Molecular Ecology (2009) **18**, 1491–1503 doi: 10.1111/j.1365-294X.2009.04116.x

Age-specific effect of heterozygosity on survival in alpine marmots, *Marmota marmota*

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Abstract

The fitness consequences of heterozygosity and the mechanisms underpinning them are still highly controversial. Using capture–mark–recapture models, we investigated the effects of individual heterozygosity, measured at 16 microsatellite markers, on age-dependent survival and access to dominance in a socially monogamous mammalian species, the alpine marmot. We found a positive correlation between standardized multilocus heterozygosity and juvenile survival. However, there was no correlation between standardized multilocus heterozygosity and either survival of older individuals or access to dominance. The disappearance of a significant heterozygosity fitness correlation when individuals older than juveniles are considered is consistent with the prediction that differences in survival among individuals are maximal early in life. The lack of a correlation between heterozygosity and access to dominance may be a consequence of few homozygous individuals attaining the age at which they might reach dominance. Two hypotheses have been proposed to explain heterozygosity-fitness correlations: genome-wide effects reflected by all markers or local effects of specific markers linked to genes that determine fitness. In accordance with genome-wide effects of heterozygosity, we found significant correlations between heterozygosities calculated across single locus or across two sets of eight loci. Thus, the genome-wide heterozygosity effect seems to explain the observed heterozygosity-fitness correlation in the alpine marmot.

Keywords: capture–mark–recapture, inbreeding, mammal, microsatellite, over-dominance

Received 3 October 2008; revision received 19 December 2008; accepted 22 December 2008

Introduction

The impact of individual genetic variability on fitness has long been of interest to both conservation and evolutionary biologists (Allendorf & Leary 1986; Mitton 1993; David 1998). Quantification of this impact in natural populations has been initially estimated from correlations between allozyme-based measures of genetic variability and potential fitness-related traits, such as growth, fluctuating asymmetry, survival or reproductive success (Allendorf & Leary 1986; Mitton 1993; Britten 1996; David 1998). The development of microsatellite markers and their associated measures of genetic variability, such as standardized multilocus heterozygosity (Coltman *et al*. 1999), mean d^2 (Coulson *et al*. 1998), or internal relatedness (Amos *et al*. 2001), have renewed the interest in this topic. Positive heterozygosity fitness correlations (HFC) have been found in a wide diversity of organisms ranging from plants to higher vertebrates (David 1998; Kempenaers 2007).

In a recent meta-analysis, Coltman & Slate (2003) confirmed that significant positive correlations between microsatellite-based measures of heterozygosity and fitness-related traits were widespread but weak. However, the magnitude of HFCs may vary with the selective pressures individuals experience, which may depend on both the environmental conditions and on their state (Husband & Schemske 1996; David 1998; Keller & Waller 2002; Armbruster & Reed 2005). Recent studies indicate that HFCs can be sensitive to environmental conditions and generally

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support the hypothesis that HFCs are stronger in more stressful conditions (see Kempenaers 2007 for a review). In contrast, state-specific HFCs have been largely overlooked (but see Meagher *et al*. 2000; Merilä *et al*. 2003; Bonneaud *et al*. 2006; for examples of sex-specific HFCs). Age is an individual state likely to impact the magnitude of HFCs (Von Hardenberg *et al*. 2007; Keller *et al*. 2008). Indeed, other things being equal, natural selection places a greater relative weight on changes in early survival or reproductive success than on changes at later ages (Medawar 1952; Hamilton 1966). HFCs can thus be expected to decrease with age (David & Jarne 1997) because among-individual differences in fitness (especially survival) are maximal early in life and because unfit genotypes are selectively eliminated in ageing cohorts (Koehn & Gaffney 1984).

Here, we investigated HFCs in a population of alpine marmots (*Marmota marmota*), a socially monogamous mammal living in family groups, with reproduction highly skewed towards the dominant pair (King & Allainé 2002). Specifically, we used capture–recapture analyses to investigate the effect of individual heterozygosity, measured across 16 microsatellite loci, on two important fitness components: survival and the probability of becoming dominant. In addition, we investigated whether the magnitude of HFC is age-dependent. If the correlation between individual heterozygosity and survival decreases with age, the mean heterozygosity is expected to increase with age whereas the variance is expected to decrease.

Heterozygote advantage resulting from overdominance at the scored loci per se (the direct effect hypothesis) was largely advocated to explain HFCs involving allozyme-based measures of heterozygosity (Mitton 1993; Hansson & Westerberg 2002). However, this mechanism is unlikely for studies using supposedly neutral markers such as microsatellites (Queller *et al*. 1993). Hansson & Westerberg (2002) reviewed two other hypotheses that may explain HFCs with microsatellite-based measures of heterozygosity. The local effect hypothesis states that apparent heterozygote advantage at neutral markers results from effects of homozygosity at closely linked fitness loci. The general effect hypothesis states that the apparent heterozygote advantage at markers results from effects of homozygosity at genome-wide fitness loci (a potential consequence of inbreeding depression).

Alpine marmots have undergone a severe bottleneck at the end of the last glaciation period, which is hypothesized to be the cause of the low genetic diversity found in extant populations of alpine marmots (Preleuthner & Pinsker 1993; Kruckenhauser *et al*. 1997). This severe bottleneck likely has generated genome-wide inbreeding effects as well as considerable linkage disequilibrium (Nei 1975; Reich *et al*. 2001). We investigated which of the two hypotheses (local vs. general effect) was more likely to explain the observed HFCs in our population. To this end, we tested whether heterozygosity was correlated across individual loci and across sets of loci, which would indicate a general effect, or whether heterozygosity at single loci explained variation in fitness-related traits better than multilocus heterozygosity, indicating a local effect.

