Master of Science Thesis

Effects of the early life environment on the morphology, behaviour and reproductive success of male bank voles (*Myodes glareolus*)

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Mikkonen, A-M.: Varhaiselämän elinolosuhteiden vaikutukset morfologiaan, käyttäytymiseen sekä lisääntymismenestykseen koirasmetsämyyrillä (*Myodes glareolus*)

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TIIVISTELMÄ

Yksilöt ovat herkkiä varhaiselämän kasvuympäristössä tapahtuville muutoksille, jotka voivat vaikuttaa myös ilmiasuun aikuisena. Varhaiselämän elinolosuhteilla voikin olla kauaskantoisia vaikutuksia yksilön elinkiertoon. Olosuhteiltaan vaihtelevassa ympäristössä elävillä populaatioilla varhaiselämä voi olla hyvin tärkeä yksilön sopeutumisessa sen tulevaan elinympäristöön. Vaikka varhaiseämän elinolosuhteiden vaikutuksista yksilön ilmiasuun on tutkittu paljon, tutkimus sen kauaskantoisista vaikutuksista koirasjälkeläisten menestymiseen on ollut vähäistä. Tutkielmassani käytin mallilajina metsämyyrää (*Myodes glareolus*) selvittääkseni miten varhaiselämän epäsuotuisat elinolosuhteet vaikuttavat aikuisten koirasjälkeläisten ilmiasuun ja kelpoisuuteen. Fennoskandiassa metsämyyrillä esiintyy populaatiotiheyden vaihteluita ja populaatiotiheyden huipun aikana kilpailu ravinnosta sekä lajitoverien välisten kohtaamisten oletetaan kasvavan. Tätä korkeaa populaatiotiheyttä simuloitiin täydelläyhdistelykokeella, jossa tiineet ja imettävät emot altistettiin yhdelle tai kahdelle ekologisesti olennaiselle käsittelylle, proteiinin rajoitukselle sekä sosiaaliselle konfrontaatiolle, näin muuttaen jälkeläisten varhaiselämän elinympäristöä. Jälkeläisiä ei altistettu millekään käsittelylle tämän tiineydenaikaisen ja synnytyksen jälkeisen ajanjakson jälkeen ja vaikutukset jälkeläisten ruumiinpainoon, käyttäytymiseen sekä kelpoisuuteen arvioitiin. Proteiinin rajoituksella oli negatiivinen vaikutus koiraiden aikuisruumiinpainoon ja sekä proteiinin rajoitus että sosiaalinen konfrontaatio muuttivat koiraiden käyttäytymistä. Naaraat eivät suosineet koiraita niiden varhaiselämän perusteella, eikä sillä ollut vaikutusta koiraiden lisääntymismenestykseen. Tiivistettynä, vaikka koirasmetsämyyrien varhaiselämä vaikutti merkittävästi niiden morfologiaan sekä käyttäytymiseen, niin tällä ei ollut vaikutusta yksilön kelpoisuuden osalta. Lisääntymismenestys on vain yksi puoli yksilön kelpoisuudessa ja lisätutkimusta tarvitaan varhaiselämän mahdollisista vaikutuksista muihin kelpoisuuteen vaikuttavista piirteistä, kuten selviytymisestä.

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ABSTRACT

The early life period of an individual is sensitive to changes in its environment and can potentially exert changes to its phenotype. Thus, the early life environment can have long-term effects on the individual's life-history. For populations living in a fluctuating environment, the early life can be especially important, as it has the potential of preparing an individual to its future environment. Even though research on the effects of a specific early life environment is growing, there has not been much focus on the long-term consequences of male individuals. In this thesis, I used the bank vole (*Myodes glareolus*) as a model species to investigate the effects of an adverse early life environment on adult male offspring phenotype and fitness. Bank voles living in Fennoscandia experience population density fluctuations, and food and encounters with conspecifics are expected to be higher during population density peaks. This situation was simulated by conducting a two by two factorial design which exposed pregnant and nursing mothers to one or two ecologically relevant treatments, protein restriction and social confrontation, thereby changing the offspring's early life environment. Offspring did not receive any treatment after this pre- and early postnatal period and effects on offspring body mass, behaviour and fitness were assessed. Protein restriction had a negative effect on male adult body mass and both protein restriction and social confrontation altered male behaviour. The early life environment did not have an effect on female preference nor male reproductive success. In conclusion, while the early life of male bank voles had significant effects on their morphology and behaviour, this did not affect the individual fitness. Reproductive success is only one aspect of an individual's fitness and further research should be done on how the early life environment might affect other fitness related traits, such as survival.

CONTENTS

1. INTRODUCTION

1.1. Early life environment

In evolutionary ecology, the origin of individual phenotypic variation and its implications are of particular interest as it is this variation that provides the material for natural selection to act upon (Mousseau and Fox 1998). Natural selection favours individuals that are better adapted to their current environment. In addition to the effects genes have on an individual's phenotype, environmental factors can have profound impacts on development (Beaman *et al.* 2016), even when experienced during very early stages of life (Paaby and Testa 2018). Several studies suggest that the early life period, defined hereafter as the period extending from preconception to early postnatal development, is particularly sensitive to the induction and intergenerational (from one generation to the next) transmission of environmental effects (reviewed in Burton & Metcalfe 2014; Drummond & Ancona 2015). The prenatal and early life for most animals is largely dictated by the maternal environment, as paternal care is absent in most animals, particularly mammals (Woodroffe and Vincent 1994). As such, a large and growing amount of laboratory studies in mammals indicate that the maternal environment can elicit long-term changes in the phenotype and behaviour of the offspring (Barbazanges *et al.* 1996; De Kloet *et al.* 2005; Weinstock 2008; Glover *et al.* 2010; Burton and Metcalfe 2014).

Many studies have shown that a wide range of maternal effects can influence offspring phenotypes, *i.e.* early life environment quality (Pravosudov and Kitaysky 2006), predation risk (Sheriff *et al.* 2009, 2010) and social environment (Landys *et al.* 2011). A variety of taxa show developmental sensitivity to the maternal environment *i.e.* growth, behaviour and physiology (fish: Mccormick 1998; McCormick 1999; reptiles: Sinervo & DeNardo 1996; Meylan & Clobert 2005; birds: Hayward & Wingfield 2004; Love *et al.* 2005; Saino *et al.* 2005; Love & Williams 2008; mammals: Seckl 2004; Dantzer *et al.* 2013). Furthermore, researchers are now understanding that phenotypic responses to maternal effects in offspring can be more than just inevitable negative outcomes, but can rather serve an adaptive function to prepare individuals to postnatal and even future environments frequently exposed to those ecological stressors (Meylan and Clobert 2005; Preisser 2009; Sheriff *et al.* 2010; Love *et al.* 2012; Sheriff and Love 2013). For example, elevation in corticosterone (stress related hormone) levels in pregnant European common lizard (*Zootoca vivipara*, formerly *Lacerta vivipara*) females increased the survival of their male offspring (Meylan and Clobert 2005). Even as the adaptive value of the early life environment of an individual has become more apparent over the years, studies are frequently focused on short-term proximate effects on offspring phenotype (Marshall and Uller 2007), without fully considering the results in the context of the organism's life-history or environment (Love *et al.* 2009, 2012). Additionally, as many of the ecological and environmental stressors rarely occur alone, the interaction between two physiological stressors, *i.e.* social confrontation and resource availability, has been massively understudied.

1.2. The adaptiveness of the early life environment

Whether or not a maternal effect is adaptive is dependent on whether the effect will be adaptive, *i.e.* increase offspring fitness. In order to study fitness effects of the early life, it is necessary to use ecologically relevant early life cues. One of the most significant environmental factor affecting populations is food resource accessibility (Krebs *et al.* 1995; Clinchy *et al.* 2004). Especially nutritional availability during an individual's early life can have a profound role in shaping the individual's phenotype and is directly associated with the development, growth and function of an organism (Laus *et al.* 2011).

For example, maternal caloric restriction in rats exerted negative developmental effects on hypothalamic structure and function of the male offspring (Konieczna *et al.* 2013). Furthermore, maternal caloric restriction decreased offspring birth weight and neonatal growth and resulted in early onset of reproductive maturation in female rats (Sloboda *et al.* 2009). Some studies suggest that it is not so much caloric restriction, but rather the restriction in dietary protein that has the most significant impact on an individual's development (Hsueh *et al.* 1967; Atinmo *et al.* 1974). For example, maternal protein restriction in mice promoted anxiety and depression-related behaviours in offspring (Belluscio *et al.* 2014) and delayed maturation, reduced testosterone concentration and decreased sperm count in male rats (Zambrano *et al.* 2005). In addition, early life protein restriction exerted changes in exploratory behaviour and anxiety (Almeida *et al.* 1996; Reyes-Castro *et al.* 2012; Belluscio *et al.* 2014).

While laboratory studies mainly focus on negative effects of caloric or protein restriction, it is assumed that natural populations are often confronted with nutritional stress (Desy and Batzli 1989). This is especially the case for populations undergoing cyclic fluctuations in population density. The changes in density can lead to periods where food becomes scarce, and thus competition for high-quality food is increased. That food is an important factor for these populations has been made clear in several rodent studies. For example, field supplementation of high-quality food significantly increased body growth rates, adult male body size, reproductive activity and population density in prairie voles (*Microtus ochrogaster*) (Desy and Batzli 1989). In meadow voles (*Microtus pennsylvanicus*) female territory size was inversely correlated with the available forage suggesting that the quantity/quality of available food has an effect on population density (Jones 1990). It is possible that, for wild individuals born in these nutritional stressful periods, the effects are not so much negative, as they are adaptive. In other words, individuals born in these environments might be better suited to survive in such conditions. For example, male mice exposed to early life protein restriction increased their food intake, were hyperactive and had an increased metabolic rate when exposed to high-fat, post weaning diet (Whitaker *et al.* 2012), indicating an adaptive response to environmental conditions where high-quality food is scarce.

