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The genetic similarity between pair members influences the frequency of extrapair paternity in alpine marmots

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Extrapair paternity is widespread in birds and mammals. In particular, the alpine marmot, *Marmota marmota*, has a high frequency of extrapair paternity that seems to be explained by the genetic compatibility hypothesis. We investigated whether the number and proportion of extrapair young depend on the heterozygosity (individual genetic diversity) of the social male, or on the genetic similarity between the social male and his mate (relatedness). Both the number and the proportion of extrapair young increased with both high similarity and dissimilarity between the social pair. In combination with previous results, our study suggests that patterns of extrapair paternity in alpine marmots can best be explained by the genetic compatibility hypothesis, and more precisely its optimal outbreeding variant. Our results indeed suggest that extrapair paternity is a mechanism to avoid both in- and outbreeding depression. We discuss which proximal mechanisms may be involved in extrapair paternity in this species.

Keywords: alpine marmot; cooperative breeding; inbreeding; *Marmota marmota*; mating system; miscrosatellite; relatedness; reproductive skew

The genetic compatibility hypothesis states that females may benefit from extrapair paternity (EPP) if their genes are more compatible with those of the extrapair male than those of their pair mate (Zeh & Zeh 1996; Tregenza & Wedell 2000). Inbreeding is one form of genetic incompatibility, so females may benefit from EPP by decreasing the inbreeding level of their offspring (Zeh & Zeh 1996; Tregenza & Wedell 2000). Although still poorly supported (Kempenaers 2007), this inbreeding avoidance hypothesis has received some support in both socially monogamous birds (Blomqvist et al. 2002; Foerster et al. 2003) and mammals (Sillero-Zubiri et al. 1996).

The genetic benefits that females actually gain from their extrapair mate choice depend on their ability to bias egg fertilization in favour of the best male. Theoretically, this could be achieved via pre- and postcopulatory choice mechanisms (Pizzari & Birkhead 2002). The number and proportion of extrapair young (EPY) should then increase as the genetic quality or the genetic compatibility of the social mate decreases relative to that of other available sexual partners. If females are not able to adjust fertilization relative to male quality/compatibility, or if females seek

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extrapair copulations (EPCs) to increase their offspring's genetic diversity, this relation is no longer expected. Thus, to understand better the evolution of EPP, it is crucial to examine the distribution of EPY within and among litters in relation to the characteristics of both social and extrapair males.

The alpine marmot, *Marmota marmota*, is a socially monogamous, territorial species where only dominant individuals are thought, from behavioural observation, to obtain access to reproduction (King & Allainé 2002; Hackländer et al. 2003) and EPP occurs frequently (Goossens et al. 1998; Cohas et al. 2006). Female choice appears to be an important determinant of EPP in alpine marmots as indicated by the nonrandom distribution of EPP across males (within-pair males and extrapair males, Cohas et al. 2006). Therefore, the alpine marmot is a suitable species for investigating the underlying ultimate causes that drive EPP and previous results seem to support the genetic compatibility hypothesis. Indeed, (1) the occurrence of EPP among litters depends on the genetic similarity between social mates (Cohas et al. 2006), (2) extrapair mates are more heterozygous and less genetically similar to the female than the corresponding within-pair mate (social partner; Cohas et al. 2006, 2007a), and (3) EPY survive better and have better access to reproduction than within-pair young (WPY) (Cohas et al. 2007b).

In alpine marmots, dispersal is costly because the probability of surviving and acquiring a breeding vacancy decreases rapidly with dispersal distance (Frey-Roos 1998). Consequently, 12–22% of subordinates become dominant in their natal territory and about 50% of subordinates become dominant in the immediate neighbourhood (Frey-Roos 1998; Magnolon 1999). This dispersal pattern leads to higher relatedness among pair mates than among random pairs (Cohas et al. 2006). Moreover, homozygous juveniles survive less well than heterozygous ones, especially under harsh winter conditions (Da Silva et al. 2006). Thus, the conditions for inbreeding avoidance to be a strong constraint driving EPP are met in the alpine marmot.

We focused on the inbreeding avoidance variant of the genetic compatibility hypothesis, specifically on the distribution of EPY among and within litters in relation to the male's genetic characteristics. In particular, we investigated whether the presence, the number and the proportion of EPY depended on within-pair mate heterozygosity (as an indicator of individual quality) and within-pair mate genetic similarity to the female.

