Maternal pathogen exposure causes diet- and pathogen-specific transgenerational costs

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(Abstract)

Transgenerational effects, whereby the environment experienced by a parent leads to an altered offspring phenotype, have now been described in a variety of taxa. In invertebrates, much of the research on these effects has concentrated on the role of parental exposure to pathogens or immune elicitors in determining offspring immune investment or disease resistance. To date, however, studies of transgenerational effects in invertebrates have generally been restricted to single infections or immune elicitors in ideal laboratory environments. Animals in field situations will commonly experience sub-optimal environments and co-infection by multiple species of parasites and pathogens, leading to increased relative costs of immune investment and changing fitness benefits from offspring responses to the parental environment. Here we investigate a more ecologically realistic scenario involving both multiple infections and resource limitation, using the Indian meal moth *Plodia interpunctella* as a model host, challenged with the entomopathogenic bacterium *Bacillus thuringiensis* and fungus *Beauveria bassiana*. Mothers were exposed to low doses of one or both pathogens, or a control. Offspring from each family were reared on either good- or poor-quality food and then exposed to one or both pathogens. Maternal exposure to pathogens led to reduced pathogen resistance in offspring, depending on the combination of maternal and offspring pathogen-specific infections and resource limitation in the offspring generation. Much research to date has focussed on trans-generational immune priming, in which parental exposure to pathogens or immune elicitors leads to upregulated immune reactivity in their offspring. The lack of any such effects in our system suggests that the production of less resistant offspring following parental exposure to pathogens might be an important alternative, driven by costs of resistance rather than adaptive benefits.

Introduction

Ecologists are increasingly appreciating the role of parental environment in determining the phenotype of their offspring, with reports of parental diet, thermal environment and exposure to predators altering offspring biology or behaviour (Dunn and Bale 2009, Storm and Lima 2010, Hafer et al. 2011, Salinas and Munch 2012). When the parental environment is relatively harsh, some transgenerational effects are likely to be simple consequences of the reduced ability of the parents to invest in their offspring, but other effects can be seen as adaptive examples of phenotypic plasticity that extend over more than one generation. If the parent detects a relevant feature of the environment and transmits that information to offspring, which then adjust their phenotype accordingly, then offspring fitness can be increased by the transgenerational effect (Shea et al. 2011). The evidence for anticipatory parental effects is currently generally weak (Uller et al. 2013), however, with the exception of a series of recent studies of transgenerational effects in invertebrates exposed to pathogens or to immune elicitors (Little et al. 2003, Sadd et al. 2005, Roth et al. 2010, Zanchi et al. 2011).

This phenomenon of transgenerational immune priming (TGIP) in invertebrates occurs when parents who are exposed to an immune stimulus produce offspring with raised pathogen resistance or increased levels of constitutive immunity (Little *et al.* 2003; Sadd *et al.* 2005; Moret 2006; Sadd & Schmid-Hempel, 2007). This process is usually interpreted as an anticipatory, protective parental effect analogous to the maternal transfer of antibodies in vertebrates (Kurtz and Armitage 2006, Hasselquist and Nilsson 2009). In invertebrates, offspring from challenged mothers have been found to possess upregulated antibacterial activity (Sadd et al. 2005, Moret 2006), increased phenoloxidase activity (Freitak et al. 2009), or greater resistance to infection by pathogens (Tidbury et al. 2011, Hernández López et al. 2014). If TGIP is widespread across invertebrate taxa, then there are important implications for our understanding of

host-pathogen dynamics, for example, TGIP will provide a new source of heterogeneity in host susceptibility and therefore influence infection prevalence (Tate and Rudolf 2012).

Research into the transgenerational effects of immune challenge and pathogen exposure is in its infancy and there is still much work to do to determine its extent and contribution to whole-organism fitness. TGIP has been the main focus of research so far but has been reported in a limited number of invertebrate species, including *Bombus terrestris, Plodia interpunctella, Manduca sexta, Tribolium castaneum*, *Trichoplusia ni* and *Tenebrio molitor* (Sadd *et al.* 2005; Tidbury *et al.* 2011; Zanchi *et al.* 2011; Trauer & Hilker 2013; Eggert, Kurtz & Diddens-de Buhr 2014), and there are some species where TGIP has not been found, namely *Aedes aegypti* (yellow fever mosquito: (Voordouw et al. 2008) and *Drosophila* (Linder & Promislow 2009). Furthermore, the majority of studies that have found TGIP to date have been carried out under standardised laboratory conditions, providing little insight as to whether protective effects will always occur when individuals are faced with greater environmental variation. Diet quality, infection with multiple pathogens, and the competitive environment have all been shown to dramatically alter within-generation immune responses, because the upregulation of immunity is a costly trait (Triggs and Knell 2011, Sternberg et al. 2012), and it is to be expected that such environmental variation should also influence transgenerational effects on immunity resulting from parental infection. Additionally, most studies of transgenerational effects on immunity have focussed on quantifying changes in the constitutive immune system in the offspring (Sadd et al. 2005, Sadd and Schmid-Hempel 2007, Voordouw et al. 2008, Freitak et al. 2009) with fewer measuring survival outcomes (although see Tidbury *et al.* 2011; Hernández López *et al.* 2014). The degree to which transgenerational effects will actually affect resistance against parasites needs to be clarified because constitutive