Methods

Study species

Alpine marmots are territorial, socially monogamous and cooperative breeding mammals (Allainé 2000). The basic social unit is the family group composed of two to 20 individuals with a dominant breeding pair, sexually mature subordinates of at least 2 years old, yearlings, and juveniles (Perrin *et al*. 1993). Reproduction is highly skewed towards dominant individuals and subordinates rarely reproduce (Goossens *et al*. 1996; Cohas *et al*. 2006). Subordinates rarely inherit dominance in their natal territory (only about 5% and 12% of males and females, respectively; Magnolon 1999) but generally disperse from 2 years of age in search of an available territory (Frey-Roos 1998; Magnolon 1999). Subordinates often delay dispersal beyond 2 years of age, thus beyond sexual maturity, and act as helpers (males) in their natal family group. The presence of subordinate males (1 year or older) indeed increases offspring winter survival through social thermoregulation (Arnold 1993; Allainé *et al*. 2000; Allainé & Theuriau 2004).

Study site and sampling procedure

The study site is located in La Grande Sassière Nature Reserve (French Alps, 45°29′N, 6°59′E, 2300 m a.s.l.) and consists of 40 ha of open alpine meadows. From 1990 to 2006, individuals were monitored by a capture–mark–recapture protocol. They were captured from early April to mid-July during at least 45 days a season. Marmots were trapped using two-door, live-capture traps. Traps were baited with dandelion *Taraxacum densleonis* and placed near the entrance of the main burrows of each family group in order to assign trapped individuals to their family. Once captured, individuals were tranquillized with Zolétil 100 (0.1 mL/kg), and individually marked with a numbered ear tag and a transponder (model ID100, Trovan) for permanent individual recognition. In addition, a piece of coloured plastic was fixed to the other ear for visual identification. Trapped individuals were sexed, aged, and their social status was confirmed through examination of sexual characteristics (scrotum for males and nipples for females). We collected plucked hairs (1992–2006) and tissue biopsies (1998–2006) of all captured individuals for genetic analyses.

The composition of the 20 families present in the study site was determined from capture–recapture data combined with intensive visual observations. Each family was observed from a distance of 80–200 m with 10 × 50 binoculars and 20 × 60 telescopes. Each family was observed on average 1 h per day for a minimum of 30 h per year with 1-h sessions being randomly distributed during the period of activity from 8:00–12:00 AM and from 5:00–9:00 PM. We recorded the number of yearlings, 2-year-olds and adults of each sex and social status for each family. Size allowed us to age individuals (up to 3 years of age) while scent-marking behaviour and aggressive interactions were used to categorize individuals as subordinates or dominants (Bel *et al*. 1999).

Genotyping and estimating genetic diversity

Individuals were typed at 16 microsatellite loci: SS-Bibl1, SS-Bibl18, SS-Bibl20, SS-Bibl31, SS-Bibl4 (Klinkicht 1993); MS41, MS45, MS47, MS53, MS56, MS6, ST10 (Hanslik & Kruckenhauser 2000); Ma002, Ma018, Ma066, Ma091 (Da Silva *et al*. 2003). Genotyping protocols were described in Cohas *et al*. (2008) and microsatellite characteristics are summarized in Table 1. To estimate the genotyping error rate, 96 randomly chosen individuals were typed twice for each microsatellite locus. Since no discrepancy between the two genotypes was found, the probability of an error on one allele should not exceed 0.0003.

Using the library 'adegenet' for R (Jombart 2008), we conducted tests of Hardy–Weinberg on dominant adults to avoid potential bias caused by family structure and on all cohorts pooled to ensure sufficient sample size ($N = 160$). Except for Ma002 ($\chi^2 = 308.90$, $P = 0.027$, 10 000 replicates), none of the loci showed deviation from Hardy–Weinberg equilibrium ($1 > P > 0.064$).

For each individual, we calculated standardized multilocus heterozygosity (SH) because 0 to 3% of individuals could not be typed at some loci (Table 1). SH is defined as the ratio of the heterozygosity of an individual to the mean heterozygosity of the loci at which the individual was typed, and avoids potential bias that may be introduced by some individuals not being typed at some loci (Coltman *et al*. 1999).

Heterozygosity and age

We investigated how the mean and variance in heterozygosity varied with age using generalized least-square models (GLS, Pinheiro & Bates 2000). GLS models can test for differences in average heterozygosity among age classes and, at the same time, account for heteroscedasticity in heterozygosity among the different age-classes. Thus, based on a sample of 693 individuals, we tested whether mean SH varied with age (we predicted an increase with age) using standard *F*-tests and whether variance in SH varied with age (we predicted a decrease with age) using likelihood-ratio tests (Pinheiro & Bates 2000).

Table 1 Characteristics of the 16 microsatellites used in this study

	SS-Bibl1		SS-Bibl18		SS-Bibl20		SS-Bibl31		SS-Bibl4		Ma002		Ma018		Ma066		Ma091		MS41		MS45		MS47		MS53		MS56		MS6		ST10	
	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq	Alleles	Freq
	95	0.16	133	< 0.01	206	< 0.01	157	0.50	175	0.12	271	0.21	296	0.27	231	0.63	159	0.13	184	0.18	107	0.37	176	0.04	132	0.13	104	0.02	142	0.06	116	0.16
	97	0.21	137	0.01	208	0.18	159	0.28	188	0.15	279	0.48	298	0.73	233	< 0.01	167	0.09	186	0.82	109	0.52	180	0.23	140	0.46	106	0.28	158	0.88	118	0.28
	101	0.45	143	0.34	216	0.39	161	0.17	190	0.70	281	0.31			241	0.37	169	0.05			111	0.11	182	0.19	142	0.41	108	0.70	160	0.07	120	0.19
	103	< 0.01	145	0.14	218	0.33	163	0.05	192	0.03	283	< 0.01					171	< 0.01					184	0.18	144	< 0.01	110	< 0.01			130	0.05
	107	0.14	147	0.41	220	0.08											173	0.17					186	0.33	148	< 0.01					132	0.14
	109	0.04	149	0.10	222	< 0.01											175	0.46					188	0.02							134	0.14
																	177	0.02					190	< 0.01							136	0.04
																	179	0.09														
Number of individuals typed	692		689		684		692		690		670		675		683		686		680		689		684		686		685		683		683	
No. of alleles	6		6		6		4		4		4		2		3		8		2		3		7		5		4		3		7	

Effect of heterozygosity on survival and dominance

For these analyses, we considered 693 individuals (1446 captures) of known genotypes captured in the 20 families followed between 1990 and 2006.