METHODS

Study Species and General Procedures

Alpine marmots are territorial cooperative breeders with reproduction highly skewed towards dominant individuals (King & Allainé 2002; Hackländer et al. 2003). The basic social unit is a family group of 2–20 individuals with a dominant breeding pair, sexually mature subordinates (at least 2 years old), yearlings and juveniles (Perrin et al. 1993). The physiological reproductive functions of the great majority of sexually mature subordinate females (Hackländer et al. 2003) and of sexually mature subordinate males (Arnold 1990; Goossens et al. 1998; Cohas et al. 2006) are inhibited by aggressive behaviour by the dominant individual of the same sex. Hence most subordinates disperse from 2 years old onwards and become transient individuals in search of a breeding territory (Frey-Roos 1998; Magnolon 1999).

The study site is located in La Grande Sassière Nature Reserve (French Alps, 45°29′N, 6°59′E, 2300 m above sea level). It covers 40 ha of alpine open meadows. From 1990 to 2006, we captured marmots from early April to late July on at least 45 days a year. We used two-door, live-capture traps baited with dandelion, *Taraxacum densleonis*. We placed the traps near the entrance of the main burrows of each family group to assign trapped individuals to their family and checked them every half hour to limit the time an individual was trapped. Once captured, individuals were tranquillized with Zolétil 100 (0.1 ml/kg) and individually marked with a numbered eartag (1 cm × 3 mm) and a transponder (model ID100, 0.9 cm long, <0.1 cm in diameter, Trovan Ltd, www.Trovan.com) injected under the skin of the neck for permanent individual recognition. In addition, a piece of coloured plastic (<1 cm^2) was fixed to one ear. Trapped individuals were sexed and aged by size (up to 3 years of age). Morphological characteristics (presence of visible testis for males, developed teats for females, development of scent glands for both sexes) were used to confirm individual social status determined from observation. For genetic analyses, we collected hair from all individuals captured since 1992, and tissue biopsies from the flank of individuals since 1997; a piece of skin (<1 mm^3) was removed with a biopsy punch (Alcyon, Lyon, France). The marking and biopsies did not cause any bleeding. Handling lasted a maximum of 10 min and individuals were absent from their territory for a maximum of 30 min. We never observed exclusion from the territory for any individual of any age following capture.

We determined the composition of 20 families from capture histories combined with intensive observations using 10 × 50 binoculars and 20 × 60 telescopes from a distance of 80–200 m. 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 0800 to 1200 hours and from 1500 to 2100 hours. We recorded the number of yearlings, 2-year-olds and adults of each sex and their social status for each family. Size allowed us to categorize individuals as yearlings, 2-year-olds or adults, and scent-marking behaviour and aggressive interactions allowed us to categorize individuals as subordinates or dominants (Bel et al. 1999). From additional daily observations, we recorded the date and the litter size at emergence ($X \pm SD = 3.6 \pm 1.2$, range 1–7). Virtually all emerged juveniles were trapped within 3 days of emergence (Allainé et al. 2000; Allainé & Theuriau 2004), and none were rejected by their mother or family group after capture. All manipulations were approved by the Centre National de la Recherche Scientifique and the Vanoise National Park Authority.

Genetic Analyses

Genotyping and microsatellite characteristics

In total, 797 individuals were typed at 16 microsatellite loci: SSBibl1, 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). Details of the genotyping method are given in the Appendix. Depending on the locus, 97.1–99.8% of all individuals were genotyped (Table 1). To estimate the genotyping error rate, we typed 96 randomly chosen individuals for each microsatellite locus. No discrepancy between the two genotypes was found, so the probability of finding an error for one allele should not exceed 0.0003.

Table 1 summarizes the microsatellite characteristics. Using the library 'adegenet' for R (Jombart 2007), we carried out Hardy–Weinberg tests on dominant adults only, to avoid potential bias caused by family structure, and on all cohorts pooled to ensure sufficient sample size ($N = 160$). Except for Ma002 ($\chi^2 = 308.90$, 10 000 replicates, $P = 0.027$), none of the loci showed deviation from Hardy–Weinberg equilibrium ($1 > P > 0.064$).

