International Journal for Parasitology 49 (2019) 481–488

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Effects of maternal and grandmaternal flea infestation on offspring quality and quantity in a desert rodent: evidence for parasite-mediated transgenerational phenotypic plasticity

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ARTICLE INFO

Article history:
Received 12 August 2018
Received in revised form 31 January 2019
Accepted 6 February 2019
Available online 13 April 2019

Keywords:
Maternal effects
Grandmaternal effects
Meriones crassus
Rodentia
Siphonaptera
Xenopsylla ramesis

ABSTRACT

Parasites can cause a broad range of sublethal fitness effects across a wide variety of host taxa. However, a host's efforts to compensate for possible parasite-induced fitness effects are less well-known. Parental effects may beneficially alter the offspring phenotype if parental environments sufficiently predict the offspring environment. Parasitism is a common stressor across generations; therefore, parental infestation could reliably predict the likelihood of infestation for offspring. However, little is known about relationships between parasitism and transgenerational phenotypic plasticity. Thus, we investigated how maternal and grandmaternal infestation with fleas (*Xenopsylla ramesis*) affected offspring quality and quantity in a desert rodent (*Meriones crassus*). We used a fully-crossed design with control and infested treatments to examine litter size, pup body mass at birth, and pup mass gain before weaning for combinations of maternal and grandmaternal infestation status. No effect of treatment on litter size or pup body mass at birth was found. However, maternal and grandmaternal infestation status significantly affected pre-weaning body mass gain, a proxy for the rate of maturation, in male pups. Pups gained significantly more weight before weaning if maternal and grandmaternal infestation statuses matched, regardless of the treatment. Thus, pups whose mothers and grandmothers experienced similar risks of parasitism, either both non-parasitized or both infested, would reach sexual maturity more quickly than those pups whose mothers' infestation status did not match that of their grandmothers. These results support the contention that parents can receive external cues such as the risk of parasitism, that prompt them to alter offspring provisioning. Therefore, parasites could be a mediator of environmentally-induced maternal effects and could affect host reproductive fitness across multiple generations.

1. Introduction

Parasites can cause a broad range of sublethal fitness effects across a wide variety of host taxa. Among these are indirect effects of parasites on host reproduction (Dunn et al., 2012). For example, avian parasites can impact the host time of breeding, clutch size, reproductive success, offspring quality, and the number of reproductive bouts per season in passerine birds (Møller et al., 1990). Gregarine parasites of damselflies reduce host fat content and thus impede the ability of their hosts to obtain territories necessary for breeding adults (Siva-Jothy and Plaistow, 1999). Similarly, monarch butterflies with high densities of protozoan parasites sustain shorter adult lifespans and smaller adult size, thus reducing their reproductive fitness (Altizer and Oberhauser, 1999). Additionally, although the effect of parasites on host reproduction remains an active area of research, a host's efforts to compensate for possible parasite-induced fitness effects are less well-known (but see Tripet and Richner, 1997).

Indeed, parents may increase their own fitness by investing resources to increase their offsprings' fitness (Lalonde, 1991).

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https://doi.org/10.1016/j.ijpara.2019.02.004
0020-7519/

These transgenerational parental effects are taxonomically widespread. In a review of transgenerational plasticity, Salinas et al. (2013) found that a range of variables in the parental environment such as temperature, precipitation, conspecific density, and predation, influenced key offspring traits such as mass, growth rate, fecundity, and age/size at maturity, in 63 plant and animal species spanning 32 orders and nine phyla. One way to accomplish this is via maternal effects, where females induce functionally important changes in offspring traits that may improve offspring survival or reproduction (Badyaev, 2008). Maternal effects that specifically increase offspring fitness and, concomitantly, maternal fitness, are called anticipatory maternal effects (Marshall and Uller, 2007) and have been documented in plant (e.g. Galloway and Etterson, 2007; Herman et al., 2012; Rasmann et al., 2012), invertebrate (Moret, 2006; Burgess and Marshall, 2011; Vehmaa et al., 2012), and vertebrate (e.g. Tschirren et al., 2004; Salinas and Munch, 2012; Dantzer et al., 2013; Schade et al., 2014; Shama et al., 2014) taxa. Many of these cases include some type of environmental matching, where offspring fitness increases when reared under the same type of environmental conditions that the parents experienced. This environmental matching requires that: (i) mothers would have accurate environmental cues, and (ii) the maternal environment would reliably predict offspring environment (Fischer et al., 2011; Ezard et al., 2014; Kuijper and Hoyle, 2015).

