Ann. Zool. Fennici 57: 99–114 ISSN 0003-455X (print), ISSN 1797-2450 (online)
Helsinki 17 April 2020 © Finnish Zoological and Botanical Publishing Board

Intra- and trans-generational phenotypic responses of the greater wax moth, *Galleria mellonella*, to a low-nutrition larval diet

Katariina Kangassalo[1,*], Jouni Sorvari[2], Ilkka Nousiainen[3], Mari Pölkki[1], Terhi M. Valtonen[1], Indrikis Krams[4,5,6] & Markus J. Rantala[1]

[1)] *Department of Biology, Section of Ecology, FI-20014 University of Turku, Finland (*corresponding author's e-mail: mkkang@utu.fi)*
[2)] *Department of Environmental and Biological Sciences, University of Eastern Finland, FI-70211 Kuopio, Finland*
[3)] *Department of Aquaculture, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, EE-51014 Tartu, Estonia*
[4)] *Latvian Biomedical Research and Study Center, Rātsupītes iela 1, LV-1067 Rīga, Latvia*
[5)] *Department of Zoology and Animal Ecology, Faculty of Biology, University of Latvia, Jelgavas iela 1, LV-1004 Rīga, Latvia*
[6)] *Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, EE-51014 Tartu, Estonia*

Received 21 Oct. 2019, final version received 1 Apr. 2020, accepted 6 Apr. 2020

Kangassalo, K., Sorvari, J., Nousiainen, I., Pölkki, M., Valtonen, T. M., Krams, I. & Rantala, M. J. 2020: Intra- and trans-generational phenotypic responses of the greater wax moth, *Galleria mellonella*, to a low-nutrition larval diet. — *Ann. Zool. Fennici* 57: 99–114.

We investigated the intra- and trans-generational effects of larval diet on immune function, body size and development time of the greater wax moth, *Galleria mellonella* (Lepidoptera : Pyralidae). We found that moths reared on a diet diluted with cellulose (a low-nutrition diet) were about one-third smaller, had about one-fifth longer development time and exhibited about 10% stronger encapsulation responses as compared with moths reared on the standard diet. The low-nutrition parental diet prolonged the development time of male offspring that were fed the low-nutrition diet by about 4% and the development time of female offspring that were fed the standard diet by about 1%. However, females that were fed the low-nutrition diet attained about 6% greater body mass when their parents were reared on the low-nutrition diet. Our results add to the growing number of studies demonstrating that the nutritional history of parents can affect the performance of their offspring.

Introduction

Insects exhibit considerable phenotypic plasticity in response to variation in quality and quantity of their diet (Whitman 2009). Perhaps most consistently, this can be observed as diet-induced differences in growth-related characteristics (Tammaru 1998, Blanckenhorn 1999, Teder *et al.* 2014). Due to variation in nutritional conditions, a negative relationship between age and size at maturity has been observed in nearly all taxonomic and ecological categories

of insects (Teder *et al.* 2014): under favourable conditions, maturity is reached sooner and at a larger size. The diet-induced changes in size and development time can have significant fitness consequences: prolonged juvenile period may decrease the likelihood of survival to maturity, while body size is closely linked to fecundity (Roff 1992, Stearns 1992). A strong positive correlation between body size and fecundity is expected especially in capital breeding insects, in which all resources available for egg production are present at the time of adult eclosion (Honek 1993, Tammaru & Haukioja 1996, Tammaru *et al.* 2002, Calvo & Molina 2005).

Nutrition is found to affect immune function in insects, yet the study results have been variable: in some studies, nutritional stress was found to increase disease resistance or other measures of immune function (Klemola *et al.* 2007, Kelly & Tawes 2013, Krams *et al.* 2015), while in others opposite was found (Suwanchaichinda & Paskewitz 1998, Boots 2000, Muturi *et al.* 2011). Differences in the methods of reducing quality or quantity of nutrition as well as in the measured immune parameters may contribute to the dissimilar results, particularly as different components of the immune system are found to have different nutritional requirements (Cotter *et al.* 2011, Ponton *et al.* 2011). In insects, immune function is found to be costly and to carry a risk of autoimmunity; the factors thought to create and maintain variation in immune defence (Rolff & Siva-Jothy 2003). Immune function is increasingly recognized as a life-history trait, subject to trade-offs with other important physiological functions such as reproduction, somatic maintenance or starvation resistance (Sheldon & Verhulst 1996, Moret & Schmid-Hempel 2000, Hoang 2001, Armitage *et al.* 2003, Ye *et al.* 2009, Schwenke *et al.* 2016).

The effects of nutrition may transcend generations, whereby the nutritional conditions experienced by an individual affect its offspring or even later descendants. Indeed, parental effects — which can be defined as the direct effect of a parent's phenotype on the phenotype of its offspring (Bernardo 1996, Youngson & Whitelaw 2008, Bonduriansky & Day 2009) — are considered an important source of phenotypic variation in organisms (Mousseau & Dingle 1991, Mousseau & Fox 1998). In invertebrates, parental diet is found to affect several life-history traits of the offspring, such as immune function (Rotem *et al.* 2003, Mitchell & Read 2005, Myers *et al.* 2011, Stjernman & Little 2011, Boots & Roberts 2012, Triggs & Knell 2012, Saastamoinen *et al.* 2013), development rate (Bonduriansky & Head 2007, Valtonen *et al.* 2012, Franzke & Reinhold 2013, Zirbel & Alto 2018), body size (Bonduriansky & Head 2007, Valtonen *et al.* 2012, Cahenzli & Erhardt 2013, Franzke & Reinhold 2013) and fecundity (Futuyma *et al.* 1993, Frago & Bauce 2014). Although transgenerational effects of maternal nutrition have been studied more extensively, diet-induced paternal effects may also be significant, even in species that lack conventional forms of paternal investment (Bonduriansky & Head 2007, Valtonen *et al.* 2012). Mechanisms of diet-induced parental effects are, for example, diet-induced variation in maternal egg provisioning (Fox & Czesak 2000, Vijendravarma *et al.* 2010) or in male nuptial feeding (which comprises any form of nutrient transfer from the male to the female during or after copulation or courtship; Parker & Simmons 1989, Simmons & Parker 1989, Vahed 1998, Gwynne 2008). Parental effects may also occur independently of variation in nutritional provisioning; for example, epigenetic marks, such as DNA methylation or histone modifications, can be transferred via the gametes to the offspring (Youngson & Whitelaw 2008, Anaka *et al*. 2009, Jablonka & Raz 2009, Friberg *et al*. 2012).

Although parental nutrition is known to affect offspring characteristics in insects, little is still known about how parental and offspring nutrition interact in their effect on offspring phenotype. It is thought, that parental effects may act as a mechanism for adaptive phenotypic response to environmental variation; in literature, this has been referred to as 'adaptive transgenerational plasticity' or 'anticipatory parental effects' (Mousseau & Fox 1998, Marshall & Uller 2007, Uller *et al.* 2013). According to this hypothesis, parental effects may increase the performance of the offspring in an environment similar to the one experienced by the parents, which would be beneficial if parental and offspring environments are likely to correlate (Mousseau & Fox 1998, Marshall & Uller 2007, Uller *et al.* 2013). This

view is supported by several studies in invertebrates, showing that exposure of parents to, for example, extreme diets (*Pieris rapae*; Rotem *et al.* 2003) or low food levels (*Daphnia*; Gliwicz & Guisande 1992) improves resistance of the offspring to nutritional stress. However, such transgenerational plasticity may come with a cost if parental and offspring environments do not match — for example, in humans, offspring of parents who experience undernutrition may have increased capacity to withstand undernutrition, but in turn have an increased risk of obesity and diabetes in an energy-rich environment (Gluckman & Hanson 2008). A recent meta-analysis found that the evidence for anticipatory parental effects is rather weak (Uller *et al.* 2013), and some parental effects are considered more likely to be physiological side effects with no adaptive value.

The greater wax moth, *Galleria mellonella* (Lepidoptera : Pyralidae), is a pest of apiculture found in most of the world. Females of *G. mellonella* lay eggs inside beehives, and the larvae feed on honeycomb, pollen, propolis and honey, creating tunnels lined with silk inside the honeycomb. In the process, the bee larvae and emerging bees are starved and entangled, which leads to a reduction in the bee population of the colony and in some cases destruction of the whole colony. Adult moths lack functional mouthparts (Kwadha *et al.* 2017) and accumulate the resources needed for reproduction during the larval stage (the capital breeding strategy; Houston *et al.* 2007).