Parentage analysis

The genotypes of each offspring and of the supposed parents were compared to check maternity. For 16 × 645 mother–offspring comparisons, only one mismatch at one locus (SS-Bibl20) between the supposed mother (usually the dominant female) and one of its offspring was found. We then defined young as WPY if no mismatch was observed with the dominant male genotype (553 WPY of 595 offspring). We defined young as EPY if at least one mismatch was observed with the dominant male genotype (42 EPY: 1–8 mismatches). For 13 offspring, exclusions of paternity were based on only one mismatch with the social male. We consider it unlikely that these young were WPY, because (1) the genotyping error rate was low (0.0003), (2) all these offspring and their parents were retyped and their genotypes confirmed, (3) the average mutation rate for microsatellites is low (1.67×10^{-4} per generation in *M. marmota*, Rassmann et al. 1994) and (4) only a single mismatch with the putative mother was found (see above).

The genotypes of EPY were then compared to the genotypes of all known sexually mature males and two types of EPY were defined. Within-group EPY had genotypes compatible with the genotype of a subordinate male in their family but incompatible with the genotypes of all males from outside their family (13 within-group EPY). Extragroup EPY had a genotype incompatible with those of all subordinate males of their family. The biological father was known for 13 extragroup EPY and unknown for 16.

Paternity analysis was repeated using the Cervus 3.0.3 software (Kalinowski et al. 2007), with the following settings: 20 candidate parents of each sex per offspring; 95% of candidate parents sampled; error rate of 1% to allow for mistyping and for mutations or null alleles (Table 2); and assignment at a 95% confidence level. For

Table 1. Characteristics of the 16 microsatellites

	Bibl1		Bibl18		Bibl20		Bibl31		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	Total
	95	0.16	133	<0.01	206	0.01	157	0.47	175	0.12	265	<0.01	296	0.27	231	0.62	159	0.13	184	0.17	107	0.36	176	0.04	132	0.13	104	0.02	142	0.06	116	0.14	
	97	0.20	137	0.01	208	0.22	159	0.30	178	<0.01	271	0.21	298	0.73	233	0.03	167	0.09	186	0.83	109	0.53	180	0.23	140	0.47	106	0.26	158	0.87	118	0.29	
	101	0.45	143	0.36	216	0.38	161	0.18	188	0.18	273	<0.01			241	0.35	169	0.05			111	0.11	182	0.19	142	0.39	108	0.72	160	0.07	120	0.19	
	103	<0.01	145	0.13	218	0.31	163	0.05	190	0.67	279	0.46					171	0.02					184	0.17	144	<0.01	110	<0.01			130	0.05	
	107	0.14	147	0.39	220	0.08			192	0.03	281	0.32					173	0.15					186	0.34	148	<0.01					132	0.15	
	109	0.05	149	0.11	222	<0.01					283	<0.01					175	0.43					188	0.03							134	0.15	
																	177	0.02					190	0.01							136	0.04	
																	179	0.09														
																	188	<0.01														
N	795		792		786		795		793		774		777		786		789		783		792		787		789		787		785		785		
No. of alleles	6		6		6		4		5		6		2		3		9		2		3		7		5		4		3		7		4.88
PIC	0.67		0.63		0.65		0.59		0.46		0.57		0.32		0.39		0.72		0.24		0.49		0.73		0.53		0.35		0.22		0.79		0.523
NE-1P	0.70		0.73		0.72		0.77		0.87		0.79		0.92		0.88		0.63		0.96		0.83		0.63		0.81		0.91		0.97		0.54		0.020
NE-2P	0.52		0.57		0.55		0.61		0.72		0.65		0.84		0.79		0.44		0.88		0.71		0.45		0.68		0.81		0.88		0.37		0.0007
NE-PP	0.34		0.39		0.37		0.44		0.56		0.50		0.75		0.68		0.24		0.80		0.57		0.27		0.54		0.70		0.78		0.19		<0.0001

N: number of individuals typed; PIC: polymorphic information content; NE-1P: nonexclusion probability of the first parent; NE-2P: nonexclusion probability of the second parent; NE-PP: nonexclusion probability of the pair of parents.

Table 2. Assignment rate and reliability of assignment (%) at a 95% level of confidence given the set of 16 microsatellites used in the parentage analysis and assuming 20 candidate parents for each sex, 95% of candidate parents sampled and an error rate of 1%

	Father, given known mother	Mother, given known father	Parent pair with sex known
Assignment rate	91.0	95.0	93.0
Identity of most likely candidate			
True parent(s)	98.3	98.6	95.0
Nonfather/mother/parents (true parent(s) sampled)	0.005	0.8	0.7
Nonfather/mother/parents (true parent(s) unsampled)	0.01	0.6	2.1*, 2.2†, 0.02‡

*True mother unsampled.
†True father unsampled.
‡Neither true parent sampled.

