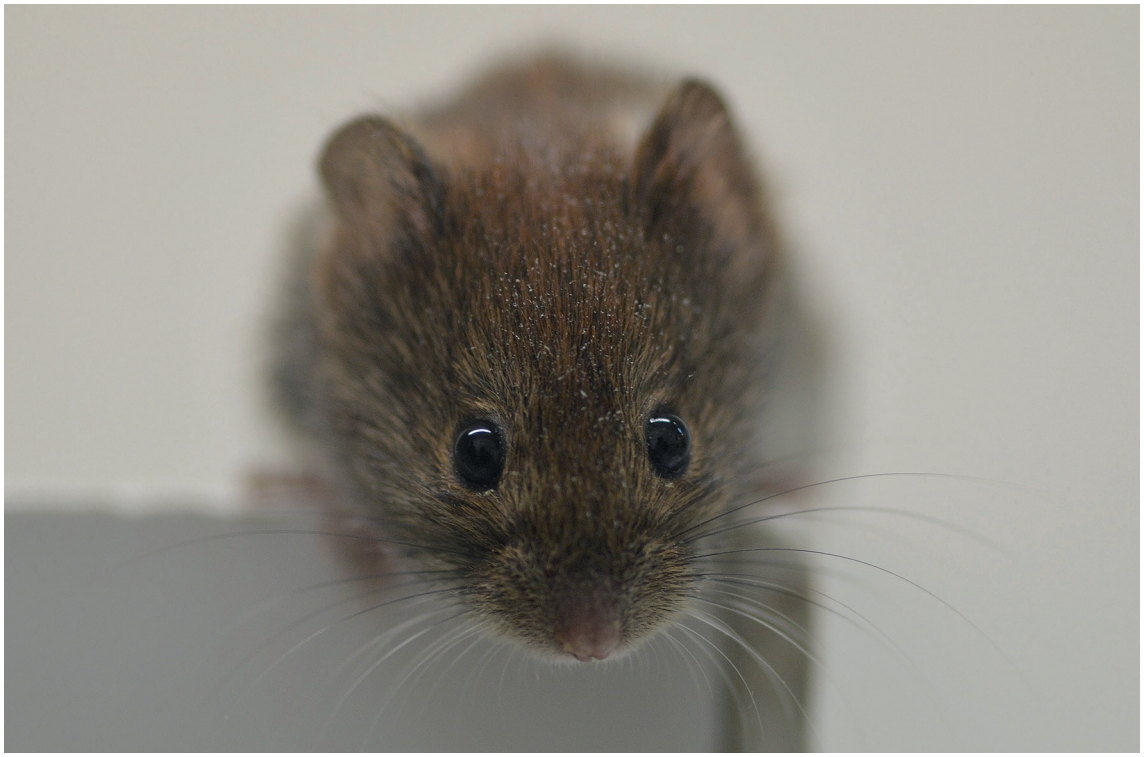
JYU DISSERTATIONS 119

Joannes van Cann

Intergenerational Responses to a Changing Environment

Maternal and Paternal Early Life Shape Fitness Components in the Bank Vole (*Myodes glareolus*)



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

JYU DISSERTATIONS 119

Joannes van Cann

Intergenerational Responses to a Changing Environment

Maternal and Paternal Early Life Shape Fitness Components in the Bank Vole (*Myodes glareolus*)

Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen luentosalissa YAA303
syyskuun 28. päivänä 2019 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
in building Ambiotica, hall YAA303, on September 28, 2019 at 12 o'clock noon.

JYVÄSKYLÄN YLIOPISTO
UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2019

Editors
Jari Haimi
Department of Biological and Environmental Science, University of Jyväskylä
Ville Korkiakangas
Open Science Centre, University of Jyväskylä

Cover photo by Joannes van Cann.

Permanent link to this publication: http://urn.fi/URN:ISBN:978-951-39-7834-1

ISBN 978-951-39-7834-1 (PDF)
URN:ISBN:978-951-39-7834-1
ISSN 2489-9003

Dedicated to the two most important women in my life

ABSTRACT

Van Cann, Joannes
Intergenerational responses to a changing environment: maternal and paternal early life shape fitness components in the bank vole (*Myodes glareolus*)
Jyväskylä: University of Jyväskylä, 2019, 56 p.
(JYU Dissertations
ISSN 2489-9003; 119)
ISBN 978-951-39-7834-1 (PDF)
Diss.

An individual's early life environment can have a significant influence over the adult phenotype, and these effects can potentially extend to their offspring. From an eco-evolutionary standpoint, the question arises whether these intergenerational effects can be adaptive *i.e.* increase offspring fitness. As most mammals lack paternal care, the most likely pathway for intergenerational effects is through the maternal line, as the mother provides the intrauterine environment but is also responsible for the post-natal environment. However, there is increasing evidence for paternal environment-dependent effects. In this thesis, it was explored how the early life environment of a wild rodent can have intra- and intergenerational effects, both through the maternal and paternal line. Bank voles (*Myodes glareolus*) were used to explore early life intergenerational effects by receiving high population density cues during their pre- and postnatal life and their growth and reproductive success were measured. Both in the maternal and paternal experiment, intergenerational effects and offspring fitness were assessed in semi-natural outdoor enclosures. Through the maternal line, the adaptive value was investigated by placing the offspring in different population densities. Through the paternal line, the focus was to determine *if* there is evidence for paternal intergenerational effects on offspring. The results showed significant effects on offspring fitness through the maternal and paternal line, with maternal effects being potentially adaptive. Additionally, two molecular mechanisms were explored, gene methylation and ribosomal RNA gene copy number. While no clear relationship between the early life and gene methylation was found, ribosomal RNA gene copy number was significantly related to early life protein diet. This thesis provides concrete evidence that the environment in which an individual is born directly affects the fitness of their offspring, and these effects can potentially be adaptive, at least through the maternal line.

Keywords: Bank vole; early life; intergenerational; maternal effects; paternal effects; rRNA.

Joannes Van Cann, University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland

TIIVISTELMÄ

Van Cann, Joannes
Vaikuttaako muuttuva ympäristö tuleviin sukupolviin? Metsämyyrän varhainen kasvuympäristö säätelee sen jälkeläisten elämää monen sukupolven päähän.
Jyväskylä: University of Jyväskylä, 2019, 56 p.
(JYU Dissertations
ISSN 2489-9003; 119)
ISBN 978-951-39-7834-1 (PDF)
Diss.

Yksilön varhainen kasvuympäristö voi vaikuttaa merkittävästi siihen, millaiseksi aikuinen yksilö kehittyy ja edelleen siihen, minkä laatuisia jälkeläisiä se tuottaa seuraaviin sukupolviin. Evolutiivisesti mielenkiintoinen kysymys on, ovatko nämä yli sukupolvien siirtyvät vaikutukset adaptiivisia. Useimmilla nisäkkäillä isät eivät hoida poikasia, joten emon kasvuympäristö vaikuttaa merkittävämmin sen poikasten menestykseen. Tässä väitöskirjassa tutkittiin kokeellisesti, onko äidin ja isän varhaisella kasvuympäristöllä sukupolvien yli siirtyviä vaikutuksia jälkeläisiin. Tutkimuksissa manipuloitiin korkeaan populaatiotiheyteen liittyvää kasvuympäristöä käyttäen malliorganismina metsämyyrää (*Myodes glareolus*). Kokeissa emoja ja isiä altistettiin sosiaaliselle stressille ja/tai heikkolaatuisemmalle ravinnolle. Myöhemmin testattiin, oliko näillä manipulaatioilla vaikutusta siihen, miten hyvin poikaset selviytyvät vastaavista korkeaan populaatiotiheyteen liittyvistä stresseistä. Tulokset osoittivat, että sekä emon että isän varhainen kasvuympäristö vaikutti niiden poikasten myöhempään menestykseen. Erityisesti äidin kasvuympäristön vaikutukset viittasivat siihen, että nämä emovaikutukset ovat adaptiivisia, eli ne auttavat poikasia sopeutumaan ympäristöön. Lisäksi testattiin epigeneettisiä mekanismeja (geenien metylaatio ja ribosomaalisen RNA:n geenien kopiolukumäärä), jotka voisivat selittää miten ei-hoitavan isän kasvuympäristö voisi vaikuttaa sen siittämiin poikasiin. Tämä tutkimus antoi viitteitä siitä, että ribosomaalisen RNA:n geenien kopiolukumäärä voi muuttua isän altistuessa sosiaaliselle stressille. Tulevissa tutkimuksissa on testattava, onko tämä havainto nyt löydettyjä isävaikutuksia selittävä geneettinen mekanismi. Väitöskirjan tulokset osoittivat, että sekä emon että isän varhaiset kasvuympäristöt vaikuttavat selkeästi niiden poikasten myöhempään kelpoisuuteen. Lisäksi varsinkin äidin välittämät vaikutukset poikasiin näyttivät olevan adaptiivisia.

Avainsanat: Emovaikutukset; isävaikutukset; Metsämyyrä; ribosomi-RNA; varhainen kasvuympäristö; ylisukupolviset vaikutukset.

Joannes Van Cann, Jyväskylän yliopisto, Bio- ja ympäristötieteiden laitos, PL 35, 40014 Jyväskylän yliopisto

Author's address Joannes van Cann
Department of Biological and Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä
Finland
Joannes.j.vancann@jyu.fi

Supervisors Docent Esa Koskela
Department of Biology & Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä, Finland

Docent Tapio Mappes
Department of Biology & Environmental Science
P.O. Box 35
FI-40014 University of Jyväskylä, Finland

Prof. Phillip C. Watts
Ecology and Genetics
FI-90014 University of Oulu, Finland

Reviewers Docent Suvi Ruuskanen
Section of Ecology
Department of Biology
University of Turku
FIN-20014

Prof. Tobias Uller
Department of Biology
Lund University
SWE-22362

Opponent Professor Michael Sheriff
Biology Department
Umass Dartmouth
MA 02747, USA

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I 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. *Journal of Animal Ecology* 00: 1–11. doi.org/10.1111/1365-2656.13039

II Van Cann J., Koskela E., Mappes T., Mikkonen A-M., Mokkonen M., Watts P.C. 2019. Early life of fathers affects offspring fitness in a wild rodent. *Journal of Evolutionary Biology*. In press. doi.org/10.1111/jeb.13516

III Van Cann J., Jernfors T., Koskela E., Mappes T., Watts P.C. 2019. Early life protein restriction reduces 18s rRNA copy numbers in a mammal. Manuscript

The following table shows the contributions to the original papers.

	I	**II**	**III**
Original idea	JVC, EK, TM, AS, PW	JVC, EK, TM, PW	PW
Experimental work	JVC, EK, TM, AS	JVC, EK, TM, AMM, PW	JVC, TJ
Statistical work	JVC, EK	JVC, EK	JVC, TJ, EK
Manuscript	JVC, EK, TM, AS, PW	JVC, EK, TM, MM, PW	JVC, TJ, EK, TM, PW

JVC = Joannes Van Cann, EK = Esa Koskela, TM = Tapio Mappes, AS = Angela Sims, PW = Phillip Watts, AMM = Anne-Mari Mikkonen, MM = Mikael Mokkonen, TJ = Toni Jernfors.

1 INTRODUCTION

1.1 Early life environment

The environment in which an individual develops, from zygote to adult, has the potential to cause irreversible changes to its phenotype (Gilbert 2001). These changes in the phenotype, also referred to as 'developmental plasticity' (Paaby and Testa 2018), have grasped the attention of biologists for centuries. For example, August Weismann (1875) showed that map butterflies (*Araschnia levana*) produced different coloured wings depending on the season in which they hatch from the pupae, and the different morphs could be replicated by cooling or warming the pupae. While early studies focussed on striking, whole body changes, research advancements in molecular techniques in the 1960's (e.g. Astbury 1961) shifted the focus to smaller, more cryptic environmental effects on the developing individual.

A large body of literature about early life effects is derived from studies focussing on humans *in utero* or born during historic stressful periods, such as the Dutch hunger winter (1944-1945). Various morphological measurements were found to be (negatively) affected by caloric restriction during the Dutch hunger winter *e.g.* placental and birthweight, length at birth and head circumference (Stein and Susser 1975). These early life environmental effects persisted to adulthood resulting in *e.g.* increased abnormalities in the central nervous system (Stein and Susser 1975). Similar results were found in experimental studies of laboratory rodents during that time, mostly focussing on effects of undernutrition (*e.g.* Cravioto *et al.* 1966, Frankova and Barnes 1968). For example, repeated early life food deprivation was found to decrease the learning capacity in laboratory mice (Cravioto *et al.* 1966), with the authors going as far as suggesting a permanent "negative spiral" of suboptimal adaptation due to the early life environment.

From an eco-evolutionary perspective, it is obvious that if the early life environment can induce significant phenotypical changes, it can influence the fitness of an individual. There are three (main) ways the early life environment

can associate with the fitness: firstly, the early life quality can be directly related to fitness, the so-called 'silver spoon effect' (Grafen 1988, Monaghan 2008) (Fig. 1a). This means that individuals growing in an optimal environment have their fitness permanently enhanced and *vice versa*. Many examples of silver spoon effects have been found, especially in relation to availability of food (Lindström 1999) and this phenomenon appears to be common in diverse taxa, including mammals and many invertebrates (Hayward and Lummaa 2013, Wong and Kölliker 2014); for example, early life habitat quality in great tits (*Parus major*) is positively associated with body size (Verhulst *et al.* 1997). Recently, the term 'silver spoon' has been criticised for implying that this effect is limited to fitness increases due to beneficial early life environments, while not being appropriate for decreases in fitness due to poor early life environments. Therefor the alternative term 'condition transfer' is sometimes used (Bonduriansky and Crean 2018). Second, the early life environment could stimulate a phenotype that is adapted to the environment, called an 'adaptive predictive response' (Gluckman *et al.* 2005) (Fig. 1b). Adaptation in an eco-evolutionary sense can be defined as a change in the phenotype of an individual to an environment that increases the individual's fitness in that specific environment (Sober 2000). In this situation, the fitness of an individual would be raised if the individuals' future environment is similar to that of their early life. For example, in the small crustacean *Daphnia pulex*, two distinct phenotypes, one benefitting survival against predation and the other reproduction, can be developed from the same larvae. If the environment contains predators, the 'survival' phenotype will develop and will give a fitness benefit by decreasing mortality. However, if there are no predators present, the 'survival' phenotype will have lower reproductive success, thus lowering its fitness (Laforsch *et al.* 2006). Lastly, environmental cues might be different in the early life, but a fully plastic response in the developing and adult phenotype will eventually lead to the same fitness regardless of the early life environment (Weiner *et al.* 1997, Ergon *et al.* 2001) (Fig. 1c). While these three theoretical outcomes of the early life create very distinct responses, it is also possible that the early life cues will generate a mix of these three scenarios (Bonduriansky and Crean 2018).

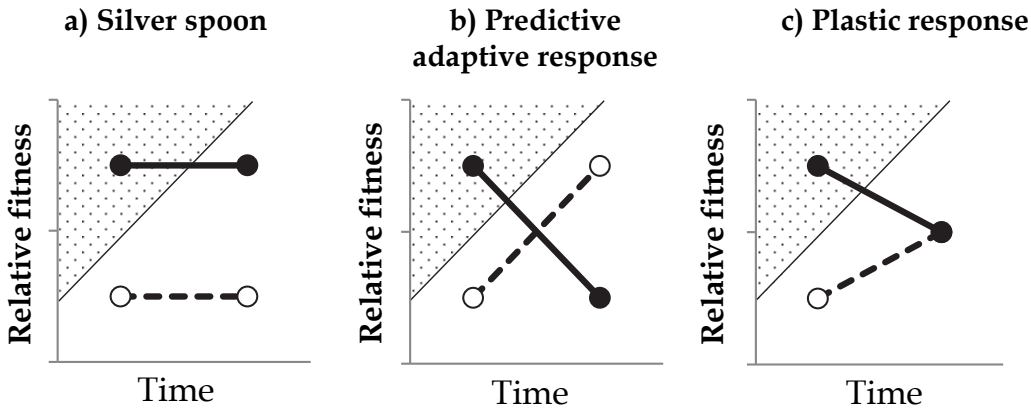


FIGURE 1 Three possible theoretical outcomes of the early life environment in regards to relative future fitness. a) a silver spoon effect will cause the (relative) adult fitness to be directly related to the quality of the early life. b) a predictive adaptive response will lead to a (relative) fitness gain if the adult environment matches that of the early life, and to a fitness decrease if the environment mismatches. c) a fully plastic response will lead to an equal (relative) fitness regardless of the quality of the early life. Closed circles and full lines indicate individuals growing up in a high quality environment and open circles with dashed lines indicate individuals growing up in a low quality environment. Dotted surface demarcated with line represents high quality environment, while the remaining surface represents a low quality environment. This figure represents individuals coming from either a high or low quality environment going to a low quality environment but a similar figure could be made with individuals going to a high quality environment. In this case a) and c) would remain the same but b) would change by increasing the relative fitness of the closed circle with time, while decreasing the relative fitness of the open circle with time

Regardless of the outcome of the early life, the phenotype of a reproducing adult individual has the potential to also influence the phenotype its offspring. These intergenerational effects might be due to different life-history strategies, or more complex early life environment-dependent heritable mechanisms, such as epigenetics (see 1.2).

1.2 Theoretical framework of intergenerational effects

1.2.1 History of intergenerational effects

Environmental effects that lead to heritable phenotypes have long been regarded as heretic in evolutionary biology (Jablonka and Lamb 1995, Rando 2012). Indeed, Darwin's theory of natural selection (Darwin 1859) has been chosen over Lamarck's inheritance of acquired traits (Lamarck 1809), Trofim Lysenko's crops never got adapted to the cold (Soyfer 2001, Kolchinsky *et al.*

2017) and Weismann's rats never developed shorter tails (Weismann 1891), even after cutting them off for five generations[1].

However, during the 1970's the concept of intergenerational effects, defined here as environmental cues exhibited on one generation having effects on a subsequent generation (*sensu* Emanuel 1986), began to gather scientific interest (Weinstein and Haas 1977). For instance, Zamenhof *et al.* (1971) showed that *in utero* protein restriction in the first generation (F1) of rats (*Rattus norvegicus*) led to offspring (F2) that have significantly reduced brain weight, brain protein content and brain cell number. In the 1980's, ecologically relevant studies started investigating evidence for possible intergenerational effects (Stearns 1989). For example, in the poeciliid fish *Heterandria formosa* it was found that there was a trade-off between number of broods and offspring size, hence creating an intergenerational effect on the offspring (Henrich 1988). It was later shown that this trade-off was due to environmental factors, namely population density (Leips *et al.* 2009).

Nowadays evidence for intergenerational effects has been acquired in diverse taxa, including plants (*e.g.* parental temperature leads to different seed/coat mass in *Plantago lanceolate* (Lacey *et al.* 1997)), invertebrates (*e.g.* food availability in the soil mite *Sancassania berlesei* affects egg size up to three generations later (Plaistow *et al.* 2006)) and vertebrates (*e.g.* glucocorticoid concentrations in offspring due to predator density in maternal environment (Sheriff *et al.* 2010); reviewed in Drake and Walker 2004). Intergenerational effects can be caused by anthropogenic environmental effects such pollutants, *e.g.* vinclozolin affects mice offspring patrilineally at least up to three generations (Brieño-Enríquez *et al.* 2015). However, for the purpose of this thesis, I will focus on two common environmental factors present in many vertebrate populations: diet quality (protein content) and territory defence (social confrontation).

1.2.2 Dietary protein content

Most vertebrate populations are limited by food availability (Lack 1954, Boutin 1990); for instance, the reproductive success of female bank voles (*Myodes glareolus*) living in outdoor enclosures is increased when food is supplemented (Jonsson *et al.* 2002). However, it has been suggested that protein content within the diet, not food availability, is a limiting factor for most natural vertebrate populations (McAdam and Millar 1999). Protein restriction (PR) experienced during the early life, defined here as the period from zygote to adulthood, has been shown to reduce birthweight in *e.g.* rats (Zambrano *et al.* 2006), mice (Goettsch 1960) and deer mice (*Peromyscus maniculatus*) (McAdam and Millar 1999). Protein restriction can have several persistent effects on the adult phenotype. For instance, in zebra finch (*Taeniopygia guttata*) males, a low protein

[1] With our current understanding of how stress hormones and its potential intergenerational effects work, Weismann might have found the behavior of the offspring to be different, thus proving intergenerational environmental effects.

diet during development leads to shorter adult lives (Alonso-Alvarez *et al.* 2006). Furthermore, early life protein restriction also leads to a delay in the production of the first clutch for both male and female zebra finches (Gil *et al.* 2004, Monaghan 2008). In rodents, effects of early life protein restriction include changes in adult behaviour (reviewed in Laus *et al.* 2011) and changes in male reproductive organs (in rats; Zambrano *et al.* 2006).

Besides effects on the generation experiencing the protein restriction during its early life, PR can also have effects that last beyond the F1 generation. For example, in the F2 grandoffspring of pregnant F0 rats exposed to PR, methylation of the hepatic gene was altered, blood pressure raised and insulin sensitivity increased (reviewed in Drake and Liu 2010). Similarly, in mice, F1 exposed to PR *in utero* had male F2 offspring with *e.g.* increased insulin levels (Peixoto-Silva *et al.* 2011) among other metabolic disorders (although the F2 did not have a different body mass).

1.2.3 Social confrontation

Apart from some critically endangered species, all animals are expected to meet conspecifics at some point in their life. For territorial animals, these meetings can lead to aggressive displays (Kaufmann 1983) and are expected to be stressful for both parties (von Holst 1998). Territory defence is common in vertebrate species (Maher and Lott 2000), and specifically in rodents the intrinsic effects of intruding or defending a territory have been extensively studied. For example, in pharmaceutical research the 'resident-intruder' system, a system where one individual acts as the territory defender and another as the intruder, is widely used for testing drugs for *e.g.* depression (Golden *et al.* 2011). The resident-intruder system is also often used in ecological research *e.g.* to study aggressive behaviour in California mice *Peromyscus californicus* (Trainor and Marler 2001). But in natural populations, confrontations between conspecifics are not necessarily similar to the resident-intruder system (Martinez *et al.* 1998). For instance, most rodents not only have a territory which they actively defend, but also a home-range (*e.g.* used for feeding) which surrounds the 'core territory' (Burt 1943). While core territories of rodents are unlikely to overlap (*e.g.* core territories do not overlap in bank voles (*Myodes glareolus*) (Jonsson *et al.* 2002)), the home-ranges of rodents can overlap, and this overlap happens more frequently when population densities are high (*e.g.* in *Peromyscus leucopus noveboracensis* and *Peromyscus maniculatus nubiterrae* (Wolff 1985); in bank voles (Koskela *et al.* 1997, 1999, Jonsson *et al.* 2002)). The main difference between conspecific encounters in the resident-intruder system and home-range encounters is that in the home-range both individuals can be considered residents (or intruders). The distinction between resident-intruder system and home-range encounters is important as residents and intruders suffer different amounts of stress (in rats (Raab *et al.* 1986); in mice (Bartolomucci *et al.* 2001)). In this thesis, I will use the term 'social confrontation' to signify aggressive conspecific encounters in which both individuals can be considered as residents. This type of social confrontation is

rarely investigated (Kaiser and Sachser 2005), but both behavioural (decreased attraction of stressed females to adult males) and morphological consequences (lower intrauterine weight) have been reported in bank voles (Marchlewska-Koj *et al.* 2003a, b)

1.3 Mechanisms of intergenerational effects

1.3.1 Maternal effects

The following paragraphs describe different mechanisms that can cause intergenerational effects. Other mechanisms exist that are common to *e.g.* bacteria, fungi and plants (*e.g.* structural templating such as prions) (Heard and Martienssen 2014). However, I will limit the discussion of mechanisms of intergenerational effects to sexually reproducing animals.

Maternal effects are broadly defined as the causal influence of the maternal genotype or phenotype on the offspring phenotype (Wolf and Wade 2009). Maternal effects were already described in the 1930's (Dobzhansky 1935) but this phenomenon did not gain much scientific interest until the 1980's (reviewed in Mousseau *et al.* 2009). Its gain in popularity was mostly due to the increased interest in quantitative genetics, where maternal effects were deemed a 'nuisance parameter' (Wolf and Wade 2009) that had to be corrected for (Riska *et al.* 1984, Newman *et al.* 1989). However, as the universality of maternal effects became clear, so did its potential adaptive value (Mousseau and Fox 1998) and hence its importance as an eco-evolutionary process (Sheriff and Love 2013, Sheriff *et al.* 2017).

Maternal effects may be delivered by maternal traits such as nursing (Champagne *et al.* 2003) or provisioning (Plaistow *et al.* 2007) to the offspring; or maternal effects can be more indirect by *e.g.* choosing a nesting site (Radder *et al.* 2009) or passive transfer of hormones (Sheriff *et al.* 2009). Maternal effects can increase offspring fitness (Mousseau and Fox 1998) but they can also reduce their fitness (Marshall and Uller 2007). The balance between the current offspring's fitness and the hypothetical future offspring is part of the parent-offspring conflict (Trivers 1974, Haig 2014). In this thesis, I will limit the definition of maternal effects to those effects that are due to the environment. This definition means that I do not consider certain mechanisms such as cytoplasmic inheritance and genomic imprinting to be maternal effects (Wolf and Wade 2009).

Besides the aforementioned pathways of maternal effects (*e.g.* nursing, provisioning), epigenetic mechanisms have emerged as possible maternal effects (see 1.3.3 for more details). It has been shown that epigenetic signals (*e.g.* methylation patterns) can be inherited through 'meiotic inheritance' (i.e. inheritance of epigenetic patterns through gametes (Richards 2006)) but also through 'mitotic inheritance', *i.e.* somatic inheritance. An example of the latter was shown in laboratory mice, where a lack of maternal licking and grooming

led to a different methylation pattern in regulatory region of the oxytocin gene, which in turn led to a behavioral phenotype that gives less maternal care to its own offspring (Weaver *et al.* 2004).

1.3.2 Paternal effects

Paternal effects here are defined similarly to maternal effects (*i.e.* a causal influence of the paternal genotype or phenotype on the offspring phenotype), and hence my discussion will be limited to environment related paternal effects (and not cytoplasmic inheritance and genomic imprinting). Outside of fitness differences in offspring due to paternal care, which is absent in most species of mammals (*e.g.* less than 10% of mammals display paternal care (Woodroffe and Vincent 1994)), there has not been as much attention for paternal effects as there has been for maternal effects. This apparent lack of interest into paternal effects is partly because, prior to the discovery of heritable epigenetics, only paternal care, indirect paternal effects (Ratikainen and Kokko 2010) and nuptial gifts (*e.g.* Thornhill 1983) were thought to convey information about the paternal environment to the offspring. Indirect paternal effects are effects due to the mother allocating resources differently to her offspring based on the quality of the male (differential allocation hypothesis; Burley 1988, Ratikainen and Kokko 2010). The paternal effect is referred to as indirect because it works in essence through maternal effects; as such, not everyone agrees that this is a true paternal effect (Ratikainen and Kokko 2010).