Modelling of survival and probability of becoming dominant. We used multistate capture–recapture models (MS–CR, Lebreton & Pradel 2002) to investigate both marmot survival and social state transition given that recapture probability of individuals was lower than 1 (recapture probability varied between 0.37 and 0.92, Farand *et al.* 2002). By using MS–CR, we could estimate both the probability of survival for subordinate and dominant individuals and the probability that an individual changed from subordinate (s) to dominant state (D) (see Cohas *et al.* 2007).

An MS-CR model corresponds to a transition matrix and associated vectors of survival and capture probabilities (Nichols *et al.* 1994) as follows:

$$\begin{bmatrix} \Psi^{ss} & 1-\Psi^{ss} \\ 1-\Psi^{DD} & \Psi^{DD} \end{bmatrix}_t \begin{bmatrix} \Phi^{s} \\ \Phi^{D} \end{bmatrix}_t \begin{bmatrix} p^{s} \\ p^{D} \end{bmatrix}_t \qquad \text{(eqn 1)}$$

where capture (p), apparent survival (Φ), and state transition conditional to survival (ψ) probabilities are defined as: p_t^a, the probability that an individual in state a in year t is captured during that year, Φ_t^a, the probability that an individual in state a in year t survives and does not permanently emigrate from the study area between t and $t + 1$ and ψ_t^{ab}, the probability that an individual in state a in year t is in state b in year $t + 1$, given that it survived and did not permanently emigrate from the study area between t and $t + 1$, where t is the time of marking.

Since marmots younger than 3 years of age have never been identified as dominant (Farand *et al.* 2002; Stephens *et al.* 2002; Grimm *et al.* 2003), the transition between social states only concerned individuals older than 2 years. So, for individuals younger than 2 years, the model simplified to:

$$(\Phi^{s})_t (p^{s})_t \qquad \text{(eqn 2)}$$

Moreover, since dominant marmots never revert to subordinate state (Arnold 1993; Farand *et al.* 2002; Stephens *et al.* 2002; Grimm *et al.* 2003), we fixed transition probabilities from dominant to subordinate at zero ($\psi^{Ds} = 1 - \psi^{DD} = 0$) for individuals older than 2 years. Since the probabilities over the state transition matrix lines sum to 1, this *de facto* constrained transition probabilities from dominant to dominant to 1 ($\psi^{DD} = 1 - \psi^{Ds} = 1$). We thus had only one transition probability to estimate (i.e. the probability of staying subordinate ψ^{ss}). For individuals older than 2 years, the model can be simplified to:

$$\begin{bmatrix} \Psi^{ss} & 1-\Psi^{ss} \\ 0 & 1 \end{bmatrix}_t \begin{bmatrix} \Phi^{s} \\ \Phi^{D} \end{bmatrix}_t \begin{bmatrix} p^{s} \\ p^{D} \end{bmatrix}_t \qquad \text{(eqn 3)}$$

Data analyses followed two steps (Lebreton *et al.* 1992). In the first step, we tested whether a general model compatible with our biological knowledge fitted our data (Burnham *et al.* 1987). We thus performed a multi-state goodness-of-fit (GOF) test (Pradel *et al.* 2003) using U-CARE (Choquet *et al.* 2005). The second step was to select the most parsimonious model from our global model, which did not include effects of heterozygosity. Following the recommendation of Burnham & Anderson (2002), we reduced the number of parameters of the general model by considering only *a priori* biological hypotheses based on our field experience and the literature (Arnold 1993; Farand *et al.* 2002; Stephens *et al.* 2002; Grimm *et al.* 2003; Allainé & Theuriau 2004). We thus considered age, time and sex effects and their interactions on all capture, survival and state transition probabilities as well as the effect of the presence of helpers on juvenile survival (Allainé *et al.* 2000; Allainé & Theuriau 2004). We considered age as a categorical variable (juveniles, yearlings, 2-year-olds and adults), because individuals are aged based on their size and > 2-year-olds have reached their adult size. Moreover, since the amount of time spent in the field changed twice over the course of the study, we considered the time as a categorical variable (three periods: 1990–1991, 1992–1996, 1997–2006) to model capture probability. Consequently, the most general model was denoted $p^{s}_{age*t*sex}\Phi^{s}_{[age1*h+age2-4]*t*sex}\Psi^{ss}_{age3-4*t*sex}, p^{D}_{age3-4*t*sex}\Phi^{D}_{age4*t*sex}\Psi^{sD}_{age3-4*t*sex}$ (all terms are defined in Table 2). We used the Akaike's information criterion (AIC, see Burnham & Anderson 2002) corrected for small sample size (noted AICc, Burnham *et al.* 1995) to select models (e.g. Johnson & Omland 2004).

Test of the effect of heterozygosity. After finding the most parsimonious global model describing survival and state transition probabilities (see above), we included individual standardized heterozygosity as an individual covariate in this model. Effects of heterozygosity were then tested by comparing the most parsimonious global model with related models that included the heterozygosity effect on (i) survival, and on (ii) transition probabilities. We tested for: (i) an age-independent effect of heterozygosity by considering an additive effect of SH and age on survival and transition probabilities; (ii) an age-dependent effect of SH by considering an interactive effect of heterozygosity and age on survival and transition probabilities; (iii) age-specific effects of heterozygosity by testing the effect of SH on survival and transition probabilities for each age-class separately.

