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Article type : Research Papers

Early life of fathers affects offspring fitness in a wild rodent

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Intergenerational fitness effects on offspring due to the early life of the parent are well studied from the standpoint of the maternal environment, but intergenerational effects owing to the paternal early life environment are often overlooked. Nonetheless, recent laboratory studies in mammals and ecologically relevant studies in invertebrates predict that paternal effects can have a major impact on the offspring's phenotype. These non-genetic, environment-dependent paternal effects provide a mechanism for fathers to transmit environmental information to their offspring, and could allow rapid adaptation. We used the bank vole *Myodes glareolus*, a wild rodent species with no paternal care, to test the hypothesis that a high population density environment in the early life of fathers can affect traits associated with offspring fitness. We show that the protein content in the diet and/or social environment experienced during the father's early life (prenatal and weaning) influence the phenotype and survival of his offspring and may indicate adaptation to density-dependent costs. Furthermore, we show that experiencing multiple environmental factors during the paternal early life can lead to a different outcome on the offspring phenotype than stimulated by experience of a single environmental factor, highlighting the need to study developmental experiences in tandem rather than independent of each other.

KEYWORDS: Paternal effect, fitness, early life environment, intergenerational effects, adaptation, protein restricted diet, winter survival, population density, Myodes glareolus, social confrontation

Introduction

An individuals' phenotype is a complex interaction between its genotype and the environment (Paaby & Testa, 2018). In particular, the early life environment of an individual can have a profound and lasting impact on the adult phenotype (Burton & Metcalfe, 2014) highlighting the important role of environmental experience during development. Moreover, the early life environment experienced by one generation can continue to exert phenotypic effects in the subsequent generation through parental effects, even in the absence of exposure to further stimuli (Burton & Metcalfe, 2014; Soubry *et al.*, 2014). Such intergenerational environmental effects, *i.e.* when an early life environment exhibited on one generation has some effect on a subsequent generation (Emanuel, 1986), are well-documented in several taxa, such as fish (Shama & Wegner, 2014), birds (Naguib & Gil, 2005), rodents (Drake & Walker, 2004; Skinner *et al.*, 2013; Van Cann *et al.*, 2019) and humans (Pembrey *et al.*, 2006, 2014). There is clear evidence of lasting, intergenerational phenotypic impacts derived from, for example, the nutritional environment (Drake & Walker, 2004; Harrison & Langley-Evans, 2009), disease burden (Drake & Liu, 2010; Denham, 2018), social environment (Franklin *et al.*, 2010) and exposure to pollutants (Soubry *et al.*, 2014). Intergenerational environmental effects can have important evolutionary consequences, as the contemporary environment may not be the only relevant influence on the phenotype. It is therefore essential to understand whether environmental effects can persist across generations to have a lasting impact on fitness traits and thus the action of selection (Bossdorf *et al.*, 2008; Burton & Metcalfe, 2014).

An important issue is whether both sexes are capable of transmitting environmental effects to offspring. In placental mammals, there is a typical gender bias in the level of parental investment into the early life experience of their offspring: an inevitably high level of

maternal investment into offspring development presents a marked contrast with typically low paternal effort into offspring development, as paternal care is absent in 90-95% of mammalian species (Woodroffe & Vincent, 1994). Mammalian offspring have ample opportunity to receive information about their environment through maternal effects during prenatal (*e.g*. nutrition provided by ova and during intra-uterine development (Wu *et al.*, 2004; Abu-Saad & Fraser, 2010)) and postnatal (*e.g.* nursing and social care such as grooming (Curley *et al.*, 2012; Liu *et al.*, 2012)) development, and accordingly there is widespread evidence that the maternal environment can have intergenerational environmental effects (Mousseau & Fox, 1998; Meaney, 2001; Curley *et al.*, 2008; Wolf & Wade, 2009). However, there is growing evidence that the paternal environment, particularly the father's nutritional experience, social environment and/or exposure to toxins (Pembrey *et al.*, 2006; Soubry *et al.*, 2014), can also have a multigenerational impact through paternal effects on the offspring phenotype, even though most mammalian fathers contribute little more than spermatozoa to the production of offspring.

Understanding the evolutionary role of intergenerational paternal effects requires knowledge about the mechanism(s) by which paternal effects can be transmitted. Paternal effects can be conveyed directly via the father's germ line, *i.e.* via spermatozoa, in the form of various epigenetic marks (*e.g*. DNA methylation (Crean & Bonduriansky, 2014), histone modifications (Richards, 2006), non-coding RNAs (Rassoulzadegan *et al.*, 2006)) and/or changes in gene copy number (Aldrich & Maggert, 2015). These direct paternal effects can be due to the father´s contemporary environment; for example, obese adult male humans have specific epigenetic marks in their sperm that alter after weight loss due to a gastric bypass (Donkin *et al.*, 2016); or paternal effects can originate in the father's early life (Kaati

et al., 2007); *e.g.* caloric deprivation during the *in utero* development of F1 mouse males led to F2 offspring with increased lipid abundance (Radford *et al.*, 2014). Alternatively, paternal effects may be transmitted indirectly. For example in mice, mothers can adjust pre- and post-natal investment into their offspring based on quality of the father's nutrition prior to mating (Mashoodh *et al.*, 2018).

We determined whether paternal early life experiences could influence fitness traits (body mass and long-term survival) of their offspring through intergenerational paternal effects, using bank voles (*Myodes glareolus*), common rodents inhabiting coniferous forests in the Palearctic region, as our model system. We achieved this by exposing bank vole males to environmental factors associated with high population density during their early lives and following the development of their offspring. As is common for small rodents, bank voles are polygynous and do not display paternal care (Gromov & Osadchuk, 2013). Furthermore, female bank voles can sire litters with multiple fathers (Ratkiewicz & Borkowska, 2000) and female voles do not adjust their investment into offspring according to male quality (Oksanen *et al.*, 1999). Early life population density is relevant to bank voles as high latitude populations of microtine rodents typically experience population density cycles (Kallio *et al.*, 2009; Korpela *et al.*, 2013), where high population density phases coincide with greater intraspecific competition for resources, such as for breeding territories and for food (Huitu *et al.*, 2003; Forbes *et al.*, 2014a; b), than the low population density phases. Due to this natural demographic variation and the evidence that diet (Drake & Walker, 2004; Harrison & Langley-Evans, 2009; Radford *et al.*, 2014) and social encounters (Franklin *et al.*, 2010) elicit parental effects in rodents, we quantified the effects of two population density related

factors: (1) protein restriction (PR) and/or (2) frequent social confrontation (SC) in a full factorial design (figure 1).

