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Genetic diversity-fitness correlation revealed by microsatellite analyses in European alpine marmots (*Marmota marmota*)

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Abstract

The relationship between individual genetic diversity and fitness-related traits are poorly understood in the wild. The availability of highly polymorphic molecular markers, such as microsatellites, has made research on this subject more feasible. We used three microsatellite-based measures of genetic diversity, individual heterozygosity H, mean d^2 and mean $d^2_{\text{outbreeding}}$ to test for a relationship between individual genetic diversity and important fitness trait, juvenile survival, in a population of alpine marmots (*Marmota marmota*), after controlling for the effects of ecological, social and physiological parameters that potentially influence juvenile survival in marmots. Analyses were conducted on 158 juveniles, and revealed a positive association between juvenile survival and genetic diversity measured by mean H. No association was found with mean d^2 and with mean $d^2_{\text{outbreeding}}$. This suggests a fitness disadvantage to less heterozygous juveniles. The genetic diversity-fitness correlation (GDFC) was somewhat stronger during years with poor environmental conditions (i.e. wet summers). The stressful environmental conditions of this high mountain population might enhance inbreeding depression and make this association between genetic diversity and fitness detectable. Moreover the mating system, allowing extra pair copulation by occasional immigrants, as well as close inbreeding, favours a wide range of individual genetic diversity (mean H ranges from 0.125 to 1), which also may have facilitated the detection of the GDFC. The results further suggest that the observed GDFC is likely to be explained by the "local effect" hypothesis rather than by the "general effect" hypothesis.

Introduction

The relationship between individual genetic variability and fitness, or more often fitness-related traits, has long been of interest to evolutionary and conservation biologists (Allendorf and Leary 1986; Houle 1989; Lacy 1993; Mitton 1993; David 1998; Hansson and Westerberg 2002; Coltman and Slate 2003). Because of the difficulty of obtaining pedigrees in the wild, studies commonly use molecular markers to measure multilocus heterozygosity, in order to infer individual inbreeding coefficients. This approach examining the association between marker heterozygosity and fitness traits is sometimes termed genetic diversity–fitness correlation (GDFC) approach. The strength of GDFCs in the wild is still debated. Studies reporting negative results exist (Rowe and Beebee

2001; Duarte et al. 2003) and may be underrepresented because of publication bias in favour of positive results. On the other hand, many recent studies report a correlation between heterozygosity at neutral microsatellite markers and individual fitness-related traits such as survival (Coltman et al. 1998; Coulson et al. 1998, 1999; Rossiter et al. 2001; Markert et al. 2004), reproductive success and recruitment (Slate et al. 2000; Höglund et al. 2002; Foerster et al. 2003; Hansson et al. 2001, 2004; Hoffman et al. 2004; Markert et al. 2004; Seddon et al. 2004), and disease resistance (Coltman et al. 1999). In a meta-analysis, Coltman and Slate (2003) found that positive associations between neutral marker heterozygosity and traits or components of individual fitness were common but weak.

In addition to the controversy over the strength or existence of GDFCs, the underlying mechanisms causing GDFCs are also still debated. Three hypotheses currently prevail (Hansson and Westerberg 2002): first, under the *direct effect* hypothesis, selection acts directly on the markers to induce GDFC; because microsatellites are thought to be selectively neutral (Queller et al. 1993; Jarne and Lagoda 1996; but see Kashi and Soller 1999) GDFCs detected by microsatellites are unlikely caused by direct effects. Second, in partially inbred populations, multilocus heterozygosity (MLH) at marker loci might reflect variation in the individual inbreeding coefficient (Weir and Cockerham 1973). Under this *general effect* hypothesis (Weir and Cockerham 1973; David 1998), a GDFC is generated as a result of effects of homozygosity at loci distributed genome-wide (Hansson and Westerberg 2002; Slate et al. 2004). Third, MLH at marker loci may reflect heterozygosity at overdominant or dominant fitness trait loci in linkage disequilibrium (LD) with them (Hansson and Westerberg 2002; Slate et al. 2004). Under this *local effect* hypothesis (Hill and Robertson 1968; Otah 1971; David 1998), a GDFC can arise because heterozygosity will tend to be the same both at marker and at fitness loci in the local chromosomal vicinity (Slate et al. 2004).