Although maternal effects occur in many different taxa (Uller, 2008), the mechanisms behind those could vary depending on the system in question. Most of these mechanisms are related to some facet of epigenetic programming (Cropley et al., 2006; Skinner et al., 2010; Zucchi et al., 2012, Wade, 1998). Initiation of this epigenetic programming is likely due to transmission of maternal factors such as hormones, nutrients, antibodies, and small RNAs to offspring (Boulinier and Staszewski, 2008; Dantzer et al., 2013; Liebers et al., 2014; Kuijper and Hoyle, 2015). Transmitting these factors to offspring essentially provides environmental induction of phenotypic traits by means of a phenotype (i.e. the mother) that is already functioning within that environment (Badyaev, 2008).

Despite the ubiquity of parasitism, anticipatory maternal effects are not well-studied in host-parasite systems (but see Schwanz, 2008) and host grandmaternal effects in response to parasitism are unknown. However, maternal and grandmaternal infection risks are likely good predictors of offspring infection risk and parents could increase their net reproductive success by producing offspring that are better able to cope with infection (Moret, 2006). Processes related to maternal immunity and physiology could also provide cues that trigger mechanisms for transgenerational plasticity. For example, parental challenge with lipopolysaccharide, a bacterial molecule that prompts an immune response, induced offspring production of antimicrobial peptides in the hemolymph of mealworm beetles (Moret, 2006). Evidence suggests that when mothers are exposed to parasites, they are more likely to invest in offspring phenotypes that mitigate the impact of infection by either improving the chances of offspring survival or reproductive success (Sorci and Clobert, 1995; Tschirren et al., 2004; Schwanz, 2008; Warburton et al., 2017). However, the "vicious circles" described by Beldomenico et al. (2008) where a host's poor body condition predisposes it to infection, which then further decreases body condition, could theoretically be extended to generational effects, even though this framework has only been developed for single individuals.

Although not as well studied as anticipatory maternal effects, there is experimental evidence for anticipatory grandmaternal effects in plants (Herman et al., 2012), invertebrates (Plaistow et al., 2006; Hafer et al., 2011; Lock, 2012), and vertebrates (Mech et al., 1991; Monteith et al., 2009; Shama and Wegner, 2014). Grandmaternal effects can be linked to environments that influence maternal and grandmaternal resource availability. For example, in deer, second generation fawns had higher body mass and survival when their grandmothers experienced environments with high quality nutrition (Mech et al., 1991; Monteith et al., 2009). However, grandmaternal effects are not limited to resource-associated settings and can also be associated with challenging or stressful environments. For example, the experimental effects of drought on plants (*Polygonum persicaria*) were cumulative over two generations (Herman et al., 2012). Offspring whose parents and grandparents experienced dry conditions had higher survival and reproductive performance under dry conditions than those individuals originating from parents or grandparents under wet conditions (Herman et al., 2012). Similar effects also occurred in invertebrates such as soil mites (Plaistow et al., 2006) and springtails (Hafer et al., 2011), where the significant effects of high and low food environments influenced life history traits over three generations. Environmental challenges need not be nutritional in nature to elicit adaptive transgenerational plasticity, however, and other conditions could play an important role in anticipatory grandmaternal effects. Marine sticklebacks reached larger body sizes when they were reared in temperatures that matched their maternal and grandmaternal environments (Shama et al., 2014). Thus, if offspring in challenging environments had mothers or grandmothers that experienced similar environments, anticipatory effects might confer an advantage such as early reproductive maturity via faster growth and maturation (Hafer et al., 2011). Therefore, fitness consequences of transgenerational plasticity could strongly depend on the context of environmental challenges and the system in question (Shama et al., 2014).

