

Extra-pair paternity in alpine marmots, *Marmota marmota*: genetic quality and genetic diversity effects

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Abstract Assuming that a male's genetic characteristics affect those of his offspring, extra-pair copulation has been hypothesized to increase heterozygosity of the progeny—the “genetic compatibility” hypothesis—and the genetic diversity within litters—the “genetic diversity” hypothesis. We tested these two hypotheses in the alpine marmot (*Marmota marmota*), a socially monogamous mammal showing a high rate of extra-pair paternity (EPP). In a first step, we tested the assumption that a male's genetic characteristics (heterozygosity and genetic similarity to the female) affect those of his offspring. Genetic similarity between parents influenced offspring heterozygosity, offspring genetic similarity to their mother, and litter genetic diversity. The father's heterozygosity also influenced litter genetic diversity but did not affect offspring heterozygosity. Hence, heterozygosity seems not to be heritable in the alpine marmot. In a second step, we compared genetic characteristics of extra-pair young (EPY) and within-pair young (WPY). EPY were less genetically similar to their mother but not more heterozygous than WPY. EPY siblings were also less genetically similar than their WPY half siblings. Finally, the presence of EPY promoted genetic diversity within the litter. Thus, our data support both the “genetic compatibility” and the “genetic diversity” hypoth-

eses. We discuss further investigations needed to determine the primary causes of EPP in this species.

Keywords Genetic similarity · Heterozygosity · Diversity · Compatibility hypothesis · Mate choice

Introduction

In socially monogamous species, males are expected to increase their reproductive success by adopting a mixed reproductive tactic consisting in establishing pair bonds with a social partner while seeking extra-pair copulations (EPC) with other females (Trivers 1972). Conversely, females, which invest much more in their offspring than males, are expected to be selective for the (genetic) quality of their mate (Trivers 1972). However, females constrained to mate with low quality males should also adopt a mixed reproductive tactic. That is, they should seek EPC with males of higher quality than their social partner. Such a tactic is expected to produce offspring of higher quality (Trivers 1972). In the last two decades, an increasing number of studies has reported the occurrence of EPC in socially monogamous species (for reviews, see Møller and Birkhead 1993; Birkhead and Møller 1995; Griffith et al. 2002) and evidence that females actively take part in EPC is accumulating (for reviews, see Westneat et al. 1990; Westneat and Stewart 2003). These observations suggest that females may obtain benefits, and it is increasingly accepted that some of these benefits are genetic (Birkhead and Møller 1992; Zeh and Zeh 1996, 1997; Jennions and Petrie 2000; Tregenza and Wedell 2000; Griffith et al. 2002).

However, the nature of genetic benefits accruing to females remains unclear and Brown (1997) suggested that

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empiricists should now shift their focus from asking whether females select males for their genetic quality to determining the nature of that genetic quality. Whether the main genetic benefits are good genes, compatible genes or diverse genes is currently debated (Mays and Hill 2004; Neff and Pitcher 2005). Under the “good genes” hypothesis, females may gain superior genes that confer higher viability and/or attractiveness to their offspring. Under this hypothesis, females are expected to mate with an absolute best male exhibiting an indicator of good genes. Under the “compatibility” hypothesis (Brown 1997; Zeh and Zeh 1996, 1997), females may gain superior combinations of alleles (overdominance) that increase vigor, viability, and/or attractiveness of the progeny (for reviews, see Allendorf and Leary 1986; Mitton 1993). By maximizing genome-wide heterozygosity of offspring, females also avoid the cost of inbreeding (reduced offspring performance and genetic load of deleterious recessive alleles, Pusey and Wolf 1996; Keller and Waller 2002). Females may raise offspring heterozygosity by mating (1) with dissimilar males, (2) with heterozygous males (assuming some heritability of heterozygosity, Mitton 1993), (3) with males having rare alleles (Farr 1980; Masters et al. 2003). Under the “genetic diversity” hypothesis (Williams 1975), females may gain higher genetic diversification of their litters. Genetic diversity may reduce sibling competition (Loman et al. 1988; Ridley 1993), or may buffer against environmental uncertainty (genetic bet-hedging, Yasui 1998). Studies show that genetic diversity enhances viability of the colony (Liersch and Schmid-Hempel 1998) or hatching success (Tregenza and Wedell 1998) in insects. Hence, females are expected to mate with males dissimilar both to themselves and to their social partner.

These three hypotheses are nonexclusive. They all assume that female’s choice affects the genetic characteristics of their offspring and make the following predictions regarding the genetic characteristics of extra-pair young (EPY) and within-pair young (WPY) half siblings:

- (1) Under the good genes hypothesis: EPY should possess better genes than WPY
- (2) Under the compatibility hypothesis: extra-pair paternity (EPP) should promote genetic diversity at the level of the individual. Thus, EPY should be more heterozygous than WPY, and/or EPY should be less genetically similar to their mother than WPY and/or EPY should possess more rare alleles than WPY
- (3) Under the genetic diversity hypothesis: EPP should promote genetic diversity at the level of the litter.