Until recently, GDFCs were mostly attributed to inbreeding depression effects (i.e. the general effect hypothesis). Balloux et al. (2004) and Slate et al. (2004) revealed that molecular metrics are often only weakly correlated with individual inbreeding coefficients (f), suggesting that correlations between MLH and fitness might require a new interpretation (Pemberton 2004). If multilocus heterozygosity shows limited power to detect variance in inbreeding, the most parsimonious explanation for studies reporting GDFC would be the local effect hypothesis rather than the general effect hypothesis (Balloux et al. 2004; Slate et al. 2004), unless the population shows particular patterns (such as strong inbreeding, for example through selfing or strong population structure and/or high levels of polygyny) (Balloux et al. 2004). Recently, substantial linkage disequilibrium has been found in vertebrates (Reich et al. 2001; McRae et al. 2002), and the local effect hypothesis has received some support as in the great reed warbler *Acrocephalus arundinaceus* (Hansson et al. 2001, 2004). Analyses were conducted with dyads of full siblings of which only one individual survived to adult age. This ensured that each member of a pair had the same f, and allowed to exclude the general effect hypothesis. Hansson et al. (2001, 2004) thus revealed that recruited individuals showed significantly higher MLH than their non recruited siblings.

This study has two aims. First, we investigate GDFC in the alpine marmot, *Marmota marmota*, using the classical measure of heterozygosity (H), and measures introduced by Coulson et al. (1998, 1999) (d^2 and $d^2_{\text{outbreeding}}$). GDFC remains difficult to investigate in the wild, and few studies have considered ecological variables in their analysis. However, there is some evidence that GDFC may be related to the harshness of the environment (Danzmann et al. 1988; Dudash 1990; Borsa et al. 1992; Audo and Diehl 1995; Meagher et al. 1997; Crnokrak and Roof 1999; Lesbarrères et al. 2005). In this context, the alpine marmot is particularly interesting because populations occur in high alpine meadows, and must cope with a harsh climate and occasionally stressful conditions. Moreover, the mating system of alpine marmots is socially monogamous, but extra pair paternity (EPP) occurs frequently (in about 33% of litters, Goossens et al. 1998). Extra pair males often come from other groups or populations, which could increase genetic diversity among individuals, and hence the power to detect GDFC. Then, we tested the prediction that a GDFC would be detected in our population, particularly during stressful years. For that, we investigated for the relationship between juvenile survival, a major fitness-related trait in this species (Farand et al. 2002) and individual

genetic diversity after controlling for the effects of ecological, social and physiological parameters assumed to potentially influence juvenile survival. Hence, the conclusions reached here will have practical use, and particularly for the management of fragmented population or endangered other marmot species, such as *Marmota vancouverensis* or *M. menzbieri*, that are relatively more difficult to study.

Second, we investigate more specifically the underlying mechanism of GDFC in our population. We tested the local effect hypothesis using the same approach as Hansson et al. (2001, 2004): in alpine marmots, litters of 3–6 juveniles are common, it is then possible to compare surviving and not surviving full-sibs, and to check if survivors have higher genetic diversity. We also tested the general effect hypothesis using Balloux et al.'s analytical approach (2004), based on the principle that if average heterozygosity reflects *f*, then the heterozygosity of loci within an individual should be correlated.

Materials and methods

Study site and population

The study site is in La Sassière Nature Reserve, in the eastern part of the Vanoise National Park in the French Alps. The elevation is about 2300 m and the climate is typical of high alpine areas. From 1990 to 2004, alpine marmots were caught from the beginning of April to the end of July during a minimum of 45 days a year. Marmots were trapped using two-door, live-capture traps baited with dandelion *Taraxacum densleonis* and placed near the entrance of the main burrow of each group in order allowing captured individuals to be assigned to a family group. Once caught, individuals were tranquillised with Zolétil 100 (0.1 ml kg^{-1}) and individually marked with a numbered ear tag and a transponder (model ID100, Trovan^{TM}, Germany) injected under the skin of the neck for permanent individual recognition. Each individual trapped was sexed, aged, weighed, and measured for morphological variables such as body length. From 1992 to 1997, hairs were collected, and since 1998, tissue biopsies were used for genetic analyses.