Direct paternal effects (excluding paternal care and nuptial gifts; Crean and Bonduriansky 2014) can only work through epigenetic mechanisms (see 1.3.3 for more details) such as inherited methylation patterns (Fullston *et al.* 2013, Radford *et al.* 2014, Donkin *et al.* 2016, Weyrich *et al.* 2016) and miRNA transfer (Crean and Adler, Rassoulzadegan *et al.* 2006, Crean and Bonduriansky 2014). These direct paternal effects can be the consequence of the father's current (adult) environment or can be the legacy of its early life. For example, in humans, adult obesitas (i.e. the paternal *current* environment) can induce epigenetic changes in sperm (Donkin *et al.* 2016) and thus theoretically be inherited to the offspring. This effect can be reversed through weight loss as a consequence of gastric-bypass surgery. In laboratory rodents, early life protein restriction in males has been shown to lead to paternal effects such as low birthweight, impaired glucose tolerance or elevated hypertension in the offspring (in rats: Harrison and Langley-Evans 2009, in mice: Jimenez-Chillaron *et al.* 2009).

1.3.3 Epigenetics

1.3.3.1 DNA methylation

Epigenetics refers to changes in gene function that do not require changes in the genetic code (Berger *et al.* 2009), although copy number variation is an exception (see 1.3.3.3). Epigenetic processes such as DNA methylation were originally associated with cell differentiation (Waddington 1942) but new

discoveries in the early 1990's made it clear that specific epigenetic patterns can be induced by the environment and inherited (Holliday 2006). The environmental influence and inheritance also means epigenetics could potentially play an important role in eco-evolutionary processes (Bossdorf *et al.* 2008, Jablonka and Raz 2009, Ledón-rettig *et al.* 2012); epigenetic variants can exist at higher frequencies than genetic variants by being present in multiple individuals from the moment the environment changes. The collective, epigenetically controlled, change in gene expression could lead to adaptive traits that can then go into fixation much faster than genetic mutations (Ledón-rettig *et al.* 2012).

The following sections describe specific heritable epigenetic mechanisms that have been shown to be relevant, or have the potential to be relevant, for natural populations. For a full review of all known heritable epigenetic mechanisms, see Heard and Martienssen (2014).

Methylation of cytosine is the best studied epigenetic mechanism (Heard and Martienssen 2014). Transcription factors are less likely to bind to sites where a methyl group is attached to a cytosine that itself is followed by a guanine base (CpG) (Boyes and Bird 1991). Variation in methylation is often found in regulatory sequences of genes, and thus greater methylation will ultimately reduce the expression of a gene. Methylation patterns have been shown to be heritable in animals to some extent (McRae *et al.* 2014) and environmentally inducible (*e.g.* early life stress leads to persistent changes in the expression of vasopressin in mice (Murgatroyd *et al.* 2009)). Evidence for the ecological relevance of inducible and heritable methylation in animals has been growing but is still limited (in contrast to methylation in plants (Ledón-rettig *et al.* 2012)). For example, in male guinea pigs (*Cavia aperia*) increased ambient temperature can induce specific, heritable methylation patterns in its male offspring (Weyrich *et al.* 2016).

1.3.3.2 miRNA

There are a multitude of coding and non-coding RNAs (*e.g.* long non-coding RNA, small interfering RNA, micro RNA (Heard and Martienssen 2014)) that have been implicated in epigenetic inheritance. Of these, micro RNA (miRNA) has received a lot of attention as miRNA expression has been linked to gene expression (Rassoulzadegan *et al.* 2006, Grandjean *et al.* 2009). Micro RNA can be inherited paternally for multiple generation by sperm transfer (Brieño-Enríquez *et al.* 2015). The ecological relevance of miRNA has become clear in recent years as it seemingly reflects the paternal environment and hence can influence the offspring phenotype, even in the absence of paternal care (Rodgers *et al.* 2013); although studies in natural populations are still lacking.

1.3.3.3 Copy number variation (CNV)

Multiple copies of certain genes (and whole chromosomes) exist in most eukaryote genomes (*e.g.* at least 11700 CNVs exist in the human genome (Redon *et al.* 2006)). Inter-individual (*e.g.* in human twins (Bruder *et al.* 2008)), inter-

population (*e.g.* in *Drosophila melanogaster* (Aldrich and Maggert 2015)) and inter-species (*e.g.* in primates and humans (Dumas *et al.* 2007)) differences exist in CNV. Specific environmental cues have been found to induce specific copy number variations and the copy number has been shown to be heritable. For example, protein restriction in adult male *D. melanogaster* led to reduced rRNA copy numbers in their male offspring (the rRNA gene in question was located on the Y-chromosome and thus could only affect male offspring) (Aldrich and Maggert 2015). Moreover, CNV can be related to the expression of the gene (Stranger *et al.* 2007). Being environmentally inducible, heritable and impacting the phenotype are all traits of an epigenetic mechanism, which is why copy number variation is often considered as such, although it technically is not *epi*genetic.

1.4 Eco-evolutionary consequences of intergenerational effects

Most studies into the drivers and consequences of intergenerational effects focus on how the current environment, *i.e.* the environment that is experienced during mating, gestation and nursing, can impact fitness traits in offspring via maternal or paternal effects (*e.g.* Leips *et al.* 2009, Crean *et al.* 2013). However, the early life environment of an individual can have a permanent effect on the adult phenotype (see 1.1). It is likely, therefore, that the parental early life environment will lead to intergenerational effects on the offspring (sometimes referred to as transgenerational plasticity (Donelson *et al.* 2018)). For example, the early life nutritional environment in birds and mammals can have persistent effects on adult body size (Lindström 1999) and subsequently influence the offspring body size. Likewise, while DNA methylation is reversible in some cases, methylation patterns can be formed during early life and remain stable to the adult life (Messerschmidt *et al.* 2014) where it can potentially influence the next generation either through changes in parent phenotype or via epigenetic inheritance (Bird 2002).

Intergenerational effects on offspring fitness could be very similar to the intra-generational effects described in Fig. 1. The first possible intergenerational outcome is that (1) the relative fitness of the offspring reflects the quality of the parental environment (intergenerational silver spoon; *sensu* Fig. 1a). Evidence for silver spoon has been shown in oystercatchers (*Haematopus ostralegus*) where the quality of the rearing environment of the parental generation is directly related to the fitness of the offspring (Van De Pol *et al.* 2006). A second possible intergenerational effect is that the (2) offspring are best prepared to the quality of their environment and have increased fitness, if that environment is similar that of their parents (intergenerational predictive adaptive response; *sensu* Fig. 1b). Empirical examples for intergenerational predictive adaptive responses are rare and often not sufficiently tested (Burton and Metcalfe 2014). One example of predictive adaptive response comes from *D. melanogaster* where larvae were raised on either low or high food quality, and after hatching they received

standard food until they successfully mated and laid eggs. Their offspring were subsequently themselves reared on low or high quality food. Vijendravarma *et al.* (2010) found that offspring raised on low food quality pupated earlier if their parents were also raised on low food quality, while not being affected by being reared with high quality food. Finally, (3) the early life of the parents might not affect the offspring fitness because the parents can either not adjust their life-history or alternatively, the offspring's own early life environment is a bigger determinator than their parents' early life (intra-generational plastic response; Fig. 1c). It should be noted that any intra-generational response does not have to match the intergenerational response. In other words, while the parental environment can have a silver spoon effect, it could still elicit an intergenerational adaptive response. Likewise, a predictive adaptive response might increase the fitness of the parental generation in a specific environment, it could just lead an increase in the offspring fitness, regardless of their environments (intergenerational silver spoon).

1.5 The bank vole as a study species

The bank vole *Myodes glareolus* is a small rodent that is common in broadleaved deciduous and coniferous woodland areas in most of Europe and some of Asia, and is regarded as a model species in eco-evolutionary research (Mokkonen *et al.* 2011, Lonn *et al.* 2017). In Northern Europe, where this research took place, bank voles typically breed from early spring to early autumn. During this period, females become territorial and defend their territories aggressively (Koskela *et al.* 1997, 2000, Gromov and Osadchuk 2013), while males move between the female territories to find potential mates. Bank voles have no paternal care and have a promiscuous mating system with both females and males having multiple partners and females can give birth to litters sired by multiple males, making sperm competition important (Klemme *et al.* 2007, Mills *et al.* 2007a). Lastly, bank voles in Northern Europe have a typical three year population density cycle (Hanski *et al.* 1991, Kallio *et al.* 2009), indicating that variation in population density and associated environmental pressures are common. Although the most likely driver of these cycles is predator pressure, it has been suggested that food limitation and maternal effects (Boonstra *et al.* 1998, Huitu *et al.* 2003) are an important aspect of the cycles.

Bank voles are an ideal study species to investigate effects of the early life environment and the possible intergenerational effects of that early life through the maternal and paternal side. This is because, like most rodents, they are short lived (in the wild one to two years), easily kept in laboratory cages and easily bred with litters commonly being between four to five pups (Oksanen *et al.* 2001). Pregnancy takes approximately 19-21 days and new-born pups are independent at around 20 days of age and start to be sexually active ten days later. However, it is specifically their natural population cyclicity and mating system that makes them an excellent model to study early life effects. The cyclic

population density, where population density can increase 300% between years (Kallio *et al.* 2009), means that some individuals are born in a "relaxed" year where intra-specific competition for resources is low (low density phase); while other individuals are born in a "competitive" year (high density phase) (Norrdahl and Korpimaki 1995).

The bank vole's mating system makes it possible to study maternal and paternal effects separately because of three reasons. Bank voles (1) do not have paternal care (Gromov and Osadchuk 2013), (2) can have litters with multiple fathers (Ratkiewicz and Borkowska 2000) thus knowledge by females about which male has fathered which offspring within a litter is low and (3) even if females know who the father is they do not adjust their investment into offspring according to male quality (Oksanen *et al.* 1999). These three characteristics mean that when offspring of treated females are studied, we can minimize any potential paternal effects as the maternal environment is the sole determinator of the pre- and postnatal environment. Likewise, when studying treated males, their lack of contribution to the offspring's life, would mean that any paternal effects would most probably be the result of direct paternal effects through sperm.

1.6 Aims and scopes of the thesis

The aim of my thesis is to quantify (1) the intra-generational effects that the early life environment has on the fitness of a wild rodent, and (2) whether the early life environment can have intergenerational effects on offspring growth, either through the maternal or the paternal line. Furthermore, I want to relate these findings in a larger eco-evolutionary framework by investigating (3) the potential effects on offspring fitness and its adaptive potential. Finally, (4) I want to explore potential molecular mechanisms that respond to variation in early life environmental cues.

Intra-generational effects were investigated by exposing F1 offspring of F0 mothers from conception up to the weaning phase to high-density population density cues (protein restriction and social confrontation) and monitoring the growth and reproductive success of the F1. I expected that the intra-generational effects of protein restriction on F1 would be (hypothesis 1a) a reduction of growth and reduced reproductive success (silver spoon effect; Fig. 1a). As protein is an important aspect of the bank vole diet (Hansson 1971) a restriction in dietary protein should reduce growth both in F1 females and F1 males, comparable to studies in humans (reviewed in Hörnell *et al.* 2013) and rodents (*e.g.* Ozanne *et al.* 2003, Bieswal *et al.* 2006). As body size is related to reproductive success in bank voles (Boratynski and Koteja 2010), the success should be reduced due to a protein restricted early life. Although the effects of social confrontation are less well studied than effects of protein restriction, I expected the intra-generational effects of early life social confrontation to also (hypothesis 1b) reduce the growth and reduce the reproductive success of both

male and female F1. One possible pathway that fitness effects are mediated is through increased glucocorticoids as a result of the aggressive encounters (Summers and Winberg 2006) which, in turn, can negatively impact body mass (*e.g.* Machado *et al.* 2013).

Intergenerational effects of F1 early life cues on their F2 offspring were investigated by following F2 offspring growth either in the field (maternal experiment) or in the laboratory (paternal experiment). If F1 early life treatments reduced the body mass of the F1 individuals, I was expecting there to be (hypothesis 2a) negative intergenerational effects on F2 growth due to protein restriction in the maternal experiment, as F1 mothers will have reduced energy reserves to transfer to their F2 offspring (intergenerational silver spoon). The same mechanism does not apply through the paternal line and not much is known about intergenerational effects due to paternal early life nutrition. Regardless, I expected paternal effects to be transferred to the F2 and thus F2 growth to be negatively affected, possibly through inherited epigenetic patterns. As I expected social confrontation to also have a negative intra-generational effect on F1 growth (hypothesis 2b) I expected social confrontation to also elicit negative intergenerational effects on F2 growth, both through the maternal and paternal line. It should be noted that there could be a certain (*i.e.* silver spoon or predictive adaptive response) or no response in the first generation, yet an entirely different response in the second generation. For example, intergenerational responses have been found in human populations exposed to famines, where the generation directly exposed to famine in the early life suffer permanent growth retardation and lowered body mass (silver spoon; Stein and Susser 1975), while their offspring's body mass is permanently increased (Veenendaal *et al.* 2013).

The potential intergenerational effects on fitness were investigated by monitoring the survival of F2 offspring during the winter in semi-natural, outdoor enclosures. For the maternal experiment, the adaptive potential was tested by placing F2 offspring either in low or high population densities. I theorized that the intergenerational effects of social confrontation, but not protein restriction, would (hypothesis 3a) lead to an adaptive response (intergenerational predictive adaptive response) by increasing the survival in a matching environment (*i.e.* a high density for F2 whose mother was exposed to social confrontation). I expected the intergenerational effects on F2 fitness due maternal protein restriction to either continue to negatively impact the fitness (intergenerational silver spoon) or, alternatively, not affect F2 at all as the offspring can plastically adapt their life-history traits to cope with the new environment. Conversely, I expected (hypothesis 3b) that both protein restriction as social confrontation during the paternal early life will affect F2 fitness by increasing the survival. While I have not tested the true adaptive values of the resulting F2 phenotypes in different winter densities, I would regard this an intergenerational predictive adaptive response, as winter survival can be considered a competitive situation.

Two molecular mechanisms were explored in relation to the intra-generational effects of the early life treatments: methylation of the oxytocin gene (and receptor) and ribosomal DNA copy number, with the latter only being analysed in response to protein restriction. Various molecular mechanisms could be responsible for both intra- and intergenerational responses to the environment. However, oxytocin was chosen as it is linked to relevant behaviours in mammals (Lee *et al.* 2009), such as maternal care, and has been shown to have transgenerational effects in mice (Champagne 2008, Curley *et al.* 2008). Therefore, I expected (hypothesis 4a) the methylation of the oxytocin hormone and its receptor to be increased (hence the theoretical expression to be decreased) in F1 individuals due to both protein restriction and social confrontation. Likewise, rRNA copy number has recently been shown to be affected by diet (Aldrich and Maggert 2015) and could potentially be related to growth of an individual. Hence, I expected (hypothesis 4b) the rRNA copy number of protein restricted F1 individuals to be reduced.

2 METHODS

2.1 Animal care

During the course of all experiments, individuals kept in the lab were always exposed to the same ambient conditions. Cages consisted of clear polyethylene (43x26x15 cm) with wood shavings and hay provided as bedding. The photoperiod consisted of 16L:8D and the temperature was maintained at 20±2°C. Water was provided *ad libitum* and standard laboratory food was provided *ad libitum* (Labfor 36; Lactamin AB, Stockholm, Sweden), except during the experiments (see 2.2). All adult animals that were used for the experiments were electronically chipped using Trovan tags (EID Aalten BV, Aalten, Holland) for identification. Individuals born during the experiments were toe-tagged at day 0 for identification.

2.2 Early life treatments

The aim was to create a generation that received different environmental cues during their early life, defined as the period from zygote to weaning (20 days for bank voles). Two cues associated with high population density in bank voles (see 1.2.1, 1.2.2 and 1.5) were exhibited to pregnant and nursing mothers (hence referred to as F0). Protein restriction (PR) was chosen to simulate increased food competition and social confrontation (SC) was chosen to simulate increased territory defence. Both treatments were given in a full factorial setup (Fig. 2), meaning that there was one group not receiving any treatments (control; PR-SC-), one group receiving only protein restriction (PR+SC-), one group receiving only social confrontation (PR-SC+) and a group receiving both treatments (PR+SC+). The latter group, the 'interaction group', was included to examine the relative importance of dietary cues vs. social cues which have both been proposed to be (in part) responsible for the population fluctuations of

bank voles (Boonstra *et al.* 1998). As a morphological measurement, the birth mass and adult mass (i.e. 30 days old) of all F1 individuals was recorded.

Protein restriction (PR+) consisted of feeding F0 females a 9% protein diet (protein restricted; Envigo, WI, USA) while feeding all other F0 females a control 18% protein diet (PR-, control diet; Envigo, WI, USA). Both diets had nearly the same energy content (9% protein: 3.2 kCal/g; 18% protein: 3.1 kCal/g).

Social confrontation was exhibited on pregnant and nursing F0 mothers by taking the F0 individual out of her homecage and placing her in an empty polyethylene cage (43x26x15 cm) together with another SC+ F0 female. Some sawdust from both F0 individuals was distributed in the new empty cage to convey odour of the homecage in the new cage. Each confrontation lasted for ten minutes and was performed every other day for each SC+ female. After the confrontation, females were returned to their respective cages. The combinations of which females were paired was randomized for the first confrontation, and afterwards female combinations were cycled to make sure specific individuals did not get used to each other. Confrontations were always performed between 09:00 and 17:00 but the exact time was different every day to avoid habituation.

An important aspect to realize about these treatments is that the goal was to emulate a competitive (or relaxed) early life environment for the F1 individuals, even though both treatments were exhibited on the F0 generation. It is assumed that the F0 mothers convey information about the treatments through (1) adjusting milk nutrients (Derrickson and Lowas 2007); (2) adjusting maternal care (Champagne and Meaney 2006) and (3) transferring hormones through the womb and milk (Sheriff *et al.* 2010, Macrì *et al.* 2011). While we did not investigate the specific mechanisms involved in the passing of environmental information, it remains that the early life environment of F1, the generation of interest, is changed.

	NO PR	PR
NO SC	PR- SC- *Control*	PR+ SC-
SC	PR- SC+	PR+ SC+

FIGURE 2 Factorial setup of the early life treatments for all experiments. PR= Protein Restriction; SC= Social Confrontation. Minus (-) signifies absence of treatment; plus (+) signifies presence of treatment.

2.3 Overview of the maternal experiment

The maternal experiment was performed to investigate whether the maternal early environment could induce adaptive intergenerational effects (chapter I). This was achieved by exposing F1 females to the early life treatments (see 2.1; Fig. 3a) and then placing them (as adults) in outdoor enclosures (40mx50m) in either low population densities (which matched the *control* early life treatment; see 2.4; Fig. 3b) or high population densities (which matched the protein and social confrontation treatment; see 2.4; see Sheriff *et al.* (2018) for a theoretical framework of this type of experiment). The low population density consisted of four females per enclosure and the high population density consisted of eight females per enclosure. The intergenerational adaptiveness was tested by checking the growth and winter survival of their F2 offspring, also in low and high population densities. If the early life cues of the F1 mother indeed could induce an intergenerational adaptive response, the expected result was that F2 individuals would survive and grow better in an environment that matches the treatment of their F1 mother. Apart from the winter survival of F2, the whole maternal experiment was replicated the following year, using completely new and unrelated individuals.

Before testing the winter survival of the F2, half of the F2 individuals were transplanted from low population densities to high population densities and *vice versa*. This was done to investigate whether the growing environment of the F2 (i.e. F2 early life) could have an *intra-generational* effect. If this effect was adaptive (*sensu* Fig. 1b), it would mean that F2 individuals born in a low

population density would survive better in an adult low population density, but worse in a high population density and *vice versa* (cf. Fig. 1). The results of the F2 transplant indicate what the relative importance is of the F2 early life versus their maternal F1 early life.

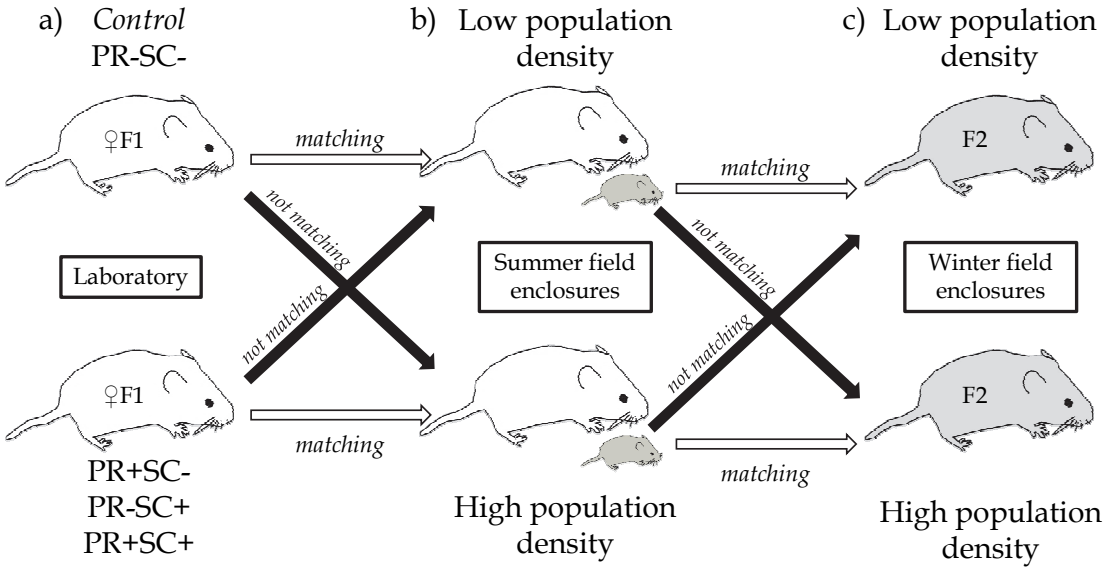


FIGURE 3 Overview of the maternal experiment (chapter I). (a) Female F1 individuals receive treatments, protein restriction (PR) and/or social confrontation (SC), during their early life in laboratory conditions. (b) Their reproductive success is subsequently tested in natural field enclosures in either a low population density (hypothetically matching the early life of the control group) or a high population density (hypothetically matching the early life of the remaining treatment groups). F1 females are allowed to breed in the field and (c) the resulting F2's are transplanted to different natural field enclosure during the winter to monitor their survival. By dividing the F2's to either a matching or a mismatching winter population density, it is possible to assess the relative importance of the F2 early life versus their parents' early life.

2.4 Overview of the paternal experiment

The aim of the paternal experiment was to investigate whether the early life of an F1 male bank vole could result in intergenerational paternal effects on F2 fitness (chapter II). This was done by exposing F1 male bank voles to early life treatments (see 2.1; fig 4a) prenatally and pre-weaning and subsequently breeding them as adults. The growth of the F2 offspring was studied in the laboratory and afterwards their winter survival in field enclosures (see 2.4; fig 4d). Paternal effects could be mediated through the mother through *e.g.* differential allocation (see 1.3.2). Therefore, two extra experiments were done to see if there was any evidence that females recognized, or showed any preference towards, the male early life treatments.

The first experiment consisted of four males (fig 2b), one of each treatment, being placed in a free competitive situation for two, untreated

females. The competitive situation was created by interlinking four standard, polyethylene cages (43x26x15 cm) with a PVC tube that allowed free passage of males and females throughout the cages. The second experiment (fig 2c) removed the competition and placed the same four males as previously used in the first experiment, in small cages in an arena (Fig. 2; middle bottom). A completely new, untreated and post-partum female was then placed in the arena and her movements were tracked using tracking software (Noldus Ethovision XT 8.5; Noldus *et al.* 2001). Female bank voles are almost always fertile one to two days post-partum and hence are presumed to be selective in their mate preference. The small cages allowed female bank voles to see and smell the males, but restricted touching and mating. By measuring the female's number of "visits" and time spend near a male it was possible to determine her preference for males of different treatments. Both part of the first experiment and part of the second experiment were replicated with different females. All females used in the female preference experiment were completely new to the males.

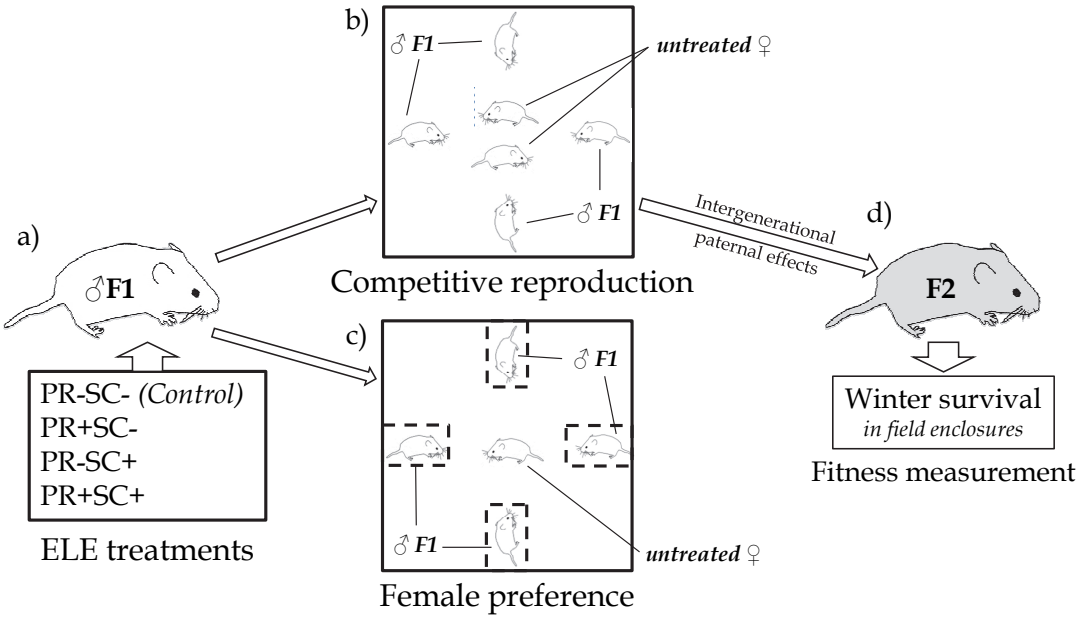


FIGURE 4 Overview of the paternal experiment. (a) Male F1 individuals were exposed to protein restriction (PR) and/or social confrontation (SC) during their early life environment. (b) As adults, the F1's competitive reproduction was tested by placing four males, one of each treatment, with two untreated females in a free competitive situation (experiment 1). (c) Preference of untreated females, not previously used, was tested by placing four males (same individuals as used in experiment 1) in small cages in an arena and tracking the female (experiment 2). (d) Intergenerational paternal effects on growth and fitness were tested on the F2 born out of experiment 1, by placing them as adults in natural outdoor enclosures during the winter.

2.5 Enclosure experiments

Field experiments were performed during the summer to investigate the reproductive success of F1 females and growth of their F2 offspring in different population densities (chapter I). Field experiments were also performed during the winter to investigate the winter survival of F2 offspring from both F1 females and F1 males (chapter I & II). All field experiments were performed in the same 23 large (40m x 50m) outdoor enclosures located in Central Finland (11 Peltokangas enclosures: 62°37'30"N 26°14'38"E; 12 Pukara enclosures: 62°39'23"N 26°09'32"E). Enclosures were fenced with 1.25m high galvanized sheet metal that was buried 0.5 m in the ground. This prevented immigration and emigration of bank voles and immigration of other small vertebrates but did not prevent possible predation by avian predators during summer and winter. Vegetation within the enclosures was completely natural and unmanaged, apart from a 50cm border zone next to the fencing which was kept clear of plants to prevent bank voles from using high plants to escape/enter.

The release of animals was always done in the middle of an enclosure by hand. In the case of individual release (first part of the female experiment and winter releases), animals were first put into cages per four individuals (never males and females together) prior to transport to the field enclosures. In the case of nursing mothers (second part female experiment), cages with mothers and pups were put on their sides around the middle of the enclosure. This allowed the mother to exit the cage on her own time and move her pups to a location of her choice (Mappes *et al.* 1995, Koskela *et al.* 2000, Oksanen *et al.* 2002).