27 young from 20 litters, some of the WPY could also be assigned to both the dominant and a subordinate male. However, for 14 young the dominant male had a higher likelihood of being the father than the subordinate male. Moreover, in all cases, the sexual organs of the subordinate male showed no sign of development at capture, and the rest of the litter could always be assigned to the dominant male. Thus, we parsimoniously considered all these young as WPY. There were no ambiguities in the assignments of EPY.

Estimates of heterozygosity and genetic similarity

Individual genetic diversity was estimated as the standardized individual heterozygosity (SH), which is defined as the proportion of heterozygous loci divided by the mean heterozygosity of the scored loci (Coltman et al. 1999). This method is used to account for the fact that few individuals were scored at fewer than 16 loci.

Three estimators of genetic similarity between mates were calculated using functions written in R (R Development Core Team 2007): Queller & Goodnight's estimator (R_{QG}, Queller & Goodnight 1989), Lynch & Ritland's estimator (R_{LR}, Lynch & Ritland 1999) and Identity (I, Belkhir et al. 2002). The three estimators were highly correlated (R_{QG} versus I: $r = 0.75$, R_{QG} versus R_{LR}: $r = 0.86$, R_{LR} versus I: $r = 0.86$, $N = 316\,410$ pairs) and the results found were independent of the estimator used. Therefore, we present only the results using Queller & Goodnight's estimator.

Definitions and Data Analyses

For this study, we limited the analyses to 103 litters where dominant individuals and all offspring were known and genotyped ($N = 369$), and where the number of mature male subordinates present in the family group was known. Litters composed of only WPY were defined as within-pair litters, those composed exclusively of EPY were defined as extrapair litters, and those containing both WPY and EPY are referred to as mixed litters.

We investigated the effect of fixed terms (number of mature male subordinates present in the family group, male heterozygosity, genetic similarity between social partners, litter size) on the presence, number and proportion of EPY within litters (three related variables). To account for repeated measures (26 pairs were present in 2–5 years), we used generalized estimating equations (GEE) to estimate the parameter of generalized mixed models (Liang & Zeger 1986; Zeger & Liang 1986; Diggle et al. 2002). This procedure is likely to be more robust than likelihood-based estimation for generalized mixed models because it makes broad hypotheses about data structure, and is better adapted to departure from normality of random effects and small sample sizes within clusters (Carlin et al. 2001). We used GEE with the pair as the clustering factor. We chose an exchangeable correlation matrix to specify the same correlation between all observations of the same cluster (this is analogous to the correlation structure derived from assuming a random factor in a mixed model; Horton & Lipsitz 1999). Moreover, we included the number of sexually mature subordinate males present in the family group as a fixed variable in all models since it affects the occurrence of EPP in alpine marmots through male–male competition (Cohas et al. 2006).

In the model with the number of EPY as the dependent variable, we used GEE with a logarithm link and a variance given by a gamma distribution to account for overdispersion (see Results, Venables & Ripley 2002). In the other two models with the presence or the proportion of EPY as the dependent variable, we also used GEE but with a logit link and a variance given by a binomial distribution (i.e. same link and variance function as in a logistic regression). The significance of fixed terms was assessed using the robust z statistics of parameter estimates (Diggle et al. 2002).

For all statistical analyses we used R 2.5.1 software and the gee library (R Development Core Team 2007). Unless otherwise stated, all tests were two tailed, the level of significance was set to 0.05, and parameter estimates are given ±SE.

RESULTS

Frequency of Extrapair Paternity

For litters where dominant individuals and all offspring were known and genotyped and where the number of mature male subordinates present in the family group was known, 36 (9.7 ± 1.5%) of 369 offspring were EPY and 20 (19.4 ± 3.9%) of 103 litters contained at least one EPY (Table 3). Ten (50.0 ± 11.1%), six (30.0 ± 10.2%), three (15.0 ± 8.0%) and one (5.0 ± 4.9%) of these litters contained one, two, three and five EPY, respectively, the mean number of EPY per litter was 1.8 ± 0.2 and half of these litters containing at least one EPY contained more than 50% of EPY.