We studied the intra- and transgenerational effects of larval nutrition (low-nutrition *vs.* standard) on three life-history traits in *G. mellonella*: egg-to-adult development time, adult body mass and immune function. Larvae of the parental and offspring generation were reared on a diet diluted with cellulose or on a standard diet, and the effects of parental and offspring nutrition on offspring characteristics were assessed. While the within-generation effects of nutrition have been studied extensively in insects, the transgenerational effects of nutrition remain less well understood. The aim of this study was to shed more light on the subject, particularly on how parental and offspring nutrition together affect the offspring phenotype.

Material and methods

Laboratory population

The laboratory population of *G. mellonella* used in this study originated from the moths collected in the field in Novosibirsk, Russia. A laboratory population had been originally established from these moths at the Siberian Branch of the Russian Academy of Sciences in Novosibirsk, from which a new laboratory population based on a few thousand of these moths was established at the University of Turku, Finland. Before the study, a standing population of around 5000–10 000 moths was maintained at our laboratory for several generations. The moths were kept at 28 ± 1 °C in several large plastic boxes in constant darkness, and the same conditions were maintained during the experiment. Stock larvae were fed *ad libitum* the standard diet described below.

Diets

The ‘standard diet’ used in the experiment was similar to the diets developed by Beck (1960) and Balanzs *et al.* (1958) with some minor adjustments to the ingredients or their proportions (Table 1). This type of diet has been found to be excellent in supporting the growth and survival of *G. mellonella* larvae. To reduce quality of the diet, 25% (by mass) of beeswax, honey, cornmeal, infant formula powder, wheat flour and dry yeast were replaced by cellulose (α-cellulose powder; Sigma-Aldrich Chemie GmbH, Munich, Ger-

Table 1. Composition of the diets; values are grams per 100 g.

	Standard diet	Low-nutrition diet
Beeswax	11.9	5.8
Cellulose	–	12.1
Corn meal	21.4	10.5
Distilled water	11.9	23.3
Dry yeast	4.8	2.3
Glycerol	14.5	28.4
Honey	11.9	5.8
Infant formula	11.9	5.8
Wheat flour	11.9	5.8

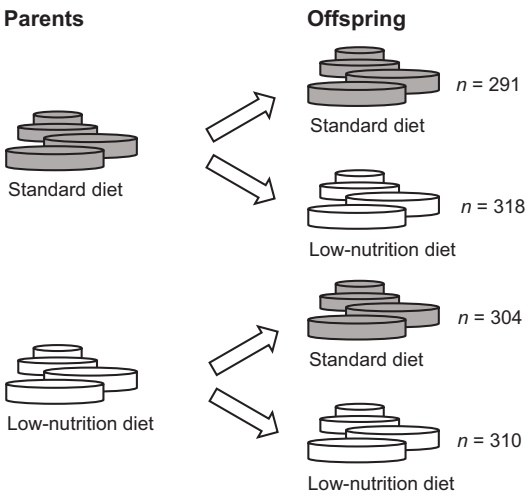

Fig. 1. Study design. *Galleria mellonella* larvae of the 'parental generation' were reared on a standard and a low-nutrition diets. Crosses were made within each diet group to produce the 'offspring generation'. The offspring were divided into standard- and low-nutrition-diet groups at an early stage of the larval development. This resulted in four parental-diet/offspring-diet combinations. Egg-to-adult development time, adult dry body mass and strength of adult encapsulation response were assessed from individuals of the offspring generation.

many). Due to the hydrophilic nature of cellulose, to achieve similar consistency of both diets, more glycerol and water had to be added to the low-nutrition diet relative to the standard diet. Cellulose acts as a non-nutritive bulking agent as most insects cannot digest it (Martin 1983, 1991). Cellulose may significantly alter the physical texture and water retentivity of a diet, which may cause mortality in *G. mellonella* larvae, unless the water content of the diet is increased (Dadd 1966). In our preliminary experiment, mortality of *G. mellonella* larvae did not differ between the standard and low-nutrition diets (results not shown). However, the used cellulose concentration was found in our previous study to have a negative effect on development rate and body size in *G. mellonella* (Kangassalo *et al.* 2018).

Study design

Parental generation

The 'parental generation' for the study was produced by allowing 40 males and 40 females of *G. mellonella* to interact in pairs for 24 hours, after which the eggs (laid in folds of filter paper) were collected (Fig. 1). The eggs were distributed evenly among standard size Petri plates containing standard (10 plates) or low-nutrition (10 plates) diets. Larvae were provided with their respective diets *ad libitum* throughout their larval period. To control for density, 35 two-week-old larvae were randomly selected from each plate and transferred to a new standard size Petri plate, while the rest of the larvae were euthanised. Later in their development, the larvae were moved to larger glass jars (400 ml) in which they remained until pupation. Pupae were collected and placed individually into vials (48 ml) with a small piece of kitchen paper providing gripping surface and cover.

Offspring generation

The 'offspring generation' for the study was produced by randomly selecting 50 males and 50 females per diet treatment from the individuals of the parental generation. The males and females were allowed to interact for 24 hours. The eggs were collected and distributed evenly among standard size Petri plates, creating 10 plates per each diet treatment (20 plates in total).

During the first two weeks of their development, all larvae were provided with the standard diet *ad libitum*. At two weeks of age, from each plate, 35 larvae were randomly selected and transferred to a new standard size Petri plate containing the standard diet, and 35 larvae to a new plate containing the low-nutrition diet (40 plates in total; henceforth referred to as 'rearing vials'), and the rest of the larvae were euthanized. The larvae were provided with their respective diets *ad libitum* for the rest of the larval period. They were later transferred to more spacious glass jars (400 ml), in which they pupated. After pupation, the insects were transferred individually into vials (48 ml) containing a small piece of kitchen paper, and they were observed every 24 hours for emerged adults. Ten females and ten males per each rearing vial were randomly selected for the measurement of encapsulation response, which was conducted on the day following adult emer-

gence. All moths were euthanized in a –80 °C freezer on the day following adult emergence. Dry body mass was later determined from all individuals by drying the defrosted moths in an oven at 60 °C for 24 hours, after which they were weighed to the nearest 0.01 mg on electronic microbalance.

Additional remarks on the study design

Our study design did not allow for rigorous control of genetic differences between the individuals (the study design was chosen for logistic reasons over more specific breeding designs, such as full- or half-sib designs). To compensate for this, a large number of individuals (80–100 per generation) were used to produce the next generation at each step. In addition, great caution was taken in distributing the eggs equally between each diet treatment group as well as each plate, so that when possible, eggs from each female or each egg cluster would be represented in each plate. For these reasons, we find it unlikely that there were significant genetic differences between the groups of individuals in the various treatment groups. It should be emphasized at this point, that the 'rearing vial' does not represent a genetically related family, but rather a shared growth environment.

Encapsulation response

Encapsulation response is the main defence mechanism of insects against large pathogens such as nematodes, protozoan parasites and parasitoids, although this defence mechanism is also used against bacteria and fungi (Hoffmann 1995, Carton *et al.* 2008, Strand 2008). Through encapsulation response, the pathogen is isolated from the haemocoel by haemocytes and/or melanin, whereupon the intruder dies by suffocation or from toxic molecules formed during the melanogenesis (Gillespie *et al.* 1997, Carton *et al.* 2008, Strand 2008). Melanin synthesis is catalysed by the phenol oxidase (PO) enzyme, which also has other important functions in the insect body (González-Santoyo & Córdoba-Aguilar 2012). The substrate for melanin synthesis is the amino acid tyrosine (or its precursor phenylalanine), which insects must obtain directly from food (Brunet 1963, Gillespie *et al.* 1997). The strength of encapsulation response can be measured by inserting into the haemocoel a standardised, artificial object, such as a piece of monofilament, to which an insect's immune system reacts by attempting to encapsulate the object (Rantala & Roff 2007, Pölkki *et al.* 2012, Prokkola *et al.* 2013, Krams *et al.* 2015). The object darkens as it is encapsulated with haemocytes and melanin, therefore the strength of encapsulation response can be quantified by measuring the darkness of the implant after its removal from the haemocoel. The strength of encapsulation response to artificial objects has been found to correlate with resistance to real pathogens, highlighting the biological relevance of this method (Gorman *et al.* 1998, Rantala & Roff 2007).