During the summer, trapping of the animals always started 14 days after the release of the F1 females and traps were checked at least twice per day for four days in a row to ensure that all living animals were trapped (animals not trapped were considered dead). All trapped animals were returned to the laboratory. Trapping started after 14 days to ensure that females would not give birth in the field as their gestation time is approximately 18 days.

For the winter experiment, animals were released into the enclosures in October. For the female experiment (chapter I) all living individuals were recaptured in March. For the male experiment (chapter II) trapping happened once a month and was otherwise exactly the same as summer trapping, with the exception that animals were immediately released after capture, and traps were set a maximum of five times per trapping period to avoid food supplementation.

Trapping of animals was done using Ugglan live traps baited with sunflower seeds and small potato pieces at the moment of trapping. At any other time, traps were left unbaited and open. All traps (20 per enclosure, placed in a 4x5 grid) were situated in a large metal box with a small hole for entering/exiting. These metal boxes protected the traps against elements of the weather such as rain and snow.

2.6 Pyrosequencing

Pyrosequencing is a generally short-read sequencing technique that allows for CpG (see 1.3.3.1) methylation analysis (Tost and Gut 2007). The aim was to investigate the methylation of oxytocin (OT) and the oxytocin receptor (OTR) in the paraventricular nucleus (PVN) of the brain. The PVN was chosen as the main (brain) production of OT is located here, as well as a high density of OTR linked to maternal care behaviour (Dumais and Veenema 2016) among other behaviours such as aggression, sexual solicitation and milk ejection.

Twenty-three F1 females (see 2.1) were dissected as adults and their brains were sliced into 2 mm cuts using a brain matrix (ZIVIC instruments, Pittsburgh, USA; Fig. 6a). Circular punches of 1mm diameter were made in the area containing the PVN (Fig. 6b) and DNA was extracted from these samples (DNeasy kit blood and tissue kit, Qiagen, Sweden) following the manufacturers protocol. A draft genome of the bank vole has previously been assembled (GenBank GCA_001305785.1) and was used to determine the promoter regions of OT and OTR. In the OT promoter, 19 CpG sites were identified, of which 10 (CpG 8-17) were amplified using a standard PCR protocol. In the OTR promoter, 57 CpG sites were identified and 20 (CpG 14-34) were amplified using custom designed primers (table 1) using Primer3 (Untergasser *et al.* 2012). After bisulfite conversion (Epitect bisulfite kit, Qiagen, Sweden), amplified strands were pyrosequenced utilizing a pyromark Q24 (Qiagen, Sweden) using the standard protocol.

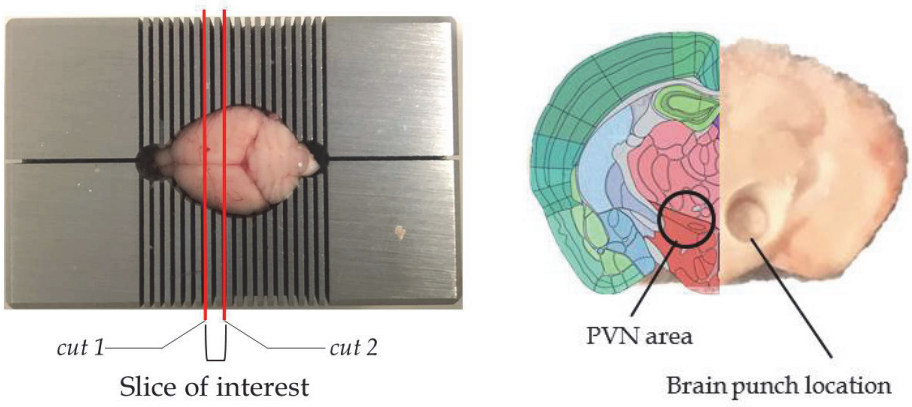


FIGURE 5 (left) Picture of dissected vole brain in brain matrix. Schematic lines indicate the cuts made in order to extract the slice of interest containing the paraventricular nucleus (PVN). (right) left side: schematic drawing of a mouse brain showing differentiable brain regions. Circle indicates general area of PVN. Right side: picture of dissected brain slice after the brain punch to extract the PVN area; Schematic drawing courtesy of The Mouse Brain Library (www.mbl.org)

TABLE 1 Primer sequences used for PCR amplifictation and pyrosequencing of oxytocin (OT) and oxytocin receptor (OTR) promoter regions.

Gene	CpG sites	Sequence	Type	Biotinylated
OTR	14-34	AGTTTTGGATTGTGGGAAAG	Forward	No
OTR	14-34	CCCAACCACCTAAAAAAAACCTA	Reverse	Yes
OTR	14-34	GGATTGTGGGAAAGT	Sequencing	No
OT	8-11	AAGTTGAAGGAAGATGGGATTAT	Forward	Yes
OT	8-11	CACTCAAAAACAAACCCTTCATT	Reverse	No
OT	8-11	AATCTAAACTAAAATCAAAATCAC	Sequencing	No
OT	12-17	AGGGTTTGTTTTTGAGTGG	Forward	No
OT	12-17	ACCCACCAAAAATAATAATTTCTCC	Reverse	Yes
OT	12-17	TGAGTGGTGTGGAGT	Sequencing	No

After sequencing, methylation percentages were averaged per gene and subsequently statistically analysed using R (Team R Development Core 2011). The percentages were not normally distributed so to test the possible interacting effects from PR and SC, a Scheirer-Ray-Hare non-parametric rank test (Sokal and Rohlf 1969) was performed. Afterwards, effects of PR and SC were tested separately using a Mann-Whitney test.

2.7 Ribosomal DNA copy number

Effects of the F1 early life on the ribosomal RNA (rRNA) gene copy number were assessed by comparing F1 individuals from the protein restriction treatment (PR+SC-; Fig. 3) to control F1. Furthermore, the sensitive time period to protein restriction was assessed by comparing F0 individuals from the protein restriction treatment to control F0. Samples (10 mm^2 ear pieces) were taken from 35 F0 mothers (after separation from their pups) and 62 of their F1 offspring (when older than 30 days). Where possible, one female and one male offspring were sampled from each nest. If there was only one sex was present in the nest, two offspring of the same sex were sampled.

DNA from the ear pieces was extracted using DNeasy Blood & Tissue Kit (QIAGEN) according to manufacturer's protocol. The 18s rRNA gene locations were determined using the bank voles genome (GenBank GCA_001305785.1) and the mouse 18s rRNA as a reference (NR_003278.3). The single-copy gene 36b4 was used as a reference to calculate the relative copy number (O'Callaghan and Fenech 2011). The extracted DNA was then analysed using qPCR (see Chapter III for full details on qPCR protocol) of both the reference 36b4 gene and the 18s rRNA gene of every individual. The relative number of rRNA copies was then calculated *sensu* (Pfaffl 2001) as:

$$ratio = \frac{(Etarget)^{\Delta Cqtarget(control-sample)}}{(Eref)^{\Delta Cqref(control-sample)}}$$

Differences in the average rRNA copy number between the two treatments, both for F0 and F1 separately, were then analysed statistically in R using linear models (Team R Development Core 2011). For the analysis of F1, sex was included as an interaction with the protein treatment in the model. Model reduction was performed until the lowest AIC was achieved.

3 RESULTS AND DISCUSSION

3.1 The intra-generational response to early life cues

3.1.1 F1 birth mass and adult body mass

All experiments (Chapters I-III) had the same early life treatments and hence the data can be analysed together. Over the period of three years, 1060 F1 individuals were born of which 940 reached adulthood. A linear mixed model, taking into account the treatments, sex, littersize and mother ID, revealed that only protein restriction (PR+) had a significant negative effect on the birth mass of F1 (table 2). A second linear mixed model, using the same variables as the birth mass model, showed that both PR+ and social confrontation (SC+) independently led to significant lower adults masses (table 2). Females were also lighter in both birth and adult mass, and being born in a large litter size had a significant negative effect on both birth and adult mass. These results confirm the effects found in both chapter I and II on smaller sample sizes.

TABLE 2 F1 morphology in relation to their early life treatments. Reduced linear mixed models (REML estimation) of F1 for birth mass (n=1060) and adult mass (n=940). Random variables include the F0 mother ID and the month of birth. PR= protein restriction treatment; SC= social confrontation treatment; * indicates interaction; (+) indicates presence of treatment; Est= estimated value. Bold p-values indicate $p < 0.05$ and are considered significant.

	F1 birth mass		F1 adult mass	
	Est	*p*	*Est*	*p*
(Intercept)	2.136	**<0.001**	17.574	**<0.001**
PR (+)	-0.098	**<0.001**	-1.174	**<0.001**
SC (+)	-0.016	0.590	-0.837	**0.016**
Interaction: PR*SC	0.042	0.327	0.720	0.136
Sex (Male)	0.064	**<0.001**	1.577	**<0.001**
Littersize	-0.068	**<0.001**	-0.507	**<0.001**

The intra-generational response to protein restriction was as expected (hypothesis 1a; see 1.6), and the results are consistent with studies on other rodents in the laboratory (*e.g.* rats (Goettsch 1960, Zambrano *et al.* 2006), mice (Goettsch 1960)) and in nature (*e.g.* deer mice *Peromyscus maniculatus borealis* (McAdam and Millar 1999)), including humans (Polberger *et al.* 1989). As protein is an important part of the bank vole's diet (Dróżdż 1968, Hansson 1971), it is not surprising that restriction leads to reduced body mass.

The intra-generational results from receiving early life social confrontation partly confirms the expected negative effect on growth (hypothesis 1b; see 1.6). The fact that social confrontation only led to a lowered adult body mass while not affecting birth mass could indicate that social cues only acted through maternal care or via lactation (*i.e.* through hormonal transfer), although there is also prenatal passive hormonal transfer. Social confrontation, as exhibited in these experiments, is not often performed and hence not much is known about the effects of social confrontation during the early life. However, in general social stresses exhibited to pregnant mothers seem to have negative effects on the offspring phenotype (Tamashiro *et al.* 2005) and have been shown to reduce body mass in rats and mice (Harvey and Chevins 1987, Ward *et al.* 1994). Similarly, social distress during pregnancy in humans can lead to offspring growth retardation (Rondó *et al.* 2003).

Overall, it seems that high population density cues exhibited pre- and postnatally lead to negative effects on growth and could perhaps be considered a silver spoon effect (Fig. 1a). However, silver spoon effects should impact fitness and body mass alone is not a sufficient fitness-related trait to prove this, although body mass is related to several life-history traits in bank voles.

3.1.2 F1 reproductive success

The female (chapter I) and male (chapter II) experiments were very different in setup to measure the reproductive success. Nevertheless, in both cases one sex was able to make a free choice and compete freely for a mate (for the female experiment this was in outdoor enclosures while for the male experiment this was in interlinked cages). In both the female and male experiment no significant influence was found on the reproductive success of F1 due to the early life cues, although the chance of reproducing was lowered for females breeding in a high population density.

The results indicate that the hypotheses that protein restriction and social confrontation would lower the reproductive success were wrong (hypothesis 1a & 1b; see 1.6). Early life cues clearly had a negative influence on the body size, but females and males do not seem to suffer from this effect, at least not as far as reproductive success goes. In the female experiment there was also no interacting effect between the early life treatments and the population density, indicating that they certainly were not "adapted" to living in a certain density (*sensu* Fig. 1b) but also did not "suffer" from the early life treatments (*sensu* Fig. 1a). Simultaneously, the chance of reproduction did depend on the population

density, indicating that the reproductive success is plastically changed depending on the adult environment (*sensu* Fig. 1c). It should be noted that in natural populations, bank vole females have multiple litters and so early life effects might only become apparent in later litters or at a later age.

The male experiment (chapter II) did not specifically address the reproductive success in either a "good" or "bad" environment, hence it is impossible to adequately address the adaptive value. Nevertheless, the reproductive setup that had competition and the female preference, in which there was no direct competition, both indicated that there was no difference in male reproductive success due to the early life cues. These results indicate that the early life environment either has no effect at all on male reproductive success or it did have an effect, but all individuals were able to plastically respond to the competitive situation (*sensu* Fig. 1c).

The combined results from 3.1.1 and 3.1.2 indicate that, while early life cues can impact the morphology of male and female bank voles, it does not seem to meaningfully impact the reproductive success. Nevertheless, it is possible that the early life cues impacted fitness-related traits not addressed in this study. For example, I did not address the link between early life cues and parasite load and infection (Hoeijmakers *et al.* 2015). Yet parasite load and infection are important aspects in a bank vole's life (Cayol *et al.* 2017) and can impact reproductive success (Cayol *et al.* 2018).

3.2 The inter-generational response to early life cues

3.2.1 F2 growth

The female and male experiments had different setups with the former (chapter I) having the added treatment of density while the latter (chapter II) did not. Furthermore, F2 individuals born in the female experiment were born and raised in the field, and those from the male experiment were born and raised in the laboratory. Nevertheless, the result in both cases was that social confrontation during the early life environment of its parent had effects on the F2's adult body mass. In both cases, ignoring the density treatment for the maternal experiment, social confrontation F2 individuals had lower adult body masses. Maternally, social confrontation interacted with the population density, negating the detrimental effect of growing up in a high population density (chapter I). Protein restriction on the other hand had a negative, but insignificant effect on offspring body mass. Paternally, the PR treatment did significantly and negatively affect the F2's adult body mass (chapter II). So overall, high population density cues in the early life of parents lead to intergenerational negative effects on body mass offspring, but can also lead to an adaptive response given that the environment matches that of the mother.

My predictions were partly confirmed *i.e.* protein restriction in the parental generation leading to a negative effect on the offspring body mass

(hypothesis 2a; see 1.6), although not always significantly. From a maternal standpoint, lowered offspring body mass could be due to lowered maternal investment (Oksanen *et al.* 1999, Koskela *et al.* 2004). The negative effect on F2 body mass due to paternal protein restriction could be explained through several paternal effects (see 1.3.2) but most likely it is due an epigenetic mechanism, rather than *e.g.* differential maternal investment, as females did not seem to recognize male treatment groups.

The prediction that social confrontation would have a negative intergenerational effect on F2 body mass (hypothesis 2b; see 1.6) was also somewhat confirmed in both experiments, at least as far as the adult body mass goes (paternal social confrontation had no effect on birth mass). However, in the paternal experiment, protein restriction and social confrontation significantly interacted and actually increased F2 adult body mass, indicating that specific environmental cues can lead to complex effects. The mechanism through which the changes of F2 body mass happened could again be explained either through lowered maternal investment or possibly intrinsic epigenetic mechanism (in both maternal and paternal experiment).

The general trend of the intergenerational effects of the F1 early life on F2 body mass, excluding the interaction in the paternal experiment, seem to follow the pattern of an intergenerational silver spoon. In a natural population, this could lead to amplifying effect of high population density effect, by both inducing intra-generational negative effects on body mass (see 3.1.1) but also inheriting negative effects from previous generations. However, in the maternal experiment it was obvious that, although in both densities the social confrontation F2 individuals had a lower body mass compared to control individuals in the low density enclosures, in the high density enclosures the treated F2 were a lot less affected by the density compared to controls. As such, this results more resembles the pattern of an intergenerational predictive adaptive response (*sensu* Fig. 1b), except that there was no negative effect for social confrontation F2 growing in a low density environment. It is possible that individuals are able to plastically adapt to a low density environment and not a high density environment, hence creating a mix of plastical adaptation and predictive adaptive response (Bonduriansky and Crean 2018).

3.2.2 F2 survival

The main fitness measurement for the F2 individuals, besides growth, was winter survival in both the female and male experiment. Again, the setup in both experiments was not completely similar as in the female experiment the F2 individuals were in different population densities. Nevertheless, social confrontation during the early life of the parent had similar effects in both experiments and increased the chance of survival. In the maternal experiment, social confrontation interacted with population density (p= 0.0537), so the apparent negative effect on survival is negated when social confrontation was present during the mother's early life environment. Protein restriction had no

detectable effect on the winter survival of F2 in either the female or male experiment.

The results confirm that indeed there is an intergenerational predictive adaptive response through the maternal line due to social confrontation (*sensu* Fig. 1b), especially combined with the results from the F2 growth in high population densities (hypothesis 3a; see 1.6). Similarly, although the true adaptive value was not investigated, the results confirm that the paternal early life can impact the offspring fitness (hypothesis 3b; see 1.6). As I only investigated some aspects of F2 fitness, it is possible that other aspects were negatively affected (*e.g.* post-winter reproductive success). Regardless, the F2 individuals in both the maternal and paternal experiment were born and released just in time for the winter, which means that the next springtime is their first opportunity to mate, hence making winter survival an essential fitness trait. Protein restriction did not seem to have any effect on the F2 survival in either the female or male experiment. Thus, while having some effect on the F2 phenotype, there does not seem to be any effect on the fitness, at least not in the traits measured in this thesis.

3.3 Molecular mechanisms

3.3.1 Pyrosequencing

Neither the oxytocin gene promoter region (OT) nor the oxytocin receptor gene promoter region (OTR) showed significantly different methylation percentages between treatment groups (all p-values > 0.1; Fig. 6). Although it is possible that the lack of significant differences is due to sample size, overall variation in percentage methylated per CpG site was low (results not shown). These results indicate that neither a protein restricted diet nor an early life environment with social confrontation has an effect on the oxytocin regulation, although more extensive research is necessary to confirm this.

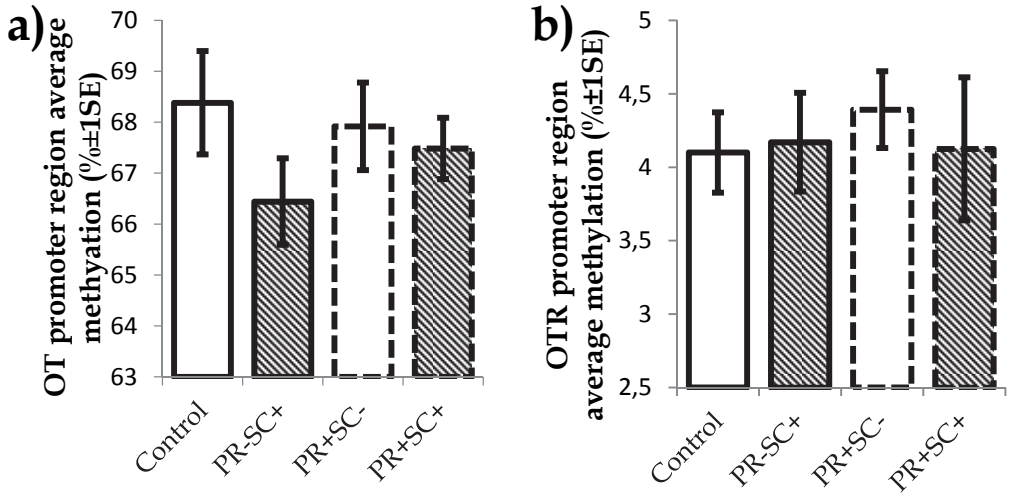


FIGURE 6 (a) average methylation of F1 oxytocin gene promoter region (10 CpG sites) per treatment group (n=23). (b) average methylation of F1 oxytocin receptor gene promoter region (20 CpG sites) per treatment group (n=23). Shaded bars indicate early life social confrontation; dashed lines indicate presence of early life protein restriction. Control= PR-SC-; OT= Oxytocin gene; OTR= Oxytocin receptor gene; PR= Protein Restriction; SC= Social Confrontation. (+) indicates presence of treatment; (-) indicates absence of the treatment

The results indicate that the prediction was wrong (hypothesis 4a; see 1.6) and oxytocin (receptor) expression is not related to the early life treatments. One reason why there is so little to no difference could be because oxytocin is abundant and also impacts various homeostatic physiological processes (Gimpl and Fahrenholz 2001) besides just behavioural traits and reproductive traits. While there does not seem to be a real difference between treatments, the results could be expanded and additionally, expression data could be added to confirm potential methylation differences.

3.3.2 rRNA gene copy number variation

Protein restriction given to a pregnant and nursing F0 mother did not change its 18s rRNA gene copy number. However, intra-uterine and pre-weaning exposure of F1 to a protein restricted environment caused significantly less copy numbers than control individuals as adults, and thus confirms my prediction (hypothesis 4b; see 1.6). The gametes which eventually led to F1 were already developed prior to the protein restriction and fathers were not treated. Hence, the observed change in 18s rRNA gene copy number most likely happened during the intra-uterine or early life development of the F1.

The observed change in rRNA gene copy numbers indicates that growing individuals are able to rapidly change their ribosomal genetic architecture as a response to specific environmental cues. Although it was not tested, it is possible that the loss of rRNA is linked to the reduced birth and body mass seen in the PR+ treatment (see 3.1.1) as has been observed in humans (Zafiropoulos *et al.* 2005) and to some extend in poultry (Su and Delany 1998). It is difficult to say how this mechanism could function within natural populations. In this case,

it would indicate that in high population densities, bank voles will have decreased rRNA copy numbers. On one hand, DNA is heritable and hence copy number variation will have an intergenerational impact. On the other hand, our results show that rRNA can plastically decrease to the growing environment. As we can assume that all bank voles have at least a functioning amount of rRNA copies, we can also assume that there cannot be an infinite decrease in rRNA copies. Indeed, there exists a mechanism (Hawley and Tartof 1985) that can extend the number of ribosomal RNA copies. If this mechanism can also respond intra-generationally to environmental cues, it would mean that rRNA copy number is solely an intra-generational plastic mechanism that might, or might not, relate to the growth of an individual. Further research is needed to confirm this last point, perhaps with protein supplemented diet and/or a multi-generational study.

4 CONCLUSION AND FUTURE DIRECTIONS

In this thesis, I have investigated the intra- and intergenerational effects of the early life environment in bank voles. The use of ecologically relevant cues allowed me to conclude that both the dietary environment and the social environment have persistent effects on the individual experiencing the cues. By mating the focal individuals I could further confirm that these early life effects influence the phenotype and fitness of their F2 offspring, partly in an adaptive manner. Finally, the investigation of molecular mechanisms that respond to the same early life treatments allowed me to assess possible underlying mechanisms.

My results showed that a common mammal, home to most of Europe and Asia, is able to respond (adaptively) to a sudden change in environment. Both implicit (protein) and explicit (social environment) cues seem to be able to independently evoke morphological changes and intergenerational changes, yet both cues together can lead to complex responses. Overall, it seems that the social environment has more significant consequences, and is able to influence basic fitness traits. It is strange that an arguably "subjective" measure of population density (social confrontation) has a bigger impact than an "objective" measure as dietary quality. It is of course possible that protein is not a limiting factor in natural populations of bank voles (Dróżdż 1968, Hansson 1971) and as such cannot act as a trustworthy signal of population density. Proper diet analyses in natural bank vole populations are still lacking and so it is hard to assess this point. However, most literature in vertebrates does agree that protein is important (Goettsch 1960, Pinheiro *et al.* 2008, Laus *et al.* 2011, Forbes *et al.* 2014b) and so I argue that even though protein restriction leads to (negative) intra- and intergenerational silver spoon effects, eventually a population will (intra-generationally) plastically adapt to the protein content of their environment. When thinking about the cyclic population fluctuations in Northern bank vole populations, this result would indicate that at least protein content is probably not an important aspect of these fluctuations (but see Forbes *et al.* 2014). In a broader sense, perhaps my results indicate that the focus on protein restriction in laboratory studies on rodents is not necessarily as (eco-

evolutionary) relevant for natural populations, including humans, as it is presumed to be.

Social confrontation, on the other the hand, led to an intergenerational predictive adaptive response. And this response was similar through the maternal and paternal line, indicating that there would not be a sexual conflict limiting this effect on offspring survival. Furthermore, it would mean that in natural populations body mass and survival would increase with increasing population density (*sensu* Chitty effect (Oli 1999)), thereby creating a positive feedback loop. It is possible that this indeed happens in the natural population cycles until the population carrying capacity is reached, after which the population crashes and is "resetted" as far as intergenerational programming goes. Of course, my results stem from two separate experiments and hence any conclusions on the absence of a sexual conflict might be presumptious. Furthermore, even though I found an adaptive response, I did not find any trade-of (Zahavi 1977). Without knowing the trade-of, be it in the fitness of the parents or offspring, any predictions on a larger timescale should be made with care.

My results showed that at least one molecular mechanism responded to early life protein restriction. While the plasticity of ribosomal DNA copy number has not been shown in many species, it is doubtful that it is limited to fruit flies (Aldrich and Maggert 2015) and bank voles and is probably present in nearly all animal species. The relation between rRNA copy number and the phenotype (and fitness) is still very unclear in animals. However, as ribosomal DNA has such a basic and essential role in all of the cellular activity, it is likely that it could have some impact. As such, it is interesting that protein restriction alone is already a strong enough cue to reduce the copy numbers and it would be interesting to study in the future how the social confrontation treatment affected the rRNA copy number. Moreover, it would be interesting to see whether the F2 generation inherited the copy numbers to some degree, or if it is "resetted" every generation.

Although I did not find the exact mechanism behind the various intra- and intergenerational effects, it is likely a combination of behavioural, physiological and/or (epi)genetic mechanisms. It is probable that other rodents, mammals or even other animals could respond in a similar fashion, *i.e.* have a primary (intra-generational) response to the immediate environmental signals but also a secondary, intergenerational and adaptive response. This is good news in light of the rapidly changing climate and could indicate that even within-generational changes in *e.g.* diet availability would not mean a population is doomed.

The results I found in my thesis should motivate researchers to look beyond one generation. Fitness is rarely assessed across generations in vertebrates, yet my results from the female experiment would not have found any adaptive value if I only looked at the F1, their reproductive success and the birthweight of F2. In the same sense, I hope my research encourages research into paternal effects, even though they seem unlikely, as they initially did in the

bank vole. If we truly hope to fully understand *how* individuals, populations and species can change when faced with a novel environment, we have to account for the facts that change could take multiple generations, and can be transmitted through less obvious mechanisms, such as paternal pathways.

Acknowledgements

It is weird that a thesis in Finland is traditionally written in the "I" form as there is nearly nothing I have really done all by myself. From the actual science to my mental, and at one stage physical, health, my thesis was a collaboration of great minds and good friends. And more often than not, people fell in both those categories.

First of all, I am enormously grateful to Michael Sheriff for reviewing my thesis and acting as the opponent and Suvi Ruuskanen and Tobias Uller for pre-examining my thesis.

Esa Koskela, my custos, my supervisor and foremost my friend. Thank you for having faith in me from the start and guiding me through my PhD. You were always ready for a discussion and a laugh. I will never forget you singing "*Meat is murder*" from the Smiths, whilst handling voles.

Tapio Mappes, you have taught me the finer details of academics and I will never forget that you taught me "never to read too much or you won't have any original ideas" and "reviewers are stupid". Most of all, you taught me that sometimes saying nothing is better than saying too much (both in papers and in real life).

Phill Watts, I think the greatest thing you (tried to) teach me is (1) ~~how I could become a better writer scientifically~~ how to write like a scientist, (2) take a step back and look at my data, and (3) think about what people will find interesting. Your practicality and writing style will hopefully be reflected in my work for many years to come.

Angela Sims, I cannot thank you enough for getting me through the first year of my PhD. I think I would have been lost without a mentor like you to toughen me up against Esa, Tapio and Phill. Both one of the most challenging and fun times I had during my thesis was the first summer experiment, where alcohol and voles went hand-in-hand, but sleep did not.

