed populations, inbreeding increases over time at a higher rate in small than in large populations (Frankham *et al.* 2002). Genetic drift in finite populations leads to the loss of genetic diversity, fixation of alleles within populations and genetic differentiation after population fragmentation (Falconer & Mackay 1996; Frankham *et al.* 2002). For these reasons, small and isolated populations often show reduced levels of allelic and genotypic variability in comparison with large and admixed populations (Falconer & Mackay 1996; Frankham 1997; Frankham *et al.* 2002; White & Searle 2007; Ortego *et al.* 2008). Accordingly, several studies have documented a lower genetic diversity after population fragmentation and demographic bottlenecks (Groombridge *et al.* 2000; Keller *et al.* 2001; Taylor *et al.* 2007; Broquet *et al.* 2010). Changes in allele frequencies over time due to genetic drift are also expected to result in an increasing genetic differentiation with time between cohorts (i.e. isolation-by-time *sensu* Demandt 2010), a pattern which is also expected to be more pronounced in small populations (Leberg 2005). Effective population size (N_e) is usually one of the most important factors determining the levels of genetic variability in natural populations, a parameter which is generally smaller than census size (N) due to several factors such as unequal sex ratios, mating systems, overlapping generations or fluctuations in population size (Frankham

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et al. 2002; Leberg 2005). Genetic impoverishment due to small effective population size and isolation can reduce fitness (Charlesworth & Charlesworth 1987; Falconer & Mackay 1996) and the ability of populations to respond to changing environmental conditions (Willi *et al.* 2006), ultimately compromising population viability (Saccheri *et al.* 1998; Frankham 2005; Pertoldi *et al.* 2007). For these reasons, the study of genetic variability in natural populations is a central topic in both evolutionary and conservation biology (Lande 1988; Frankham 1996, 2003; Hedrick 2001).

Population dynamics (e.g. demographic fluctuations, bottlenecks, immigration, etc.) can also have profound effects on the levels of genetic variability in natural populations. Populations experiencing fluctuating dynamics or population bottlenecks are expected to show lower levels of genetic diversity than stable populations (Motro & Thomson 1982; Kaeuffer *et al.* 2007a). Patterns of immigration are also expected to have important consequences on the levels of genetic diversity within a population: genetic variability can decrease over time in isolated populations (i.e. in absence of immigration) even when they undergo demographic expansions (e.g. decrease in observed heterozygosity, Bensch *et al.* 2006), whereas the arrival of immigrants can increase the genetic diversity of populations experiencing strong demographic declines (e.g. enhanced allelic diversity, Ruokonen *et al.* 2010). Thus, the negative genetic consequences of population fragmentation can be compensated by the 'rescue' effect of immigration (Ingvarsson 2002; Tallmon *et al.* 2004; Hedrick 2005). Accordingly, some studies have reported a progressive genetic recovery (i.e. more heterozygous offspring) after the arrival of sometimes just a few immigrants carrying novel alleles (Hansson *et al.* 2000; Keller *et al.* 2001; Vilà *et al.* 2003; Ortego *et al.* 2007; Johnson *et al.* 2010; Ruokonen *et al.* 2010). Particularly in genetically depauperate populations, effective immigration (i.e. the arrival of immigrants genetically contributing to the next generation) can increase the genetic variability of local populations, prevent inbreeding depression and lead to demographic recovery (Westemeier *et al.* 1998; Madsen *et al.* 1999; Vilà *et al.* 2003; Hogg *et al.* 2006; Johnson *et al.* 2010). On the other hand, selection against homozygous individuals can increase or maintain genetic variability in the absence of immigration (Coltman *et al.* 1999; Bensch *et al.* 2006; Kaeuffer *et al.* 2007b).

The impact of population dynamics on genetic variability should vary with lifespan: a long generation time can buffer temporal changes in population genetic diversity or structure (Petit *et al.* 2002; Hailer *et al.* 2006). Therefore, long-term studies are necessary to understand the factors modulating genetic diversity in long-lived organisms. However, there have been few attempts to assess the links between demography and temporal dynamics of genetic variability in long-lived wild organisms, probably because such studies require logistically challenging long-term demographic and genetic surveys. Longitudinal studies of intensively monitored populations allow the comparison of genetic patterns with detailed demographic data over time, information not usually available in most comparative studies considering different populations sampled at the same time (Kaeuffer *et al.* 2007b).