Materials and methods

a) Study species

Bank vole populations in Northern Europe undergo seasonal and multi-annual population density fluctuations (Kallio *et al.*, 2009; Korpela *et al.*, 2013). The first breeding opportunity for animals in a cohort born during mid- to late-summer is usually the following spring and the population size can reduce up to 50% during winter (Prévot-Julliard *et al.*, 1999; Kallio *et al.*, 2009) indicating that winter survival for that cohort is a key fitness factor (Koskela, 1998) (see (e) F2 phenotype and winter survival). In the laboratory all individuals were kept in polyethylene cages (43x26x15 cm), except during the F1 trials (see (b) F1 reproductive success), and maintained on a 16L:8D photoperiod at 20±2°C, with wood shavings and hay provided as bedding. Water was provided *ad libitum* and standard food (Labfor 36; Lactamin AB, Stockholm, Sweden) was provided *ad libitum*, except during early life environment treatments (see (b) F1 early life environment).

b) F1 early life environment

Unrelated males and females (hereafter referred to as the F0 generation) were chosen randomly from a second-generation laboratory colony originally captured in Central Finland (62°36'59"N 26°20'45"E). F0 females were randomly assigned to four treatment groups in a

two by two factorial design (Fig. 1a): (1) a control group (PR18/SC-), (2) a protein restricted group (PR9/SC-), (3) a social confrontation group (PR18/SC+) and (4) a group receiving both protein restriction and social confrontation (PR9/SC+). F0 individuals were mated with randomly chosen partners to produce gravid F0 mothers. At the start of the breeding, F0 females receiving the PR18 treatment received a control diet (18% protein; 3.1 kCal/g; Envigo, WI, USA) that contained a protein content representative of the diet of wild bank voles (Droždž, 1968) while females from the PR9 treatment were given a restricted protein diet (9% protein; 3.2 kCal/g; Envigo, WI, USA). This diet was maintained from the pairing of females and males, up to the weaning of the offspring (at the age of 20 days). After seven days, males were removed (and hereafter not used again) and F0 females receiving the SC+ treatment started receiving social confrontation. Social confrontation consisted of confronting each female in a new, empty cage with another SC+ female every second day (Marchlewska-Koj *et al.*, 2003) for 10 minutes. New pairs of females were used every day to avoid habituation. As the setup was fully factorial, it was possible to study impacts of both factors separately and to quantify any interaction (PR9/SC+; figure 1a). The protein restricted and control diet began when the F0 males and F0 females were paired, the social confrontation treatment started when the F0 male and F0 females were separated (seven days later). Treatments lasted throughout the pregnancy and nursing period and ended when the F1 pups were 20 days old (weaning age). Body mass of the F1 individuals was measured at 30 days of age (young adults reaching their maturity) using an electronic scale. After body mass measurements, F1 females were no longer included in the experiments.

c) F1 reproductive success

To assess the reproductive success of F1 males in a competitive situation, 49 reproductive trials were set up consisting of four sexually mature (at least 30 day old, approximately all the same age) F1 males, one from each treatment (n=196), and two non-experimental, unrelated females (*i.e.* females who did not experience early life treatments and who had no prior experience of the experimental F1 males). These reproductive trials were carried out in an experimental cage system that consisted of four polyethylene cages (43x26x15 cm) that were interlinked using a PVC tube (SI figure 1) which allowed individuals to move freely between all cages. Each trial lasted nine days to ensure at least two oestrus cycles in the females and afterwards all F1 individuals were kept in separate cages. Twenty-five reproductive trials were replicated with half of the F1 males (n=100) and different, non-experimental females (once again with two females per reproductive trial). Paternity of the pups was determined using microsatellite genotypes (Mills *et al.*, 2007) (SI methods).

d) Female preference test

To investigate the attractiveness of F1 males, preference of naïve, post-partum oestrus females for F1 males was determined. Per preference trial, four F1 males were used. The four males per preference trial were the same combinations as used for the reproductive trials, *i.e.* one from each treatment. F1 males were placed in mesh-wire boxes randomly at different sides of a 60cm by 60cm open field arena (SI figure 2) and, after five minutes of habituation, one female in post-partum oestrus was placed in the middle. This female did not receive any form of experimental treatment and was not previously used for any other

experiment. The mesh-wire boxes allowed transfer of smell and sound of the F1 males, but prevented direct physical contact (and mating) with the females. The movement of the female was tracked to analyse whether she showed preference towards a specific male. Tracking was done using Noldus Ethovision XT 8.5 (Noldus *et al.*, 2001) for twenty minutes and a zone was (virtually) demarcated around each F1 male, which was considered the visitation zone (SI figure 2). Both time spent near each male and the number of visits were documented automatically by the tracking software. In total, 17 preference trials were performed (n=68), which were all replicated using the same combinations of four males (n=68) but different post-partum oestrus females (SI table 1) several days later.

e) F2 phenotype and winter survival

To study whether the paternal early life environment of F1 males affected the F2 offspring phenotype (born from the F1 reproductive success experiment), F2 body mass was recorded in the laboratory at birth and as young adults (30 days old) using an electronic scale.

To determine whether the F2 fitness traits could be affected by potential paternal effects, winter survival of 72 F2 offspring (at least 50 days old) was determined in semi-natural outdoor enclosures. Individuals were chosen equally between treatments, in an equal sex ratio and from different litters (to assure minimum relatedness). Over winter survival was measured from October to March, during which the temperature mostly remained below zero and the ground was covered with snow. Prior to release, individuals were acclimatised to outdoor temperatures and light-dark cycles by keeping them in cages placed in a semi-open outdoor hall for ten days. During those ten days, F2 diet was supplemented with

plants, flowers and mushrooms picked in the vicinity of the enclosures. After the acclimatisation, all individuals were released to nine large (40mx50m), outdoor enclosures located in Konnevesi, Finland (62°37'30"N 26°14'38"E) in an equal sex ratio (four males and four females) and equal treatment ratio (one male and one female of each treatment group). All individuals were monitored using a capture-and-release method once a month (SI methods) until March, as individuals started showing signs of fertility. In March, all enclosures were trapped exhaustively (at least three trapping with no individuals captured). Individuals not captured during any of the trapping were considered to have died during that month.

f) Statistical methods

All statistical analyses were performed using R (R Core Team, 2018). Body mass analyses (F1 and F2) were done using linear mixed models. Reproductive success measured as siring at least one pup (determined via microsatellite analysis; see SI methods) was analysed using binomial generalized mixed model (GLMM; package lme4 (Bates *et al.*, 2015)). The number of pups sired per F1 male was analysed using a zero-truncated Poisson GLMM (package glmmTMB (Brooks *et al.*, 2017)). The number of visits by a female during the male preference test and the over winter survival of F2 (measured as number of months survived) were analysed using a Poisson GLMM (package lme4 (Bates *et al.*, 2015)). The time a female spent near a male in the preference test was divided by the total time of the experiment (twenty minutes) and this ratio was analysed using a binomial GLMM (package lme4 (Bates *et al.*, 2015)). Model selection, where applicable, began with a full model that had stepwise reduction until the model with the lowest AIC was achieved, after which the model fit was

examined. For the analyses of both F1 fathers and F2 offspring, treatments (PR and SC) were always included in the final model as well as the interaction (PR*SC), regardless of the significance of the terms in the reduced model. Litter size was included as a categorical covariate (Mappes & Koskela, 2004; Schroderus *et al.*, 2012) in initial model for the analysis of the F1 and F2 body mass. For all body mass measurements, the random factors included litter ID to account for litter effects. In the male reproductive trials, male body mass was included as a covariate (Boratynski & Koteja, 2010). As certain male trials were repeated, male ID was also included as a random factor for the male preference test. In the female preference trials, where one female had to choose between four males, the analysis considered the male individual as the statistical unit but the ID for the female was included as a random factor to account for non-independence. For the winter survival analysis, the enclosure number was included as a random factor.