A big thank you to all of the "technical staff" who has helped me in the laboratory and in the field! Sami, Juho, the coolest lab techs in the field, thanks for putting up with my lack of laboratory knowledge. Anja, Susanne and Anna, my sample size would have been pretty small without your enormous help. Anja, I'm sorry for breeding so many animals and subsequently not really knowing what to do with them. Aija, Sandra and Suvi, thank you for keeping the voles alive! And also a big thank you to the technical staff in Konnevesi Research Station, especially Helenä, for keeping the voles happy during their holidays in Konnevesi.

To all my Finland friends, who can probably be collectively called the "poker-sauna group and friends", thank you for the great evenings in Finland. It takes a lot of friends to survive a PhD during the long, dark winters, and I certainly had enough of them. At the risk of forgetting some people, a special thanks to (in alphabetical order): Aigi, Anbu, David, Emily, Jaakko, Jaanna, Jeff & Ati, Jenna, Jimi, Manoj, Marina, Maz & Zara, Oopi, Otto, Stephen, Sebastiano and so many others! And of course, also my two pretend-sisters who used to be

in my office and research group, but have loosened the shackles of PhD some time ago: Claire and Eija!

Besides my “Finland friends” I would also like to thank my friends on the home front for always making sure that every time I went to Belgium there was ample room for fun and relaxation. Specifically, I want to mention Heyde, Smets and Thoelen. I am sorry for making so many nights in Belgium way too late.

I was lucky enough to get the opportunity to go to the UK for a research stay in the lab of Christopher Murgatroyd at the MMU and I am very grateful for that period as it taught me how stream lined a lab *could* run, at least when qiagen decided they could be bothered to deliver the reagents. A special thanks to my office-mates there, who showed me a very welcome time. Also a special thanks to Michael Carroll and Stephane Berneau, who at the time of writing should still be looking at the penisbones from my voles. I would also like to thank Laura Lahtinen and the Central Hospital Central Finland for allowing me to continue my methylation analyses.

Second to last, I would like to thank my family. My studies did not always go straightforward but I always had the feeling that I could accomplish whatever I wanted, and that is totally because of my parents, my brother and my sister. And look at me now! My sister, Marie-Ann, taught me how to have fun in life and be sociable. My brother, Dries, taught me to think for myself and not to care too much about what authorities, including teachers, have to say. My father, Dominique, taught me how to be self-reliant and proud of myself, without gloating, skills which are absolutely necessary for a PhD abroad. My mother, Reinhilde, who was herself a PhD in Biology, taught me how to be a critical thinker and scientist; but most of all, it was her love and support that made me feel confident to even start a PhD.

Lastly, and most importantly, I would like to thank my fiancée, friend and recently PhD Nathalie. There is absolutely no way I would have gotten to this point in my life if it was not for her. Words cannot express how grateful I am for sticking with me. Not only during our studies, but also during the four years living in different countries. This PhD is as much the result of my efforts as it is of her effort. I could not wish for a better partner and I will eternally grateful for you for joining me on my adventure.

Undoubtedly, I will have forgotten to thank people. If you feel you deserve praise, you probably do.

This thesis is the accumulation of work and friendship, and some beers on the side. Thank you everyone, and thank you, Jyväskylä.

SAMENVATTING (RÉSUMÉ IN DUTCH)

Intergenerationele antwoorden op een veranderende omgeving: de vroege omgeving van moeder en vader bepalen de fitness van de rosse woelmuis (*Myodes glareolus*)

De omgeving waarin een individu opgroeit, van bevruchte eicel tot volwassene, kan een aanzienlijke invloed hebben op het verdere leven van dat individu. Deze effecten kunnen een negatieve impact hebben op de fitness (bv. ondervoeding verlaagt het lichaamsgewicht permanent) of een positieve impact (bv. een omgeving met weinig predatoren leidt tot een hogere overlevingskans). Daarnaast kan de omgeving tijdens het jonge leven ook leiden tot een aanpassing van het individu aan zijn omgeving zodat het individu geen fitnessverlaging ondervindt, op voorwaarde dat die omgeving constant is. Onze kennis over deze *early life effects* is ondertussen uitgebreid, van laboratoriumexperimenten tot observaties in wilde populaties. Wat echter nog niet duidelijk is, is in welke mate deze effecten worden doorgegeven naar de volgende generatie, i.e. intergenerationele effecten.

Onderzoek naar intergenerationele effecten waren tot nu toe beperkt tot bv. ouderlijke zorg en nestlocatie. Aangezien vaderlijke zorg eerder zeldzaam is bij de meeste soorten met ouderzorg, werden traditioneel vooral maternale effecten bestudeerd. De moeder kan reeds invloed uitoefenen op haar nakomelingen in de baarmoeder nog vóór hun geboorte, en ook erna. De recente vooruitgang in epigenetica doet echter vermoeden dat intergenerationele effecten veel algemener zouden kunnen zijn. Epigenetica omvat een groep van moleculaire mechanismen die de werking van DNA kunnen veranderen, zonder het DNA zelf te veranderen. Epigenetische mechanismen kunnen worden vastgelegd gedurende het vroege leven en vervolgens worden doorgegeven naar de volgende generatie, net zoals DNA. Bijgevolg kunnen deze mechanismen ook via de vader werken. Vanuit een eco-evolutionair standpunt is de vraag of de invloed die ouders uitoefenen op hun nakomelingen (voor of na de geboorte), al dan niet een adaptief voordeel kan bieden voor hun latere leven. Een dergelijk mechanisme zou immers een nakomeling een fitnessvoordeel kunnen geven, zonder zelf aan die omgeving blootgesteld te zijn geweest tijdens de ontwikkeling. Op deze manier zouden individuen of populaties zich op een korte tijd kunnen aanpassen aan een nieuwe omgeving.

In deze thesis heb ik bestudeerd hoe specifieke omgevingssignalen tijdens de vroege ontwikkeling van de rosse woelmuis (*Myodes glareolus*) de groei en voortplanting van het individu zelf beïnvloeden (*i.e.* effecten in dezelfde generatie). Daarnaast heb ik ook bestudeerd welke effecten deze invloeden tijdens het vroege leven op de volgende generatie kunnen hebben, zowel vanuit het perspectief van de moeder als van de vader (i.e. effecten in de volgende generatie(s)). Ten slotte heb ik ook twee achterliggende epigenetische mechanismen onderzocht: DNA-methylatie en ribosomaal RNA gen *copy*

number variation, waarvan geweten is dat ze overerfbaar zijn en veranderen t.g.v. omgevingssignalen. De rosse woelmuis is uitermate geschikt om dit onderwerp te bestuderen aangezien het een veel voorkomend zoogdier is in Europa en Azië, en dus ecologisch relevant, en daarnaast bekend staat voor zijn cyclische populatiedensiteit. Hun densiteit fluctueert doordat in verschillende jaren natuurlijke populaties aan verschillende omgevingssignalen worden blootgesteld. Dit laat toe experimentele signalen te kiezen die ecologisch relevant zijn: competitie voor voeding en territoriumverdediging.

Om het belang van maternale en paternale intergenerationele effecten te bestuderen heb ik twee grote experimenten uitgevoerd waarbij individuen gedurende hun vroege ontwikkeling experimenteel werden blootgesteld aan twee omgevingssignalen: beperkt proteïnegehalte in de voeding en/of sociale confrontatie. Beiden zijn gelinkt aan een hoge populatiedensiteit.

In het maternaal experiment werden volwassen vrouwtjes in het laboratorium blootgesteld aan de experimentele omgevingssignalen tijdens hun vroege leven, en daarna in semi-natuurlijke omheinde gebieden gezet waarin ze konden voortplanten met niet-experimentele mannetjes. De gebieden hadden ofwel een lage, ofwel een hoge populatiedensiteit. Hierdoor konden we de effecten op het voortplantingssucces van de vrouwtjes bestuderen en vervolgens het effect op hun nakomelingen. De groei van de nakomelingen werd gevolgd en uiteindelijk, op volwassen leeftijd, werd het overlevingssucces van de nakomelingen in de winter gevolgd. Winteroverleving wordt gezien als één van de belangrijkste factoren voor het bepalen van de fitness in rosse woelmuizen in Fennoscandinavië. De hypothese is dat de vroege levenssignalen van de ouders hun nakomelingen voorbereiden op een dichtheid die overeenkomt met de signalen en dus hun fitness, hier winteroverleving, hoger is dan die van de andere groepen. De nakomelingen werden daarom voor de winter opnieuw in de twee populatiedensiteiten verdeeld, waardoor vier groepen gecreëerd werden. Twee van deze groepen van nakomelingen zaten in de winter in dezelfde dichtheid als waarin ze zijn opgegroeid en de twee andere groepen werden in de andere dichtheid geplaatst. Hierdoor kon het relatieve effect onderzocht worden van (1) de densiteit in de winter, (2) de densiteit waarin de nakomelingen opgegroeid zijn en (3) de vroege levenssignalen waaraan hun ouders werden blootgesteld. De resultaten tonen aan dat nakomelingen van ouders die werden blootgesteld aan sociale confrontatie inderdaad beter overleefden in een hoge densiteit dan de andere groepen, maar een laag proteinegehalte had geen intergenerationale effecten. De omgeving waarin nakomelingen zelf waren opgegroeid was niet belangrijk, maar dus wel die van hun ouders. Dit illustreert duidelijk het belang van de *inter*generationele effecten en kan beschouwd worden als een adaptieve respons.

Het paternale experiment was eenvoudiger: de blootstelling aan vroege levenssignalen waren dezelfde als in het maternale experiment maar ze werden achteraf niet aan verschillende populatiedensiteiten blootgesteld. In het paternale experiment werden experimentele volwassen mannetjes in een

competitieve seminatuurlijke omgeving gezet met niet-experimentele vrouwtjes. De groei en winteroverleving van de nakomelingen werd onderzocht om na te gaan of hun vader signalen kon doorgeven en zo het latere leven van de nakomelingen kon beïnvloeden. Dit was onwaarschijnlijk om twee redenen: 1) rosse woelmuizen hebben geen vaderlijke zorg en 2) vrouwtjes kunnen mannetjes niet onderscheiden op basis van hun vroege levenssignalen en dus ook niet meer (of minder) investeren in de nakomelingen van deze mannetjes. Desalniettemin blijkt dat nakomelingen van mannetjes die blootgesteld werden aan sociale confrontatie, een hogere overlevingskans hebben, en dus beïnvloed worden door hun vaders. Een laag proteinegehalte had ook hier geen intergenerationeel effect op overleving van de nakomelingen. De specifieke experimentele structuur laat toe te besluiten dat deze effecten hoogstwaarschijnlijk via epigenetische mechanismen werken.

Uit onderzoek naar de twee moleculaire mechanismen, DNA-methylatie en variatie in het aantal ribosomaal RNA gen-kopieën, bleek dat het methylatiepatroon van het oxytocinegen en het oxytocine receptorgen niet veranderde door het proteinegehalte of de sociale confrontatie. Oxytocine was geselecteerd omdat het een belangrijk gedragshormoon is voor bv. maternale zorg. Het aantal kopieën van het 18s ribosomaal RNA gen daartegenover veranderde wel door proteïnebeperking, waarbij het aantal kopieën werd verlaagd. Ribosomaal DNA is een belangrijk gen voor celactiviteit en is gelinkt aan veroudering en verschillende ziektes, o.a. bepaalde vormen van kanker.

In deze thesis heb ik aangetoond dat milde, ecologisch relevante signalen in de vroege ontwikkeling van een wild knaagdier, gevolgen kunnen hebben voor de groei en reproductie van het individu zelf, maar ook voor de nakomelingen, en dat dit zowel via de moeder als via de vader gestuurd kan worden. Vanuit het standpunt van de moeder kan dit adaptief zijn. Ik heb ook aangetoond dat deze signalen de genetische code van een zeer conservatief gen, ribosomaal DNA, kan veranderen. Wat nog verder onderzocht moet worden, is in welke mate dit overerfbaar is en welke impact dit mechanisme op het latere leven van het individu en zijn/haar nakomelingen kan hebben.

REFERENCES

Aldrich J.C. & Maggert K.A. 2015. Transgenerational Inheritance of Diet-Induced Genome Rearrangements in Drosophila. *PLoS Genet.* 11: 1–21.

Alonso-Alvarez C., Bertrand S., Devevey G., Prost J., Faivre B., Chastel O. & Sorci G. 2006. An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution (N. Y).* 60: 1913–1924.

Astbury W.T. 1961. Molecular biology or ultrastructural biology? *Nature* 190: 1124.

Bartolomucci A., Palanza P., Gaspani L., Limiroli E., Panerai A.E., Ceresini G., Poli M.D. & Parmigiani S. 2001. Social status in mice: Behavioral, endocrine and immune changes are context dependent. *Physiol. Behav.* 73: 401–410.

Berger S.L., Kouzarides T., Shiekhattar R. & Shilatifard A. 2009. An operational definition of epigenetics. *Genes Dev.* 23: 781–783.

Bieswal F., Ahn M.T., Reusens B., Holvoet P., Raes M., Rees W.D. & Remacle C. 2006. The importance of catch-up growth after early malnutrition for the programming of obesity in male rat. *Obesity* 14: 1330–1343.

Bird A. 2002. DNA methylation patterns and epigenetic memory. *Genes Dev.* 16: 6–21.

Bonduriansky R. & Crean A.J. 2018. What are parental condition-transfer effects and how can they be detected? *Methods Ecol. Evol.* 9: 450–456.

Boonstra R., Krebs C.J. & Stenseth N.C. 1998. Population cycles in small mammals: the problem of explaning the low phase. *Ecology* 79: 1479–1488.

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.

Bossdorf O., Richards C.L. & Pigliucci M. 2008. Epigenetics for ecologists. *Ecol. Lett.* 11: 106–115.

Boutin S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. *Can. J. Zool.* 68: 203–220.

Boyes J. & Bird A. 1991. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell* 64: 1123–1134.

Brieño-Enríquez M.A., García-López J., Cárdenas D.B., Guibert S., Cleroux E., Děd L., Hourcade J.D.D., Pěknicová J., Weber M. & Mazo J. Del. 2015. Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of microRNAs in primordial germ cells. *PLoS One* 10: 1-19.

Bruder C.E.G., Piotrowski A., Gijsbers A.A.C.J., Andersson R., Erickson S., Diaz de Ståhl T., Menzel U., Sandgren J., Tell D. von, Poplawski A., Crowley M., Crasto C., Partridge E.C., Tiwari H., Allison D.B., Komorowski J., Ommen G.J.B. van, Boomsma D.I., Pedersen N.L., Dunnen J.T. den, Wirdefeldt K. & Dumanski J.P. 2008. Phenotypically Concordant and Discordant Monozygotic Twins Display Different DNA Copy-Number-Variation Profiles. *Am. J. Hum. Genet.* 82: 763–771.

Burley N.T. 1988. The differential-allocation hypothesis: an experimental test. *Am. Nat.* 132: 611–628.

Burt W.H. 1943. Territoriality and Home Range Concepts as Applied to Mammals. *J. Mammal.* 24: 346.

Burton T. & Metcalfe N.B. 2014. Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B Biol. Sci.* 281: 20140311.

Cayol C., Koskela E., Mappes T., Siukkola A. & Kallio E.R. 2017. Temporal dynamics of the tick *Ixodes ricinus* in northern Europe: epidemiological implications. *Parasites and Vectors* 10: 1-11.

Cayol C., Giermek A., Gomez-Chamorro A., Hytönen J., Kallio E.R., Mappes T., Salo J., Voordouw M.J. & Koskela E. 2018. *Borrelia afzelii* alters reproductive success in a rodent host. *Proc. R. Soc. B Biol. Sci.* 285: 20181056

Champagne F.A. 2008. Epigenetic mechanisms and the transgenerational effects of maternal care. *Front. Neuroendocrinol.* 29: 386–397.

Champagne F.A, Francis D.D., Mar A. & Meaney M.J. 2003. Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol. Behav.* 79: 359–371.

Champagne F.A. & Meaney M.J. 2006. Stress During Gestation Alters Postpartum Maternal Care and the Development of the Offspring in a Rodent Model. *Biol. Psychiatry* 59: 1227–1235.

Cravioto J., DeLicardie E.R. & Birch H.G. 1966. Nutrition, Growth and Neurointegrative Deveopment: an Experimental and Ecologic Study. *Pediatrics* 38: 319–320.

Crean A.J. & Adler M.I. 2016. Seminal Fluid and Mate Choice : New Predictions. *Trends Ecol. Evol.* 31:253-255

Crean A.J. & Bonduriansky R. 2014. What is a paternal effect ? *Trends Ecol. Evol.* 29: 554–559.

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.

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.

Darwin C. 1859. *On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life.* Murray, London.

Derrickson E.M. & Lowas S.R. 2007. The Effects of Dietary Protein Levels on Milk Protein Levels and Postnatal Growth in Laboratory Mice (*Mus musculus*). *J. Mammal.* 88: 1475–1481.

Dobzhansky T. 1935. Maternal effect as a cause of the difference between the reciprocal crosses in *Drosophila pseudoöbscura*. *Proc Nat Acad Sci USA* 21: 443–446.

Donelson J.M., Salinas S., Munday P.L. & Shama L.N.S. 2018. Transgenerational plasticity and climate change experiments: Where do we go from here? *Glob. Chang. Biol.* 24: 13–34.

Donkin I., Versteyhe S., Ingerslev L.R., Qian K., Mechta M., Nordkap L., Mortensen B., Appel E.V.R., Jørgensen N., Kristiansen V.B., Hansen T., Workman C.T., Zierath J.R. & Barrès R. 2016. Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. *Cell Metab.* 23: 369–378.

Drake A.J. & Liu L. 2010. Intergenerational transmission of programmed effects: Public health consequences. *Trends Endocrinol. Metab.* 21: 206–213.

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.

Dróżdż A. 1968. Digestibility and assimilation of natural foods in small rodents. *Acta Theriol. (Warsz).* XIII: 367–389.

Dumais K.M. & Veenema A.H. 2016. Vasopressin and oxytocin receptor systems in the brain: Sex differences and sex-specific regulation of social behavior. *Front. Neuroendocrinol.* 40: 1–23.

Dumas L., Kim Y.H., Karimpour-Fard A., Cox M., Hopkins J., Pollack J.R. & Sikela J.M. 2007. Gene copy number variation spanning 60 million years of human and primate evolution. *Genome Res.* 17: 1266–1277.

Emanuel I. 1986. Maternal Health during Childhood and Later Reproductive Performance. *Ann. N. Y. Acad. Sci.* 477: 27–39.

Ergon T., Lambin X. & Stenseth N.C. 2001. Life-history traits of voles in a fluctuating population respond to the immediate environment. *Nature* 411: 1043–1045.

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

Frankova S. & Barnes R.H. 1968. Influence of malnutrition in early life on exploratory behavior of rats. *J. Nutr.* 96: 477–484.

Fullston T., Teague E.M.C.O., Palmer N.O., Deblasio M.J., Mitchell M., Corbett M., Print C.G., Owens J.A. & Lane M. 2013. Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB J.* 27: 4226–4243.

Gil D., Heim C., Bulmer E., Rocha M., Puerta M. & Naguib M. 2004. Negative effects of early developmental stress on yolk testosterone levels in a passerine bird. *J. Exp. Biol.* 207: 2215–2220.

Gilbert S.F. 2001. Ecological Developmental Biology: Developmental Meets the Real World. *Dev. Biol.* 233: 1–12.

Gimpl G. & Fahrenholz F. 2001. The Oxytocin Receptor System: Structure, Function, and Regulation. *Physiol. Rev.* 81: 629–683.

Gluckman P.D., Hanson M.A. & Spencer H.G. 2005. Predictive adaptive responses and human evolution. *Trends Ecol. Evol.* 20: 527–533.

Goettsch M. 1960. Comparative Protein Requirement of the Rat and Mouse for Growth, Reproduction and Lactation Using Casein Diets. *J. Nutr.* 70: 307-312.

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

Grafen A. 1988. On the uses of data on lifetime reproductive success. In: Clutton-Brock T. (ed.), *Reproductive success*, University of Chicago Press, Chicago, IL, pp. 454–471.

Grandjean V., Gounon P., Wagner N., Martin L., Wagner K.D., Bernex F., Cuzin F. & Rassoulzadegan M. 2009. The miR-124-Sox9 paramutation: RNA-mediated epigenetic control of embryonic and adult growth. *Development* 136: 3647–3655.

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.

Haig D. 2014. Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity (Edinb).* 113: 96–103.

Hanski I., Hansson L. & Henttonen H. 1991. Specialist Predators, Generalist Predators, and the Microtine Rodent Cycle. *J. Anim. Ecol.* 60: 353.

Hansson L. 1971. Small rodent food, feeding and population dynamics A comparison between granivorous and herbivorous species in Scandinavia. *Oikos* 22: 183–198.

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.

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.

Hawley R.S. & Tartof K.D. 1985. A two-stage model for the control of rDNA magnification. *Genetics* 109: 691–700.

Hayward A.D. & Lummaa V. 2013. Testing the evolutionary basis of the predictive adaptive response hypothesis in a preindustrial human population. *Evol. Med. Public Heal.* 2013: 106–117.

Heard E. & Martienssen R.A. 2014. Transgenerational epigenetic inheritance: Myths and mechanisms. *Cell* 157: 95–109.

Henrich S. 1988. Variation in Offspring Sizes of the Poeciliid Fish *Heterandria formosa* in Relation to Fitness. *Oikos* 51: 13.

Hoeijmakers L., Lucassen P.J. & Korosi A. 2015. The interplay of early-life stress, nutrition, and immune activation programs adult hippocampal structure and function. *Front. Mol. Neurosci.* 7.

Holliday R. 2006. Epigenetics: A historical overview. *Epigenetics* 1: 76–80.

Holst D. von. 1998. The Concept of Stress and Its Relevance for Animal Behavior. *Adv. Study Behav.* 27: 1–131.

Hörnell A., Lagström H., Lande B. & Thorsdottir I. 2013. Protein intake from 0 to 18 years of age and its relation to health: a systematic literature review for the 5th Nordic Nutrition Recommendations. *Food Nutr. Res.* 57: 21083.

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.

Jablonka E. & Lamb M. 1995. Epigenetic inheritance and evolution - The Lamarckian dimension. *J. Evol. Biol.*: 364.

Jablonka E. & Raz G. 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *Q. Rev. Biol.* 84: 131–176.

Jimenez-Chillaron J.C., Isganaitis E., Charalambous M., Gesta S., Pentinat-Pelegrin T., Faucette R.R., Otis J.P., Chow A., Diaz R., Ferguson-Smith A. & Patti M.E. 2009. Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. *Diabetes* 58: 460–468.

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. *Evol. Ecol.* 16: 455–467.

Kaiser S. & Sachser N. 2005. The effects of prenatal social stress on behaviour: Mechanisms and function. *Neurosci. Biobehav. Rev.* 29: 283–294.

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 defenitions and functions of dominance and territoriality. *Biol. Rev.* 58: 1–20.

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

Kolchinsky E.I., Kutschera U., Hossfeld U. & Levit G.S. 2017. Russia's new Lysenkoism. *Curr. Biol.* 27: R1042–R1047.

Koskela E., Mappes T. & Ylönen H. 1997. Territorial behaviour and reproductive success of bank vole *Clethrionomys glareolus* females. *J. Anim. Ecol.* 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. *J. Anim. Ecol.* 68: 513–521.

Koskela E., Juutistenaho P., Mappes T. & Oksanen T.T.A. 2000. Offspring defence in relation to litter size and age: experiment in the bank vole *Clethrionomys glareolus*. *Evol. Ecol.* 14: 99–109.

Koskela E., Huitu O., Koivula M., Korpimäki E. & Mappes T. 2004. Sex-biased maternal investment in voles: Importance of environmental conditions. *Proc. R. Soc. B Biol. Sci.* 271: 1385–1391.

Lacey E.P., Smith S. & Case A.L. 1997. Parental effects on seed mass: Seed coat but not embryo/endosperm effects. *Am. J. Bot.* 84: 1617–1620.

Lack D. 1954. The Significance of Migration BT - The Natural Regulation of Animal Numbers. In: *The Natural Regulation of Animal Numbers*.

Laforsch C., Beccara L. & Tollrian R. 2006. Inducible defenses: The relevance of chemical alarm cues in Daphnia. *Limnol. Oceanogr.* 51: 1466–1472.

Lamarck J.B. 1809. *Philosophie Zoologique, ou, Exposition des considérations relative à l'histoire naturelle des animaux*. Paris

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. *Int. J. Environ. Res. Public Health* 8: 590–612.

Ledón-rettig C.C., Richards C.L. & Martin L.B. 2012. Epigenetics for behavioral ecologists. *Behav. Ecol.* 24: 311–324.

Lee H.J., Macbeth A.H., Pagani J.H. & Scott Young W. 2009. Oxytocin: The great facilitator of life. *Prog. Neurobiol.* 88: 127–151.

Leips J., Richardson J.M.L., Rodd F.H. & Travis J. 2009. Adaptive maternal adjustments of offspring size in response to conspecific density in two populations of the least killifish, *Heterandria formosa*. *Evolution (N. Y).* 63: 1341–1347.

Lindström J. 1999. Early development in birds and mammals. *Trends Ecol. Evol.* 14: 343–348.

Lonn E., Koskela E., Mappes T., Mokkonen M., Sims A.M. & Watts P.C. 2017. Balancing selection maintains polymorphisms at neurogenetic loci in field experiments. *Proc. Natl. Acad. Sci.* 114: 201621228.

Machado T.D., Dalle Molle R., Laureano D.P., Portella A.K., Werlang I.C.R., Benetti C.D.S., Noschang C. & Silveira P.P. 2013. Early life stress is associated with anxiety, increased stress responsivity and preference for 'comfort foods' in adult female rats. *Stress* 16: 549–556.

Macrì S., Zoratto F. & Laviola G. 2011. Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. *Neurosci. Biobehav. Rev.* 35: 1534–1543.

Maher C.R. & Lott D.F. 2000. A Review of Ecological Determinants of Territoriality within Vertebrate Species. *Am. Midl. Nat.* 143: 1–29.

Mappes T., Koskela E. & Ylonen H. 1995. Reproductive costs and litter size in the bank vole. *Proc. R. Soc. B Biol. Sci.* 261: 19–24.

Marchlewska-Koj A., Kapusta J. & Kruczek M.S. 2003a. Prenatal stress modifies behavior in offspring of bank voles (*Clethrionomys glareolus*). *Physiol. Behav.* 79: 671–678.

Marchlewska-Koj A., Kruczek M., Kapusta J. & Pochroń E. 2003b. Prenatal stress affects the rate of sexual maturation and attractiveness in bank voles. *Physiol. Behav.* 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. *Aggress. Behav.* 24: 241–256.

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.

McRae A.F., Powell J.E., Henders A.K., Bowdler L., Hemani G., Shah S., Painter J.N., Martin N.G., Visscher P.M. & Montgomery G.W. 2014. Contribution

of genetic variation to transgenerational inheritance of DNA methylation. *Genome Biol.* 15: R73.

Messerschmidt D.M., Knowles B.B. & Solter D. 2014. DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos. *Genes Dev.* 28: 812–828.

Mills S.C., Alatalo R. V, Koskela E., Mappes J., Mappes T. & Oksanen T.A. 2007. Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution* 61: 1748–1757.

