

Extrinsic and intrinsic constraints interact to drive extra-pair paternities in the Alpine marmot

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Abstract

To reproduce, animals have to form pairs and large variations in the degree of mate switching are observed. Extrinsic and intrinsic factors can constrain individual's mate switching. Among intrinsic factors, genes involved in pair-bonding, such as *Avpr-1a*, receive increasing attention. The length of microsatellites present in the regulatory region of *Avpr-1a* determines the neural densities and distributions of the vasopressin receptors known to impact pair-bonding behaviours. For the first time, we investigated whether and how the genetic makeup at *Avpr-1a*, an intrinsic factor, and the social context, an extrinsic factor, experienced by wild Alpine marmot (*Marmota marmota*) females affect the proportion of extra-pair young. This proportion was positively correlated with the length of their *Avpr-1a* regulatory region but only when the social constraints were relaxed, *that is* when mature male subordinates were present. When ignoring the interactive effect between the length of their *Avpr-1a* regulatory region and the social constraints, the genetic makeup at *Avpr-1a* was not associated with the proportion of extra-pair young. Under natural conditions, the genetic regulation of pair-bonding could be hidden by extrinsic factors constraining mate choice.

Introduction

To reproduce, animals have to mate and often form pairs that could vary in strength and duration. Even across socially monogamous species, a large variation in the degree of mate switching within and between mating seasons is observed (Rowley, 1983). Similarly, mate switching (e.g. divorce or extra-pair mating) rates are highly variable between individuals (Westneat & Stewart, 2003; Culina *et al.*, 2015). Why individuals vary in their degree of mate switching is still debated (Forstmeier *et al.*, 2014). The adaptive hypotheses of mate switching predict that the decision to stay or leave depends on the balance between the costs and benefits of each strategy. But the opportunities for an individual to switch mates could be influenced by both extrinsic and intrinsic factors (Griffith *et al.*, 2011). Extrinsic temporal, environmental, density-dependent or social

constraints can reduce the accessibility and/or the availability of potential partners, limiting mate switching (Choudhury, 1995; Isvaran & Clutton-Brock, 2007). And given that some individuals are more likely than others to engage in mate switching, intrinsic constraints are also expected to play a role (Forstmeier, 2007).

In this context, specific genes involved in pair-bonding have received increasing attention (Lim & Young, 2006) and specifically in prairie voles (*Microtus ochrogaster*). The gene *Avpr-1a*, and more specifically the length of repeated sequences (microsatellites) located in its upstream regulatory region, determines the neural density and distribution patterns of the vasopressin 1a receptor (V1aR). This receptor binds the arginine vasopressin, a neuropeptide regulating behaviours involved in mate choice and pair-bonding (Lim *et al.*, 2004; Young & Wang, 2004; Lim & Young, 2006; Hammock, 2007; and see Appendix S2). Microsatellite length can either up- or down-regulate gene expression. In some brain regions of male prairie voles, a long allele confers a higher level of V1aR binding, whereas in other regions, it confers a lower level of receptor binding (Hammock & Young, 2005). Microsatellites thus alter

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the pattern of gene expression across brain regions, rather than globally altering total levels of expression (Hammock & Young, 2005). Long microsatellites can alter transcription via changes in DNA conformation (e.g. Z-DNA structures, Rothenburg *et al.*, 2001) or by physically separating the regulatory element from the transcription start site (Filippova *et al.*, 2001). The nature of the repeated sequence can also modify gene expression. It was found for instance that regulatory *Avpr-1a* sequences with more Cytosine-phosphate-Guanine (CpG) sites lead to low gene expression (Okhovat *et al.*, 2015), supposedly because these sites are common targets of DNA methylation which reduces gene expression (Razin & Riggs, 1980).

