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Parental diet has strong transgenerational effects on offspring immunity

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Summary

1. Transgenerational effects of immune stimulation on offspring immunity are widely reported from insects, but we know very little of how other aspects of the parental environment affect offspring immune reactivity.

2. We reared male and female moths *Plodia interpunctella* on either good- or poor-quality food and then also raised their offspring on one of the two diet qualities. We found strong transgenerational effects on immunity: in general, if only one parent received the poor diet, reductions in immunity were observed whether that parent was the mother or the father, and the lowest offspring immune reactivity was observed when both parents received the poor diet.

3. The mechanism behind these effects is not known, but they could be caused either by imprinting, whereby the parent gives the offspring a cue such as an epigenetic mark that changes the offspring phenotype, or by the mother allocating fewer resources to their offspring when the diet was poor. Two lines of evidence point towards imprinting: the strong paternal effects and the observation that the size of these effects was either unchanged or increased when the offspring were fed a good-quality diet themselves.

4. Weight was also reduced when either parent was fed a poor diet, except when both parents had a poor diet and the offspring were raised on good food, contrasting with the increased rates of obesity seen in vertebrates when either parent is raised on a restricted diet.

5. Overall, the effects of parental diet on offspring weight and immune reactivity are substantial and in some cases are equivalent to that of the diet that the offspring itself consumes.

Key-words: diet, epigenetics, haemocyte, immunity, phenoloxidase, transgenerational effects, weight

Introduction

Transgenerational effects occur when parental experience determines offspring phenotype. Such effects have only recently become the focus of much research, but it is rapidly becoming clear that they are important and widespread, with serious implications for both animal and human health and welfare. In vertebrates, for example, if either the mother or the father eats a poor diet, the offspring are more likely to become obese and develop diabetes (Curley, Mashoodh & Champagne 2011; Ferguson-Smith & Patti 2011). Transgenerational effects are also potentially important in ecological systems. At the individual level, they will have obvious effects on the relationship between environment and phenotype, and at the population level, they can introduce delayed effects, including, potentially, delayed density-dependent effects, leading to wide-ranging and fundamental changes in population dynamics (Benton *et al.* 2001; Plaistow, Lapsley & Benton 2006; Benton, St Clair & Plaistow 2008).

In invertebrates, most research on transgenerational effects has looked at exposure to pathogens or other immune system elicitors and the phenomenon of 'transgenerational immune priming', whereby offspring immune defences are stronger when the parent has been exposed to a parasite or pathogen has now been described from a number of invertebrate species (Sadd *et al.* 2005; Moret 2006; Sadd & Schmid-Hempel 2007, 2009; Roth *et al.* 2010; Tidbury, Pedersen & Boots 2011; Zanchi *et al.* 2011), although not in all cases (Voordouw, Lambrechts & Koella 2008; Linder & Promislow 2009), as well as several vertebrates (Hasselquist & Nilsson 2008; Sandell, Tobler & Hasselquist 2009; Tobler *et al.* 2009; Walke *et al.* 2011). In most instances, these transgenerational effects have been

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investigated as a maternal effect, and in vertebrates, there seems to be little in the way of paternal effects on offspring immunity (Reid *et al.* 2006). There is, however, some evidence of paternal exposure to immune stimulation leading to increased offspring immunity in insects (Roth *et al.* 2010).

Investment in immunity is determined by many environmental factors other than the simple presence of parasites and pathogens, of course, and food quality in particular is known to have powerful effects on immune reactivity (Siva-Jothy & Thompson 2002; Srygley *et al.* 2009; Myers *et al.* 2011; Triggs & Knell 2012). Poor food will lead to fewer resources being available, and immune defences are costly, so an animal that invests in its immune system will have to trade that investment off against other life-history traits; hence, poor food is generally associated with reduced immune reactivity (Siva-Jothy & Thompson 2002; Myers *et al.* 2011; Triggs & Knell 2012).