Mokkonen M., Kokko H., Koskela E., Lehtonen J., Mappes T., Martiskainen H. & Mills S.C. 2011. Negative Frequency-Dependent Selection of Sexually Antagonistic Alleles in *Myodes glareolus*. *Science (80-.).* 334: 972–974.

Monaghan P. 2008. Early growth conditions, phenotypic development and environmental change. *Philos. Trans. R. Soc. B Biol. Sci.* 363: 1635–1645.

Mousseau T. & Fox C. 1998. The adaptive significance of maternal effects. *Trends Ecol. Evol.* 13: 403–407.

Mousseau T.A., Uller T., Wapstra E. & Badyaev A. V. 2009. Evolution of maternal effects: past and present. *Philos. Trans. R. Soc. B Biol. Sci.* 364: 1035–1038.

Murgatroyd C., Patchev A. V, Wu Y., Micale V., Bockmühl Y., Fischer D., Holsboer F., Wotjak C.T., Almeida O.F.X. & Spengler D. 2009. Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat. Neurosci.* 12: 1559–1566.

Newman S., Rutledge J.J. & Riska B. 1989. Estimation of prenatal maternal genetic effects. *J. Anim. Breed. Genet.* 106: 30–38.

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.

Norrdahl K. & Korpimaki E. 1995. Mortality Factors in a Cyclic Vole Population. *Proc. R. Soc. B Biol. Sci.* 261: 49–53.

O'Callaghan N.J. & Fenech M. 2011. A quantitative PCR method for measuring absolute telomere length. *Biol. Proced. Online* 13: 1-10.

Oksanen T.A., Koskela E. & Mappes T. 2002. Hormonal manipulation of offspring number: maternal effort and reproductive costs. *Evolution* 56: 1530–1537.

Oksanen T.A., Jonsson P., Koskela E. & Mappes T. 2001. Optimal allocation of reproductive effort: manipulation of offspring number and size in the bank vole. *Proc. Biol. Sci.* 268: 661–666.

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.

Oli M.K. 1999. The Chitty Effect: A Consequence of Dynamic Energy Allocation in a Fluctuating Environment. *Theor. Popul. Biol.* 56: 293–300.

Ozanne S.E., Olsen G.S., Hansen L.L., Tingey K.J., Nave B.T., Wang C.L., Hartil K., Petry C.J., Buckley A.J. & Mosthaf-Seedorf L. 2003. Early growth

restriction leads to down regulation of protein kinase C zeta and insulin resistance in skeletal muscle. *J. Endocrinol.* 177: 235–241.

Paaby A.B. & Testa N.D. 2018. *Developmental Plasticity and Evolution*. Oxford University Press.

Peixoto-Silva N., Frantz E.D.C., Mandarim-De-Lacerda C.A. & Pinheiro-Mulder A. 2011. Maternal protein restriction in mice causes adverse metabolic and hypothalamic effects in the F1 and F2 generations. *Br. J. Nutr.* 106: 1364–1373.

Pfaffl M.W. 2001. A new mathematical model for relative quantification in RT-PCR. *Nucleic Acids Res.* 29: 16–21.

Pinheiro A.R., Salvucci I.D.M., Aguila M.B. & Mandarim-de-Lacerda C.A. 2008. Protein restriction during gestation and/or lactation causes adverse transgenerational effects on biometry and glucose metabolism in F1 and F2 progenies of rats. *Clin. Sci.* 114: 381–392.

Plaistow S.J., Lapsley C.T. & Benton T.G. 2006. Context□Dependent Intergenerational Effects: The Interaction between Past and Present Environments and Its Effect on Population Dynamics. *Am. Nat.* 167: 206–215.

Plaistow S.J., Clair J.J.H. St., Grant J. & Benton T.G. 2007. How to Put All Your Eggs in One Basket: Empirical Patterns of Offspring Provisioning throughout a Mother's Lifetime. *Am. Nat.* 170: 520–529.

Pol M. Van De, Bruinzeel L.W., Heg D., Jeugd H.P. Van Der & Verhulst S. 2006. A silver spoon for a golden future: Long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). *J. Anim. Ecol.* 75: 616–626.

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.

Raab A., Dantzer R., Michaud B., Mormede P., Taghzouti K., Simon H. & Moal M. Le. 1986. Behavioural, physiological and immunological consequences of social status and aggression in chronically coexisting resident-intruder dyads of male rats. *Physiol. Behav.* 36: 223–228.

Radder R.S., Pike D.A., Quinn A.E. & Shine R. 2009. Offspring Sex in a Lizard Depends on Egg Size. *Curr. Biol.* 19: 1102–1105.

Radford E.J., Ito M., Shi H., Corish J.A., Yamazawa K., Isganaitis E., Seisenberger S., Hore T.A., Reik W., Erkek S., Peters A.H.F.M., Patti M.E. & Ferguson-Smith A.C. 2014. In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science (80-.).* 345.

Rando O.J. 2012. Daddy issues: Paternal effects on phenotype. *Cell* 151: 702–708.

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.

Ratikainen I.I. & Kokko H. 2010. Differential allocation and compensation: Who deserves the silver spoon? *Behav. Ecol.* 21: 195–200.

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.

Redon R., Ishikawa S., Fitch K.R., Feuk L., Perry G.H., Andrews T.D., Fiegler H., Shapero M.H., Carson A.R., Chen W., Cho E.K., Dallaire S., Freeman J.L., González J.R., Gratacòs M., Huang J., Kalaitzopoulos D., Komura D., MacDonald J.R., Marshall C.R., Mei R., Montgomery L., Nishimura K., Okamura K., Shen F., Somerville M.J., Tchinda J., Valsesia A., Woodwark C., Yang F., Zhang J., Zerjal T., Zhang J., Armengol L., Conrad D.F., Estivill X., Tyler-Smith C., Carter N.P., Aburatani H., Lee C., Jones K.W., Scherer S.W. & Hurles M.E. 2006. Global variation in copy number in the human genome. *Nature* 444: 444–454.

Richards E.J. 2006. Inherited epigenetic variation - Revisiting soft inheritance. *Nat. Rev. Genet.* 7: 395–401.

Riska B., Atchley W.R. & Rutledge J.J. 1984. A genetic analysis of targeted growth in mice. *Genetics* 107: 79–101.

Rodgers A.B., Morgan C.P., Bronson S.L., Revello S. & Bale T.L. 2013. Paternal Stress Exposure Alters Sperm MicroRNA Content and Reprograms Offspring HPA Stress Axis Regulation. *J. Neurosci.* 33: 9003–9012.

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.

Sheriff M.J. & Love O.P. 2013. Determining the adaptive potential of maternal stress. *Ecol. Lett.* 16: 271–280.

Sheriff M.J., Krebs C.J. & Boonstra R. 2009. The sensitive hare: Sublethal effects of predator stress on reproduction in snowshoe hares. *J. Anim. Ecol.* 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., Dantzer B., Love O.P. & Orrock J.L. 2018. Error management theory and the adaptive significance of transgenerational maternal-stress effects on offspring phenotype. *Ecol. Evol.* 8: 6473–6482.

Sheriff M.J., Bell A., Boonstra R., Dantzer B., Lavergne S.G., McGhee K.E., MacLeod K.J., Winandy L., Zimmer C. & Love O.P. 2017. Integrating Ecological and Evolutionary Context in the Study of Maternal Stress. *Integr. Comp. Biol.* 57: 437–449.

Sober E. 2000. *Philosophy of Biology*. Daniels & Lehrer, Oxford.

Sokal R.R. & Rohlf F.J. 1969. *Biometry. The Principles and Practice of Statistics in Biological Research*. Freeman, San Fransisco.

Soyfer V.N. 2001. The consequences of political dictatorship for Russian science. *Nat. Rev. Genet.* 2: 723–729.

Stearns S.C. 1989. Trade-Offs in Life-History Evolution. *Funct. Ecol.* 3: 259.

Stein Z. & Susser M. 1975. The Dutch Famine, 1944–1945, and the Reproductive Process. II. Interrelations of Caloric Rations and Six Indices at Birth. *Pediatr. Res.* 9: 76–83.

Stranger B.E., Forrest M.S., Dunning M., Ingle C.E., Beazley C., Thorne N., Redon R., Bird C.P., Grassi A. de, Lee C., Tyler-Smith C., Carter N., Scherer S.W., Tavare S., Deloukas P., Hurles M.E. & Dermitzakis E.T. 2007. Relative Impact of Nucleotide and Copy Number Variation on Gene Expression Phenotypes. *Science (80-.).* 315: 848–853.

Su M.H. & Delany M.E. 1998. Ribosomal RNA Gene Copy Number and Nucleolar-Size Polymorphisms Within and among Chicken Lines Selected for Enhanced Growth. *Poult. Sci.* 77: 1748–1754.

Summers C.H. & Winberg S. 2006. Interactions between the neural regulation of stress and aggression. *J. Exp. Biol.* 209: 4581–4589.

Tamashiro K.L.K., Nguyen M.M.N. & Sakai R.R. 2005. Social stress: From rodents to primates. *Front. Neuroendocrinol.* 26: 27–40.

Team R Development Core. 2011. R: a language and environment for statistical computing.

Thornhill R. 1983. Cryptic Female Choice and Its Implications in the Scorpionfly *Harpobittacus nigriceps*. *Am. Nat.* 122: 765–788.

Tost J. & Gut I.G. 2007. DNA methylation analysis by pyrosequencing. *Nat. Protoc.* 2: 2265–2275.

Trainor B.C. & Marler C.A. 2001. Testosterone, paternal behavior, and aggression in the monogamous California mouse (*Peromyscus californicus*). *Horm. Behav.* 40: 32–42.

Trivers R.L. 1974. Parent-Offspring Conflict. *Am. Zool.* 14: 249–264.

Untergasser A., Cutcutache I., Koressaar T., Ye J., Faircloth B.C., Remm M. & Rozen S.G. 2012. Primer3-new capabilities and interfaces. *Nucleic Acids Res.* 40: e115

Veenendaal M.V.E., Painter R.C., Rooij S.R. De, Bossuyt P.M.M., Post J.A.M. Van Der, Gluckman P.D., Hanson M.A. & Roseboom T.J. 2013. Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine. *BJOG An Int. J. Obstet. Gynaecol.* 120: 548–553.

Verhulst S., Perrins C.M. & Riddington R. 1997. Natal dispersal of Great Tits in a patchy environment. *Ecology* 78: 864–872.

Vijendravarma R.K., Narasimha S. & Kawecki T.J. 2010. Effects of parental larval diet on egg size and offspring traits in *Drosophila*. *Biol. Lett.* 6: 238–241.

Waddington C.H. 1942. The epigenotype. *Endeavour*: 18–20.

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.

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. *Nat. Neurosci.* 7: 847–854.

Weiner J., Martinez S., Muller-Scharer H., Stoll P. & Schmid B. 1997. How Important are Environmental Maternal Effects in Plants? A Study with *Centaurea Maculosa*. *J. Ecol.* 85: 133.

Weinstein R.S. & Haas J.D. 1977. Part two: Early Stress and Later Reproductive Performance Under Conditions of Malnutrition and High Altitude Hypoxia. *Med. Anthropol.* 1: 25–54.

Weismann A. 1875. *Studien zur descendenz-theorie: I. Ueber den saison-dimorphismus der schmetterlinge*. Engelmann. Leipzig.

Weismann A. 1891. Essays Upon Heredity and Kindred Biological Problems. *Oxford Clarendon Press* 1: 1–5.

Weyrich A., Lenz D., Jeschek M., Chung T.H., Rübensam K., Göritz F., Jewgenow K. & Fickel J. 2016. Paternal intergenerational epigenetic response to heat exposure in male Wild Guinea pigs. *Mol. Ecol.* 25: 1729–1740.

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

Wolff J.O. 1985. The effects of density, food, and interspecific interference on home range size in *Peromyscus leucopus* and *Peromyscus maniculatus*. *Can. J. Zool.* 63: 2657–2662.

Wong J.W.Y. & Kölliker M. 2014. Effects of food restriction across stages of juvenile and early adult development on body weight, survival and adult life history. *J. Evol. Biol.* 27: 2420–2430.

Woodroffe R. & Vincent A. 1994. Mother's little helpers: Patterns of male care in mammals. *Trends Ecol. Evol.* 9: 294–297.

Zafiropoulos A., Tsentelierou E., Linardakis M., Kafatos A. & Spandidos D.A. 2005. Preferential loss of 5S and 28S rDNA genes in human adipose tissue during ageing. *Int. J. Biochem. Cell Biol.* 37: 409–415.

Zahavi A. 1977. The cost of honesty. *J. Theor. Biol.* 67: 603–605.

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. *J. Physiol.* 571: 221–230.

Zamenhof S., Marthens E. van & Grauel L. 1971. DNA (cell number) in neonatal brain: second generation (F2) alteration by maternal (F0) dietary protein restriction. *Science* 172: 850–851.

ORIGINAL PAPERS

I

INTERGENERATIONAL FITNESS EFFECTS OF THE EARLY LIFE ENVIRONMENT IN A WILD RODENT

by

Joannes Van Cann, Esa Koskela, Tapio Mappes, Angela Sims, Phillip C. Watts
2019

Journal of Animal Ecology 00: 1–11.

doi.org/10.1111/1365-2656.13039

TITLE: Intergenerational fitness effects of the early life environment in a wild rodent

AUTHORS: Joannes Van Cann[1]*, Esa Koskela[1], Tapio Mappes[1], Angela Sims[1], Phillip C. Watts[1,2]

Authors after the first author are listed in alphabetical order

[1] Department of Biological and Environmental Science, University of Jyväskylä, FI-40014 Jyväskylä, Finland

[2] Ecology and Genetics, University of Oulu, FI-90014, Oulu, Finland

*email: joannes.j.vancann@jyu.fi

JOURNAL: Journal of Animal Ecology

Summary

1. The early life environment can have profound, long-lasting effects on an individual's fitness. For example, early life quality might (1) positively associate with fitness (a silver spoon effect), (2) stimulate a predictive adaptive response (by adjusting the phenotype to the quality of the environment to maximize fitness) or (3) be obscured by subsequent plasticity. Potentially, the effects of the early life environment can persist beyond one generation, though the intergenerational plasticity on fitness traits of a subsequent generation is unclear.

2. To study both intra- and inter-generational effects of the early life environment, we exposed a first generation of bank voles to two early life stimuli (variation in food and social environment) in a controlled environment. To assess possible intra-generational effects, the reproductive success of female individuals was investigated by placing them in large outdoor enclosures in two different, ecologically relevant environments (population densities).

3. Resulting offspring were raised in the same population densities where they were conceived and their growth was recorded. When adult, half of the offspring were transferred to opposite population densities to evaluate their winter survival, a crucial fitness trait for bank voles.

4. Our setup allowed us to assess: (1) do early life population density cues elicit an intra-generational adaptive response i.e. a higher reproductive success when the density matches the early life cues and (2) can early life stimuli of one generation elicit an intergenerational adaptive response in their offspring i.e. a higher growth and winter survival when the density matches the early life cues of their mother.

5. Our results show that the early life environment directly affects the phenotype and reproductive success of the focal generation, but adaptive responses are only evident in the offspring. Growth of the offspring is maintained only when the environment matches their mother's early life environment. Furthermore, winter survival of offspring also tended to be higher in high population densities if their mothers experienced an competitive early life. These

results show that the early life environment can contribute to maintain high fitness in challenging environments, but not necessarily in the generation experiencing the early life cues.

KEYWORDS: early life, intergenerational plasticity, maternal effect, population density, predictive adaptive response, protein restriction, silver spoon, social environment

INTRODUCTION

Many organisms adjust their phenotype in response to environmental stimuli (Burton & Metcalfe, 2014; Nettle, Frankenhuis, & Rickard, 2013; Paaby & Testa, 2018). The early life environment is of particular importance as it can have a profound, and often irreversible, impact upon the phenotype (Burton & Metcalfe, 2014). A high quality early life environment can elicit high adult fitness – the so called 'silver spoon effect' (Grafen, 1988; Monaghan, 2008) or 'condition transfer' (Bonduriansky & Crean, 2018) which appears to be a common phenomenon in animals (Cartwright, Nicoll, Jones, Tatayah, & Norris, 2014; Lindström, 1999; Wong & Kölliker, 2014). Alternatively, either a high or a low quality early life environment might stimulate a certain phenotypic trajectory that maximizes the fitness in a matching (i.e. high or low quality) environment – a 'predictive adaptive response' (Duckworth, Belloni, & Anderson, 2015; Galloway & Etterson, 2007; Gluckman, Hanson, & Spencer, 2005). For example, in the marine polychaete *Ophryotrocha labronica*, maternal temperature during oogenesis can lead to increased temperature tolerance when the offspring are reared in a similar ('matching') environment (i.e. an environment with thermal variation; Massamba-N'Siala, Prevedelli, & Simonini, 2014). The evolutionary benefits of a predictive adaptive response are unclear when the future environment is not predictable, as the early life environment can stimulate a phenotype that performs poorly in later conditions (Bateson, Gluckman, & Hanson, 2014; Vickers et al., 2000).

Traditionally the focus of early life effects has been on within-generational plastic responses because considering the length of time between the environmental cue and the expressed phenotype, the effects might be expected to be more apparent within (F1 early environment affecting F1 fitness) than between generations (F1 early environment affecting F2 fitness) (Burton & Metcalfe, 2014). Indeed, laboratory studies on rodents (Bateson et al., 2014; Vickers et al., 2000) and insects (Raveh, Vogt, & Kölliker, 2016; Vijendravarma, Narasimha, & Kawecki, 2010) show how the similarity in nutritional environment during early (development) and late (mature) life (i.e. occurring within one generation) determines the fitness of phenotype. With this in mind, it is intriguing that

the phenotypic consequences of an F1 individual's early life environment can persist to the next generation (F2) (Burton & Metcalfe, 2014; Donelson, Salinas, Munday, & Shama, 2018; Donohue, 1999), as depending on environmental autocorrelation, intergenerational effects can either limit or enhance the ability of an individual to express the optimal phenotype (Guillaume, Monro, & Marshall, 2016; Heckwolf, Meyer, Döring, Eizaguirre, & Reusch, 2018). Of course, the F2 adult phenotype might be plastic (Ergon, Lambin, & Stenseth, 2001; Piersma & Drent, 2003) (i.e. able to reversibly change the phenotype based on immediate environmental cues) and capable of mitigating the consequences of the early life environment (Beaman, White, & Seebacher, 2016). The processes that impact phenotype have obvious eco-evolutionary relevance and yet it has proved difficult to quantify their relative impact in natural populations (Monaghan, 2008). The challenge in identifying the eco-evolutionary relevance of the early life environment (Mousseau & Fox, 1998) on subsequent generations lies in assessing the fitness consequences, which in turn depend on the degree of environmental variation.

Numerous examples exist of F1 early life exposure resulting in intergenerational F2 responses. For example, F1 early life ambient temperature for certain fish species (*Acanthochromis polyacanthus* Donelson, Munday, McCormick, & Pitcher, 2012; *Gasterosteus aculeatus* Shama & Wegner, 2014; Shama et al., 2016) and lizards (*Lacerta vivipara* Marquis, Massot, & Le Galliard, 2008) can lead to changes in offspring body size. Evidence in mammals for intergenerational phenotypic effects of F1 early life environment on F2 is derived largely from biomedical studies of humans (Gluckman et al., 2005) and laboratory rodents (Bateson et al., 2014), whereby conditions experienced during pregnancy can impact the birth characteristics and health of both the focal generation (F1) and the next generation (F2) (Lumey & Stein, 1997; Painter et al., 2008; Rickard et al., 2012). Intergenerational effects may occur through several different pathways, such as differential parental investment into F2 offspring and/or by direct environmental influence on the F2 through epigenetic processes that occur during prenatal or early life (Burton & Metcalfe, 2014). Despite unambiguous evidence of intergenerational early life effects in humans and laboratory animal models, it is unclear

whether intergenerational effects have fitness consequences in natural settings (Sheriff & Love, 2013) and the extent to which any (mal)adaptive effects are mediated in different ecological circumstances. For example, intergenerational phenotypic effects could allow populations to cope with climate change (Donelson et al., 2018) by facilitating individuals to rapid changes in temperature and precipitation (e.g. Marquis et al., 2008; Shama & Wegner, 2014).

The bank vole *Myodes glareolus* has several life-history traits that make it an ideal species to study the within- and between-generation consequences of the early life environment through the maternal line: females are promiscuous, males do not display paternal care, and females are territorial during the breeding season (Gromov & Osadchuk, 2013). The maternal environment is therefore presumed to be the main contributor to the early life environment of the next generation (but see Vaiserman, Koliada, & Jirtle, 2017; Weyrich et al., 2016 for potential environmental paternal effects in other study systems). Furthermore, varying (early life) environments are relevant to bank voles as natural populations typically undergo cyclic fluctuations in density (Kallio et al., 2009; Korpela et al., 2013; Korpimaki et al., 2005; Prévot-Julliard, Henttonen, Yoccoz, & Stenseth, 1999). As a consequence, density-related factors, such as nutritional quality (Helle, Koskela, & Mappes, 2012) and population density itself (Prévot-Julliard et al., 1999) impact several life-history traits including maturation, reproductive success and susceptibility to the costs of reproduction (Koskela, Jonsson, Hartikainen, & Mappes, 1998; Mappes et al., 2008; S. C. Mills et al., 2007; Prévot-Julliard et al., 1999). Some life-history traits follow a delayed density pattern and a predictive adaptive response has previously been hypothesized to be partly responsible (Oksanen, Koivula, Koskela, Mappes, & Soulsbury, 2012). An important aspect of the bank vole's life cycle is the overwintering survival where populations can easily decrease by 50% during winter (Kallio et al., 2009). For voles born during the summer the first mating opportunity is after the winter, making winter survival a key fitness trait. Intra-generationally, it has been shown that bank voles born in a certain density have a higher winter survival if the adult winter density matches that early life density (Oksanen et al., 2012). So far, no efforts have been made to investigate potential intergenerational effects of the

early life density. In theory, intergenerational effects that 'prepare' offspring for an increasing population density could be beneficial for bank voles due to the cyclicity of the population fluctuations.

Our aim was to quantify the role of the early life environment in mediating fitness traits in natural conditions both within and between generations and in different environments. We predict that (1) if early life environmental cues have a silver spoon effect (Grafen, 1988; Monaghan, 2008), then a low quality early life reduces adult fitness independent of the adult environmental quality; (2) if early life cues elicit a predictive adaptive response (Gluckman et al., 2005), then a low quality early life leads to a lower adult fitness only if the adult environmental quality does not match the early life quality and (3) if the phenotype is plastic and can compensate for any effects of the early life environment, then our treatments will have little to no effect on adult fitness. To test these predictions, we varied the early life environment, which is the intra-uterine development and nursing period of female bank voles (termed F1), by exposing them to two population density-related cues in a factorial setup in the laboratory (Fig. 1a). The chosen early life cues were (1) social confrontation, relevant to bank voles as gravid female bank voles fiercely defend their territories (Gromov & Osadchuk, 2013; Koskela, Juutistenaho, Mappes, & Oksanen, 2000; Koskela, Mappes, & Ylönen, 1997), and (2) variation in dietary protein, as the nutritional environment is related to population density (Brook & Bradshaw, 2006; Forbes et al., 2014; Tanner, 1966) and protein is an important component of the bank vole diet (Hansson, 1971). To test the effects of the early life in either a matching or a non-matching adult environment, the F1 animals were released into experimental field-enclosures at two different population densities, where they freely competed and reproduced (Fig. 1b). Reproduction of F1 in the field produced a second generation (F2) whose growth and overwintering survival was quantified at different population densities, allowing us to test whether there was an intergenerational response (Fig. 1c). We quantified the phenotypes (i.e. fitness-related traits - growth, reproductive characteristics and survival) of the focal (F1) and second (F2) generations at both low and high population densities under a full factorial design (Fig. 1).

METHODS

Starting colony

Three hundred and eighty two bank voles females (F0) (Table S1) were bred in spring of 2014 (first replicate) and 2015 (second replicate) from a random mix of first or second generation wild-caught animals that had been trapped in Central Finland (62°8379 N; 26°8209 E). All bank voles were kept in polyethylene cages (43x26x15 cm) 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 prior to the experiment standard food (Labfor 36; Lactamin AB, Stockholm, Sweden) was provided *ad libitum*. Animals were chipped with electronic Trovan tags (EID Aalten BV, Aalten, Holland) for identification.

Early life environment– laboratory manipulation

All F0 females were randomly assigned to four groups in a two by two factorial design (Fig. 1; Table S1): (1) a control group (PR-SC-; Fig. 1), (2) a protein restricted group (PR+SC-), (3) a social confrontation group (PR-SC+) and (4) a group which received both protein restriction and social confrontation (PR+SC+). There were no significant differences in average body mass (one way ANOVA; $F_{3,477}$=0.359; p=0.783) between groups. Mature males (from the laboratory stock, non-kin with the females) were randomly grouped with F0 females.

At the start of the breeding, F0 females receiving the PR- treatment received a control diet (18% protein; 3.1 kCal/g; Envigo, WI, USA) that has a similar protein content as found in the wild (Droždž, 1968) while females from the PR+ treatment were given a restricted protein diet (9% protein; 3.2 kCal/g; Envigo, WI, USA). After seven days, males were removed and 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, Kruczek, Kapusta, & Pochroń, 2003) for 10 minutes. New combinations of females were used every day to avoid habituation. A more detailed explanation of the social confrontation treatment can be found

in the supplementary information. All treatments were carried out throughout the pregnancy and continued until the pups (F1) had weaned (20 days post-partum). Upon weaning, F1 individuals were transferred to separate cages per litter where they received water and standard food *ad libitum* (Labfor 36; Lactamin AB, Stockholm, Sweden). At the age of 30 days, litters were separated per sex until they were released to the field enclosures.

Population density treatments – field experiments

At the age of two months, i.e. the beginning of July 2014 or 2015 for individuals born in 2014 or 2015 respectively, fitness of F1 females from all treatment groups was assessed by releasing them to field enclosures twice for approximately three weeks (Fig. 1b). F1 individuals mated and carried litters in the first three weeks and nursed their litters in the second three weeks. Twenty three enclosures (40m x 50m) were used, located in Central Finland (62°37'30"N 26°14'38"E and 62°39'23"N 26°09'32"E; Fig. S2). Enclosures were fenced with 1.25m high galvanized sheet metal buried 0.5 m in the ground, which prevented immigration and emigration of bank voles but did not prevent predation by avian predators. Trapping was performed by placing 20 Ugglan live traps (Grahnab, Hillerstorp, Sweden) per enclosure, organized in a grid, baited with sunflower seeds and potato. Traps were not set and contained no food during any other period. Throughout the field experiments, individuals relied on natural resources for food and water.

Over two years, F1 females were released in enclosures in one of two population densities (termed 'population density 1'; PD1): (1) a low population density treatment, consisting of four female individuals (one from each early life treatment group), or (2) a high population density treatment, consisting of eight female individuals (two from each early life treatment group). Densities corresponded to the variation of natural bank vole populations over the multiannual density cycle (Rikalainen, Aspi, Galarza, Koskela, & Mappes, 2012; Yoccoz, Stenseth, Henttonen, & Prévot-Julliard, 2001). In total, the two replicates consisted of nineteen low and twelve high density enclosures. After one day, experimentally naive males, previously caught from wild populations, were added to

the enclosures to produce a 1:1 sex ratio, and all individuals were allowed to mate. Individuals were exhaustively trapped (individuals not caught were considered dead) and brought to the laboratory just before their parturitions, where they could give birth to the F2 generation (Fig. 1b).