Three additional analyses were done to investigate whether females showed any preference towards reproductively successful males (reproductive success having been measured in the 'F1 reproductive success' experiment, see (c) F1 reproductive success). Three new models were constructed containing the same fixed and random factors as the previous female preference analysis, but with the addition of one of three measurements of male reproductive success: absolute number of pups sired per father, relative number of pups sired per father or whether a father sired at least one pup (SI tables 3-5). All statistical analyses performed in this study are reported in SI table 8.

Results

Intra-generational effects on F1 phenotype and reproductive success

a) Early life treatments affected F1 adult body mass

Different early life treatments elicit significant variation in the F1 phenotype (figure 1a). The presence of a protein restricted diet (PR9) or social confrontation (SC+) during the F1's early life (*i.e*. during gestation and nursing; see methods for detailed description) had significant negative effects on adult body mass (table 1; mean±SD mass (g) of F1: PR18 = 17.5±2.5; PR9 = 16.6±2.4; SC- = 17.5±2.6; SC+ = 16.7±2.4), but there was no significant interaction among the early life treatments. Furthermore, male F1 were significantly heavier than the females (mean±SD mass (g) females=16.2±2.1; male=17.9±2.6) but there was no significant interaction between sex and the early life treatments. Individuals born to larger litters (litter sizes did not differ between treatments: one-way ANOVA: $F_{(3,252)} = 0.214$; p= 0.887) typically weighed significantly less than individuals from smaller litters, but litter size did not have a significant interaction with the early life treatments (table 1).

b) Early life treatments did not influence F1 male reproductive success

During reproductive success trials, 41% of all F1 males (n=99) sired at least one pup and 372 F2 individuals were born in 90 litters (figure 1b; SI table 1). Multiple paternity was common with 37% of all litters sired by more than one father. None of the early life treatments had a notable effect on reproductive success (either having sired at least one pup or the total number of pups sired) of F1 males (table 1). By contrast, F1 adult body mass, which is associated with the early life treatments (see above), significantly influenced F1 male

reproductive success: heavier males had a significantly greater probability of siring at least one pup. However, this effect of body size was somewhat countered by an effect whereby lighter males had significantly more pups on the condition that the male had sired at least one pup (table 1).

c) Naïve females did not prefer males of a certain treatment

Mate choice experiments with experimentally naive females showed no clear preference (either the number of visits or the time spent near a male) for F1 males from any of the four treatment groups (table 2; figure 1b). Male body mass was not retained during model selection, indicating that these experimentally naive female bank voles did not prefer any obvious adult phenotype associated with the early life treatments (although females could identify reproductively successful males, and preferred to visit them; SI table 3-5).

Intergenerational effects of paternal early life on offspring phenotype and fitness

a) Paternal early life treatments changed offspring adult mass

At birth, F2 males were significantly heavier than F2 females, but paternal treatment did not have a significant effect (table 3). In contrast, F2 adult body mass was significantly affected by the paternal early life treatments, with the increase or decrease of body mass conditional on the treatment (table 3; figure 1c). Similar to the F1 generation, adult body mass of the F2 (figure 2a) was lower when the F1 father's early life consisted of either only protein restriction (mean±SD mass (g) PR9SC-= 16.2±2.5) or only social confrontation

(PR18SC+=16.5g±2.5) compared with control individuals (PR18SC- =17.1g±2.1). It is notable that the body mass of the adult offspring whose F1 fathers had experienced both PR9 and SC+ during their early life was significantly greater than all other treatment groups (PR9/SC+=17.7g±2.9; table 3). Thus, experience of multiple early life environmental factors does not exert a simple extrapolation of individual effects on bank vole phenotype (figure 2a).

b) Paternal early life social confrontation increased offspring overwinter survival

Over winter survival of the F2 offspring (figure 2b) was associated significantly with the early life of the father and sex (table 3; figure 1c). F2 individuals whose father had experienced social confrontation during gestation and nursing (*i.e*. SC+ treatment) survived on average one month more (mean±SD= 3.0 months±0.4) compared with F2 individuals that came from SC- fathers (mean±SD= 2.1 months±0.4; figure 1c). Paternal early life protein restriction on the other hand had no significant effect on F2 winter survival. Intergenerational paternal effects were exhibited both in male and female offspring, even though there was a significant difference in overwinter survival between sexes; on average, females survived for a longer period (mean±SD= 3.2 months±0.4) than males did (mean±SD= 1.9 months±0.4; table 3).

Table 1: Effects of early life environment treatments on F1 phenotype and F1 (fathers) reproductive success in bank voles. a) Reduced linear mixed models (REML estimation) of adult body mass of F1 individuals belonging to different treatment groups (n=624); full model can be found in SI table 6. b) Zero truncated Poisson generalized linear mixed model (GLMM) of amount of pups sired by F1 males on the condition that they had at least one pup (n=99). c) Binomial GLMM whether or not an F1 male managed to sire at least one pup (n=240). Random factors included for body mass model is litter identity. Random factors included for the reproductive success are male identity (to account for repeated measures; see SI table 1 for details), trial and female ID. Lsize = litter size. Bold p-values indicate $p < 0.05$ and are considered significant.

		Estimate	**Std. Error**	**t-value**	**p-value**
a) F1 Adult body mass	PR (PR9)	-1.2476	0.4208	-2.9646	**0.0036**
	SC (SC+)	-1.0025	0.4263	-2.3520	**0.0200**
	PR x SC (interaction)	0.7731	0.6222	1.2425	0.2161
	Lsize	-0.4519	0.1159	-3.8976	**0.0001**
	Sex (male)	1.6035	0.1356	11.8280	**<0.0001**
		Estimate	**Std. Error**	**z-value**	**p-value**
b) F1 Pups sired	SC (SC+)	-0.1681	0.2149	-0.7820	0.4342
	PR (PR9)	-0.1351	0.2105	-0.6420	0.5208
	PR x SC (interaction)	0.2580	0.2942	0.8770	0.3805
	Body mass	-0.0641	0.0260	-2.4660	**0.0137**
		Estimate	**Std. Error**	**z-value**	**p-value**
c) F1 Sired at least one pup	PR (PR9)	-0.1706	0.4687	-0.3640	0.7158
	SC (SC+)	-0.0303	0.4736	-0.0640	0.9489
	PR x SC (interaction)	0.6586	0.6837	0.9630	0.3354
	Body mass	0.1291	0.0626	2.0620	**0.0392**

Table 2: Preference shown by experimentally naïve females towards F1 males in relation to their early life (PR = protein restriction, SC = social confrontation) measured as time spent near a certain F1 male and number of visits in the proximity of the F1 male (n=49 trials; replicated for 25 trials). a) Poisson GLMM of number of visits. b) Binomial GLMM of time spent near F1 male relative to the total time spent in the testing arena (twenty minutes). Random factors included for all models are litter identity where the males were born into and female identity.

		Estimate	**Std. Error**	**z-value**	**p-value**
a) Number of visits	PR (PR9)	0.0179	0.1232	0.1450	0.8850
	SC (SC+)	0.0956	0.1225	0.7800	0.4350
	PR x SC (interaction)	-0.1150	0.1738	-0.6610	0.5080
		Estimate	**Std. Error**	**z-value**	**p-value**
b) Time spent near male	PR (PR9)	-0.3730	0.6448	-0.5790	0.5630
	SC (SC+)	0.2892	0.6442	0.4490	0.6530
	PR x SC (interaction)	0.2779	0.9104	0.3050	0.7600

Table 3: Intergenerational effects of paternal early life (PR = protein restriction, SC = social confrontation) on the F2 (offspring of F1 males) phenotype and winter survival. a) Reduced linear mixed models (REML estimation) of intergenerational paternal effects on F2 birth mass (n=372) and b) F2 adult body mass (n=215); full models can be found in SI table 7a,b. c) Poisson GLMM (number of months survived) of winter survival (n=72). For all models the litter identity is included as a random factor. For F2 winter survival the enclosure identity is included as a random factor as well. Lsize = litter size. Bold p-values indicate $p < 0.05$ and are considered significant.