EPY were not randomly distributed (i.e. did not follow a Poisson distribution) both among all litters

Table 3. Distribution of within- and extrapair offspring among the different types of litters

		EPY			
	WPY	Within-group*	Extragroup†	Unidentified‡	Total
Within-pair litters	298 (83)	—	—	—	298 (83)
Mixed litters	35 (15)	4 (2)	8 (4)	10 (9)	57 (15)
Extrapair litters	—	9 (4)	5 (1)	0 (0)	14 (5)
Total	333 (98)	13 (6)	13 (5)	10 (9)	369 (103)

Numbers of litters are given in parentheses. WPY: within-pair young; EPY: extrapair young.
*Young sired by a subdominant male within the social group.
†Young sired by a known male from outside the social group.
‡Young sired by an unidentified extrapair male outside the social group.

(Kolmogorov–Smirnov test: $D = 1.89$, $N = 103$, $P < 0.001$) and among litters containing at least one EPY ($D = 3.55$, $N = 20$, $P < 0.001$).

Factors Influencing Loss of Paternity

The presence of EPY within a litter increased with the number of sexually mature subordinate males present in the family group (Table 4, Fig. 1). The presence of EPY in a litter also depended on a quadratic relation with the genetic similarity between pair mates. Thus, the probability of observing EPY in a litter was higher for extreme (low and high) values of genetic similarity between mates than for intermediate ones (Table 4, Fig. 1). Similarly, both the number and the proportion of EPY per litter followed a quadratic relation with the genetic similarity between pair mates: EPY were more numerous for extreme (low and high) values of genetic similarity between mates than for intermediate values (Table 4, Fig. 2).

The presence of EPY within a litter and the number and proportion of EPY were independent of within-pair male heterozygosity and of litter size (Table 4). The number and proportion of EPY within a litter were also independent of the presence of sexually mature subordinate males in the family group (Table 4).

Table 4. Generalized estimating equation models showing the effects of terms on the presence of extrapair young (EPY) in a litter, the number of EPY per litter and the proportion of EPY per litter

	Coefficients	SE	*P*
Presence of EPY in litter			
Number of subordinate males	0.980	0.284	<0.001
Within-pair male heterozygosity	1.708	1.565	0.275
Genetic similarity between pair mates	−1.171	1.130	0.300
(Genetic similarity between pair mates)^2	6.709	2.654	0.011
Litter size	0.191	0.185	0.302
Number of EPY per litter			
Number of subordinate males	0.285	0.167	0.087
Within-pair male heterozygosity	1.176	0.962	0.221
Genetic similarity between pair mates	−1.333	0.548	0.015
(Genetic similarity between pair mates)^2	3.603	1.516	0.017
Litter size	0.188	0.164	0.252
Proportion of EPY per litter			
Number of subordinate males	0.383	0.209	0.067
Within-pair male heterozygosity	1.278	2.622	0.238
Genetic similarity between pair mates	−1.637	0.893	0.067
(Genetic similarity between pair mates)^2	5.181	2.279	0.023
Litter size	−0.107	0.217	0.620

Patterns of Paternity Gain

Of the 20 litters containing at least one EPY, two were fathered by three males, whereas all others were fathered by two males. The within-pair male sired none of the young in only five ($4.5 \pm 2.1\%$) litters (Table 3). Among the five within-pair males with complete paternity loss only one never sired young in other years.

Thirteen of 36 EPY ($36.1 \pm 8.0\%$), born in three ($15.0 \pm 8.0\%$) of the 20 litters containing at least one EPY, were fathered by an extrapair male within the same family. The remaining 23 ($63.9 \pm 8.0\%$) EPY, born in 17

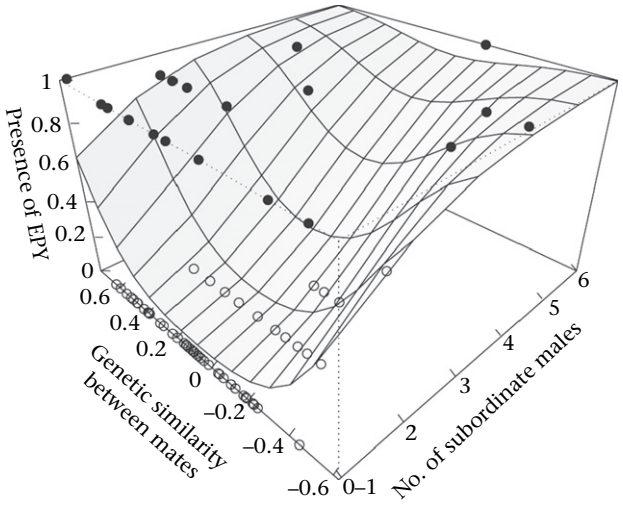

Figure 1. Presence of extrapair young (EPY) in litters as a function of genetic similarity between pair mates and the number of sexually mature subordinate males present in the family group. ●: Litters with EPY; ○: litters without EPY; the surface represents the fitted model.