immune titres do not always reflect survival rates after infection (Graham et al. 2011, Auld et al. 2012).

Under an adaptive interpretation of TGIP, benefits will accrue to parents as TGIP increases the likelihood of offspring surviving an encounter with a parasite. But when parents are exposed to pathogens, they can also suffer damaging fitness costs. The energetic and nutritional costs of mounting a within-generation response have been extensively investigated, from the deployment and maintenance of the immune system, to the risk of self-reactivity (Stearns 1989, Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000, Schmid-Hempel 2003, Wilson and Cotter 2013). The expression of antiparasitic defences can result in trade-offs with other somatic traits, such as development time, competitive ability and pupal mass (Kraaijeveld and Godfray 1997, Freitak et al. 2007), or with other components of the immune system (Wilson and Cotter 2013, Cotter et al. 2013). Infection can also result in serious costs to reproductive success, including reduced output and viability of offspring, as well as the mother's future breeding bouts (Zuk and Stoehr 2002, Rolff and Siva-Jothy 2003, Luong and Polak 2007). It is therefore reasonable to expect that parental exposure to pathogens will be another source of transgenerational effects which may result in costs for the offspring. For example, *Tenebrio molitor* (yellow mealworm beetle) offspring exhibited increased development time and reduced pupal mass when parents were challenged (Zanchi et al. 2011).

Classical life history theory predicts that such costs might be revealed under resource limitation, which has been traditionally used to study trade-offs made within a single organism's life history strategy (Boggs and Freeman 2005, Diamond and Kingsolver 2011). Food is a heterogeneously distributed resource, and animals have developed a variety of adaptive and non-adaptive phenotypically plastic responses to a lack of food. For example, in response to resource depletion, animals may produce fewer eggs, grow

to a smaller size, or decrease the organism's ability to resist infection (Urabe and Sterner 2001, Lee et al. 2006, Graham et al. 2014). Resource depletion could also affect how offspring respond to transgenerationally inherited stress, for example parental starvation or infection. Although difficult to demonstrate experimentally, well-provisioned offspring may be able to compensate for a poor start in life in a similar manner to compensation for stress in early development (Metcalfe and Monaghan 2001).In order to test how transgenerational effects arising from parental pathogen exposure are affected by multiple pathogens, and to investigate the role of resource limitation, we investigated the links between parental exposure and offspring resistance using a stored product pest, the pyralid moth *Plodia interpunctella*, and two pathogens, the bacterium *Bacillus thuringiensis* (Bt) and the fungus *Beauveria bassiana* (Bb). Bt is a gram-positive soil dwelling bacteria, naturally transmitted by ingestion, and active against a wide range of invertebrate hosts. Its mode of action involves the creation of pores in the gut using Cry and Cyt toxins, resulting in systemic septicaemia and death (Soberón et al. 2009). Bb is a generalist fungal entomopathogen which affects many insect orders (de Faria and Wraight 2007). Entomopathogenic fungi attack insects by penetrating the cuticle through mechanical or enzymatic means, eventually invading the haemocoel and internal organs (Shah and Pell 2003). Bb also produces a variety of entomopathogenic secondary metabolites such as beauvericin, which disrupt the host immune response and progression to metamorphosis (Grove and Pople 1980, Boucias et al. 1995). Both pathogens in this study have been developed as successful commercial biopesticides, and by 2011, 100 million accumulated hectares of Bt transgenic crops had been planted in a bid to control mostly lepidopteran pest species (James 2011), contributing an additional reason to study these pathogens in *P. interpunctella*. Finally, there are very few instances of vertical transmission of fungi, and no evidence that Bt can be vertically transmitted, which makes them appropriate models for investigating

transgenerational effects as determined by parental investment or other signals (Raymond et al. 2008, Hesketh et al. 2010).