		Estimate	**Std. Error**	**t-value**	**p-value**
a) F2 birth mass	PR (PR9)	-0.0213	0.0278	-0.7675	0.4434
	SC (SC+)	0.0377	0.0342	1.1009	0.2719
	PR x SC (interaction)	-0.0091	0.0453	-0.2002	0.8415
	F2 Lsize	-0.0757	0.0125	-6.0521	**<0.0001**
	F2 Sex (male)	0.0638	0.0142	4.5028	**<0.0001**
		Estimate	**Std. Error**	**t-value**	**p-value**
b) F2 adult mass	PR (PR9)	-0.9732	0.4553	-2.1376	**0.0341**
	SC (SC+)	-1.1725	0.5776	-2.0300	**0.0441**
	PR x SC (interaction)	1.9414	0.8020	2.4208	**0.0167**
	F2 Lsize	-0.5003	0.1964	-2.5475	**0.0137**
	F2 Sex (male)	1.8048	0.2498	7.2256	**<0.0001**
		Estimate	**Std. Error**	**z-value**	**p-value**
c) F2 winter survival	PR (PR9)	-0.0234	0.2589	-0.0900	0.9280
	SC (SC+)	0.4802	0.2415	1.9890	**0.0467**
	PR x SC (interaction)	-0.2978	0.3450	-0.8630	0.3881
	F2 Sex (male)	-0.5422	0.1586	-3.4190	**0.0006**

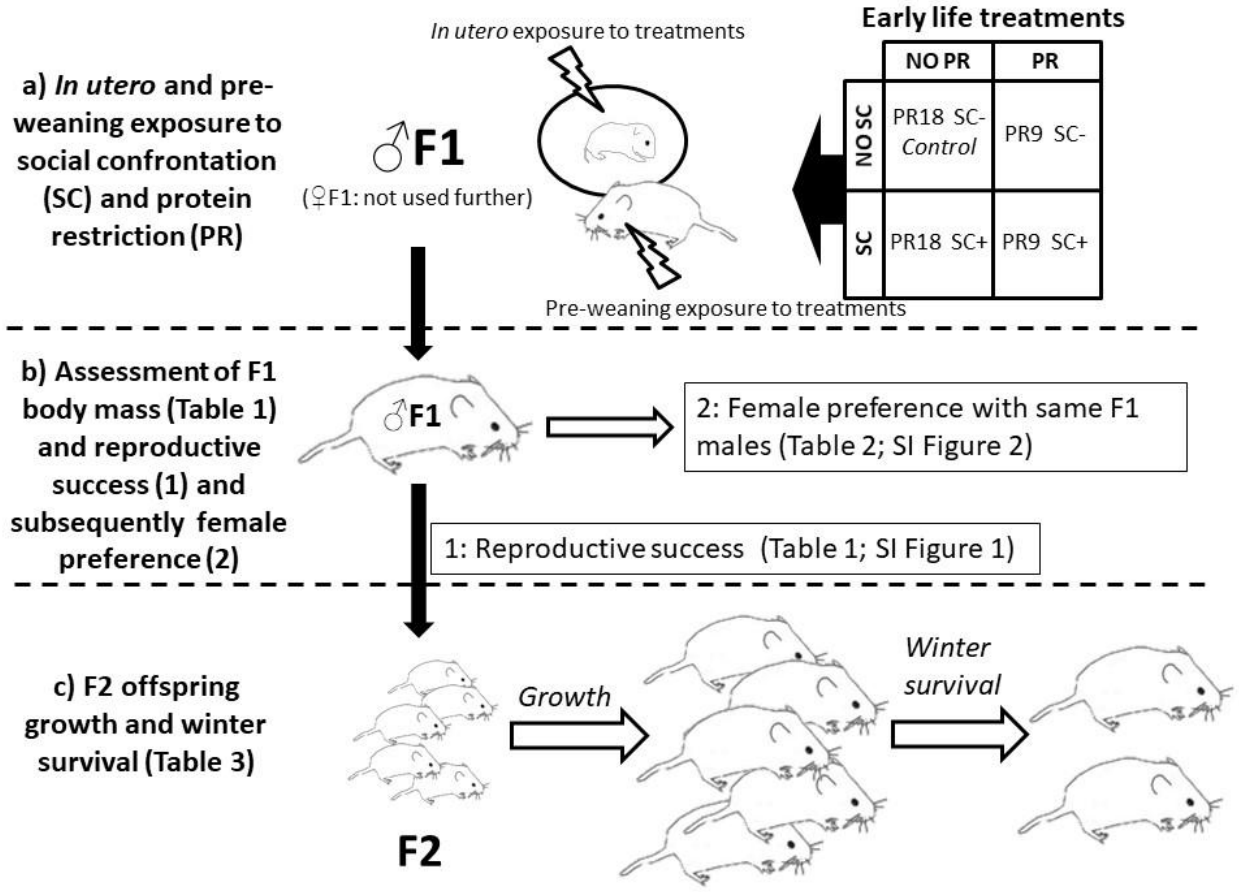

Figure 1: Overview of the experimental setup to investigate whether a F1 male bank vole's early life environment impacts growth and survival of its F2 offspring through paternal effects. (a) Early life environment treatments (protein restriction (PR) and/or social confrontation (SC) in a full factorial setup; PR18 signifies control diet and PR9 signifies protein restricted diet; SC- sign signifies absence of social confrontation, SC+ signifies presence of social confrontation) are presented to the F1 individuals during the intra-uterine development and nursing period. (b) Effects on F1 body mass are checked and subsequently the males' reproductive success (SI figure 1) and preference of non-experimental females towards males (SI figure 2). (c) Growth of the F2 offspring produced in the competitive reproduction trials is checked at birth and as adults (30 days old) and their over winter survival is tracked in semi-natural outdoor enclosures.