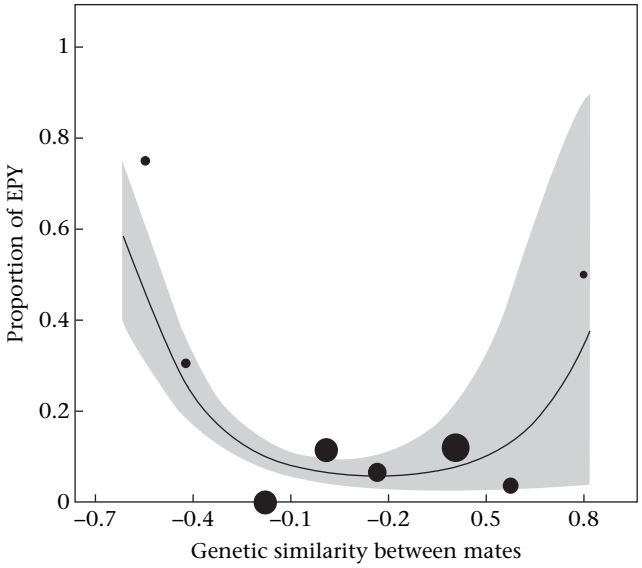

Figure 2. Proportion of extrapair young (EPY) per litter as a function of the genetic similarity between pair mates. The black circles represent observed data averaged over classes of genetic similarity between mates (class width 0.2) and their size is proportional to the number of litters within each class. The solid line shows the fitted model and the grey surface represents standard errors of the fitted model.

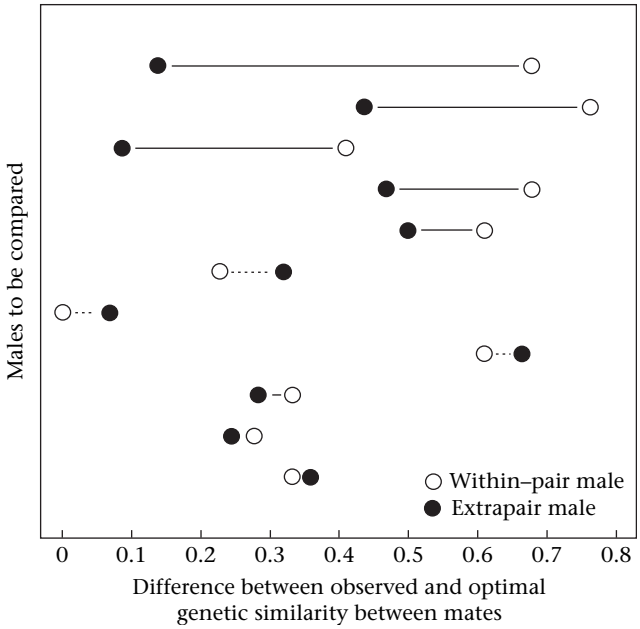

Figure 3. Absolute difference between the observed and optimal genetic similarity between the female and the within-pair and corresponding extrapair males. Optimal genetic similarity equals 0.16, as obtained from the generalized estimating equation model of extrapair young proportion within a litter (see Results).

(85.0 ± 8.0%) of the 20 litters containing at least one EPY, were known or inferred to be fathered by a transient male (Table 3). None of the EPY was fathered by a dominant or a subordinate male from a neighbouring group. Extrapair mates were never closely related to the dominant female, but sometimes they were related to the dominant male: one extrapair male was a brother of the dominant male and sired two young in a litter and five were sons of the dominant male and sired a total of seven young in four litters.

From theory, there are clear expectations with regard to the direction of the difference in heterozygosity and in genetic similarity between extrapair and within-pair males. We thus tested only for extrapair males being more heterozygous and more optimally similar to the females and used one-tailed instead of two-tailed tests. Among the 11 identified extrapair males, eight (72 ± 10.9%) showed higher heterozygosity than that of the corresponding within-pair males: four of six extragroup extrapair males and four of five within-group extrapair males were more heterozygous than the corresponding within-pair males. However, the extrapair males were not significantly more heterozygous than the corresponding with-pair males (one-tailed Wilcoxon signed-ranks test: $V = 27$, $N = 11$, $P = 0.31$).