Methods

A stock population of *Plodia interpunctella* has been maintained at Queen Mary since December 2011, which originated from an outbred population at the University of Leeds. The population is maintained on unlimited standard lab food consisting of organic wheat bran (Mount Pleasant Mill, Lincolnshire), brewer's yeast and glycerol in a 10:1:1 ratio at 27°C on a 12:12 hour light/dark cycle. To create the next generation, over 200 mixed adults are placed in a funnel with both ends secured with net, and allowed to mate. The resulting eggs are collected and placed on standard laboratory food, and the larvae are allowed to grow until adulthood.

Preparation and infection with pathogen cultures

Bb cultures were obtained from Rothamsted Research, Harpenden, UK. Stocks were maintained in 50% glycerol solution at -80°C. The fungus was cultured on Sabouraud Dextrose Agar in Petri dishes sealed with parafilm. After three weeks of growth, the fungus was harvested in sterile conditions with an autoclaved scraper, and vortexed for 10 minutes in 5ml autoclaved distilled water and the resulting solution filtered with a Buchner funnel. The appropriate dilution of spore suspension was created by diluting the initial suspension with distilled water and counting spores with a Neubauer brightline haemocytometer. Larvae were infected by dipping the whole animal into the spore suspension for 15 seconds, and dried on a paper towel before being returned to their own individual Petri dish. Bb suspension was stored at 4° C and a fresh dilution for larval dosing made up each day. The solution was vortexed thoroughly each day and sonicated for two minutes before use to prevent the spores clumping together. Suspensions of Bt (DiPel® formulation, ProGreen Weed Control Solutions Ltd, South Fen Business Park, Lincs) were created using sugar saturated water and 10% blue food

dye. Larvae were infected by feeding each one a 1µl droplet of this suspension, and observed until all the solution had been consumed. Larvae were discarded from the experiment if they did not finish it.

Dose-response assays were used in preliminary experiments to determine the doses causing 5% (LD05) and 33% mortality (LD33) in fourth instar larvae for both Bb and Bt.

Parental generation

To create the parental generation in this experiment, approximately 200 adult *P. interpunctella* were taken from the stock population and allowed to mate (Fig. 1). After 24 hours their eggs were placed on the standard laboratory diet. 16-20 days after egg-laying, fourth instar female larvae were separated out and randomly assigned to one of six pathogen exposure or control treatments: either dipped in 7900 spores/ul Bb spore suspension (corresponding to an approx. LD05 dose), droplet dosed with an 0.01 mg/ml suspension of Bt (approx. LD05), exposed to LD05 doses of both pathogens, dosed with a control droplet of 10% food colouring in sugar solution, dipped in distilled water, or handled with forceps only. The low LD05 doses were used to infect the parental generation so as not to select for resistance in the offspring, while still stimulating the immune system, whereas higher LD33 doses were used for the bioassays of the offspring to give more accurate estimates of resistance. Larvae were stored at 26°C in an incubator on a 12:12 light: dark cycle in individual 55mm Petri dishes with *ad libitum* lab diet. The dishes were checked a week after infection and any dead larvae were removed from the experiment.

Mating and egg collection

Dishes containing pupae were checked daily for eclosion. To create the offspring generation, freshly eclosed females were mated with a freshly eclosed virgin male from the stock population. Final sample sizes at the family level ranged from n = 31 (control

dip in distilled water) to n = 38 (naïve with handling only). Eggs were counted after 48 hours and each family's eggs were split into two groups and grown on either good (10:1:1 wheat bran, brewer's yeast, glycerol) or poor (20:1:1) diet. This latter diet was chosen because although it leads to some phenotypic changes in traits such as development time, it does not impose significant mortality in preliminary experiments – thus the larvae reared on it are experiencing nutritional stress but are not starving. This offspring generation was allowed to develop in the incubator at 26°C, and the diet was systematically searched on days 18, 19, 20 and 21 after egg laying for fourth instar larvae. Larvae were assigned to 3 groups: 1) infection with a 0.35mg/ml dose of Bt (corresponding to an approximate LD33, n = 913 giving an average of 152 larvae dosed per treatment), 2) 20000 spores/µl dose of Bb (also approximately an LD33, n = 921 giving an average of 154 larvae dosed per treatment), or 3) coinfection with both pathogens (n = 912 giving an average of 152 larvae dosed per treatment). After infection, larvae were placed in 25 cell Petri dishes supplied with either good or poor diet as appropriate, and assayed for mortality eight days later. Preliminary experiments showed that this is the optimum time to assay complete mortality from the fungus. Mortality from Bt primarily occurs within the first 48 hours after exposure.