The vast majority of studies investigating the influence of vasopressin and its receptors in pair-bonding have focused on male voles (Genus *Microtus*). Male voles with longer microsatellites or presenting specific single nucleotide polymorphisms in the regulatory region of the *Avpr-1a* gene show different V1aR density and distribution that in turn leads to more common monogamous behaviours such as social engagement, bonding behaviours, preferences towards familiar females and pup grooming (Hammock & Young, 2005; Phelps, 2010; Okhovat *et al.*, 2015). Few studies, conducted under laboratory settings, have examined affiliative and parental behaviour in females. Laboratory studies have shown that female rats (*Rattus norvegicus*) with greater expression of V1aR in particular brain regions display more maternal behaviour than those with less V1aR expression (Bosch & Neumann, 2008; Bosch *et al.*, 2010). There was also an association with maternal sensitivity in humans where maternal sensitivity was positively correlated with a longer microsatellite allele within the *Avpr-1a* gene (Bisceglia *et al.*, 2012). In semi-natural populations, female voles that had summed *Avpr-1a* microsatellite allele lengths greater than its median produced more litters and more offspring than females with summed *Avpr-1a* lengths less than its median (Harris *et al.*, 2014). In this last study, the authors hypothesized that female *Avpr-1a* microsatellite length should correlate with V1aR expression in a similar way as in male prairie voles (Hammock & Young, 2005; Okhovat *et al.*, 2015).

Despite the fact that mate switching is now widely recognized as a common reproductive strategy in females (Jennions & Petrie, 2000; Griffith *et al.*, 2002; Westneat & Stewart, 2003), little is known regarding intrinsic factors such as the potential impact of the genetic makeup at the upstream regulatory region of the gene *Avpr-1a* on mate switching in females. Harris *et al.* (2014) searched for an impact of the females' *Avpr-1a* genotype on mate switching and found that female prairie voles with long *Avpr-1a* microsatellite alleles produced offspring sired by more males. Under natural conditions, extrinsic factors such as temporal, environmental, density-dependent or social factors

constraining mate switching had been repeatedly investigated (Richardson & Burke, 2001; Cohas & Allainé, 2009; Botero & Rubenstein, 2012). However, little is known, especially for females, about intrinsic constraints such as an individual's own genetic makeup and even less about possible interactions between extrinsic and intrinsic mate choice constraints (Solomon *et al.*, 2009; Martin *et al.*, 2014).

In this study, we investigate whether and how the genetic makeup at *Avpr-1a* in combination with social constraints is associated with females' extra-pair mating behaviour in free-ranging Alpine marmots (*Marmota marmota*). In this socially monogamous species, social pair-bonding lasts until the death or eviction of one member of the dominant pair by a third individual and divorce is never observed (Lardy *et al.*, 2010). Dominant males never reproduce with any female other than the dominant female, but a strategy for a female to counteract a suboptimal social mate choice is to obtain extra-pair paternities (EPPs) (Cohas *et al.*, 2008; Ferrandiz-Rovira *et al.*, 2016). Marmots live in families, and the social context is an extrinsic factor strongly constraining the proportion of extra-pair young (EPY) (Cohas *et al.*, 2006). In the Alpine marmot, previous findings indicated that the occurrence of extra-pair paternity is driven by both genetic characteristics, especially the within-pair relatedness (Cohas *et al.*, 2008), and the presence of mature male subordinates (Cohas *et al.*, 2006, 2008; Bichet *et al.*, 2016). Thus, we predict that females with longer *Avpr-1a* microsatellites should have a higher propensity to mate switching (as found in female prairie voles) and therefore should obtain a higher proportion of EPY, especially under a permissive social context (i.e. presence of mature male subordinates, Cohas *et al.*, 2006).