The aim of this study was to investigate how EPPs affect the genetic characteristics of offspring in the alpine marmot *Marmota marmota*. The alpine marmot is an excellent model for such a purpose because it is a socially monogamous

mammal with a high frequency of EPP: 31% of litters contain EPY and 16% of juveniles are born to extra-pair father (Goossens et al. 1998a; Cohas et al. 2006). The basic social unit is a family group of 2–20 individuals, composed of a territorial dominant breeding pair, mature subordinates of 2–4 years, yearlings, and juveniles (Perrin et al. 1993). Although sexually mature, subordinate females are reproductively suppressed (Arnold 1990; Goossens et al. 1996) and subordinate males rarely sire extra-pair young (Arnold 1990; Goossens et al. 1998a). EPP seems mainly to concern transient males (Cohas et al. 2006). EPP is infrequent in the absence of subordinate males in the family group and occurs particularly when the social partners are genetically similar, whereas EPP is almost systematic when subordinates are present in the family group (Cohas et al. 2006). This suggests that (1) resident males are able to prevent EPC in the absence of competitors in the family group, (2) that females play an active role in EPC (because the genetic similarity of the social partner is not an absolute attribute of the male determining his ability to prevent EPC), and (3) that extra-pair males were less genetically similar to the female than the social male although Cohas et al. (2006) were unable to test for this. Instead, Cohas et al. (2006) found some evidence that extra-pair fathers are more heterozygous than the social partner.

Given these previous results, we assumed that extra-pair mates (EPM) are more heterozygous and/or less genetically similar to the females than their social mates. We investigated how these assumed extra-pair mate characteristics affected the genetic characteristics (heterozygosity, genetic similarity, and possession of rare alleles) of the offspring, and the genetic diversity of the litter. We then tested two nonexclusive evolutionary causes of EPP: the “compatibility” and the “genetic diversity” hypotheses. To test the genetic compatibility hypothesis, we investigated whether EPY were more heterozygous than WPY (prediction 1), and whether EPY were less genetically similar to their mother than WPY (prediction 2); we also investigated whether EPY had more rare alleles than WPY (prediction 3). To test the genetic diversity hypothesis, we investigated whether EPY promoted genetic diversity within the litter (prediction 4).

Materials and methods

Study site and field methods

The study site is located in La Grande Sassièr Nature Reserve (French Alps, 45°29'N, 6°59'E). From 1990 to 2002, alpine marmots were caught from early April to late July. Marmots were trapped using two-door, live-capture traps baited with dandelion *Taraxacum densleonis*. Traps

were placed near the entrance of main burrows for each group to allow assignation of captured individuals to a family group. Once trapped, individuals were tranquilized with Zolétil 100, and individually marked with a numbered ear tag and a transponder (Trovan™, Germany). Trapped individuals were sexed using anogenital distance, aged from their size up to three years of age, weighed, and measured for several morphological variables. In addition, hairs were collected from 1992 to 1997, and tissue biopsies thereafter for genetic analyses. Virtually all emerged juveniles were trapped within three days after emergence (Allainé et al. 2000; Allainé 2004).

Genetic analysis

All individuals were not typed at the same number of loci due to development of new microsatellite markers during the 12 years of this study. Hence, in the subset of parents and their offspring considered in the subsequent analyses, 135 were typed at five microsatellite loci: SS-Bib11, SS-Bib14, SS-Bib18, SS-Bib120, and SS-Bib131 (Klinkicht 1993), 86 were typed at three additional microsatellite loci: MS45, MS47, and MS53 (Hanslik and Kruckenhauser 2000), and 51 were typed at four more loci: Ma001, Ma018, Ma066, and Ma091 (Da Silva et al. 2003). Details on microsatellite methods and characteristics can be found in Goossens et al. (1998a); Hanslik and Kruckenhauser (2000), and Da Silva et al. (2003).

Tests of Hardy–Weinberg equilibrium and of linkage disequilibrium, performed using GENEPOP v3.3 (Raymond and Rousset 1995), on dominant adults to avoid bias due to family structure and on all cohorts gathered to ensure adequate sample size ($N=69$ for SS-Bib11, SS-Bib14, SS-Bib18, SS-Bib120, and SS-Bib131, $N=31$ for MS45, MS47, and MS53, $N=11$ for Ma001, Ma018, Ma066, and Ma091) did not evidence departure from Hardy–Weinberg equilibrium for any of the loci (all $p>0.05$) nor from gametic linkage equilibrium among any of the loci (all $p>0.05$).

Paternity analysis

The genotypes of each young and of the dominant pair were used to check maternity of the dominant female (always the case in our study) and then, paternity of the dominant male. We defined a young as within-pair young (WPY) if its genotype matched the dominant male's genotype and as extra-pair young (EPY) if it did not. Hereafter, litters composed only of WPY are called within-pair litters (WPL) and those containing both WPY and EPY are called mixed litters.

Even if many paternity exclusions were based on only one difference between the genotype of the young considered and its potential father (22 offspring), we can

be confident in our results because we can discard the possibility of both false exclusion and false inclusion of a young because average exclusion probability was very high (from 0.926 when considering individuals typed at five loci to 0.995 for individuals typed at 12 loci) and genotyping errors were unlikely (below 0.002%) as mutations (average mutation rate of microsatellite loci is 1.67×10^{-4} per generation in *M. marmota*, Rassmann et al. 1994) (for discussion of these problems, see Goossens et al. 1998b; Cohas et al. 2006).