Statistical analysis

Analysis was performed in R version 3.0.1 (R Development Core Team 2013) using the package lme4 (Bates et al. 2014). Three separate generalised mixed effects models with binomial errors were fitted for each of the types of offspring infection, i.e. infection with Bb, Bt, or coinfection with both pathogens. Models were initially fitted with two-way interactions between offspring sex, maternal Bt treatment, maternal Bb treatment and offspring diet quality. Higher order interactions were generally not included to avoid over parameterisation of the models (Zuur et al. 2009), although the interactions between maternal Bt treatment, maternal Bb treatment and offspring sex, and maternal

Bt treatment, maternal Bb treatment and offspring diet quality were included to test whether the coinfection treatment interacts with offspring diet or sex. Family ID and the date of offspring infection were included as crossed random effects. Non-significant terms were sequentially removed from the models, interaction terms first, and the nested models were compared with likelihood ratio tests until a minimal adequate model was achieved (Zuur et al. 2009), which was then refitted using a REML algorithm. Additional models were used to further investigate the significant interaction between parental exposure to Bb and offspring diet quality. Separate binomial mixed effects models were fitted for each diet quality, with and without the effect of maternal exposure. The likelihood ratio test was used to evaluate the effect of priming within each level of diet quality.

Results

For full tables of statistical tests, refer to Appendix 1.

Mortality in offspring challenged with Bt (Supplementary material Appendix 1 Table A2)

Larvae had greater survival against Bt infection when they were fed a high-quality diet (Fig. 2, back-transformed values: good diet = 72.3% survival, poor diet = 63.2% survival, $\chi^2 = 6.65$, $p = 0.010$). However, there was no indication of TGIP: offspring mortality from Bt was unaffected by maternal exposure to Bt or to Bb or to both pathogens (maternal Bt treatment: $\chi^2 = 2.10$, $p = 0.147$, maternal Bb treatment: $\chi^2 = 0.04$, $p = 0.835$, maternal Bt × Bb interaction (coinfection treatment): $\chi^2 = 0.56$, $p = 0.458$). Female offspring were not significantly better at resisting the Bt infection than males ($\chi^2 = 0.09$, $p = 0.770$).

Mortality in offspring challenged with Bb (Supplementary material Appendix 1 Table A3)

There was no effect of the heterologous maternal pathogen exposure (Bt) on offspring mortality when infected with Bb ($\chi^2 = 0.68$, $p = 0.411$). When mothers had not received a fungus treatment, 75.2% offspring survived when fed the good diet and similarly 76.5% survived when fed the bad diet (back-transformed values). However, offspring mortality was affected by the interaction between maternal infection with Bb and diet quality ($\chi^2 = 6.40$, $p = 0.011$, Fig. 3). Maternal exposure to Bb had a slight beneficial effect on the survival on the offspring when they were reared on the good diet, with 81% surviving the dose of Bb, but when the larvae experienced nutritional stress maternal fungal priming had a negative effect on progeny survival with only 66.3% surviving. This negative effect was the same for offspring of mothers given a single pathogen exposure and also for those from mothers exposed to Bt as well as Bb, as indicated by the non-significant three-way association between the two maternal pathogen exposures and diet quality ($\chi^2 = 1.06$, $p = 0.304$). Examination of effect sizes and confidence intervals suggests that this significant interaction is largely a consequence of the increased mortality in the maternal exposure-poor food treatment group, which is supported by the observation that when a model was fitted to data from offspring on poor diets only there was a significant effect of maternal exposure ($\chi^2 = 4.71$, $p = 0.0299$), but a separate model fitted to data from offspring on good diets only found no significant effect of maternal exposure ($\chi^2 = 2.03$, $p = 0.154$).

Mortality in offspring challenged with both pathogens (Supplementary material Appendix 1 Table A4)

When offspring were challenged with both pathogens simultaneously, maternal Bt treatment (including both the single and coinfection dose) decreased offspring survival by 10.8% (Fig. 4, $\chi^2 = 6.50$, $p = 0.011$). There was no effect of maternal Bb treatment on survival ($\chi^2 = 0.77$, $p = 0.380$), and no interaction between maternal Bt exposure and maternal Bb exposure ($\chi^2 = 0.21$, $p = 0.651$). Sex did not influence larval survival ($\chi^2 =$

0.01, $p = 0.930$). Although food quality was a determinant of offspring survival when infected with Bt alone, this was not apparent when offspring were coinfected ($\chi^2 = 1.62$, $p = 0.203$).