Materials and methods

Studied species

Alpine marmots are territorial, socially monogamous and cooperative breeding ground-dwelling squirrels inhabiting alpine meadows (Allainé, 2000). Marmots live in family groups of two to 16 individuals composed of a dominant pair, sexually mature (≥ 2 years) and immature (1 year) subordinates of both sexes, and pups of the year (1–7 pups) (Allainé, 2000). Each family group occupies a territory defended by a dominant pair. At sexual maturity (i.e. 2 years of age), subordinates of both sexes may either keep their subordinate status, attempt to become dominants in their natal groups (15% of the dominant males, 35% of the dominant females) or disperse to attempt to gain dominance in another territory (Lardy *et al.*, 2012). Dominance is established for several years and lasts until the dominant is evicted by another individual or dies (Lardy *et al.*, 2011). Once an individual reaches dominance, it

cannot reverse to subordinate status. During the 23 years of our study, only three males and one female lost their dominant status but managed to establish dominance in another territory, whereas all other dominants died following eviction (Lardy *et al.*, 2011). Male subordinates are considered as helpers. The presence of helpers in a family group increases the survival probability of offspring during their first hibernation (Allainé & Theuriau, 2004) through their role in social thermoregulation during hibernation (Arnold, 1988).

Alpine marmots hibernate from mid-October to early April, and mating occurs shortly after the end of the hibernation. Although socially monogamous, EPP occur. The majority of the extra-pair fathers are within-group mature male subordinates and the others are transient males, dispersing in search for a breeding vacancy, or are dominant males from another neighbouring family (Cohas *et al.*, 2006; Bichet *et al.*, 2016; Appendix S1). The occurrence of EPP is associated with social factors and more precisely increases with the presence and the number of mature male subordinates in the family group (Cohas *et al.*, 2006; Bichet *et al.*, 2016). Extra-pair behaviours are also associated with genetic characteristics, especially the relatedness of the dominant pair measured with microsatellites. Higher EPP levels are observed for both low and high within-pair relatedness, whereas lower EPP levels are observed at intermediate within-pair relatedness (Cohas *et al.*, 2008; Ferrandiz-Rovira *et al.*, 2016).

Field methods

Marmots were monitored from 1992 to 2015 in La Grande Sassièrre Nature Reserve (2340 m a.s.l., French Alps, 45°29'N, 65°90'). Marmots belonging to 39 family groups were captured annually, after the mating season, from mid-April to mid-July using two-door live-capture traps (see Cohas *et al.*, 2008 for details). Traps were placed near the entrance of the main burrows of each group to assign trapped individuals to their family. Once captured, individuals were tranquillized with Zolétel 100, sexed, aged, and their social status (dominant or subordinate) was determined. Four age classes can be distinguished according to body size: pup, yearling, two-year-old and adult individuals. Social status was determined through examination of sexual characteristics (scrotum for dominant males and teats for dominant females) and was confirmed by behavioural observations. Scent-marking behaviour was used to discriminate subordinates and dominants since only the dominants actively mark and defend their territory (Bel *et al.*, 1999). Individuals were marked permanently with a transponder and a numbered ear tag, placed on the right ear of females and the left ear of males. An additional coloured plastic ear tag was placed on the opposite ear of dominant individuals. Hair and skin biopsies were collected for genetic analyses.

The annual composition of each family was determined by combining individuals' capture histories with daily observations. The litter size at first emergence from the natal burrow was obtained from additional observations and pups were trapped within the 3 days after the first emergence from the natal burrow.

Parentage analyses

A total of 1428 individuals, including dominants, subordinates and pups, were genotyped at sixteen microsatellites: SS-Bib11, SS-Bib18, SS-Bib20, SS-Bib31, SS-Bib4 (Klinkicht, 1993); MS41, MS45, MS47, MS53, MS56, MS6, ST10 (Hanslik & Kruckenhauser, 2000); Ma002, Ma018, Ma066, Ma091 (Da Silva *et al.*, 2003) (see Appendix S1 for detailed description of microsatellites genotyping and microsatellites' characteristics).