Parental diet is known to have transgenerational effects on life-history traits such as egg size, age at maturity and fecundity in invertebrates (Plaistow, Lapsley & Benton 2006; Hafer *et al.* 2011), but no previous research of which we are aware has directly tested the effects of parental diet on offspring immunity. Two studies of parental diet effects on offspring resistance to pathogens have returned mixed results that are not easy to relate to immune reactivity. The offspring of *Daphnia magna* mothers raised on a poor diet and in a crowded environment are less susceptible to infection by the bacterium *Pasteuria ramose* (Mitchell & Read 2005; Stjernman & Little 2011), but this effect may be simply because the offspring of the mothers reared in poor conditions were smaller and therefore less likely to acquire infection by filter-feeding. By contrast, the offspring of *Malacosoma pluviale californicum* (the Western tent caterpillar) raised on a restricted diet showed no difference in resistance to a nuclear polyhedrosis virus (Myers *et al.* 2011).

We investigated the importance of transgenerational effects of diet on immunity by raising a parental generation of Indian meal moths *Plodia interpunctella* on either a normal laboratory diet or a restricted diet with reduced amounts of protein, lipid and micronutrients. Animals from the parental generation were paired with members of the opposite sex in all four possible combinations of parental treatment (both parents raised on good food, one parent raised on good food and the other on poor food and both parents raised on poor food), allowing us to measure both maternal and paternal effects plus any interaction.

In order to investigate whether the effect of parental experience interacts with the environment experienced by the offspring, the eggs from each parental pair were themselves allocated to either the normal diet or the poor diet and larvae raised individually. Once these larvae reached the late 5th instar, shortly before metamorphosis, two components of the immune system, haemocyte count and phenoloxidase (PO) activity, were assayed. Haemocytes are the effector cells of the insect immune system, and their density has been found to correlate with the ability to encapsulate parasitoid eggs (Eslin & Prevost 1998; Kraaijeveld & Godfray 2001; Kacsoh & Schlenke 2012) and artificial implants (Wilson *et al.* 2003). PO is an important enzyme in the melanization cascade, which is itself an important component of encapsulation, and PO activity is correlated with resistance to pathogens in a variety of species (Hagen, Grunewald & Ham 1994; Nigam *et al.* 1997; Reeson *et al.* 1998; Wilson *et al.* 2001; Cotter *et al.* 2004), although we should note here that Saejeng *et al.* (2010) found that PO activity did not predict resistance to a baculovirus in *P. interpunctella*. PO is found in insect haemolymph as both the active PO form and an inactive dimer (prophenoloxidase or proPO). We chose to assay just the active PO present in the haemolymph for two reasons: first, the assay is simpler, an important consideration when dealing with very large numbers of samples, and secondly, the majority of studies that have found relationships between PO activity and pathogen resistance have assayed active PO only (Nigam *et al.* 1997; Reeson *et al.* 1998; Wilson *et al.* 2001; Cotter *et al.* 2004).

Methods

STUDY ANIMAL

Plodia interpunctella is a small (*c.* 1 cm length) pyralid moth that is a global pest of stored food commodities. A stock culture of *P. interpunctella* was started using moths from 3 stock cultures from other laboratories in the UK in 2006. The resulting population was reared under a 12L:12D light regime at 27 °C and fed on 10 : 1 : 1 ratio of wheat bran/brewers yeast/glycerol for 14 generations. Each generation eggs were collected from at least 200 adults, and these eggs were allowed to grow to adulthood with unlimited food. There has been no disease observed in the colony to date.

THE PARENTAL GENERATION

For each block, *c.* 200 adult moths from the stock culture were placed in one container and allowed to mate and oviposit and their eggs collected. The eggs to be used in the experiment were then allocated to one of two food treatments, normal (10 : 1 : 1 wheat bran/brewers yeast/glycerol) or poor food (20 : 1 : 1 wheat bran/brewers yeast/glycerol), and placed in individual 30-ml plastic pots with *ad libitum* food of the appropriate quality. Larvae were raised until the end of their 5th instar when they exhibit 'wandering' behaviour, looking for suitable pupation sites. At this point, they were sexed (on the basis of the absence or presence of visible testes) and weighed, and a 3-μL sample of haemolymph was extracted by piercing each larva between the final thoracic legs and the first prolegs with a fine needle and allowing a small amount of haemolymph to pool onto Parafilm. The larvae were then transferred back into their original pot and allowed to pupate and emerge as adults.