Intergenerational effect on F2 phenotype – field experiments

After parturitions, F1 mothers and their new-born F2 litters were returned to the same densities as before. Releases were done by placing the cage in which the mother gave birth in the enclosure on its side, giving the mother opportunity to relocate her nest (after which the cages were removed). This method to trap late pregnant bank vole females to give birth in the lab and then promptly return them with their new-born pups back to the enclosures makes it possible to determine the phenotypes of new-born pups (Koskela, Juutistenaho, Mappes, & Oksanen, 2000; Oksanen, Koskela, & Mappes, 2002). New experimentally naïve males, not used for the experiment so far, were added after one day, in a 1:1 sex ratio. At weaning (ca. three weeks old), the F2 individuals were exhaustively trapped and brought to the laboratory where they were weighed and kept in standard cages until tested for winter survival. All F1 mothers and naïve males were also trapped and not used subsequently in the experiment.

Intergenerational effects on F2 survival – winter survival

When F2 individuals were at least 50 days old their winter survival was assessed in outdoor enclosures in either low or high population densities (termed 'population density 2' (PD2); Fig. 1c). Half of the F2 individuals were designated to the density treatment by matching their early life density (PD1), and half were designated by not matching their early life density. This reciprocal transplant experiment meant that every F2 individual now had three factors for which their winter survival could be tested: (1) the intergenerational effects of the F1 early life (PR and/or SC), (2) the intra-generational effect of the density in which the F2 was conceived and raised (PD1) and (3) the direct effect of the density in which an individual resides during the winter (PD2). There were 10 low

density populations consisting of 8 individuals (one female and one male F2 individual from each F1 early life treatment group) and 5 high density populations with 16 individuals in each (two female and two male F2 individuals from each F1 early life treatment group). Enclosures never contained individuals from the same litter. The winter survival experiment began in October, when the temperature started dropping below zero (average temperature at day of release was 6°C), and long-term survival was determined by trapping all enclosures exhaustively in April, as the snow started melting and this means the onset of the next breeding season.

Morphological measurements

All body masses were measured using electronic scales. Body mass of F0 was measured at the start of the experiment, at the birth of F1 and at F1's weaning age (20 days). F1 body mass was recorded at birth and as adults (30 days). F2 individuals' body mass was recorded at the age of recapture (25 days).

Statistical analyses

Statistical analyses were performed using R v.3.4.2 (R Core Team, 2017). All analyses of body mass and the F2 winter survival (logit link) were performed using the package lme4 (Bates, Mächler, Bolker, & Walker, 2015), and p-values were calculated with the package lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017) using Satterthwaite's method for approximating degrees of freedom. The litter size (number of offspring) that F1 produced in the field was analysed as a zero-truncated Poisson generalized mixed model using glmmTMB (Brooks et al., 2017) (log link). Whether or not a F1 mother reproduced at all ("Reproduced"; binomial yes/no; logit link) was analysed using glmmTMB (Brooks et al., 2017). All model selections 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. The F1 early life treatments, PR and SC, and their interaction term (PR x SC) were purposely retained during model selection. For the F1 enclosure experiments, density (PD1) was

always included as a fixed factor in the model. For the analysis of F2 winter survival, the full model included the F2 winter density (PD2), as well as the F2 summer density (PD1), in which the F2 were conceived and raised, as fixed factors (in addition to PR, SC and PR x SC). For all analyses, sex (Koivula, Koskela, Mappes, & Oksanen, 2003; Koskela, Mappes, Niskanen, & Rutkowska, 2009; Schulte-Hostedde, 2007) and the litter size the individual was born in (Koivula et al., 2003; Mappes & Koskela, 2004; Oksanen et al., 2002; Schroderus et al., 2012) were included as covariates in the full model. As individuals were born in different years and weeks, all F1 analyses included the 'batch number' as a random factor. F2 individuals were released in two groups during the first year and this was included as a random factor for the summer F2 analyses. All analyses also included 'litter number' as a random factor, which groups siblings to account for litter effects (either for F1 or F2 individuals). All field enclosure analyses included 'enclosure number' as a random factor, which accounts for local differences between the enclosures. Random factors were always included in the initial full model and retained during model selection. Standard deviations of all included random factors are reported in the supplementary tables (Tables S4, S5 and S6).

RESULTS

Early Life Environment

Both the social confrontation and protein restriction treatments affected the phenotype of F1 individuals (Table 1). In total, 643 F1 individuals (Table S2) were born to 158 F0 mothers. A restricted protein diet significantly lowered both the F1 birthmass (mean±SD(g), PR-: 1.90±0.23; PR+: 1.83±0.22) as well as the F1 adult body mass (mean±SD(g), PR-: 16.71±2.21; PR+: 16.11±2.48) compared with the control group (Fig. S1). F1 individuals receiving the SC treatment had a significantly lower adult body mass (mean±SD(g), SC-:16.57±2.51; SC+:16.21±2.21), while not having a significantly different body mass at birth (mean±SD(g), SC-:1.85±0.23; SC+:1.88±0.23), compared

with the control group. The final model did not reveal a significant interaction of the PR and SC treatment indicating that their effects were independent of each other (Tables 1 and S3).

F1 reproductive success

Neither a restricted protein or social confrontation early environment had a significant influence on the reproductive success of F1 females in the field (Tables 1 and S4). Only PD1 density had a significant effect on the probability of breeding with high density populations significantly lowering the chance of reproduction. 88% of all F1 females originally released survived during the six weeks in the field enclosures (no difference in survival between treatments: one way ANOVA; $F_{3,168}$=0.407; p=0.748), of which 105 (68%) were gravid.

Intergenerational effects on F2 phenotype

A high population density had a negative effect on the adult body mass of the F2 individuals if the mother did not receive the SC treatment (SC-; low density: mean±SD(g)=12.57±1.08; SC-; high density: mean±SD(g)=11.12±1.98; Tables 2 and S5; Fig. 2a), but if the mother did receive the SC treatment, this negative effect associated with a high population density disappeared (SC+; low density: mean±SD(g)=12.41±1.33; SC+; high density: mean±SD(g)=12.06±1.53). Hence, if the mother received a high density related cue during her early life (*i.e.* social confrontation), her offspring were not affected by a high population density. Litter size in which the F2 individual was born had a significant negative correlation with the F2 body mass which is common in bank voles (Mappes & Koskela, 2004; Schroderus et al., 2012) (Table S5).

Intergenerational effects on F2 survival

F2 Individuals in high population densities had a lower winter survival, but this was somewhat countered if their mothers had received social confrontation during their early life. Of the 160 F2 individuals that went to the enclosures in October, 37 survived (23%) until the start of the next breeding season in April (and no unknown immigrants were captured). The density in which the F2

were conceived and raised (PD1) had no significant effect on winter survival (Tables 2 and S6). However, the early life treatment of their F1 mothers had a near statistically significant (p=0.0537) interaction effect with density on the winter survival (PD2) (Tables 2 and S6; Fig. 2b) indicating that effects of the maternal early life persisted to the F2 generation and provided a fitness benefit if the winter environment matched that of the maternal early life environment. Neither the restricted protein treatment, nor its interaction with SC, presented a statistically detectable effect upon F2 winter survival.

Table 1: Phenotypic and reproductive characteristics of F1 individuals in relation to manipulation of early life environment and population density (PD1). Reduced table of body mass of F1 individuals at birth (*n*=643) and at 30 days old (*n*=565) in the laboratory and F1 reproductive success (littersize and reproduced (*n*=172)) in the field enclosures. PR= protein restriction; SC= social confrontation; PD1= population density in which F1 females reproduced; HD= high population density. Note that estimated values for reproduced represent the probability of not breeding. Bold *p*-values indicate $p < 0.05$. Est is estimated value; Std error is standard error; x indicates interaction between variables. Full results including random effects are provided in tables S3 and S4.

		Est	Std error	DF	t value	p
F1 mass at birth	PR (+)	-0.1124	0.0394	144	-2.8540	**0.0050**
	SC (+)	-0.0213	0.0404	143	-0.5280	0.5982
	PR x SC	0.0601	0.0551	145	1.0900	0.2774
F1 mass at 30d	PR (+)	-1.0768	0.4024	137	-2.6760	**0.0084**
	SC (+)	-0.8706	0.4157	136	-2.0940	**0.0381**
	PR x SC	0.6812	0.5623	138	1.2110	0.2278
Reproduced	PR (+)	-0.6638	0.4752		-1.3970	0.1624
	SC (+)	-0.2307	0.4657		-0.4950	0.6204
	PD1 (HD)	0.9005	0.3794		2.3740	**0.0176**
	PR x SC	-0.0093	0.6781		-0.0140	0.9891
Litter size	PR (+)	-0.1002	0.1195		-0.8390	0.4016
	SC (+)	-0.2177	0.1288		-1.6910	0.0909
	PD1 (HD)	-0.1013	0.0856		-1.1830	0.2369
	PR x SC	0.2147	0.1716		1.2510	0.2109

Table 2: Adult body mass and winter survival of F2 individuals in relation to: early life treatments of their F1 mothers (PR and/or SC), their own early life (PD1) population density and their adult/winter (PD2) population density. Reduced results of 25 day body mass (*n*=414) and winter survival (*n*=160). PR= protein restriction; SC= social confrontation; PD1= population density in which the F2 individuals were raised; PD2= population density during winter survival; HD= high population density; Lsize= size of litter in which the F2 individual was born. Bold *p*-values indicate $p < 0{,}05$. Est is estimated value; Std error is standard error; x indicates interaction between variables. Full results including random effects are provided in tables S5 and S6.

		Est	Std error	DF	t value	p
F2 Mass at 25 days	PR (+)	-0.2998	0.3459	75	-0.8670	0.3888
	SC (+)	-0.9242	0.8082	75	-1.1440	0.2564
	PD1 (HD)	-1.7794	0.4303	59	-4.1350	**0.0001**
	Lsize	-0.4591	0.1139	94	-4.0310	**0.0001**
	PR x SC	-0.1284	0.4970	77	-0.2580	0.7969
	PD1 x SC	1.0753	0.5085	77	2.1150	**0.0377**
F2 Winter survival	PR (+)	0.1178	0.6447		0.183	0.8551
	SC (+)	0.3216	0.6691		0.481	0.6307
	PR x SC	-0.6692	0.8195		-0.817	0.4141
	PD2 (HD)	-1.3193	0.8055		-1.638	0.1014
	SC x PD2	1.7024	0.8824		1.929	0.0537

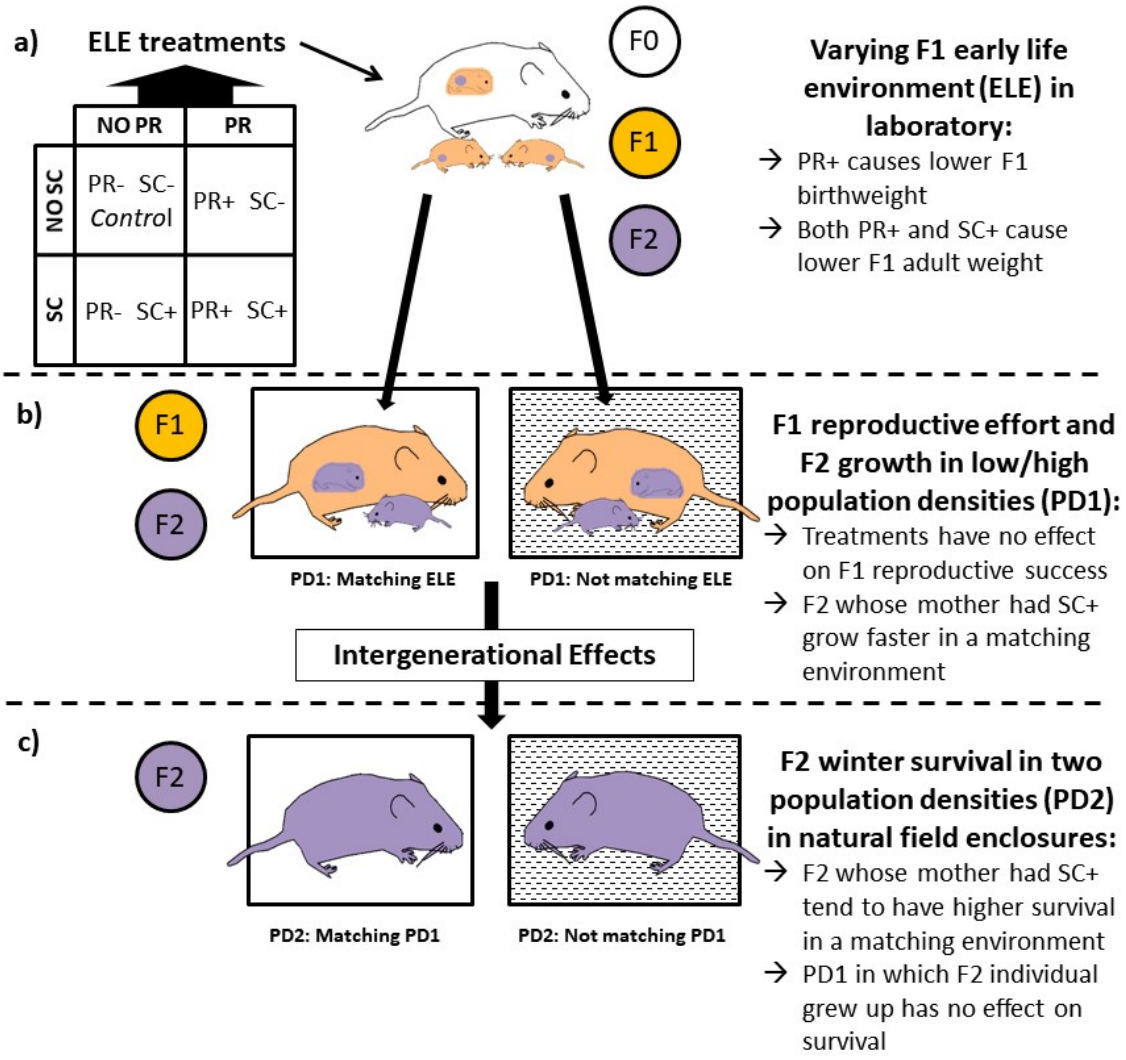


Fig. 1: Overview and summary of the experimental design to investigate the effects of population density related cues during the early life environment (ELE) of F1 on the morphology and reproductive success of F1, as well as the intergenerational effects on the phenotype and winter survival of F2. a) early life treatments (protein restriction (PR) and/or social confrontation (SC) in a factorial setup) are presented to the F1 individuals during the intra-uterine development and nursing period. b) The (adaptive) response is tested by placing the female F1 in natural outdoor enclosures in different population densities (low/high population density; blank square is population density that matches the ELE and shaded square is population density that does not match the ELE). c) Winter survival of F2 offspring is tested in a reciprocal transplant experiment where the population density (low/high population density; PD2) matches or does not match the population density in which the

F2 individual was born (blank square represents a PD2 that matches the PD1 and shaded square represent a PD2 that does not match the PD1). The setup allows winter survival of F2 to be tested for: the population density during the winter (PD2; (c)), the population density in which the F2 was born and grew up in (PD1; (b)), and the intergenerational effects of the maternal F1 ELE (a). Uncoloured voles indicate F0 individuals; orange colour indicates F1 as pups and adults; purple indicates F2 as egg cells, pups and adults.

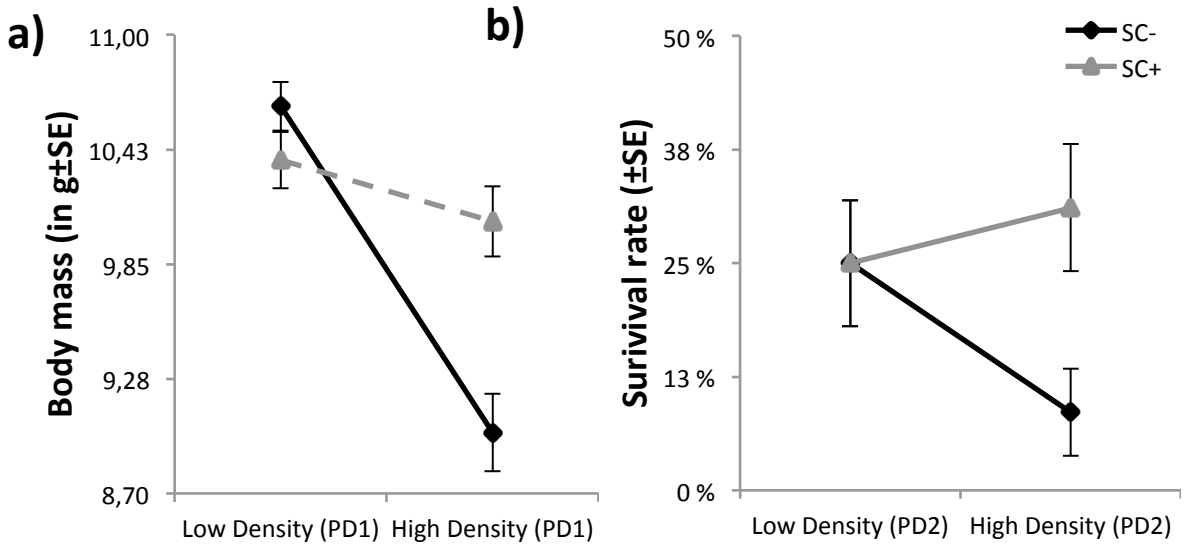


Fig. 2: Variation in F2 bank vole fitness traits (adult body mass and survival) whose maternal early life environment either had frequent social confrontation or not, competing in different population densities during the summer (PD1) and winter (PD2). a) 25 day old body mass of 414 F2 individuals (111 SC- and 121 SC+ in low density; 91 SC- and 91 SC+ in high density) in the field during the summer; b) winter survival percentage of 160 F2 individuals (40 SC- and 40 SC+ in low density of which 10 SC- and 10 SC+ survived; 35 SC- and 45 SC+ in high density of which 3 SC- and 14 SC+ survived). Diamond shaped black dots represent the average value for SC- F2 individuals. Triangular shaped grey dots represent average value for SC+ F2 individuals.

DISCUSSION

The early life environment, defined here as the period between fertilization and weaning, can have a profound influence on the adult phenotype and thus impact fitness in different ways. For example, the quality of the early life environment may associate with fitness (silver spoon effect) or an individual's fitness might be conditional upon the match in the environment experienced during early and late life (predictive adaptive response) or even a combination of both silver spoon effects and a predictive adaptive response (Bonduriansky & Crean, 2018). What is unclear, however, is the extent to which the early life experienced by the target individuals (i.e. first generation) (Burton & Metcalfe, 2014) can have intergenerational effects on the fitness of the next generation and whether these effects can be adaptive or not (Monaghan, 2008; Nussey, Wilson, & Brommer, 2007). This is especially important as we try and predict animal population response under climate change and extreme weather events (Donelson et al., 2018; Ghalambor, McKay, Carroll, & Reznick, 2007; Yeh & Price, 2004). By manipulating dietary protein and social confrontation during the early life of female bank voles we show that (1) maternal early life effects can persist to the subsequent generation (F2) where it influences adult body mass in an adaptive manner; moreover (2) winter survival of the F2 offspring tends to depend on the maternal early life environment (F1), rather than its own early life environment.

Social confrontation was exerted on pregnant bank voles during the period that wild female bank voles would be most likely to aggressively defend their territory. The effects of social confrontation on body mass were similar to a previous study in bank voles (Marchlewska-Koj et al., 2003), but did not have any effects on the reproductive success. Social confrontation during early life also had a notable intergenerational phenotypic effect on the F2 that persisted to the adult stage. F2 individuals were captured from the field not long after they are old enough to be independent from the mother, hence their body mass is largely dependent on the feeding of the mother. The ability of F2 to cope with the high population density could therefore be due to increased maternal care,

signifying that there would be a predictive adaptive response (Nettle et al., 2013) in the F1 maternal behaviour, although further investigation is needed to confirm this.

Social confrontation during the early life of the F1 generation had a nearly statistically significant positive effect (p = 0.0537) on the F2 winter survival, indicating a predictive adaptive response in F2 winter survival. In contrast, the early life environment of the F2 (i.e. PD1 density) had little effect on winter survival, implying that the maternal early life environment is of greater importance for the F2 winter survival than the F2 individuals' own early life environment. Two mechanisms could be mediating this effect (Burton & Metcalfe, 2014): (1) F1 mothers from the SC+ treatment changed some aspect of their parental care, leading to a higher survival rate of their offspring or (2) SC+ during the early life of F1 had *in utero* effects on the F2 egg cells. An example of the second possibility occurs in laboratory rats where *in utero* exposure to social stress has been linked to lower mass at birth and subsequent cardiometabolic diseases (Drake & Walker, 2004). While it might be intuitive to argue that the difference in F2 body mass relates to their winter survival, and hence maternal care would be the most likely mechanism, body mass in bank voles is not always related to winter survival (Helle et al., 2012). The specific mechanism by which the F2 survival is increased could due to selective predation, e.g. Tengmalm's owls (*Aegolius funereus funereus*) have been shown to prefer lighter prey that are in good condition (Koivunen, Korpimäki, Hakkarainen, & Norrdahl, 1996). Differences in physiology e.g. metabolic rate (Boratyński & Koteja, 2009) might also contribute to the difference in survival rate.

It is important to note that, while SC+ F2 individuals had higher survival in the high density environment (i.e. a matching environment) compared to SC- individuals, SC+ individuals did not have a lower survival in the low density environment (i.e. a mismatching environment) compared to SC- individuals (Fig. 2b). This response is not fully in line with a predictive adaptive response (*sensu* Gluckman et al., 2005) and perhaps indicates a co-occurrence of more than one type of response (Bonduriansky & Crean, 2018). It is possible that the SC+ individuals were able to plastically adapt to

the less competitive, low density environment; while the SC- individuals were not able to plastically adapt to the more competitive, high density environment. Alternatively, it is possible that the costs of living in a mismatched low density environment were too small to be detected, at least in the variables measured in this study.

Body size in female bank voles is positively correlated to fitness-related traits such as a faster onset and higher probability of breeding (Mappes & Koskela, 2004; Mappes, Ylonen, & Viitala, 1995). Additionally, dietary protein content has a major impact on mammalian phenotypes, such as mass at birth (Zambrano et al., 2005), hypertension (Harrison & Langley-Evans, 2009), and epigenetic patterns (Burdge et al., 2007; Lillycrop, Phillips, Jackson, Hanson, & Burdge, 2005). Changes in epigenetic patterns could potentially lead to intergenerational effects (Bonduriansky & Day, 2009; Burton & Metcalfe, 2014; Heard & Martienssen, 2014; Skvortsova, Iovino, & Bogdanovi, 2018; Youngson & Whitelaw, 2008). In our study, protein restriction during the early life of bank voles elicited negative effects on F1 body size both as new-borns and as adults. However, we did not find any impact on the reproductive success in F1 and no detectable impact on the F2 adult phenotype or overwintering survival probability. This is in contrast with laboratory rats (Pinheiro, Salvucci, Aguila, & Mandarim-de-Lacerda, 2008), where protein restriction increased F2's mass at birth and decreased male F2 adult body mass. While we did not find intergenerational effects due to protein restriction, we might have overlooked other phenotypic traits, such as pathogen susceptibility (Monaghan, 2008), which can affect fitness-related traits in F2 besides winter survival, e.g. reproduction (Hakkarainen et al., 2007; Kallio, Helle, Koskela, Mappes, & Vapalahti, 2015; J. N. Mills, 2006). We propose that a protein restricted early life impacts bank vole phenotype, but it is of little consequence to fitness in natural settings. As such, protein restriction cannot be considered to have a 'silver spoon effect', at least not for the fitness traits measured here.

Conclusion

High population density related cues experienced during the early life environment influence the phenotype of both the generation that experiences it (F1), and their offspring (F2). Social confrontation during the early life of the F1 individuals caused a predictive adaptive response that enabled their offspring to better compete (i.e. grow and survive) in a high population density. That the juvenile environment of F2 was less important for winter survival than the prenatal/early life environment of their F1 mothers, underlines the eco-evolutionary importance of intergenerational effects. Protein restriction during the early life of F1 had a negative effect on the body mass, but otherwise did not lead to a silver spoon effect or a predictive adaptive response. Protein restriction might affect other fitness-related traits or its impact can be overcome later in life via plasticity. This study provides clear evidence of the maternal early life environment having direct adaptive influences on the phenotype and fitness of the subsequent generation in an ecologically relevant setting of intraspecific competition at different population densities.

ACKNOWLEDGEMENTS

We would like to thank the animal care staff at the University of Jyväskylä and at the Konnevesi research station. This work was supported by the Academy of Finland and the University of Jyväskylä Graduate School. 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). The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

J.V.C., E.K., T.M., A.S and P.W. designed the research and wrote the paper. J.V.C., E.K., T.M. and A.S performed the research and analysed the data.

DATA ACCESSIBILITY

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.1040834 (Van Cann et al., 2019).

CITATION LIST

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 51. doi:10.18637/jss.v067.i01

Bateson, P., Gluckman, P., & Hanson, M. (2014). The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. *The Journal of Physiology*, *592*(11), 2357–2368. doi:10.1113/jphysiol.2014.271460

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*(3), 237–249. doi:10.1016/j.tree.2016.01.004

Bonduriansky, R., & Crean, A. J. (2018). What are parental condition-transfer effects and how can they be detected? *Methods in Ecology and Evolution*, *9*(3), 450–456. doi:10.1111/2041-210X.12848

Bonduriansky, R., & Day, T. (2009). Nongenetic Inheritance and Its Evolutionary Implications. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 103–125. doi:10.1146/annurev.ecolsys.39.110707.173441

Boratyński, Z., & Koteja, P. (2009). The association between body mass, metabolic rates and survival of bank voles. *Functional Ecology*, *23*(2), 330–339. doi:10.1111/j.1365-2435.2008.01505.x

Brook, B. W., & Bradshaw, C. J. (2006). Strength of evidence for density dependence in abundance time series of 1198 species. *Ecology*, *87*, 1445–1451.

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

Burdge, G. C., Slater-Jefferies, J., Torrens, C., Phillips, E. S., Hanson, M. A., & Lillycrop, K. A. (2007).

Dietary protein restriction of pregnant rats in the F0 generation induces altered methylation of hepatic gene promoters in the adult male offspring in the F1 and F2 generations. *British Journal of Nutrition*, *97*(3), 435. doi:10.1017/S0007114507352392

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*(1785). doi:10.1098/rspb.2014.0311

Cartwright, S. J., Nicoll, M. A. C., Jones, C. G., Tatayah, V., & Norris, K. (2014). Anthropogenic natal environmental effects on life histories in a wild bird population. *Current Biology*, *24*(5), 536–540. doi:10.1016/j.cub.2014.01.040

Donelson, J. M., Munday, P. L., McCormick, M. I., & Pitcher, C. R. (2012). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change*, *2*(1), 30–32. doi:10.1038/nclimate1323

Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. S. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology*. doi:10.1111/gcb.13903

Donelson, J. M., Wong, M., Booth, D. J., & Munday, P. L. (2016). Transgenerational plasticity of reproduction depends on rate of warming across generations. *Evolutionary Applications*, *9*(9), 1072–1081. doi:10.1111/eva.12386

Donkin, I., Versteyhe, S., Ingerslev, L. R., Qian, K., Mechta, M., Nordkap, L., … Barrès, R. (2016). Obesity and bariatric surgery drive epigenetic variation of spermatozoa in humans. *Cell Metabolism*, *23*(2), 369–378. doi:10.1016/j.cmet.2015.11.004

Donohue, K. (1999). Seed Dispersal as a Maternally Influenced Character: Mechanistic Basis of Maternal Effects and Selection on Maternal Characters in an Annual Plant. *The American*

Naturalist, *154*(6), 674–689. doi:10.1086/303273

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 Introduction: the early life origins of disease. *Journal of Endocrinology*, *180*, 1–16. doi:10.1677/joe.0.1800001

Droždž, A. (1968). Digestibility and Assimilation of Natural Foods in Small Rodents. *Acta Theriologica*, *13*(21), 367–389.