Discussion

In this study we investigate transgenerational effects of pathogen exposure using multiple infections and resource depletion. Mothers were exposed to low doses of one or both pathogens, or a control. Offspring from each family were reared on either good- or poor-quality food and then exposed to one or both pathogens. Offspring survival was dependent on the combination of maternal and offspring infections and resource limitation, but there was no evidence of a priming effect whereby maternal pathogen exposure causes improved survival when offspring are exposed to the same pathogen. Instead, in some treatment combinations maternal exposure led to a reduction in offspring survival, as when mothers were infected with Bt and offspring were coinfected with both pathogens. In other cases maternal exposure led to no changes in offspring survival, as when mothers were infected with Bt and offspring were exposed to a single infection of either Bt or Bb. Diet also interacted with maternal pathogen exposure: when mothers and offspring were infected with Bb offspring had lower survival rates when they were resource depleted.

Diet quality was an important factor in determining response to infection. When offspring were infected with Bt, those consuming the more nutritious diet had a 9.1% higher survival rate than those consuming the poor diet. This is possibly a consequence of improved immune reactivity in those individuals fed the better diet. There is generally a positive relationship between diet quality and levels of immune markers (Siva-Jothy and Thompson 2002) and this has previously been shown in *P. interpunctella* (Triggs and Knell 2012). Parasite resistance is energetically expensive and often associated with a protein cost due to the production of immune cells and

peptides (Wilson and Cotter 2013). When a fixed amount of resources is allocated between costly traits (Sheldon and Verhulst 1996), if the total pool of acquired resources is decreased under nutritional stress, this may result in trade-offs with other life-history traits or between different components of the immune system (Krams et al. 2012, Cotter et al. 2013). Selection experiments using Bt have shown that resistance is traded-off with development time or pupal weight, and is also lost when selection pressure is relaxed, indicating the costly nature of defence against this pathogen (Oppert et al. 2000, Janmaat and Myers 2003).

An alternative to the link between diet and immunity is that nutritionally stressed individuals could be more susceptible to infection if the composition of the poor diet resulted in upregulated nutrient processing to maximise the macronutrients from their poor diet. The poor diet in this experiment was created by diluting the macronutrient-rich components of the diet (protein-rich yeast and glycerol) with wheat bran, which is not as nutrient-rich and acts as a bulking agent. Invertebrates have a wide array of plastic pre- and post-digestive processes to obtain the required nutrients from the diet in response to food deprivation or nutrient imbalance (Douglas et al. 2005, Arrese and Soulages 2010, Simpson and Raubenheimer 2012). For example, when macronutrients in the diet are diluted, or when they are present in an uneven ratio, the size of the gastrointestinal tract has been shown to increase (Sørensen et al. 2010, Clissold et al. 2012). In *Drosophila*, increased levels of stem cell divisions have been shown to mediate gut remodelling when nutrient levels change (O'Brien et al. 2011), which could facilitate this plasticity. However, a side effect of gastro-intestinal enlargement might be that there are more receptors for Bt to attack the midgut, resulting in an increased level of mortality. Eco-immunology studies using oral inoculation as a route to infection should take note of factors affecting gut size as possible variables to control when administering the desired dose.

Maternal infection with Bt was costly when offspring were infected with both pathogens: 10.8% more offspring died when mothers were exposed to Bt and offspring were coinfected with both pathogens than when mothers that had experienced the fungus or control treatments. One possible mechanism as to why this effect is only seen when offspring are coinfected lies in the increased cost of upregulating defences against both bacteria and fungi due to the requirement for pathogen-specific immune molecules, such as antifungal peptides (Mak et al. 2010, Arvanitis et al. 2013). If maternal Bt exposure alters immune investment or resource availability this could interact with this requirement to produce a wider variety of immune effectors and cause reduced survival. An alternative possibility is that this is simply a dosage effect – in these experiments the larvae given a coinfection were exposed to more infective units of pathogen (the coinfective dose is more lethal than the single doses, producing 47.5% mortality in this experiment, in comparison to 34.7% for Bt and 24.9% for Bb), which would also require increased resources for immune defence. By comparing doses that controlled for the number of infective units in additives doses, and using combinations of different pathogens to allow for virulence differences in mixed infections, future experiments could separate these two possible explanations.

When larvae were infected with Bb, maternal exposure to the fungus was detrimental, leading to 33.7% mortality in comparison to 23.5% mortality in larvae descended from mothers who experienced the Bt and control treatments. Unlike the costs paid by coinfected offspring, however, maternal exposure to infection in fungus-infected progeny only decreased survival when the larvae were nutritionally stressed. There was also a suggestion that larvae received a small beneficial effect of maternal immune priming from mothers infected with Bb (i.e. the single maternal fungus treatment and the coinfected maternal treatment) when they experienced plentiful resources, although the effect is weak and not significantly different from control maternal treatments.

Overall, our results are indicative of pathogen-specific costs depending on the nature of the challenge received by both mothers and offspring. Maternal exposure to Bb is costly when offspring are infected with the homologous pathogen and experiencing nutritional stress, and maternal exposure to Bt is costly when offspring are coinfected with both pathogens.