We analysed the temporal dynamics of genetic variability in a population of mountain goats, *Oreamnos americanus*, a long-lived alpine ungulate (generation time = 8 years) with a fragmented distribution (Festa-Bianchet & Côté 2008; Shafer *et al.* 2011) and low genetic variability at both neutral (Mainguy *et al.* 2005; Poissant *et al.* 2009) and coding loci (Mainguy *et al.* 2007). The study population at Caw Ridge, west-central Alberta, Canada, has been intensively monitored over two decades (Festa-Bianchet & Côté 2008). This population is one of the largest in Alberta and is geographically isolated from other populations by 20 km or more (Hamel *et al.* 2006). Although the population is geographically isolated (Hamel *et al.* 2006), effective immigration has been documented (Festa-Bianchet & Côté 2008). Over the last 20 years, the number of adults (≥3 years old) increased by 53%, from 46 in 1991 to 98 in 2008 (Fig. 1). Adult sex ratio also changed over time, from a proportion of males to females of 0.44 in 1991 to 0.75 in 2008 (Fig. 1). Thus, this population offers an interesting system to study the relative influence of genetic drift, immigration and demographic changes on population genetic variability and structure.

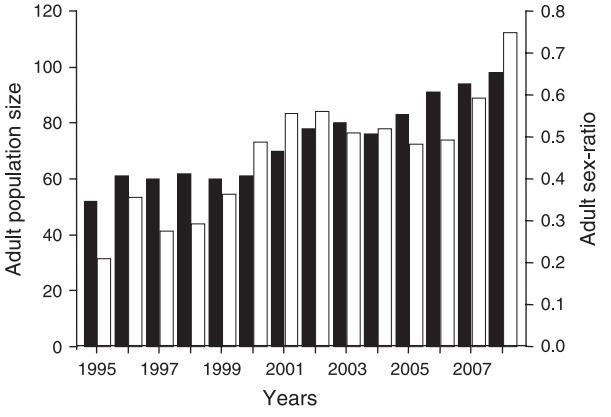

Fig. 1 Adult population size (black bars, left *y*-axis) and adult sex ratio (open bars, right *y*-axis) over 14 years in the mountain goat (*Oreamnos americanus*) population at Caw Ridge, Alberta, Canada.

We combined information from long-term population monitoring and extensive microsatellite data to estimate effective population size (N_e) and analyse patterns of genetic diversity over 14 cohorts. We expected a low effective population size (N_e) due to small census population size (N_c), low effective immigration rates and a polygynous mating system (Festa-Bianchet & Côté 2008; Mainguy *et al.* 2009a), and tested the following predictions: (i) although adult population size and sex-ratio increased over time, offspring heterozygosity (H_O), allelic richness (A_R) and expected heterozygosity (H_E) have decreased, and mate relatedness and inbreeding coefficients (F_{IS}) increased over the study period due to low N_e; (ii) immigrants have carried novel alleles into the population and matings between immigrant and residents have resulted in more heterozygous offspring; and (iii) due to the population isolation and low N_e, genetic drift has led to an 'isolation-by-time' pattern of genetic structure (Bunje *et al.* 2007; Calderón *et al.* 2009; Demandt 2010).

Methods

Study area

Caw Ridge (54°N, 119°W), west-central Alberta, Canada, is in the front range of the Rocky Mountains. The mountain goat population uses about 28 km^2 of alpine tundra, small cliffs and open subalpine forest from 1750 to 2170 m altitude. The population has been intensively studied since 1989 (Festa-Bianchet & Côté 2008). A few immigrant males are occasionally observed, and emigration by individuals born at Caw Ridge has also been documented (Festa-Bianchet & Côté 2008).

Population monitoring and sampling

Mountain goats were captured from late May to mid-September 1989–2009 in traps baited with salt (Festa-Bianchet & Côté 2008). Because of the risk of abandonment, after 1997 we did not capture kids and most of our cohort-specific data are from animals first captured in their second year of life (i.e. yearlings). We refer to a cohort as a group of individuals born in the same year. All individuals were marked with a unique combination of plastic ear tags and visual or radio-collars. From 1994 to 2009 a tissue sample was taken from each captured goat using an ear punch and kept in a solution of 20 percent dimethylsulfoxide saturated with NaCl at −20°C. Goats were observed with spotting scopes (15–45×) most days from mid-May to mid-September. For each group sighted, we noted the identity of all individuals and determined whether females were lactating by observations of nursing behaviour (Côté & Festa-Bianchet 2001).

Microsatellite genotyping and basic genetic statistics

Genomic DNA was extracted from ear tissue with QIAGEN DNeasy extraction kits. We amplified 28 polymorphic microsatellite loci (Mainguy *et al.* 2005; Table 1). Twenty six of these microsatellite loci are located in 17 different chromosomes, whereas loci RT9 and RT27 have not been yet assigned to any chromosome (Table 1). We followed the protocols described in Mainguy *et al.* (2005) and obtained an overall genotyping success of 99.98% (Mainguy *et al.* 2009a,b). Microsatellite markers were tested for departure from Hardy–Weinberg equilibrium using an exact test (Guo & Thompson 1992) based on 900 000 Markov chain iterations as implemented in the program ARLEQUIN 3.1 (Excoffier *et al.* 2005). We also used ARLEQUIN 3.1 to test for linkage equilibrium within each pair of loci using a likelihood-ratio statistic, whose distribution was obtained by a permutation procedure (Excoffier *et al.* 2005). Bonferroni corrections were applied to account for multiple comparisons.