The composition of 20 family groups was assessed from capture–recapture data on 607 trapped individuals from 1992 to 2002, and from intensive observation from April to July with 10–50× binoculars and 20–60× telescopes from a distance of 80–200 m. Each group was observed on average 1 h per day for a minimum of 30 h per year. For each group, the number of yearlings, 2-year-olds and adult individuals of each sex and their social status were recorded. Individuals were classified as yearlings, 2-year-olds or adult individuals from their size. The sex was determined from ano-genital distance. Each year, we recorded the date and the litter size at emergence. Virtually all emerged juveniles were trapped within 3 days after emergence (Allainé et al. 2000; Allainé 2004). Despite social monogamy of *Marmota marmota*, extra pair copulations (EPC) occurred, and approximately 20% of juveniles would be born from EPC (Goossens et al. 1998). Males implicated in EPC are from other groups or other populations. Indeed, the population studied is not isolated, and exchanges of individuals occur between neighbouring populations (Goossens et al. 2001).

Juvenile survival

Juvenile survival (i.e. survival from birth to 1 year) was determined as the proportion of juveniles still present as yearlings in the family group, the spring 1 year after birth. Juvenile survival was estimated from field observations and from capture–recapture data. The number of yearlings is a reliable indicator of juvenile survival because dispersal does not occur before 2-years-old, except in very rare cases (Arnold 1990; Perrin et al. 1993). Trapping effort was important and the mean recapture rate of yearlings was 0.92 (Farand et al. 2002). Non-recaptured yearlings were sometimes identified from visual observation and from counts of number of yearling alive in the family group. Juveniles that were known to have been killed following territory take-overs by new males and those of unknown fate were not included in the analyses.

Non-genetic terms

To examine GDFC, it might be necessary to correct for potential confounding non-genetic factors.

Non-genetic factors may include both environmental and individual characteristics. Because the deleterious effects of homozygosity may be more apparent under stressful conditions (Danzmann et al. 1988; Dudash 1990; Borsa et al. 1992; Audo and Diehl 1995; Meagher et al. 1997; Crnokrak and Roof 1999; Lesbarrères et al. 2005), we considered two environmental factors: the cohort and the territory quality. We considered two individual characteristics: the sex and the body condition, and a social factor: the presence of helpers.

Cohort (categorical variable, two levels)

Cohorts from 1991 to 2000 were considered. Farand et al. (2002) showed that the only environmental factor to which juvenile survival seemed to be sensitive was summer rainfall. Wet summers induce stressful climatic conditions because juveniles spend less time feeding and thus gaining weight. We have used "Météo France" data (France weather service), on daily readings of precipitation at the Tignes station (elevation, 1800 m high), the nearest station to our study site (7 km to the reserve). Because precipitation increases with altitude (Ozenda 1985), data from Tignes station underestimated precipitation at our study site. However the Tignes station is in the same valley system as the study site and therefore accurately reflects annual weather variability. We separated years into two groups ("good" years and "bad" years), based on summer precipitation. The median (2.8 mm day^{-1} over the period July–September) was used to determine the two groups: good = dry years (1990, 1991, 1992, 1993, 1995 and 1997), with a mean summer rainfall lower than the median, and bad = wet years (1994, 1996, 1998, 1999 and 2000), with a mean summer rainfall greater than the median. Using cohort as a categorical covariate helped investigating interactions with genetic variables.

Territory quality (categorical variable, two levels)

The quality of each territory was estimated from the date of snowmelt because early snow melt favoured early access to food resources (Bibikov 1996; Schwartz et al. 1998). During 2 years, photographs of each territory were regularly taken during May and June. Territories were then ranked according to the date at which 20% of the surface territory was accessible (no more covered by snow), and also, 50%, 75%, and 100%. For each territory, the ranks for the four percentages were summed, and the sum was used as a proxy of territory quality. Again, to keep large sample size, the 19 territories were divided into two groups: "high quality" territories (12 territories) and "poor quality" territories (7 territories). Territories of high quality had a sum lower than 40, whereas those of low quality had a sum from 55 to 84.

Body condition (continuous variable)

The juvenile body condition was the residual of the multiple regression of juvenile body mass on body size, age, sex and litter size. Body condition thus corresponded to body mass corrected for (i) structural size effect, and (ii) the difference in date of capture among individuals, and (iii) sexual dimorphism (Allainé et al.1998), and (iv) the trade-off between size and number of juveniles (Allainé et al. 1998). Body size was the body length from head to tail. Age was determined from the date of emergence from the natal burrow. A positive residual indicated a juvenile in relatively good condition whereas a negative residual indicated a juvenile in relatively poor condition.