To prepare the monofilament implants, 0.8 mm diameter nylon filament was rubbed against sandpaper to remove the smooth outermost layer of the filament. Knotted pieces of 2 ± 0.1 mm were then cut from the filament and stored in 70% ethanol. To insert and remove the implants, moths were anesthetised using carbon dioxide. A small puncture was made with a disinfected insect needle in the cuticle on the right side of the thorax, after which the implant was inserted through this puncture into the haemocoel. The insects were kept at 28 ± 1 °C for 60 minutes, after which the implants were removed and stored in a freezer at –80 °C. The moths were then euthanised and stored at –80 °C for later analyses. The monofilament implants were photographed from two angles with a digital camera (Delta-Pix Invenio 3S, Smørum, Denmark) attached to a stereomicroscope (Olympus SZ-CTV, Tokyo, Japan). The level of grey of the implants was measured using the ImageJ program (ImageJ 1.42; National Institutes of Health, Bethesda, MD, USA). An average of the grey values obtained from the two photographs of each implant was subtracted from the average grey value of a clear, unencapsulated implant. The resulting value, expressed in artificial units, indicated darkness of the encapsulated implant. This value was thus used to quantify the strength of encapsulation response, a higher value indicating stronger encapsulation response.

Statistical analyses

Normality and homoscedasticity of residuals were assessed visually from residual plots, and when heteroscedasticity was detected, the results were adjusted for unequal variances. Males (n = 632) and females (n = 591) were analysed separately, as *G. mellonella* shows sexual dimorphism in size, development time and immune function (Kecko *et al.* 2017, Kwadha *et al.* 2017, Kangassalo *et al.* 2018). Stepwise backward elimination method was used for all models to remove the least significant interaction terms one at a time until only the main effects and significant interaction terms remained. When significant results were obtained, post-hoc pairwise comparisons were conducted using Tukey's test.

The analyses were carried out using SAS ver. 9.4 (SAS Institute Inc., Cary, North Carolina, USA).

Dry body mass

Linear mixed model (SAS 'Proc Mixed') was used to analyse the effects of parental diet and offspring diet on adult dry body mass. The full model included dry body mass as a response variable, offspring diet and parental diet, as well as their interaction, as explanatory variables and rearing vial as a random variable. In females, heteroscedasticity was detected in body mass among the offspring diet groups, which was taken into account in the model (run with the command line 'REPEATED/group = Offspring diet' in linear mixed model code in SAS).

Egg-to-adult development time

Linear mixed model (SAS 'Proc Mixed') was used to evaluate the effects of parental diet and offspring diet on egg-to-adult development time. The full model included egg-to-adult development time as the response variable, offspring diet and parental diet, as well as their interaction, as explanatory variables and rearing vial as a random variable. Heteroscedasticity was detected in development time among the offspring diet groups, which was adjusted in the final model (implemented with 'REPEATED/group = Offspring diet' statement).

Next, we assessed the effect of larval diet on development time within the body mass range in which the body masses of moths reared on the low-nutrition and standard diet overlapped (females over 55 mg, males 35 mg or more). The purpose of the analysis was to determine the effect of larval diet on development time independent of the effect of body mass. In the model (linear mixed model; SAS 'Proc Mixed'), offspring diet was included as the explanatory variable, rearing vial as a random variable and development time as the response variable. Heteroscedasticity among the offspring diet groups was taken into account in the model (implemented with 'REPEATED/group = Offspring diet' statement).

Encapsulation response

Encapsulation response was analysed using a generalized linear mixed model (SAS 'Proc Glimmix'; identity link, normal distribution). Previous literature suggests that in insects, the strength of encapsulation response can be affected by body size (Rantala & Roff 2005). Hence, we included dry body mass as a covariate in the model for encapsulation response. However, due to the strong association between quality of larval diet and body size in this species (Krams *et al.* 2015, Kangassalo *et al.* 2018), the inclusion of body mass as such could have confounded the results and made it difficult to answer the research questions set forth. Thus, a dry body mass variable free from the effect of larval diet (henceforth referred to as 'resDBM') was created using the residuals from a linear mixed model predicting dry body mass based on larval diet. The full model included strength of encapsulation response (indicated as the darkness of an encapsulated implant) as a response variable, offspring diet, parental diet and resDBM, as well as their interactions, as explanatory variables and rearing vial as a random variable. In a subsequent analysis the sex difference in the strength of encapsulation response was assessed with generalized linear mixed model (SAS 'Proc Glimmix'; identity

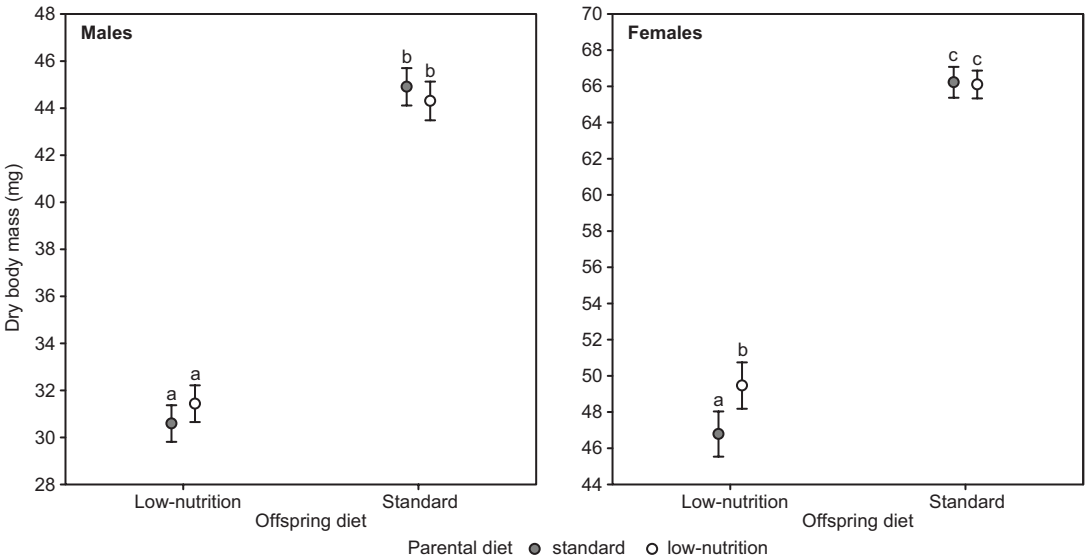

Fig. 2. Adult dry body mass (marginal mean ± 95%CI) of *G. mellonella* males and females with different parental and larval diets as predicted by linear mixed model (SAS 'Proc Mixed'). Different letters indicate a significant pairwise difference (Tukey's test, $p < 0.05$).

link, normal distribution). This model included strength of encapsulation response as a response variable, sex and offspring diet, as well as their interaction, as explanatory variables and rearing vial as a random variable.

Results

Moths reared on the low-nutrition diet were smaller than those reared on the standard diet (Table 2, Fig. 2 and Appendix). In females, parental diet also had a significant effect on body mass (Table 2). Furthermore, in both sexes, the interaction 'offspring diet × parental diet' predicted adult dry body mass (Table 2), suggesting that parental nutrition (type of diet) affected offspring body mass. The females fed the low-nutrition diet and originating from parents fed the low-nutrition diet were significantly heavier than those fed the low-nutrition diet and originating from parents fed the standard diet (Fig. 2). In males, body mass did not differ between groups with different parental diet but the same larval diet (Fig. 2).

Development time was longer in female moths reared on the low-nutrition diet as com-

Table 2. Adult dry body mass (linear mixed model; SAS 'Proc Mixed') of *G. mellonella* males and females; *p* values indicating significant results ($p < 0.05$) are in boldface.

	df1, df2	*F*	*p*
Males			
Offspring diet	1, 620	1401.20	< **0.0001**
Parental diet	1, 616	0.11	0.74
Offspring diet × parental diet	1, 618	3.97	**0.0469**
Females[a]			
Offspring diet	1, 496	1116.55	< **0.0001**
Parental diet	1, 496	5.62	**0.0182**
Offspring diet × parental diet	1, 496	6.72	**0.0098**

[a] To account for heteroscedasticity between the offspring diet groups, the model was run with a command line 'REPEATED/group = Offspring diet' in the linear mixed model code in SAS.