Following eclosion, females were randomly allocated to a male raised on either normal or low-quality food. Each pair was placed in a clean 55-mm Petri dish and left to mate and lay eggs for 48 h. These eggs were then allocated to one of the two diet treatments and placed individually into a plastic pot with an *ad libitum* diet of the appropriate food quality. Larvae were reared to the late 5th instar as before, and each larva was weighed and had a sample of

haemolymph extracted. A total of 2722 larvae from 122 mated pairs of *P. interpunctella* were successfully reared, in seven separate blocks.

HAEMOCYTE COUNT

EDTA anticoagulant in phosphate-buffered saline was prepared by dissolving 10 mM EDTA and 10 mM citric acid in 80 mL PBS, 1 M hydrochloric acid was added a drop at a time until the pH reached 7·4, and the solution was made up to 100 mL with PBS. One microlitre of haemolymph was transferred to a 0·2-mL PCR tube using a capillary tube and thoroughly mixed with 3 μL of EDTA anticoagulant in PBS. Four microlitres of glycerol was added to each haemolymph sample, allowing them to be frozen without disrupting the haemocytes (Cotter *et al*. 2004). Once the samples had been defrosted, 8 μL of this mixture was then pipetted onto a haemocytometer. All the squares on the centre of the haemocytometer were counted and summed to give an estimate of the haemocyte density for each individual.

PHENOLOXIDASE ACTIVITY ASSAY

One microlitres of haemolymph was transferred to a 0·2-mL PCR tube using a capillary tube, 10 μL phosphate-buffered saline (PBS), pH 6·8, was added, and the samples were frozen for *c*. 8 weeks. Defrosted samples were vortexed and transferred to a 96-well U bottom microtitre plate (Sterilin) kept on ice. 100 μL of 5 mM dopamine was added to each sample, and the absorbance was measured at 492 nm at 28 °C in a temperature-controlled plate reader (ASCENT SOFTWARE Version 2.6; Thermo Labsystems Multiskan Ascent, Thermo Scientific, Basingstoke, UK). Absorbance was measured at the start of the reaction and again after 20 min, and the change in PO activity over time was used because preliminary experiments indicated that this provided a good estimate of activity during the linear phase of the reaction (Pomfret & Knell 2006).

STATISTICAL ANALYSIS

Because there were multiple offspring from each pair of parents, offspring immunity was analysed using mixed-effects models. These were initially fitted with 'family' as a random factor, with random intercepts and random slopes within families for the relationships between offspring weight and the response variables. The goodness-of-fit of these models was compared with models with only random intercepts and with models with no random effects using the methods outlined by Zuur *et al*. (2009), and the random effects were only retained where they led to a significant increase in explanatory power: as examples, for the PO activity model, both random intercepts and slopes were retained, hence the inclusion of larval weight in the 'random effects' section of the results, whereas for haemocyte count, only the random intercepts were retained. Block was included as a fixed effect because of the low number of factor levels (7), and offspring weight was included as a covariate.

Maternal treatment, paternal treatment, offspring sex and offspring treatment were included in the initial models as explanatory factors plus their two-way interactions. When fitting complex models, higher-order interactions are often difficult to interpret and frequently only lead to small increases in explanatory power, so it is considered good practice to avoid fitting these unless there is reason to believe that they are important in the interpretation of the data (Zuur *et al*. 2009): this was the case for the larval weight data where three-way interactions were also included in the initial model following preliminary analysis. The models were initially fitted using maximum likelihood to allow likelihood ratio tests to compare model fits (Crawley 2002; Zuur *et al*. 2009) and minimal adequate models produced by sequential removal of nonsignificant terms, after which the minimal model was re-fitted using REML. The haemocyte count data were square-root-transformed to correct a moderate amount of heteroscedasticity and positive skew seen in the residuals, and because offspring weight was not included in the final model for haemocyte count as a fixed effect, it was removed from the random term as well.