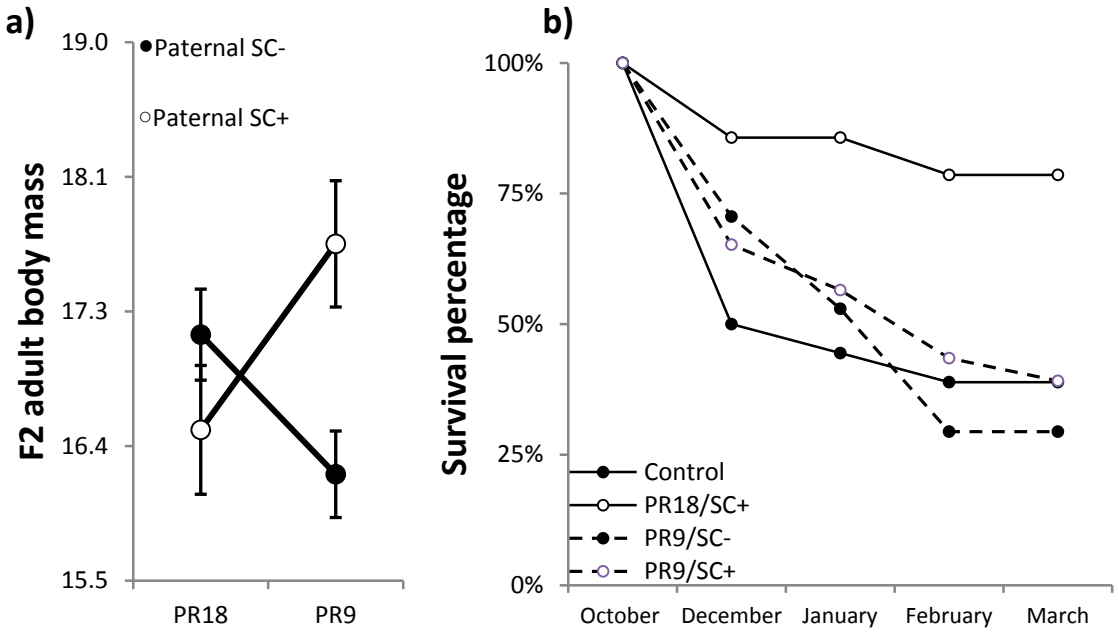

Figure 2: Effects of the F1 early life environment on the F2 offspring's adult body mass and over winter survival in the field. a) F2 adult body mass (30 days old; n=215); b) F2 winter survival (October to March) in semi-natural outdoor enclosures (n=72). Error bars represent ±1SE; closed circles indicate no paternal early life social confrontation (SC-); open circles indicate paternal early life social confrontation (SC+). In b) solid lines indicate absence of paternal early life protein restriction (PR18); dashed lines indicate presence of paternal early life protein restriction (PR9).

Discussion

Intergenerational effects can influence the outcome of selection as phenotypes can, at least in part, be conditional on the historic environment. Here, we find that paternal early life diet and social environment can induce intergenerational fitness effects in their offspring. The results show that the early life environment of the father can persist to affect their offspring's (adult) phenotype and, crucially, aspects of their fitness. However, the relation between the paternal early life and their offspring's phenotype is complex and depends on which paternal early life environmental factors were experienced.

a) Effects on F1 phenotype and reproductive success

The early life environment is a critical period for development and expression of the adult phenotype (Burton & Metcalfe, 2014; Paaby & Testa, 2018). That protein restriction during bank vole early life has detrimental effects on growth (*i.e*. adult body size) is consistent with studies on insects (*e.g. D. melanogaster*; Piper & Partridge, 2007), on humans (Polberger *et al.*, 1989), rodents in the laboratory (*e.g*. rats (Zambrano *et al.*, 2006); bank voles (Van Cann *et al.*, 2019)) and rodents in nature (*e.g*. deer mice *Peromyscus maniculatus borealis* (McAdam & Millar, 1999)). Likewise, social stresses exhibited to pregnant mothers seem to have negative effects on the offspring phenotype (Tamashiro *et al.*, 2005); for example, crowding exhibited to pregnant dams (rats and mice) results in offspring with lower body mass (Harvey & Chevins, 1987; Ward *et al.*, 1994). These effects are not restricted to rodents alone, for example, high early life population densities in brown trout (*Salmo trutta*) reduces survival and lowers cognitive abilities (Brockmark & Johnsson, 2010). Distress during pregnancy correlates with growth retardation in humans (Rondó *et al.*, 2003), although the analogy between human perception of stress and the effects of interspecific

confrontation in bank voles is obviously different. However, fewer studies have explored potential intergenerational effects of social confrontation on 'neutral grounds', as opposed to intruder setups. Two studies in bank voles, one by Marchlewska-Koj *et al.* (2003) and one by Van Cann *et al.* (2019) had a similar setup but only the latter found a negative relation between maternal social stress and offspring body mass.

To have an evolutionary impact, the early life environment should impact fitness. Early life experiences can have significant effects on male reproductive success (*e.g*. artificial versus wild early life of Atlantic salmon (*Salmo salar*); (Fleming *et al.*, 1997)). In male bank voles, body size positively correlates with social dominance that, in turn, is often associated with greater reproductive success (Klemme *et al.*, 2007; Kruczek & Styrna, 2009; Mokkonen *et al.*, 2011), so it is a reasonable prediction that early life treatments would impact reproductive success via body mass. By contrast we found little evidence that the early life of F1 males, nor their body size, affected their reproductive success, as opposed to a previous study by Klemme *et al.* (2007). It is possible that our lack of finding a relation between male body mass and reproductive success is due to our more "natural" setup where there was free competition between multiple males and females and which allowed for different behavioural tactics (*e.g.* sneaky males (Stockley *et al.*, 1994)), as opposed to Klemme *et al*. (2007), which used trials between two males and one female and only lasted 30 minutes. Another study on bank voles, which looked at natural island populations, also failed to find male body mass as an important predictor of reproductive success (Boratynski & Koteja, 2010). Overall, our data indicate that male adult phenotype is sensitive to the early life experience, but without notable consequences for male reproductive success.

b) Intergenerational paternal effects on phenotype and winter survival

Studies on intergenerational paternal effects have mostly focused on how a father's current environment can affect offspring; e.g. exposure of male laboratory mice (*Mus musculus*) to stress alters their offspring's behaviour (Saavedra-Rodríguez & Feig, 2013) and paternal population density in the marine tunicate *Styela plicata* directly affects offspring fitness (Crean *et al.*, 2013). But studies of intergenerational paternal effects due to the early life environment are rare and have focused on invertebrates (*e.g.* Bonduriansky & Head, 2007) or human health (*e.g*. Pembrey *et al.*, 2014). For example, a higher quality larval diet (early life environment) in males had led to larger offspring (Bonduriansky & Head, 2007) in the fly *Telostylinus angusticollis*. In humans, for example, poor quality early life nutrition experienced by some male inhabitants of Överkalix, northern Sweden, was associated with an increase in mortality rate in their grandsons (Pembrey *et al.*, 2014). Our data strengthens the view that paternal early life has an important and persistent context, manifest by an impact on offspring adult body mass. Crucially, we also show that the direction of the phenotypic response depends on the environmental experience during paternal early life. Paternal effects are generally studied in relation to a single environmental factor (*e.g*. paternal diet (Ng *et al.*, 2010; Zajitschek *et al.*, 2017)) and yet interactions among multiple environmental factors are inevitable in natural populations and can lead to complex phenotypes (*e.g.* in cichlid fishes (Fischer *et al.*, 2017)). While the growth pattern of the first generation (F1) resembles a 'developmental constraint', as both social confrontation and protein restriction lead to impaired growth, the growth pattern of the second generation (F2) is more in line with 'adaptive developmental plasticity' (Nettle & Bateson, 2015). This is surprising as it could indicate that the non-experimental mother had provided differently for

F2 offspring coming from fathers that had none, one or two treatments in their early life. We argue that this is unlikely as 1) females did not show any notable behavioural preference towards any males based on their early life treatments, but did prefer males that had more reproductive success in previous trials; 2) female bank voles have previously been shown not to invest differently in offspring based on mate quality (Oksanen *et al.*, 1999); and 3) there is little evidence that mothers are able to differentiate between young of different fathers within the same litters (Alonzo & Klug, 2012). Alternatively, the paternal early life could have caused an internal response (*e.g.* epigenetic changes) in the F2 offspring which either led to an inherent growth retardation in PR9/SC- and PR18/SC+ offspring, but not PR9/SC+, and/or it led to a change in F2 feeding behaviour. While it has been shown that paternal early life stress can lead to both body mass and behavioural changes in the offspring (*e.g.* Gapp *et al.*, 2014), the pattern observed in the F2 offspring's growth (with interacting effects causing non-additive effects, figure 2a) has to our knowledge never been observed as a consequence of intergenerational paternal effects in any species.