Only the litters for which the genotype of the dominant pair (82 females and 91 males) and all of their 865 pups were known were subjected to parentage analyses by both exclusion and paternity analyses (see Appendix S1 for detailed description on parentage analyses). Briefly, the genotypes of each pup and the dominant female were compared to confirm maternity. For 82 × 865 mother–pup comparisons, only one mismatch at one locus between the putative mother and one of its pups was found (with no other female of the group matching with the pup's genotype) and was highly likely due to a mutation within the pup's genotype. The mother was always considered to be the dominant female. The genotypes of each pup, its mother and the dominant male of their family were then compared to check paternity. A pup was considered as within-pair young (WPY) if no mismatch was observed with the dominant male's genotype and, otherwise, as EPY. Parentage analyses were repeated using the software CERVUS 3.0.7 (Kalinowski *et al.*, 2007), including the identity of the known mother and all potential fathers. We considered as potential fathers all sexually mature males present (captured or observed at the year t) and potentially present (captured or observed at the year $t - 1$ and/or $t + 1$) in the studied population. This analysis confirmed the results obtained with the exclusion analyses.

Characterization of the *Avpr-1a* genotypes

Three polymorphic microsatellites (loci 1, 2 and 3 with, respectively, 3, 6 and 6 alleles) were identified within the promoter of the *Avpr-1a* gene and genotyped for 82 dominant females (see Appendix S2). Since variations in the 5'-UTRs could regulate gene expression by affecting transcription and translation, we calculated, for each locus, its length by summing the length of its two alleles for each dominant female (Harris *et al.*, 2014). We further calculated the total length of the microsatellites located in the promoter by summing the lengths of

all alleles at the three loci for each dominant female (Harris *et al.*, 2014). The length of loci 1, 2 and 3 as well as the total length (locus 1 + locus 2 + locus 3) was then used as explanatory variables in the statistical analyses. The locus lengths are correlated with one another (Spearman's correlation: locus 1–locus 2: $r_{77} = 0.88$; locus 1–locus 3: $r_{77} = 0.62$; locus 2–locus 3: $r_{80} = 0.45$; all P -values < 0.001) and are strongly correlated with the total length (Spearman's correlation: locus 1: $r_{77} = 0.92$; locus 2: $r_{77} = 0.82$; locus 3: $r_{77} = 0.82$; all P -values < 0.001).

Characterization of the social constraints

In Alpine marmots, the social environment is known to influence EPP (Cohas *et al.*, 2006, 2008). Both the occurrence of EPP and the proportion of EPY positively correlate with the presence of sexually mature male subordinates within a family group (Cohas *et al.*, 2006, 2008). Thus, the presence of sexually mature male subordinates within a family was used as a measure of the social constraints experienced by the dominant female.

Confounding variables

The proportion of EPY presents a quadratic relationship with the relatedness of the social pair: the high proportion of EPY is observed at both high and low within-pair relatedness, and the low proportion of EPY is observed at intermediate within-pair relatedness (Cohas *et al.*, 2006, 2008). Within-pair relatedness thus was estimated by the Queller & Goodnight's index (Queller & Goodnight, 1989) calculated over the 16 microsatellites used for parentage analyses and considered in subsequent analyses as a potential confounding variables.

Statistical analyses

We investigated whether the female *Avpr-1a* genotype had an effect on the proportion of EPY using generalized linear mixed models (GLMMs) performed with the package 'lme4' (Bates *et al.*, 2015) of R 3.3.1 (R Core Team, 2014). By litter, the number of EPY was entered as the response variable and the total number of pups as the binomial denominator. The standardized lengths of each locus (either length of locus 1, length of locus 2, length of locus 3) or the standardized total length (locus 1 + locus 2 + locus 3) of the *Avpr-1a* microsatellites, the presence/absence of mature male subordinates and the first-order interaction between the presence/absence of sexually mature male subordinates and any of the length of the *Avpr-1a* microsatellites were entered as explanatory variables in four different GLMMs (one for each length of the *Avpr-1a* microsatellites). The quadratic effect of within-pair relatedness, known to have an effect on the EPP occurrence, was also included in our models. Female identity was added

as a random intercept. Models were fitted by maximum likelihood. If nonsignificant, interactions were removed following a backward selection procedure. Significance was assessed using the normal approximation of the Z -value with the level of significance set to $\alpha = 0.05$ and parameter estimates are given as mean \pm SE.