Given that the fundamental relationship between transgenerational phenotypic effects and parasitism is virtually unknown, here we investigate host grandmaternal effects in response to parasitism using a rodent, *Meriones crassus*, and fleas, *Xenopsylla ramesis*. Although grandmaternal effects in host-parasite systems are unknown, it is reasonable to expect that they would act in a similar manner as previously documented maternal effects, as is the case in other systems (Mech et al., 1991; Plaistow et al., 2006; Monteith et al., 2009; Hafer et al., 2011; Herman et al., 2012; Lock, 2012; Shama and Wegner, 2014). Thus, we asked if a grandmother's infestation status impacts, either alone or in combination with a mother's infestation status, metrics of offspring quality (i.e. mass at birth, mass gain) and quantity (i.e. litter size). We desired to test whether grandmaternal effects would be anticipatory, and thus if offspring quality and quantity would be strongest when mothers and grandmothers experience the same, rather than mismatched, infestation statuses. If these effects were indeed anticipatory, we hypothesized that litter size, pup body mass at birth, and/or pup proportional mass gained before weaning would be highest in litters where both mothers and grandmothers were uninfested. Similarly, given that infested mothers have been found to have pups that gain more mass before weaning than those from uninfested mothers under certain circumstances (Warburton et al., 2017), we hypothesized that these same variables would be next highest in litters where both mothers and grandmothers experienced flea infestation.

2. Materials and methods

2.1. Study animals

Fleas and rodents originated from our laboratory colonies. Prior to experiments, rodents were individually housed in plastic cages (33 cm × 23 cm × 13 cm at 25 °C ± 1 °C and 12 h:12 h dark:light) with wood shavings as bedding material. They were fed whole mil-

let seeds ad libitum and fresh alfalfa as a water source; therefore, drinking water was not offered. Fleas were maintained on *M. crassus* males within our insectarium under the same conditions (i.e. rodents housed in plastic cages (33 cm × 23 cm × 13 cm) at 25 °C ± 1 °C and 12 h:12 h dark:light). When rodents are infested with fleas, either for rearing fleas or for experimental procedures, they are placed in an individual plastic cage (33 cm × 23 cm × 13 cm) with the bottom covered by a 1 cm sand layer and a set of three wire screens separating the animal from the sandy floor. This design created a refugium for fleas that provided them with a hiding place between blood meals and an area for oviposition of eggs. Sand was collected every 2 weeks and transferred to incubators where fleas could continue their lifecycle. Newly emerged imagoes were then collected after hatching and either transferred to experiments or returned to the insectarium to produce more offspring. Further specific details regarding rearing procedures and colony maintenance are published elsewhere (e.g. Krasnov et al., 2001, 2002; Khokhlova et al., 2009a,b).

2.2. Experimental design

A general outline of the experimental design is presented in Fig. 1. More specifically, first generation, nulliparous *M. crassus* females between 5 and 6 months of age were assigned to one of two treatments: control (C) or infestation with *X. ramesis* (I). Of 32 females, 16 were assigned to group C and 16 were assigned to group I. Newly emerged flea imagoes were randomly selected from laboratory colonies and released into home cages of individual rodents (100 fleas for each *M. crassus*). As rodents were free to groom, approximately 50% of fleas per week were expected to be dislodged and killed by a host (Hawlena et al., 2007; Krasnov, 2008). Therefore, every week 100 newly emerged flea imagoes were added to each animal's cage to keep flea pressure more or less constant. Female rodents were weighed every day during the experimental period.

Two weeks after initial infestation, males were introduced to female cages and fleas were added so that a sufficient number of newly emerged flea imagoes could infest both males and females (100 fleas for each *M. crassus* or 200 fleas total). Males were housed with females for 2 weeks to allow successful copulation. One week after introducing a male, another set of 200 newly emerged flea imagoes was added to each rodent pair. After 2 weeks, we removed the males and collected all fleas from their bodies via brushing their coats with a toothbrush. Then, males were returned to the laboratory colony while females were placed individually in new cages and again infested with 100 fleas. Cages were changed and a new group of 100 newly emerged flea imagoes was added every 2 weeks when the females' cages were cleaned. This continued until shortly before parturition, approximately the nineteenth day of pregnancy for *M. crassus*, as determined by the pattern of female mass gain during pregnancy (Krasnov et al., 1996). Fleas were also removed from females using a toothbrush and females were transferred to clean, flea-free cages before giving birth. Control animals experienced the same conditions; however, they were kept in flea-free cages.