Statistical analyses

We used linear mixed models and generalized linear mixed models specifying father within mother as the grouping levels to account for nonindependence of the data (25 mothers repeated 3 to 21 times with 51 fathers repeated 1 to 15 times). Nonidentified fathers of EPY were considered as the same individual for a given litter and as different individuals for different litters. We included predictor variables and their interactions in all models. The effects of the predictor variables were estimated by using the restricted log-likelihood (REML) for linear mixed models and the penalized quasi-likelihood (PQL) for generalized linear mixed models. The use of random factors in a generalized linear model (i.e., a generalized linear mixed model fitted using penalized quasi-likelihood) allows for overdispersion as the random factors will add extra sources of variation to the binomial variance. We then assessed the significance of the predictor variables' effects and their interactions with other variables included in the model (partial test).

To test the effect of parental genetic characteristics on offspring genetic characteristics we limited the analyses to WPY because genotypes of both parents were needed.

To compare the genetic characteristics of WPY and EPY, we limited the analyses to mixed litters. Both females paired with an attractive pair mate and females paired with an unattractive male but lacking EPC opportunities produced WPL. Some variability in WPY genetic characteristics may arise: females mated to attractive males are expected to have high quality offspring while those constrained in their mate choice are expected to have low quality offspring (Sheldon and Ellegren 1996). To avoid such possible confounding effects and to be sure that any differences between EPY and WPY can be attributed to differential paternal genetic contribution, we limited the comparison to maternal half siblings only.

All the statistical analyses were performed using R 2.0.0 software (R Development Core Team 2003), linear mixed models were fitted using the function `lme` in the library `nlme` (Pinheiro and Bates 2002) and the generalized mixed models were fitted using the function `glmmPQL` in the

library MASS (Venables and Ripley 2002). Unless otherwise stated, all tests are two-tailed, the level of significance set to 0.05 and parameter estimates are given with $\pm 95\%$ CI.

Offspring heterozygosity

Heterozygosity was measured by standardized heterozygosity H (Coltman et al. 1999), mean d^2 (Coulson et al. 1998) and internal relatedness IR (Amos et al. 2001a). Only the results dealing with standardized heterozygosity are presented because the results found with the two other estimators were similar. Because all individuals were not typed for the same number of loci, we checked for the 96 individuals typed for 12 loci that standardized heterozygosity obtained from 5, 8, and 12 loci were highly correlated (H_{12} vs H_5 : $r=0.81$, $t=8.7059$, $df=96$, $p<0.001$; H_8 vs H_5 : $r=0.88$, $t=12.1893$, $df=96$, $p<0.001$; H_{12} vs H_8 : $r=0.94$, $t=20.2117$, $df=96$, $p<0.001$). Moreover, analyses were also performed over the subset of individuals typed for 8 and 12 loci. Because the results were similar, we only present the results obtained over all individuals.

We ran a linear mixed model to investigate how heterozygosity of each parent and their genetic similarity affected the heterozygosity of the offspring (Table 1) and another one to compare EPY and WPY heterozygosity (Table 2).

Offspring inbreeding

The pedigrees of juveniles were unknown, so we used genetic similarity between parents to estimate the coefficient of individual inbreeding. However, because extra-pair fathers were rarely identified, we used genetic similarity between the mother and her offspring as a proxy of the genetic similarity between parents: the genetic similarity between a mother and her offspring is assumed to increase with the genetic similarity between the parents (see “Results” for the test of this assumption). Three estimators of genetic similarity were considered: Queller and Goodnight’s estimator (Rqg, Queller and Goodnight 1989), Lynch and Ritland’s estimator, (Rlr, Lynch and Ritland 1999), and Identity (I, Belkhir et al. 2002). We used IDENTIX 1.0 software (Belkhir et al. 2002) to calculate all three estimators.

To check that the genetic similarity between parents affected the genetic similarity between a mother and her offspring, a linear mixed model was used (Table 1). We also investigated whether the heterozygosity of each parent affected the genetic similarity between a mother and her offspring (Table 1). We present only the results from Queller and Goodnight’s estimator because estimates of the three genetic similarity estimators were highly correlated (Rqg–I: $r=0.94$, Rqg–Rlr: $r=0.89$ Rlr–I: $r=0.81$) and the results found with the two other genetic similarity estimators were similar. Another linear mixed model was done to compare EPY and WPY genetic similarity to their mother (Table 2).

Table 1 Methods used and results obtained concerning the effects of parental genetic characteristics on offspring genetic characteristics

Response variables	Predictive variables	Models	Effect
Offspring heterozygosity	Paternal heterozygosity	Linear mixed	No
	Maternal heterozygosity		No
	Parents’ genetic similarity		Negative
Offspring rarity (presence/absence)	Paternal heterozygosity	Generalized linear mixed, binomial response, logit link	No
	Maternal heterozygosity		No
	Parents’ genetic similarity		No
Offspring rarity (offspring presenting rare alleles)	Paternal heterozygosity	Linear mixed	No
	Maternal heterozygosity		No
	Parents’ genetic similarity		No
Offspring–mother genetic similarity	Paternal heterozygosity	Linear mixed	No
	Maternal heterozygosity		No
	Parents’ genetic similarity		Positive
Expected litter diversity	Paternal heterozygosity	Linear mixed	Positive
	Maternal heterozygosity		Positive
	Parents’ genetic similarity		Negative
Siblings genetic similarity	Paternal heterozygosity	Linear mixed on bootstrapped datasets	Negative
	Maternal heterozygosity		Negative
	Parents’ genetic similarity		Positive

All analyses were done on within-pair young only and we used father within mother as grouping factors.