Despite emerging interest in paternal effects (Rando, 2012; Crean & Bonduriansky, 2014), few studies have determined their impact on fitness traits (Crean & Bonduriansky, 2014). The increase in winter survival of F2 due to the experienced social confrontation of their fathers demonstrates intergenerational fitness consequences of the paternal early life: the few individuals (Kallio *et al.*, 2009) who can extend a typically short life-span (Petrusewicz, 1983), by surviving a winter until the breeding season, have a clear opportunity to increase their lifetime reproductive success. Social confrontation may simulate high summer densities in bank vole populations, which is normally followed by crash of populations in the next winter (Krebs & Myers, 1974; Johnsen *et al.*, 2017). Moreover, survival of bank voles is

very low during the following crash winter due to density-dependent factors (intra-specific competition for food, diseases etc. (Krebs, 1996)) and consequently, the survival benefits shown here could indicate adaption to these density-dependent costs. However, as bank vole overwinter survival depends on multiple environmental factors such as predation, weather (Korpela *et al.*, 2013) and/or metabolic rate (Boratyński *et al.*, 2010), much work remains to understand the mechanisms by which intergenerational effects have fitness consequences in natural environments.

Conclusion

In this study, we show that paternal early life environment has intergenerational consequences for the phenotype (adult body size) and fitness (survival) of its offspring, in a wild mammal that lacks paternal care. Moreover, expression of these intergenerational effects depends on the type of early life environment (*i.e*. whether the father received one or two treatments during the period of prenatal and postnatal development). Our results suggest that recent findings for non-genetic paternal effects in humans, invertebrates and rodents under laboratory setting can be extended to studies of natural populations in mammals. As our early life treatments administered to the F1 fathers during their intrauterine and early life environment were ecologically relevant, our results should encourage further research towards the potential long-term consequences of non-genetic paternal effects in other wild animal populations.

DATA ACCESIBILITY STATEMENT: The datasets generated during and analysed during the current study are available in the Zenodo repository via the link https://doi.org/10.5281/zenodo.1288354.

ETHICS STATEMENT: Use of study animals followed the ethical guidelines for animal research in Finland and all institutional guidelines and was conducted under permissions from the National Animal Experiment Board (ESAVI/7256/04.10.07/2014).

REFERENCES

Abu-Saad, K. & Fraser, D. 2010. Maternal nutrition and birth outcomes. *Epidemiol. Rev.* **32**: 5–25. http://doi: 10.1093/epirev/mxq001.

Aldrich, J.C. & Maggert, K.A. 2015. Transgenerational Inheritance of Diet-Induced Genome Rearrangements in Drosophila. *PLoS Genet.* **11**: 1–21. http://doi: 10.1371/journal.pgen.1005148.

Alonzo, S.H. & Klug, H. 2012. Paternity, maternity, and parental care. In: *The Evolution of Parental Care* (N. J. Royle et al., eds), pp. 189–205. Oxford, UK, Oxford University Press. http://doi: 10.1093/acprof:oso/9780199692576.003.0011.

Bates, D., Mächler, M., Bolker, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**: 51. http://doi: 10.18637/jss.v067.i01.

Bonduriansky, R. & Head, M. 2007. Maternal and paternal condition effects on offspring phenotype in Telostylinus angusticollis (Diptera: Neriidae). *J. Evol. Biol.* **20**: 2379–2388.

http://doi: 10.1111/j.1420-9101.2007.01419.x.

Boratyński, Z., Koskela, E., Mappes, T. & Oksanen, T.A. 2010. Sex-specific selection on energy metabolism - selection coefficients for winter survival. *J. Evol. Biol.* **23**: 1969–1978. http://doi: 10.1111/j.1420-9101.2010.02059.x.

Boratynski, Z. & Koteja, P. 2010. Sexual and natural selection on body mass and metabolic rates in free-living bank voles. *Funct. Ecol.* **24**: 1252–1261. http://doi: 10.1111/j.1365-2435.2010.01764.x.

Bossdorf, O., Richards, C.L. & Pigliucci, M. 2008. Epigenetics for ecologists. *Ecol. Lett.* **11**: 106–15. http://doi: 10.1111/j.1461-0248.2007.01130.x.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., *et al.* 2017. Modeling Zero-Inflated Count Data With glmmTMB. *bioRxiv* 132753. http://doi: 10.1101/132753.

Burton, T. & Metcalfe, N.B. 2014. Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B Biol. Sci.* **281**. http://doi: 10.1098/rspb.2014.0311.

Crean, A.J. & Bonduriansky, R. 2014. What is a paternal effect ? *Trends Ecol. Evol.* **29**: 554–559. http://doi: 10.1016/j.tree.2014.07.009.

Crean, A.J., Dwyer, J.M. & Marshall, D.J. 2013. Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology* **94**: 2575–2582. http://doi: 10.1890/13-0184.1.

Curley, J.P., Champagne, F. a., Bateson, P. & Keverne, E.B. 2008. Transgenerational effects of

impaired maternal care on behaviour of offspring and grandoffspring. *Anim. Behav.* **75**: 1551–1561. http://doi: 10.1016/j.anbehav.2007.10.008.

Curley, J.P., Jensen, C.L., Franks, B. & Champagne, F. a. 2012. Variation in maternal and anxiety-like behavior associated with discrete patterns of oxytocin and vasopressin 1a receptor density in the lateral septum. *Horm. Behav.* **61**: 454–61. http://doi: 10.1016/j.yhbeh.2012.01.013.

Denham, J. 2018. Exercise and epigenetic inheritance of disease risk. *Acta Physiol.* **222**. http://doi: 10.1111/apha.12881.

Donkin, I., Versteyhe, S., Ingerslev, L.R., Qian, K., Mechta, M., Nordkap, L., *et al.* 2016. Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. *Cell Metab.* **23**: 369–378. http://doi: 10.1016/j.cmet.2015.11.004.

Drake, A.J. & Liu, L. 2010. Intergenerational transmission of programmed effects: Public health consequences. *Trends Endocrinol. Metab.* **21**: 206–213. http://doi: 10.1016/j.tem.2009.11.006.

Drake, A.J. & Walker, B.R. 2004. The intergenerational effects of fetal programming: Non-genomic mechanisms for the inheritance of low birth weight and cardiovascular risk. *J. Endocrinol.* **180**: 1–16. http://doi: 10.1677/joe.0.1800001.

Droždž, A. 1968. Digestibility and Assimilation of Natural Foods in Small Rodents. *Acta Theriol. (Warsz).* **13**: 367–389.

Emanuel, I. 1986. Maternal Health during Childhood and Later Reproductive Performance. *Ann. N. Y. Acad. Sci.* **477**: 27–39. http://doi: 10.1111/j.1749-6632.1986.tb40318.x.

Fischer, S., Bohn, L., Oberhummer, E., Nyman, C. & Taborsky, B. 2017. Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder. *Proc. Natl. Acad. Sci.* 201705934. http://doi: 10.1073/pnas.1705934114.