Since these models were nested within the global model, we used log-likelihood ratio tests (LRT) between the global model and the models including a heterozygosity effect to assess the significance of these effects. Effect size of heterozygosity on survival was estimated by computing the odds ratio (Agresti 2002, p. 166). The odds ratio gives the changes in the odds of an event (e.g. survival) for a unit

Table 2 Abbreviations used in model notations

Abbreviation	Biological significance
p	Capture probability
Φ	Survival probability
ψ	State transition conditional on survival probability
Subscript	
Age	Age as a four modalities categorical variable
Age1	Age from 0 to 1 year
Age2	Age from 1 to 2 years
Age3	Age from 2 to 3 years
Age4	Age of 3 years and older
Agex–y	Age from age x to age y
Agex,y	Age x and age y
Sex	Sex
Agex†	Age x and older
t	Year
tcl	Period as three modalities categorical variable (1990 and 1991, 1992 to 1996, 1997 to 2006)
h	Helpers as a three modalities categorical variable (presence, absence, unknown)
SH	Standardized multilocus heterozygosity
*	Interactive effect
+	Additive effect
Superscript	
s	Subordinate status
D	Dominant status

change in the dependent variable which, in the case of SH, almost covers the full range of variation in heterozygosity.

General vs. local effects of heterozygosity

Characteristics of the microsatellites. Under the general effect but not under the local effect hypothesis, positive correlations between heterozygosity measured at different loci are expected.

We tested this prediction by calculating Spearman rank correlations (because heterozygosity does not follow a normal distribution when measured at a single locus) for all pairs of loci (Slate & Pemberton 2002). The sign and significance of each correlation was recorded. Since each microsatellite is involved 15 times, pseudoreplication arises among the 120 (calculated as $16 \times 15/2$) possible correlations. We thus performed a randomization-based test. Genotypes were randomized 9999 times without replacement across individuals for each locus. Each time, the correlation between the 120 possible pairs of loci was recalculated. The statistical significance of the correlation between loci was then assessed as the proportion of the 9999 replicates for which the number of positive correlations exceeded that obtained from the real data set.

To confirm the results obtained via the previous method, we divided the sixteen loci in 6435 ($0.5 \times \{16!/[(16-8)! \times 8!]\}$) combinations of two sets of eight loci and calculated for each combination the Pearson's correlation coefficient between SH measured on both set of loci (as proposed by Balloux *et al*. 2004). The statistical significance of the correlation between sets of loci was then calculated as the proportion of the 6435 correlation coefficients that exceeded zero.

Effect of heterozygosity at single locus on survival and probability of becoming dominant. Whenever we found a significant effect of heterozygosity on survival or dominance, we tested whether one particular locus significantly influenced this effect. For this purpose, we replaced, in the appropriate models, SH calculated over all loci by (a) by the heterozygosity calculated at each single locus, and (b) by SH calculated after omitting each of the loci one at a time.

All statistical analyses were performed using R 2.5.1 software (R development core team, 2007). All MS–CR models were fitted with the generalized logit link function, using MARK 5.1 (White & Burnham 1999). Unless otherwise stated, all tests are two tailed, the level of significance set to 0.05 and all parameter estimates are given as mean ± standard error and 95% confidence intervals.

Results

Heterozygosity and age

Mean individual standardized heterozygosity increased with age ($F = 3.18$, d.f. = 3, 1265, $P = 0.02$, Fig. 1). The mean heterozygosity was 0.925 ± 0.011 for juveniles, with an average increase of 0.031 ± 0.017 for yearlings ($t = 1.82$, d.f. = 1265, $P = 0.07$), of 0.047 ± 0.020 for 2-year olds ($t = 2.35$,

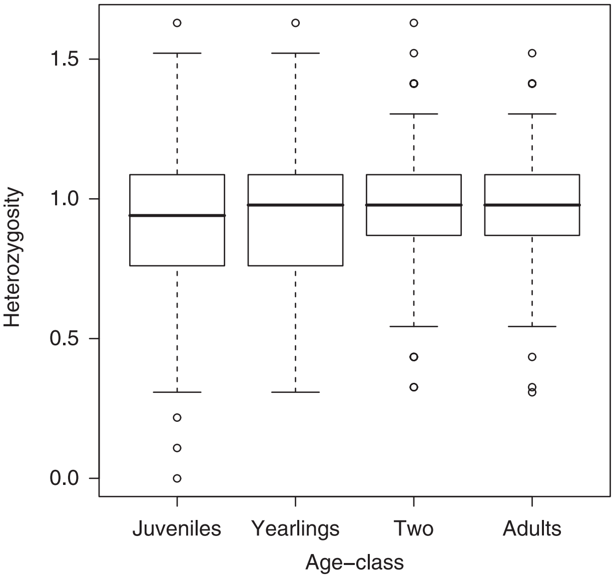

Fig. 1 Variation in the distribution of individual SH with age. The boxplot shows an increase in mean SH (generalized least square models: $F = 3.18$; d.f. = 3, 1265; $P = 0.02$) and a decrease in the variance of SH with age starting from juveniles, to yearlings, 2 years old (two) and adults of 3 years of age and older. Box plots show the data by the median (black horizontal line) embedded in the 25% and 75% of the SH distribution (box). Dashed lines encompass the 5% and 95% of the SH distribution, and empty circles represent extreme values.

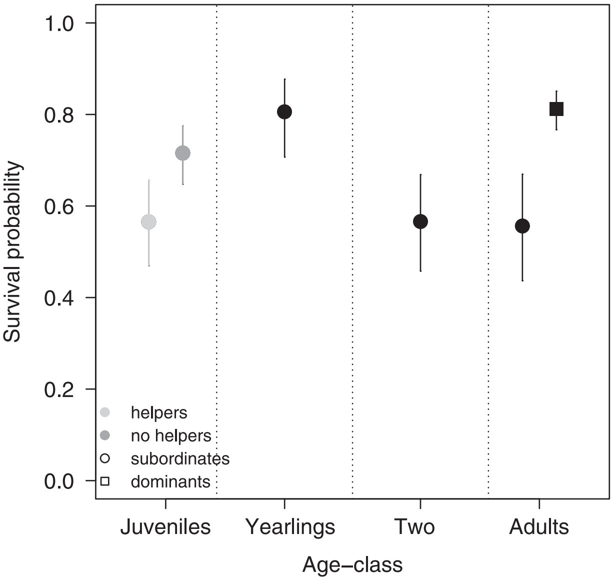

Fig. 2 Survival probabilities and their 95% confidence intervals of four age classes: juveniles (juv.), yearlings (year.), 2-years old (two) and adults of 3 years of age and older (adu). Survival probabilities for juveniles are presented in the absence and in the presence of helpers. Survival probabilities for adults of 3 years of age and older are presented separately for subordinate and dominant individuals.

d.f. = 1265, $P = 0.02$) and of 0.048 ± 0.019 for adults ($t = 2.42$, d.f. = 1265, $P = 0.01$). There were no significant differences between age-classes older than juveniles ($F = 0.468$, d.f. = 2, 748, $P = 0.62$).