Results

The poor diet had substantial effects on the weight and the immune reactivity of the parental generation (Fig. 1). Females' weight was reduced by 30% and the weight of males by 23% (treatment by sex interaction, $F_{1,276}$ = 12·35, P = 0·0005), and both measures of immune reactivity were reduced by about half, with haemocyte count reduced by 47% (untransformed values) and PO activity by 52% in animals raised on the poor diet (diet main effects: haemocyte count $F_{1,276}$ = 119, P < 0·0001, PO activity $F_{1,276}$ = 590, P < 0·0001). The sexes responded differently to diet quality: in both cases, males had lower immune reactivity than females when food was good but higher values when food was poor, although this interaction effect only reached statistical significance in the case of PO activity (haemocyte count, sex by food quality interaction, $F_{1,276}$ = 3·71, P = 0·0551, PO activity, sex by food quality interaction, $F_{1,276}$ = 4·14, P = 0·0428).

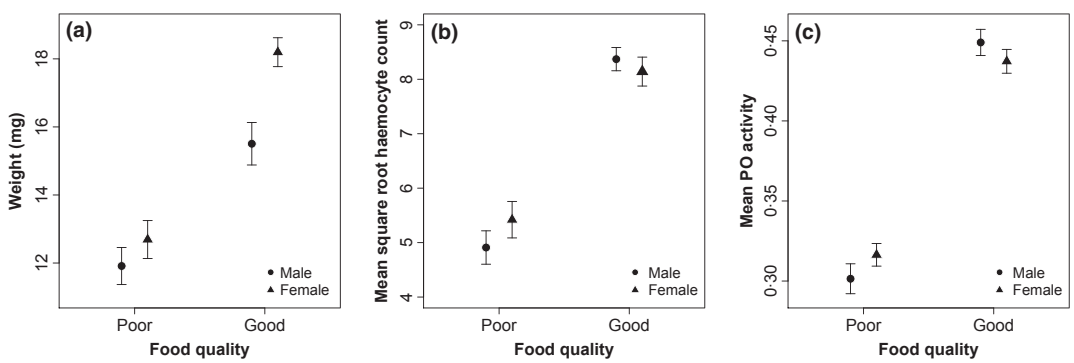

Fig. 1. Effects of diet on weight (a), haemocyte count (b) and PO activity (c) in the parental generation of *Plodia interpunctella*. Error bars indicate 95% confidence intervals.

Parental diet led to substantial transgenerational effects on offspring weight and on both measures of immune investment. The most dramatic effects on immunity were seen in the PO data (Fig. 2, Table 1), where a poor parental diet reduced offspring PO activity by *c.* 11% on average if the father received the poor diet, by around 15% if the mother received it and by around 26% if both parents received the poor diet. The highly significant paternal diet by offspring diet and maternal diet by offspring diet interactions indicate that when the offspring were fed a good diet, poor parental diet led to a greater reduction in PO activity than when offspring were fed a poor diet (i.e. the effect was even stronger with a good offspring diet). When offspring were fed a good diet, a poor paternal diet caused