Table 2 Methods used and results obtained concerning the effects of type of offspring on offspring genetic characteristics

Hypotheses	Predictions	Response variables	Predictive variables	Grouping factors	Data	Models	Results	Conclusions
Compatibility	Heterozygosity (1) EPY>WPY	Offspring heterozygosity	Young type (EPY vs WPY)	Father within mother	ML	Linear mixed	EPY=WPY	Partial
	Inbreeding (2) EPY<WPY	Offspring-mother genetic similarity	Young type (EPY vs WPY)	Father within mother	ML	Linear mixed	EPY<WPY	
	Rare alleles (3) EPY>WPY	Offspring rarity (presence/absence)	Young type (EPY vs WPY)	Father within mother	ML	Generalized mixed, binomial response, logit link	EPY=WPY	Support
		Offspring rarity (offspring with rare alleles)	Young type (EPY vs WPY)	Father within mother	ML	Linear mixed	EPY=WPY	
Genetic diversity	Litter diversity (4) ML>WPL	Observed litter genetic diversity			ML	Pairwise comparison: observed vs expected litter genetic diversity without EPP	ML>WPL	Support
	Siblings diversity (4) EPY-EPY>WPY-WPY	Siblings genetic similarity	Pair type (EPY-EPY vs WPY-WPY)		ML	Linear mixed on bootstrapped datasets	EPY-EPY>WPY-WPY	

Numbers in brackets referred to the predictions mentioned in the text. EPY Extra-pair young, WPY within-pair young, ML mixed litter, WPL within-pair litter

Offspring rarity

To quantify rarity of the genotype, following the method used by Masters et al. (2003), we computed the genetic similarity between an offspring and a hypothetical genotype comprising the most common allele(s) found in the population at each locus (Queller and Goodnight 1989). We considered as rare all alleles with frequencies lower than 0.05, all other alleles of a given locus being treated as a single allele in the hypothetical genotype. We discarded the loci MS53, Ma001, Ma018, and Ma066 because none of their alleles had a frequency below 0.05.

Rarity departed markedly from normality (Kolmogorov–Smirnov test $D=0.367$, $p<0.001$) because of the high frequency of individuals carrying no rare alleles. Thus, we first considered two classes of offspring rarity: offspring carrying rare alleles (rarity less than one), and offspring carrying no rare alleles (rarity equal to one). We then ran a generalized linear mixed model with a logit link and a binomial variance with rarity encoded as presence/absence as the response variable and heterozygosity of each parent and their genetic similarity as the predictor variables (Table 1). Second, we considered only individuals having rare alleles (rarity less than one). Then, using a linear mixed model with rarity as the response variable, we tested for the effects of maternal heterozygosity, paternal heterozygosity, and genetic similarity between parents (Table 1).

To test the prediction that EPY carry more rare alleles, we proceeded in the same way. First, we used a generalized linear mixed model specifying a logit link and a binomial variance to investigate whether the type of offspring (EPY vs WPY) affected their rarity (encoded as presence/absence) (Table 2). Second, we used a linear mixed model for individuals carrying rare alleles with rarity as the response variable and the type of offspring as the predictor variable (Table 2).

All three genetic similarity estimators were considered. Because the results were similar, only the results with Queller and Goodnight's estimator are presented.

Genetic diversity within the litter

Litter genetic diversity was assessed as the variance of the genetic distance between offspring within a litter:

$$\frac{1}{n} \sum_{ij} (d_{ij} - \mu)^2$$

where n is litter size, d_{ij} is the genetic distance between offspring j and offspring i , and μ is the mean genetic distance over all offspring pairs. Genetic distance between two offspring was the sum of allelic differences over all loci. Allelic difference was rated 1 if the two offspring did

not share any allele for the considered locus, 0.5 if the two offspring shared only one allele and 0 if the two offspring shared both alleles.

To test how parental genetic characteristics affected the litter genetic diversity, we ran a linear model with expected litter genetic diversity for each pair of parents as the response variable and maternal heterozygosity, paternal heterozygosity, and genetic similarity between parents and their interactions as predictor variables (Table 1). Expected litter genetic diversity for each pair of parents was defined as the mean of genetic litter diversity given a litter size of four. The distribution of genetic diversity is obtained, for each pair, by calculating, over the five loci typed for all individuals, the genetic litter diversity of 10,000 simulated litters of four offspring (mean litter size of the studied population = 3.7 ± 0.14 , $n=63$). Offspring genotypes were derived from the genotypes of the mother and the father. Litter genetic diversity was square root transformed to obtain a symmetrical distribution.