After parturition, pups were weighed approximately 2 h after birth and litter sizes as well as numbers of male and female pups were recorded. Pups were marked to allow for individual identification. Pups were weighed daily until weaning at 30 days of age. Pup sex was confirmed at weaning with no difference in sex recorded at birth and sex recorded at weaning for any individual. These pups were then considered the second generation and once they reached reproductive maturity (5–6 months of age) the above procedures were repeated in the second generation. We implemented a fully crossed design in which each treatment combination (CC, CI, IC, II; first letter represents the first generation treatment, second letter represents the second generation treatment) had eight replicates. Again, females were subjected to *X. ramesis* infestation or control conditions and resulting pups were monitored in the same fashion until weaning. In the end, females gave birth to 34 CC pups, 35 CI pups, 23 IC pups, and 31 II pups. All experimental protocols met the requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel and were approved by the Ben-Gurion University (Israel) Committee for the Ethical Care and Use of Animals in Experiments (Permit IL-72-10-2012).

2.3. Statistical analyses

We analyzed the effects of maternal and grandmaternal treatment (C or I), as four treatment groups, on litter size (LS; the number of offspring in a litter produced by an individual female), body mass of a pup at birth (BMB), and proportional body mass of a pup gained before weaning (PBMG; proportional difference between body mass at weaning and body mass at birth), on the third generation of *M. crassus*. PBMG was transformed using the logit transformation prior to analysis (Warton and Hui, 2011). The effects of treatment on litter size were analyzed using linear mixed-effects models using a Poisson distribution for count data with a log link in the R Statistical Environment (R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org). In the analyses of the effect of parasitism on LS (dependent variable), explanatory variables were female mass at pairing as well as maternal and grandmaternal treatment group. The effects of treatment on BMB and PBMG were also analyzed via linear mixed-effects models. All mixed-effects models included maternal and grandmaternal identity as random effects with variable intercepts and were applied using package "lme4" (Bates et al., 2015) implemented in R.

As rodents used in this study were characterized by sexual dimorphism in body size and growth rate (Koffler, 1972), BMB and PBMG were also analyzed separately for male and female pups if best fit models included an effect of both treatment and pup sex. Each model also included LS and maternal body mass at pairing (a proxy for female body condition at fertilization; Trivers and Willard, 1973; Huck et al., 1988) as fixed effects. Grandmaternal and maternal identity were included as random effects. To ensure that separating the datasets for male and female offspring did not meaningfully change model selection, we performed mixed-effects models that included random effects of grandmaternal and maternal identity as well as pup sex. We then calculated the $\log_{10}$ evidence ratio (LER) for the competing models and, being below 0.5, their differences in weight were classified as minimal (Snipes and Taylor, 2014). Confirming that there was no meaningful difference between the two methods, we considered data for males and females separately to highlight the biological differences in growth and development between the sexes. Visual inspection of residual plots did not show deviations from homoscedasticity or normality. Initially, a model was constructed with all possible terms and interactions for each dependent variable. Then, the best model was selected using Akaike Information Criterion (AIC) corrected for sample size with function "dredge" of the R package "MuMIn" (Barton, K., 2016. MuMIn: Multi-model inference. R package version. 1.15.6. http://CRAN.R-project.org/package=MuMIn). If the best fit model for a dependent variable was an intercept-only model, or if the model did not include any terms related to experimental treatment, further analysis of that dependent variable was not pursued. We also used Cohen's *d* (Cohen, 1977) to calculate effect sizes for maternal or grandmaternal treatment (i.e. C or I) and the interaction between them. If either maternal or grandmaternal treatment was statistically significant, we used multiple