Duckworth, R. A., Belloni, V., & Anderson, S. R. (2015). Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science*, *347*(6224), 875–877. doi:10.1126/science.1260154

Ergon, T., Lambin, X., & Stenseth, N. C. (2001). Life-history traits of voles in a fluctuating population respond to the immediate environment. *Nature*, *411*(6841), 1043–1045. doi:10.1038/35082553

Forbes, K. M., Stuart, P., Mappes, T., Hoset, K. S., Henttonen, H., & Huitu, O. (2014). Diet quality limits summer growth of field vole populations. *PLoS ONE*, *9*(3). doi:10.1371/journal.pone.0091113

Fullston, T., Teague, E. M. C. O., Palmer, N. O., Deblasio, M. J., Mitchell, M., Corbett, M., … Lane, M. (2013). Paternal obesity initiates metabolic disturbances in two generations of mice with incomplete penetrance to the F2 generation and alters the transcriptional profile of testis and sperm microRNA content. *FASEB Journal*, *27*(10), 4226–4243. doi:10.1096/fj.12-224048

Galloway, L. F., & Etterson, J. R. (2007). Transgenerational plasticity is adaptive in the wild. *Science*, *318*(5853), 1134–1136. doi:10.1126/science.1148766

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments.

Functional Ecology, *21*(3), 394–407. doi:10.1111/j.1365-2435.2007.01283.x

Gluckman, P. D., Hanson, M. A., & Spencer, H. G. (2005). Predictive adaptive responses and human evolution. *Trends in Ecology and Evolution*, *20*(10), 527–533. doi:10.1016/j.tree.2005.08.001

Grafen, A. (1988). On the uses of data on lifetime reproductive success. In T. Clutton-Brock (Ed.), *Reproductive success* (pp. 454–471). Chicago, IL: University of Chicago Press.

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*(1), 114–118. doi:10.1134/S1062359013010056

Guillaume, A. S., Monro, K., & Marshall, D. J. (2016). Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer? *Functional Ecology*, *30*(7), 1175–1184. doi:10.1111/1365-2435.12604

Hakkarainen, H., Huhta, E., Koskela, E., Mappes, T., Soveri, T., & Suorsa, P. (2007). Eimeria-parasites are associated with a lowered mother's and offspring's body condition in island and mainland populations of the bank vole. *Parasitology*, *134*(Pt 1), 23–31. doi:10.1017/S0031182006001120

Hansson, L. (1971). Small rodent food, feeding and population dynamics A comparison between granivorous and herbivorous species in Scandinavia. *Oikos*, *22*(2), 183–198.

Harrison, M., & Langley-Evans, S. C. (2009). Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *British Journal of Nutrition*, *101*(7), 1020. doi:10.1017/S0007114508057607

Heard, E., & Martienssen, R. A. (2014). Transgenerational Epigenetic Inheritance: myths and mechanisms. *Cell*, *157*(1), 95–109. doi:10.1007/s10439-011-0452-9.Engineering

Heckwolf, M. J., Meyer, B. S., Döring, T., Eizaguirre, C., & Reusch, T. B. H. (2018). Transgenerational plasticity and selection shape the adaptive potential of sticklebacks to salinity change.

Evolutionary Applications, *11*(10), 1873–1885. doi:10.1111/eva.12688

Helle, H., Koskela, E., & Mappes, T. (2012). Life in varying environments: Experimental evidence for delayed effects of juvenile environment on adult life history. *Journal of Animal Ecology*, *81*(3), 573–582. doi:10.1111/j.1365-2656.2011.01937.x

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*(2), 101–7. doi:10.1016/j.epidem.2009.03.002

Kallio, E. R., Helle, H., Koskela, E., Mappes, T., & Vapalahti, O. (2015). Age-related effects of chronic hantavirus infection on female host fecundity. *Journal of Animal Ecology*, *84*(5), 1264–1272. doi:10.1111/1365-2656.12387

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*(2), 398–405. doi:10.1890/0012-9658(2003)084[0398:CORITW]2.0.CO;2

Koivunen, V., Korpimäki, E., Hakkarainen, H., & Norrdahl, K. (1996). Prey choice of Tengmalm's owls (*Aegolius funereus funereus*): preference for substandard individuals? *Canadian Journal of Zoology*, *74*(5), 816–823. doi:10.1139/z96-094

Korpela, K., Delgado, M., Henttonen, H., Korpimäki, E., Koskela, E., Ovaskainen, O., … Huitu, O. (2013). Nonlinear effects of climate on boreal rodent dynamics: Mild winters do not negate high-amplitude cycles. *Global Change Biology*, *19*(3), 697–710. doi:10.1111/gcb.12099

Korpimaki, E., Norrdahl, K., Huitu, O., Klemola, T., Korpimaki, E., Norrdahl, K., … Klemola, T. (2005). Predator-induced synchrony in population oscillations of coexisting small mammal species. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1559), 193–202. doi:10.1098/rspb.2004.2860

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*(1401), 1129–1134.

Koskela, E., Juutistenaho, P., Mappes, T., & Oksanen, T. T. A. (2000). Offspring defence in relation to litter size and age: experiment in the bank vole Clethrionomys glareolus. *Evolutionary Ecology*, *14*(2), 99–109. doi:10.1023/A:1011051426666

Koskela, E., Mappes, T., Niskanen, T., & Rutkowska, J. (2009). Maternal investment in relation to sex ratio and offspring number in a small mammal - A case for Trivers and Willard theory? *Journal of Animal Ecology*, *78*(5), 1007–1014. doi:10.1111/j.1365-2656.2009.01574.x

Koskela, E., Mappes, T., & Ylönen, H. (1997). Territorial behaviour and reproductive success of bank vole Clethrionomys glareolus females. *Journal of Animal Ecology*. doi:10.2307/5980

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13), R package version 2.0-6. doi:10.18637/jss.v082.i13

Lillycrop, K. A., Phillips, E. S., Jackson, A. A., Hanson, M. A., & Burdge, G. C. (2005). Nutrient-Gene Interactions Dietary Protein Restriction of Pregnant Rats Induces and Folic Acid Supplementation Prevents Epigenetic Modification of Hepatic Gene Expression in the Offspring 1. *The Journal of Nutrition*, *135*(December 2004), 1382–1386.

Lindström, J. (1999). Early development in birds and mammals. *Trends in Ecology and Evolution*, *14*(9), 343–348.

Lumey, L. H., & Stein, A. D. (1997). Offspring birth weights after maternal intrauterine undernutrition: a comparison within sibships. *American Journal of Epidemiology*, *146*(10), 810–819. doi:10.1093/oxfordjournals.aje.a009198

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*(2). doi:10.1371/journal.pone.0001687

Mappes, T., & Koskela, E. (2004). Genetic basis of the trade-off between offspring number and quality in the bank vole. *Evolution*, *58*(3), 645–650. doi:10.1111/j.0014-3820.2004.tb01686.x

Mappes, T., Ylonen, H., & Viitala, J. (1995). Higher reproductive success among kin groups of bank voles (Clethrionomys glareolus). *Ecology*, *76*(4), 1276–1282. doi:10.2307/1940934

Marchlewska-Koj, A., Kruczek, M., Kapusta, J., & Pochroń, E. (2003). Prenatal stress affects the rate of sexual maturation and attractiveness in bank voles. *Physiology & Behavior*, *79*(2), 305–310. doi:10.1016/S0031-9384(03)00099-4

Marquis, O., Massot, M., & Le Galliard, J. F. (2008). Intergenerational effects of climate generate cohort variation in lizard reproductive performance. *Ecology*, *89*(9), 2575–2583. doi:10.1890/07-1211.1

Massamba-N'Siala, G., Prevedelli, D., & Simonini, R. (2014). Trans-generational plasticity in physiological thermal tolerance is modulated by maternal pre-reproductive environment in the polychaete Ophryotrocha labronica. *Journal of Experimental Biology*, *217*(11), 2004–2012. doi:10.1242/jeb.094474

Mills, J. N. (2006). Infectious Diseases from Nature: Mechanisms of Viral Emergence and Persistence. *Archives of Virology*, *19*, 45–47. doi:10.1007/3-211-29981-5

Mills, S. C., Alatalo, R. V, Koskela, E., Mappes, J., Mappes, T., & Oksanen, T. a. (2007). Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution; International Journal of Organic Evolution*, *61*(7), 1748–57. doi:10.1111/j.1558-5646.2007.00145.x

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1497), 1635–1645. doi:10.1098/rstb.2007.0011

Mousseau, T., & Fox, C. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, *13*(10), 403–407. doi:10.1016/S0169-5347(98)01472-4

Nettle, D., Frankenhuis, W. E., & Rickard, I. J. (2013). The evolution of predictive adaptive responses in human life history. *Proceedings. Biological Sciences / The Royal Society*, *280*(1766), 20131343. doi:10.1098/rspb.2013.1343

Nussey, D. H., Wilson, A. J., & Brommer, J. E. (2007). The evolutionary ecology of individual phenotypic plasticity in wild populations. *Journal of Evolutionary Biology*, *20*(3), 831–844. doi:10.1111/j.1420-9101.2007.01300.x

Oksanen, T. A., Koivula, M., Koskela, E., Mappes, T., & Soulsbury, C. D. (2012). Interactive effects of past and present environments on overwintering success-a reciprocal transplant experiment. *Ecology and Evolution*, *2*(5), 899–907. doi:10.1002/ece3.82

Oksanen, T. A., Koskela, E., & Mappes, T. (2002). Hormonal manipulation of offspring number: maternal effort and reproductive costs. *Evolution; International Journal of Organic Evolution*, *56*(7), 1530–1537. doi:10.1554/0014-3820(2002)056

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

Painter, R. C., Osmond, C., Gluckman, P., Hanson, M., Phillips, D. I. W., & Roseboom, T. J. (2008). Transgenerational effects of prenatal exposure to the Dutch famine on neonatal adiposity and health in later life. *BJOG: An International Journal of Obstetrics and Gynaecology*, *115*(10), 1243–1249. doi:10.1111/j.1471-0528.2008.01822.x

Piersma, T., & Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution*. doi:10.1016/S0169-5347(03)00036-3

Pinheiro, A. R., Salvucci, I. D. M., Aguila, M. B., & Mandarim-de-Lacerda, C. a. (2008). Protein restriction during gestation and/or lactation causes adverse transgenerational effects on biometry and glucose metabolism in F1 and F2 progenies of rats. *Clinical Science*, *114*, 381–392. doi:10.1042/CS20070302

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*(4), 684–697. doi:10.1046/j.1365-2656.1999.00307.x

R Core Team. (2017). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Radford, E. J., Ito, M., Shi, H., Corish, J. A., Yamazawa, K., Isganaitis, E., … Ferguson-Smith, A. C. (2014). In utero undernourishment perturbs the adult sperm methylome and intergenerational metabolism. *Science*, *345*(6198). doi:10.1126/science.1255903

Raveh, S., Vogt, D., & Kölliker, M. (2016). Maternal programming of offspring in relation to food availability in an insect (*Forficula auricularia*). *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828), 20152936. doi:10.1098/rspb.2015.2936

Rickard, I. J., Courtiol, A., Prentice, A. M., Fulford, A. J. C., Clutton-Brock, T. H., & Lummaa, V. (2012). Intergenerational effects of maternal birth season on offspring size in rural Gambia. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1745), 4253–4262. doi:10.1098/rspb.2012.1363

Rikalainen, K., Aspi, J., Galarza, J. a, Koskela, E., & Mappes, T. (2012). Maintenance of genetic diversity in cyclic populations-a longitudinal analysis in Myodes glareolus. *Ecology and*

Evolution, *2*(7), 1491–502. doi:10.1002/ece3.277

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*(1), 44. doi:10.1186/1471-2148-12-44

Schulte-Hostedde, A. I. (2007). Sexual size dimorphism in rodents. In J. O. Wolff & P. W. Sherman (Eds.), *Rodent Societies: An Ecological and Evolutionary Perspective* (pp. 115–128). Chicago, IL: University of Chicago Press.

Shama, L. N. S., Mark, F. C., Strobel, A., Lokmer, A., John, U., & Mathias Wegner, K. (2016). Transgenerational effects persist down the maternal line in marine sticklebacks: gene expression matches physiology in a warming ocean. *Evolutionary Applications*, *9*(9), 1096–1111. doi:10.1111/eva.12370

Shama, L. N. S., & Wegner, K. M. (2014). Grandparental effects in marine sticklebacks: Transgenerational plasticity across multiple generations. *Journal of Evolutionary Biology*, *27*(11), 2297–2307. doi:10.1111/jeb.12490

Sheriff, M. J., & Love, O. P. (2013). Determining the adaptive potential of maternal stress. *Ecology Letters*. doi:10.1111/ele.12042

Skvortsova, K., Iovino, N., & Bogdanovi, O. (2018). Functions and mechanisms of epigenetic inheritance in animals. *Nature Reviews Molecular Cell Biology*, *19*(december), 774–790. doi:10.1038/s41580-018-0074-2

Tanner, J. T. (1966). Effects of population density on growth rates of animal populations. *Ecology*, 733–745.

Vaiserman, A. M., Koliada, A. K., & Jirtle, R. L. (2017). Non-genomic transmission of longevity between generations: potential mechanisms and evidence across species. *Epigenetics &*

Chromatin, *10*(1), 38. doi:10.1186/s13072-017-0145-1

Van Cann, J., Koskela, E., Mappes, T., Sims, A., Watts, P. (2019). Data from: Intergenerational fitness effects of the early life environment in a wild rodent. *Zenodo repository*, https://doi.org/10.5281/zenodo.1040834.

Vickers, M. H., Breier, B. H., Cutfield, W. S., Hofman, P. L., Gluckman, P. D., Amiel, S., … Gluckman, P. (2000). Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *American Journal of Physiology. Endocrinology and Metabolism*, *279*(1), E83-7. doi:10.1210/jcem-72-2-277

Vijendravarma, R. K., Narasimha, S., & Kawecki, T. J. (2010). Effects of parental larval diet on egg size and offspring traits in Drosophila. *Biology Letters*, *6*(2), 238–241. doi:10.1098/rsbl.2009.0754

Weyrich, A., Lenz, D., Jeschek, M., Chung, T. H., Rübensam, K., Göritz, F., … Fickel, J. (2016). Paternal intergenerational epigenetic response to heat exposure in male Wild Guinea pigs. *Molecular Ecology*, *25*(8), 1729–1740. doi:10.1111/mec.13494

Wong, J. W. Y., & Kölliker, M. (2014). Effects of food restriction across stages of juvenile and early adult development on body weight, survival and adult life history. *Journal of Evolutionary Biology*, *27*(11), 2420–2430. doi:10.1111/jeb.12484

Yeh, P. J., & Price, T. D. (2004). Adaptive Phenotypic Plasticity and the Successful Colonization of a Novel Environment. *The American Naturalist*, *164*(4), 531–542. doi:10.1086/423825

Yoccoz, N. G., Stenseth, N. C., Henttonen, H., & Prévot-Julliard, A.-C. (2001). Effects of food addition on the seasonal density- dependent structure of bank vole Clethrionomys glareolus populations. *Journal of Animal Ecology*, *70*(70), 713–720. doi:10.1046/j.0021-8790.2001.00531.x

Youngson, N. A., & Whitelaw, E. (2008). Transgenerational Epigenetic Effects. *Annual Review of*

Genomics and Human Genetics, *9*(1), 233–257. doi:10.1146/annurev.genom.9.081307.164445

Zambrano, E., Rodriguez-Gonzalez, G. L., Guzman, C., Garcia-Becerra, R., Boeck, L., Diaz, L., … Nathanielsz, P. W. (2005). A maternal low protein diet during pregnancy and lactation in the rat impairs male reproductive development. *Journal of Physiology-London*, *563*, 275–284. doi:10.1113/jphysiol.2004.078543

Supplementary file

Early life of fathers affects offspring fitness in a wild rodent

Joannes Van Cann[1]*, Esa Koskela[1], Tapio Mappes[1], Angela Sims[1], Phillip C. Watts[1,2]

[1]Department of Biological and Environmental Science, University of Jyväskylä, FI-40014 Jyväskylä, Finland

[2]Ecology and Genetics, University of Oulu, FI-90014, Oulu, Finland

Supplementary methods: Social confrontation treatment

The social confrontation treatment consisted of pairing individuals from the SC+ treatment in a novel, empty cage for ten minutes every second day. Initially, pairs were randomized and afterwards all individuals were cycled so that contact between the same two individuals was minimized. The time of day during which the confrontations were done was always randomized but always between 9AM and 4PM. Prior to putting both individuals in the new, empty cage, the cage was wiped clean with a 70% ethanol-water solution. After cleaning, a handful of sawdust from the top layer of both individuals' cage was taken and distributed equally over the floor of the new cage to convey territorial odours. At the start of the confrontation, one female was placed in one corner and immediately afterwards the second female was placed in the opposite corner. The order of which female was placed first, as well as in which corner, was random. If females already had pups, the pups remained in the home cage while the mother was away for ten minutes. After the confrontation, each individual was returned to their home cage, avoiding immediate contact with her pups, if present. The social confrontations were carried out throughout the pregnancy and continued until the pups (F1) had weaned (20 days post-partum).

Supplementary figure 1

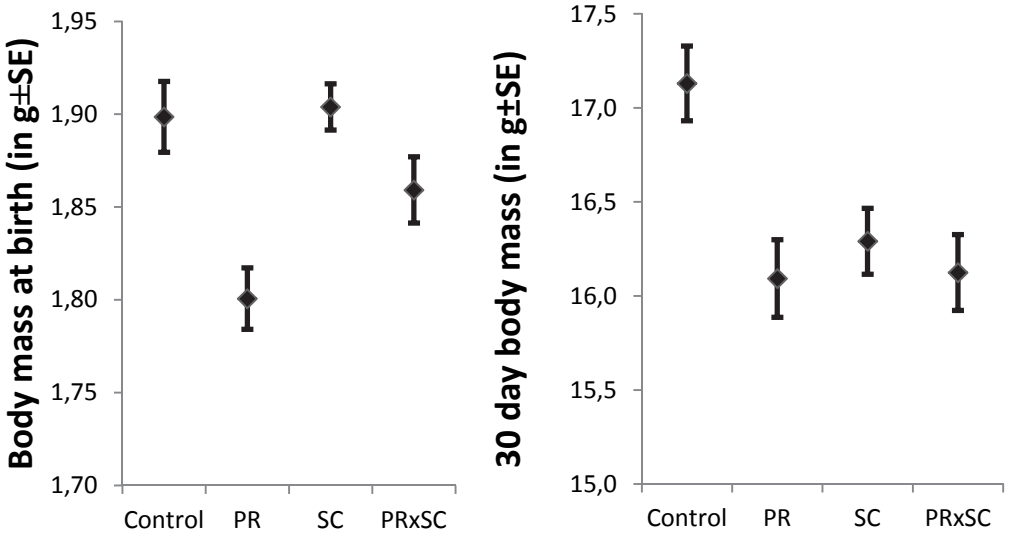


Fig. S1. Body mass (in grams) of F1 individuals at different life stages (birth and age of sexual maturity) per treatment group. SC= social confrontation treatment (PR-SC+); PR= protein restriction treatment (PR+SC-); PRxSC= both treatments (PR+SC+). For details see main text table 1 and supplementary table 3. Error bars represent mean ± standard error.

Supplementary figure 2

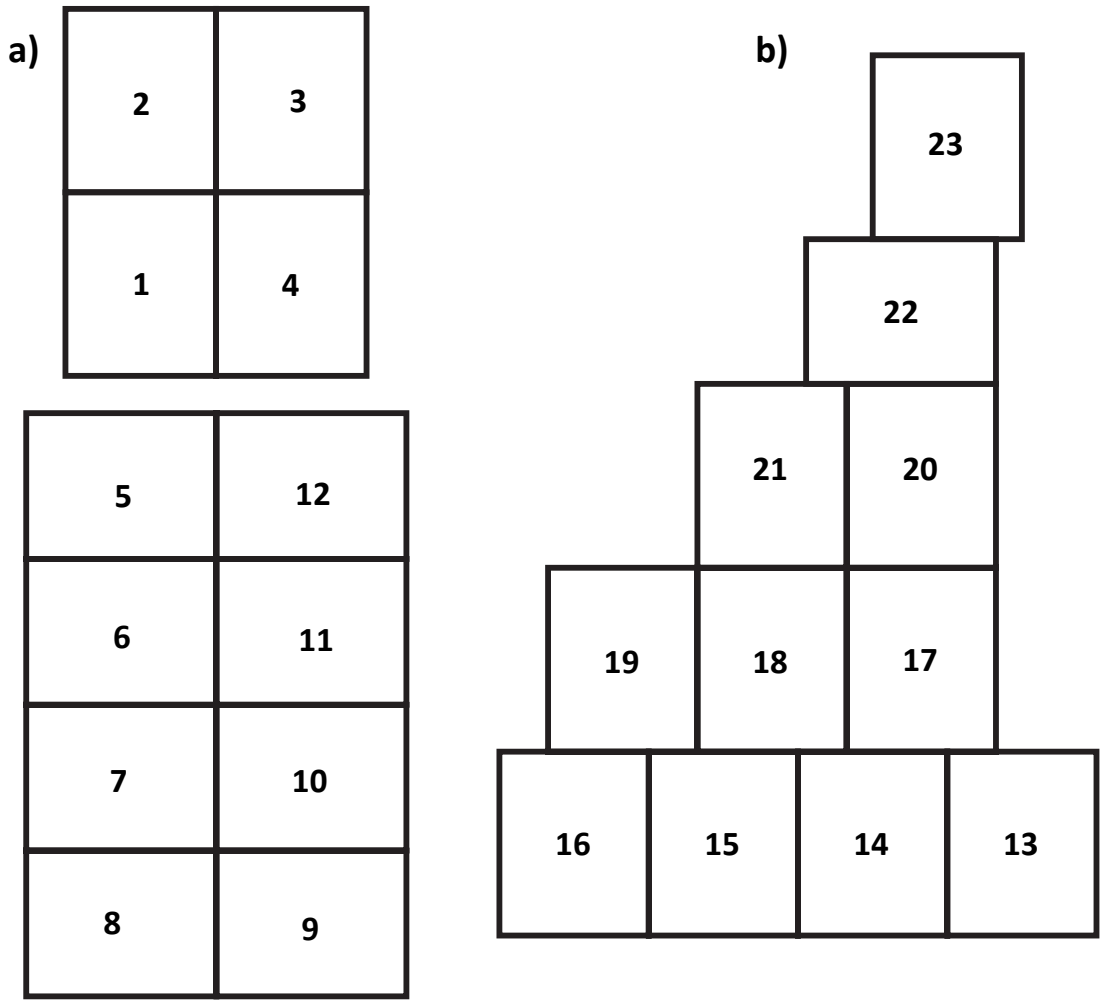


Fig. S2. Orientation of enclosures used for both summer and winter field experiments in 2014 and 2015. Each enclosure measures 40m by 50m. a) 12 enclosures situated close to Pukara (62°39'23"N 26°09'32"E); b) 11 enclosures situated close to Peltokangas (62°37'30"N 26°14'38"E)

Supplementary table 1

Table S1. Descriptives of the number of mothers (F0) that were used to create the F1 generation whose early life environment differed by receiving high population density cues (social confrontation and/or protein restriction). PR= protein restriction treatment; SC= social confrontation treatment; plus sign indicates presence of the treatment; minus sign indicates absence of the treatment. Total = total number of F0 that were mated per treatment group; Gravid = total number of F0 individuals that got pregnant; %Gravid = percentage of F0 individuals that got pregnant

Treatment	Total	Gravid	%Gravid
Control (PR-SC-)	86	35	41
PR-SC+	99	39	39
PR+SC-	95	45	47
PR+SC+	102	39	38

Supplementary table 2

Table S2. Descriptives of individuals of each generation that were either used (F0) or were born (F1 and F2) during the experiment, divided per sex and generation.