Because we expected that EPPs increase litter genetic diversity, we compared the mean of the litter genetic diversity distribution to the observed litter genetic diversity for each mixed litter with a paired *t* test (Table 2). To obtain the mean of the litter genetic diversity distribution, we used the same procedure as the one described above with the following modifications. From the genotypes of the mother and the within-pair father, we derived *x* possible offspring genotypes, with *x* corresponding to the size of the litter considered and we considered all typed loci because we used pairwise comparisons. The same analysis conducted over the five loci common to all individuals gave the same results.

In addition, if EPCs promote genetic diversity within litters, the genetic similarity between siblings should also be affected. We tested whether parental genetic characteristics affected the genetic similarity between siblings (Table 1). Because a given individual occurred in different sibling pairs, WPY sibling pairs are not independent. We thus bootstrapped offspring 5,000 times to provide independent replicates. We then ran the linear model on all bootstrapped datasets ($N=192$, number of WPY) with genetic similarity between siblings as the response variable and maternal heterozygosity, paternal heterozygosity, and genetic similarity between parents as the predictor variables (Table 1).

We then tested the hypothesis that the genetic similarity among EPY siblings should be lower than among their WPY half siblings. Again sibling pairs cannot be assumed independent due to the repeated presence of individuals in different sibling pairs (EPY–EPY or WPY–WPY pairs). We thus bootstrapped offspring 5,000 times, keeping the sample size of EPY and WPY as observed ($N=27$ and $N=36$). We then ran the linear model on all bootstrapped datasets with genetic similarity between siblings as the response variable

and the type of offspring pair (EPY–EPY or WPY–WPY) (Table 2) as the predictor variable.

Results

We considered only litters where potential parents and all offspring were known and typed ($N=63$). Forty-six were WPL comprising 156 offspring and 17 were mixed litters comprising 63 offspring (36 WPY and 27 EPY).

Effect of parental genetic characteristics on offspring genetic characteristics

Offspring heterozygosity and offspring genetic similarity to the mother depended neither on maternal heterozygosity nor on paternal heterozygosity while both depended on genetic similarity between parents (Table 3): offspring heterozygosity increased with decreasing genetic similarity between parents and thus, as assumed, offspring genetic similarity to the mother increased with genetic similarity between parents. Offspring rarity did not depend on the genetic characteristics considered (Table 3). Expected litter genetic diversity increased with both maternal and paternal heterozygosity and decreased with genetic similarity between parents (Table 4). Offspring genetic similarity to their siblings decreased with both maternal and paternal heterozygosity, and increased with genetic similarity between parents (Table 3).

Comparison of EPY and WPY

Predictions 1 and 3 were not supported (Table 2): EPY were neither more heterozygous nor carried more rare alleles than WPY (Table 4, Fig. 1a,b).

Prediction 2 was supported (Table 2): as expected, EPY were less genetically similar to the mother than WPY (Table 4, Fig. 1c).

Prediction 4 was also supported (Table 2): litter genetic diversity increased ($t=3.201$, $df=16$, $P=0.006$, Fig. 2) in litters with EPY (mean difference between observed litter genetic diversity and mean of expected litter genetic diversity = 0.202 ± 0.134). Moreover, EPY were less genetically similar to their siblings than WPY (Table 4, Fig. 1d).

Discussion

Effect of parental genetic characteristics on offspring characteristics

The “compatibility” hypothesis suggests that females should not mate preferentially with an absolute best male in the population but rather with a male possessing alleles

Table 3 Effect of parental genetic characteristics on offspring genetic characteristics obtained from mixed models with each offspring genetic characteristics as the response variable and the parental genetic characteristics (maternal heterozygosity, paternal heterozygosity, and genetic similarity between parents) as the fixed variables and father within mother as the random variables

Genotype characteristics	β	<i>t</i>	<i>p</i>
Heterozygosity			
Maternal heterozygosity	-0.025±0.210	0.233	0.818
Paternal heterozygosity	0.023±0.172	0.266	0.796
Parents'genetic similarity	-0.406±0.137	5.802	<0.001*
Rarity			
Presence/absence of rare alleles			
Maternal heterozygosity	0.526±3.119	0.331	0.745
Paternal heterozygosity	-1.194±3.309	0.708	0.506
Parents'genetic similarity	0.056±2.581	0.042	0.968
Rarity for individuals presenting rare alleles			
Maternal heterozygosity	-0.011±0.544	0.032	0.975
Paternal heterozygosity	-0.232±0.698	0.837	0.424
Parents'genetic similarity	0.117±0.448	0.513	0.620
Genetic similarity to the mother			
Maternal heterozygosity	-0.046±0.119	0.756	0.457
Paternal heterozygosity	-0.039±0.096	0.790	0.450
Parents'genetic similarity	0.241±0.078	6.033	<0.001*
Expected litter diversity			
Maternal heterozygosity	0.451±0.265	3.340	<0.003*
Paternal heterozygosity	0.518±0.207	4.911	<0.001*
Parents'genetic similarity	-0.298±0.165	3.538	<0.006*
Siblings' genetic similarity (from bootstrap)			
Maternal heterozygosity	-0.092±0.054		<0.001*
Paternal heterozygosity	-0.096±0.047		<0.001*
Parents'genetic similarity	0.241±0.066		<0.001*