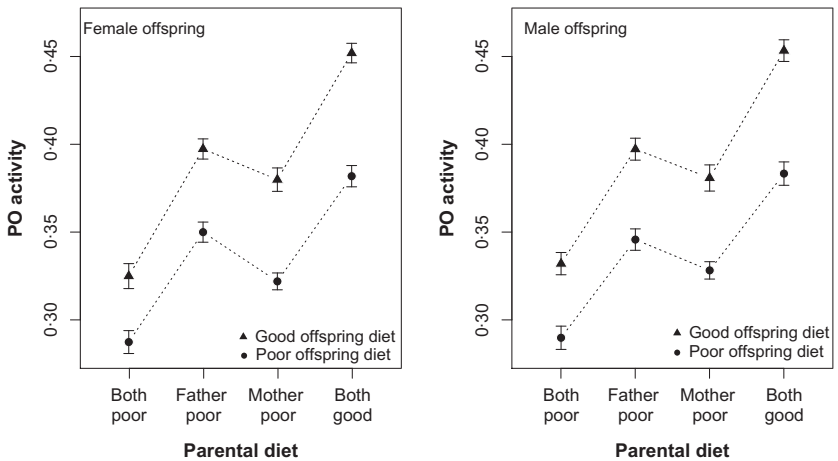

Fig. 2. Effects of parental and offspring diet on phenoloxidase activity in 5th-instar *Plodia interpunctella* larvae. Triangles indicate means of offspring that received a good diet themselves, and circles are those that received a poor diet. Error bars indicate 95% confidence limits. Female offspring are shown on the left and males on the right, and the parental diet is indicated on the *x*-axis.

Table 1. Final model details for PO activity

Random effects					SD
Intercepts					0·076313517
Larval weight					0·004716806
Residual					0·016632317
Fixed effects					
Variable	Estimated coefficient (treatment contrasts)	SE	d.f.	Likelihood ratio from deletion test using models fitted with ML rather than with REML.	*P*
Intercept	0·258	0·00867	2513		
Larval weight	0·00245	0·000491	2513		
Larval sex (male)	0·00495	0·000975	2513	25·5	<0·0001
Maternal treatment (good diet)	0·0528	0·00641	137		
Paternal treatment (good diet)	0·0382	0·00626	137		
Offspring treatment (good diet)	0·0343	0·00413	2513		
Offspring weight: offspring treatment (good diet)	−0·000830	0·000272	2513	9·28	0·0023
Maternal treatment (good diet): offspring treatment (good diet)	0·0202	0·00255	2513	62·2	<0·0001
Paternal treatment (good diet): offspring treatment (good diet)	0·0191	0·00228	2513	68·8	<0·0001

Coefficient estimates, etc., are presented for a final model fitted using a REML algorithm. Significance tests were calculated using a maximum-likelihood fit as explained in the methods which are only presented for terms that are not included in higher-order interaction terms. (Crawley 2002; Zuur *et al.* 2009). Coefficient estimates are presented as treatment contrasts: see Grafen & Hails (2002) for more information on interpreting these results.

an estimated reduction in PO activity of 13%, a poor maternal diet a change of 17% and PO activity being reduced by 31% when both parents received the poor diet.

Larval weight also explained some of the variance in PO activity, but less so in those animals fed a poor diet as indicated by the significant offspring weight by offspring diet interaction (slope relating PO activity to weight = 0·0024 when offspring diet was poor but 0·0016 when it was good). Finally, there was a small but highly significant effect of sex, with male larvae having PO activity that was 1–1·5% greater than females.

Offspring diet had a very pronounced effect on haemocyte count (Fig. 3, Table 2), with those larvae fed a poor diet having haemocyte counts reduced from an overall average of 63 (corresponding to $5{\cdot}04 \times 10^6$ haemocytes per mL^{-1}) to one of 19 ($1{\cdot}52 \times 10^6.mL^{-1}$). Within the offspring diet treatments, however, there were clear transgenerational effects of parental diet. When the offspring were fed a poor diet, transgenerational effects were only evident when both parents received the same diet, as indicated by the significant paternal treatment by maternal treatment interaction in the final model. When the offspring were fed a good diet, however, a poor maternal diet led to a reduction in offspring haemocyte count, shown by the significant offspring diet × maternal diet interaction. There was also a significant main effect of offspring sex in the opposite direction to that found for the PO data, with male larvae having mean haemocyte counts that tended to be between 2 and 4 haemocytes per count less than females depending on the diet the offspring received.

Offspring weight also showed a strong but complex response to parental environment (Fig. 4, Table 3). In males, both paternal and maternal diet negatively affected offspring weight, but in females, the effect of maternal diet was more pronounced, and when females were themselves raised on a poor diet, there was a little effect of paternal diet. Most notably, and in contrast with the effects of parental environment on immune indicators, when the offspring were fed a good diet, those larvae with a single parent raised on a poor diet had a lower weight than those with both parents raised on a poor diet, the latter weighing only slightly less than those with neither parent raised on poor food.