In addition to nutritional environment, the social environment can be important to an individual's early life development as it is directly linked to population density. In high population densities, encounters with conspecifics are expected to be more frequent. High population density has been found to affect postnatal development of offspring (Dantzer *et al.* 2013; Tschirren 2015). For example, simulation of high population density cues induced adaptive increases in offspring growth rate in North American red squirrels (*Tamiasciurus hudsonicus*) which increased their first winter survival (Dantzer *et al.* 2013). Moreover, at high social densities female great tits (*Parus major*) allocate higher concentrations of androgens to eggs producing fast-growing sons (Tschirren 2015), which have been found to increase competitiveness in house sparrows (*Passer domesticus*) (Strasser and Schwabl 2004). Social interactions and conflicts have long been shown to act as a source of environmental stress in vertebrates (Creel 2001; Creel *et al.* 2013). The social environment of an individual is often a complex mix of several factors. However, for an individual that relies on maternal care, the early life social environment is completely dictated by the mother, and is communicated as such through maternal care (Weaver *et al.* 2004; Meaney *et al.* 2007; Love *et al.* 2012) and maternal hormones during intrauterine development and nursing (reviewed in Fowden & Forhead 2004). For many species, the social environment can depend on the number of conspecifics in the area, *i.e.* population density. In territorial animals, territory defence is common (Maher and Lott 2006) and encounters with conspecifics can lead to aggressive displays (Kaufmann 1983) and can be

stressful for both parties (von Holst 1998). The effects of intruding or defending a territory have been well studied particularly in rodents (Golden *et al.* 2011). However, in some species, confrontations between conspecifics do not always represent the resident-intruder system (Martinez *et al.* 1998). For example, in most rodents, individuals do not only have a core territory (does not overlap with other conspecifics), which they actively defend, but also occupy a home-range, which is used *e.g.* for foraging (Burt 1943). As opposed to core territory, home-ranges can overlap with other individuals. The encounters with conspecifics in the home-range area differ from the intruder-resident system in that in home-range encounters, both individuals can be considered as residents (or intruders). This overlap in home-ranges is expected to happen more frequently during high population densities (*e.g.* in deer mice (Wolff 1985) and in bank voles (*Myodes glareolus*) (Koskela *et al.* 1997, 1999; Jonsson *et al.* 2002)).

1.3. Bank vole (*Myodes glareolus*)

The bank vole (*Myodes glareolus*) is a microtine rodent species and is commonly found in the boreal forest regions of the northern hemisphere (Stenseth 1985). In Fennoscandia, bank vole populations experience 3-4-year population density fluctuations with peaks and crashes (Kallio *et al.* 2009; Korpela *et al.* 2013) associated with differences in food quality (Koskela *et al.* 1998, 1999) and the expected frequency of social confrontation with conspecifics (Schirmer *et al.* 2019). Population density has been shown to have an effect on the physiology of the bank vole (Norrdahl and Korpimäki 2002; Nieminen *et al.* 2015). For example, bank voles exhibit higher body masses during the phases of increasing population densities (Nieminen *et al.* 2015) whereas declining population density phases are associated with lower body masses (Norrdahl and Korpimäki 2002; Nieminen *et al.* 2015). The life-history of bank voles is characterised by a short life span (Innes and Millar 1994), early maturation (Mappes and Koskela 2004; Oksanen *et al.* 2007), high fecundity (Koivula *et al.* 2003; Mappes and Koskela 2004; Schroderus *et al.* 2012) and multiple reproductive events within a breeding season (Koivula *et al.* 2003). Many bank vole life-history traits show high plasticity and are either increased or decreased depending on the population density (Bujalska 1985; Kruczek and Marchlewska-Koj 1986; Koskela *et al.* 1999; Prévot-Julliard *et al.* 1999; Oksanen *et al.* 2007; Mappes *et al.* 2008), which may be advantageous in fluctuating environments.

Bank voles have a polygynandrous mating system (Mills *et al.* 2007), meaning both males and females mate with multiple partners, and where fathers do not provide paternal care to their offspring (Horne and Ylönen 1996; Gromov and Osadchuk 2013). Bank vole females can discriminate males by their social status and consistently prefer dominant males over subordinate ones as mates (Horne and Ylönen 1996). Additionally, olfactory cues have been shown to have an important role in female bank vole mate choice (Kruczek 1997; Marchlewska-Koj *et al.* 2003; Radwan *et al.* 2008), where females use olfactory signals in the male's urine to evaluate its dominance status. Females can produce litters sired by multiple males (Ratkiewicz and Borkowska 2000) and young are dependent on the mother until weaning at approximately 20 days old. Females are territorial during the breeding season and become highly aggressive towards the end of pregnancy (Koskela *et al.* 1997). Due to the life-history traits of bank voles, combined with the naturally changing environment, the bank vole is an ideal study species to investigate early life effects in.

1.4. Study hypotheses and predictions

The objective of this study was to examine the effects of the early life environment, which simulated a high population density, on the phenotypic characteristics and the fitness of male bank voles. The focus on male bank voles was chosen as males have received less

attention in previous research. Specifically, in this thesis I aimed to answer two questions strongly linked to the bank vole ecology.

i) How does the manipulation of early life environment conditions (protein restriction and /or social confrontation) influence the phenotypic characteristics (morphology and behaviour) of the offspring?
ii) Does the early life environment affect attractiveness and reproductive success of male offspring?

My first question was designed to determine the effects of the early life environment on the male offspring's phenotype. In previous studies, adverse early life environments have been linked to changes in both the morphology and behaviour of the offspring (see 1.2.). In accordance to these previous findings, I predicted that the stressful early life conditions (protein restriction/social confrontation) would have a negative impact on the male offspring body mass. Low protein content in diet would lead to impaired foetal and juvenile development, and the available resources would be allocated to forming and maintaining vital organs and body parts while compromising on growth. I also expected to see an increase in anxiety-related behaviours in offspring exposed to protein restriction during their early life. Anxiety-related behaviour in conditions of scarce food resources would help conserve vital energy resources to increase the chance of survival. Moreover, I predicted that adverse early life social environment would also decrease the offspring body mass.

My second question was to determine whether the effects of early life environment on the male offspring are adaptive; i.e. whether they lead to an increase in survival or reproductive success. By testing both the competitive success and the attractiveness of each male, it would be possible to see if any potential fitness difference is due to changes in behaviour or due to olfactory signals emitted by stressed males. In a high population density situation associated with increased competition for resources, I predicted that early life protein restriction will negatively affect the attractiveness of males to females, as bank vole females have been shown to prefer larger males as mates. I also predicted that early life protein restriction will have a negative effect on the males' reproductive success, as smaller individuals are less likely to be dominant and to win fights with larger males.
Earlier research has shown that females are less attracted to males prenatally exposed to social stress. In accordance to this finding, I predicted that early life social confrontation would reduce male attractiveness. Conversely, being bolder and more active, males exposed to social stress during early life could have higher success in finding mates and reaching them before other males. Thus, I predicted early life social confrontation to have a positive impact on the males' reproductive success regardless of their attractiveness.

2. MATERIALS AND METHODS

2.1. 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).

2.2. Housing conditions

All experimental individuals were raised and kept in the facilities of the University of Jyväskylä (Finland). Individuals were housed in polycarbonate cages (Type 3, 425 x 265 x 150 mm, Makrolon) with wood shavings and hay as bedding material and maintained on a

16L:8D photoperiod at 20±2°C. Water was available *ad libitum* and standard food (Labfor 36; Lactamin AB, Stockholm, Sweden) was provided *ad libitum*, except during early life treatments (see details below).

2.3. Experimental setup

2.3.1. F1 early life environment treatments

To study the effect of different early life environments on the offspring, a parental line (hence referred to as the F0 generation) was established. All F0 individuals were chosen from unrelated, non-experimental first- or second-generation laboratory males (N=241) and females (N=241) originally captured in Central Finland (62°36'59"N 26°20'45"E). These individuals were paired randomly to produce gravid F0 females, and the males and females were kept together a period of seven days before separation. In order to change the prenatal and early life of the F1 offspring, a two by two factorial experimental design was established (Figure 1). F0 females were randomly and equally assigned to one of four treatment groups before mating: a control group, a protein restricted group, a socially confronted group and an interaction group (getting both protein restriction and social confrontation). The effects of early life environment on the subsequent F1 generation males were assessed through morphological measurements, reproductive success and behavioural tests (Figure 2, see details further on).