Linear mixed models were used for offspring heterozygosity, offspring rarity for individuals presenting rare alleles, offspring genetic similarity to the mother and offspring genetic similarity to siblings. Generalized linear mixed models were used for offspring presence/absence of rare alleles. Slopes ($\pm 95\%$ CI) are given. Significant factors at a level of 0.05 are indicated in bold and marked with an asterisk.

that complement her own genetic makeup (Brown 1997; Zeh and Zeh 1996, 1997; Tregenza and Wedell 2000). Some recent studies examining the adaptive value of extra-pair mating support this “genetic compatibility” hypothesis by revealing that EPP occurs whenever social mates are homozygous and/or genetically similar to the female (Blomqvist et al. 2002; Eimes et al. 2005; Cohas et al. 2006 but see Keller et al. 2002; Schmoll et al. 2005). These studies thus suggest that extra-pair mates (EPM) are more heterozygous and/or more dissimilar to the female than the corresponding within-pair mate (WPM). Moreover, recent studies explicitly contrasting EPM and WPM genetic

characteristics report that EPM were more dissimilar to the female (Amos et al. 2001b; Landry et al. 2001; Masters et al. 2003) or more heterozygous (Aparicio et al. 2001) than WPM. EPM are supposed to be genetically dissimilar to the female as they often come from distant groups or are transient individuals (Dunn et al. 1994; Otter et al. 1998; Leisler et al. 2000; Amos et al. 2001b; Foerster et al. 2003).

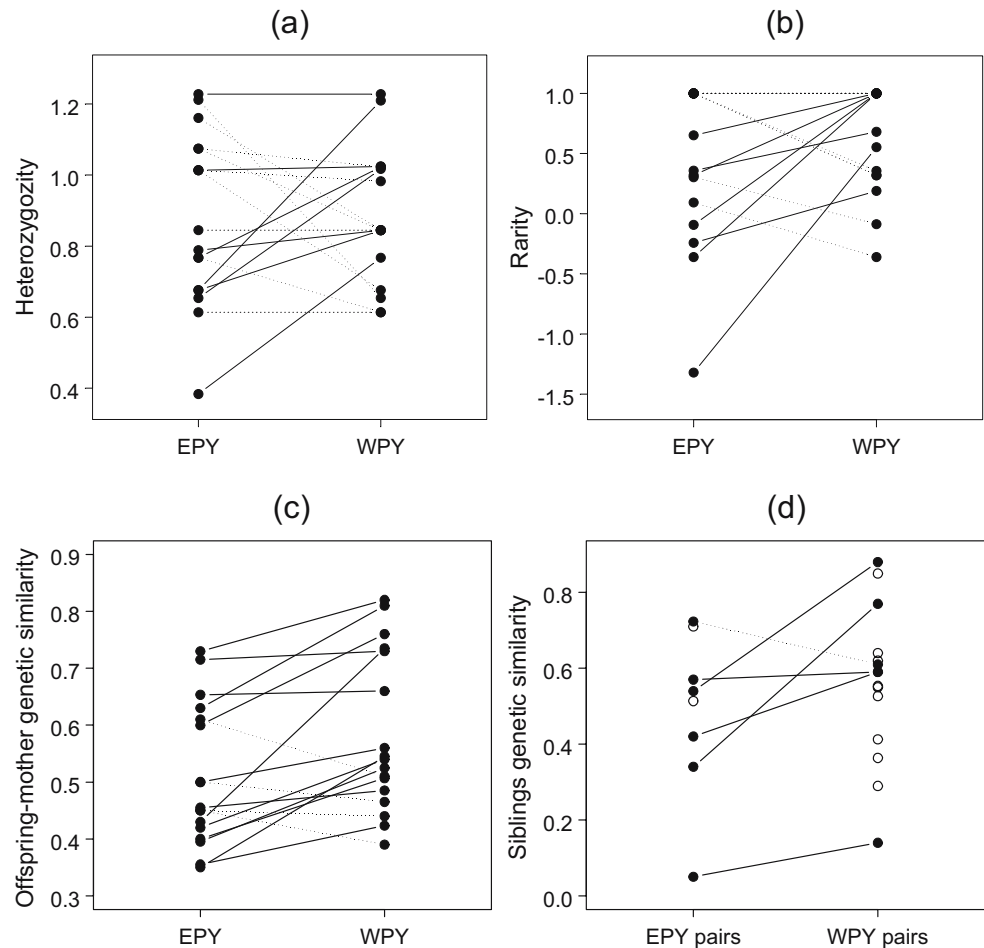
These studies implicitly assume that mating with dissimilar and/or heterozygous EPM enhances offspring heterozygosity and thus reduces inbreeding depression (Hansson and Westerberg 2002; David 1998). However, evidence that mate characteristics affect the genetic char-

Table 4 Comparison of the genotype characteristics of within-pair young (WPY) and extra-pair young (EPY)

Genotype characteristics	WPY	EPY	β	<i>t</i>	<i>p</i>
Heterozygosity	0.936±0.520	0.879±0.625	0.018±0.070	0.502	0.621
Rarity: presence/absence of rare alleles	0.593±0.121	0.510±0.123	0.378±1.216	0.610	0.549
Rarity: individuals presenting rare alleles	-0.047±0.752	-0.203±0.950	0.068±0.1533	0.866	0.397
Genetic similarity to the mother	0.560±0.306	0.520±0.289	0.032±0.027	2.371	0.028*
Siblings' genetic similarity (from bootstrap)			0.085±0.083		0.036*

Mean values ($\pm 95\%$ CI) and slopes ($\pm 95\%$ CI) are given. Significant factors at a level of 0.05 are indicated in bold and marked with an asterisk.