These data contrast with a number of studies reporting transgenerational immune priming (TGIP). As mentioned in the introduction, TGIP does not seem to be ubiquitous and appears not to operate in some important systems (Voordouw et al. 2008, Linder and Promislow 2009). With regards to the specific organisms used in this experiment, maternal exposure to a granulosis virus in *P. interpunctella* has been reported to lead to increased virus resistance in their offspring (Tidbury et al. 2011). Moreover, several studies have reported TGIP in insects exposed to Bt including *Tribolium* beetles (Roth et al. 2010, Eggert et al. 2014, Tate and Graham 2015) and *Trichoplusia ni* (cabbage looper) (Shikano et al. 2015). On the basis of these previous studies it might be expected that we should have found evidence for TGIP and we must therefore reconcile our findings in this study both generally and specifically with previous results.

One possibility to explain the lack of discovery of TGIP in this study is that it might be dose-dependent or life stage-dependent in this system, and our design might not have covered doses or life stages where TGIP might be evident. This begs the question of how many doses and life stages need to be tested before any result can be thought of as being well supported. Ultimately, we have been unable to detect TGIP while using doses and development points that previous studies have indicated as being appropriate. We have found evidence for parental effects in some cases, but they are in the direction of increased rather than decreased offspring susceptibility. The sample sizes used in the experiment are large and there is nothing to suggest that the lack of TGIP might be a type 2 error. If we are to consider the possibility of TGIP operating when parents are

dosed at a different developmental stage, or with a larger or a smaller dose of pathogen, then we also have to explain a system where the direction of effect changes depending on the timing or magnitude of the dose.

Transgenerational pathogen transmission may play a key role in TGIP, both in terms of pathogen infectivity and mode of host exposure. Live doses of virus can be vertically transmitted: Burden *et al* showed that RNA transcripts of *Plodia interpunctella* granulovirus persist in the ovaries, testes and offspring of individuals that survived a viral infection (Burden et al. 2002). It seems probable that this vertical transmission of the virus is associated with the increased resistance found in *P. interpunctella* larvae when mothers are primed with the virus. We chose Bt and Bb for our study as neither are known to be passed on to offspring in this way.

With regards to examples of TGIP in insects exposed to Bt, the majority of these studies challenged parents with heat-killed bacteria injected into the haemocoel (Roth et al. 2010, Eggert et al. 2014, Tate and Graham 2015), which is very different from the normal oral route of infection of Bt. Animals challenged in this way will not be exposed to the effects of the Bt toxin, which needs to be cleaved by protease enzymes in the gut to be activated and which operates on the midgut epithelium (Soberón et al. 2009). Mechanisms of Bt resistance are associated with toxin action in the gut, such as the reduction in the receptor affinity in the brush border membrane of the midgut epithelium (Van Rie et al. 1990, Ferré et al. 1991) or downregulation of protease production that interferes with Cry/Cyt protoxin activation (Ibrahim et al. 2010). It seems unlikely that a haemolymph injection of dead bacteria will elicit a response from these mechanisms, leaving the question of how TGIP naturally occurs in a Bt-insect host system open. Nonetheless, the lack of TGIP we report here in *P. interpunctella* after parents were challenged orally with live Bt is in contrast to Shikano *et al* who found evidence of TGIP in *Trichoplusia ni*, in the only other study to use this mode of

exposure (Shikano et al. 2015). No transgenerational immune priming studies have yet been carried out with Bb, and the results from two within-generation studies differ, demonstrating both no evidence for within-generation immune priming (Reber and Chapuisat 2012), and that a previous encapsulation response improves survival during a Bb infection relative to handling treatment (Krams et al. 2013).

In general, it is important to consider that transgenerational priming against pathogens will only be adaptive when the parental environment predicts the environment that the offspring experience (Burgess and Marshall 2014). In species where most organisms disperse to new environments every generation it is possible that inducing offspring to invest more in costly immunity would lead to reduced, rather than enhanced fitness. The origins of *P. interpunctella* are obscure but given its preferences for dried fruits, nuts and grains (Mohandass et al. 2007), we can speculate that in its natural state it was an opportunistic feeder on fallen fruit and other patches of ephemeral vegetable matter, and as such it is likely that adults would have dispersed to new patches in most generations. TGIP would not necessarily be adaptive for an animal with such a lifestyle, especially when considering pathogens like Bt and Bb that are encountered mostly from contaminated environments rather than from contact with infected conspecifics. Similar arguments can be made for *Drosophila melanogaster* and *Aedes aegypti*, the other two species that have been reported not to show TGIP.

