Functional Ecology

British Ecological Society

Functional Ecology 2014, **28**, 954–962 doi: 10.1111/1365-2435.12231

Long-term effects of litter sex ratio on female reproduction in two iteroparous mammals

Raquel Monclús*†[1,2], Dietrich von Holst[3], Daniel T. Blumstein[1,4] and Heiko G. Rödel[2,]*

[1] *Department of Ecology & Evolutionary Biology, University of California, 621 Charles E. Young Dr. South, Los Angeles, California 90095, USA;* [2] *Laboratoire d'Ethologie Expérimentale et Comparée E.A. 4443 (LEEC), Université Paris 13, Sorbonne Paris Cité, 99 Av. J.B. Clément F-93430, Villetaneuse, France;* [3] *Department of Animal Physiology, University of Bayreuth, Universitätsstrasse 30 D-95440, Bayreuth, Germany; and* [4] *The Rocky Mountain Biological Laboratory, Box 519, Crested Butte, Colorado 81224, USA*

Summary

1. Female mammals that develop in male-biased litters show signs of masculinization because they are exposed to the testosterone produced by their male siblings in utero. The early exposure to testosterone can affect female morphology, physiology, behaviour and life-history traits.

2. Masculinization is commonly seen as the collateral effect of developing in a male-biased litter. However, when resources are limiting, masculinization might be adaptive because androgenized females could monopolize resources.

3. We investigated whether females from male-biased litters, and therefore likely to be masculinized, showed adaptive advantages under different competing scenarios. For that, we used data from two long-term field projects studying yellow-bellied marmots (*Marmota flaviventris* Audubon and Bachman, 1841) and European rabbits (*Oryctolagus cuniculus* Linnaeus, 1758). We explored whether litter sex composition affected fitness-related reproductive traits: the onset of breeding, offspring production and the probability of reproduction in a given year.

4. Our study shows long-term consequences of litter sex composition on reproduction in European rabbits and yellow-bellied marmots. Females from male-biased litters from both species delayed the onset of breeding, suggesting the maladaptive nature of masculinization. However, in European rabbits, this effect was density-dependent. Under higher-density conditions, female rabbits from male-biased litters brought forward the onset of breeding, which might increase the probability of offspring overwinter survival and therefore their fitness. These females under limiting conditions might have a higher resource holding potential.

5. We conclude that prenatal maternal effects in natural populations should be taken into account when studying life-history variation. Moreover, it is notable that small variations in naturally occurring prenatal exposure to androgens have profound impacts on individuals, social groups and the population structure.

Key-words: density dependence, European rabbit, litter size, *Marmota flaviventris*, masculinization, *Oryctolagus cuniculus*, yellow-bellied marmot

Introduction

Events that occur during early development can have long-term consequences on an animal's fitness, and maternal effects may be particularly important (Stearns 1992; Lindström 1999). Mothers can modify an individual's early environment through genetic and non-genetic effects (Mousseau & Fox 1998). For instance, mothers might attempt to increase their fitness by differential investment in sons and daughters, resulting in litters that differ from the 1 : 1 sex ratio expected by chance (Trivers & Willard 1973; Silk & Brown 2008). Apart from the obvious direct effects to the unrepresented sex, indirect effects are apparent as well. It is long known that when sex ratio is biased to sons, female siblings become androgenized (vom Saal & Bronson 1978; Ryan & Vandenbergh 2002). This

---

*Correspondence author. E-mails: raquel.monclus@uam.es or heiko.rodel@leec.univ-paris13.fr

†Present address. Departamento de Biología, Universidad Autónoma de Madrid, 28049 Madrid, Spain.

androgenization is an inevitable consequence of male foetuses producing testosterone (vom Saal 1989), and this exogenous testosterone has organizational effects on female development. Thus, females from male-biased litters show morphological, physiological and behavioural signs of masculinization (Cantoni, Glaizot & Brown 1999; Ryan & Vandenbergh 2002; Monclús, Cook & Blumstein 2012), with life-history consequences on different levels (Monclús & Blumstein 2012).

Whether masculinization might be adaptive or maladaptive remains an open question (Uller *et al.* 2004). To date, most of the studies have been carried out in the laboratory, and the results strongly support the maladaptive point of view. In short, androgens modify the hypothalamic–pituitary–gonadal (HPG) axis function (vom Saal 1989), resulting in masculinized females being more aggressive and less receptive to males, having later puberty onset and longer oestrous cycles, as well as being less preferred by males (reviewed in Ryan & Vandenbergh 2002). Moreover, they enter senescence at an earlier age (vom Saal 1989). Together, this results in lower fecundity and fertility, and thus, in lower lifetime reproductive success (Zehr, Gans & McClintock 2001; Uller *et al.* 2004). Nevertheless, some studies suggest that under high population density, when resources are limited, masculinization might be adaptive and favour resource holding potential, because masculinized females are more aggressive and might be better at monopolizing resources (Zielinski & Vandenbergh 1991). However, empirical evidence of this hypothesis is scarce, and thus, we conducted a study based on two long-term data sets to test for the effects of litter sex ratio on reproduction under different population densities. For that, we focused on those reproductive parameters that might have strong fitness consequences, such as the probability of reproduction, the onset of breeding and the number of offspring produced. We followed a comparative approach using two different study species: European rabbits (*Oryctolagus cuniculus*) and yellow-bellied marmots (*Marmota flaviventris*). Both are social, group-living species that have been studied for long periods of time in temperate climates; marmots for 51 years in Colorado (Ozgul *et al.* 2010; Armitage 2012; Blumstein 2013), and European rabbits for 25 years in a field enclosure in Germany (von Holst *et al.* 2002; Rödel, von Holst & Kraus 2009b). These long-term data provide a natural experimental set-up because population density has varied over time. Both species are polytocous with known litter sex ratio effects (Bánszegi, Altbäcker & Bilkó 2009; Monclús & Blumstein 2012). They form matrilineal societies, where breeding burrows (or favourable sites for building a breeding burrow) constitute limiting resources, in particular under high-density conditions, and at the beginning of the breeding season females compete for access to them (Myers & Poole 1961; Johns & Armitage 1979; von Holst *et al.* 1999). Additionally, for both species, the onset of breeding has important lifetime fitness consequences. Individuals born earlier in the season have a higher probability to survive until maturity and show a higher lifetime reproductive success (Schwartz & Armitage 2005; Rödel, von Holst & Kraus 2009b). However, yellow-bellied marmots and European rabbits differ in several life-history traits. Marmots are obligate hibernators with a short active season and start reproducing as 2 year olds. They have one breeding attempt in any given year and most do not reproduce every year (Armitage 2003b). On the other hand, rabbits in our study site attained sexual maturity after their first winter and could have several reproductive events in any reproductive season (von Holst *et al.* 2002). We expected to find differences between both species as annual reproductive requirements and competition for resources might differ.