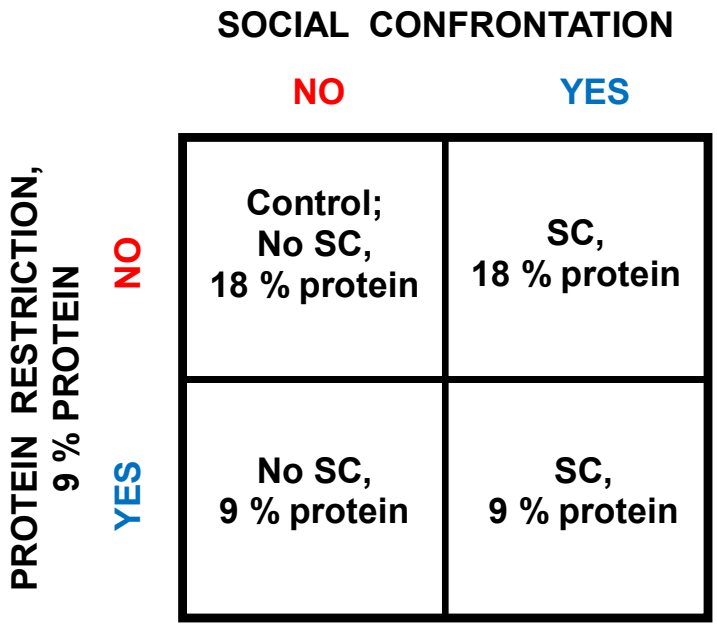

Figure 1. The F1 early life treatment groups were assigned as follows in a two by two factorial design: gravid F0 females that received neither protein restriction (9 % protein diet) nor social confrontation treatment were in the control group (Control), females that received protein restriction treatment, but no social confrontation treatment were in the protein restriction group (PR), females receiving social confrontation treatment but no protein restriction were in the social confrontation group (SC) and females receiving both treatments were in the interaction group (SC*PR). The two by two factorial setup design allowed the examination of the effect of both treatments separately as well as to study for the interaction between them.

2.3.2. Protein restriction

F0 females assigned to the protein restriction (PR) treatment group were provided *ad libitum* low protein diet (9% protein dry weight, Envigo, WI, USA), versus the control diet (18 % protein dry weight, Envigo, WI, USA) provided to the control (C) and social confrontation (SC) treatments (Figure 2). This diet started from pairing of the F0 females until the weaning of the F1 offspring at 20 days old.

2.3.3. Social confrontation

The social confrontation treatment consisted of two females in similar stages of pregnancy/nursing put together into a cage (Type 3H, 425 x 265 x 180mm, Makrolon) by placing each individual in opposing corners simultaneously. Individuals were kept in the cage for 15 minutes every other day after which they were returned to their home cages. Prior to placing the females, a small handful of the top layer of the sawdust from the females' cages was spread to the floor to elicit territorial odours. Pairs were changed every time as well as the time of day (between 10am and 5pm) at which the confrontation happened to avoid habituation. The social confrontation treatments were started after the separation of the F0 females and males. Treatment lasted throughout the pregnancy and nursing period until the weaning age of the offspring (20 days old).

Females in the control group did not receive any treatments. However, to account for handling and cage changes during the early life treatments, females in the control group were handled and transferred alone to an empty, clean cage with a small handful of bedding from their own housing cage for 15 minutes before returned to their own cages. This procedure was done once every week from mating of the females up to weaning of the offspring.

2.3.4. F1 generation morphological measurements

The F1 individuals were weighed using an electronic microscale. The individuals were weighed at three time points in their life: at birth (0 days old), at weaning (20 days old) and at sexual maturity (30 days old). At 30 days old, the male offspring were also separated from their female siblings to avoid potential inbreeding.

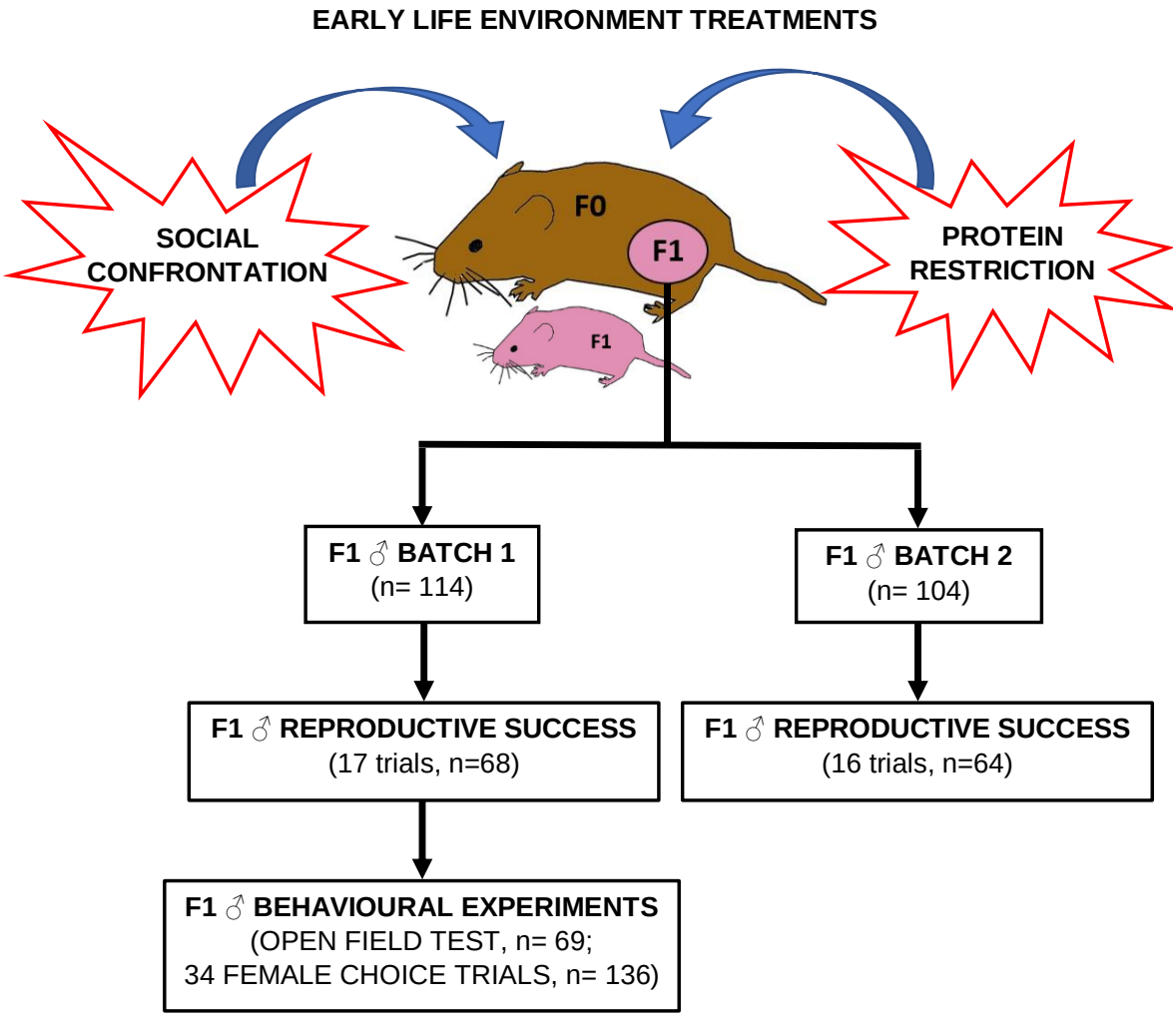

Figure 2. The experimental design of the study. Gravid mothers were subjected to one of or both of two treatments (social confrontation and/or protein restriction) throughout their pregnancy and nursing period. The reproductive success of the subsequent generation (F1) males was determined, and behavioural experiments were conducted to study the effects of the treatments on various behaviour characters of the F1 males. Reproductive success and behavioural experiments were replicated.

2.4. F1 male reproductive success

To assess whether the different early life treatments influence the reproductive success of the males in the F1 generation, the males were put into a competitive situation, where they were allowed to compete over two females (hence referred as a trial). The paternity of the resulting offspring (F2 generation) was then analysed to determine the reproductive success of the F1 males.

Between 40 and 45 days old, a random set of F1 males from each treatment was chosen for the trials. All chosen males were sorted by weight and treatment and subsequently assigned to a trial based on their weight rank so that the amount of variation in weight was reduced within the trials. The trials were done in two rounds due to the differences in maturation times between the born F1 batches, with 17 trials in the first round and 16 trials in the second (Figure 2).

Trials were conducted in an experimental cage system consisting of four polyethylene cages (425 x 265 x 150 mm, Makrolon) interlinked with a PVC tube allowing free movement of animals between all four cages (Figure 3). One F1 male from each

treatment group was put randomly to one of the interlinked cages (four males in total) and they had a one-day settlement period. Two non-experimental, unrelated females were then introduced to the experimental cage system. The females were put randomly in to one of the four cages. Each trial lasted for nine days to ensure at least two oestrus cycles in the females (oestrus cycles in bank voles last three days) before separating the males and females into their own cages. After the resulting F2 offspring were born, the reproductive success of the F1 males was determined as the proportion of offspring a male sired in a trial over the total number of offspring born within the trial it was in. Paternity was determined using microsatellite markers (see 2.5) extracted from ear tissue taken from the F1 males prior to the experiments and tail tissue taken from the F2 offspring at birth.