Sex (categorical variable, two levels)

Because the alpine marmot is primarily monogamous (Goossens et al. 1998), the juvenile survival is expected to be independent of the sex of individuals (see also analyses of Farand et al. 2002). Indeed, many studies on mammals found a correlation between sexual dimorphism and sexual bias in survival. The sex investigating more energy in development (generally males) would suffer higher mortality, (Clutton-Brock et al. 1982; Loison 1995; Jorgenson et al. 1997; Van Horne et al. 1997). Consequently, a sexual bias in survival should be preferentially found in polygynous species in which sexual dimorphism is important (Promislow 1992).

Helpers (categorical variable, two levels)

The presence of helpers (subordinate males) in the hibernaculum was found to be an important factor for juvenile winter survival, probably because they actively warm juveniles (Arnold 1993) and facilitate thermoregulation (Allainé et al. 2000; Allainé and Theuriau 2004). For a given year, each group was encoded by 1 (helpers presence) or by 0 (helpers absence).

Molecular markers and genetic terms

We typed 187 individuals (101 males and 86 females) at up to 9 microsatellites: SS-Bibl1, SS-Bibl4, SS-Bibl18, SS-Bibl20, SS-Bibl25, SS-Bibl31 (Klinkicht 1993) and MS45, MS47, MS53 (Hanslik and Kruckenhauser 2000). PCR amplification conditions in *Marmota marmota* are described, for SS-Bibl1, SS-Bibl4, SS-Bibl18, SS-Bibl20, SS-Bibl25, SS-Bibl31 in Goossens et al. (1998) and for MS45, MS47, MS53 in Hanslik and Kruckenhauser (2000). Amplified fragments were loaded on 5% Long Ranger polyacrylamide gel and electrophoresis was run for 3 h on an automated sequencer ABI 377^{TM} (Applied Biosystems). Microsatellite patterns were examined with Genotyper® 2.0 (Applied Biosystem).

Tests for deviation from Hardy–Weinberg proportions and linkage disequilibrium between markers were implemented using GENEPOP v3.3 (Raymond and Rousset 1995). Tests were performed using all dominant adults (to avoid bias due to family group structure) and on all cohorts gathered (to ensure adequate sample size). The analyses of SS-Bibl1, SS-Bibl4, SS-Bibl18, SS-Bibl20, SS-Bibl25, SS-Bibl31, were conducted on 69 dominant adults (cohorts from 1992 to 2000); whereas analyses of MS45, MS47 and MS53 were conducted on 31 dominant adults (cohorts from 1997 to 2000), because these markers are only recently used in our population (i.e. less individuals are typed with them and older DNAs were no more available).

Three metrics of genome-wide diversity were used:

H: mean individual heterozygosity. Individual heterozygosity is classically measured by the proportion of heterozygous loci.

In addition, we used two measures derived from mean d^2, i.e. the squared difference in number of repeat units between the two alleles of an individual at a microsatellite locus, averaged over all loci at which the individual was scored (Coulson et al. 1998):

$\mathrm{LD} = \log_{10}(\text{mean } d^2 + 1)$.

$\mathrm{LDO} = \log_{10}(\text{mean } d^2_{\text{outbreeding}} + 1)$ where mean $d^2_{\text{outbreeding}}$ is the mean d^2 score excluding homozygous loci (Coulson et al. 1999).

The distributions of mean d^2 and mean $d^2_{\text{outbreeding}}$ were right-skewed. Consequently and following Coltman et al. (1998) the logarithmic transformation was used to improve the spread of the variables.

Assuming that microsatellites evolve under the stepwise mutation model (SMM) (Valdes et al. 1993), mean d^2 and mean $d^2_{\text{outbreeding}}$ provide a measure of the genetic distance between parental gamete genomes and appear particularly sensitive to outbreeding (Coulson et al. 1998, 1999). The fit of each locus distribution to expected distribution under three different mutation models, the SMM, the IAM (infinite allele model) and an intermediate two-phase model (TPM) was tested using the program BOTTLENECK (Cornuet and Luikart 1996). These analyses provide a test statistic, the Wilcoxon sign-rank test, for the probability that an observed allele distribution with a given heterozygosity (gene diversity) was generated under each of the three mutation models (SMM, IAM, TPM).

Individuals were typed with approximately the same number of microsatellites (see Results). Hence, no standardisation transformation was necessary for measures of genetic diversity.

Statistical modelling of survival

We used generalized linear mixed models with the penalized quasi-likelihood (PQL) procedure (Breslow and Clayton 1993) and a binomial error to examine the terms that affected juvenile survival. GLMMs were appropriate for these analyses because data were not independent (McCullagh and Nelder 1989). Indeed, mothers may have several litters of one to seven juveniles, so we used litters nested within mothers as random terms (Steele and Hogg 2003).