We used the proportion of male siblings in the litter as a proxy of masculinization, as it has been shown before that females from male-biased litters are likely to become masculinized (Cantoni, Glaizot & Brown 1999; Monclús & Blumstein 2012). We expected to find differences in the reproductive performance of females coming from female or male-biased litters. Indeed, it has been reported that females from male-biased litters were less likely to reproduce (Ryan & Vandenbergh 2002), might delay the onset of breeding (vom Saal & Bronson 1978) and/or have smaller litter sizes (Kinsley *et al.* 1986). Moreover, we expected to find that litter sex composition under different density scenarios might represent an advantage or a disadvantage. In particular, under high-density conditions, if masculinized females monopolized resources, they should have an earlier onset of breeding, larger litters and, in the case of the rabbits, more reproductive events throughout the season. On the other hand, under low-density conditions, and thus, relaxed resource competition, females from male-biased litters might be in disadvantage. Therefore, these females might be less likely to reproduce as they are less receptive to males, or they might delay the onset of breeding, or as other studies support, they might produce less offspring (vom Saal & Bronson 1980; Bánszegi *et al.* 2012). We predicted that such a result might be more pronounced in rabbits than in marmots, because rabbits were studied in a field enclosure where population density was eventually high, and thus, competition among females could be higher.

Materials and methods

YELLOW-BELLIED MARMOTS

Study animals and study site

Marmots were studied in and around the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA, from 2001 to 2011, as part of a long-term project (Armitage 2012). Individually marked animals from 10 different colonies have been regularly followed since 1962. The social and family relationships were known as well as the fate of the marmots from weaning to death or dispersal (whatever comes first). The different study colonies were located along an altitudinal gradient, and we could divide them in two distinct classes: up- and down-valley colonies (five colonies each), which differed up to 165 m in elevation (Van Vuren & Armitage 1991).

Monitoring reproduction

Marmots were regularly trapped in 2-week intervals during the summer growing season. Trapped animals were weighed and their reproductive status was checked. Female marmots show clear signs of gestation and lactation, apparent by the size and state of the nipples (Armitage 2003a). As we could not access the breeding burrows, successful reproduction was assessed by the emergence of weaned pups above-ground. The day of parturition could then be calculated by subtracting the 25 days of the weaning period (Armitage 1981). We assumed that birth litter composition did not differ from weaned litter composition, which is mostly the case in other small mammals, as pup mortality most frequently affects the whole litter (Millar, Havelka & Sharma 2004; Rödel *et al.* 2009a). We observed the colonies on a daily basis to determine the exact date of emergence of the pups. All the pups were live-trapped in Tomahawk traps, sexed and weighed, and we plucked some hair from the back for later DNA parentage assignment (for a detailed description of the methods see Blumstein *et al.* 2010).

Animals from colonies located up-valley and down-valley differed markedly in their emergence from hibernation. Animals from down-valley colonies emerged on average 15 days earlier, started reproducing earlier, and the vegetation period down-valley was longer (Van Vuren & Armitage 1991). Therefore, for each location (up- or down-valley), we calculated the individual onset of breeding relative to the date the first pups emerged in any given year. To control for further purported differences between these two locations, we included a fixed factor with two levels (up- and down-valley) into our statistical models. This factor, however, never reached statistical significance ($P > 0{\cdot}10$) and thus was removed from the final models.

Note that female yellow-bellied marmots do not produce more than one litter per season, whereas rabbits of the study population produce up to 7 litters per season (von Holst *et al.* 2002).

Population and group densities

We differentiated between colony and social group membership. A social group was defined as the number of females living in a colony site that socially interacted among them and that overlapped in space use. In addition, the total female population of the colony site was considered. In some instances, both densities coincided. Social group density and colony density were assessed through direct observation of the social interactions of marmot females, which were all individually known. For that, all the colonies were regularly observed in the mornings (7–9 AM) and in the afternoons (4–6 PM) with the help of spotting scopes. During the study period, the number of adult females per colony ranged between 1 and 21, and there were 1–14 adult females in each social group. See below for more information.

EUROPEAN RABBITS

Study animals and study site

Rabbits were studied in a field enclosure of two hectares in size, situated at the campus of the University of Bayreuth (Franconia, Germany). The animals were descendants of rabbits caught in the wild in South Germany (for details see von Holst *et al.* 2002). Data presented here were collected from 1991 to 2007 as part of a long-term project, which had started in 1985 (von Holst *et al.* 2002; Rödel, von Holst & Kraus 2009b). The number of animals at the onset of the breeding season ranged between 13·5 and 44·0 (on average: 25·1 ± 2·2 SE) adults per hectare. According to field data, these densities are high but within the range of wild rabbit population densities (Palomares 2001; Petrovan *et al.* 2011).

Vegetation in the enclosures consisted grassland with interspersed bushes and trees. This was the animals' only food source during the vegetation period. Additional food (hay and fodder beets) was only provided during the winter season when the access to the ground vegetation was hampered by ice and snow cover. The enclosure contained 16 artificial burrow systems made out of concrete with interconnected chambers and removable tops. However, the rabbits were not prevented from digging their own burrows (around 40–50 per year). Similar to the natural burrow systems, the nests in the artificial burrows were around 20–50 cm below-ground. The whole study site could be observed from two towers, and all animals could be identified by their individual ear tags.