The variance of standardized heterozygosity tended to decrease with age, being 1.07 (95% CI: 0.98–1.17) larger in juveniles and yearlings than in older age classes ($\chi^2 = 2.77$, d.f. = 1, $P = 0.09$, Fig. 1).

The generalized determination coefficient (R^2 as measured by the correlation between observed and predicted heterozygosity values, Zheng & Agresti 2000), was equal to 8.6% (95% CI: 4.3–13.1%) for the heteroscedastic GLS model.

Effect of standardized heterozygosity on survival and probability of becoming dominant

GOF test. The multistate GOF tests showed that the two individual component tests are accepted: (i) 'behavioural equivalence' of individuals released together regardless of their past capture history is verified (test 3G: $\chi^2 = 86.26$, d.f. = 79, $P = 0.27$); (ii) 'equivalence' among those individuals that are eventually recaptured (on a subsequent occasion) conditional on whether or not they are encountered at the present occasion (test M: $\chi^2 = 28.28$, d.f. = 28, $P = 0.45$) (see Pradel *et al*. 2003 for full details about these tests). The full time-dependent model ($p_t^s \Phi_t^s \Psi_t^{ss}, p_t^D \Phi_t^D \Psi_t^{sD}$ or Jolly-Movement model) adequately fitted our data set ($\chi^2 = 114.53$, d.f. = 107, $P = 0.29$).

Selection of the global model excluding SH. The model of capture probability with the lowest AICc included differences among age classes, sex and period (Table 3a). This model was better supported than a model that included an additional status effect (AICc weight 0.44 vs. 0.28, Table 3a) and was thus retained as the most parsimonious. The model of survival with the lowest AICc included effects of age, year, status for adults, and effect of the presence of helpers on juveniles (Table 3b). This model was three times better supported than the next model (AICc weight 0.74 vs. 0.26, Table 3b). Finally, we modelled the transition from subordinate to dominant status. The model with the lowest AICc included only the effects of age class (Table 3c). In summary, the best model (model B) included age-, sex- and period-dependent capture probabilities, age- and time-dependent survival probabilities for all age classes and helper-dependent survival probabilities for juveniles, and age-dependent transition probabilities (Table 3, for details see Table S2, Supporting information).

Survival probability increased with age: 0.64 ± 0.03 for juveniles, 0.81 ± 0.04 for yearlings, and 0.81 ± 0.02 for dominant adults (Fig. 2, for details see Table S1, Supporting information). Apparent survival was 0.57 ± 0.05 for 2 years

Table 3 Model selection based on AICc for the five best capture models (a), survival models (b), and state transition models (c) nested in the most general model $p^{s}_{age*t*sex}\Phi^{s}_{[age1*h+age2-4]*t*sex}\Psi^{ss}_{age3-4*t*sex}, p^{D}_{age3-4*t*sex}\Phi^{D}_{age4*t*sex}\Psi^{sD}_{age3-4*t*sex}$ (see Table 2 for explanation of abbreviations)

(a) Capture model	k	Deviance	AICc	AICc weight
B $P_{age+sex*tcl}$	33	2578.067	2645.66	0.44
$p2$ $P^{s}_{age+sex*tcl}P^{D}_{age4+sex*tcl}$	34	2576.846	2646.53	0.28
$p3$ $P_{age*sex+sex*tcl}$	36	2573.675	2647.57	0.17
$p4$ $P^{s}_{age*sex+sex*tcl}P^{D}_{age4*sex+sex*tcl}$	38	2572.232	2648.23	0.04
$p5$ $P_{age+tcl}$	30	2590.854	2652.17	0.02
(b) Survival model	k	Deviance	AICc	AICc weight
B $\Phi^{s}_{age1*h+age2-4+t}\Phi^{D}_{age4+t}$	33	2578.067	2645.66	0.74
$\Phi2$ $\Phi^{s}_{age1*h+age2-4+sex+t}\Phi^{D}_{age4+sex+t}$	34	2578.039	2647.73	0.26
$\Phi3$ $\Phi^{s}_{[age1*h+age2-4]*sex+t}\Phi^{D}_{age4*sex+t}$	40	2575.046	2657.38	0.00
$\Phi4$ $\Phi^{s}_{age1*h+age2-4+sex*t}\Phi^{D}_{age4+sex*t}$	49	2565.855	2667.37	0.00
$\Phi5$ $\Phi^{s}_{[age1*h+age2-4]*sex+sex*t}\Phi^{D}_{age4*sex+sex*t}$	55	2562.724	2677.16	0.00
(c) State transition model	k	Deviance	AICc	AICc weight
B $\Psi^{ss}_{age3-4}, \Psi^{sD}_{age3-4}$	33	2578.067	2645.66	0.65
$\Psi2$ $\Psi^{ss}_{age3-4*sex}\Psi^{sD}_{age3-4*sex}$	35	2575.258	2647.05	0.32
$\Psi3$ $\Psi^{ss}_{age3-4+sex}\Psi^{sD}_{age3-4+sex}$	31	2588.692	2652.09	0.03
$\Psi4$ $\Psi^{ss}_{age3-4+t}\Psi^{sD}_{age3-4+t}$	45	2574.698	2667.65	0.00
$\Psi5$ $\Psi^{ss}_{age3-4+sex+t}\Psi^{sD}_{age3-4+sex+t}$	46	2573.825	2668.92	0.00

old and 0.56 ± 0.06 for subordinate adults (Fig. 2, Table S1). The presence of helpers had a positive effect on juvenile survival, increasing it from 0.57 ± 0.05 for juveniles born without helpers to 0.72 ± 0.03 for juveniles born with helpers (Fig. 2, Table S1).