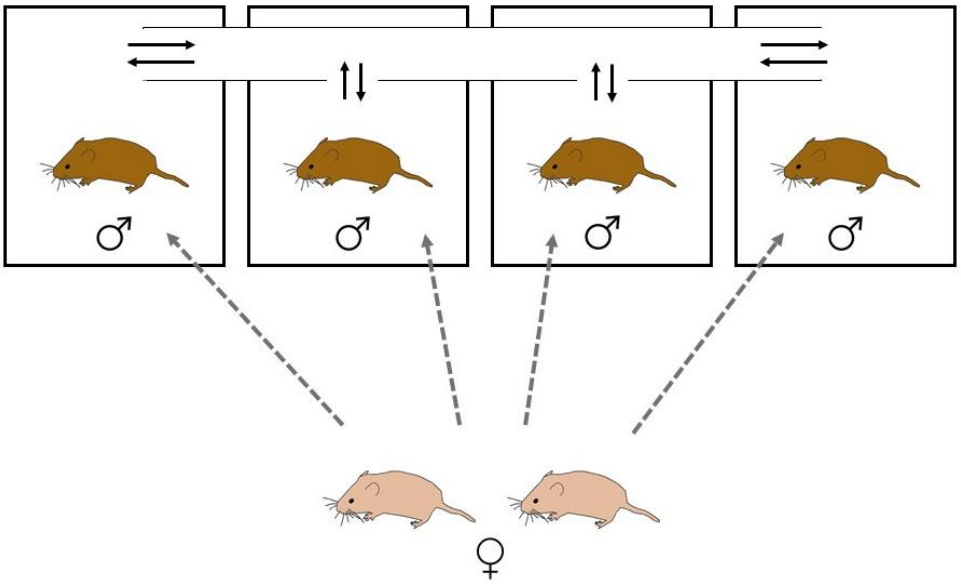

Figure 3. Reproductive success experiment design. One male from each early life treatment (four males in total) was put into the experimental cage system consisting of four interlinked cages allowing free movement of animals between them. Two experimentally naïve, unrelated females were introduced to the cage system. Males and females were separated to their respective cages after nine days ensuring at least two oestrus cycles in the females. The reproductive success experiments were replicated one week after the first experiments with two new experimentally naïve, unrelated females.

2.5. Paternity analysis

Paternity analysis of all F2 pups born in the "reproductive success" trials (see 2.4) was conducted by first genotyping the DNA extracted from tissue samples taken from the tip of the tail of each F2 pup at birth (about 2-3 mm piece) and from the F1 male ear biopsies. Both the F1 and F2 individuals were genotyped at six microsatellite markers (Gockel *et al.* 1997; Rikalainen *et al.* 2008) and the DNA extractions were done by using the following protocol. To prepare the 50µl lysis mix for one sample, 3.75 µl of Proteinase K was added to 46.25 µl of buffer ATL and vortexed. Tissue sample was added to the lysis mix. The sample mix was incubated for three hours at 56 °C. 50 µl of buffer Al was mixed with 50 µl of 100 % ethanol and added to the incubated samples. Samples were incubated in 56 °C for another three minutes.

Paternity was determined by matching microsatellite lengths between fathers and offspring. Six different microsatellite loci were used (13G2, 10A11, 15F7, 16E2, 6G11,

17E9) (Rikalainen *et al.* 2008). Polymerase chain reaction (PCR) amplifications were done separately for each locus and the reactions were then mixed with two different ABI premixes and finally combined to one ABI run. The PCR amplifications were performed in 20 µl reactions consisting of 3 µl of DNA template, 2 µl of 10xBuffer with 20 mM $MgCl_2$ (Fermentas), 2 µl of 2 mM dNTPs (Fermentas), 2 µl of BSA (5 mg/ml), 0.3 µl of 10 µM unlabelled forward primer, 0.1 µl of 10 µM fluorescence-labelled forward primer, 0.4 µl of 10 µM reverse primer, 0.1 µl of 5U/µl DreamTaq (Fermentas) and H_2O to fill the 20 µl. Amplified fragments were then detected with ABI Prism 3130xl -sequencer and read using GENEMAPPER version 5.0 software (AB).

Once the genotypes were constructed, paternity was assigned by calculating a natural logarithmic of the likelihood-odds ratio (LOD) for each F1 male with a 95% confidence level using the software Cervus 3.0.7 (Kalinowski *et al.* 2007), with the genotype of the mother known (Mills *et al.* 2007a, 2009). All F1 males from the same reproductive success trial were considered as potential fathers for the F2 pups born within that trial. The male with the highest LOD score was considered as the father. The simulation was run using 10000 cycles, 100% of candidate parents sampled, 98% loci typed and a genotyping error of 1%. No mismatches were allowed between offspring and either of the parents.

2.6. Behavioural tests

To test whether the different early life environments of the F1 individuals influence their behaviour, open field tests (OFT) were conducted. An arena (60 x 60 cm), made from white plywood, was used for the tests. The arena was evenly lit by a central lamp with a light intensity of approximately 60 lumen (Gould *et al.* 2009). Each animal was carefully removed from its housing cage and placed into the centre of the open field arena in a non-transparent container. To minimise disturbance, all trials were recorded with a digital video camera placed straight above the arena and the experimenter was not present in the room during recording. The OFT lasted for 10 minutes (starting from the removal of the container) after which the animal was weighed with electronical microscale and returned to its housing cage. After each trial, the arena was wiped down using 35 % ethanol, a concentration enough to remove the scent of previous animals but not to influence the behaviour (Gould *et al.* 2009). The short time length emphasizes exploratory behaviour and response to novelty (Gould *et al.* 2009).

From the video recordings, the movement of each individual was tracked by the centre point of the body starting from the removal of the container using a video tracking software EthoVision XT 8.5 (Noldus Information Technology, Wageningen, Netherlands). Activity (total distance moved) and the time spent close to the walls of the arena (thigmotaxis) were analysed automatically by the software. Four behaviours were scored manually: unsupported rearing, supported rearing, self-grooming and jumping (described in Table 1) (Gould *et al.* 2009). The duration and frequency of these behaviours were analysed. 32 of the total 68 animal recorded were randomly chosen (eight individuals from each treatment) for repeated measurements.

Table 1. Detailed descriptions of the behaviours recorded in the open field test (OFT), the ways of measurement and their analyses. Behaviours are defined as being either exploratory or anxiety related behaviours. Total experimental time in the OFT was 10 minutes during which the behaviours were recorded. The analyses were done using R programming software (R Core Team 2018).

Behaviour	Description	Measurement	Analyses
Unsupported rearing (exploratory)	Standing on hind legs, front paws off the floor unsupported	Frequency (number of occurrences)	Poisson GLMM
Supported rearing (exploratory)	Standing on hind legs, front paws off the floor supported against the wall	Frequency (number of occurrences)	Poisson GLMM
Self-grooming (anxiety)	Licking paws followed by washing nose/face/head/body/tail	Duration (duration of behaviour/the total time of experiment)	Binomial GLMM
Jumping (exploratory)	All paws off the floor	Frequency (number of occurrences)	Poisson GLMM
Thigmotaxis (anxiety)	The time spent along the wall of the open field arena	Duration (duration of behaviour/the total time of experiment)	Binomial GLMM
Activity	The total distance an individual moved during the total experimental time	Distance (cm)	LMM

2.7. Female choice trials

F1 males from the first batch of the early life treatments and subsequently from the first reproductive success trials (cf. Figure 1) were used in these female choice trials. Males (one from each early life treatment) from the same reproductive trial (cf. Figure 3) were used in the same female choice trial.

The experiments were conducted in an open field arena and were video recorded (cf. Behavioural tests). The arena was wiped with 35 % ethanol before each trial to remove odours. The males were placed into their own respective small mesh-wire cages (Figure 4A), which had been previously cleaned with 35 % ethanol and water respectively to remove the smell of previous voles. The cages were then placed in the centre of each side of the arena (Figure 4B) in a random order adjusted to ensure that the same treatments were not consistently next to one another. Males were given a five-minute habituation period before introducing a non-experimental female in postpartum oestrus to the centre of the arena. The female was transferred to the arena in an opaque transport container. The transport container was gently removed, and the recording started. The female was left in the arena with the males for 20 minutes. The female could freely move inside the arena and see, smell and limitedly touch the males through the mesh-wire, but the males could not make physical contact with each other. After 20 minutes, the recording was stopped; the female was removed from the arena and subsequently the males. All individuals were returned to their respective cages. No experimenter was present during the recording. All female-preference trials were repeated later with different non-experimental females in postpartum oestrus.

The movements of each female in the recordings were tracked by the video tracking software EthoVision XT 8.5 (Noldus *et al.* 2001)(cf. Behavioural tests) and any missing or incorrect track data were manually completed and corrected respectively. Areas encircling the males (zones) were created using the tracking software so that a zone covered the small mesh-wire cage and the area in its vicinity (Figure 4C). The size of these zones was chosen so that the centre point of the female, as the tracking software tracks the centre point of the animal, could be recorded being inside the zone when the female is near the cage. The time that the female spent in each zone and the number of times the female entered the zones were recorded.