Table 1 Microsatellite loci used to genotype mountain goats: chromosome location, number of alleles (K), expected heterozygosity (H_E) and observed heterozygosity (H_O) for each locus

Locus	Chromosome location	K	H_E	H_O	Primer origin
MAF64	1	2	0.41	0.45	Crawford *et al.* (1995)
URB038	1	4	0.66	0.68	Crawford *et al.* (1995)
BM6444	2	4	0.55	0.50	Bishop *et al.* (1994)
AR028	2	4	0.47	0.50	Crawford *et al.* (1995)
McM64	2	2	0.44	0.45	Crawford *et al.* (1995)
TGLA10	2	5	0.40	0.41	Crawford *et al.* (1995)
HUJ1177	3	2	0.43	0.44	Barendse *et al.* (1994)
OARCP26	4	4	0.45	0.49	Crawford *et al.* (1995)
OARHH35	4	3	0.54	0.55	Crawford *et al.* (1995)
McM527	5	4	0.50	0.52	Crawford *et al.* (1995)
HUJ616	13	4	0.55	0.59	Barendse *et al.* (1994)
MCM152	13	2	0.39	0.41	Crawford *et al.* (1995)
BM4513	14	4	0.49	0.50	Bishop *et al.* (1994)
BM4630	14	5	0.72	0.71	Bishop *et al.* (1994)
BR3510	15	2	0.36	0.37	Bishop *et al.* (1994)
BM4025	16	2	0.12	0.13	Bishop *et al.* (1994)
OARHH62	16	2	0.33	0.35	Crawford *et al.* (1995)
ILSTS058	17	7	0.76	0.79	Kemp *et al.* (1995)
HEL10	19	4	0.52	0.54	Bishop *et al.* (1994)
BM1225	20	4	0.41	0.41	Bishop *et al.* (1994)
TGLA122	21	5	0.69	0.77	Crawford *et al.* (1995)
MAF36	22	2	0.40	0.39	Crawford *et al.* (1995)
BM1818	23	4	0.50	0.55	Bishop *et al.* (1994)
OARJMP29	24	2	0.50	0.50	Crawford *et al.* (1995)
BL6	24	2	0.45	0.39	Grosz *et al.* (1997)
OARJMP58	26	2	0.48	0.49	Crawford *et al.* (1995)
RT9	Unassigned	3	0.52	0.54	Wilson *et al.* (1997)
RT27	Unassigned	4	0.39	0.42	Wilson *et al.* (1997)

Parental assignments

Many mother–offspring pairs (n = 77) were determined when the yearling was associated with its mother in the spring (Gendreau *et al.* 2005). All these mother–offspring associations were genetically confirmed using CERVUS 3.0 (Kalinowski *et al.* 2007). Using the same software, we inferred maternity for 46 additional goats for which mother identity could not be determined by field observations (Mainguy *et al.* 2009a,b). We inferred genetic paternity for 123 offspring with a known genotyped mother at strict (95%) statistical confidence (Mainguy *et al.* 2009a,b). See Mainguy *et al.* (2009a,b) for further details on parental assignment analyses.

Effective population size

Demographic method. We used demographic data and detailed individual life-history information to estimate the expected rate of loss of heterozygosity per generation (H_{loss}) with VORTEX 9.9 (Lacy *et al.* 2005). We estimated N_e as:

$$N_e = 1/(2\times H_{loss}), \quad (1)$$

or more generally,

$$N_e = 1/(2\times(1 - H_{rem}^{(1/t)}), \quad (2)$$

where H_{rem} is the remaining observed heterozygosity after t simulated generations (Harris & Allendorf 1989; Andrén 2006). We ran VORTEX 9.9 with 1000 iterations considering a single isolated population, a time step of 50 years, and a carrying capacity of 200 individuals. We considered a polygynous mating system (Mainguy *et al.* 2008, 2009a) and the following reproductive parameters calculated from data obtained over the 14 study years (1995–2008): age of first offspring for males and females = 3 years; maximum age of reproduction = 17 years; maximum number of broods per year = 1; maximum number of progeny per brood = 2; sex-ratio at birth (% males) = 51.9%; mean percentage of reproductive females breeding in a given year (±SE) = 41 ± 14%; percentage of males in the breeding pool = 31%. We also specified the distribution of number of offspring per female per brood: 1 offspring = 99.6% and 2 offspring = 0.4%. Initial population size was set considering the age distribution observed in 1995. Age-specific mortality rates for each sex were calculated from survival data obtained over the 14 study years. No population supplementation or genetic management has been applied to the studied population and goats have not been hunted at Caw Ridge since 1969 (Festa-Bianchet & Côté 2008). For this reason, these parameters were not considered in the simulations. The simulation output provides the remaining degree of heterozygosity (H_{rem}) and generation time, i.e. all the information necessary to calculate N_e according to equation (2) (Andrén 2006).