The probability of becoming dominant the following year was 0.43 ± 0.05 for 2 years old vs. 0.29 ± 0.06 for older subordinates (Table S1).

Effect of SH on survival and probability of becoming dominant. We found a significant interaction effect of heterozygosity and age on survival (Table 4a). Standardized heterozygosity had a strong effect on juvenile survival [$\beta \pm SE = 1.243 \pm 0.499$, CI(0.265; 2.223), Fig. 3], but no effect on the survival of older individuals [$\beta \pm SE = -0.018 \pm 0.352$, CI(–0.708; 0.671), Table 4a]. This was also true when we considered each age-class separately [yearling survival: $\beta \pm SE = -0.172 \pm 1.006$ CI(–2.144; 1.780), 2 years old survival: $\beta \pm SE = -0.742 \pm 1.074$ CI(–2.847; 1.364), adult survival: $\beta \pm SE = 0.233 \pm 0.547$ CI(–0.837; 1.305), Table 4a]. The effect of heterozygosity on juvenile survival was independent of the presence of helpers within the family and was also constant over years (Table 4a). Our results indicate that for juveniles, the odds of surviving increases by 3.46 [CI(1.02; 9.23)] for an increase of 1 in heterozygosity [standardized coefficient: 1.34 CI(1.06; 1.69)]. In other words, an increase in heterozygosity of 0.1 increases the probability of survival of young marmots by approximately 13% (Fig. 3). The partial generalized coefficient of determination for discrete models (Nagelkerke 1991) yields a value of $R^2 = 1\%$.

Individual heterozygosity did not affect the probability of becoming dominant. There was no additive effect of SH, no interactive of SH with age and no age-specific effect of SH on the probability of becoming dominant (Table 4b). No effect of individual standardized heterozygosity was evidenced on the probability of becoming dominant neither for 2 years old [$\beta \pm SE = -0.877 \pm 0.961$ CI(–2.761; 1.007)] nor for older individuals [$\beta \pm SE = -1.170 \pm 1.353$ CI(–3.821; 1.481)].

General vs. local effects of heterozygosity

There were more observed positive correlations of heterozygosity between pairs of loci than expected by chance (72 positive correlations out of 120, 9999 simulations, $P = 0.017$) and more significant positive correlations than expected by chance (25 out of 120, 9999 simulations, $P < 0.001$). Thus, heterozygosity was positively correlated across loci. This was confirmed by a significant positive correlation of SH between two sets of eight loci ($P = 0.006$), although the median correlation coefficient was quite low: 0.143 (2.5 and 97.5 percentiles: 0.038, 0.226).

Table 4 Effect of heterozygosity on survival probabilities (a) and state transition probabilities (b) tested by likelihood ratio tests and models including effects of heterozygosity (see Table 2 for explanation of abbreviations)

(a)

Effect tested	Model	χ^2	d.f.	*P*
Additive effect between SH and helper on juvenile survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[[age1*h+SH]+age2-4]+t},\Phi^{D}_{age4+t}$	6.349	1	0.01
Interactive effect between SH and helper on juvenile survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[[age1*h*SH]+age2-4]+t},\Phi^{D}_{age4+t}$	7.650	3	0.06
Additive effect of SH on all age classes survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-4]+t+SH},\Phi^{D}_{age4+t+SH}$	2.210	1	0.14
Interactive effect of SH contrasting juvenile and older individuals survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-4]+[age1+age2^{+}]*SH+t},\Phi^{D}_{age4+t}$	6.532	2	0.04
Interactive effect of SH on all age classes survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-4]*SH+t},\Phi^{D}_{age4*SH+t}$	6.836	4	0.14
Effect of SH on 2-year-old survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2+age3*SH+age4]+t},\Phi^{D}_{age4+t}$	0.210	1	0.65
Effect of SH on dominant adult survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4*SH+t}$	0.141	1	0.71
Effect of SH on adult survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-3+age4*SH]+t},\Phi^{D}_{age4*SH+t}$	0.106	1	0.30
Effect of SH on yearling survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2*SH+age3-4]+t},\Phi^{D}_{age4+t}$	0.027	1	0.87
Effect of SH on subordinate adult survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-3+age4*SH]+t},\Phi^{D}_{age4+t}$	0.002	1	0.96
Interactive effect of SH on time-dependent juvenile survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+t*SH]+[age2-4+t]},\Phi^{D}_{age4+t}$	16.389	16	0.43
Interactive effect of SH on time-dependent adult survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2-3+t]+[age4+t*SH]},\Phi^{D}_{age4+t*SH}$	16.331	16	0.43
Interactive effect of SH on time-dependent 2-year-old survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age2+age4+t]+[age3+t*SH]},\Phi^{D}_{age4+t}$	10.745	16	0.82
Interactive effect of SH on time-dependent yearling survival	$\Phi^{s}_{[age1*h+age2-4]+t},\Phi^{D}_{age4+t}\text{ vs. }\Phi^{s}_{[age1*h+age3-4+t]+[age2+t*SH]},\Phi^{D}_{age4+t}$	2.395	16	0.99

(b)