Seven (63 ± 14.6%) of the 11 identified extrapair males showed a genetic similarity to the female that was closer to the optimum (optimal genetic similarity estimated from the GEE model on EPY proportion = 0.16) than that of the corresponding within-pair mate: three of six extragroup extrapair males and four of five within-group extrapair males were closer to the optimal genetic similarity with the female than the corresponding social males (Fig. 3). However, the extrapair males only tended to show a more optimal level of genetic similarity to the female than the corresponding social males (one-tailed Wilcoxon signed-ranks test: $V = 50$, $N = 11$, $P = 0.07$).

DISCUSSION

Previous investigations on the ultimate causes driving EPP in the alpine marmot supported the genetic compatibility hypothesis, and more specifically its inbreeding avoidance variant: the comparison of the genetic characteristics of WPY and EPY half-siblings indicated that extrapair males were less genetically similar to the female than the corresponding within-pair males (Cohas et al. 2007a), and EPY outperformed WPY as indicated by their higher probability of surviving and becoming dominant (Cohas et al. 2007b). Our results matched the prediction of the optimal outbreeding hypothesis. Indeed, the probability that EPY were present and the number and proportion of EPY within a litter increased with high similarity and dissimilarity between the social pair mates. Such a pattern has rarely been reported, and only in bird species such as the pied flycatcher, *Ficedula hypoleuca* (Rätti et al. 1995) or the house sparrow, *Passer domesticus* (Bonneaud et al. 2006).

Female choice for extrapair mates does not seem to target heterozygous males ('heterozygosity as good genes', Brown 1997) in the alpine marmot. Indeed, (1) neither the probability that EPY were present nor the number and proportion of EPY within a litter increased with the homozygosity of the social mate; (2) extrapair males were not more heterozygous than the corresponding within-pair

males; (3) finally, contrary to a central assumption of this hypothesis (Mitton 1993; Brown 1997), heterozygosity is not heritable in alpine marmots (Cohas et al. 2007a).

Precopulatory mechanisms are important in determining the pattern of EPP in alpine marmots for at least two reasons. First, the fact that the probability that EPY are present depends on the number of sexually mature subordinate males present in the family group suggests that the social setting affects the conditions for EPCs to occur (Cohas et al. 2006). Dominant male marmots (i.e. the within-pair mate) may limit the opportunity of females to find extrapair mates either through reproductive suppression of male competitors or through mate guarding (Arnold & Dittami 1997). Females may thus also be limited by the availability of adequate extrapair males. Second, there is additional evidence that alpine marmots avoid inbreeding before copulation. For example, the level of testosterone of subordinate males decreases with their relatedness to the dominant female but not to that of the dominant male (Magnolon 1999), a pattern also found in prairie voles, *Microtus ochrogaster* (Carter et al. 1986) and in white-footed mice, *Peromyscus leucopus* (Wolff 1992). Similarly, the dispersal rate of subordinate females increases with their relatedness to the dominant male but not to that of the dominant female (Magnolon 1999). Moreover, owing to the social structure of the alpine marmot, different potential EPC candidates exist: (1) subordinate males of the family group, (2) subordinate or (3) dominant males of other family groups and (4) transient males in search of a territory. Among these potential extrapair males, we showed that EPP preferentially involved transient individuals, probably originating from distant family groups, and hence less related to the female. Furthermore, the few subordinate males of the family group that obtained EPP were all unrelated to the female. Finally, incestuous matings are extremely rare: in 20 years of study, only one mating involving a mother and her son was observed.

The fact that the number and proportion of EPY within litters depended on the genetic similarity between pair mates can be explained by several mechanisms. First, females may copulate more frequently with an extrapair male or less frequently with their own mate when the latter is more genetically similar (precopulatory process). This assumes that females can assess their genetic similarity with their mate. Alternatively, the frequency of EPP in alpine marmots may also partly be explained by postcopulatory 'choice' mechanisms. For example, if sperm from different males are present within the female's reproductive tract, the sperm of the more compatible male may have an advantage, that is, a higher probability of fertilizing the eggs. More litters with EPY and more identified extrapair males are needed to compare the proportion of young sired in relation to the genetic similarity of the female with both within-pair and extrapair males (e.g. Foerster et al. 2006).