None of our GLMMs showed sign of over-dispersion (locus 1: $\text{Chi}^2 = 74.58$, ratio = 0.33, $P = 1.00$; locus 2: $\text{Chi}^2 = 100.53$, ratio = 0.42, $P = 1.00$; locus 3: $\text{Chi}^2 = 84.83$, ratio = 0.36, $P = 1.00$; total length: $\text{Chi}^2 = 67.47$, ratio = 0.30, $P = 1.00$).

Results

Fifty-eight ($6.7 \pm 0.9\%$) of the 865 pups were EPY, and 30 ($12.2 \pm 2.1\%$) litters among 246 litters born to 82 females contained at least one EPY (Table 1). Litters with EPY were produced in 16 families that had sexually mature male subordinates and in 14 families that did not have any, whereas litters without EPY were produced in 81 families that had sexually mature male subordinates and in 135 families that had none.

We did not find any main effect of the lengths of the *Avpr-1a* loci on the proportion of EPY (Table 2, Appendix S3). However, the proportion of EPY depended on an interaction between the lengths of the *Avpr-1a* loci (loci 1, 2, 3 and total length) and the presence of sexually mature male subordinates (Table 2). When no sexually mature male subordinates were present in a family, the proportion of EPY was low and independent of the length of any of the loci (loci 1, 2 and 3) (Table 2, Fig. 1a–c, slopes = -0.002 , 0.001 and -0.016 , respectively), as well as for the total length (Table 2, Fig. 1d, slope = -0.010). When sexually mature male subordinates were present, the proportion of EPY increased, particularly when the *Avpr-1a* microsatellites were long (Table 2, Fig. 1a, b, d, slopes = 0.039 , 0.085 and 0.036 for locus 1, locus 2 and

Table 1 Distribution of the litters and the different kinds of pups as a function of the number of EPY per litter (A) and distribution of the litters depending on the number of fathers (B). WPY: within-pair young, EPY: extra-pair young.

A	Number of EPY per litter						Total
	0	1	2	3	4	6	
Number of litters	216	16	6	4	3	1	246
Numbers of WPY	748	44	11	4	0	0	807
Number of EPY	0	16	12	12	12	6	58

B	Number of fathers				Total
	1	2	3	4	
Number of litters with WPY	216	21	1	1	239
Number of litters with EPY only	4	3	0	0	7

Table 2 Effects of the *Avpr-1a* promoter region's characteristics (lengths at each locus and total length of the three loci) on the proportion of EPY. Nonsignificant interactions were removed from the models. Significant effects are in bold.

Explanatory variable	Length of locus 1			Length of locus 2			Length of locus 3			Total length		
	Estimate ± SE	Z	P-value	Estimate ± SE	Z	P-value	Estimate ± SE	Z	P-value	Estimate ± SE	Z	P-value
Intercept	-4.35 ± 3.33	-1.31	0.19	-3.69 ± 3.29	-1.12	0.26	-0.77 ± 3.72	-0.21	0.84	-4.18 ± 3.46	-1.21	0.23
Explanatory variable	-0.14 ± 0.57	-0.24	0.81	-0.14 ± 0.57	0.25	0.80	-1.40 ± 0.77	-1.82	0.07	-0.47 ± 0.63	-0.75	0.46
Absence/presence of mature male subordinates	1.49 ± 0.54	2.76	< 0.01	1.26 ± 0.52	2.47	0.01	1.80 ± 0.62	2.93	< 0.01	1.58 ± 0.58	2.71	< 0.01
Relatedness (linear)	-14.90 ± 11.92	-1.25	0.21	-16.93 ± 11.54	-1.47	0.14	-33.50 ± 14.55	-2.30	0.02	-16.89 ± 12.54	-1.35	0.18
Relatedness (quadratic)	19.33 ± 10.59	1.83	0.07	21.75 ± 10.01	2.17	0.03	37.23 ± 12.99	2.87	< 0.01	21.37 ± 11.20	1.91	0.06
Explanatory variable: absence/presence of mature male subordinates	1.15 ± 0.47	2.43	0.02	1.31 ± 0.56	2.22	0.02	1.81 ± 0.66	2.74	< 0.01	1.57 ± 0.53	2.98	< 0.01
Random effect (variance ± SD)	14.36 ± 3.79			12.53 ± 3.54			19.39 ± 4.40			16.31 ± 4.04		