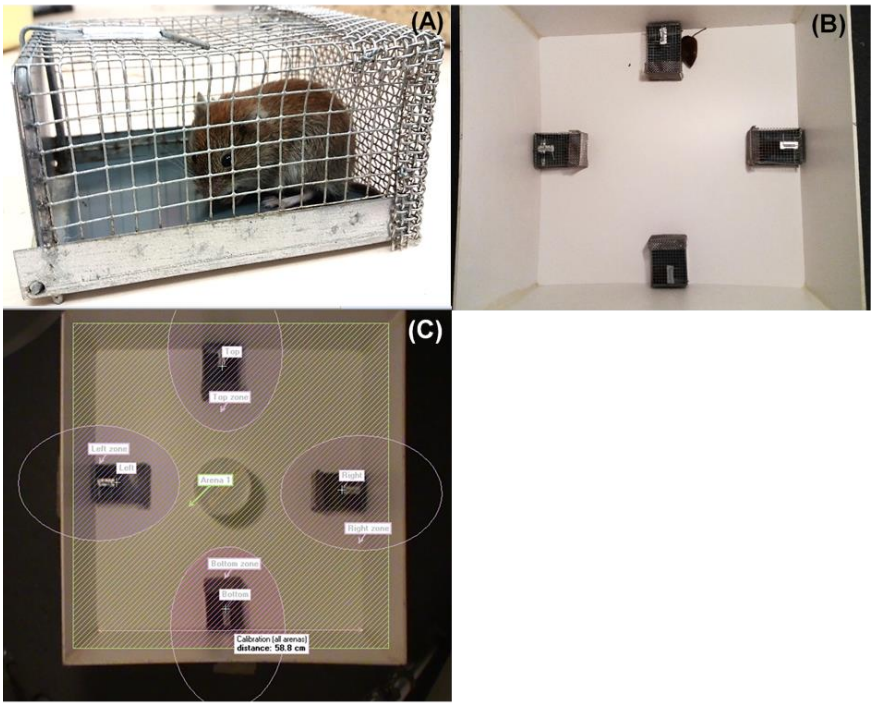

Figure 4. Female choice trials consisted of one male from each early life treatment (four in total); each put in a small mesh-wire cage and placed into a square open field arena. Cages were positioned randomly in the centre of each side of the arena. A female was introduced to the setup and allowed to move freely in the arena. The experiment lasted 20 minutes and was recorded with a video camera. (A) Male in the mesh-wire cage. (B) The orientation of the males in the female choice trials where the order of the males was randomised for every trial. (C) Showing the areas encircling the males (zones; purple ovals) created with a video tracking software for the female choice trials. To determine female preference the female's movements was recorded in the whole area of the arena (green square) and the number of entries and time spent in the zones were recorded by the tracking software.

2.8. Statistical analyses

All statistical analyses were done using R (R Core Team 2018). As multiple fixed and random factors were to be taken into account, mixed models were chosen as appropriate statistical tests. Due to the experimental design, both treatments (social confrontation and protein restriction) as well as their interaction (social confrontation*protein restriction) were always included as fixed factors in all the statistical models. All models were checked for the normality of residuals.

F1 generation male morphological measurements were analysed with linear mixed

models (LMM; package nlme; (Pinheiro *et al.* 2016)). Litter size was included as a covariate as litter size is known to have negative influence on the weight of the offspring. The F0 mother and batch number were set as random factors to account for relatedness and possible differences in environmental conditions due to date of birth.

F1 generation male reproductive success was measured as the proportion of pups an individual F1 male sired, divided by the total number of pups that male did not sire in that trial using a generalised linear mixed model (GLMM; package lme4; (Bates *et al.* 2015)) with binomial distribution (logit link). As the reproductive success trials were replicated, male trial number nested in the replicate was added as a random factor. To account for relatedness, the F0 mother was also included as a random factor.

F1 generation manually scored male behaviours were analysed with GLMMs (Table 1). In all behaviour analyses, the F0 mother and the male trial number were included as random factors to account for relatedness and for the non-independent environment due to the trial setup. The number of occurred unsupported and supported rearing as well as the number of jumps were analysed with Poisson GLMM with log link. The time spent self-grooming was measured as a proportion of the time spent grooming over the total experimental time (10min) and analysed using a binomial GLMM. Activity of an individual was determined as the total distance moved during the total experimental time and was analysed with LMM where one extreme outlier was removed from the data. The time spent close to the border walls of the arena (thigmotaxis) was analysed proportional to the total experiment time with binomial GLMM.

Female preference was determined by the number of visits a female made to any one male and by the duration a female spent in the vicinity of a male proportional to the total experiment time (20 min). The number of visits was analysed with Poisson GLMM and the duration of visits with binomial GLMM. To account for relatedness, the F0 mother was used as a random effect. Since the trials were replicated with different females, the male trial number nested in the replicate was also included in the random effects.

3. RESULTS

3.1. Morphological measurements

The early life treatments did not have a significant effect on the body mass of F1 males at birth (Table 2). Birth body mass of F1 males from the protein restriction group was on average 3.3% lighter compared to the birth body mass of the control group and males from the social confrontation group were on average 0.5% lighter. Males from the interaction group (protein restriction*social confrontation) were on average 1.2% heavier compared to the control group.

The early life treatments did not significantly affect the weanling body mass (Table 2). Based on model estimates, protein restricted males at weaning age weighed on average 4.3% less than the individuals in the control group, social confrontation group males were on average 2% lighter, and males receiving both treatments were 1% lighter.

Protein restricted males were significantly lower adult body mass, on average 6.4%, compared to the control individuals (Table 2). Other early life treatments did not have a significant effect on adult body mass. According to the model, social confrontation group males were on average 4.8% lighter whereas males receiving both treatments were 4.4% heavier than the control group individuals. In all age groups, litter size had a significant negative effect on the F1 males' body mass (Table 2). Based on the statistical model, the body mass decreased roughly 0.06g for every increase in litter size at birth, 0.27g in weanlings and 0.4g in adult individuals.

Table 2. Linear mixed model results of the effects of the early life environment treatments (protein restriction and/or social confrontation) and litter size on the body mass (in grams) of F1 generation bank vole males at birth (0d), at weaning age (20d) and adulthood (30d). Statistically significant (<0.05) results are in bold.

	F1 birthweight				F1 weaning weight (20d)				F1 adult weight (30d)			
Early life treatments	Est	S.E.	df	*P*	Est	S.E.	df	*P*	Est	S.E.	df	*P*
Intercept	2.22	0.06	189	**<0.001**	13.48	0.54	189	**<0.001**	20.67	0.74	189	**<0.001**
Protein restriction (PR)	-0.07	0.04	133	0.087	-0.59	0.38	133	0.130	-1.31	0.52	133	**0.013**
Social confrontation (SC)	0.01	0.04	133	0.809	-0.27	0.38	133	0.483	-1.00	0.51	133	0.055
Protein restriction by social confrontation (PR*SC)	-0.03	0.06	133	0.676	-0.14	0.56	133	0.805	0.90	0.77	133	0.248
Litter size	-0.06	0.01	133	**<0.001**	-0.27	0.10	133	**0.020**	-0.41	0.14	133	**0.006**

3.2. Reproductive success

The early life treatments did not have a significant effect on the F1 males' reproductive success (Table 3). The total number of F2 pups sired in the reproductive success trials was 356, of which the control treatment group F1 males sired 93, the social restriction group males sired 81, the protein restriction group males sired 76 and the interaction group males sired 106.

Table 3. Generalised linear mixed model results of the effects of the early life environment treatments (protein restriction and/or social confrontation) on the reproductive success of the F1 generation males. Reproductive success was determined as the proportion of pups sired by one male divided by the total number of pups sired in its trial that were not sired by the male. Statistically significant (<0.05) results are in bold. Data was used from years 2015 and 2016.

	Reproductive success			
Early life treatments	Est	S.E.	*n*	*P*
Intercept	-2.184	0.617	240	**<0.001**
Protein restriction (PR)	-0.789	0.660	240	0.232
Social confrontation (SC)	-0.892	0.873	240	0.307
Protein restriction by social confrontation (PR*SC)	2.175	1.146	240	0.058

3.3. Behaviour

3.3.1. Unsupported rearing

The early life environment treatments did not affect unsupported rearing behaviour (Table 4). Based on the statistical model, control group males reared unsupported on average 3.6 times. Both protein restriction and social confrontation group males reared unsupported 1.5

times more, on average, than the control males. Males that had received both early life treatments reared unsupported 0.4 times less compared to the control group.

3.3.2. Supported rearing

The early life treatments did not have a significant effect on supported rearing (Table 4). According to the model, supported rearing occurred on average 29.6 times in the control group. The protein restriction group males reared supported 1.1 times more and social confrontation group males 1.6 more than the control males. The males in the interaction group reared supported 0.5 less than the control males on average.

3.3.3. Jumping

The protein restriction treatment affected significantly to the number of jumps F1 males made (Table 4). Other early life treatments did not have a significant effect on the number of jumps. Based on model estimates, jumping occurred on average 0.2 times in the control group. Protein restricted males jumped significantly more, on average 5.8 times more, compared to the control males. Social confrontation group males jumped on average 3.4 times more than control individuals whereas the interaction group males jumped 0.1 times less compared to the control group.

3.3.4. Self-grooming

The early life treatments did not significantly affect the time spent grooming during the experiment (Table 4). According to model estimates, the both control group males and protein restricted males spent on average 4.5% of the experimental time grooming. The social confrontation group males groomed themselves the least, on average 2.5% of the total experimental time. The interaction group males spent 3.8% of the experimental time self-grooming.

3.3.5. Thigmotaxis

Social confrontation treatment had a significant effect on the time spent along the arena wall (thigmotaxis) during the total experimental time (Table 5). Other early life treatments did not affect thigmotaxis significantly. Based on model estimates, control males spent 89.1% of the total experimental time close to the wall on average. Protein restriction group males spent the most time along the arena wall of all the treatment groups, 93.5% on average. Social confrontation group males spent significantly less time along the arena walls, 69.9% on average. The interaction group males spent 86.4% of the total experimental time along the walls on average.

3.3.6. Activity

Social confrontation treatment increased the total distance moved during the experiment (Table 5). Other early life treatments did not have a significant effect. According to the model estimates, the protein restricted males covered on average 9.2% shorter distance than the control males during the experiment. Social confrontation group males covered significantly longer distance (40.6%) than the control males, whereas the interaction group were the least active covering 38.3% shorter distance on average compared to the control group.