Monitoring reproduction

Every morning during the breeding season (starting between mid-February and mid-April, Rödel, Hudson & von Holst 2008a), we checked for new born litters. To do this, all natural warrens and breeding burrows dug by the animals were prepared with artificial vertical openings to the nest chambers, which we covered with concrete flagstones. By checking the nests daily, we could record the birth of all litters within 24 h and considered this post-natal day 1.

The animals were trapped once a month. For this, peanut-baited live traps made of wood were used (own construction: 90 × 30 × 30 cm). The traps were set overnight and checked at dawn the next morning. They were then set again and left open until noon and checked every 1–2 h. Between trapping sessions, the traps were left open and were frequently used by the rabbits as overnight resting sites. Animals were removed from the traps and placed singly in gunny sacks. The abdominal fur of the adult females was dyed with different colours (silk colour; Marabu, Germany), and 1–2 h later, the animals were returned to the enclosure.

Because female rabbits pluck out abdominal hair to build their nests (González-Mariscal & Rosenblatt 1996), we were able to determine the mother of each litter by the location of the nest in combination with the colour of the hair found in it. Identity of the mothers was additionally confirmed by the analysis of females' reproductive status during the regular trapping sessions (detection of pregnancies by abdominal palpation) and by behavioural observations, such as breeding burrow use, post-partum copulations and nest defence against other females (Rödel *et al.* 2008b).

Population and group densities

The composition of all social groups of the study population was determined by regular direct observations. Observations were performed during 2–4 h before dusk when the rabbits show a peak in their social activity (Wallage-Drees 1989). Every adult female was observed for 3–8 h per month, and all social interactions were recorded. The fate of each female in our enclosure was known enabling us to calculate group and population densities during the early breeding season (mid-March). During the study period, the total number of adult females of the 2-ha population ranged between 16 and 47 individuals, and there were one to nine adult females per group. See below for more information.

DATA ANALYSIS

Model structure

For both species, we tested the effects of litter sex ratio on reproduction under higher- and lower-density situations (relative to the average densities) by applying multivariate generalized linear mixed-effects models generalized linear mixed model (GLMM). Analyses were performed using the package LME4 of the program R, version 2.15.0 (R Development Core Team 2012).

We calculated separate models using different reproductive parameters as response variables. For both species, we used the *individual onset of breeding* (=day of parturition) per year, calculated as the delay in days after the first female of the annual population gave birth. In marmots, we used the *litter size*, as this species only produces one litter per season. In rabbits, we analysed the *number of litters* as well as the *number of offspring per season* as response variables. These analyses were performed using GLMM for Poisson error distribution with a log link. In rabbits, we did not calculate the average litter size per season, because the size of the litters produced by individual females strongly changes along the season, and thus, the average litter size will be biased by seasonal effects (Eccard & Rödel 2011). In marmots, we analysed the *probability that a female reproduced* during a given year, using GLMM for binomial error distribution with a logit link. This variable was not used in rabbits, because almost all females of the population were reproductively active (96·3%, Rödel, von Holst & Kraus 2009b).

Our models included four predictor variables: litter sex ratio, female density, the females' reproductive year (i.e. first, secondly and thirdly potentially active reproductive season) and their original litter size. Whereas litter sex ratio is known to affect female's phenotype in a continuous way (Monclús & Blumstein 2012), we transformed it into a factor with three levels [<0·25, from 0·25 (excluding it) to 0·5 and >0·5] to reduce complexity and to reach model convergence while maintaining a high variability in litter sex ratio. We tested for all two-way and three-way interactions among these different predictors. These interactions were removed in a reverse stepwise procedure when they were non-significant and the models were re-calculated. *Litter sex ratio* was calculated as the proportion of males within the females' original litters. This predictor variable showed a right-skewed distribution, and thus, we transformed it into a factor with three interval levels (see Figs 1 and 2) to homogenize variances of the GLMM for Poisson models. *The density of females* was used as a proxy of the females' competitive environment, which might be influenced by the number of female competitors within the social group but also by the overall density of the colony or population (e.g. Rödel *et al.* 2004b). For this, we calculated a new variable '*Female density*' using the first component of a PCA based on measurements of adult female colony density and group density in yellow-bellied marmots and of adult female population density and group density in European rabbits. The resulting first components, after transformation into factors with two levels by median cut ('high' and 'low' density for marmots and rabbits, respectively), still captured 65·5% of the variation in the two original density variables in marmots and 46·2% of the variation in rabbits. We included the *female (reproductive) age* as a factor with up to three levels (note that not all of the females reproduced or survived during these 3 years) to test for differences during a considerable part of most of the females' reproductive life. In addition, we included the *females' litter size of origin* as a covariate to correct for potential biases, because the litter sex ratio might at least partly depend on the total litter size. We found significant, although marginal correlations between original litter size and litter sex ratio in yellow-bellied marmots (R^2 = 0·046, F = 8·67, P = 0·004) and in European rabbits (R^2 = 0·027, F = 6·90, P = 0·009), indicating negligible collinearities between these two covariates.

All GLMMs included the year of birth, the identity of the mother, litter identity and social group identity as random factors to correct for the same origin of different clusters of females. In addition, we used female identity as a random factor to allow for repeated measurements in cases where models included more than one female age class.