Fleming, I.A., Lamberg, A. & Jonsson, B. 1997. Effects of early experience on the reproductive performance of Atlantic salmon. *Behav. Ecol.* **8**: 470–480. http://doi: 10.1093/beheco/8.5.470.

Forbes, K.M., Stuart, P., Mappes, T., Henttonen, H. & Huitu, O. 2014a. Food resources and intestinal parasites as limiting factors for boreal vole populations during winter. *Ecology* **95**: 3139–3148. http://doi: 10.1890/13-2381.1.

Forbes, K.M., Stuart, P., Mappes, T., Hoset, K.S., Henttonen, H. & Huitu, O. 2014b. Diet quality limits summer growth of field vole populations. *PLoS One* **9**: 1–8. http://doi: 10.1371/journal.pone.0091113.

Franklin, T.B., Russig, H., Weiss, I.C., Gräff, J., Linder, N., Michalon, A., *et al.* 2010. Epigenetic transmission of the impact of early stress across generations. *Biol. Psychiatry* **68**: 408–15. http://doi: 10.1016/j.biopsych.2010.05.036.

Gapp, K., Jawaid, A., Sarkies, P., Bohacek, J., Pelczar, P., Prados, J., *et al.* 2014. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. *Nat. Neurosci.* **17**: 667–669. http://doi: 10.1038/nn.3695.

Gromov, V.S. & Osadchuk, L. V. 2013. Parental care and testosterone in males of the bank vole (Myodes glareolus): Sensitization and androgenic stimulation of paternal behavior. *Biol. Bull.* **40**: 114–118. http://doi: 10.1134/S1062359013010056.

Harrison, M. & Langley-Evans, S.C. 2009. Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *Br. J. Nutr.* **101**: 1020–1030. http://doi: 10.1017/S0007114508057607.

Harvey, P.W. & Chevins, P.F. 1987. Crowding during pregnancy delays puberty and alters estrous cycles of female offspring in mice. *Experientia* **43**: 306–308. http://doi: 10.1007/BF01945561.

Huitu, O., Koivula, M., Korpimäki, E., Klemola, T. & Norrdahl, K. 2003. Winter food supply limits growth of northern vole populations in the absence of predation. *Ecology* **84**: 2108–2118. http://doi: 10.1890/02-0040.

Johnsen, K., Boonstra, R., Boutin, S., Devineau, O., Krebs, C.J. & Andreassen, H.P. 2017. Surviving winter: Food, but not habitat structure, prevents crashes in cyclic vole populations. *Ecol. Evol.* **7**: 115–124. http://doi: 10.1002/ece3.2635.

Kaati, G., Bygren, L.O., Pembrey, M. & Sjöström, M. 2007. Transgenerational response to nutrition, early life circumstances and longevity. *Eur. J. Hum. Genet.* **15**: 784–790. http://doi: 10.1038/sj.ejhg.5201832.

Kallio, E.R., Begon, M., Henttonen, H., Koskela, E., Mappes, T., Vaheri, A., *et al.* 2009. Cyclic hantavirus epidemics in humans--predicted by rodent host dynamics. *Epidemics* **1**: 101–107. http://doi: 10.1016/j.epidem.2009.03.002.

Klemme, I., Eccard, J.A. & Ylönen, H. 2007. Why do female bank voles, Clethrionomys glareolus, mate multiply? *Anim. Behav.* **73**: 623–628. http://doi: 10.1016/j.anbehav.2006.07.010.

Korpela, K., Delgado, M., Henttonen, H., Korpimäki, E., Koskela, E., Ovaskainen, O., *et al.* 2013. Nonlinear effects of climate on boreal rodent dynamics: Mild winters do not negate high-amplitude cycles. *Glob. Chang. Biol.* **19**: 697–710. http://doi: 10.1111/gcb.12099.

Koskela, E. 1998. Offspring growth, survival and reproductive success in the bank vole: A litter size manipulation experiment. *Oecologia* **115**: 379–384. http://doi: 10.1007/s004420050531.

Krebs, C.J. 1996. Population Cycles Revisited. *J. Mammal.* **77**: 8–24. http://doi: 10.2307/1382705.

Krebs, C.J. & Myers, J.H. 1974. Population Cycles in Small Mammals. *Adv. Ecol. Res.* **8**: 267–399. http://doi: 10.1016/S0065-2504(08)60280-9.

Kruczek, M. & Styrna, J. 2009. Semen quantity and quality correlate with bank vole males' social status. *Behav. Processes* **82**: 279–285. http://doi: 10.1016/j.beproc.2009.07.009.

Liu, S., Sun, K., Jiang, T., Ho, J.P., Liu, B. & Feng, J. 2012. Natural Epigenetic variation in the female great roundleaf bat (Hipposideros armiger) populations. http://doi: 10.1007/s00438-012-0704-x.

Mappes, T. & Koskela, E. 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution (N. Y).* **58**: 645–650. http://doi: 10.1111/j.0014-3820.2004.tb01686.x.

Marchlewska-Koj, A., Kruczek, M., Kapusta, J. & Pochroń, E. 2003. Prenatal stress affects the rate of sexual maturation and attractiveness in bank voles. *Physiol. Behav.* **79**: 305–310.

http://doi: 10.1016/S0031-9384(03)00099-4.

Mashoodh, R., Habrylo, I.B., Gudsnuk, K.M., Pelle, G. & Champagne, F.A. 2018. Maternal modulation of paternal effects on offspring development. *Proc. R. Soc. B Biol. Sci.* **285**: 2120–2125. http://doi: 10.1098/rspb.2018.0118.

McAdam, A.G. & Millar, J.S. 1999. Dietary protein constraint on age at maturity: An experimental test with wild deer mice. *J. Anim. Ecol.* **68**: 733–740. http://doi: 10.1046/j.1365-2656.1999.00320.x.

Meaney, M.J. 2001. Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity Across Generations. *Annu. Rev. Neurosci.* **24**: 1161–1192. http://doi: 10.1146/annurev.neuro.24.1.1161.

Mills, S.C., Grapputo, A., Koskela, E. & Mappes, T. 2007. Quantitative measure of sexual selection with respect to the operational sex ratio: a comparison of selection indices. *Proc. R. Soc. B Biol. Sci.* **274**: 143–150. http://doi: 10.1098/rspb.2006.3639.

Mokkonen, M., Kokko, H., Koskela, E., Lehtonen, J., Mappes, T., Martiskainen, H., *et al.* 2011. Negative Frequency-Dependent Selection of Sexually Antagonistic Alleles in Myodes glareolus. *Science (80-.).* **334**: 972–974. http://doi: 10.1126/science.1208708.

Mousseau, T. & Fox, C. 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**: 403–407. http://doi: 10.1016/S0169-5347(98)01472-4.

Naguib, M. & Gil, D. 2005. Transgenerational effects on body size caused by early developmental stress in zebra finches. *Biol. Lett.* **1**: 95–97. http://doi: 10.1098/rsbl.2004.0277.

Nettle, D. & Bateson, M. 2015. Adaptive developmental plasticity: what is it, how can we recognize it and when can it evolve? *Proc. R. Soc. B Biol. Sci.* **282**: 20151005. http://doi: 10.1098/rspb.2015.1005.

Ng, S.-F., Lin, R.C.Y., Laybutt, D.R., Barres, R., Owens, J.A. & Morris, M.J. 2010. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. *Nature* **467**: 963–966. http://doi: 10.1038/nature09491.