Table 4. GLMMs of the effects of the early life environment treatments (protein restriction and/or social confrontation) on the behaviour of the F1 generation males. Behaviour experiments were conducted using the open field test. Four types of behaviour were recorded: unsupported rearing (standing on hind legs unsupported), supported rearing (standing on hind legs, front paws supported against the arena wall) and jumping (all legs off the ground) and self-grooming (licking face/body), where the behaviours were determined as the number of times (counts) the behaviour occurred during the total experiment time (10min) and as the duration of the occurred behaviour over the total experiment time (proportion), respectively. Statistically significant (<0.05) results are in bold.

	Unsupported rearing				Supported rearing				Jumping				Self-grooming			
Early life treatments	Est	S.E.	*n*	*P*	Est	S.E.	*n*	*P*	Est	S.E.	*n*	*P*	Est	S.E.	*n*	*P*
Intercept	1.267	0.491	69	**0.009**	3.388	0.224	69	**<0.001**	-1.671	0.722	69	**0.021**	-3.060	0.659	69	**<0.001**
Protein restriction (PR)	0.432	0.576	69	0.453	0.089	0.269	69	0.740	1.756	0.791	69	**0.026**	-0.005	0.769	69	0.994
Social confrontation (SC)	0.427	0.587	69	0.466	0.465	0.276	69	0.092	1.216	0.825	69	0.141	-0.592	0.807	69	0.463
Protein restriction by social confrontation (PR*SC)	-0.841	0.856	69	0.325	-0.752	0.407	69	0.064	-2.128	1.126	69	0.058	0.417	1.146	69	0.716

Table 5. GLMM and LMM of the effects of the early life environment treatments (protein restriction and/or social confrontation) on the behaviour of the F1 generation males. Behaviour experiments were conducted using open field test. Thigmotaxis (being close to the arena wall) was determined as of time spent close to the arena wall proportional to the total experimental time. Activity was defined by the total distance (cm) an individual moved during the experimental time. A clear outlier was removed from the activity data. Statistically significant (<0.05) results are in bold.

	Thigmotaxis				Activity			
Early life treatments	Est	S.E.	*n*	*P*	Est	S.E.	*n*	*P*
Intercept	2.105	0.529	69	**<0.001**	3131.915	445.230	68	**<0.001**
Protein restriction (PR)	0.565	0.560	69	0.313	-289.425	596.771	68	0.632
Social confrontation (SC)	-1.268	0.570	69	**0.026**	1272.409	614.275	68	**0.048**
Protein restriction by social confrontation (PR*SC)	0.445	0.873	69	0.610	-1199.448	856.065	68	0.169

3.4. Female choice

3.4.1. Number of visitations

No significant effect of early life treatment on the number of female visitations was found (Table 6). Based on the statistical model, females visited the control males 4.8 times on average. Females visited the protein restricted males 1.02 times more and social confrontation group males 1.1 times more compared to the control males. However, females visited the interaction group males 0.89 times less than the control males.

3.4.2. Duration of visitations

The early life treatments did not have a significant effect on the duration of the female visitations (Table 6). According to the statistical model, females spent, on average, 10.4% of the total experimental time in the vicinity of the control males. Females spent most time in the vicinity of social confrontation group males, on average 13.4% of the total experimental time whereas the least time was spent in the vicinity of the protein restricted males, 7.4% on average. The males that had received both treatments were visited by the females, on average, 12.3% of the total experimental time.

Table 6. The effect of the early life environment treatments (protein restriction and/or social confrontation) on female choice. Female choice was determined as the number of visitations a female made to each of the F1 treatment males (one of each) and as the duration of those visits. Statistically significant (<0.05) results are in bold.

	Female choice							
	Number of visitations				Duration of visitations			
Early life treatments	Est	S.E.	*n*	*P*	Est	S.E.	*n*	*P*
Intercept	1.572	0.232	68	**<0.001**	-2.155	0.467	68	**<0.001**
Protein restriction (PR)	0.018	0.123	68	0.885	-0.373	0.645	68	0.563
Social confrontation (SC)	0.096	0.122	68	0.435	0.289	0.645	68	0.654
Protein restriction by social confrontation (PR*SC)	-0.115	0.174	68	0.508	0.278	0.911	68	0.760

4. DISCUSSION

This study was designed to explore the effects of the early life environment on certain phenotypic characteristics (morphology and behaviour) and fitness traits (reproductive success and attractiveness) of male bank voles (*Myodes glareolus*). Here, I find that some F1 male phenotypic traits, but not fitness traits, were affected by different early life environment treatments.

4.1. Effects of early life environment on F1 male body mass

None of the early life environment treatments had a significant effect on F1 male body mass at birth or at weaning age. This could indicate that maternal provisioning buffers the effects of any nutritional deficit. Alternatively, this thesis suggests that the effects of

stressful environment during pre- and early postnatal development become more apparent later in an individual's lifetime.

The effects of the early life environment on the F1 male body mass did not become evident until adulthood, where protein restriction significantly reduced body mass. The reduction in adult body mass due to protein restriction treatment was expected and is consistent with several previous laboratory studies (Zambrano *et al.* 2006; Zhang *et al.* 2010; Reyes-Castro *et al.* 2012; Belluscio *et al.* 2014)even though compensatory (or catch-up) growth can occur when conditions improve (reviewed in Arendt 1997; Metcalfe & Monaghan 2001). For the bank vole ecology, this would indicate that protein restriction, *i.e.* a high population density, during early life can result in populations exhibiting lower body mass. However, this is contradictory to the findings in natural populations where high population density is associated with heavier individuals (Yoccoz et al. 2000). This contradiction might indicate that, although food is an important factor for bank vole population, protein restriction has less impact, even in high population densities, or possibly interact with another ecological factor not tested in this study.

Maternally derived social confrontation during F1 male early life did not have a statistically significant effect on the F1 male body mass, although there was a negative trend on F1 adult mass owing to social confrontation. As the frequency of social confrontations is expected to increase with an increase in population density, this could lead to a decrease in territory size (Wolff 1985; Koskela *et al.* 1997, 1999; Jonsson *et al.* 2002). With fewer resources to deploy, this could lead to reductions in body mass although this reduction is not evident in natural populations.

While not significant, the presence of both early life treatments (protein restriction and social confrontation) displayed a positive trend with adult body mass. This finding of increased body mass is more in line with previous findings in natural populations, where high density populations exhibit heavier individuals than during low population densities. The result could indicate adaptiveness to high population density environment, as large body mass positively correlates with other traits previously linked to dominance, higher reproductive success and female preference in male bank voles (Horne and Ylönen 1996, 1998) although the interdependences between the traits remain to be ascertained. Investment in larger body mass could be advantageous in environments where females have a vast number of potential mates to choose from and consequently can afford to be choosier.

4.2. Effects of early life environment on F1 male behaviour

Early life environment treatments did not have a significant effect on exploratory behaviour of F1 males. However, protein restriction significantly increased jumping, related to anxiety behaviour, in the F1 males. Jumping is not commonly used in behavioural studies as a proxy for anxiety related behaviour so comparing results is difficult. From observations, this jumping behaviour did not seem consistent with exploratory behaviour but was more sporadic and random which indicates a more anxiety based explanation for the behaviour. From an evolutionary perspective, changes in coping behaviour are an individual's response against challenging circumstances; whether to avoid threats or exploit scarce resources. Implications for vole ecology could be that protein restriction in early life causes an increase in anxiety related behaviours, and as such individuals would be more susceptible to environmental stimuli. This in turn could help with e.g. predator avoidance, avoiding conflict with conspecifics as smaller males would be more likely to suffer injury in fight with other males or through being more alert for sudden changes in the surroundings increasing their survival probability.

The time spent along the wall (thigmotaxis) and the total distance moved were both significantly affected by the social confrontation treatment. Social confrontation treatment affected negatively to the amount of time spent along the arena wall over the overall experimental time indicating that social confrontation treated males were less fearful under novel circumstances. The total distance moved during the experiment was significantly increased by the social confrontation treatment indicating higher overall activity. In high population density conditions, the tendency for bold behaviour would be advantageous when competition for mates and food is high. Boldness could help males find new food sources first and get access to more females. General activity was also higher in socially confronted males. This in turn could help with resource gathering by covering larger area when there are more conspecifics to compete with.

4.3. Effects of early life environment on the attractiveness and reproductive success of F1 males

There was no statistically significant effect between any of the early life treatments and female preference. Moreover, the early life treatments did not have a statistically significant effect on the F1 male reproductive success. An interesting note was, however, how both social confrontation treatment and protein restriction treatment separately had a decreasing trend on the F1 male reproductive success, but when experienced together; their interaction increased the F1 males' reproductive success. It is possible that this is related to the increase in body mass.

Nevertheless, there was no statistically significant preference or increased reproductive success was found due to early life treatments. This is somewhat surprising as larger bank voles would be expected to be more dominant and thus secure more matings with the females resulting in better reproductive success. Possibly, this is due to the fact that males were intentionally put in the same weight class together in the reproductive success trials and subsequently in the female choice trials and females were not able to pick up on this small difference. Also, no direct male-male competition, which is an important factor in female mate choice, was able to take place due to our female choice trial setup. It is possible that without direct competition between males, dominance rank could not be established and thus females could not differentiate between males. Alternatively, it is possible that our reproductive success trial setup did not allow females to escape males, and hence they were not able to escape unwanted males.