(i) The significance of the genetic terms was first tested by fitting each genetic term separately and without non-genetic factors, into the model. Identically the significance of non-genetic terms was tested by fitting them separately and without genetic terms into the model.

(ii) Then the significance of genetic terms was assessed after taking into account potential effects of non-genetic factors on juvenile survival. In this approach, we selected a non-genetic minimal model as described by Coltman et al. (2001): All of the non-genetic terms were fitted into the model. The non-genetic full model was

then reduced by removing each non-significant terms. Following reduction, the resulting model consisted of only significant term (the non-genetic minimal model). In order to test for an association between genetic diversity and juvenile survival after having corrected for non-genetic variables, genetic terms were fitted separately in the non-genetic minimal model. Interactions in the non-genetic model and between genetic and non-genetic terms (that remained in the non-genetic minimal model) were also assessed.

The significance of explanatory fixed (non-genetic and genetic) terms was assessed by their Wald statistics which are distributed as a χ^2. We used R 1.8.0 (Ihaka and Gentleman 1996) for all analyses.

Underlying mechanisms of GDFC

To test for the local effect hypothesis we used the approach described by Hansson et al. (2001, 2004) applied to great reed warblers. Dyads of siblings marmots of which only one individual survived to one year were selected. Siblings within dyads were confirmed to have the same genetic parents (all litters with EPP – presence of mismatch with the social father – were excluded). We tested the differences in H, LD and LDO between siblings within dyads with two-tailed (non-parametric) Wilcoxon signed-rank test because the number of dyads was small.

To test for the general effect hypothesis, we randomly divided our sample of loci into two groups and asked whether the heterozygosity (H) of the first group across individuals was correlated with the heterozygosity (H) of the second group, as recommended by Balloux et al. (2004). Then by resampling the data we repeated the procedure many times to obtain a mean and a standard error for the correlation. All of the possible combinations with the eight microsatellites used were realised.

Results

Preliminary analyses

Analyses were performed on 158 juveniles (85 males and 73 females), because survival or identity of mother was undetermined for 29 juveniles in the initial sample of 187 individuals. About 54 litters bred by 27 mothers were considered. The sample size for each variable was 158, except for body condition (151 juveniles) and for the "helpers presence" (140 juveniles). Mean juvenile survival rate was 0.705 and the range across years was 0.55 to 0.95.

Microsatellite analyses revealed that one locus, SS-Bibl25, deviated significantly from Hardy–Weinberg proportions (deficit of heterozygotes, $P = 0.0015$, see Table 1); perhaps due to non amplifying (null) allele. Further analyses, conducted with all typed juveniles, revealed that at this locus, mean heterozygosity was only 0.094 ($SE = 0.023$, $n = 101$) for males, and 0.622 ($SE = 0.404$, $n = 86$) for females. This locus was therefore excluded from all analyses. Hence anal-

Table 1. Polymorphism characteristics of microsatellite loci used to type juvenile marmots

Locus name	Na	Size range (bp)	Ho	He	HWE	Mean d^2
SS-Bibl11	6	100–114	0.61	0.65	0.10	26.12
SS-Bibl14	6	175–193	0.51	0.52	0.67	45.75
SS-Bibl18	6	138–150	0.68	0.71	0.06	11.46
SS-Bibl20	5	206–220	0.29	0.29	0.99	28.21
SS-Bibl25	5	145–155	0.41	0.62	0.0015	1.82
SS-Bibl131	4	167–173	0.25	0.32	0.14	5.29
MS45	3	109–113	0.52	0.59	0.54	2.25
MS47	7	178–190	0.68	0.74	0.07	11.81
MS53	3	135–145	0.52	0.61	0.09	15.28

PCR amplification conditions in *Marmota marmota* are described, for SS-Bibl1, SS-Bibl4, SS-Bibl18, SS-Bibl20, SS-Bibl25, SS-Bibl31 in Goossens et al. (1998) and for MS45, MS47, MS53 in Hanslik and Kruckenhauser (2000).
Na: number of alleles; Ho: observed Heterozygosity; He: unbiased expected heterozygosity (Nei 1978); HWE: significance level of deviation in expected genotype frequencies from Hardy–Weinberg expectation calculated by Fisher's exact test (Raymond and Rousset 1995).

yses were conducted with eight microsatellites. A total of 66% of juveniles were successfully typed on 8 microsatellites and 34% on 7 microsatellites.