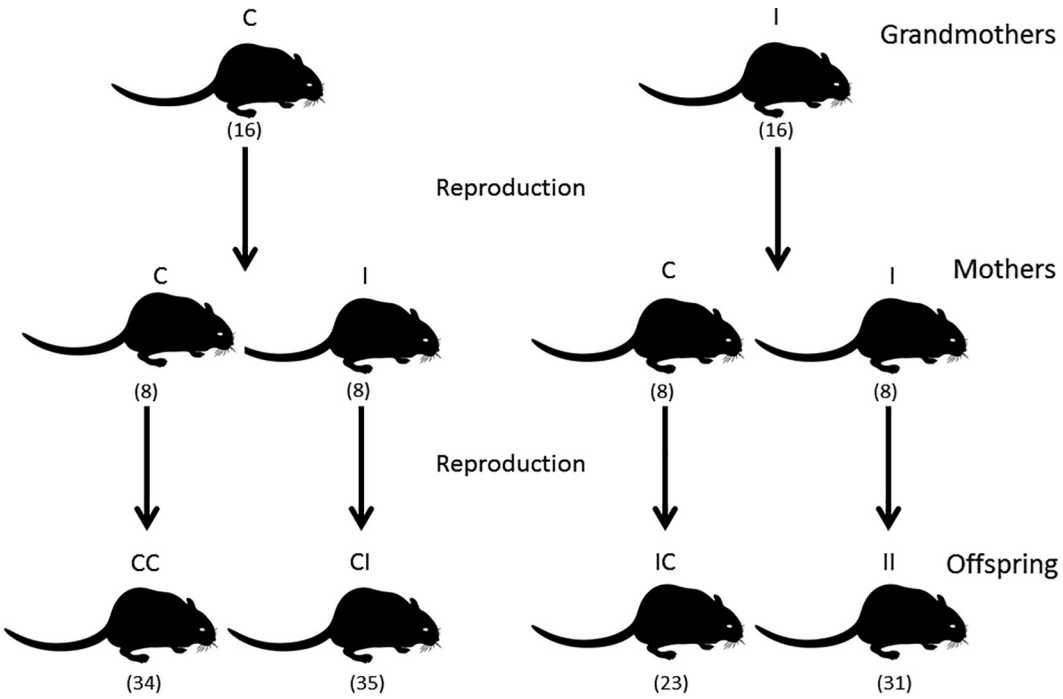

Fig. 1. A general diagram of the experimental design utilized in the experiment. *Meriones crassus* grandmothers and mothers were assigned to either control (C) or infested by *Xenopsylla ramesis* (I) treatment groups. Grandmaternal treatment is listed first followed by maternal treatment. Numbers of animals in each step in the experiment are listed under each treatment group as (*n*). The quality and quantity of resultant offspring from each treatment group (grandmother group, mother group) were then compared.

comparisons via R package "multcomp" (Hothorn et al., 2008), function glth with Tukey contrasts, to determine between-treatment differences in a given dependent variable for best fit models.

3. Results

We found no effect of either maternal or grandmaternal treatment on LS as the intercept-only model had the best fit (Table 1). In general, litter size, the number of male pups and the number of female pups were similar between C and I groups in both generations (Table 2). No terms related to the maternal or grandmaternal treatment group were included in the best fit model of pup BMB and BMB was similar for all treatment groups for both sexes (Table 3). However, we found a significant effect of maternal and grandmaternal infestation statuses on pup PBMG. In addition to these terms, pup sex was also included in the best fit model; thus, separate models were created for male and female pups. However, the significant effects of maternal and grandmaternal treatment only occurred in male offspring (Table 4). Male CC pups exhibited a median PBMG of 26.52 (interquartile range (IQR) = 7.94) and male II pups exhibited a median PBMG of 25.94 (IQR = 6.71) while male CI pups exhibited a median PBMG of 15.35 (IQR = 15.91) and male IC pups exhibited a median PBMG of 16.01 (IQR = 14.60) (Fig. 2). This pattern occurred regardless of whether their status was CC or II. Male offspring PBMG was significantly higher ($P = 0.044$) when mothers and grandmothers shared infestation statuses and this interaction had a large effects size (Cohen's $d = 1.06$) (Table 4). This did not occur in female offspring. Female CC pups exhibited a median PBMG of 19.76 (IQR = 12.60) and female II pups exhibited a median PBMG of 12.72 (IQR = 4.68) while female CI pups exhibited a median PBMG of 12.16 (IQR = 1.89) and female IC pups exhibited a median PBMG of 9.60 (IQR = 9.08) (Fig. 3). Female offspring PBMG was not significantly higher ($P = 0.41$) when mothers and grandmothers shared infestation statuses and, correspondingly, this interaction had a small effects size (Cohen's $d = 0.41$) (Table 4). Further analysis via multiple comparisons of the effects of grandmaternal and maternal treatment on male pups' PBMG before weaning revealed that the two variables were not significant on their own (Table 5) and thus suggests that a significant interaction between the two variables was driving their inclusion in the best fit model described in Table 1.