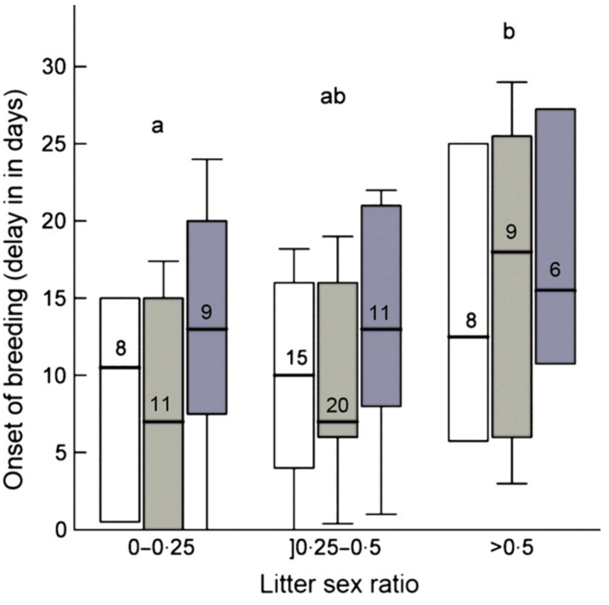

Fig. 1. Litter sex ratio and onset of breeding in yellow-bellied marmots of different age classes (white boxes: 2 year old; light grey boxes: 3 year old; dark grey boxes: 4 year old). Females with a higher proportion of male siblings in their litter of origin showed a consistently later onset of breeding. Different letters indicate significant *post hoc* comparisons after Bonferroni correction; sample sizes are given inside the boxes. Note that litter sex ratios of 0·25 are included in the first interval of the *x*-axis and excluded from the second one, denoted by 0·25. Medians with 25th/75th percentiles (box) and 10th/90th percentiles (whiskers) are shown. Note that female yellow-bellied marmots reach reproductive maturity as 2 year olds.

Whenever GLMM for Poisson error distribution showed indications of over-dispersion, we included case-level random effects (Browne *et al.* 2005). We also checked for homogeneity of variances and goodness-of-fit by plotting residuals vs fitted values. *P*-values were calculated by likelihood-ratio tests based on changes in deviance when each term was dropped from the full model (Faraway 2006).

Sample sizes

Yellow-bellied marmots. We used 182 measurements on reproduction from 78 adult females born during nine different years, stemming from 38 different mothers and 53 different litters. Animals from 10 colonies were used, and there were one to three different social groups per colony.

European rabbits. We used 246 measurements on reproduction from 125 adult females born during 10 different years. These females stemmed from 65 different mothers and 101 different litters. Every year, there were 7–12 different social groups within the population.

We analysed up to three subsequent reproductive seasons per individual female. In yellow-bellied marmots, this sequence started with the third year of life when females of our study population started to reproduce (Armitage 2003a). In European rabbits, it started with the second year of life, when female rabbits of our study population had their first litters (von Holst *et al.* 2002).

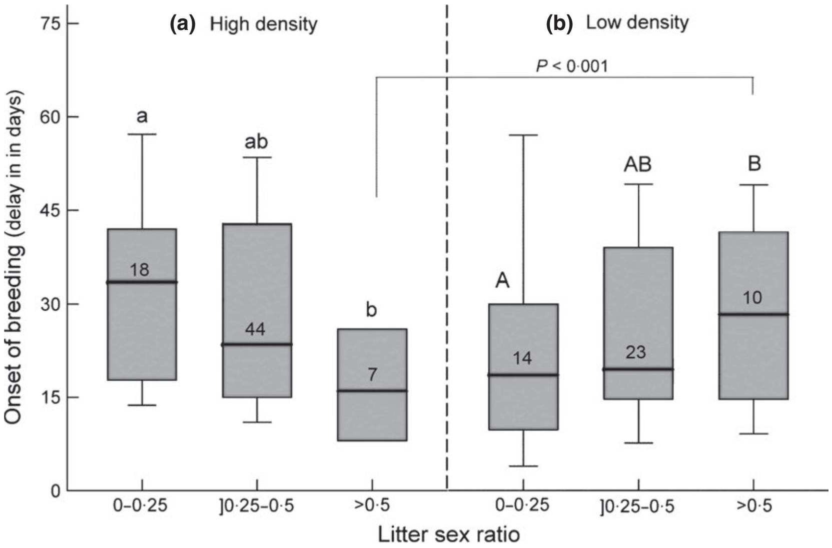

Fig. 2. Litter sex ratio and onset of breeding in 1-year-old European rabbits at (a) higher and (b) lower female density conditions. Females with a higher proportion of male siblings in their litter of origin delayed the onset of breeding when at low-density situations, whereas they brought forward the onset of breeding at high-density situations. Different letters indicate significant *post hoc* comparisons after Bonferroni correction within (a) and (b); sample sizes are given inside the boxes. Note that litter sex ratios of 0·25 are included in the first interval of the *x*-axis and excluded from the second one, denoted by 0·25. Medians with 25th/75th percentiles (box) and 10th/90th percentiles (whiskers) are shown.

Results

YELLOW-BELLIED MARMOTS

Onset of breeding

Litter sex ratio affected the onset of breeding in female yellow-bellied marmots ($\chi^2_2 = 9{\cdot}02$, $P = 0{\cdot}011$). Such effects occurred consistently during the first three reproductive years, as evident by the non-significant interaction between female age × litter sex ratio ($\chi^2_4 = 0{\cdot}97$, $P = 0{\cdot}91$). *Post hoc* analyses revealed a significantly later onset of reproduction in females from more male-biased litters compared with females from litters with a low proportion of brothers (Fig. 1). There were no further significant interactions between litter sex ratio, female age, female density and the females' original litter size ($P > 0{\cdot}10$).

Female age also affected the onset of breeding ($\chi^2_2 = 8{\cdot}64$, $P = 0{\cdot}013$), and *post hoc* comparisons indicated a significantly earlier onset in 2-year-old than in 4-year-old females ($\chi^2_1 = 16{\cdot}87$, $P < 0{\cdot}001$, see Fig. 1). Furthermore, female marmots in low-density environments started to reproduce slightly earlier, on average by 2 days, than females experiencing higher densities ($\chi^2_1 = 4{\cdot}81$, $P = 0{\cdot}028$).

Probability of seasonal reproduction and litter size

Female litter sex ratio, the original litter size and the density of females had no effect (including two-way or three-way interactions) on the probability of reproduction or on the size of the litters they gave birth to (all $P > 0{\cdot}10$). However, the probability of reproduction differed significantly among female marmots of different age classes ($\chi^2_2 = 14{\cdot}87$, $P < 0{\cdot}001$). A significantly lower proportion of 2-year-old females (51·3%) reproduced (Bonferroni-corrected *post hoc* tests: $P < 0{\cdot}01$) compared with 3 year old (75·4%) and 4 year olds (78·0%). The latter two did not differ significantly ($P > 0{\cdot}10$). There were no female age effects with respect to litter size ($P > 0{\cdot}10$).