Despite a recent focus on the beneficial and possibly adaptive effects of TGIP (Sadd et al. 2005, Hernández López et al. 2014), our study highlights the burden of infection on a mother's fitness. Although TGIP has been the main focus of research into parental effects on the immune system of some invertebrate species, it is likely that the transgenerational costs that mothers suffer to their fitness are equally if not more widespread and important in defining the evolutionary dynamics of response to infection. It is interesting to consider that our experiments showed that costs for mothers

in terms of increased offspring mortality were present even though the maternal dose of pathogen was low. While studies that measure immune titres such as phenoloxidase or antimicrobial peptides are useful to determine the action of TGIP in the immune system, it is only by measuring offspring ability to resist or tolerate pathogen attack that we can see whether TGIP translates into real costs or benefits for individuals, as survival outcomes will ultimately determine whether offspring will live to reproductive age. Few studies have emphasised the costly nature of pathogen exposure in terms of higher levels of parasitic infection in offspring or dependence on nutritional resource levels. If host-pathogen systems are only tested under ideal conditions of optimal food then we may be building a false picture of how common parental effects are in such systems, and whether they are costly or beneficial. We suggest that the production of less resistant offspring and related costs may be more widespread than is currently realised and could play a more prominent role in defining host-pathogen dynamics than the beneficial effects of TGIP.

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Author contributions

JL and RK designed the study, JL and AML carried out the lab work, JL carried out the data analysis and drafted the manuscript. All authors gave final approval for publication.

Data accessibility

Should this manuscript be accepted, data will be uploaded to Dryad. Full statistical results, including non-significant terms from models, can be found in the Supporting information.

References

Arrese, E. L. and Soulages, J. L. 2010. Insect fat body: energy, metabolism, and regulation. - Annu. Rev. Entomol. 55: 207–225.

Arvanitis, M. et al. 2013. Invertebrate models of fungal infection. - Biochim. Biophys. Acta 1832: 1378–1383.

Auld, S. K. J. R. et al. 2012. Elevated haemocyte number is associated with infection and low fitness potential in wild *Daphnia magna*. - Funct. Ecol. 26: 434–440.

Bates, D. et al. 2014. _lme4: Linear mixed-effects models using Eigen and S4_. R package version 1.1-7.

Boggs, C. L. and Freeman, K. D. 2005. Larval food limitation in butterflies: Effects on adult resource allocation and fitness. - Oecologia 144: 353–361.

Boucias, D. G. et al. 1995. Comparative analysis of the in vivo and in vitro metabolites produced by the entomopathogen *Beauveria bassiana*. - Can. J. Bot. 73: 1092–1099.

Burden, J. P. et al. 2002. Vertical transmission of sublethal granulovirus infection in the Indian meal moth, *Plodia interpunctella*. - Mol. Ecol. 11: 547–555.

Burgess, S. C. and Marshall, D. J. 2014. Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. - Oikos 123: 769–776.

Clissold, F. J. et al. 2012. Protein-induced weight increase of the gastrointestinal tract of locusts improves net nutrient uptake via larger meals rather than more efficient nutrient absorption. - J. Exp. Biol. 216: 329–337.

Cotter, S. C. et al. 2013. A direct physiological trade-off between personal and social immunity. - J. Anim. Ecol. 82: 846–853.

de Faria, M. R. and Wraight, S. P. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. - Biol. Control 43: 237–256.

Diamond, S. E. and Kingsolver, J. G. 2011. Host plant quality, selection history and trade-offs shape the immune responses of *Manduca sexta*. - Proc. R. Soc. B Biol. Sci. 278: 289–297.

Dorai-Raj, S. 2014. binom: Binomial confidence intervals for several parameterizations. R package version 1.1-1. http://CRAN.R-project.org/package=binom.

Douglas, S. J. et al. 2005. The neurogenetics and evolution of food-related behaviour. - Trends Neurosci. 28: 644–652.

Dunn, G. A. and Bale, T. L. 2009. Maternal high-fat diet promotes body length increases and insulin insensitivity in second-generation mice. - Endocrinology 150: 4999–5009.

Eggert, H. et al. 2014. Different effects of paternal trans-generational immune priming on survival and immunity in step and genetic offspring. - Proc. R. Soc. B Biol. Sci. 281: 20142089.

Ferré, J. et al. 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. - Proc. Natl. Acad. Sci. U. S. A. 88: 5119–5123.

Freitak, D. et al. 2007. Immune system responses and fitness costs associated with consumption of bacteria in larvae of *Trichoplusia ni*. - BMC Biol. 5: 56.