SE, standard error; SD, standard deviation.

total length, respectively). Locus 3 was the only exception and did not impact the proportion of EPY (Table 2, Fig. 1c, slope = -0.014). Within-pair relatedness has a significant quadratic effect on the proportion of EPY (Table 2).

Discussion

In this study, we show for the first time in a wild mammal that the proportion of EPY produced by females did not depend on the genetic makeup of their *Avpr-1a* regulatory region, except under particular social contexts. Specifically, when sexually mature male subordinates were present, the proportion of EPY increased with the lengths of the *Avpr-1a* regulatory region. Under natural conditions, the genetic regulation of pair-bonding could be hidden by extrinsic factors constraining EPP, notably the social context.

The only other study investigating the effects of the *Avpr-1a* promoter on females' mating behaviour also reported an influence of the *Avpr-1a* promoter length on pair-bonding in female prairie voles under semi-natural conditions (Harris *et al.*, 2014). Similar to female Alpine marmots, female voles with summed allele lengths within the regulatory region of the *Avpr-1a* gene greater than the median produced offspring sired by more than twice as many males relative to females with summed lengths less than the median (Harris *et al.*, 2014). In males, previous studies on humans and voles also revealed an impact of the gene *Avpr-1a* on pair-bonding. In men, repeat polymorphism in the 5' flanking region of the *Avpr-1a* gene is associated with behaviours such as partner bonding, perception of marital problems, and marital status (Walum *et al.*, 2008). Fifteen per cent of the men carrying no 334 allele did not report marital crisis, whereas 34% of the men carrying two copies of this allele reported marital crisis, suggesting that being homozygous for the 334 allele doubles the risk of marital crisis compared with having no 334 allele (Walum *et al.*, 2008). In male prairie voles under experimental settings, the *Avpr-1a* regulatory region polymorphism, via its impact on the location and density of V1aR in the brain, predicts both pair-bonding and paternal care (Pitkow *et al.*, 2001; Hammock & Young, 2005; Hammock *et al.*, 2005). Males with longer microsatellites in the 5' region have a greater probability of social engagement and bonding behaviour, prefer a familiar over an unknown female and groom pups more frequently (Hammock & Young, 2005; Okhovat *et al.*, 2015). Okhovat *et al.* (2015) indicated that specific alleles are robust predictors of V1aR levels in memory structures, levels that predict whether males will gain extra-pair paternity. They further suggest that these specific SNPs (single nucleotide polymorphisms) within regulatory sequences differ in CpG abundance and methylation status and correspond to low-expressing alleles (Okhovat *et al.*, 2015).