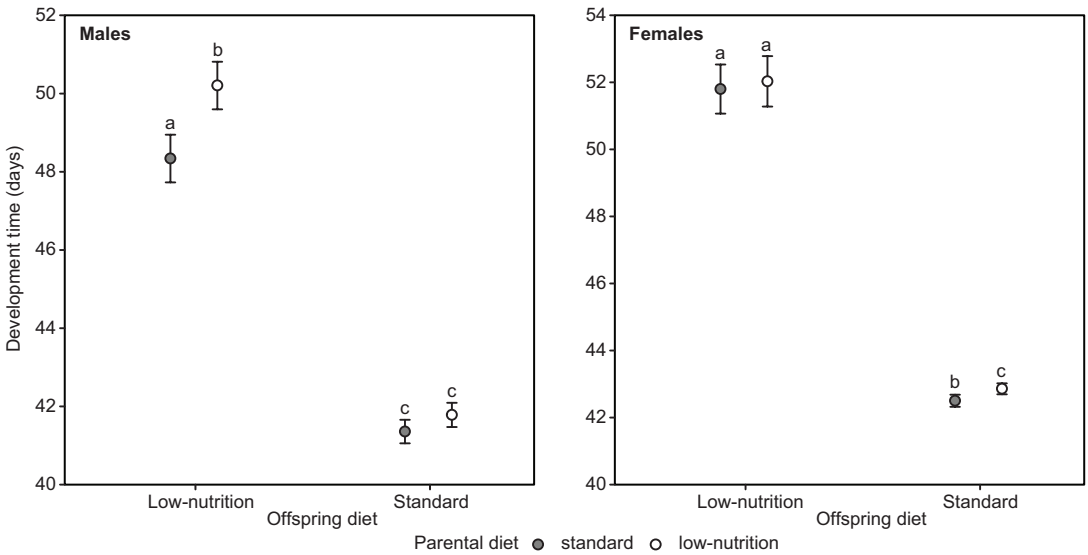

Fig. 3. Egg-to-adult development time (marginal mean ± 95%CI) of *G. mellonella* males and females with different parental and larval diets as predicted by linear mixed model (SAS 'Proc Mixed'). Different letters indicate a significant pairwise difference (Tukey's test, $p < 0.05$).

pared with that of female moths reared on the standard diet (Table 3 and Fig. 3). Parental diet also had a significant effect on development time; the females whose parents were fed the low-nutrition diet had longer development time than the females whose parents were fed the standard diet. However, among the offspring diet groups there was a significant difference between the parental diet groups only in females reared on the standard diet (Fig. 3). In females, the offspring diet and the parental diet did not have an interactive effect on egg-to-adult development time (Table 3). Development time was longer in males reared on the low-nutrition diet as compared with that in males reared on the standard diet (Table 3 and Fig. 3). Parental diet also had a significant effect on development time in males (Table 3). The interaction 'offspring diet × parental diet' significantly predicted egg-to-adult development time in males (Table 3),

Table 3. Egg-to-adult development time (linear mixed model; SAS 'Proc Mixed') in *G. mellonella* males and females. Stepwise backward elimination method was used to remove non-significant interaction terms ($p \geq 0.05$) from the final model. Step 1 in the last column is the non-significant interaction term before its removal, and 'final model' is the model after the removal of a non-significant interaction term; *p* values indicating significant results ($p < 0.05$) are in boldface.

	df1, df2	*F*	*p*	Model step
Males				
Offspring diet	1, 425	1167.55	< **0.0001**	Final model[a]
Parental diet	1, 423	25.96	< **0.0001**	
Offspring diet × parental diet	1, 423	10.24	**0.0015**	
Females				
Offspring diet × parental diet	1, 328	0.05	0.82	Step 1
Offspring diet	1, 329	1140.14	< **0.0001**	Final model[a]
Parental diet	1, 322	8.47	**0.0039**	

[a] To account for heteroscedasticity in development time between the offspring diet groups, the final models were run with a command line 'REPEATED/group = Offspring diet' in the linear mixed model code in SAS.

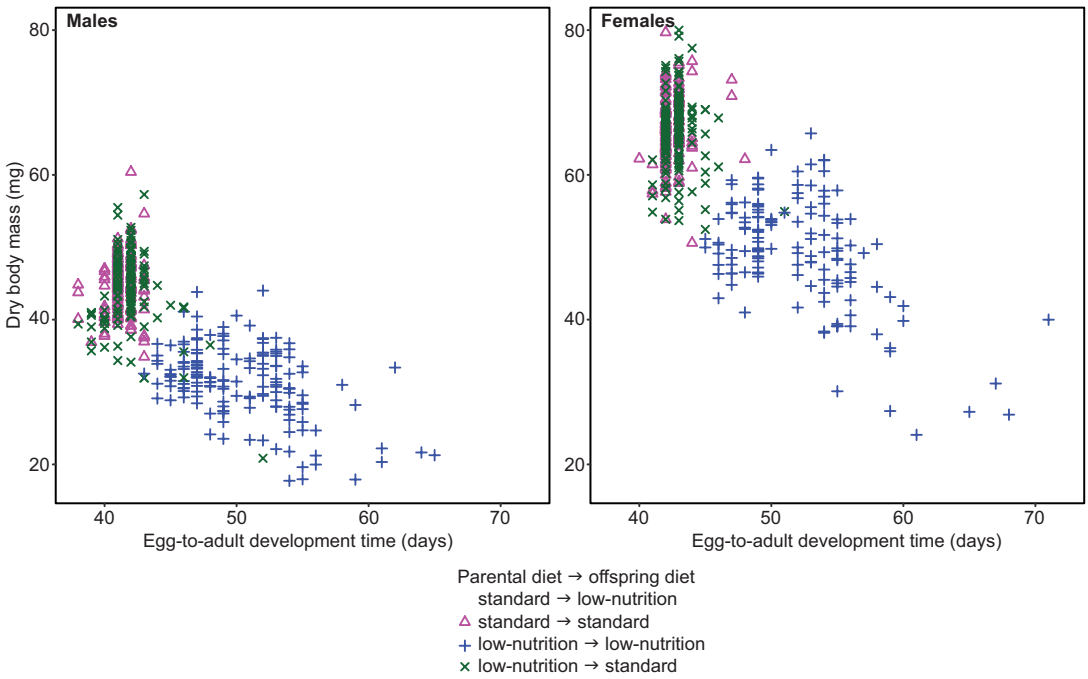

Fig. 4. Development time and body mass of *G. mellonella* females and males with different larval and parental diets.

indicating that the effect of parental diet on offspring development time was different under low-nutrition and standard conditions. The males fed the low-nutrition diet and originating from parents fed the low-nutrition diet had longer development time than the those fed the low-nutrition diet and originating from parents fed the standard diet (Fig. 3).

The relationship between development time and body mass (*see* Fig. 4) was, in general, more negative in moths reared on the low-nutrition diet than in moths reared on the standard diet. However, as the moths reared on the low-nutrition diet were significantly smaller, this difference is likely to be a consequence of a different association between body size and development time at different ends of the body size spectrum rather than a consequence of the larval diet *per se*.

To assess the effect of larval diet on development time within the body mass range at which there was an overlap between the larval diet groups (Fig. 4), we considered only females weighing over 55 mg and males weighing 35 mg or more. In both sexes, development time was longer in moths reared on the low-nutrition diet than in moths reared on the standard diet, indicating that low-nutrition conditions prolonged development time required to attain the same adult body mass (estimated marginal mean ± 95%CI; females: low-nutrition diet: 50.62 ± 0.75 days; standard diet: 42.67 ± 0.14 days; $F_{1,48.5} = 449.93$, $p < 0.0001$; males: low-nutrition diet: 48.78 ± 0.78 days; standard diet: 41.46 ± 0.30 days; $F_{1,66.1} = 369.55$, $p < 0.0001$).