Noldus, L.P.J.J., Spink, A.J. & Tegelenbosch, R.A.J. 2001. EthoVision: A versatile video tracking system for automation of behavioral experiments. *Behav. Res. Methods, Instruments, Comput.* **33**: 398–414. http://doi: 10.3758/BF03195394.

Oksanen, T.A., Alatalo, R. V, Horne, T.J., Koskela, E., Mappes, J. & Mappes, T. 1999. Maternal effort and male quality in the bank vole, Clethrionomys glareolus. *Proc. R. Soc. B* **266**: 1495–1499. http://doi: 10.1098/rspb.1999.0806.

Paaby, A.B. & Testa, N.D. 2018. *Developmental Plasticity and Evolution*. Oxford University Press. http://doi: 10.1007/978-3-319-33038-9_110-1.

Pembrey, M., Saffery, R. & Bygren, L. 2014. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. *J. Med. Genet.* **51**: 563–572. http://doi: 10.1136/jmedgenet-2014-102577.

Pembrey, M.E., Bygren, L.O., Kaati, G., Edvinsson, S., Northstone, K., Sjöström, M., *et al.* 2006. Sex-specific, male-line transgenerational responses in humans. *Eur. J. Hum. Genet.* **14**: 159–166. http://doi: 10.1038/sj.ejhg.5201538.

Petrusewicz, K. 1983. *Ecology of the Bank Vole*. Polish Scientific Publishers.

Polberger, S.K., Axelsson, I.A. & Räihä, N.C. 1989. Growth of very low birth weight infants on varying amounts of human milk protein. *Pediatr. Res.* **25**: 414–419. http://doi: 10.1203/00006450-198904000-00022.

Prévot-Julliard, A.C., Henttonen, H., Yoccoz, N.G. & Stenseth, N.C. 1999. Delayed maturation in female bank voles: Optimal decision or social constraint? *J. Anim. Ecol.* **68**: 684–697. http://doi: 10.1046/j.1365-2656.1999.00307.x.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2018. R Foundation for Statistical Computing. http://doi: ISBN 3-900051-07-0.

Radford, E.J., Ito, M., Shi, H., Corish, J.A., Yamazawa, K., Isganaitis, E., *et al.* 2014. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science (80-.).* **345**. http://doi: 10.1126/science.1255903.

Rando, O.J. 2012. Daddy issues: Paternal effects on phenotype. *Cell* **151**: 702–708. http://doi: 10.1016/j.cell.2012.10.020.

Rassoulzadegan, M., Grandjean, V., Gounon, P., Vincent, S., Gillot, I. & Cuzin, F. 2006. RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. *Nature* **441**: 469–474. http://doi: 10.1038/nature04674.

Ratkiewicz, M. & Borkowska, A. 2000. Multiple paternity in the bank vole (Clethrionomys glareolus): field and experimental data. *Zeitschrift Fur Saugetierkunde-International J. Mamm. Biol.* **65**: 6–14.

Richards, E.J. 2006. Inherited epigenetic variation - Revisiting soft inheritance. *Nat. Rev.*

Genet. **7**: 395–401. http://doi: 10.1038/nrg1834.

Rondó, P.H.C., Ferreira, R.F., Nogueira, F., Ribeiro, M.C.N., Lobert, H. & Artes, R. 2003. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur. J. Clin. Nutr.* **57**: 266–272. http://doi: 10.1038/sj.ejcn.1601526.

Saavedra-Rodríguez, L. & Feig, L.A. 2013. Chronic social instability induces anxiety and defective social interactions across generations. *Biol. Psychiatry* **73**: 44–53. http://doi: 10.1016/j.biopsych.2012.06.035.

Schroderus, E., Koivula, M., Koskela, E., Mappes, T., Oksanen, T. a & Poikonen, T. 2012. Can number and size of offspring increase simultaneously? - a central life-history trade-off reconsidered. *BMC Evol. Biol.* **12**: 44. http://doi: 10.1186/1471-2148-12-44.

Shama, L.N.S. & Wegner, K.M. 2014. Grandparental effects in marine sticklebacks: Transgenerational plasticity across multiple generations. *J. Evol. Biol.* **27**: 2297–2307. http://doi: 10.1111/jeb.12490.

Skinner, M.K., Guerrero-Bosagna, C., Haque, M., Nilsson, E., Bhandari, R. & McCarrey, J.R. 2013. Environmentally induced transgenerational epigenetic reprogramming of primordial germ cells and the subsequent germ line. *PLoS One* **8**: e66318. http://doi: 10.1371/journal.pone.0066318.

Soubry, A., Hoyo, C., Jirtle, R.L. & Murphy, S.K. 2014. A paternal environmental legacy: Evidence for epigenetic inheritance through the male germ line. *BioEssays* **36**: 359–371. http://doi: 10.1002/bies.201300113.

Stockley, P., Searle, J.B., Macdonald, D.W. & Jones, C.S. 1994. Alternative reproductive tactics in male common shrews: relationships between mate-searching behaviour, sperm production, and reproductive success as revealed by DNA fingerprinting. *Behav. Ecol. Sociobiol.* **34**: 71–78. http://doi: 10.1007/BF00175460.

Tamashiro, K.L.K., Nguyen, M.M.N. & Sakai, R.R. 2005. Social stress: From rodents to primates. *Front. Neuroendocrinol.* **26**: 27–40. http://doi: 10.1016/j.yfrne.2005.03.001.

Van Cann, J., Koskela, E., Mappes, T., Sims, A. & Watts, P.C. 2019. *Intergenerational fitness effects of the early life environment in a wild rodent*. http://doi: 10.1111/1365-2656.13039.

Ward, I.L., Ward, O.B., Winn, R.J. & Bielawski, D. 1994. Male and female sexual behavior potential of male rats prenatally exposed to the influence of alcohol, stress, or both factors. *Behav. Neurosci.* **108**: 1188–1195. http://doi: 10.1037/0735-7044.108.6.1188.

Wolf, J.B. & Wade, M.J. 2009. What are maternal effects (and what are they not)? *Philos. Trans. R. Soc. B* **364**: 1107–1115. http://doi: 10.1098/rstb.2008.0238.

Woodroffe, R. & Vincent, A. 1994. Mother's little helpers: Patterns of male care in mammals. *Trends Ecol. Evol.* **9**: 294–297. http://doi: 10.1016/0169-5347(94)90033-7.

Wu, G., Bazer, F.W., Cudd, T.A., Meininger, C.J. & Spencer, T.E. 2004. Maternal nutrition and fetal development. *J. Nutr.* **134**: 2169–2172. http://doi: 134/9/2169 [pii].

Zajitschek, F., Zajitschek, S. & Manier, M. 2017. High-protein paternal diet confers an advantage to sons in sperm competition. *Biol. Lett.* **13**: 20160914. http://doi: 10.1098/rsbl.2016.0914.

Zambrano, E., Bautista, C.J., Deás, M., Martínez-Samayoa, P.M., González-Zamorano, M., Ledesma, H., *et al.* 2006. A low maternal protein diet during pregnancy and lactation has sex- and window of exposure-specific effects on offspring growth and food intake, glucose metabolism and serum leptin in the rat. *J. Physiol.* **571**: 221–230. http://doi: 10.1113/jphysiol.2005.100313.