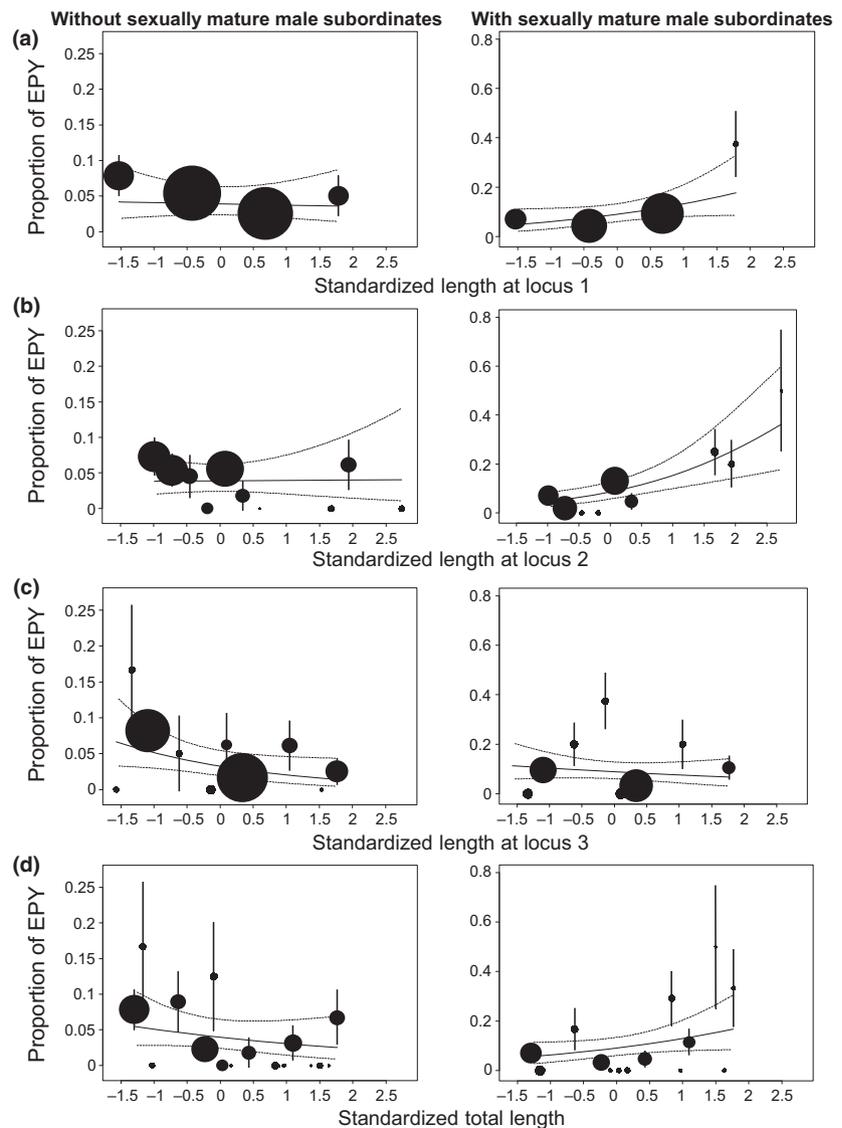


Fig. 1 Effect of the *Avpr-1a* microsatellite loci 1 (a), 2 (b), 3 (c) and total (d) lengths on the proportion of EPY, without (left side) or with (right side) sexually mature male subordinates in the family. Dots represent the mean proportion of EPY by standardized locus lengths. Dot size is proportional to the number of pups. Standard errors around each means are represented by the black bars. Black lines represent model's predictions, and associated standard errors appear in dashed.

In species where social mate choice is highly constrained, female intrinsic genetic characteristics can drive extra-pair behaviour but only when extrinsic constraints are relaxed. In the Alpine marmots, female social mate choice is strongly constrained (Lardy *et al.*, 2010) and we only found an impact of the females' genetic makeup on the proportion of EPY when sexually mature males were present in the family groups. In this species, abiotic and biotic factors interact to strongly constrain extra-pair mating opportunities (Bichet *et al.*, 2016). The annual proportion of extra-pair litters increases directly with earlier springs and indirectly with increased snow in winters. Earlier spring snowmelt is expected to relax energetic, movement and time constraints linked to mate searching. Snowier winters result in a higher proportion of families with sexually mature male subordinates (Bichet *et al.*, 2016;