There was no evidence for allelic disequilibrium between any loci ($P > 0.05$ for all pairs of loci). Mean H was 0.54 ± 0.035, and ranged from 0.125 to 1; mean d^2 was 1.1026 ± 0.133 and ranged from 0.3 to 1.9, and mean $d^2_{\text{outbreeding}}$ was 1.365 ± 0.112 and ranged from 0.7 to 2.1.

Tests of mutation model (BOTTLENECK analyses) showed that the distribution of the 8 microsatellites included into the analyses did not depart from the distribution expected under the SMM model ($P = 0.25$, n = 158). On the contrary it departed from distribution expected under the two other models (IAM: $P = 0.0039$, n = 158, TPM: $P = 0.004$, n = 158).

Modelling juvenile survival

When considered separately, sex ($\chi^2 = 1.22$, $P = 0.27$), nor territory quality ($\chi^2 = 0.11$, $P = 0.73$), nor juvenile body condition ($\chi^2 = 0.44$, $P = 0.50$) affected juvenile survival. The cohort significantly affected juvenile survival ($\chi^2 = 7.97$, $P = 0.0086$). In particular, juvenile survival was higher during dry years ("good" years, mean survival = 0.88) than during wet years ("bad" years, mean survival = 0.62). The presence of helpers affected juvenile survival ($\chi^2 = 5.45$, $P = 0.02$); mean juvenile survival appeared to be higher when helpers were present (0.76 vs. 0.59). However, when all the non-genetic terms were considered together, the "cohort" was the only non-genetic term significantly associated with juvenile survival (Table 2). The "cohort" was consequently the unique factor included into the minimal non-genetic model.

Genetic factors were also considered separately and alone. In this case, H showed a positive association with juvenile survival ($\chi^2 = 5.04$, $P = 0.026$), whereas no significant association was found with LD ($\chi^2 = 2.57$, $P = 0.11$), and with LDO ($\chi^2 = 1.32$, $P = 0.25$).

To assess the influence of genetic factors after taking into account the effect of non-genetic terms, we built three models by adding each of the three genetic terms (H, LD and LDO) separately into the non-genetic minimal model (i.e. with the factor "cohort"). H affected juvenile survival significantly ($\chi^2 = 5.74$, $P = 0.018$). This disadvantage to homozygous individuals was stronger during bad "years", with an interaction between the "cohort" and H ($\chi^2 = 3.31$, $P = 0.036$, one-tailed test). No association appeared between LD and juvenile survival or between LDO and juvenile survival (Table 2).

Underlying mechanisms of GDFC

We tested if one particular locus significantly influenced our results. We fitted, separately, d^2 and H values based on each individual locus, and mean d^2 and H scores calculated by omitting a single locus, in the selected non-genetic model (Table 3). Significant associations were found between

Table 2. Generalised linear mixed model of juvenile survival

Model terms	Wald statistic (χ^2)	Coefficient (SE) (with numbers of degrees of freedom associated to each parameter)	*P*-value	Direction of associations
Non-genetic terms				
Cohort	7.97	2.29 (±0.814) df = 27	**0.0086**	Better survival during "good" years
Sex	1.40	−0.44 (±0.373) df = 99	0.23	
Territory quality	0.006	0.07 (±0.970) df = 27	0.93	
Birth weight	0.60	0.0026 (±0.003) df = 96	0.44	
helpers	2.11	0.93 (±0.64) df = 23	0.15	
Genetic terms				
H	5.74	2.90 (±1.212) df = 99	**0.018**	Disadvantage to homozygotes
LD	2.55	0.909 (±0.585) df = 99	0.10	
LDO	1.04	0.657 (±0.642) df = 99	0.31	

Sample Size = 158. The significance of each genetic and non-genetic term is assessed taking into account for the factor "cohort".

survival and H values of MS45 ($P = 0.017$, $\chi^2 = 5.838$), SS-Bibl20 ($P = 0.034$, $\chi^2 = 4.615$) and SS-Bibl131 ($P = 0.019$, $\chi^2 = 5.666$). However, when MS45, SS-Bibl20 and SS-Bibl131, were excluded (each one separately), P-values were less than 0.10 (with H scores respectively: without MS45, $P = 0.078$, $\chi^2 = 3.158$; without SS-Bibl20 $P = 0.002$, $\chi^2 = 9.826$; and without SS-Bibl131, $P = 0.089$, $\chi^2 = 2.933$). Moreover, when MS45, SS-Bibl20 and SS-Bibl131 were all excluded at the same time, the P-value for H score was still less than 0.1 ($P = 0.075$, $\chi^2 = 3.12$). These results indicated that these three loci (and particularly MS45 and SS-Bibl131) have certainly a predominant influence on our results but, globally most or all the loci seemed to play a role in the final result. When omitting the other loci (one at a time), association between H and juvenile survival was always significant (Table 3). Analyses with d^2 revealed that when the d^2 value was based on each individual locus, a significant association appeared with MS45 ($P = 0.003$, $\chi^2 = 9.264$), and when we excluded loci one by one no association was found significant.