EUROPEAN RABBITS

Onset of breeding

In European rabbits, the significant three-way interaction (litter sex ratio × female density × female age class: $\chi^2_4 = 25{\cdot}95$, $P < 0{\cdot}001$) indicated that the effects of litter sex ratio on the onset of breeding were modulated by female density, although not consistently across the three tested consecutive breeding seasons (i.e. age classes). Further analyses within age classes showed a significant interaction between litter sex ratio and female density during the first breeding season ($\chi^2_2 = 10{\cdot}86$, $P = 0{\cdot}004$). *Post hoc* analyses revealed that females coming from male-biased litters started reproducing later when female density was low but earlier when density was high (*post hoc* comparisons in Fig. 2a,b). There were no significant differences between females originating from litters with different sex ratio during the second or third breeding season ($P > 0{\cdot}10$).

Moreover, female age significantly explained the onset of breeding: as females became older, they started reproducing earlier in the season ($\chi^2_2 = 149{\cdot}47$, $P < 0{\cdot}001$, all *post hoc* comparisons after Bonferroni correction: $P < 0{\cdot}010$). One-year-old females started to reproduce the latest, and on average 28·0 (±1·4 SE) days after the onsets of breeding of the first female of the annual population, 2-year-old females showed an average delay of 18·6 (±1·8 SE) days and 3-year-old females a delay of only 11·8 (±2·2 SE) days.

Number of litters and number of offspring per season

The maternal litter sex ratio had no significant effect on the number of litters or on the number of offspring produced during the different seasons ($P > 0{\cdot}10$). All two-way or three-way interactions with litter size, female density and female age were not significant ($P > 0{\cdot}10$).

However, the average number of offspring born to female rabbits during high-density situations (10·0 ± 0·5 SE offspring/season) was significantly lower than during years of low densities (14·0 ± 0·5 SE offspring/season; $\chi^2_1 = 13{\cdot}20$, $P < 0{\cdot}001$). In addition, the number of offspring was significantly different among females of different age classes ($\chi^2_2 = 10{\cdot}68$, $P = 0{\cdot}005$), with 1-year-old females giving birth to significantly fewer offspring (10·5 ± 0·5 SE; both *post hoc* tests: $P < 0{\cdot}001$) than 2-year-old (13·4 ± 0·5 SE) and 3-year-old females (14·1 ± 0·5 SE). Two- and three-year-old females did not differ significantly in their reproductive performance ($P > 0{\cdot}10$). There were no such effects of maternal age and of density on the number of litters per season ($P > 0{\cdot}10$).

Discussion

We have shown, in two different free-living species, that prenatal effects had direct and long-lasting fitness consequences on offspring, because litter sex composition affected the onset of breeding in marmots and in rabbits. This reproductive component is a strong predictor of juvenile overwinter survival in many mammals (Clutton-Brock *et al*. 1987; Gaillard *et al*. 2000; Neuhaus 2000; European rabbits: Rödel *et al*. 2004a; yellow-bellied marmots: Armitage 2012). The probability of overwinter survival, especially for young animals, strongly depends on the energetic reserves stored during the previous vegetation period, which directly depends on the length of time to fatten or to grow (Rödel *et al*. 2004a; Armitage 2012). Additionally, it has been shown in European rabbits that females born earlier in the season have higher lifetime reproductive success, independently of the effects on their own survival probability to maturity (Rödel, von Holst & Kraus 2009b). To the best of our knowledge, this is the first study under natural conditions showing that litter sex composition is associated with later reproduction and therefore fitness.

EFFECTS OF LITTER SEX COMPOSITION

The effect of litter sex ratio on the onset of breeding differed between both species studied. In marmots, females from male-biased litters reproduced later in the season than same-age females from non-masculinized litters, and this effect was still apparent 4 years after birth. In rabbits, masculinized females delayed the onset of breeding as well, but only under lower female population density. However, when the density of other adult females in the population was high, masculinized 1-year-old females reproduced sooner. There might be different proximal explanations as to why females from male-biased litters reproduce sooner. For instance, it might be driven by the comparatively higher levels of testosterone in masculinized females (vom Saal & Bronson 1980), which are also known to show higher levels of aggression (Kinsley *et al*. 1986; Zielinski & Vandenbergh 1991). More aggressive females might be able to acquire and defend limited resources more efficiently than less aggressive ones. Indeed, in alpine marmots (*Marmota marmota*), females from male-biased litters were more likely to become the dominant female of the group through agonistic behaviour (Hackländer & Arnold 2012). Apart from the potential benefits of holding more resources, masculinization in these species might also serve to prevent infanticide, because masculinized females are also more aggressive towards intruders during lactation (Kinsley *et al*. 1986).

An important difference between both studied species is the probability of reproduction in a given season. Sexually mature European rabbits produce young nearly every breeding season throughout their lives (Rödel *et al*. 2004b; Tablado, Revilla & Palomares 2009). In contrast, female marmots reach maturity as 2 year olds but many of them do not reproduce, and once marmots start reproducing, most do not produce young every year (Armitage 2012). Despite the litter sex ratio being associated with the onset of breeding, the probability of reproduction in a given year was independent of litter composition for both marmots and rabbits. Taken together, our results provide further evidence that masculinization has no effect on the very basic physiology of reproduction (vom Saal & Bronson 1978, 1980), or in other words, females from male-biased litters are physiologically able to reproduce. However, behavioural mechanisms, such as higher aggression rates or lower receptivity to males in masculinized females, might explain our results (Ryan & Vandenbergh 2002).