Genetic methods. We used two methods to estimate N_e from genotypic data: (i) the linkage disequilibrium (LD) method, based on a single temporal sample (Hill 1981; Waples 2006). We used the program LDNE (Waples & Do 2008) to estimate N_e from genotypic data based on the LD method and implementing the bias correction of Waples (2006). The jackknife method was employed while assuming a random-mating model. This program can calculate separate estimates using different criteria for excluding rare alleles and we tested the following critical values (P_{crit}): 0.05; 0.02; 0.01 (Waples & Do 2008). We estimated an N_e value for each cohort with five or more analysed individuals (i.e. cohorts 1998–2008). Finally, we calculated the harmonic mean of estimated N_e values over these 11 analysed cohorts (Waples & Do 2008); (ii) we also used a moment-based temporal approach which estimates N_e from allele frequency variation across generations (Waples 1989). This method requires two temporally spaced samples with a difference of one or more generations between samples. Thus, we considered cohorts spaced by one generation given the generation time (approximately 8 years) estimated using VORTEX (see Results section). We also only analysed cohorts with five or more genotyped individuals (cohorts 1998–2008). Thus, we obtained three different temporal N_e estimates considering genotypic data from the cohorts 1998 and 2006, 1999 and 2007, and 2000 and 2008. Finally, we calculated the harmonic mean of the estimated N_e values. These analyses were performed with the software NEESTIMATOR 1.3 (Peel *et al.* 2004).

Genetic diversity estimates

We used two metrics to estimate offspring heterozygosity: (i) uncorrected heterozygosity (H_O), calculated as the proportion of loci at which an individual is heterozygous; (ii) homozygosity by loci (*HL*), a microsatellite-derived measure that improves heterozygosity estimates in most natural populations (when $H_O > 0.4$) by weighting the contribution of each locus to the homozygosity value depending on its allelic variability (Aparicio *et al.* 2006). H_O and *HL* were calculated using CERNICALIN, an EXCEL spreadsheet available upon request (J. Ortego). We calculated the correlation between heterozygosity measures using Pearson's correlation. Individual inbreeding coefficients (*f*) were estimated from the pedigree using PEDIGREE VIEWER (http://www-personal.une.edu.au/~bkinghor/pedigree.htm). Parental genetic

relatedness for offspring in which both parents were unambiguously identified by parentage analyses was calculated using Lynch & Ritland's (1999) estimator with STORM (Frasier 2008), as this estimator best reflected pedigree relatedness (Mainguy *et al.* 2009b). For each cohort with at least five offspring, we calculated inbreeding coefficients (F_{IS}), expected heterozygosity (H_E) and allelic richness (A_R). Cohort inbreeding coefficients were calculated following Nei (1977). Expected heterozygosity (H_E) was calculated using CERNICALIN and allelic richness (A_R) was standardized for sample size using the program HP-RARE (Kalinowski 2005).

We analysed which variables contributed to explain offspring heterozygosity of 123 individuals born between 1995 and 2008 using Generalized Linear Mixed Models (GLMMs) with a normal error structure and an identity link function implemented within the GLIMMIX macro of SAS 9.1 (SAS Institute 2004). GLMMs allow analyses of data where the response variable is thought to be determined by both random and fixed effects (SAS Institute 2004; Bolker *et al.* 2009). Cohort, and adult population size or adult sex ratio were included as covariates. Offspring sex and parental mating status (0 = when both parents were local individuals; 1 = when the mother was a local individual and the father an immigrant) were included as fixed factors. No immigrant female has been documented at Caw Ridge (Festa-Bianchet & Côté 2008). We fitted paternal and maternal identities as random effects to control for non-independence of heterozygosity between related offspring (i.e. half or full siblings; Singer 1998; Bolker *et al.* 2009; e.g. Ortego *et al.* 2008). Parental relatedness was also analysed using a GLMM with a normal error structure and an identity link function. Male and female identities were also included as random effects because several individuals mated and reproduced for more than one breeding season and, thus, the observations of mate relatedness were often not independent (Bolker *et al.* 2009). Finally, we analysed the associations between cohort inbreeding coefficients (F_{IS}), expected heterozygosity (H_E), allelic richness (A_R) and year, adult population size and sex ratio in different Generalized Linear Models (GLMs). The precision of annual estimates may differ because sample sizes varied between cohorts. To take this into account, we used a weighted least square method, where weight equals the sample size of each cohort (e.g. Kaeuffer *et al.* 2007b; Ortego *et al.* 2007).