Females reared on the low-nutrition diet exhibited stronger encapsulation responses as adults compared with females reared on the standard diet (estimated marginal mean ± 95%CI, low-nutrition diet: 73.86 ± 2.18; standard diet: 67.24 ± 2.20; Table 4 and Fig. 5). In females, parental diet and residual dry body mass (resDBM) did not affect the strength of encapsulation response, and none of the interactions between the explanatory variables predicted the strength of encapsulation response.

Males reared on the low-nutrition diet showed stronger encapsulation responses than males reared on the standard diet (Table 4). Parental diet or resDBM did not affect the strength of encapsulation response of the male moths. However, the effect of 'resDBM × offspring diet' was significant in males, indicating that the asso-

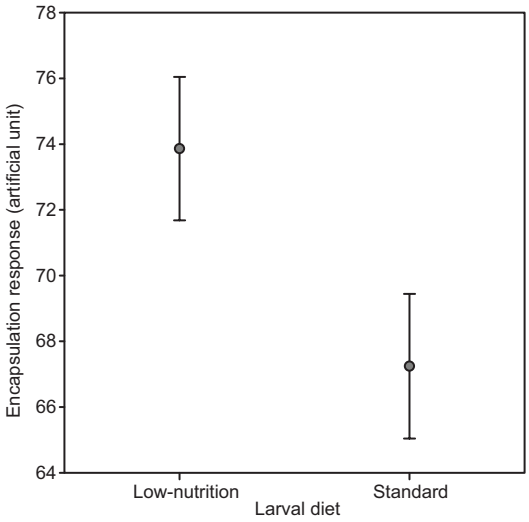

Fig. 5. Encapsulation response (marginal mean ± 95%CI) of *G. mellonella* females reared on a low-nutrition or a standard larval diet as predicted by generalized linear mixed model (SAS 'Proc Glimmix').

ciation between body mass and encapsulation response was affected by larval diet.

In males reared on the low-nutrition diet, larger individuals exhibited stronger encapsulation responses, while the trait association was reversed in males reared on the standard diet (*see* Fig. 6).

The males were found to have stronger encapsulation responses than females (estimated marginal mean ± 95%CI; males: 73.22 ± 1.56; females: 70.51 ± 1.58; $F_{1,776} = 5.76$, $p = 0.0166$; Fig. 7). Larval diet also had an effect on encapsulation response ($F_{1,776} = 53.49$, $p < 0.0001$) which was stronger in moths reared on the low-nutrition diet (estimated marginal mean ± 95%CI = 75.99 ± 1.57) compared with moths reared on the standard diet (estimated marginal mean ± 95%CI = 67.74 ± 1.57). The interaction 'sex × offspring diet' was found to have no effect on encapsulation response ($F_{1,775} = 1.97$, $p = 0.16$).

Discussion

In accordance with the previous findings in *G. mellonella* (Krams *et al.* 2015, Kangassalo *et al*. 2018), and with a generally observed trend in insects (Teder *et al*. 2014), we found that the moths reared on the low-nutrition diet had longer

Table 4. Encapsulation response in *G. mellonella* males and females (generalized linear mixed model; SAS 'Proc Glimmix'). Stepwise backward elimination method was used to remove non-significant interaction terms ($p \geq 0.05$) one at a time, until only the main effects and significant interaction terms ($p < 0.05$) remained. Steps 1, 2, 3 and 4 in the last column are least significant interactions before their removal, and 'final model' is the model after the removal of the non-significant interaction terms; *p* values indicating significant results ($p < 0.05$) are in boldface. resDBM (residual body mass) is the body mass variable free from the effect of larval diet, created using the residuals from a linear mixed model predicting dry body mass based on larval diet (*see* the text for more information).

	df1, df2	*F*	*p*	Model step
Males				
Offspring diet × parental diet × resDBM	1, 383	0.51	0.47	Step 1
Offspring diet × parental diet	1, 384	0.12	0.73	Step 2
Parental diet × resDBM	1, 385	0.78	0.38	Step 3
Offspring diet × resDBM	1, 386	5.03	**0.0255**	Final model
Offspring diet	1, 386	31.41	< **0.0001**	
Parental diet	1, 386	0.42	0.52	
resDBM	1, 386	0.07	0.79	
Females				
Offspring diet × parental diet × resDBM	1, 375	0.01	0.93	Step 1
Offspring diet × resDBM	1, 376	0.01	0.92	Step 2
Parental diet × resDBM	1, 377	0.14	0.71	Step 3
Offspring diet × parental diet	1, 378	1.04	0.31	Step 4
Offspring diet	1, 379	17.26	< **0.0001**	Final model
Parental diet	1, 379	0.05	0.82	
resDBM	1, 379	0.02	0.88	

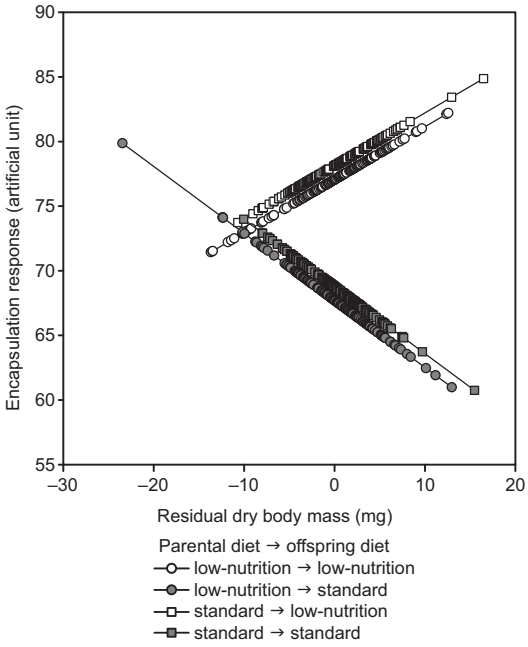

Fig. 6. Associations between residual dry body mass (body mass free from the effect of larval diet; see the main text for more information) and strength of encapsulation response in *G. mellonella* males with different parental and larval diets. The values are predictions from generalized linear mixed model (SAS 'Proc Glimmix').

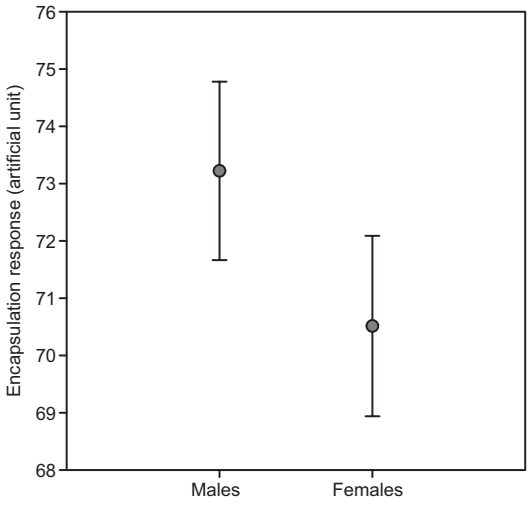

Fig. 7. Encapsulation response (estimated marginal means ± 95%CI) of *G. mellonella* males and females, as predicted by generalized linear mixed model (SAS 'Proc Glimmix').

development time and were smaller as adults compared with the moths reared on the standard diet. More specifically, adult dry body mass of the moths reared on the low-nutrition diet was about one-third smaller and their development time was about one-fifth longer compared with the moths reared on the standard diet. Furthermore, to attain the same body mass, the moths reared on the low-nutrition diet had to develop about one week longer compared with the moths reared on the standard diet, as evidenced by the analysis of moths within the size range at which the sizes of the individuals reared on different larval diets overlapped.

Adult encapsulation responses were about 10% stronger in the moths reared on the low-nutrition diet compared with those of the moths reared on the standard diet. The result is in accordance with several previous findings, showing that poor larval diet or starvation increase the strength of encapsulation response or resistance to an entomopathogenic fungus in *G. mellonella* (Kangassalo *et al.* 2015, Krams *et al.* 2015, Kecko *et al.* 2017, Kangassalo *et al.* 2018). Similarly, other stress factors such as thermal (Mowlds & Kavanagh 2008) or physical stress (Mowlds *et al.* 2008) and microbial priming (Bergin *et al.* 2006) are known to increase immune function in this species. On the other hand, Banville *et al.* (2012) found, that a period of food deprivation decreased several immune markers as well as resistance to fungal disease in *G. mellonella* larvae.