4. Discussion

We hypothesized that LS, BMB, and/or PBMG would be highest in litters where both mothers and grandmothers were from control groups and next highest in litters where both mothers and grandmothers experienced flea infestation. Previous results in this specific host-parasite system indicated that pups of parasitized mothers gained more mass before weaning than those of unparasitized mothers; however, this effect only occurred in small litters (Warburton et al., 2017) and we found no effect of LS in the present investigation. Although our results did not support the ranking given in our hypotheses, we nonetheless found evidence of higher offspring quality, at least for male pups, when mothers and grandmothers both experienced the same risk of flea parasitism. It is important to emphasize that, unlike our original hypothesis, actual infestation with fleas did not contribute to male pup mass gained before weaning but rather that this occurred when the maternal and grandmaternal environments matched. This suggests that transgenerational environmental effects, not parasitism itself, were driving pup mass gain. The distinction is vital as it indicates that over successive generations of infestation risk, females can alter offspring phenotypes in such a way that metrics of offspring quality from infested mothers are similar to those from uninfested mothers. Thus, parents could increase their net reproductive success by producing offspring that are better able to cope with infection (Moret, 2006). It is also possible that this effect could vary with offspring sex, so that male offspring might benefit more from a phenotype that invests more in growth and less in immunity,

Table 1
Top three best fit models of litter size, pup body mass at birth and proportional body mass gain until weaning in pups of *Meriones crassus*. Fixed effects include maternal mass, grandmaternal treatment, maternal treatment, pup sex (PS, for body mass at birth and proportional body mass gain), and litter size (for body mass at birth and proportional body mass gain).

Model	Terms	df	AICc	Weight
LS				
	Intercept only	3	130.4	0.530
	GMT	4	132.2	0.217
	MT	5	133.2	0.130
BMB				
	LS	4	157.2	0.974
	PS + LS	5	164.5	0.025
	PS + LS + PS*LS	6	173.8	0.001
PBMG				
	GMT + MT + PS + GMT*MT + GMT*PS + MT*PS + GMT*MT*PS	10	523.7	0.674
	MM + GMT + MT + PS + GMT*MT + GMT*PS + MT*PS + GMT*MT*PS	11	527.6	0.095
	GMT + MT + PS + LS + GMT*MT + GMT*PS + MT*PS + GMT*MT*PS	11	528.7	0.054

df, degrees of freedom; AICc, Akaike Information Criterion corrected.

Table 2
Mean *Meriones crassus* litter size, number of male pups, number of female pups, and litter mass at birth for females from control or infested groups and each generation of the experiment. Note that offspring of the maternal generation also had grandmothers both in control or infested groups. S.E.M. is also listed for each variable.

	C Group	S.E.M.	I Group	S.E.M.
Offspring of grandmaternal generation				
LS	4.17	0.37	4.38	0.32
Number of male pups	2.00	0.48	2.06	0.30
Number of female pups	2.09	0.34	2.33	0.22
Offspring of maternal generation				
LS	4.73	0.45	4.27	0.38
Number of male pups	2.55	0.41	2.18	0.30
Number of female pups	2.18	0.41	2.00	0.44

Table 3
Mean body mass at birth for male and female pups (*Meriones crassus)* from each of the four treatment groups. Note that grandmaternal infestation status is represented by the first C (control group) or I (infested group) and maternal infestation status is represented by the second C or I.

	CC	S.E.M.	CI	S.E.M.	IC	S.E.M.	II	S.E.M.
Male BMB	3.36	0.10	3.29	0.14	3.22	0.09	3.35	0.09
Female BMB	3.42	0.06	3.21	0.16	3.54	0.14	3.59	0.12

Table 4
Summary of maternal treatment (M Treat) and grandmaternal treatment (GM Treat) on proportional body mass gain until weaning (PBMG) in male and female pups of *Meriones crassus* using the best fit model of PBMG. Cohen's *d* is a measure of effect size (0.20 ≥ small, 0.50 ≥ moderate, 0.80 ≥ large). The reference level for categorical independent variables M Treat and GM Treat was the control group.

	Value	S.E.	*df*	*P*	Cohen's *d*
Male					
M Treat	−9.425	4.088	14	0.037	0.50
GM Treat	−10.072	5.198	14	0.073	0.66
M Treat * GM Treat	−17.191	9.362	47	0.044	1.06
Female					
M Treat	−1.487	3.486	19	0.674	0.52
GM Treat	−3.053	4.372	28	0.491	0.41
M Treat * GM Treat	1.369	4.807	28	0.778	0.41
d					

df, degrees of freedom.

similar to the terminal investment hypothesis (Clutton-Brock, 1984). This agrees with other work indicating that maternal effects can attenuate the impact of parasitism on offspring fitness (Sorci and Clobert, 1995; Tschirren et al., 2004; Schwanz, 2008).