Simulations

We used simulations in BOTTLESIM 2.6 to estimate the expected change in observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficients (F_{IS}) and allelic richness (A_R) over the 14 years of study (Kuo & Janzen 2003; e.g. Ruokonen *et al.* 2010). We used the 'multilocus with variable population size' module which considers observed population size and adult sex-ratio for each year (Kuo & Janzen 2003). We performed 1000 iterations considering observed population allele frequencies and assuming random mating and completely overlapping generations. Age at maturity was set at 3 years (Festa-Bianchet & Côté 2008). Considering the age of death as the last sighting of the individual, we estimated that the mean longevity was 8.3 years (SD ± 4.1 years). However, accurate estimates of longevity are difficult to obtain for this species due to its long generation time and the difficulty to distinguish emigration from mortality (Festa-Bianchet & Côté 2008). For this reason, we simulated two different scenarios considering longevity ranging between 8 and 9 years. BOTTLESIM does not take into account selection, migration or mutation in the simulation models (Kuo & Janzen 2003). We used one-way ANCOVAS to test if the slope of the association between cohort and observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficients (F_{IS}) and allelic richness (A_R) differed between observed and simulated data.

Genetic structure and 'isolation-by-time'

We analysed patterns of population genetic structure over 14 cohorts (1995–2008). First, we calculated overall genetic differentiation (F_{ST}) among cohorts using PCAGEN (http://www.unil.ch/izea/softwares/pcagen.html) with 10 000 randomizations. We also calculated pairwise F_{ST} between cohorts and assessed significance with Fisher's exact tests after 10 000 permutations in ARLEQUIN 3.1 (Excoffier *et al.* 2005). We used Bonferroni corrections to account for multiple comparisons. Finally, we analysed patterns of 'isolation-by-time', i.e. the association between genetic similarity and number of years between cohorts. For this purpose, we first generated a matrix of genetic similarity, calculating for each individual its relatedness with all other individuals using Lynch & Ritland's (1999) estimator in MARK (K. Ritland). Second, we obtained a matrix of temporal distances, calculating the number of years elapsed between the different cohorts considering each pair of individuals (Demandt 2010). We used a Mantel test to analyse the association between these two matrices using ZT software with 10 000 permutations (Bonnet & Van de Peer 2002).

Results

Observed heterozygosity at each locus ranged from 0.36 to 0.82 and allelic diversity was low (two to seven alleles; Table 1). After corrections for multiple tests, no

locus departed from Hardy–Weinberg equilibrium. There was strong LD within chromosomes: 6 of 12 locus-pairs (50%) located on the same chromosome had significant LD. As expected, LD was less pronounced for loci located in different chromosomes, where 121 of 316 comparisons (38%) showed significant LD (see Bensch *et al.* 2006 for very similar values of LD in a small and inbred wolf, *Canis lupus*, population). The pedigree revealed that only three individuals that descended from local–local matings had inbreeding coefficients (f) above zero: a male ($f = 0.125$) and a female ($f = 0.031$) born in 2004 and a female ($f = 0.125$) born in 2005. These individuals had lower heterozygosity ($H_O = 0.429$) than 27 non-inbred goats ($H_O = 0.485$) born in the same 2 years (t-test; $t = 3.21$, $P = 0.003$).

Effective population size

VORTEX simulations estimated a generation time of 7.78 years and 86.9% of remaining heterozygosity (H_{rem}) after 50 years (6.43 generations), producing an estimated N_e of 23.2 individuals. The LD method estimated an N_e of approximately 32 individuals. Estimates of N_e using this method and considering different criteria for excluding rare alleles produced very similar results (harmonic means of N_e values for the 11 analysed cohorts; $P_{crit\ (0.05)}$: 30.8; $P_{crit\ (0.02)} = 32.9$; $P_{crit\ (0.01)} = 32.9$). Variation in allele frequency between generations yielded higher estimates of N_e, from 44.8 (95% CI: 9.6-∞) in the generation elapsed between the 1999 and 2007 cohorts and 65.9 (95% CI: 5.2–∞) between 2000 and 2008 cohorts. The harmonic mean for the estimated N_e values using the moment-based temporal approach was 53.3 individuals.

Offspring heterozygosity, parental relatedness and inbreeding

H_O and HL were highly correlated (Pearson $r = -0.966$, $P < 0.001$), thus statistical analyses are only presented for H_O. HL data provided analogous results and are available from the authors. After controlling for parental identity (male identity: $Z = 0.47$, $P = 0.32$; female identity: $Z = 0.00$, $P = 1.00$), offspring heterozygosity decreased over the 14 cohorts (GLMM, $F_{1,120} = 8.16$, $P = 0.005$; Fig. 2) and was higher in offspring of immigrant fathers than in those with both parents born at Caw Ridge ($F_{1,120} = 4.75$, $P = 0.03$; Fig. 2). Both adult sex ratio ($r = 0.871$, $P < 0.001$, $n = 14$ years) and adult population size ($r = 0.965$, $P < 0.001$, $n = 14$ years) were highly correlated with study year (or cohort) (Fig. 1). Sex ratio and population size were also highly correlated ($r = 0.844$, $P < 0.001$, $n = 14$ years; Fig. 1). To avoid colinearity between independent variables, we excluded