In their natural environment, *G. mellonella* larvae may be less protected from pathogens when food is limited, as components of the natural diet of this species, especially honey, possess antimicrobial properties (Viuda-Martos *et al.* 2008, Israili 2014). Low food levels may also be associated with a weaker bee colony with a smaller number of bees, which may allow for easier access of parasitic intruders to the nest. In addition, longer juvenile period typically associated with poor nutrition may increase the risk of infection before maturity is reached (Roff 1992, Stearns 1992). Therefore, it can be speculated that increased investment in immune function in response to poor nutrition may be favoured in the natural habitat of this species. Indeed, insects are often found to increase their immune defence in response to conditions of an elevated risk of disease (Rolff & Siva-Jothy 2003).

We found a negative association between size (residual dry body mass) and encapsulation response in *G. mellonella* males fed the standard diet. However, under low-nutrition conditions (smaller body sizes) this association was positive. This result is somewhat similar to an earlier finding by Krams *et al.* (2015) who observed a negative association between body mass and larval encapsulation rate in *G. mellonella* fed high-energy diet, while on an average- or low-energy diet there was no such association. Furthermore, a study by Kecko *et al.* (2017) suggests that high growth rates are associated with weaker encapsulation responses in *G. mellonella* larvae subjected to a period of fasting. Our findings as well as those of the previous studies raise a question as to whether high growth rate leads to a reduction in immune function in *G. mellonella*. Studies in other animals indicate that there may be physiological or genetic trade-offs between growth and immunity (Rantala & Roff 2005, Cotter *et al.* 2008, Vijendravarma *et al.* 2009, van der Most *et al.* 2011), and the association between body size and immunity may vary depending on which component of the immune system is measured (Rantala & Roff 2005).

The males of *Galleria mellonella* exhibited about 4% stronger encapsulation responses compared with the females. However, comparison of the present and previous studies on *G. mellonella* suggests that the sex difference in encapsulation rate in this species is not fixed but may depend on factors such as larval nutritional conditions (Kecko *et al.* 2017) and previous immune challenges (Kangassalo *et al*. 2018).

Our results indicate that the effects of the parental diet on development time and body mass of the offspring are sex-specific. The low-nutrition parental diet prolonged the development time of the male offspring by about 4% under low-nutrition conditions and of the female offspring by about 1% under standard conditions. On the other hand, the females with the low-nutrition parental diet were about 6% heavier under low-nutrition — but not standard — conditions compared with the females with the standard parental diet. Thus, in accordance with the hypothesis of anticipatory parental effects (Mousseau & Fox 1998, Marshall & Uller 2007, Uller *et al.* 2013), under low-nutrition conditions, the female offspring appeared to benefit from a low-nutrition diet of their parents. However, the same was not true for the males. It is unclear why the effect of parental nutrition differed between sexes; however, sex-specific parental effects on body size and development time were observed also in some previous studies (e.g. *Drosophila melanogaster* [Valtonen *et al.* 2012], *Orchesella cincta* [Zizzari *et al.* 2016]).

The mechanisms behind the observed parental effects on body mass and development time can only be speculated. One possible mechanism is diet-induced variation in egg size, as in insects, offspring hatching from larger eggs typically have shorter development time or larger adult size (Rossiter 1991, Fox 1994, Azevedo *et al.* 1997, Fox & Czesak 2000, Vijendravarma *et al.* 2010). In *G. mellonella*, egg size is found to correlate positively with female body size (Marston & Campbell 1973), which could explain the shorter development times we observed in the offspring of parents reared on the standard diet. Furthermore, studies on invertebrates suggest that progeny hatching from large eggs may have an advantage specifically in low-quality environments due to their higher capacity to withstand environmental stresses (Fox & Czesak 2000), which could account for why in the males the effect on development time was only observed under low-nutrition conditions. The finding that the parental effects on body mass and development time were mainly observed under low-nutrition conditions can also reflect the fact that variability in body size or growth rate is typically higher under low-quality environments (Teder *et al.* 2008, Tammaru & Teder 2012). Although further studies are needed to draw any definite conclusion in this regard, the results of our study suggest that the life-history characteristics of *G. mellonella* may, to some extent, be determined by the nutritional conditions experienced by parental populations. Our results add to the growing number of studies demonstrating an interaction between parental and offspring nutrition in determining offspring performance.

In our study, we found no effect of the parental diet on the strength of encapsulation response in the offspring. Earlier, Kangassalo *et al.* (2015) found that low-nutrition maternal diet increased the survival time of *G. mellonella*

larvae after infection by an entomopathogenic fungus *Beauveria bassiana*; however, parental diet did not affect the total mortality from the fungal infection (Kangassalo *et al.* 2015). Studies on the effect of parental diet on offspring immune function in other invertebrates yielded variable results (Rotem *et al.* 2003, Mitchell & Read 2005, Myers *et al.* 2011, Stjernman & Little 2011, Boots & Roberts 2012, Triggs & Knell 2012, Valtonen *et al.* 2012, Saastamoinen *et al.* 2013). Furthermore, the effect of parental diet on offspring immune function may be more evident at the early developmental stages, as was found in *Melitaea cinxia*, in which the effect of parental food deprivation on offspring immune function was observed in young larvae only (Saastamoinen *et al.* 2013).

Acknowledgements

We are thankful to all who helped us at the laboratory during this experiment. Special thanks to Ivan M. Dubovskiy for providing us the stock population of *Galleria mellonella* and for advice on the stock maintenance. We are also grateful to Toomas Tammaru and an anonymous reviewer for their valuable comments and suggestions. This study was funded by the Jenny and Antti Wihuri Foundation and the Turku University Foundation personal grants to KK and by the Academy of Finland grant to MJR.

References

Anaka, M., Lynn, A., McGinn, P. & Lloyd, V. K. 2009: Genomic imprinting in *Drosophila* has properties of both mammalian and insect imprinting. — *Development Genes and Evolution* 219: 59–66.

Armitage, S., Thompson, J., Rolff, J. & Siva-Jothy, M. T. 2003: Examining costs of induced and constitutive immune investment in *Tenebrio molitor*. — *Journal of Evolutionary Biology* 16: 1038–1044.

Azevedo, R., French, V. & Partridge, L. 1997: Life-history consequences of egg size in *Drosophila melanogaster*. — *American Naturalist* 150: 250–282.

Balanzs, A. 1958: Nutritional and nervous factors in the adaption of *Galleria mellonella* to artificial diet. — *Acta Biologica Hungarica* 9: 47–69.

Banville, N., Browne, N. & Kavanagh, K. 2012: Effect of nutrient deprivation on the susceptibility of *Galleria mellonella* larvae to infection. — *Virulence* 3: 497–503.

Beck, S. D. 1960: Growth and development of the greater wax moth, *Galleria mellonella* (L.). — *Transactions of the Wisconsin Academy of Sciences, Arts, and Letters* 49: 137–148.

Bergin, D., Murphy, L., Keenan, J., Clynes, M. & Kavanagh, K. 2006: Pre-exposure to yeast protects larvae of *Galleria mellonella* from a subsequent lethal infection by *Candida albicans* and is mediated by the increased expression of antimicrobial peptides. — *Microbes and Infection* 8: 2105–2112.

Bernardo, J. 1996: Maternal effects in animal ecology. — *American Zoologist* 36: 83–105.

Blanckenhorn, W. 1999: Different growth responses to temperature and resource limitation in three fly species with similar life histories. — *Evolutionary Ecology* 13: 395–409.

Bonduriansky, R. & Head, M. 2007: Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). — *Journal of Evolutionary Biology* 20: 2379–2388.

Bonduriansky, R. & Day, T. 2009: Nongenetic inheritance and its evolutionary implications. — *Annual Review of Ecology, Evolution and Systematics* 40: 103–125.

Boots, M. 2000: Density-independent resource limitation and the transmission of an insect pathogen. — *Oecologia* 124: 172–175.

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

Brunet, P. 1963: Tyrosine metabolism in insects. — *Annals of the New York Academy of Sciences* 100: 102.

Cahenzli, F. & Erhardt, A. 2013: Transgenerational acclimatization in an herbivore–host plant relationship. — *Proceedings of the Royal Society B* 280: 20122856.