We were able to define 17 dyads of juveniles after having excluded litters with EPP and litters where all juveniles died or survived. Surviving individuals showed not significantly higher H ($P = 0.57$, n = 17) nor LD ($P = 0.85$, n = 17) nor LDO ($P = 0.89$, n = 17) than their non surviving siblings.

The correlation between H based on one half of the genetic markers and H based on the other half on the 8 microsatellites used was calculated 35 times by resampling the data (all combinations were considered). The mean correlation coefficient was 0.081 (95% CI = 0.070–0.091).

Discussion

We tested for a relationship between individual genetic diversity and fitness in alpine marmots, when non-genetic (ecological, social and physiological) confounding effects were taken into account. Fitness was measured by juvenile survival, an important component of fitness in this species (Farand et al. 2002).

Non-genetic factors

The survival of juveniles was not affected by their sex. This result was expected for a primarily

Table 3. An evaluation of the influence of individual loci on the model of the relationship between heterozygosity, mean d^2 and juvenile survival

Locus name	H: loci considered one by one		d^2: loci considered one by one	
	P-value	Wald Statistic (χ^2)	P-value	Wald Statistic (χ^2)
MS45	**0.0179**	5.838	**0.003**	9.264
MS47	0.311	1.038	0.742	0.108
MS53	0.201	1.659	0.306	1.055
SS-Bibl11	0.098	2.777	0.889	0.019
SS-Bibl14	0.117	2.495	0.164	1.960
SS-Bibl18	0.484	0.493	0.405	0.699
SS-Bibl20	**0.034**	4.615	0.957	0.003
SS-Bibl131	**0.019**	5.666	0.613	0.257
MS45	*0.078*	3.158	0.556	0.348
MS47	**0.034**	4.574	0.521	0.415
MS53	**0.028**	4.951	0.367	0.819
SS-Bibl11	**0.038**	4.383	0.446	0.584
SS-Bibl14	**0.036**	4.488	0.501	0.455
SS-Bibl18	**0.003**	9.150	0.488	0.483
SS-Bibl20	**0.002**	9.826	0.516	0.423
SS-Bibl131	*0.089*	2.933	0.563	0.335

In the first part of the table we fitted, separately, d^2 and H values based on each individual locus. In the second part, mean d^2 and H scores were calculated by omitting a single locus. The significance of each genetic term is assessed taking into account for the factor "cohort" (i.e. fitted in the non-genetic minimal model). Sample Size = 158.

monogamous species without sexual dimorphism in size (Promislow 1992).

Surprisingly, the ''body condition'' did not affect juvenile survival. One possible explanation is that body condition measured a few days after weaning reflected more maternal condition than the critical body condition just before hibernation (Armitage et al. 1976). Another possibility is that body condition is weakly related to survival (Allainé et al. 1998; Wood and Armitage 2002) probably because of the importance of social thermoregulation (Arnold 1993; Allainé et al. 2000). The best way to address this question would be to weigh juveniles just before hibernation, when they are unfortunately particularly difficult to trap.

Territory quality was evaluated by the date of snow melt and, consequently, of access to food which influences fat reserves (Bibikov 1996; Schwartz et al. 1998). Contrary to our expectation, territory quality did not affect juvenile survival. It is possible that food quality (polyunsaturated fatty acids, Geiser and Kenagy 1987; Florant et al. 1993) rather than access to food should be used to measure territory quality of alpine marmots (Mysterud et al. 2001).

The presence of helpers in family groups was shown to affect juvenile survival during winter (Allainé et al. 2000; Allainé and Theuriau 2004). In the present study, survival was considered over the first year of life (i.e. including survival during winter and summer). The effect of ''helpers' presence'' was significant when considered alone but no longer when considered with the ''cohort'' effect. Likely, our dataset was not large enough to highlight an interaction between ''helpers presence'' and ''cohort'' effects. Indeed, the effect of the presence of helpers was decisive during stressful environmental conditions: during ''bad'' years (mean survival was 0.52 in absence of helpers and 0.71 in presence of helpers), but not during good years (mean survival was 0.87 in absence of helpers and 0.90 in presence of helpers).