	Model	χ^2	d.f.	*P*
Effect of SH on the probability of becoming dominant for adult individuals	$\Psi^{ss}_{age3-4},\Psi^{sD}_{age3-4}\text{ vs. }\Psi^{ss}_{age3+age4*SH}\Psi^{sD}_{age3+age4*SH}$	1.199	1	0.28
Effect of SH on the probability of becoming dominant for 2-year-old individuals	$\Psi^{ss}_{age3-4},\Psi^{sD}_{age3-4}\text{ vs. }\Psi^{ss}_{age3*SH+age4}\Psi^{sD}_{age3*SH+age4}$	1.109	1	0.29
Additive effect of SH on the probability of becoming dominant for 2-year-old and adult individuals	$\Psi^{ss}_{age3-4},\Psi^{sD}_{age3-4}\text{ vs. }\Psi^{ss}_{age3-4+SH}\Psi^{sD}_{age3-4+SH}$	1.934	1	0.16
Interactive effect of SH on the probability of becoming dominant for 2-year-old and adult individuals	$\Psi^{ss}_{age3-4},\Psi^{sD}_{age3-4}\text{ vs. }\Psi^{ss}_{age3-4*SH}\Psi^{sD}_{age3-4*SH}$	1.961	2	0.38

No significant association was found between juvenile survival and heterozygosity calculated for each locus separately ($0.065 < P < 0.984$, $-0.061 < \text{slope} < 0.460$, Fig. 4). Heterozygosity at 14 of the 16 microsatellites presented a positive effect ($0.005 < \text{slope} < 0.460$, Fig. 4) with heterozygosity at SS-Bibl4 showing the strongest effect (parameter estimate ± SD = 0.460 ± 0.249, $t = 1.847$, d.f. = 691, $P = 0.065$).

The association between SH and juvenile survival always remained significant ($0.007 < P < 0.03$), when omitting loci one at a time. SS-Bibl20, SS-Bibl31, SS-Bibl4, MS41, MS45 and ST10, each increased the global slope of SH on survival by more than 10% whereas SS-Bibl1, MS47, MS56, MS6 and Ma002 decreased the global slope by 2% to 8% (Fig. 4). These results indicated that heterozygosity at SS-Bibl20, SS-Bibl31, SS-Bibl4, MS41, MS45 and ST10 (and particularly at SS-Bibl4 and ST10) have a predominant influence on the overall result (Fig. 4).

Discussion

Evidence of age-dependent heterozygosity-fitness correlation in the alpine marmot

HFC should decrease with age because the variability in fitness components such as growth and survival is maximal early in life (David 1998) and because unfit individuals

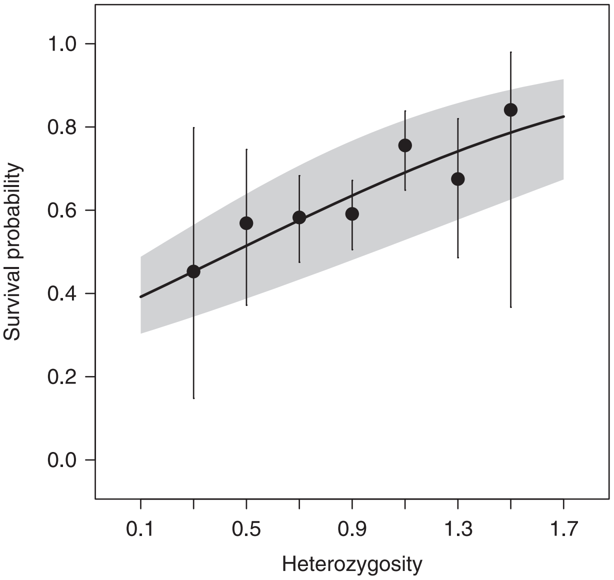

Fig. 3 Effect of standardized heterozygosity on juvenile survival. The black circles represent observed data averaged over classes of standardized heterozygosity (class width 0.2, except for the first class, which is from 0 to 0.4) and their 95% confidence intervals. The solid line shows the fitted model and the grey surface represents standard errors of the fitted model.

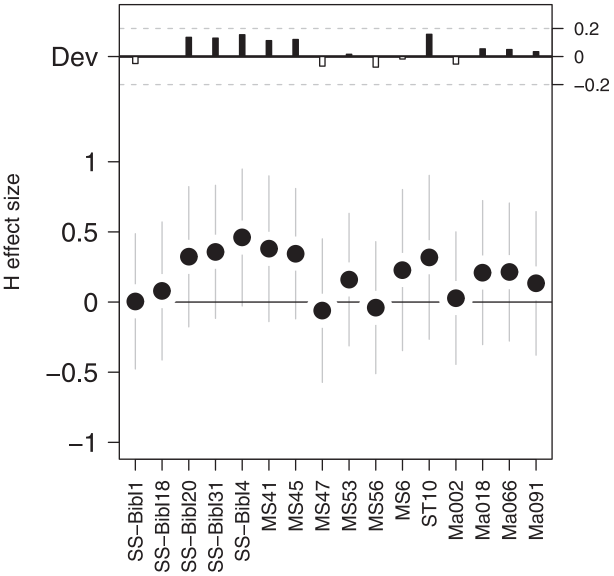

Fig. 4 Effect of heterozygosity (*H*) calculated for each locus separately on juvenile survival (parameter estimate ± SE). The top bars represent the percentage of variation of the global slope of the effect of SH on juvenile survival once the given locus is added (black bars represent positive contribution of the locus, white bars represent negative contribution of the locus).