Calvo, D. & Molina, J. 2005: Fecundity–body size relationship and other reproductive aspects of *Streblote panda* (Lepidoptera: Lasiocampidae). — *Annals of the Entomological Society of America* 98: 191–196.

Carton, Y., Poirie, M. & Nappi, A. J. 2008: Insect immune resistance to parasitoids. — *Insect Science* 15: 67–87.

Cotter, S. C., Myatt, J. P., Benskin, C. M. H. & Wilson, K. 2008: Selection for cuticular melanism reveals immune function and life-history trade-offs in *Spodoptera littoralis*. — *Journal of Evolutionary Biology* 21: 1744–1754.

Cotter, S. C., Simpson, S. J., Raubenheimer, D. & Wilson, K. 2011: Macronutrient balance mediates trade-offs between immune function and life history traits. — *Functional Ecology* 25: 186–198.

Dadd, R. H. 1966: Beeswax in the nutrition of the wax moth, *Galleria mellonella* (L.). — *Journal of Insect Physiology* 12: 1479–1492.

Fox, C. W. 1994: The influence of egg size on offspring performance in the seed beetle, *Callosobruchus maculatus*. — *Oikos* 71: 321–325.

Fox, C. & Czesak, M. 2000: Evolutionary ecology of progeny size in arthropods. — *Annual Review of Entomology* 45: 341–369.

Frago, E. & Bauce, E. 2014: Life-history consequences of chronic nutritional stress in an outbreaking insect defoliator. — *PLoS ONE* 9(2): e88039, https://doi.org/10.1371/journal.pone.0088039.

Franzke, A. & Reinhold, K. 2013: Transgenerational effects of diet environment on life-history and acoustic signals

of a grasshopper. — *Behavioral Ecology* 24: 734–739.

Friberg, U., Stewart, A. D. & Rice, W. R. 2012: X- and Y-chromosome linked paternal effects on a life-history trait. — *Biology Letters* 8: 71–73.

Futuyma, D. J., Herrmann, C., Milstein, S. & Keese, M. C. 1993: Apparent transgenerational effects of host plant in the leaf beetle *Ophraella notulata* (Coleoptera, Chrysomelidae). — *Oecologia* 96: 365–372.

Gillespie, J., Kanost, M. & Trenczek, T. 1997: Biological mediators of insect immunity. — *Annual Review of Entomology* 42: 611–643.

Gliwicz, Z. & Guisande, C. 1992: Family planning in *Daphnia*: resistance to starvation in offspring born to mothers grown at different food levels. — *Oecologia* 91: 463–467.

Gluckman, P. D. & Hanson, M. A. 2008: Developmental and epigenetic pathways to obesity: an evolutionary-developmental perspective. — *International Journal of Obesity* 32: S62–S71.

González-Santoyo, I. & Córdoba-Aguilar, A. 2012: Phenoloxidase: a key component of the insect immune system. — *Entomologia Experimentalis et Applicata* 142: 1–16.

Gorman, M. J., Schwartz, A. M. & Paskewitz, S. M. 1998: The role of surface characteristics in eliciting humoral encapsulation of foreign bodies in *Plasmodium*-refractory and -susceptible strains of *Anopheles gambiae*. — *Journal of Insect Physiology* 44: 947–954.

Gwynne, D. T. 2008: Sexual conflict over nuptial gifts in insects. — *Annual Review of Entomology* 53: 83–101.

Hoang, A. 2001: Immune response to parasitism reduces resistance of *Drosophila melanogaster* to desiccation and starvation. — *Evolution* 55: 2353–2358.

Hoffmann, J. A. 1995: Innate immunity of insects. — *Current Opinion in Immunology* 7: 4–10.

Honek, A. 1993: Intraspecific variation in body size and fecundity in insects: a general relationship. — *Oikos* 66: 483–492.

Houston, A. I., Stephens, P. A., Boyd, I. L., Harding, K. C. & McNamara, J. M., 2007: Capital or income breeding? A theoretical model of female reproductive strategies. *Behavioral Ecology* 18: 241–250.

Israili, Z. H. 2014: Antimicrobial properties of honey. — *American Journal of Therapeutics* 21: 304–323.

Jablonka, E. & Raz, G. 2009: Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. — *Quarterly Review of Biology* 84: 131–176.

Kangassalo, K., Valtonen, T. M., Roff, D., Pölkki, M., Dubovskiy, I. M., Sorvari, J. & Rantala, M. J. 2015: Intra- and trans-generational effects of larval diet on susceptibility to an entomopathogenic fungus, *Beauveria bassiana*, in the greater wax moth, *Galleria mellonella*. — *Journal of Evolutionary Biology* 28: 1453–1464.

Kangassalo, K., Valtonen, T. M., Sorvari, J., Kecko, S., Pölkki, M., Krams, I., Krama, T. & Rantala, M. J. 2018: Independent and interactive effects of immune activation and larval diet on adult immune function, growth and development in the greater wax moth (*Galleria mellonella*). — *Journal of Evolutionary Biology* 31: 1485–1497.

Kecko, S., Mihailova, A., Kangassalo, K., Elferts, D., Krama, T., Krams, R., Luoto, S., Rantala, M. J. & Krams, I. A. 2017: Sex-specific compensatory growth in the larvae of the greater wax moth *Galleria mellonella*. — *Journal of Evolutionary Biology* 30: 1910–1918.

Kelly, C. D. & Tawes, B. R. 2013: Sex-specific effect of juvenile diet on adult disease resistance in a field cricket. — *PLoS ONE* 8(4): e61301, https://doi.org/10.1371/journal.pone.0061301.

Klemola, N., Klemola, T., Rantala, M. J. & Ruuhola, T. 2007: Natural host-plant quality affects immune defence of an insect herbivore. — *Entomologia Experimentalis et Applicata* 123: 167–176.

Krams, I., Kecko, S., Kangassalo, K., Moore, F. R., Jankevics, E., Inashkina, I., Krama, T., Lietuvietis, V., Meija, L. & Rantala, M. J. 2015: Effects of food quality on trade-offs among growth, immunity and survival in the greater wax moth *Galleria mellonella*. — *Insect Science* 22: 431–439.

Kwadha, C. A., Ong'amo, G. O., Ndegwa, P. N., Raina, S. K. & Fombong, A. T. 2017: The biology and control of the greater wax moth, *Galleria mellonella.* — *Insects* 8(2), 61, https://doi.org/10.3390/insects8020061.

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

Marston, N. & Campbell, B. 1973: Comparison of nine diets for rearing *Galleria mellonella*. — *Annals of the Entomological Society of America* 66: 132–136.

Martin, M. M. 1983: Cellulose digestion in insects. — *Comparative Biochemistry and Physiology A: Physiology* 75: 313–324.

Martin, M. M. 1991: The evolution of cellulose digestion in insects. — *Philosophical Transactions of the Royal Society of London Series B* 333: 281–288.

Mitchell, S. & Read, A. 2005: Poor maternal environment enhances offspring disease resistance in an invertebrate. — *Proceedings of the Royal Society B* 272: 2601–2607.

Moret, Y. & Schmid-Hempel, P. 2000: Survival for immunity: the price of immune system activation for bumblebee workers. — *Science* 290: 1166–1168.

Mousseau, T. A. & Dingle, H. 1991: Maternal effects in insect life histories. — *Annual Review of Entomology* 36: 511–534.

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

Mowlds, P. & Kavanagh, K. 2008: Effect of pre-incubation temperature on susceptibility of *Galleria mellonella* larvae to infection by *Candida albicans*. — *Mycopathologia* 165: 5–12.

Mowlds, P., Barron, A. & Kavanagh, K. 2008: Physical stress primes the immune response of *Galleria mellonella* larvae to infection by *Candida albicans*. — *Microbes and Infection* 10: 628–634.

Muturi, E. J., Kim, C., Alto, B. W., Berenbaum, M. R. & Schuler, M. A. 2011: Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. — *Tropical Medicine & International Health* 16: 955–964.

Myers, J. H., Cory, J. S., Ericsson, J. D. & Tseng, M. L.

2011: The effect of food limitation on immunity factors and disease resistance in the western tent caterpillar. — *Oecologia* 167: 647–655.