Fig. 1 Offspring heterozygosity (a), offspring rarity (b), offspring genetic similarity to the mother (c), and offspring genetic similarity to their siblings (d) as a function of the type of offspring (WPY: within-pair young, EPY: extra-pair young) for each litter. **a–c** The black circles represent the means of observed data for each litter; **d** the black circles represent litters with both EPY pairs and WPY pairs, the open circles represent litters with only EPY pairs or WPY pairs; **a–d** the lines join WPY and EPY of a given litter with dotted lines joining EPY means higher than WPY means and solid lines joining EPY means lower than WPY means



acteristics of offspring is still scarce (but see Amos et al. 2001b; Marshall et al. 2003). Our results confirm that mating with a dissimilar male enhanced offspring heterozygosity and decreased mother–offspring genetic similarity, but also decreased full siblings’ genetic similarity and promoted genetic diversity within litters. Thus, obtaining EPP from dissimilar EPM may increase offspring’s heterozygosity, as expected under the “compatibility” hypothesis, but may also increase the genetic diversity of litters, as expected under the “genetic diversity within litters” hypothesis (Loman et al. 1988; Yasui 1998). Our results also indicate that the paternal (and maternal too) heterozygosity affected siblings’ genetic similarity and the genetic diversity of litters, but did not affect offspring heterozygosity and offspring rarity. Thus, contrary to an often made assumption (Mitton 1993), we did not find that heterozygous fathers (parents) produced heterozygous offspring, suggesting that heterozygosity is not a heritable characteristic in the alpine marmot. Thus, obtaining EPP from heterozygous EPM may not produce heterozygous offspring, as expected under the “compatibility” hypothesis, but may instead produce genetically diverse litters, as expected under the “genetic diversity within litters” hypothesis.

Comparison of EPY and WPY genetic characteristics

From the results we found concerning the effect of mate characteristics on offspring genetic characteristics, we can make the following predictions. (1) Under the “compatibility” hypothesis, EPY should be more heterozygous and less genetically similar to their mother (expected only if females mate with genetically dissimilar EPM), but should not possess more rare alleles (unexpected whatever the genetic characteristics of the EPM: heterozygosity or genetic dissimilarity), than WPY. (2) Under the “genetic diversity” hypothesis, EPY should be less genetically similar to their siblings than WPY (expected whether females mate with heterozygous or genetically dissimilar EPM), and mixed litters should be more diverse than expected without EPP (expected whether females mate with heterozygous or genetically dissimilar EPM).

The comparison between EPY and WPY half siblings confirmed that EPY did not possess more rare alleles than their WPY counterparts—however, given the high confidence interval around rarity, a lack of power can hide a higher rarity of EPY compared to WPY—and that EPY–mother genetic similarity was lower than WPY–mother

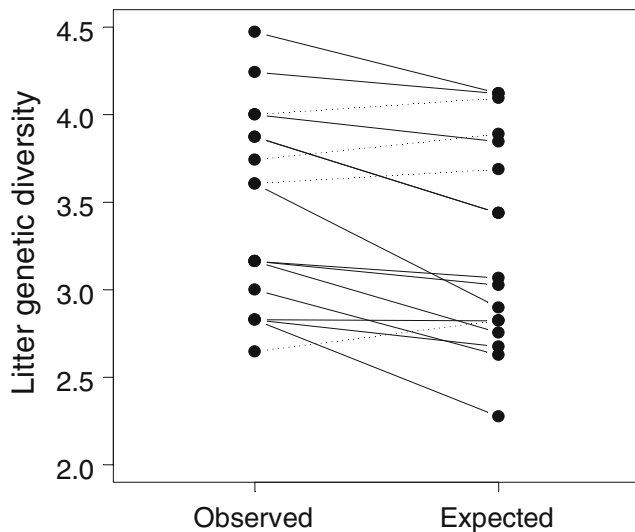


Fig. 2 Observed litter genetic diversity compared to the mean of expected litter genetic diversity for each mixed litter. The *lines* join observed and mean expected litter genetic diversity for a given litter with *dotted lines* joining observed litter genetic diversity lower than mean of expected litter genetic diversity and *solid lines* joining observed litter genetic diversity higher than mean of expected litter genetic diversity