Rézouki *et al.*, 2016), and further create a social context within which extra-pair paternity is favoured (Bichet *et al.*, 2016). Indeed, the presence of sexually mature males increases the cost of reproductive monopolization for dominant males and facilitates that dominant females escape mate guarding (Cohas *et al.*, 2006; Lardy *et al.*, 2012). Lack of investigation of environmental factors constraining pair-bonding could explain the contradictory findings reported in voles. In a study conducted on male prairie voles in semi-natural conditions, Ophir *et al.* (2008) did not observe that *Avpr-1a* genotype predicts variation in the mating system of males in this species. In agreement, again in semi-natural conditions, the effect of *Avpr-1a* promoter length on monogamous behaviour was only detected in nonresident prairie vole males, not constrained in their explorative behaviour (Solomon *et al.*, 2009). On the contrary, in a natural

population of prairie voles, males with the longest *Avpr-1a* microsatellite alleles were significantly more likely to sire offspring with more than one female (Keane *et al.*, 2014). In a more recent study, Okhovat *et al.* (2015) further stress the link between the *Avpr-1a* polymorphism, spatial behaviour and mate fidelity in male voles. Under semi-natural conditions, the spatial behaviour of males that sire young with several partners differs from that of males who sire young only with a partner (Okhovat *et al.*, 2015). The former males have larger home ranges than the later males, and they more frequently encounter extra-pair females (Okhovat *et al.*, 2015). Unfaithful males have less V1aR in the retrosplenial cortex than faithful males (also found in Ophir *et al.*, 2008), and low levels of retrosplenial cortex V1aR are also associated with high intrusion rates and poor mate guarding (Okhovat *et al.*, 2015). V1aR levels in regions implicated in spatial memory and sexual fidelity were linked to *Avpr-1a* sequence variation (Okhovat *et al.*, 2015). Together, these studies illustrate the importance of taking into account extrinsic constraints to understand the genetic drivers of mating tactics in natural populations.

In our study, when sexually mature male subordinates are present, the direction of the correlation between *Avpr-1a* promoter length and monogamy was the opposite of the experimental studies on male voles and humans (Hammock & Young, 2005; Walum *et al.*, 2008), but similar to the study conducted on male voles in a natural population (Keane *et al.*, 2014). Moreover, in line with the study conducted by Ophir *et al.* (2008) on male prairie voles, we failed to observe a significant effect of *Avpr-1a* promoter lengths on mate choice behaviour. As stated by Solomon *et al.* (2009), even if there is clear evidence that the genetic makeup at the *Avpr-1a* upstream regulatory region could influence mate choice and fidelity, the expected direction of this relationship remains unclear, as well as the complexity or even the existence of such relationship among taxa (Fink *et al.*, 2006; Young & Hammock, 2007). The underlying mechanisms between the *Avpr-1a* gene, the V1aR distribution and density, and finally pair-bonding were only investigated in male voles and humans (Hammock & Young, 2005; Walum *et al.*, 2008; Okhovat *et al.*, 2015). Genetic mechanisms driving pair-bonding behaviours could be more complex than previously thought, and extrinsic factors could further obscure our understanding of these mechanisms. We strongly recommend more investigations on the mechanisms underlying pair-bonding in other species under both laboratory and natural settings.

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Data accessibility

Data are available at <https://doi.org/10.5061/dryad.h588kh6>. DNA sequence has been deposited in GenBank under the accession number MG062731.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1 Molecular and parentage analyses.

Appendix S2 Characterization of the promoter region of the *Avpr-1a* gene and identification of the polymorphic areas in a population of Alpine marmots.

Appendix S3 Minimalistic statistical models involving the effect of female *Avpr-1a* genotype on the proportion of EPY.

Data deposited at Dryad: <https://doi.org/10.5061/dryad.h588kh6>

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