Earlier research on the effects of prenatal social stress on the attractiveness of male bank voles has found that prenatal social stress had a non-significant negative effect on male bank vole attractiveness (Marchlewska-Koj et al. 2003) whereas this study indicates the opposite, that early life social confrontation in male bank voles has a non-significant positive effect on their attractiveness. Even though the methods were not identical between these two studies, in conjunction, these non-significant results in opposite directions suggest that social confrontation during early life of male bank voles does not affect their attractiveness to females. Moreover, female preference on the other early life treatments did not significantly differ from the control.

For the male bank vole life history, my findings indicate that neither the quality of nutrition (protein content) nor the social environment during the early life development have much impact on the males reproductive success and therefore their fitness. Without having an impact on the individual's fitness, the differences in phenotypical responses to adverse early life environments in male bank voles cannot have evolutionary implications. However, reproductive success is only one part of an individual's life-history. It is possible that these early life environments could have had effects on the males over winter survival, which is another important aspect in bank vole's ecology. If any of the early life

environments positively affected the over winter survival, it could mean better chances of reproduction during spring breeding season as only a small number of individuals survives the winter. Thus, to properly determine the adaptive potential of the effects of an individual's early life environment, researchers should adopt a broader perspective integrated to the organism's life-history, examine their results both in short- and long-term environmental context and over individual's lifetime.

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REFERENCES

Almeida S.S., Tonkiss J. & Galler J.R. 1996. Prenatal protein malnutrition affects exploratory behavior of female rats in the elevated plus-maze test. *Physiology and Behavior* 60: 675–680.

Arendt J.D. 1997. Adaptive Intrinsic Growth Rates: An Integration Across Taxa. *The Quarterly Review of Biology* 72: 149–177.

Atinmo T., Pond W.G. & Barnes R.H. 1974. Effect of maternal energy vs. protein restriction on growth and development of progeny in swine. *Journal of Animal Science* 39: 703–711.

Barbazanges A., Piazza P. V, Moal M. Le & Maccari S. 1996. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16: 3943–3949.

Bates D., Maechler M., Bolker B. & Walker S. 2015. Fitting Linear Mixed-Effects Models using lme4. *Journal of Statistical Software* 67: 1–48.

Beaman J.E., White C.R. & Seebacher F. 2016. Evolution of Plasticity: Mechanistic Link between Development and Reversible Acclimation. *Trends in Ecology and Evolution* 31: 237–249.

Belluscio L.M., Berardino B.G., Ferroni N.M., Ceruti J.M. & Cánepa E.T. 2014. Early protein malnutrition negatively impacts physical growth and neurological reflexes and evokes anxiety and depressive-like behaviors. *Physiology and Behavior* 129: 237–254.

Bujalska G. 1985. Regulation of female maturation in Clethrionomys species, with special reference to an island population of C. glareolus. *Annales Zoologici Fennici* 22: 331–342.

Burt W.H. 1943. Territoriality and Home Range Concepts as Applied to Mammals. *Journal of Mammalogy* 24: 346.

Burton T. & Metcalfe N.B. 2014. Can environmental conditions experienced in early life influence future generations? *Proceedings of the Royal Society B: Biological Sciences* 281: 20140311.

Clinchy M., Zanette L., Boonstra R., Wingfield J.C. & Smith J.N.M. 2004. Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings of the Royal Society B: Biological Sciences* 271: 2473–2479.

Creel S. 2001. Social dominance and stress hormones. *Trends in Ecology and Evolution* 16: 491–497.

Creel S., Dantzer B., Goymann W. & Rubenstein D.R. 2013. The ecology of stress: Effects of the social environment. *Functional Ecology* 27: 66–80.

Dantzer B., Newman A.E.M., Boonstra R., Palme R., Boutin S., Humphries M.M. & McAdam A.G. 2013. Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science* 340: 1215–1217.

Desy E.A. & Batzli G.O. 1989. Effects of food availability and predation on prairie vole demography: a field experiment. *Ecology* 70: 411–421.

Drummond H. & Ancona S. 2015. Observational field studies reveal wild birds responding to early-life stresses with resilience, plasticity, and intergenerational effects. *The Auk* 132: 563–576.

Fowden A.L. & Forhead A.J. 2004. Endocrine mechanisms of intrauterine programming. *Reproduction* 127: 515–526.

Glover V., O'Connor T.G. & O'Donnell K. 2010. Prenatal stress and the programming of the HPA axis. *Neuroscience and Biobehavioral Reviews* 35: 17–22.

Gockel J., Harr B., Schlotterer C., Arnold W., Gerlach G. & Tautz D. 1997. Isolation and characterization of microsatellite loci from Apodemus flavicollis (rodentia, muridae) and Clethrionomys glareolus (rodentia, cricetidae). *Molecular Ecology* 6: 597–599.

Golden S.A., Covington H.E., Berton O. & Russo S.J. 2011. A standardized protocol for repeated social defeat stress in mice. *Nature Protocols* 6: 1183–1191.

Gould T.D., Dao D.T. & Kovacsics C.E. 2009. The Open Field Test. In: *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests*, pp. 1–20.

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. *Biology Bulletin* 40: 114–118.

Hayward L.S. & Wingfield J.C. 2004. Maternal corticosterone is transferred to avian yolk and may alter offspring growth and adult phenotype. *General and Comparative Endocrinology* 135: 365–371.

Holst D. von. 1998. The Concept of Stress and Its Relevance for Animal Behavior. In: *Advances in the Study of Behavior*, pp. 1–131.

Horne T.J. & Ylönen H. 1996. Female bank voles (Clethrionomys glareolus) prefer dominant males; but what if there is no choice? *Behavioral Ecology and Sociobiology* 38: 401–405.

Horne T.J. & Ylönen H. 1998. Heritabilities of Dominance-Related Traits in Male Bank Voles (Clethrionomys glareolus). *Evolution* 52: 894–899.

Hsueh A.M., Agustin C.E. & Chow B.F. 1967. Growth of Young Rats after Differential Manipulation of Maternal Diet. *The Journal of Nutrition* 91: 195–200.

Innes D.G.L. & Millar J.S. 1994. Life histories of Clethrionomys and Microtus (Microtinae). *Mammal Review* 24: 179–207.

Jones E.N. 1990. Effects of Forage Availability on Home Range and Population Density of Microtus pennsylvanicus. *Journal of Mammalogy* 71: 382–389.

Jonsson P., Hartikainen T., Koskela E. & Mappes T. 2002. Determinants of reproductive success in voles: Space use in relation to food and litter size manipulation. *Evolutionary Ecology* 16: 455–467.

Kalinowski S.T., Taper M.L. & Marshall T.C. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16: 1099–1106.

Kallio E.R., Begon M., Henttonen H., Koskela E., Mappes T., Vaheri A. & Vapalahti O. 2009. Cyclic hantavirus epidemics in humans - Predicted by rodent host dynamics. *Epidemics* 1: 101–107.

Kaufmann J.H. 1983. On the definitions and functions of dominance and territoriality. *Biological Reviews* 58: 1–20.

Kloet E.R. De, Sibug R.M., Helmerhorst F.M. & Schmidt M. 2005. Stress, genes and the mechanism of programming the brain for later life. *Neuroscience and Biobehavioral Reviews* 29: 271–281.

Koivula M., Koskela E., Mappes T. & Oksanen T.A. 2003. Cost of reproduction in the wild: Manipulation of reproductive effort in the bank vole. *Ecology* 84: 398–405.

Konieczna J., García A.P., Sánchez J., Palou M., Palou A. & Picó C. 2013. Oral leptin treatment in suckling rats ameliorates detrimental effects in hypothalamic structure and function caused by maternal caloric restriction during gestation. *PLoS ONE* 8: 1–14.

Korpela K., Delgado M., Henttonen H., Korpimäki E., Koskela E., Ovaskainen O., Pietiäinen H., Sundell J., Yoccoz N.G. & Huitu O. 2013. Nonlinear effects of climate on boreal rodent dynamics: Mild winters do not negate high-amplitude cycles. *Global Change Biology* 19: 697–710.

Koskela E., Jonsson P., Hartikainen T. & Mappes T. 1998. Limitation of reproductive success by food availability and litter size in the bank vole, Clethrionomys glareolus. *Proceedings of the Royal Society B: Biological Sciences* 265: 1129–1134.

Koskela E., Mappes T. & Ylönen H. 1997. Territorial behaviour and reproductive success of bank vole Clethrionomys glareolus females. *Journal of Animal Ecology* 66: 341–349.

Koskela E., Mappes T. & Ylönen H. 1999. Experimental manipulation of breeding density and litter size: effects on reproductive success in the bank vole. *Journal of Animal Ecology* 68: 513–521.

Krebs C.J., Boutin S., Boonstra R., Sinclair A.R.E., Smith J.N.M., Dale M.R.T., Martin K. & Turkington R. 1995. Impact of Food and Predation on the Snowshoe Hare Cycle. *Science* 269: 1112–1115.

Kruczek M. 1997. Male rank and female choice in the bank vole, Clethrionomys glareolus. *Behavioural Processes* 40: 171–176.

Kruczek M. & Marchlewska-Koj A. 1986. Puberty Delay of Bank Vole Females in a High-Density Population. *Biology of Reproduction* 35: 537–541.