Apart from litter sex ratio, offspring phenotype might be affected by other early developmental events. For instance, females coming from male-biased litters might suffer from enhanced resource competition, which might be translated into lower female body condition. However, a similar outcome might result from bigger litter sizes and we did not find any effect of litter size on future reproductive parameters. Another factor that might explain our results could be food availability during the pre- and perinatal stages. Although we could not formally check for this effect, for both our marmot and rabbit populations, the study years were quite stable and we did not record major environmental events, such as droughts. Thus, to the best of our knowledge, there was not major annual variation in food availability during the breeding seasons in both study systems. Pollution might be another source of variation. Endocrine disrupting compounds released into the environment via pesticides, wastes of paper mills or cattle farms, among others, are widespread in natural environments, and the results of different studies show compelling evidence of the long-term effects triggering phenotypic variation (Gray *et al.* 2002; Jašarević *et al.* 2013). Whereas we do not think that might have affected our results, given the location and the stability of the study areas, it is a factor worth taking into consideration when studying wild populations.

DENSITY AND AGE EFFECTS

In addition to the effects of litter sex ratio, other factors affected the reproduction of the females. Rabbit breeding was density-dependent with fewer offspring produced by the females under high-density conditions, as it has been described in previous studies (Myers & Poole 1962; Rödel *et al.* 2004b; Baayen, Davidson & Bates 2008). This might be mainly due to suppressive effects of social stress on female reproductive functions (von Holst 1998).

Moreover, reproduction in both species was age dependent with young females showing a lower performance than older ones. This was exemplified by the comparatively lower probability of reproduction in 2-year-old marmots and by the comparatively later onset of breeding and lower number of litters per season in 1-year-old rabbits (cf. Rödel *et al.* 2004b). A lower performance in young, first time breeders is a common phenomenon in many species of mammals and birds (Clutton-Brock 1988; Forslund & Pärt 1995). Apart from outright suppression, as it has been described in marmots (Armitage 2003c), older females might directly interfere with the ability of younger females to rear young. Indeed, other studies have showed how more dominant species, or dominant individuals within the species, are capable of directly interfering in the fitness of an animal (Eccard & Ylönen 2002). Reproductive interference is consistent with the finding that young females delayed the onset of breeding when the density of other adult females in the colony was high. Direct interference in the reproduction is a more subtle strategy than suppression with a potentially similar outcome, a detriment of younger females' fitness (Rödel, von Holst & Kraus 2009b). Further explanations for the lower reproductive performance are that younger females frequently are in a lower body condition and usually occupy lower social ranks (Rödel *et al.* 2004b; Dearborn, Anders & Juola 2008).

IS MASCULINIZATION ADAPTIVE?

Masculinization seemed adaptive for rabbits under high-density conditions, whereas for marmots, it did not constitute any obvious advantage, independently of female density. One potential explanation is that in our study area, marmots were not resource limited. Indeed, higher-density marmot colonies were in larger forest clearings that had more potential food and burrows (Armitage & Schwartz 2000). Thus, monopolizing resources, which is a costly strategy, might not be advantageous. On the other hand, rabbits were studied in a field enclosure. Although there was hardly any human interference and rabbits were subjected to natural predators, densities were eventually high, although within the range of wild populations (Palomares 2001; Petrovan *et al.* 2011), and therefore, resources (such as favourable sites for breeding) might have become limiting at some point.

An important difference between both species is that marmots are obligate hibernators with a short active season and a single annual reproductive event. On the other hand, rabbits have a much greater reproductive potential, which increases the need for establishing boundaries among the group members. Thus, monopolization of resources might constitute an advantageous tactic to ensure successful reproduction throughout the successive breeding attempts.

One might wonder why masculinization persists even though in many species seems maladaptive (Avdi & Driancourt 1997; Ryan & Vandenbergh 2002; Lummaa, Pettay & Russell 2007; Bánszegi, Altbäcker & Bilkó 2009). In some cases, male-biased litters arise from maternal investment in sons, and thus, it might be adaptive for the mother, because a few masculinized females are compensated by the higher fitness payoff rendered by sons (Trivers & Willard 1973). However, when conditions are unfavourable, maternal effects might be adaptive for the daughters too, by increasing their competitive potential (Boots & Roberts 2012).

Conclusions

Density-dependent effects are tightly linked to fitness components in social mammals. Our study highlights for the first time how density modulates the adaptability of prenatal maternal effects depending on the availability of resources. These maternal effects, thus, should be studied in the natural context of the species, and they might constitute essential tools to understand variability in fitness, as well as the functioning of the social group and the population structure. Therefore, they are well worth taking into account when investigating mammalian life-history traits and strategies.

Acknowledgements

We are grateful to all the colleagues, students and field assistants who contributed to the data collection over the years. We thank the comments of two anonymous reviewers that greatly improved the manuscript. R.M. was supported by postdoctoral fellowships from the Spanish Ministerio de Innovación y Ciencia and from the Fulbright programme. D.T.B was supported by the National Geographic Society, UCLA (Faculty Senate and the Division of Life Sciences), a Rocky Mountain Biological Laboratory research fellowship and by the NSF (IDBR-0754247, DEB-1119660 to D.T.B. as well as DBI 0242960 and 0731346 to the

Rocky Mountain Biological Laboratory). Field enclosure studies on European rabbits were supported by grants from the German Research Foundation DFG (Ho 443/19-1,2,3 and Ro 2431/3-1). Permission for population biology studies in European rabbits was granted by the government of Middle Franconia (211-3894 a); marmots were studied under research protocols issued by UCLA (ARC protocol 2001-191).

References

Armitage, K.B. (1981) Sociality as a life history tactic of ground squirrels. *Oecologia*, **48**, 36–49.

Armitage, K.B. (2003a) Marmots: *Marmota monax* and allies. *Wild Mammals of North America* (eds G.A. Feldhamer, B.C. Thompson & J.A. Chapman), pp. 188–210. The John Hopkins University Press, Baltimore, Maryland, USA.

Armitage, K.B. (2003b) Recovery of a yellow-bellied marmot population following a weather-induced decline. *Adaptive Strategies and Diversity in Marmots* (eds R. Ramousse, D. Allainé & M. Le Berre), pp. 217–224. International Network on Marmots, Lyon, France.

Armitage, K.B. (2003c) Reproductive competition in yellow-bellied marmots. *Adaptive Strategies and Diversity in Marmots* (eds R. Ramousse, D. Allainé & M. Le Berre), pp. 133–142. International Network on Marmots, Lyon, France.