As expected (Farand et al. 2002), juvenile survival was significantly associated with the ''cohort'', which includes climatic conditions (rainfalls). Juvenile survival was lower during wet summers, probably because of reduced efficiency in thermoregulation and access to food (juveniles stay within burrows during rainfalls).

Occurrence of a GDFC

Recent studies have suggested that ideal conditions to reveal GDFC require hundred or thousands individuals and more than 10 loci (Slate and Pemberton 2002; Coltman and Slate 2003). In spite of a relatively small sample size (n = 158), and after controlling for the effect of non-genetic terms, our results revealed a GDFC when genetic diversity was measured by *H*. Hence our results suggest a disadvantage to homozygous individuals, particularly during stressful conditions (Danzmann et al. 1988; Dudash 1990; Borsa et al. 1992; Audo and Diehl 1995; Meagher et al. 1997; Crnokrak and Roof 1999; Lesbarrères et al. 2005). Several processes may enhance the range of individual genetic variability (*H* ranges from 0.125 to 1) making possible the detection of a GDFC in our population. First, virtually subordinates can disperse over long distances (greater than 13 km, Arnold 1993; Rassman et al. 1994). Consequently, mates may be from genetically differentiated populations. Second, some subordinates are philopatric and get dominance in their natal territory. Thus, some mates may be closely related (e.g., a dominant male can be replaced by his son, who will consequently mate with his mother). Finally, immigrant males implicated in EPC could have genotypes fairly distinct from local genotypes (Goossens et al. 1998).

No association was found with *LD* and *LDO*. In recent studies, d^2 measures appeared less correlated with fitness than *H* measures (Hedrick et al. 2001; Slate and Pemberton 2002), and theoretical results (Tsitrone et al. 2001; Goudet and Keller 2002) have postulated that conditions where fitness should be more closely related with d^2 than with *H* are limited and remain to be clearly identified (admixture for example). Such conditions seem not to be met in our study where only *H* was associated with juvenile survival.

Underlying mechanisms of GDFC

Analyses of the individual contribution of each locus revealed that MS45 and SS-Bibl131 had a strong influence on the results. We can hypothesize that these loci are located near genes and reflect mainly the heterozygosity of these genes through linkage disequilibrium (Hill and Robertson 1968; Otah 1971; David 1998), which remains to be

confirmed. However the GDFC we found cannot be fully explained by these two microsatellites, and seems to be affected by most or all microsatellites. To better test the local hypothesis, we considered dyads of siblings (and hence showing the same *f*), as suggested by Hansson et al. (2001; 2004). Surviving juveniles were not more heterozygous than their dead counterparts. Thus, the local hypothesis is poorly supported. However, we were able to consider only 17 dyads, and our results may have suffered from a lack of statistical power. Finally to test the general hypothesis we investigated whether heterozygosity (*H*) at half of the loci was correlated with heterozygosity at the other half, as recommended by Balloux et al. (2004). The heterozygosity-heterozygosity correlation found in the data was significantly different from zero, indicating a possible genome wide effect (Pemberton 2004), but remained low (mean $=0.081$). We then conclude that the general effect, if it occurred in our population, is limited. It seems likely that the underlying mechanism of the observed GDFC is linkage disequilibrium as suggested by recent studies (Balloux et al. 2004; Hansson et al. 2004; Pemberton 2004; Slate et al. 2004), rather than inbreeding depression. However, further analyses with more dyads are required to clearly test the two hypotheses.

Conclusion

This study provides evidence for a positive influence of genetic diversity on juvenile survival in the European Alpine marmot, *Marmota marmota*. These results have been obtained after controlling genetic analyses for ecological variables such as results could have very practical management implications and could be generalised to other marmot species. We suggest that genetic factors should be considered, along with non-genetic factors, in the management of fragmented or endangered species such as *Marmota vancouverensis* or *M. menzbieri*, two species that are relatively difficult to study. Moreover, considering our species, EPC with males from adjacent populations could have major impact on the population by increasing offspring heterozygosity and fitness and then by reducing potential effects of inbreeding (mating between relatives). Management of alpine marmots should consider monitoring and maintaining connectivity among populations.

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