Generation		Sex	n
F0	Total	(Females only)	382
	Got pregnant	(Females only)	158
F1	Total born	Females	311
		Males	332
	Went to Field	(Females only)	172
	Survived	(Females only)	155
	Gravid in Field	(Females only)	104
F2	Total born	Females	315
		Males	370
	Went to winter enclosure	Females	77
		Males	83

Supplementary table 3

Table S3. a) Extended results of table 1: phenotypic characteristics change depending treatments during the early life of bank voles. Final reduced models for F1 morphology (LMM) at two life stages (REML estimation). b) Full models including all relevant two-way interactions of F1 morphology (LMM) at two life stages (REML estimation). n=643 for BM at birth; n=565 for 30d BM. BM = body mass; PR = protein restriction treatment; SC = social confrontation treatments; Sex = sex of the individual; Lsize = size of litter in which the F1 individual was born; x indicates interaction between variables. Random factors included in both full and reduced models are batch and litter number.

a)		**Est**	**Std error**	**DF**	**t value**	**p**
F1 mass at birth	Intercept	2.2338	0.0558	173	40.0030	**<0.0001**
	PR (+)	-0.1124	0.0394	144	-2.8540	**0.0050**
	SC (+)	-0.0213	0.0404	143	-0.5280	0.5982
	Sex (male)	0.0537	0.0130	540	4.1320	**<0.0001**
	Lsize	-0.0741	0.0101	158	-7.3510	**<0.0001**
	PR x SC	0.0601	0.0551	145	1.0900	0.2774
Random factors			**Std dev**			
	Litter number		0.1529			
	Batch		<0.0001			
		Est	**Std error**	**DF**	**t value**	**p**
F1 mass at 30d	Intercept	19.5423	0.5823	163	33.5600	**<0.0001**
	PR (+)	-1.0768	0.4024	137	-2.6760	**0.0084**
	SC (+)	-0.8706	0.4157	136	-2.0940	**0.0381**
	Sex (male)	1.4713	0.1431	484	10.2840	**<0.0001**
	Lsize	-0.6577	0.1037	149	-6.3450	**<0.0001**
	PR x SC	0.6812	0.5623	138	1.2110	0.2278
Random factors			**Std dev**			
	Litter number		1.47			
	Batch		<0.0001			

*Bold p-values indicate p <0.05

b)		Est	Std error	DF	t value	p
F1 mass at birth	Intercept	2.2320	0.0577	193	38.6610	<0.0001
	PR (+)	-0.1115	0.0423	186	-2.6340	0.0092
	SC (+)	-0.0182	0.0434	185	-0.4200	0.6750
	Sex (male)	0.0567	0.0239	543	2.3710	0.0181
	Lsize	-0.0740	0.0101	158	-7.3380	<0.0001
	PR x SC	0.0594	0.0553	146	1.0730	0.2850
	PR x Sex	-0.0007	0.0262	538	-0.0260	0.9791
	SC x Sex	-0.0052	0.0262	538	-0.2000	0.8416
Random factors			Std dev			
	Litter number		0.153			
	Batch		<0.0001			
		Est	**Std error**	**DF**	**t value**	**p**
F1 mass at 30d	Intercept	19.5675	0.6007	181	32.5760	<0.0001
	PR (+)	-1.1540	0.4340	179	-2.6590	0.0085
	SC (+)	-0.8408	0.4453	174	-1.8880	0.0607
	Sex (male)	1.4316	0.2586	484	5.5350	<0.0001
	Lsize	-0.6576	0.1036	149	-6.3480	<0.0001
	PR x SC	0.6903	0.5634	139	1.2250	0.2225
	PR x Sex	0.1529	0.2877	482	0.5320	0.5953
	SC x Sex	-0.0839	0.2871	482	-0.2920	0.7702
Random factors			Std dev			
	Litter number		1.4699			
	Batch		<0.0001			

*Bold p-values indicate p <0.05

Supplementary table 4

Table S4. a) Extended results of table 1: reproductive characteristics change depending on treatments during the early life of bank voles. Final reduced models for F1 reproductive characteristics (zero-truncated poisson gLMM for littersize; binomial gLMM for reproduced) in the field enclosures. b) Full models including all relevant two-way interactions of reproductive characteristics of F1 in field enclosures. Note that estimates for reproduced represent the probability of not breeding. n= 172. PR = protein restriction treatment; SC = social confrontation treatment; PD1 = population density in which the F1 reproduced; HD= high population density; x indicates interaction between variables. Random factors included in both full and reduced models are litter number, enclosure number and release group.

a)		**Estimate**	**Std.Error**	**t-value**	**p-value**
Reproduced	Intercept	-0.6166	0.4127	-1.4940	**0.1352**
	PR (+)	-0.6638	0.4752	-1.3970	0.1624
	SC(+)	-0.2307	0.4657	-0.4950	0.6204
	PD1 (HD)	0.9005	0.3794	2.3740	**0.0176**
	PRxSC	-0.0093	0.6781	-0.0140	0.9891
Random factors			**Std dev**		
	Litter number		<0.0001		
	Release group		<0.0001		
	Enclosure number		<0.0001		
		Estimate	**Std.Error**	**t-value**	**p-value**
Litter size	Intercept	1.8464	0.0963	19.1740	**<0.0001**
	PR (+)	-0.1002	0.1195	-0.8390	0.4016
	SC(+)	-0.2177	0.1288	-1.6910	0.0909
	PD1 (HD)	-0.1013	0.0856	-1.1830	0.2369
	PRxSC	0.2147	0.1716	1.2510	0.2109
Random factors			**Std dev**		
	Litter number		0.0002		
	Release group		<0.0001		
	Enclosure number		0.6121		

*Bold p-values indicate p <0.05

b)		Estimate	Std.Error	t-value	p-value
Reproduced	Intercept	-0.2741	0.4831	-0.5670	0.5705
	PR (+)	-1.4394	0.6657	-2.1620	**0.0306**
	SC (+)	-0.2928	0.6102	-0.4800	0.6314
	PD1 (HD)	0.2866	0.6000	0.4780	0.6329
	PRxSC	-0.0511	0.6945	-0.0740	0.9414
	PR x PD1	1.2750	0.7259	1.7570	0.0790
	SC x PD1	0.1221	0.7121	0.1720	0.8638
Random factors			**Std dev**		
	Litter number		<0.0001		
	Release group		<0.0001		
	Enclosure number		<0.0001		
		Estimate	**Std.Error**	**t-value**	**p-value**
Litter size	Intercept	1.7914	0.1156	15.4990	**<0.0001**
	PR (+)	-0.0270	0.1445	-0.1860	0.8520
	SC (+)	-0.1930	0.1566	-1.2330	0.2180
	PD1 (HD)	0.0115	0.1508	0.0760	0.9390
	PRxSC	0.2101	0.1727	1.2160	0.2240
	PR x PD1	-0.1554	0.1730	-0.8980	0.3690
	SC x PD1	-0.0543	0.1721	-0.3160	0.7520
Random factors			**Std dev**		
	Litter number		0.0002		
	Release group		<0.0001		
	Enclosure number		0.6264		

*Bold p-values indicate p <0.05

Supplementary table 5

Table S5. a) Extended result of table 2: adult body mass of F2 individuals are dependent on the maternal ELE and on the summer population density (PD1) (LMM; REML estimation). b) Full models including all relevant two-way interactions of F2 BM at 25 days in the field enclosures during the summer. Body mass at recaptured is corrected for age using multiple regression. n=414 for F2 BM at 25 days; n=685 for F2 recapture rate. BM = body mass; PR = protein restriction treatment; SC = social confrontation treatment; PD1 = population density in which the F2 were conceived and raised; Sex = sex of the individual; Lsize = litter size in which the F2 individual was born; HD= high population density; x indicates interaction between variables. Random factors included in both full and reduced models are enclosure, release group and F1 litter number.

a)		**Est**	**Std error**	**DF**	**t value**	**p**
F2 mass 25 days	Intercept	16.7470	0.9346	85	17.9190	<0.0001
	PR (+)	-0.2998	0.3459	75	-0.8670	0.3888
	SC (+)	-0.9242	0.8082	75	-1.1440	0.2564
	PD1 (HD)	-1.7794	0.4303	59	-4.1350	0.0001
	Lsize	-0.4591	0.1139	94	-4.0310	0.0001
	PR x SC	-0.1284	0.4970	77	-0.2580	0.7969
	PD1 x SC	1.0753	0.5085	77	2.1150	0.0377
Random factors			Std dev			
	Enclosure		<0.0001			
	Release group		0.6998			
	Litter number		1.0805			

*Bold p-values indicate p <0.05

b)		**Est**	**Std error**	**DF**	**t value**	**p**
F2 mass 25 days	Intercept	16.6234	0.9915	90	16.7650	<0.0001
	PR (+)	-0.3760	0.8113	74	-0.4630	0.6444
	SC (+)	-0.9989	0.8177	77	-1.2210	0.2256
	PD1 (HD)	-1.6268	0.5016	80	-3.2430	0.0017
	Sex (Male)	-0.4639	0.1146	93	-4.0470	0.0001
	Lsize	0.2849	0.3292	329	0.8650	0.3874
	PR x SC	-0.1513	0.4984	76	-0.3040	0.7623
	PR x PD1	-0.0031	0.4898	71	-0.0060	0.9950
	SC x PD1	1.0854	0.5099	75	2.1290	0.0365
	PR x Sex	0.1625	0.1970	329	0.8250	0.4101
	SC x Sex	0.1362	0.1967	330	0.6920	0.4891
	PD1 x Sex	-0.3004	0.1976	330	-1.5210	0.1293
Random factors			Std dev			
	Enclosure		<0.0001			
	Release group		0.6987			
	Litter number		1.0830			

*Bold p-values indicate p <0.05

Supplementary table 6

Table S6. a) extended results of table 2: Final model for winter survival of F2 individuals (binomial gLMM) in natural field enclosures. b) Full model including all relevant two-way interactions of winter survival of F2 individuals (binomial gLMM) after one winter in natural field enclosures. n=160. PR = protein restriction treatment; SC = social confrontation treatment; PD1= population density during the first period in which F2 were conceived and raised; PD2= population density in which the F2 are during the winter; HD= high population density; x indicates interaction between variables. Random factors included in both full and reduced models are enclosure number and F1 litter number.

a)		**Estimate**	**Std Error**	**z value**	**p**
F2 winter survival	Intercept	-1.2666	0.5534	-2.2890	**0.0221**
	PR (+)	0.1178	0.6447	0.1830	0.8551
	SC (+)	0.3216	0.6691	0.4810	0.6307
	PD2 (HD)	-1.3193	0.8055	-1.6380	0.1014
	PR x SC	-0.6692	0.8195	-0.8170	0.4141
	SC x PD2	1.7024	0.8824	1.9290	0.0537
Random factors			**Std dev**		
	Enclosure		<0.0001		
	Litter number		0.6514		

*Bold p-values indicate p <0,05

b)		**Estimate**	**Std Error**	**z value**	**p**
F2 winter survival	Intercept	-1.1664	0.5660	-2.0610	0.0393
	PR (+)	-0.1351	0.7059	-0.1910	0.8483
	SC (+)	0.3997	0.7042	0.5680	0.5703
	PD1 (HD)	0.0734	0.5998	0.1220	0.9027
	PD2 (HD)	-1.8697	1.0350	-1.8060	0.0708
	PR x SC	-0.9365	0.9143	-1.0240	0.3057
	PR x PD2	0.8984	0.8704	1.0320	0.3020
	SC x PD2	1.8324	0.9192	1.9940	0.0462
	PD1 x PD2	0.0439	0.8919	0.0490	0.9607
Random factors			Std dev		
	Enclosure		<0.0001		
	Litter number		0.6492		

*Bold p-values indicate p <0,05

II

EARLY LIFE OF FATHERS AFFECTS OFFSPRING FITNESS IN A WILD RODENT

by

Joannes Van Cann, Esa Koskela, Tapio Mappes, Anne-Mari Mikkonen, Mikael Mokkonen, Phillip C. Watts 2019

Journal of Evolutionary Biology. In press.

doi.org/10.1111/jeb.13516

MR. JOANNES VAN CANN (Orcid ID : 0000-0002-1394-804X)

Article type : Research Papers

Early life of fathers affects offspring fitness in a wild rodent

AUTHORS: Joannes Van Cann[1], Esa Koskela[1], Tapio Mappes[1], Anne-Mari Mikkonen[1], Mikael Mokkonen[1,2], Phillip C. Watts[1,3]

[1]Department of Biological and Environmental Science, University of Jyväskylä, FI-40014 Jyväskylä, Finland

[2]Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

[3]Ecology and Genetics, University of Oulu, FI-90014, Oulu, Finland

Corresponding author mail id: joannes.j.vancann@jyu.fi

EMAIL ADRESSES: Joannes Van Cann: joannes.j.vancann@jyu.fi
Esa Koskela: esa.m.koskela@jyu.fi
Tapio Mappes: tapio.mappes@jyu.fi
Anne-Mari Mikkonen: anne-mari.h.mikkonen@student.jyu.fi
Mikael Mokkonen: mikael.p.mokkonen@jyu.fi
Phillip Watts: phillip.watts@oulu.fi

CORRESPONDING AUTHOR: Joannes Van Cann
Department of Biology & Environmental Sciences
University of Jyväskylä
PO Box 35, 40014 Finland
Tel: +358 (0)401239118

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jeb.13516

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**

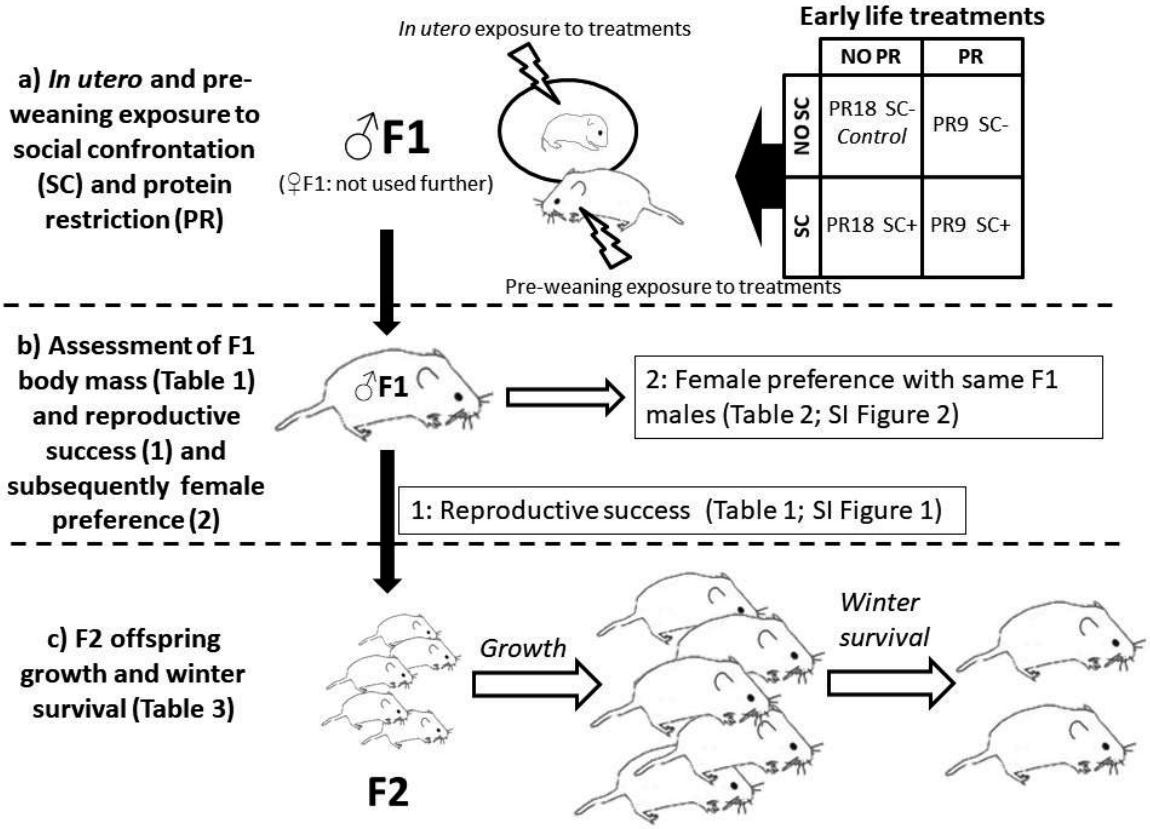


Figure 1: Overview of the experimental setup to investigate whether a F1 male bank vole's early life environment impacts growth and survival of its F2 offspring through paternal effects. (a) Early life environment treatments (protein restriction (PR) and/or social confrontation (SC) in a full factorial 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.

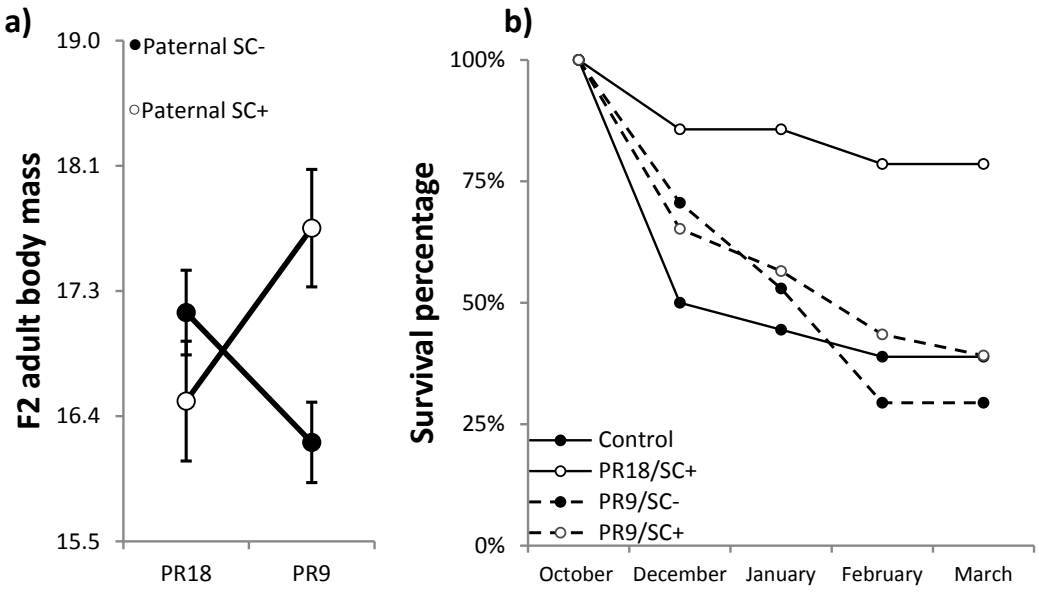


Figure 2: Effects of the F1 early life environment on the F2 offspring's adult body mass and over winter survival in the field. a) F2 adult body mass (30 days old; n=215); b) F2 winter survival (October to March) in semi-natural outdoor enclosures (n=72). Error bars represent ±1SE; closed circles indicate 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.

Supplementary file

Early life of fathers affects offspring fitness in a wild rodent

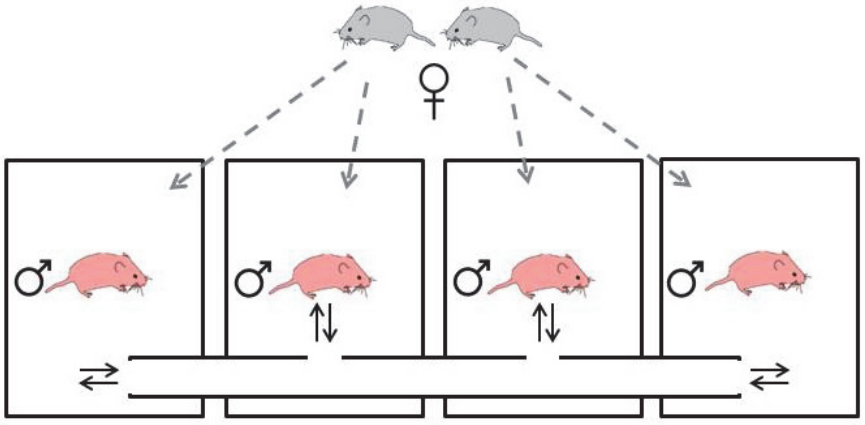
Joannes Van Cann[1]*, Esa Koskela[1], Tapio Mappes[1], Anne-Mari Mikkonen[1], Mikael Mokkonen[1,2], Phillip C. Watts[1,3]

[1]Department of Biological and Environmental Science, University of Jyväskylä, FI-40014 Jyväskylä, Finland

[2]Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

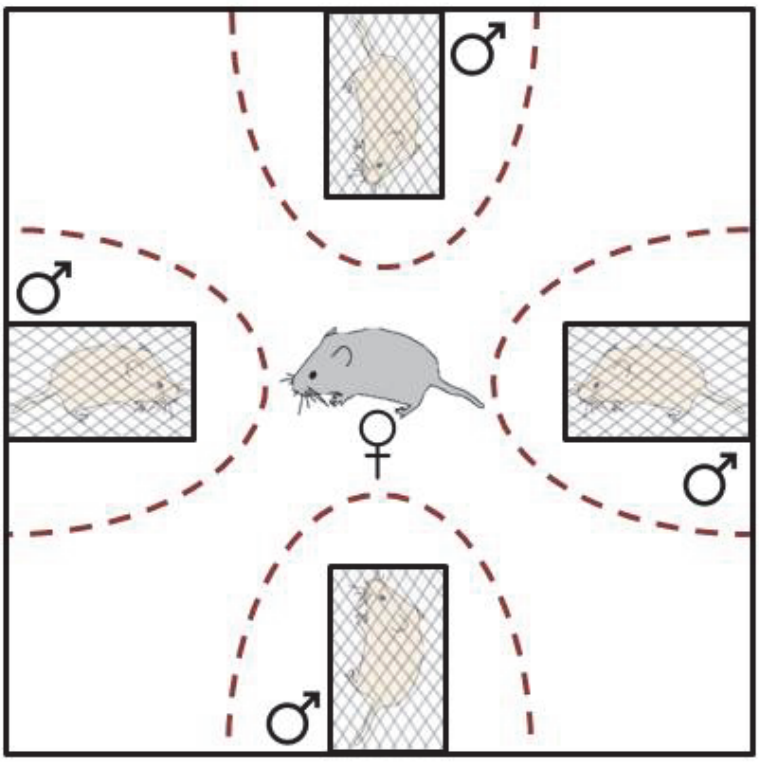
[3]Ecology and Genetics, University of Oulu, FI-90014, Oulu, Finland

Supplementary figure 1



Supplementary figure 1: setup of the male reproductive trials to assess the reproductive success of the F1 male in a competitive situation. Four F1 males (coloured red), one of each treatment, are placed (in a random order) in four cages connected by a PVC tube with holes in the sides. The PVC tube allows free passage of the males between all the cages. After one day acclimatization two naïve, non-experimental females (coloured grey) are added to the cage system; these females can also move freely between cages.

Supplementary figure 2



Supplementary figure 2: experimental setup for the female choice experiment. The setup consists of four small, wire mesh cages placed in the middle of each side of a white, square arena (60cmx60cm). These cages allowed transfer of smell, sound and vision but prevented mating. Each small, wire mesh cage has an F1 male (coloured orange) inside, one of each treatment, in a random order. After a 5-minute acclimation, a non-experimental, previously unused female in post-partum oestrus is added in the middle of the arena and tracked for 20 minutes using a video camera. Red dashed lines around the see-through cages demarcate the zone for the Noldus tracking software in which the female is considered to be "visiting" the male. This red-dashed zone is only virtual and no physical lines were present in the arena.

Supplementary table 1

Descriptives of the male reproductive success trials and the female preference trials. Rows describe the amount of trials performed, the amount of experimental F1 males used in the trials, the amount of naïve females used and the amount of F2 pups produced in the reproductive success trials. Left column describes the amount of each in the first round and right column describes the amount of trials replicated and the amount of individuals used to replicate. # = amount of.

	Original	Replicated
Reproductive success		
# Trials	58	33
# F1 males	232	132¤
# Females	116	66§
# F2 pups produced	253	126
Female preference		
# Trials	17	17
# F1 males	68	68¤
# Females*	17	17§

¤ These males were also part of the original trials

§ These females were new and not used for the original trials

*Post-partum estrus females not previously used for reproductive success trials

Supplementary table 2

Descriptives of F0 and F1 individuals used and produced during the experiment. # = number of

	n
# F0 females total	277
# F0 females pregnant	159
# Litters produced	159
# F1 males born	359
# F1 females born	325

Supplementary table 3

Preference shown by untreated females towards F1 males in relation to their ELE treatments and their reproductive success (whether or not the F1 male sired at least one pup; table 1) measured as time spent near a certain F1 male and number of visits in the proximity of the F1 male (n=49; two replications). Poisson generalized linear mixed model of number of visits. Binomial generalized linear mixed model 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 year. Est is estimated value. Bold p-values indicate p < 0.05 and are considered significant. Note that table provides the result of a similar analysis as shown in table 2, with only the measure of reproductive success added.

	Number of visits		Time spent near male	
	Est	*p*	*Est*	*p*
Intercept	1.397	**<0.001**	-3.099	**<0.001**
Protein restriction (PR)	-0.129	0.219	-0.981	0.150
Social confrontation (SC)	0.150	0.158	1.190	0.095
Interaction: PR*SC	0.044	0.769	0.247	0.802
Male sired at least one pup	0.262	**0.002**	1.751	**0.001**

Supplementary table 4

Preference shown by untreated females towards F1 males in relation to their ELE treatments and their reproductive success (number of pups sired by the F1 individual divided by all the pups sired within the trial; i.e. relative male competitive success) measured as time spent near a certain F1 male and number of visits in the proximity of the F1 male (n=49; two replications). Poisson generalized linear mixed model of number of visits. Binomial generalized linear mixed model 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 year. Est is estimated value. Bold p-values indicate p < 0.05 and are considered significant. Note that table provides the result of a similar analysis as shown in table 2, with only the measure of reproductive success added.

	Number of visits		Time spent near male	
	Est	*p*	*Est*	*p*
Intercept	1.391	**<0.001**	-3.067	**<0.001**
Protein restriction (PR)	-0.106	0.316	-0.846	0.233
Social confrontation (SC)	0.177	0.104	1.266	0.088
Interaction: PR*SC	0.034	0.823	0.260	0.799
Male competitive success	0.286	**0.002**	1.724	**0.005**

Supplementary table 5

Preference shown by untreated females towards F1 males in relation to their ELE treatments and their reproductive success (number of pups sired by the F1 individual divided by all the pups sired within the trial; i.e. relative male competitive success) measured as time spent near a certain F1 male and number of visits in the proximity of the F1 male (n=49; two replications). Poisson generalized linear mixed model of number of visits. Binomial generalized linear mixed model 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 year. Est is estimated value. Bold p-values indicate p < 0.05 and are considered significant. Note that table provides the result of a similar analysis as shown in table 2, with only the measure of reproductive success added.

	Number of visits		Time spent near male	
	Est	*p*	*Est*	*p*
Intercept	1.395	**<0.001**	-2.961	**<0.001**
Protein restriction (PR)	-0.118	0.262	-0.975	0.173
Social confrontation (SC)	0.179	0.100	1.249	0.097
Interaction: PR*SC	0.017	0.909	0.266	0.797
Male competitive success	0.055	**0.001**	0.291	**0.011**

Supplementary methods: paternity analysis

Paternity analysis of all F2 pups born in the "F1 reproductive success" trials (see main methods) was done by first genotyping the DNA from a tissue sample taken from the tail tip of each F2 pup at birth and comparing it to ear biopsies taken from all potential F1 fathers and the F2's respective mother. DNA was extracted using the standard protocol of the DNeasy kit (QIAGEN, Valencia, CA). All individuals were genotyped at six microsatellite loci: 6G11, 10A11, 13G2, 15F7, 16E2, and 17E9 (Gockel *et al.* 1997, Rikalainen *et al.* 2008). Paternity was assigned to the most likely male candidate with a confidence level of 95% using the software Cervus 3.0.7 (Kalinowski *et al.* 2007), while accounting for the known genotype of the mother (Mills *et al.* 2007a, 2009). All males within one trial were considered as potential genitors. The simulation was performed using 10000 cycles, 100% of candidate parents sampled, 98% of loci typed, a genotyping error rate of 1%, and no mismatches between offspring, neither with the known mother, nor with the assigned father were allowed.

Supplementary methods: trapping of F2 individuals during winter

Galvanized steel sheets surrounding the enclosures prevented immigration and emigration of bank voles but did not prevent possible predation by avian predators. During the experiment, individuals were not fed and they relied on natural food resources, except when being captured. To monitor the survival of the F2 Individuals over the winter, trappings were performed once per month using sunflower seeds and potato set in an Ugglan live trap. Trapped individuals were recorded and immediately released. Traps were set for a maximum of 36 hours each month and checked 3 times; except for the last trapping in March where all enclosures were trapped exhaustively. At any other point in time, traps were open (i.e. not set) and without food. The overwinter experiment ended in March as individuals started showing signs of fertility. Individuals were considered dead from the month they were no longer captured.

Supplementary references

Gockel J, Harr B, Schlötterer C, Arnolds W, Gerlach G, Tautz D. 1997 Isolation and characterization of microsatellite loci from *Apodemus flavicollis* (rodentia , muridae) and *Clethrionomys glareolus* (rodentia , cricetidae). *Mol. Ecol.* **6**, 597–599.

Rikalainen K, Grapputo A, Knott E, Koskela E, Mappes T. 2008 A large panel of novel microsatellite markers for the bank vole (*Myodes glareolus*). *Mol. Ecol. Resour.* **8**, 1164–1168.

Kalinowski ST, Taper ML, Marshall TC. 2007 Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol.* **16**, 1099–1106.

Mills SC, Alatalo RV, Koskela E, Mappes J, Mappes T, Oksanen TA. 2007 Signal reliability compromised by genotype-by-environment interaction and potential mechanisms for its preservation. *Evolution* **61**, 1748–57.

Mills SC, Grapputo A, Jokinen I, Koskela E, Mappes T, Oksanen TA, Poikonen T. 2009 Testosterone-Mediated Effects on Fitness□Related Phenotypic Traits and Fitness. *Am. Nat.* **173**, 475–487.

III

EARLY LIFE PROTEIN RESTRICTION REDUCES 18s rRNA COPY NUMBERS IN A MAMMAL

by

Joannes Van Cann, Toni Jernfors, Esa Koskela, Tapio Mappes, Phillip C. Watts

Manuscript

Request copy from author