Despite the evidence for anticipatory grandmaternal effects in our host-parasite system, results indicated significant effects only on body mass gained by male pups. A similar pattern has also been documented in deer (Mech et al., 1991; Monteith et al., 2009) and voles (Koskela et al., 2004). Although initially puzzling, two possible, although at this point speculative, explanations exist. Firstly, males might be more physiologically sensitive to the mechanisms behind transgenerational plasticity in our experiment. Male embryos tend to be more sensitive to substances such as hormones and toxicants in utero than female embryos (Vom Saal et al., 1983; Vandenbergh, 2003; Waalkes et al., 2004; Foster, 2006). Evidence supports that maternal factors such as hormones, antibodies, and micro-RNAs contribute to the epigenetic programming of maternal effects (Meaney et al., 2007; Muller et al., 2007; Boulinier and

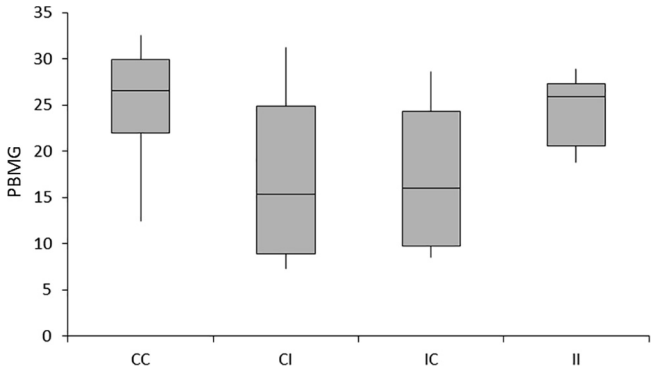

Fig. 2. Proportional pup mass gained before weaning presented in a boxplot for *Meriones crassus* male pups from different combinations of grandmaternal – maternal treatment groups. Grandmaternal treatment is listed first followed maternal treatment. Control (uninfested) groups are denoted with C while groups infested with *Xenopsylla ramesis* are denoted with I. Interbox lines indicate median values, while hinges and whiskers show interquartile ranges and full ranges, respectively.

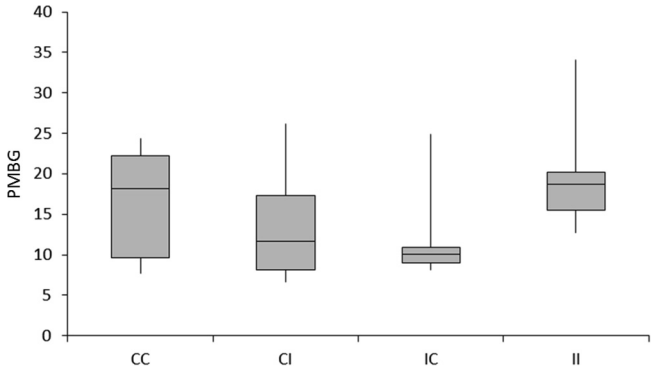

Fig. 3. Proportional pup mass gained before weaning presented in a boxplot for *Meriones crassus* female pups from different combinations of maternal – grandmaternal treatment groups. Maternal treatment is listed first followed grandmaternal treatment. Control (uninfested) groups are denoted with C while groups infested with *Xenopsylla ramesis* are denoted with I. Interbox lines indicate median values, while hinges and whiskers show interquartile ranges and full ranges, respectively.

Table 5
Independent contrasts of maternal treatment (M Treat) and grandmaternal treatment (GM Treat) for the control group (C) and the infested group (I) of male *Meriones crassus* pups' proportional body mass gained before weaning. Note that these contrasts are averages over the levels of M Treat and GM Treat, respectively, and do not take any interactions between the two variables into account.

Contrast	Estimate	S.E.	df	*P*
M Treat				
C-I	1.477	3.887	14	0.709
GM Treat				
C-I	0.829	3.887	14	0.834

df, degrees of freedom.