Armitage, K.B. (2012) Sociality, individual fitness and population dynamics of yellow-bellied marmots. *Molecular Ecology*, **21**, 532–540.

Armitage, K.B. & Schwartz, O.A. (2000) Social enhancement of fitness in yellow-bellied marmots. *Proceedings of the National Academy of Science of the United States of America*, **97**, 12149–12152.

Avdi, M. & Driancourt, M.A. (1997) Influence of sex ratio during multiple pregnancies on productive and reproductive parameters of lambs and ewes. *Reproduction Nutrition Development*, **37**, 21–27.

Baayen, R.H., Davidson, D.J. & Bates, D.M. (2008) Mixed-effects modeling with crossed random effects for subjects and items. *Journal of Memory and Language*, **59**, 390–412.

Bánszegi, O., Altbäcker, V. & Bilkó, Á. (2009) Intrauterine position influences anatomy and behavior in domestic rabbits. *Physiology and Behavior*, **98**, 258–262.

Bánszegi, O., Szenczi, P., Dombay, K., Bilkó, A. & Altbäcker, V. (2012) Anogenital distance as a predictor of attractiveness, litter size and sex ratio of rabbit does. *Physiology and Behavior*, **105**, 1126–1230.

Blumstein, D.T. (2013) Yellow-bellied marmots: insights from an emergent view of sociality. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **368**, 20120349.

Blumstein, D.T., Lea, A.J., Olson, L.E. & Martin, J. (2010) Heritability of anti-predatory traits: vigilance and locomotor performance in marmots. *Journal of Evolutionary Biology*, **23**, 879–887.

Boots, M. & Roberts, K.E. (2012) Maternal effects in disease resistance: poor maternal environment increases offspring resistance to an insect virus. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **279**, 4009–4014.

Browne, W.J., Subramanian, S.V., Jones, K. & Goldstein, H. (2005) Variance partitioning in multilevel logistic models that exhibit overdispersion. *Journal of the Royal Statistical Society. Series A, (Statistics in Society)*, **168**, 599–613.

Cantoni, D., Glaizot, O. & Brown, R.E. (1999) Effects of sex composition of the litter on anogenital distance in California mice (*Peromyscus californicus*). *Canadian Journal of Zoology*, **77**, 124–131.

Clutton-Brock, T.H. (1988) *Reproductive Success. Studies of Individual Variation in Contrasting Breeding Systems*. University of Chicago Press, Chicago, Illinois, USA.

Clutton-Brock, T.H., Major, M., Albon, S.D. & Guinness, F.E. (1987) Early development and population dynamics in red deer. I. Density-dependent effects on juvenile survival. *Journal of Animal Ecology*, **56**, 53–67.

Dearborn, D.C., Anders, A.D. & Juola, F.A. (2008) Parent age differentially influences offspring size over the course of development in Laysan albatross. *Journal of Zoology*, **276**, 14–20.

Eccard, J.A. & Rödel, H.G. (2011) Optimizing temperament through litter size in short-lived, iteroparous mammals in seasonal environments. *Developmental Psychobiology*, **53**, 585–591.

Eccard, J.A. & Ylönen, H. (2002) Direct interference or indirect exploitation? An experimental study of fitness costs of interspecific competition in voles. *Oikos*, **99**, 580–590.

Faraway, J.J. (2006) *Extending the Linear Model with R. Generalized Linear, Mixed Effects and Nonparametric Regression Models*, Chapman & Hall, New York, New York, USA.

Forslund, P. & Pärt, T. (1995) Age and reproduction in birds-hypotheses and tests. *Trends in Ecology and Evolution*, **10**, 374–378.

Gaillard, J.M., Festa-Bianchet, M., Yoccoz, N.G., Loison, A. & Toïgo, C. (2000) Temporal variation in fitness components and population dynamics of large herbivores. *Annual Review of Ecology and Systematics*, **31**, 367–393.

González-Mariscal, G. & Rosenblatt, J.S. (1996) Maternal behavior in rabbits. A historical and multidisciplinary perspective. *Advances in the Study of Behavior*, **25**, 333–360.

Gray, L.E. Jr, Lambright, C., Parks, L., Tyl, R.W., Orlando, E.F., Guillette, L.J. Jr *et al.* (2002) Emerging issues related to endocrine disrupting chemicals and environmental androgens and antiandrogens. *Endocrine Disruptors Part II* (ed. M. Metzler), pp. 209–247. Springer Verlag, Berlin.

Hackländer, K. & Arnold, W. (2012) Litter sex ratio affects lifetime reproductive success of free-living female Alpine marmots *Marmota marmota*. *Mammal Review*, **42**, 310–313.

von Holst, D. (1998) The concept of stress and its relevance for animal behavior. *Advances in the Study of Behavior*, **27**, 1–131.

von Holst, D., Hutzelmeyer, H., Kaetzke, P., Khaschei, M. & Schonheiter, R. (1999) Social rank, stress, fitness, and life expectancy in wild rabbits. *Naturwissenschaften*, **86**, 388–393.

von Holst, D., Hutzelmeyer, H., Kaetzke, P., Khaschei, M., Rödel, H.G. & Schrutka, H. (2002) Social rank, fecundity and lifetime reproductive success in wild European rabbits (*Oryctolagus cuniculus*). *Behavioral Ecology and Sociobiology*, **51**, 245–254.

Jašarević, E., Williams, S.A., Vandas, G.M., Ellersieck, M.R., Liao, C., Kannan, K. *et al.* (2013) Sex and dose-dependent effects of developmental exposure to bisphenol A on anxiety and spatial learning in deer mice (*Peromyscus maniculatus bairdii*) offspring. *Hormones and Behavior*, **63**, 180–189.

Johns, D.W. & Armitage, K.B. (1979) Behavioral ecology of alpine yellow-bellied marmots. *Behavioral Ecology and Sociobiology*, **5**, 133–157.

Kinsley, C.H., Konen, C.M., Miele, J.L., Ghiraldi, L. & Svare, B. (1986) Intrauterine position modulated maternal behaviors in female mice. *Physiology and Behavior*, **36**, 793–799.