Although the good genes hypothesis has unquestionably received more empirical support than any other hypothesis in birds (reviewed in Westneat et al. 1990; Birkhead & Møller 1992; Griffith et al. 2002), more recently, empirical support for the compatibility hypothesis, and especially the inbreeding avoidance variant of this hypothesis, has started to accumulate (Dobson et al. 1997; Kempenaers et al. 1999; Blomqvist et al. 2002; Foerster et al. 2003; Freeman-Gallant et al. 2003; Eimes et al. 2005; Tarvin et al. 2005). In birds and mammals with group living and cooperative breeding, inbreeding avoidance is a strong evolutionary force (Stacey & Koenig 1990; Jennions & Macdonald 1994; Creel & Macdonald 1995; Hoffman et al. 2007). Indeed, in these species, limited opportunities for dispersal and a scarcity of breeding vacancies result in kin of opposite sex residing in the same group (habitat saturation hypothesis: Selander 1964; Emlen 1982; Brown 1987). Consequently, inbreeding avoidance may be an especially strong evolutionary force driving EPP in group-living species (*Malurus splendens*: Brooker et al. 1990; *Canis simensis*: Sillero-Zubiri et al. 1996). Indeed, in mammalian species where paternity analyses have been undertaken, EPP is rarer among species living in solitary pairs than among species living in groups (percentage of litters with EPY: solitary pair median = 2.1; group living median = 20.5; Wilcoxon test: $W = 8$, $N = 14$, $P = 0.04$, Cohas 2006).

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Appendix

To allow polymerase chain reaction multiplexing and subsequent assessment of the allele sizes for each microsatellite locus, we used primer sets labelled with FAM, PET, NED and VIC fluorescent dyes (FAM for SSBibl1, MS45, Ma066 and Ma091, PET for SS-Bibl18, SS-Bibl20, SS-Bibl4, Ma002 and Ma018, NED for SS-Bibl31, MS47, MS53 and Ma002, and VIC for MS41, MS56, MS6 and ST10).

We extracted genomic DNA from 15–30 hairs or from tissue by placing the sample in 50 μl lysis buffer (2.0 mM Tris–HCl, 1.5 mM $MgCl_2$, 25 mM KCl, 0.5% Tween 20, 0.1 mg/ml proteinase K for hairs and 0.4 mg/ml proteinase K for tissue). The samples were incubated at 66°C for 80 min for hairs and at 56°C for 120 min for tissue and then for 20 min at 96°C.

To carry out the polymerase chain reaction (PCR) of the 16 loci we used three 10 μl reactions, Mix1, Mix2 and Mix3, containing 5 μl of Kit PCR (Quiagen, Hilden, Germany) and 1 μl of DNA extract with a DNA concentration of 25–100 ng/ml. In addition, Mix1 contains 0.03 μl of each primer for MS45, 0.1 μl of each primer for SS-Bibl31, MS41and ST10, 0.2 μl of each primer for SS-Bibl18 and SS-Bibl4 and 0.3 μl of each primer of Ma002. Mix2 contains 0.05 μl of each primer for MS56 and MS6, 0.1 μl of each primer for MS53 and Ma091, 0.14 μl of each primer for SSBibl1, and 0.2 μl of each primer for MS47, Ma018 and Ma066. Mix 3 contains 0.2 μl of each primer for SS-Bibl20. Amplifications were carried out in a Mastercycler (Eppendorf, Hamboug, Germany) thermocycler with the following cycling conditions: 15 min at 95°C, then 28 cycles for Mix1 and Mix2 and 35 cycles for Mix3 composed of 30 s denaturing at 94°C, 90 s annealing at 57°C, 60 s extension at 72°C, and finally 30 min at 60°C to ensure complete extension.

We then added 1.5 μl of Mix1 and 1.5 μl of Mix2 plus 1.5 μl Mix3 to 0.15 μl of size standard ROX 60-415 and 10 μl of formamide and loaded them separately on 5% Long Ranger polyacrylamide gel (Fric). Electrophoresis was run for 3 h on an automated sequencer ABI 3130 (Applied Biosystems Inc., Foster City, CA, U.S.A.) using the size standard, ROX 60-415, to determine allele sizes. Microsatellite patterns were examined with Genemapper 4.0 (Applied Biosystems).