Landys M.M., Goymann W. & Slagsvold T. 2011. Rearing conditions have long-term consequences for stress responsiveness in free-living great tits. *General and Comparative Endocrinology* 174: 219–224.

Laus M.F., Vales L.D.M.F., Costa T.M.B. & Almeida S.S. 2011. Early postnatal protein-calorie malnutrition and cognition: A review of human and animal studies. *International Journal of Environmental Research and Public Health* 8: 590–612.

Love O.P., Chin E.H., Wynne-Edwards K.E. & Williams T.D. 2005. Stress Hormones: A Link between Maternal Condition and Sex-Biased Reproductive Investment. *The American Naturalist* 166: 751–766.

Love O.P., Gilchrist H.G., Bêty J., Wynne-Edwards K.E., Berzins L. & Williams T.D. 2009. Using life-histories to predict and interpret variability in yolk hormones. *General and Comparative Endocrinology* 163: 169–174.

Love O.P., Mcgowan P.O. & Sheriff M.J. 2012. Maternal adversity and ecological stressors in natural populations: The role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology* 27: 81–92.

Love O.P. & Williams T.D. 2008. Plasticity in the adrenocortical response of a free-living vertebrate: The role of pre- and post-natal developmental stress. *Hormones and Behavior* 54: 496–505.

Maher C.R. & Lott D.F. 2006. A Review of Ecological Determinants of Territoriality within Vertebrate Species. *The American Midland Naturalist* 143: 1–30.

Mappes T., Koivula M., Koskela E., Oksanen T.A., Savolainen T. & Sinervo B. 2008. Frequency and density-dependent selection on life-history strategies - A field experiment. *PLoS ONE* 3: e1687.

Mappes T. & Koskela E. 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution* 58: 645–650.

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

Marshall D.J. & Uller T. 2007. When is a maternal effect adaptive? *Oikos* 116: 1957–1963.

Martinez M., Calvo-Torrent A. & Pico-Alfonso M.A. 1998. Social defeat and subordination as models of social stress in laboratory rodents: A review. *Aggressive Behavior* 24: 241–256.

Mccormick M.I. 1998. Behaviorally induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology* 79: 1873–1883.

McCormick M.I. 1999. Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia* 118: 412–422.

Meaney M.J., Szyf M. & Seckl J.R. 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends in Molecular Medicine* 13: 269–277.

Metcalfe N.B. & Monaghan P. 2001. Compensation for a bad start: Grow now, pay later? *Trends in Ecology & Evolution* 16: 254–260.

Meylan S. & Clobert J. 2005. Is corticosterone-mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. *Hormones and Behavior* 48: 44–52.

Mills S.C., Grapputo A., Jokinen I., Koskela E., Mappes T., Oksanen T.A. & Poikonen T. 2009.

Testosterone-Mediated Effects on Fitness-Related Phenotypic Traits and Fitness. *The American Naturalist* 173: 475–487.

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. *Proceedings of the Royal Society B: Biological Sciences* 274: 143–150.

Mousseau T. & Fox C.W. 1998. The adaptive significance of maternal effects. *Trends in Ecology & Evolution* 13: 403–407.

Nieminen P., Huitu O., Henttonen H., Finnilä M.A.J., Voutilainen L., Itämies J., Kärjä V., Saarela S., Halonen T., Aho J. & Mustonen A.M. 2015. Physiological condition of bank voles (Myodes glareolus) during the increase and decline phases of the population cycle. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology* 187: 141–149.

Noldus L.P.J.J., Spink A.J. & Tegelenbosch R.A.J. 2001. EthoVision: A versatile video tracking system for automation of behavioral experiments. *Behavior Research Methods, Instruments, and Computers* 33: 398–414.

Norrdahl K. & Korpimäki E. 2002. Changes in individual quality during a 3-year population cycle of voles. *Oecologia* 130: 239–249.

Oksanen T.A., Koivula M., Koskela E. & Mappes T. 2007. The cost of reproduction induced by body size at birth and breeding density. *Evolution* 61: 2822–2831.

Paaby A.B. & Testa N.D. 2018. Developmental Plasticity and Evolution. In: *Evolutionary Developmental Biology: A Reference Guide*, pp. 1–14.

Pinheiro J., Bates D., DebRoy S. & Sarkar D. 2016. nlme: Linear and Nonlinear Mixed Effects Models. *R package version 3.1-128*.

Pravosudov V. V. & Kitaysky A.S. 2006. Effects of nutritional restrictions during post-hatching development on adrenocortical function in western scrub-jays (Aphelocoma californica). *General and Comparative Endocrinology* 145: 25–31.

Preisser E.L. 2009. The physiology of predator stress in free-ranging prey. *Journal of Animal Ecology* 78: 1103–1105.

Prévot-Julliard A.C., Henttonen H., Yoccoz N.G. & Stenseth N.C. 1999. Delayed maturation in female bank voles: Optimal decision or social constraint? *Journal of Animal Ecology* 68: 684–697.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*.

Radwan J., Tkacz A. & Kloch A. 2008. MHC and preferences for male odour in the bank vole. *Ethology* 114: 827–833.

Ratkiewicz M. & Borkowska A. 2000. Multiple paternity in the bank vole (Clethrionomys glareolus): field and experimental data. *Zeitschrift für Säugetierkunde-International Journal of Mammalian Biology* 65: 6–14.

Reyes-Castro L.A., Rodriguez J.S., Charco R., Bautista C.J., Larrea F., Nathanielsz P.W. & Zambrano E. 2012. Maternal protein restriction in the rat during pregnancy and/or lactation alters cognitive and anxiety behaviors of female offspring. *International Journal of Developmental Neuroscience* 30: 39–45.

Rikalainen K., Grapputo A., Knott E., Koskela E. & Mappes T. 2008. A large panel of novel microsatellite markers for the bank vole (Myodes glareolus). *Molecular Ecology Resources* 8: 1164–1168.

Saino N., Romano M., Ferrari R.P., Martinelli R. & Møller A.P. 2005. Stressed mothers lay eggs with high corticosterone levels which produce low-quality offspring. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 303: 998–1006.

Schirmer A., Herde A., Eccard J.A. & Dammhahn M. 2019. Individuals in space: personality-dependent space use, movement and microhabitat use facilitate individual spatial niche specialization. *Oecologia* 189: 647–660.

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 Evolutionary Biology* 12: 44.

Seckl J.R. 2004. Prenatal glucocorticoids and long-term programming. *European journal of*

endocrinology 151: U49–U62.
Sheriff M.J., Krebs C.J. & Boonstra R. 2009. The sensitive hare: Sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology* 78: 1249–1258.
Sheriff M.J., Krebs C.J. & Boonstra R. 2010. The ghosts of predators past: Population cycles and the role of maternal programming under fluctuating predation risk. *Ecology* 91: 2983–2994.
Sheriff M.J. & Love O.P. 2013. Determining the adaptive potential of maternal stress. *Ecology Letters* 16: 271–280.
Sinervo B. & DeNardo D.F. 1996. Costs of Reproduction in the Wild: Path Analysis of Natural Selection and Experimental Tests of Causation. *Evolution* 50: 1299–1313.
Sloboda D.M., Howie G.J., Pleasants A., Gluckman P.D. & Vickers M.H. 2009. Pre- and postnatal nutritional histories influence reproductive maturation and ovarian function in the rat. *PLoS ONE* 4: 1–8.
Stenseth N.C. 1985. Geographic distribution of Clethrionomys species. *Annales Zoologici Fennici* 22: 215–219.
Strasser R. & Schwabl H. 2004. Yolk testosterone organizes behavior and male plumage coloration in house sparrows (Passer domesticus). *Behavioral Ecology and Sociobiology* 56: 491–497.
Tschirren B. 2015. Differential effects of maternal yolk androgens on male and female offspring: A role for sex-specific selection? *PLoS ONE* 10: e0133673.
Weaver I.C.G., Cervoni N., Champagne F.A., D'Alessio A.C., Sharma S., Seckl J.R., Dymov S., Szyf M. & Meaney M.J. 2004. Epigenetic programming by maternal behavior. *Nature Neuroscience* 7: 847–854.
Weinstock M. 2008. The long-term behavioural consequences of prenatal stress. *Neuroscience and Biobehavioral Reviews* 32: 1073–1086.
Whitaker K.W., Totoki K. & Reyes T.M. 2012. Metabolic adaptations to early life protein restriction differ by offspring sex and post-weaning diet in the mouse. *Nutrition, Metabolism and Cardiovascular Diseases* 22: 1067–1074.
Wolff J.O. 1985. The effects of density, food, and interspecific interference on home range size in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Canadian Journal of Zoology* 63: 2657–2662.
Woodroffe R. & Vincent A. 1994. Mother's little helpers: Patterns of male care in mammals. *Trends in Ecology and Evolution* 9: 294–297.
Zambrano E., Bautista C.J., Deás M., Martínez-Samayoa P.M., González-Zamorano M., Ledesma H., Morales J., Larrea F. & Nathanielsz P.W. 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. *Journal of Physiology* 571: 221–230.
Zambrano E., Rodríguez-González G.L., Guzmán C., García-Becerra R., Boeck L., Díaz L., Menjivar M., Larrea F. & Nathanielsz P.W. 2005. A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. *Journal of Physiology* 563: 275–284.
Zhang Y., Li N., Yang J., Zhang T. & Yang Z. 2010. Effects of maternal food restriction on physical growth and neurobehavior in newborn Wistar rats. *Brain Research Bulletin* 83: 1–8.