genetic similarity. But, contrary to our expectations, EPY were not more heterozygous than their WPY counterparts. At first glance, this raises a contradiction: EPY are less inbred (as indicated by genetic similarity estimators) but not more heterozygous (as indicated by measures of heterozygosity) than WPY. However, although a negative correlation is expected in theory between inbreeding and heterozygosity, empirical illustrations of such a correlation are ambiguous. For example, Hansson et al. (2001) showed, over 50 dyads of great reed warblers (*Acrocephalus arundinaceus*), that siblings with the same inbreeding coefficients differ in their heterozygosity at five microsatellite loci. Similarly, Hedrick et al. (2001) demonstrated that microsatellite measures of heterozygosity did not correlate with inbreeding coefficient in a population of wolves (*Canis lupus*) of known pedigree. Hence, one plausible explanation of the apparent contradiction we observed is that we measured heterozygosity over too few microsatellite loci to accurately express genome-wide heterozygosity (Slate and Pemberton 2002). Indeed, simulations showed that the conditions under which inbreeding and multilocus heterozygosity correlate are tremendously restricted. So, inferring inbreeding from multilocus heterozygosity is likely to fail even when very large numbers of individuals are typed (Balloux et al. 2004; Slate et al. 2004). This is why genetic similarity estimators may be preferred to assess inbreeding instead of heterozygosity (Van de Casteele et al. 2001; Rousset 2002; Blouin 2003). It is therefore possible that females seek EPC to reduce inbreeding and thus to increase offspring heterozygosity, at

many or at specific loci, but we failed to measure correctly offspring heterozygosity. In this case, our results may fully support the “compatibility” hypothesis.

The comparison between expected genetic diversity with and without EPP confirms that mixed litters were more diverse than expected without EPP. Moreover, the comparison of EPY and WPY half siblings confirms that the genetic similarity was lower between EPY siblings than between their WPY half siblings. These results indicate that EPP promotes genetic diversity not only because mixed litters presented two fathers instead of one, but also because EPY siblings present more genetic diversity than their WPY half siblings. Moreover, this later result suggests that extra-pair fathers are more heterozygous and/or less genetically similar to the female than within-pair fathers (see also Cohas et al. 2006). Hence, our results support also the “genetic diversity” hypothesis.

These two hypotheses are not mutually exclusive and it is possible that alpine marmot females search for both compatible genes and genetic diversity within litters. However, Yasui (1998) showed that genetic bet-hedging was unlikely to favor the evolution of EPP. It is therefore possible that genetic diversity is not a primary cause of EPP in the alpine marmot but only a by-product of a choice for compatible genes through mating with dissimilar EPM. Indeed, our results strongly suggest that mating with dissimilar EPM simultaneously increases offspring heterozygosity and promotes genetic diversity within litters. Finally, we cannot discard the possibility that alpine marmot females search for good genes (or good sperm, Madsen et al. 1992). We have no information on how alpine marmot females assess their genetic make-up and the genetic make-up of their mate. It is unlikely that alpine marmot females use phenotypic cues such as coloration or asymmetry (Brown 1997). Phenotypic cues used by females to choose their mate are often thought to be visual (particularly in birds). However, in mammals, and especially in rodents, individual recognition is often based on odor (i.e., Mateo 2006). It is now well established that female rodents may use MHC-determined odors to select dissimilar partners at the MHC complex (see for example Brown and Eklund 1994; Penn and Potts 1998). So, we suspect that female alpine marmots may use odor to choose genetically dissimilar mates at least at some key loci such as loci of the MHC.

Conclusion

To determine the primary cause of EPC in the alpine marmot, further investigations are still needed in three main directions. First, EPC behavior should be carefully examined. Especially, the comparison of successful and unsuc-

cessful EPCs may help in understanding the function of EPP. Second, the characteristics of EPM should be identified. Indeed, if extra-pair mate heterozygosity is an important cue in female choice, then the “genetic diversity” hypothesis becomes the most likely evolutionary cause of EPC in the alpine marmot. To our knowledge, when compared, EPM were not consistently more heterozygous than WPM (*Acrocephalus sechellensis* Richardson et al. 2005 but see *Troglodytes aedon* Masters et al. 2003) nor they were more dissimilar to the female than WPM (*Acrocephalus arundinaceus* Bensch et al. 1994, *Troglodytes aedon* Masters et al. 2003 but see *Parus caeruleus* Kempenaers et al. 1997, *Emberiza schoeniclus* Kleven and Lifjeld 2005). Third, future studies should accurately assess the fitness benefits provided to alpine marmot females by the presence of EPY (Yasui 1998). Our prediction is that if the primary evolutionary cause of EPC is the “compatibility” hypothesis, EPY should be more heterozygous and should outperform WPY as found in the bluethroat *Luscinia svecica* (Johnsen et al. 2000) or in the blue tit *Parus caeruleus* (Kempenaers et al. 1997). Alternatively, if the primary evolutionary cause of EPC is the “genetic diversity” hypothesis, EPY should not perform better than WPY in an absolute sense, but survival within mixed litters should be higher than within pair litters. Although well documented in insects (Fuchs and Schade 1994; Oldroyd et al. 1997; Baer and Schmid-Hempel 1999), this pattern is less clear in vertebrate species. To our knowledge, some studies showed that overall survival of mixed litters was higher than survival of within-pair litters but none of these studies have controlled for a higher survival of EPY compared to WPY (*Lacerta agilis* Olsson et al. 1994; *Vipera berus* Madsen et al. 1992; *Cynomys gunnisoni* Hoogland 1998) and the pattern seems inconsistent, several studies reporting no difference in within-pair and mixed litters survival (*Passer domesticus* Strohbach et al. 1998, *Crinia georgiana* Byrne and Roberts 2000, *Parus caeruleus* Charmantier et al. 2004).

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