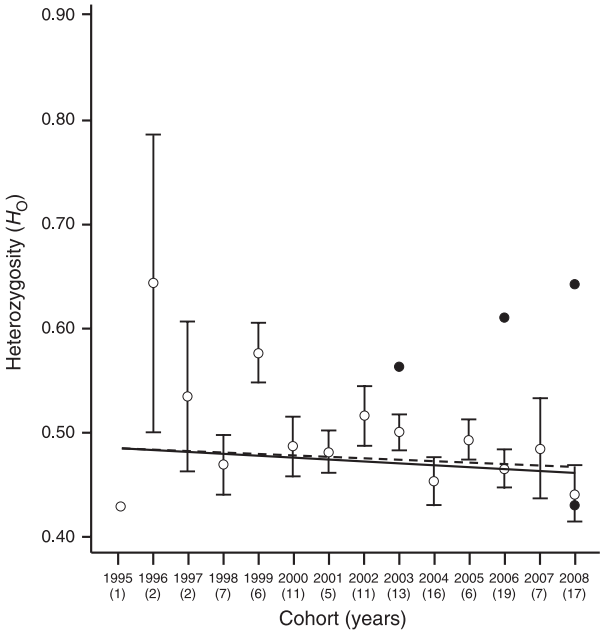

Fig. 2 Mean ± SE offspring heterozygosity (H_O) for 14 cohorts (1995–2008) of mountain goats at Caw Ridge, Alberta, Canada. Black dots represent offspring of immigrant males. Regression lines show the simulated temporal change in observed heterozygosity assuming a longevity of eight (solid line) and nine (dashed line) years. Figures in parentheses indicate sample size for each cohort.

cohort and tested the association between offspring heterozygosity and adult sex ratio/population size in different GLMMs. Heterozygosity was negatively associated with sex ratio ($F_{1,\ 120} = 6.38$, $P = 0.01$) and population size ($F_{1,\ 120} = 7.50$, $P = 0.007$). Offspring sex had no effect on heterozygosity in any analyses (all P's > 0.4). We also ran a full model using a backward selection procedure to test whether the association between heterozygosity and any of the studied variables was a correlated effect with a third parameter. We found that the final model retained cohort as a significant variable, whereas both sex ratio ($F_{1,119} = 0.14$, $P = 0.7$) and population size ($F_{1,119} = 0.00$, $P = 1.0$) were excluded. After controlling for male ($Z = 1.75$, $P = 0.04$) and female ($Z = 3.53$, $P < 0.001$) identities, genetic similarity of mating pairs increased over time ($F_{1,120} = 4.23$, $P = 0.04$; Fig. 3). Genetic similarity also tended to be positively associated with sex ratio ($F_{1,120} = 3.09$, $P = 0.08$) and population size ($F_{1,120} = 3.99$, $P = 0.048$). We also ran a full model including all independent variables and again both sex ratio ($F_{1,119} = 0.15$, $P = 0.7$) and population size ($F_{1,119} = 0.09$, $P = 0.8$) were excluded from the final model, which only retained year as a significant variable. Within cohorts, F_{IS} values were positively associated with year ($r = 0.69$; $F_{1,9} = 8.23$, $P = 0.02$), sex ratio ($r = 0.67$; $F_{1,9} = 7.48$, $P = 0.02$) and population size

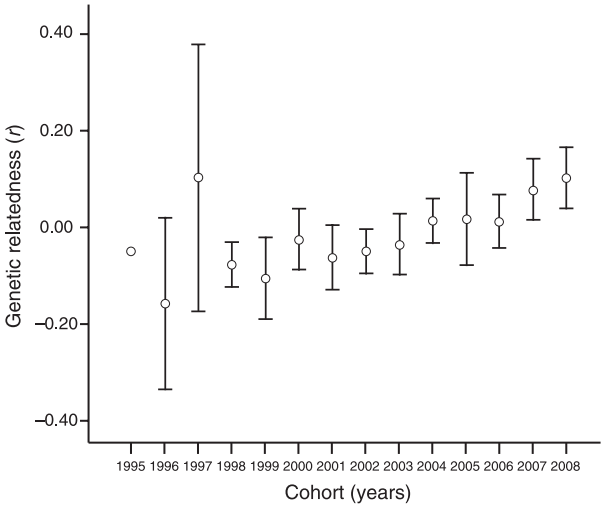

Fig. 3 Mean ± SE relatedness (r) of mating pairs for 14 cohorts (1995–2008) in the mountain goat population at Caw Ridge, Alberta, Canada.

(r = 0.66; $F_{1,9}$ = 6.94, P = 0.03). Backward selection from an initial full model retained sex ratio, whereas year ($F_{1,8}$ = 0.31, P = 0.6) and population size ($F_{1,8}$ = 0.46, P = 0.5) were excluded. Within cohort expected heterozygosity (H_E) was not associated with any of the variables examined (year: r = 0.01, $F_{1,9}$ = 0.01, P = 1.0; sex ratio: r = 0.15, $F_{1,9}$ = 0.22, P = 0.7; population size: r = 0.07, $F_{1,9}$ = 0.05, P = 0.8). Finally, within cohort allelic richness (A_R) was positively associated with year (r = 0.62; $F_{1,9}$ = 5.48, P = 0.04) but not with sex ratio (r = 0.21; $F_{1,9}$ = 0.40, P = 0.5) or population size (r = 0.51; $F_{1,9}$ = 3.21, P = 0.1).