Parker, G. A. & Simmons, L. W. 1989: Nuptial feeding in insects: theoretical models of male and female interests. — *Ethology* 82: 3–26.

Ponton, F., Wilson, K., Cotter, S. C., Raubenheimer, D. & Simpson, S. J. 2011: Nutritional immunology: a multi-dimensional approach. — *PLoS Pathogens* 7(12): e1002223, https://doi.org/10.1371/journal.ppat.1002223.

Prokkola, J., Roff, D., Kärkkäinen, T., Krams, I. & Rantala, M.J. 2013: Genetic and phenotypic relationships between immune defense, melanism and life-history traits at different temperatures and sexes in *Tenebrio molitor*. — *Heredity* 111: 89–96.

Pölkki, M., Kangassalo, K. & Rantala, M. J. 2012: Transgenerational effects of heavy metal pollution on immune defense of the blow fly *Protophormia terraenovae*. — PLoS ONE 7(6): e38832, https://doi.org/10.1371/journal.pone.0038832.

Rantala, M. J. & Roff, D. A. 2005: An analysis of trade-offs in immune function, body size and development time in the Mediterranean field cricket, *Gryllus bimaculatus*. — *Functional Ecology* 19: 323–330.

Rantala, M. J. & Roff, D. A. 2007: Inbreeding and extreme outbreeding cause sex differences in immune defence and life history traits in *Epirrita autumnata*. — *Heredity* 98: 329–336.

Roff, D. A. 1992: *The evolution of life histories: theory and analysis*. — Routledge, Chapman and Hall, New York.

Rolff, J. & Siva-Jothy, M. T. 2003: Invertebrate ecological immunology. — *Science* 301: 472–475.

Rossiter, M. 1991: Maternal effects generate variation in life-history: consequences of egg weight plasticity in the gypsy moth. — *Functional Ecology* 5: 386–393.

Rotem, K., Agrawal, A. & Kott, L. 2003: Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? — *Ecological Entomology* 28: 211–218.

Saastamoinen, M., Hirai, N. & van Nouhuys, S. 2013: Direct and trans-generational responses to food deprivation during development in the Glanville fritillary butterfly. — *Oecologia* 171: 93–104.

Schwenke, R. A., Lazzaro, B. P. & Wolfner, M. F. 2016: Reproduction-immunity trade-offs in insects. — *Annual Review of Entomology* 61: 239–256.

Sheldon, B. & Verhulst, S. 1996: Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. — *Trends in Ecology & Evolution* 11: 317–321.

Simmons, L. W. & Parker, G. A. 1989: Nuptial feeding in insects: mating effort versus paternal investment. — *Ethology* 81: 332–343.

Stearns, S. C. 1992: *The evolution of life histories*. — Oxford University Press, Oxford.

Stjernman, M. & Little, T.J. 2011: Genetic variation for maternal effects on parasite susceptibility. — *Journal of Evolutionary Biology* 24: 2357–2363.

Strand, M. R. 2008: The insect cellular immune response. — *Insect Science* 15: 1–14.

Suwanchaichinda, C. & Paskewitz, S. 1998: Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize Sephadex beads. — *Journal of Medical Entomology* 35: 157–161.

Tammaru, T. 1998: Determination of adult size in a folivorous moth: constraints at instar level? — *Ecological Entomology* 23: 80–89.

Tammaru, T. & Haukioja, E. 1996: Capital breeders and income breeders among Lepidoptera: consequences to population dynamics. — *Oikos* 77: 561–564.

Tammaru, T. & Teder, T. 2012: Why is body size more variable in stressful conditions: an analysis of a potential proximate mechanism. — *Evolutionary Ecology* 26: 1421–1432.

Tammaru, T., Esperk, T. & Castellanos, I. 2002: No evidence for costs of being large in females of *Orgyia* spp. (Lepidoptera, Lymantriidae): larger is always better. — *Oecologia* 133: 430–438.

Teder, T., Tammaru, T. & Esperk, T. 2008: Dependence of phenotypic variance in body size on environmental quality. — *American Naturalist* 172: 223–232.

Teder, T., Vellau, H. & Tammaru, T. 2014: Age and size at maturity: a quantitative review of diet-induced reaction norms in insects. — *Evolution* 68: 3217–3228.

Triggs, A. M. & Knell, R. J. 2012: Parental diet has strong transgenerational effects on offspring immunity. — *Functional Ecology* 26: 1409–1417.

Valtonen, T. M., Kangassalo, K., Pölkki, M. & Rantala, M. J. 2012: Transgenerational effects of parental larval diet on offspring development time, adult body size and pathogen resistance in *Drosophila melanogaster*. — *PLoS ONE* 7(2): e31611. https://doi.org/10.1371/journal.pone.0031611.

Vahed, K. 1998: The function of nuptial feeding in insects: review of empirical studies. — *Biological Reviews* 73: 43–78.

van der Most, P. J., de Jong, B., Parmentier, H. K. & Verhulst, S. 2011: Trade-off between growth and immune function: a meta-analysis of selection experiments. — *Functional Ecology* 25: 74–80.

Vijendravarma, R. K., Kraaijeveld, A. R. & Godfray, H. C. J. 2009: Experimental evolution shows *Drosophila melanogaster* resistance to a microsporidian pathogen has fitness costs. — *Evolution* 63: 104–114.

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: 238–241.

Viuda-Martos, M., Ruiz-Navajas, Y., Fernandez-Lopez, J. & Perez-Alvarez, J. A. 2008: Functional properties of honey, propolis, and royal jelly. — *Journal of Food Science* 73: R117–R124.

Whitman, D. 2009: *Phenotypic plasticity of insects: mechanisms and consequences*. — CRC Press, Boca Raton.

Uller, T., Nakagawa, S. & English, S. 2013: Weak evidence for anticipatory parental effects in plants and animals. — *Journal of Evolutionary Biology* 26: 2161–2170.

Ye, Y. H., Chenoweth, S. F. & McGraw, E. A. 2009: Effective but costly, evolved mechanisms of defense against a virulent opportunistic pathogen in *Drosophila melanogaster*. — *PLoS Pathogens* 5(4): e1000385, https://

doi.org/10.1371/journal.ppat.1000385.

Youngson, N. A. & Whitelaw, E. 2008: Transgenerational epigenetic effects. — *Annual Review of Genomics and Human Genetics* 9: 233–257.

Zirbel, K. E. & Alto, B. W. 2018: Maternal and paternal nutrition in a mosquito influences offspring life histories but not infection with an arbovirus. — *Ecosphere* 9(10): e02469, https://doi.org/10.1002/ecs2.2469.

Zizzari, Z. V., van Straalen, N. M. & Ellers, J. 2016: Transgenerational effects of nutrition are different for sons and daughters. — *Journal of Evolutionary Biology* 29: 1317–1327.

Appendix. Parental and offspring diets, sex, dry body mass of adults, encapsulation response and development time. Shown are mean ± SD values with sample sizes in parentheses.

Parental diet	Offspring diet	Sex	Dry body mass (mg)	Encapsulation response	Development time (days)
Standard	Standard	Male	44.94 ± 3.87 (156)	68.95 ± 16.32 (98)	41.35 ± 1.26 (159)
Standard	Low-nutrition	Male	30.56 ± 4.27 (165)	78.56 ± 15.46 (97)	48.35 ± 3.43 (165)
Low-nutrition	Standard	Male	44.31 ± 5.04 (143)	67.69 ± 19.22 (99)	41.79 ± 1.63 (143)
Low-nutrition	Low-nutrition	Male	31.44 ± 4.94 (163)	77.72 ± 13.48 (100)	50.22 ± 4.18 (165)
Standard	Standard	Female	66.22 ± 4.79 (130)	68.08 ± 15.83 (96)	42.50 ± 1.00 (132)
Standard	Low-nutrition	Female	46.78 ± 7.97 (151)	73.24 ± 13.84 (99)	51.80 ± 4.63 (153)
Low-nutrition	Standard	Female	66.10 ± 5.04 (160)	66.30 ± 15.46 (96)	42.86 ± 1.09 (161)
Low-nutrition	Low-nutrition	Female	49.46 ± 7.60 (143)	74.48 ± 15.84 (94)	52.03 ± 4.58 (145)