Discussion

Our results demonstrate powerful effects of parental environment on offspring immune reactivity, even when the important factor in the parental environment is not one that stimulates the immune system directly. Both components of the *P. interpunctella* immune response that we measured responded to parental environment, although they did not change in exactly similar ways: offspring diet had a stronger effect on haemocyte count than parental diet, whereas the reverse was true for PO activity.

Previous work on transgenerational effects on immunity has concentrated on the effect of stimulating the parental (usually the maternal) immune system on offspring immunity, and it is now widely reported that this leads to increased expression of immune system components in the offspring (Sadd *et al.* 2005; Moret 2006; Reid *et al.* 2006; Sadd & Schmid-Hempel 2007, 2009; Hasselquist & Nilsson 2008; Sandell, Tobler & Hasselquist 2009; Tobler *et al.* 2009; Roth *et al.* 2010; Tidbury, Pedersen & Boots 2011; Zanchi *et al.* 2011), although not in the mosquito *Aedes aegypti* (Voordouw, Lambrechts & Koella 2008) or *Drosophila melanogaster* (Linder & Promislow 2009). This is interpreted as an adaptive mechanism by which a parent that is in an environment where the risk of contracting an infection is high can produce offspring that have a better chance of resisting any infection that they encounter (Sadd *et al.* 2005; Moret 2006), although it should be noted that definitive tests of this prophylaxis hypothesis have not yet been carried out. Here, we find that the offspring of parents given a restricted diet that leads to reduced immune investment reduce their own investment in immunity, and

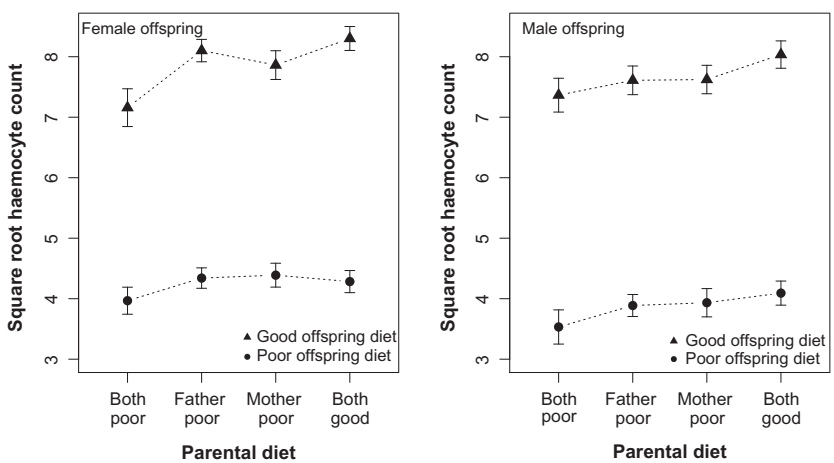

Fig. 3. Effects of parental and offspring diet on mean haemocyte count in 5th-instar *Plodia interpunctella* larvae. Triangles indicate means of offspring that received a good diet themselves, and circles are those that received a poor diet. Error bars indicate 95% confidence limits. Female offspring are shown on the left and males on the right, and the parental diet is indicated on the *x*-axis.

Table 3. : final model details for weight. Significance tests and contrasts are as for Table 1