Heterozygosity in local and immigrant-local crosses

Heterozygosity of offspring descended from immigrant males (mean ± SE = 0.56 ± 0.09) was 18.5% higher than the heterozygosity observed in offspring from local matings (mean ± SE = 0.46 ± 0.08). Three of the four offspring of immigrant males had higher heterozygosity than offspring from local matings born in the same year (t-tests within cohorts; numbers separated by a dash refer to offspring and cohort codes, respectively; ID326-2003: t = 4.04, n = 13, P = 0.002; ID390-2006: t = 7.82, n = 19, P < 0.001; ID427-2008: t = 7.45, n = 17, P < 0.001; ID412-2008: t = 0.49, n = 16, P = 0.6; Fig. 2). Immigrants introduced into the population three novel alleles from three different loci (AR028, HUJ616, and BM1225), which were all transmitted to their offspring.

Simulations

Simulations revealed an expected decline in H_O, H_E and A_R over the 14 study years (all P's < 0.001; Fig. 2). However, simulations showed that F_{IS} values were expected to first decrease and then increase over the 14 years period, with a minimum in the seventh and eighth simulated years for the lower and higher longevity scenario, respectively (all P's < 0.01). The decline of H_O was less pronounced when we considered greater longevity (interaction between year and longevity: $F_{1,24}$ = 4.60; P = 0.042; Fig. 2). However, the association between F_{IS}, H_E and A_R and year did not significantly differ between the two simulated longevity scenarios (all P's > 0.4). The slope of the association between H_O, H_E, A_R and F_{IS} and cohort neither differed between observed and simulated data considering any longevity scenario (all P's > 0.05).

Genetic structure and 'isolation-by-time'

We found significant overall genetic differentiation among cohorts (F_{ST} = 0.031, P = 0.002). However, no pair-wise F_{ST} values between cohorts remained significant after sequential Bonferroni correction (all P's > 0.05). We observed an isolation-by-time pattern of genetic structure, with a weak negative association between genetic relatedness and number of years elapsed between cohorts (r = −0.04, P < 0.001; Fig. 4). When we excluded offspring sired by immigrant individuals, we also found an overall genetic differentiation among cohorts (F_{ST} = 0.031, P = 0.005) and a significant isolation-by-time pattern of genetic structure (r = −0.03, P = 0.01).

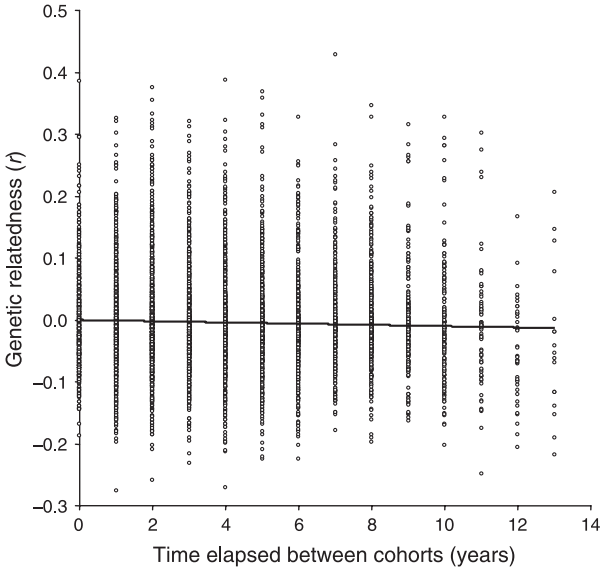

Fig. 4 Correlation between genetic relatedness (r) and time elapsed between cohorts (14 cohorts, 1995–2008) in the mountain goat population at Caw Ridge, Alberta, Canada.

Discussion

Long-term monitoring and genetic data revealed significant temporal trends in the genetic diversity of our study population of mountain goats. Offspring heterozygosity and allelic richness (A_R) decreased, whereas parental genetic similarity and inbreeding coefficients (F_{IS}) increased over time. Pedigree depth was insufficient to allow accurate estimation of inbreeding coefficients (f) for enough individuals to analyse temporal trends (e.g. Bensch *et al.* 2006). This can be in part attributable to the limited number of genetically sampled males in the first few years of the long-term study (Mainguy *et al.* 2009a). Nevertheless, the few inbred individuals identified had a lower heterozygosity than apparently non-inbred offspring (i.e. $f = 0$), suggesting that heterozygosity estimated using our panel of 28 microsatellite markers captures some of the variation in individual inbreeding (e.g. Bensch *et al.* 2006). The decline of heterozygosity over the 14 study years predicted by simulations did not differ from that observed (Fig. 2). Thus, although the study population experienced an important demographic increase and adult sex ratio is now approaching parity, its genetic variability decreased over the 14 study years.