Staszewski, 2008; Groothuis and Schwabl, 2008; Radtke et al., 2011; Liebers et al., 2014; Kuijper and Hoyle, 2015) and it is possible that male embryos require a lower amount of these factors to flip the epigenetic switch. In addition, some alleles are more vulnerable to environmental influence, and thus epigenetic effects, only if they are paternally inherited (Cropley et al., 2006). DNA methylation patterns that regulate gene expression (i.e., methylomes) might also be paternally inherited in some species (Jiang et al., 2013; Potok et al., 2013). If such alleles are sex-linked, then males might be more likely to express epigenetic traits. Indeed, some sex-specific differences in DNA methylation have been documented (Guerrero-Bosagna et al., 2008). Secondly, faster male growth, and thereby earlier onset of sexual maturity, might be more advantageous for males than females in challenging environments. The extra time gained for reproduction might be negligible from a female's standpoint because she must expend significant time and energy toward gamete production, pregnancy and lactation. However, male gametes are less costly to produce and males do not undergo the time and energy sinks of pregnancy and lactation. Many species are also non-monogamous and a single male may be able to fertilize many females within a short period of time (c.f. Trivers and Willard, 1973). As early offspring have a disproportionately high contribution toward lifetime reproductive fitness (Pianka and Parker, 1975), a male's relative fitness could be greatly increased due to an earlier onset of sexual maturity.

Maternal effects are expected to evolve when environments fluctuate across generations, the offspring environment can be predicted from maternal phenotypes, and the cost of obtaining and responding to environmental information is low compared to the benefit it produces (Uller, 2008; Kuijper et al., 2014; Kuijper and Hoyle, 2015). In addition, selection models indicate that maternal effects are quite sensitive to specific ecological and organismal features such as environmental change, strength of selection, and the amount of phenotypic plasticity (Kuijper and Hoyle, 2015). We often think about parental effects as traits that primarily impact offspring, yet it is also important to consider how selection acts on maternal effects to maximize maternal fitness rather than, or in addition to, increasing offspring fitness (Marshall and Uller, 2007). Thus, the relative importance of a maternal effect is likely to depend on the offsprings' environment; however, the nature of a maternal effect will depend on the maternal resource state, life expectancy, and cost-benefit trade-offs (Bernardo, 1996; Marshall and Uller, 2007). Although questions remain about the general ubiquity and relative power of anticipatory parental effects, selection could more strongly favor them in certain situations or taxa (Uller et al., 2013).

Even though anticipatory transgenerational phenotypic plasticity could mitigate fitness consequences of parasitism, host maternal and grandmaternal effects are not well-studied within this context. In addition to contributing general information about anticipatory maternal effects, our results provide valuable information about this type of transgenerational phenotypic plasticity within the context of parasitism. Although we cannot completely rule out the existence of effects from other possible reproductive variables, our results support existing evidence that mothers may favor phenotypes that increase offspring survival or reproductive fitness in order to mitigate infection risk (Sorci and Clobert, 1995; Tschirren et al., 2004; Moret, 2006; Schwanz, 2008). Further, our data are the first known to demonstrate that grandmaternal effects play a valuable role in host-parasite systems. Maternal effects are potentially significant sources of variation in early offspring success (Bernardo, 1996). Therefore, parasites could be a mediator of environmentally-induced maternal effects and could affect host reproductive fitness across multiple generations. This implies that interpretation of field studies examining parasite effects on host fitness should be cautious because the infection status of parents and grandparents are generally unknown. However, further study of host transgenerational phenotypic plasticity in response to parasitism can better quantify the relative strengths of these effects.

Acknowledgements

The authors thank the anonymous reviewer and the Deputy Editor for helpful comments on the manuscript. This study was partly supported by the Israel Science Foundation (Grant number 149/17 to BRK and ISK). EMW received financial support from the Blaustein Center for Scientific Cooperation, Israel. LVDM

received financial support from the Blaustein Center for Scientific Cooperation and the French Associates Institute for Agriculture and Biotechnology of Drylands, Israel. CJD received financial support from the Blaustein Center for Scientific Cooperation. EMD received financial support from the Blaustein Center for Scientific Cooperation, the Kreitman School of Advanced Graduate Studies (Israel) and the Swiss Institute for Dryland Environmental and Energy Research, Israel. This is publication 1005 of the Mitrani Department of Desert Ecology, Israel.

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