Lindström, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology and Evolution*, **14**, 343–348.

Lummaa, V., Pettay, J.E. & Russell, A.F. (2007) Male twins reduce fitness of female co-twins in humans. *Proceedings of the National Academy of Science of the United States of America*, **104**, 10915–10920.

Millar, J.S., Havelka, M.A. & Sharma, S. (2004) Nest mortality in a population of small mammals. *Acta Theriologica*, **49**, 269–273.

Monclús, R. & Blumstein, D.T. (2012) Litter sex composition affects life-history traits in yellow-bellied marmots. *Journal of Animal Ecology*, **80**, 80–86.

Monclús, R., Cook, T. & Blumstein, D.T. (2012) Masculinized female yellow-bellied marmots initiate more social interactions. *Biology Letters*, **8**, 208–210.

Mousseau, T.A. & Fox, C.W. (1998) The adaptive significance of maternal effects. *Trends in Ecology and Evolution*, **13**, 403–407.

Myers, K. & Poole, W.E. (1961) A study of the biology of the wild rabbit, *Oryctolagus cuniculus* (L.), in confined populations. II. The effects of season and population increase on behaviour. *Wildlife Research*, **6**, 1–41.

Myers, K. & Poole, W.E. (1962) A study of the biology of the wild rabbit, *Oryctolagus cuniculus* (L.), in confined populations. III. Reproduction. *Australian Journal of Zoology*, **10**, 225–267.

Neuhaus, P. (2000) Timing of hibernation and molt in female columbian ground squirrels. *Journal of Mammalogy*, **81**, 571–577.

Ozgul, A., Childs, D.Z., Oli, M.K., Armitage, K.B., Blumstein, D.T., Olson, L.E. *et al.* (2010) Coupled dynamics of body mass and population growth in response to environmental change. *Nature*, **466**, 482–485.

Palomares, F. (2001) Comparison of 3 methods to estimate rabbit abundance in a Mediterranean environment. *Wildlife Society Bulletin*, **29**, 578–585.

Petrovan, S.O., Barrio, I.C., Ward, A.I. & Wheeler, P.M. (2011) Farming for pests? Local and landscape-scale effects of grassland management on rabbit densities. *European Journal of Wildlife Research*, **57**, 27–34.

R Development Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Rödel, H.G., Hudson, R. & von Holst, D. (2008a) Optimal litter size for individual growth of European rabbit pups depends on their thermal environment. *Oecologia*, **155**, 677–689.

Rödel, H.G., von Holst, D. & Kraus, C. (2009b) Family legacies: short- and long-term fitness consequences of early-life conditions in female European rabbits. *Journal of Animal Ecology*, **78**, 789–797.

Rödel, H.G., Bora, A., Kaetzke, P., Khaschei, M., Hutzelmeyer, H. & von Holst, D. (2004a) Over-winter survival in subadult European rabbits: weather effects, density dependence, and the impact of individual characteristics. *Oecologia*, **140**, 566–576.

Rödel, H.G., Bora, A., Kaiser, J., Kaetzke, P., Khaschei, M. & von Holst, D. (2004b) Density-dependent reproduction in the European rabbit: a consequence of individual response and age-dependent reproductive performance. *Oikos*, **104**, 529–539.

Rödel, H.G., Starkloff, A., Bautista, A., Friedrich, A.C. & von Holst, D. (2008b) Infanticide and maternal offspring defence in European rabbits under natural breeding conditions. *Ethology*, **114**, 22–31.

Rödel, H.G., Starkloff, A., Seltmann, M.W., Prager, G. & von Holst, D. (2009a) Causes and predictors of nest mortality in a European rabbit population. *Mammalian Biology*, **74**, 200–211.

Ryan, B.C. & Vandenbergh, J.G. (2002) Intrauterine position effects. *Neuroscience and Biobehavioral Reviews*, **26**, 665–678.

vom Saal, F.S. (1989) Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses *in utero*. *Journal of Animal Science*, **67**, 1824–1840.

vom Saal, F.S. & Bronson, F.H. (1978) *In utero* proximity of female mouse fetuses to males: effect on reproductive performance during later life. *Biology of Reproduction*, **19**, 842–853.

vom Saal, F.S. & Bronson, F.H. (1980) Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science*, **208**, 597–599.

Schwartz, O.A. & Armitage, K.B. (2005) Weather influences on demography of the yellow-bellied marmot (*Marmota flaviventris*). *Journal of Zoology*, **265**, 73–79.

Silk, J.B. & Brown, G.R. (2008) Local resource competition and local resource enhancement shape primate birth sex ratios. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **275**, 1761–1765.

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford, UK.

Tablado, Z., Revilla, E. & Palomares, F. (2009) Breeding like rabbits: global patterns of variability and determinants of European wild rabbit reproduction. *Ecography*, **32**, 310–320.

Trivers, R.L. & Willard, D.E. (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science*, **179**, 90–92.

Uller, T., Massot, M., Richard, M., Lecomte, J. & Clobert, J. (2004) Long-lasting fitness consequences of prenatal sex ratio in a viviparous lizard. *Evolution*, **58**, 2511–2516.

Van Vuren, D. & Armitage, K.B. (1991) Duration of snow cover and its influence on life-history variation in yellow-bellied marmots. *Canadian Journal of Zoology*, **69**, 1755–1758.

Wallage-Drees, J.M. (1989) A field study of seasonal changes in circadian activity of rabbits. *Zeitschrift für Säugetierkunde*, **54**, 22–30.

Zehr, J.L., Gans, S.E. & McClintock, M.K. (2001) Variation in reproductive traits is associated with short anogenital distance in female rats. *Developmental Psychobiology*, **38**, 229–238.

Zielinski, W.J. & Vandenbergh, J.G. (1991) Increased survivorship of testosterone-treated female house mice, *Mus musculus*, in high-density field conditions. *Animal Behaviour*, **42**, 955–967.

Received 22 August 2013; accepted 27 November 2013
Handling Editor: Jonathan Blount