Random effects					SD
Intercepts					1·832
Residual					2·11
Fixed effects					
Variable	Estimated coefficient (treatment contrasts)	SE	d.f.	Likelihood ratio from deletion test using models fitted with ML rather than with REML.	*P*
Intercept	10·9	0·582	2533		
Blocks 2–7	−1·01, −0·307, −0·955, −1·98, −1·09, −1·89	0·626, 0·630, 0·585, 0·577, 0·620, 0·675	2533, 131, 131, 131, 131, 131	17·8	0·0068
Offspring sex (Male)	−3·84	0·241	2533		
Maternal treatment (good diet)	5·32	0·521	131		
Paternal treatment (good diet)	1·42	0·537	131		
Offspring treatment (good diet)	13·1	0·220	2533		
Offspring sex (male): offspring treatment (good diet)	−1·38	0·260	2533		
Offspring sex (male): paternal treatment (good diet)	3·69	0·266	2533		
Offspring sex (male): maternal treatment (good diet)	−0·197	0·305	2533		
Paternal treatment (good diet): maternal treatment (good diet)	−1·10	0·735	131		
Maternal treatment (good diet): offspring treatment (good diet)	−7·94	0·286	2533		
Paternal treatment (good diet): offspring treatment (good diet)	−8·20	0·256	2533		
Offspring sex (male): offspring treatment (good diet): maternal treatment (good diet)	−0·858	0·337	2533	6·48	0·011
Offspring sex (male): paternal treatment (good diet):maternal treatment (good diet)	−2·80	0·341	2533	66·0	<0·0001
Offspring treatment (good diet): paternal treatment (good diet): maternal treatment (good diet)	12·7	0·333	2533	1152	<0·0001

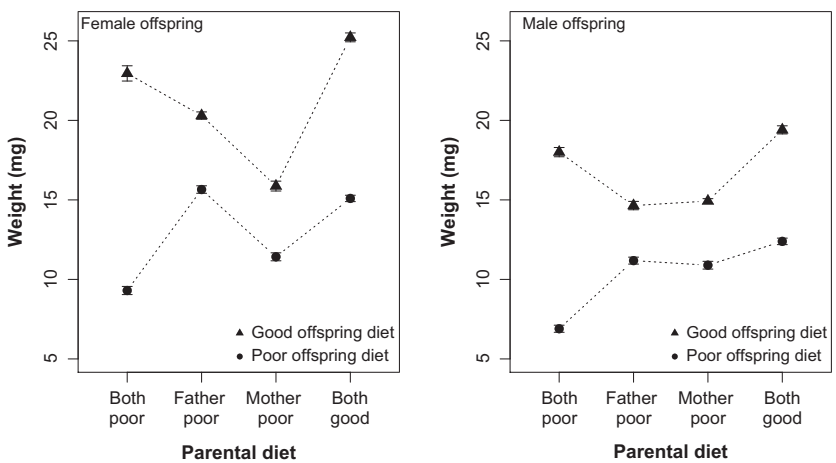

Fig. 4. Effects of parental and offspring diet on weight of 5th-instar *Plodia interpunctella* larvae. Triangles indicate means of offspring that received a good diet themselves, and circles are those that received a poor diet. Error bars indicate 95% confidence limits. Female offspring are shown on the left and males on the right, and the parental diet is indicated on the *x*-axis.

Table 2. Final model details for haemocyte count: significance tests and contrasts are as for Table 1

Random effects					SD
Intercept					0·4020454
Residual					1·3739
Fixed effects					
Variable	Estimated coefficient (treatment contrasts)	SE	d.f.	Likelihood ratio from deletion test using models fitted with ML rather than with REML.	*P*
Intercept	3·63	0·171	2517		
Blocks 2–7	0·120, 0·525, 0·473, 0·425, 0·693, 0·416	0·181, 0·178, 0·166, 0·164, 0·177, 0·194	2517, 131, 131, 131, 131, 131	21·7	0·0014
Offspring sex (male)	−0·300	0·0540	2517	30·6	<0·0001
Maternal treatment (good diet)	0·554	0·151	131		
Paternal treatment (good diet)	0·574	0·145	131		
Offspring treatment (good diet)	3·52	0·0836	2517		
Maternal treatment (good diet): offspring treatment (good diet)	0·335	0·109	2517	9·50	0·0021
Paternal treatment (good diet): maternal treatment (good diet)	−0·540	0·198	131	7·77	0·0053

it is possible that this response is adaptive: if the offspring are likely to experience the same environment as their parents, then parental manipulation of the offspring phenotype might give a fitness advantage. In this case, if resources for the offspring are scarce, then reducing immune investment might free resources which can be used for other aspects of the animal's biology.