Demographic information and the single generation genetic method yielded estimates of effective population size of mountain goats at Caw Ridge that are very low ($N_e \sim 28$ individuals) and much lower than the census population size ($N_e/N_c \sim 0.4$). Estimates of effective population size based on variation in microsatellite allele frequencies between generations were higher ($N_e \sim 50$ individuals; $N_e/N_c \sim 0.6$). However, temporal genetic methods tend to overestimate N_e for organisms with overlapping generations, particularly when samples are only separated by one or a few generations, as it is the case here (Jorde & Ryman 1995; Leberg 2005; Waples & Yokota 2007; e.g. Kaeuffer *et al.* 2007a). This N_e is lower than values reported for other small and isolated populations of large mammals (e.g. Bensch *et al.* 2006; Kaeuffer *et al.* 2007a). Low effective population size is expected to produce a rapid accumulation of inbreeding and strong genetic drift, which could explain the rapid loss of genetic variability observed over the 14 study cohorts.

Different factors probably contribute to the low effective population size in the study population. First, Caw Ridge is an 'island' of mountain goat habitat separated from other adequate areas by extensive unsuitable habitats (Festa-Bianchet & Côté 2008). Population monitoring suggests that the studied herd mostly behaves as an isolated population (Festa-Bianchet & Côté 2008). Immigration has been limited to a dozen males over 18 years, of which about 65% settled and could have potentially contributed genetically to the population (Mainguy *et al.* 2008). In contrast, emigration towards other nearby populations is relatively frequent, leading to a negative demographic impact of dispersal (Festa-Bianchet & Côté 2008). Thus, the observed demographic increase is mostly the result of intrinsic population growth with a minimal impact of immigration (Festa-Bianchet & Côté 2008; Ezard *et al.* 2009). In addition, mountain goats have a polygynous mating system in which a small proportion of males obtain most paternities (Mainguy *et al.* 2008, 2009a). The observed demographic growth and increased male to female ratio may have a limited positive impact on effective population size if a few males garner most paternities (Sugg *et al.* 1996; Parker & Waite 1997). Thus, small population size, isolation and the polygynous mating system of this species are probably the most important factors determining the very low N_e we have found. In any case, we would expect that the remarkable increase of the proportion of adult males over time to decrease the chance of extinction of the population after a hypothetical severe population crash (Kaeuffer *et al.* 2007a; Ezard *et al.* 2009).

Immigrants introduced novel alleles into the population and matings between local and immigrant individuals resulted in more heterozygous offspring in comparison with local crosses, suggesting that effective immigration can increase population genetic variability. Rare effective immigration would have a long-term positive feedback on population genetic variability (Saccheri & Brakefield 2002; Vilà *et al.* 2003). Previous research in this population provided evidence of inbreeding avoidance and increased survival of more heterozygous offspring (Mainguy *et al.* 2009b). Thus, offspring of immigrants should enjoy high survival and eventually mating success. This may ultimately favour the spread of immigrant genomes as reported in both experimental (Saccheri & Brakefield 2002; Hogg *et al.* 2006) and natural populations (Keller *et al.* 2001; Vilà *et al.* 2003). Small rates of effective immigration could potentially have a disproportionate positive impact on population genetic variability, although it would take some generations for such genetic consequences to become detectable in long-lived organisms (Saccheri & Brakefield 2002; Vilà *et al.* 2003). It should be also considered that most studies documenting a genetic rescue have been performed in extremely inbred populations (e.g. Madsen *et al.* 1999; Vilà *et al.* 2003), which may explain the limited impact of immigration and selection on the observed levels of genetic variability and the similarity between observed and expected loss of genetic variation.

Analyses of temporal genetic structure also indicate that this population has experienced significant genetic drift over time. The observed 'isolation by time' pattern of genetic structure suggests stochastic variability in reproductive success probably due to small effective

population size exacerbated by the polygynous mating system (Calderón *et al.* 2009). Since approximately 10% of adult males sire approximately 50% of offspring (Mainguy *et al.* 2009a), the death of highly successful males and their replacement with other highly competitive individuals in subsequent years could promote temporal changes of allele frequencies over generations and contribute to the observed progressive genetic differentiation over time (Demandt 2010).

Overall, our data suggest that the temporal decline of individual genetic diversity probably resulted from a combination of inbreeding and genetic drift. The substantial increase in population size and moderate immigration were apparently insufficient to counter the negative effect of small effective population size. Historical population bottlenecks during Pleistocene glaciations and subsequent demographic expansions (Shafer *et al.* 2011), together with the current small population size (present study) and limited inter-population gene flow (Shafer *et al.* 2011) could both explain the very low levels of genetic variability observed in mountain goats (Mainguy *et al.* 2005, 2007; Poissant *et al.* 2009). This study highlights the importance of long-term genetic surveys to better understand the links between demographic processes and temporal changes of genetic diversity in long-lived organisms that often experience complex ecological and evolutionary dynamics (Ezard *et al.* 2009).

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