among young ones do not survive and are absent in older age classes (filter effect hypothesis, Koehn & Gaffney 1984). A consequence of the elimination of unfit individuals in ageing cohorts is the prediction that heterozygosity should be higher in average and less variable among oldest individuals. Decreasing HFCs with age were essentially documented for morphometric traits like size or growth, and concerned mainly marine organisms (*Ostrea edulis,* Bierne *et al*. 1998; Marsic-Lucic & David 2003; *Anguilla anguilla,* Pujolar *et al*. 2006) and one mammal (*Capra ibex,* Von Hardenberg *et al*. 2007). However, to the best of our knowledge, age-dependent effects of heterozygosity on survival was not yet reported. Until now, the relationship between heterozygosity and survival has been investigated only in juveniles and positive correlations have been reported in a variety of mammals (*Cervus elaphus,* Coulson *et al*. 1998; Coulson *et al*. 1999; *Halichoerus grypus,* Bean *et al*. 2004, *Marmota marmota,* Da Silva *et al*. 2006; *Rhinolophus ferrumequinum,* Rossiter *et al*. 2001; *Phoca vitulina,* Coltman *et al*. 1998; *Phoca groenlandica,* Kretzmann *et al*. 2006; *Zalophus californianus,* Acevedo-Whitehouse *et al*. 2006 but see *Arctocephalus gazella,* Hoffman *et al*. 2006; *Capreolus capreolus,* Da Silva *et al*. 2009), and in birds (*Acrocephalus arundinaceus,* Hansson *et al*. 2001; Hansson *et al*. 2004; *Parus major,* van de Casteele *et al*. 2003; *Passer domesticus,* Jensen *et al*. 2007). The lack of age-dependent effects of heterozygosity on survival may be the consequence of a publication bias (Coltman & Slate 2003) but is more probably due to the difficulty of both collecting a sufficient amount of longitudinal data and using appropriate methodology to analyse them. Here we report for the first time an age-dependent effect of heterozygosity on survival in the alpine marmot. Specifically, we find a positive correlation between standardized multilocus heterozygosity at 16 microsatellite loci and juvenile survival, but no relationship between SH and the survival of older individuals. Although small, the effect size of this correlation (< 1%) falls within the range reported in previous studies (Coltman & Slate 2003). As expected, this HFC leads to an increase in mean heterozygosity and a decrease in the variance in heterozygosity between juveniles and older individuals.

The acquisition of a breeding vacancy is a critical determinant of reproductive success in species, like the alpine marmot, with highly skewed reproduction towards dominants. Many studies revealed an advantage to heterozygous males in territory acquisition (data concerning females are lacking). For example, in the black grouse, *Tetrao tetrix,* males that hold a lek territory, particularly in a central position, are more heterozygous than males that never obtained a territory (Höglund *et al*. 2002). Individual heterozygosity was also positively related to territory size in the subdesert mesite, *Monias benschi* (Seddon *et al*. 2004) or to territory tenure in the Antarctic fur seal, *A. gazella* (Hoffman *et al*. 2004). In the alpine marmot, we failed to find a correlation between individual heterozygosity and the probability of becoming dominant, neither for males, nor for females.

This may be explained by the small number of homozygous individuals attaining the age at which they might reach dominance (at least 3 years old in alpine marmot). Similarly, in mandrills, *Mandrillus sphinx*, no relationship was found between individual heterozygosity and the probability to reach alpha status in males, or between individual heterozygosity and social rank in females, even though more heterozygous individuals had a higher lifetime reproductive success (Charpentier *et al.* 2005). Before concluding that there is no effect of individual heterozygosity on reproductive performance in the alpine marmot, further analyses should consider other important determinants of reproductive success, such as the length of dominance tenure, or lifetime reproductive success per se.

Underlying mechanisms of HFC

Analyses of the individual contribution of each separate locus to the overall positive effect of heterozygosity on juvenile survival revealed that no single locus contributed disproportionately to the observed correlation. Thus, at least in our population of alpine marmots, the local effect hypothesis seems inappropriate to explain the observed HFC. This is in agreement with a previous study on alpine marmots that found no correlation between heterozygosity and juvenile survival among full-siblings (Da Silva *et al.* 2006), but also with most studies on HFC (review by Hansson & Westerberg 2002). Relatively few studies found support for the local effect hypothesis, in the sense that some loci contributed more to the overall HFC than others (Merilä & Sheldon 2000; Bean *et al.* 2004; Acevedo-Whitehouse *et al.* 2006; Lieutenant-Gosselin & Bernatchez 2006; Brouwer *et al.* 2007). However, this is expected by chance even under the general effect hypothesis (but see Da Silva *et al.* 2009).

The general effect hypothesis seems to better explain the observed positive correlation found between heterozygosity and juvenile survival in alpine marmots. Pairs of loci indeed showed a positive correlation in heterozygosity, more often than expected by chance, and the overall correlation of standardized heterozygosity between two sets of eight loci was significant, albeit quite low (mean = 0.14). Thus, heterozygosity was positively correlated across loci, indicating a possible genome-wide effect (Pemberton 2004). This general effect can result from inbreeding and/or from considerable linkage disequilibrium (Balloux *et al.* 2004; Hansson *et al.* 2004; Pemberton 2004; Slate *et al.* 2004). Both mechanisms are likely to occur in alpine marmots because of its low effective population sizes (Hansson & Westerberg 2002). The effective population size is typically smaller than the observed population size in alpine marmots because of the social structure that results in suppression of the reproduction by subordinates of both sexes (Cohas *et al.* 2006; Cohas *et al.* 2008). Moreover, linkage disequilibrium is likely because the species has undergone a bottleneck followed by rapid population expansion (Preleuthner & Pinsker 1993; Kruckenhauser *et al.* 1997).

Acknowledgements

We thank all students involved in the trapping of alpine marmots at the Grande Sassière Nature Reserve. We thank Alexander Girg and Sylvia Kuhn at the Max Planck Institute for Ornithology for their great help in typing marmots. We warmly thank Nigel Gilles Yoccoz for statistical advice, Raquel Page for carefully editing the manuscript and Jean-Michel Gaillard, Jon Slate and two anonymous referees for helpful comments on a previous version of the manuscript. Thanks are also extended to authorities of the Vanoise National Park for allowing us to work in the Grande Sassière Nature Reserve. Financial support was received from the ANR (ANR-08-BLAN-0214-03), the CNRS (France), the Fyssen foundation and the Max Planck Society. All experiments conducted comply with current French laws.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Parameter estimates of the MS–CMR model $p_{age+tcl*sex}\Phi^{s}_{[age1*h+age2-4]+t}\Psi^{ss}_{age3-4},\Phi^{D}_{age4+t}\Psi^{sD}_{age3-4}$

Table S2 Beta estimates of the MS–CMR model $p_{age+tcl*sex}\Phi^{s}_{[age1*h+age2-4]+t}\Psi^{ss}_{age3-4},\Phi^{D}_{age4+t}\Psi^{sD}_{age3-4}$ with their associated confidence limits at the 95% level (lower and upper bounds are provided) and standard errors