A second possibility is that this phenomenon is not adaptive and that the reduced immune responses of offspring when the parents are reared in a poor environment are the consequence of some form of constraint associated with the reduced parental investment in immunity: for example, genomic imprinting of offspring could simply be a side effect of the same process being used to reduce parental gene expression. These hypotheses could be tested by comparing the strength of transgenerational effects on immunity between species where the offspring disperse widely or have a long diapause period and species where the offspring will tend to grow in the same environment as the parent: if the transgenerational effects are adaptive, then they should be weaker when the offspring are likely to find themselves in a different environment from that experienced by the parent.

In most studies on transgenerational effects, especially in invertebrates, it is not clear whether the observed effects arise from differential resource allocation by the parents, for example by changing the size of eggs, or from 'imprinting' mechanisms that affect offspring development independently of resources (e.g. via epigenetic marks that are passed to the offspring and affect gene expression for components of the immune response). The interactions between parental diet and offspring diet give us insight into this question in the case of *P. interpunctella*: there was a larger effect of parental diet on PO activity when the offspring diet was good (i.e. the reduction in PO activity associated with a poor parental diet was greater when the offspring were fed high-quality food than when they were fed low-quality food), and the effect of maternal diet on haemocyte count is also increased in larvae fed a good-quality diet. If these transgenerational effects were caused by differential investment in offspring by parents from different environments, then we would expect to see the opposite because offspring with more resources available from their own diet would need to rely less on parentally supplied resources. This implies that these effects are not mediated by differential resource allocation by the parents but by epigenetic imprinting, a possibility that is further supported by the strong effects of paternal environment that we found. In vertebrates, epigenetic markers affecting gene expression include genomic methylation, noncoding micro-RNAs and histone markers, which are known to be affected by a wide range of dietary factors (McKay & Mathers 2011). Recent research has revealed that genomic imprinting by methylation is found in at least some insects (Anaka *et al*. 2009; Elango *et al*. 2009) including the Lepidoptera (Xiang *et al*. 2010; Glastad *et al*. 2011). It is likely that these mechanisms are responsible for the patterns seen here, although it is also possible that some other unknown imprinting mechanism is operating.

There are strong effects of parental food quality on offspring weight, and the changes in weight seen when the offspring were fed a good diet are particularly striking: if a single parent came from a poor-quality environment, weight was reduced, but if both parents were raised on poor food, this effect was much reduced and offspring weight was similar to that of larvae whose parents had a good diet. Thus, when resources are abundant but both parents had a restricted diet, larvae invest in body size, but not in immunity. This effect might be seen as an analogous response to the increase in obesity seen in vertebrates born of parents exposed to malnutrition, but it is notable that dietary restriction of a single parent is enough to induce this effect in animals such as laboratory rats (Curley, Mashoodh & Champagne 2011; Ferguson-Smith & Patti 2011).

This study makes a clear demonstration of the existence of transgenerational effects of parental food quality on offspring immunity, and this leads us to ask how important these effects might be in field systems, rather than controlled laboratory settings. There are some aspects of animal nutrition in field situations that will have important impacts on immunity that are not examined in this study, most obviously the existence of choice: insects are known to alter their diet to include more protein in response to immune challenges (Lee *et al*. 2006; Povey *et al*. 2009), and either the parental generation or offspring might be able to ameliorate the effects of a poor diet by being more selective about their food. Animals are also known to alter their diets to change their intake of antioxidants or toxic plant secondary chemicals when parasitized (Smilanich *et al*. 2011), and again, this could be important in determining immune reactivity for parents and offspring.

Moving from the individual level to the population, strong transgenerational effects of this nature have the potential to be important drivers of population dynamics (Benton *et al*. 2001; Plaistow, Lapsley & Benton 2006; Benton, St Clair & Plaistow 2008). Resource availability is likely to be a function of population density in most species, giving the possibility of delayed density-dependent effects in host–parasite systems. Delayed density-dependent effects are generally destabilizing and are likely to lead to complex dynamics such as cycles (Plaistow, Lapsley & Benton 2006 and references therein). Because the effects documented here are substantial, it is possible that transgenerational effects could have significant effects on host–pathogen dynamics.

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