Freitak, D. et al. 2009. Dietary-dependent trans-generational immune priming in an insect herbivore. - Proc. R. Soc. B Biol. Sci. 276: 2617–2624.

Graham, A. L. et al. 2011. Fitness consequences of immune responses: strengthening the empirical framework for ecoimmunology. - Funct. Ecol. 25: 5–17.

Graham, R. I. et al. 2014. Locusts increase carbohydrate consumption to protect against a fungal biopesticide. - J. Insect Physiol. 69: 27–34.

Grove, J. F. and Pople, M. 1980. The insecticidal activity of Beauvericin and the Enniatin complex. - Mycopathologia 70: 103–105.

Hafer, N. et al. 2011. Transgenerational effects of food availability on age at maturity and reproductive output in an asexual collembolan species. - Biol. Lett. 7: 755–758.

Hasselquist, D. and Nilsson, J. A. 2009. Maternal transfer of antibodies in vertebrates: trans-generational effects on offspring immunity. - Philos. Trans. R. Soc. London Ser. B, Biol. Sci. 364: 51–60.

Hernández López, J. et al. 2014. Trans-generational immune priming in honeybees. - Proc. R. Soc. B Biol. Sci. 281: 20140454.

Hesketh, H. et al. 2010. Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. - In: The Ecology of Fungal Entomopathogens. pp. 55–73.

Ibrahim, M. A. et al. 2010. *Bacillus thuringiensis*. A genomics and proteomics perspective. - Bioeng. Bugs 1: 31–50.

James, C. 2011. Brief 43: Global Status of Commercialized Biotech/GM Crops: 2011.

Janmaat, A. F. and Myers, J. 2003. Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage loopers, *Trichoplusia ni*. - Proc. R. Soc. B Biol. Sci. 270: 2263–2270.

Kraaijeveld, A. R. and Godfray, H. C. 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. - Nature 389: 278–280.

Krams, I. et al. 2012. Heterophil/lymphocyte ratios predict the magnitude of humoral immune response to a novel antigen in great tits (*Parus major*). - Comp. Biochem.

Physiol. - A Mol. Integr. Physiol. 161: 422–428.

Krams, I. et al. 2013. Previous encapsulation response enhances within individual protection against fungal parasite in the mealworm beetle *Tenebrio molitor*. - Insect Sci. 20: 771–777.

Kurtz, J. and Armitage, S. A. O. 2006. Alternative adaptive immunity in invertebrates. - Trends Immunol. 27: 493–496.

Lee, K. P. et al. 2006. Flexible diet choice offsets protein costs of pathogen resistance in a caterpillar. - Proc. R. Soc. B Biol. Sci. 273: 823–829.

Linder, J. E. and Promislow, D. E. L. 2009. Cross-generational fitness effects of infection in *Drosophila melanogaster*. - Fly (Austin). 3: 143–150.

Little, T. J. et al. 2003. Maternal transfer of strain-specific immunity in an invertebrate. - Curr. Biol. 13: 489–492.

Lochmiller, R. L. and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? - Oikos 88: 87–98.

Luong, L. T. and Polak, M. 2007. Costs of resistance in the *Drosophila-Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction. - Evolution (N. Y). 61: 1391–1402.

Mak, P. et al. 2010. A different repertoire of *Galleria mellonella* antimicrobial peptides in larvae challenged with bacteria and fungi. - Dev. Comp. Immunol. 34: 1129–1136.

Metcalfe, N. B. and Monaghan, P. 2001. Compensation for a bad start: grow now, pay later? - Trends Ecol. Evol. 16: 254–260.

Mohandass, S. et al. 2007. Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. - J. Stored Prod. Res. 43: 302–311.

Moret, Y. 2006. “Trans-generational immune priming”: specific enhancement of the antimicrobial immune response in the mealworm beetle, Tenebrio molitor. - Proc. R. Soc. B Biol. Sci. 273: 1399–1405.

O’Brien, L. E. et al. 2011. Altered modes of stem cell division drive adaptive intestinal growth. - Cell 147: 603–614.

Oppert, B. et al. 2000. Fitness costs of resistance to *Bacillus thuringiensis* in the Indianmeal moth, *Plodia interpunctella*. - Entomol. Exp. Appl. 96: 281–287.

R Development Core Team 2013. R: A language and environment for statistical computing.

Raymond, B. et al. 2008. Quantifying the reproduction of *Bacillus thuringiensis* HD1 in cadavers and live larvae of *Plutella xylostella*. - J. Invertebr. Pathol. 98: 307–313.

Reber, A. and Chapuisat, M. 2012. No evidence for immune priming in ants exposed to a fungal pathogen. - PLoS One 7: e35372.

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

Roth, O. et al. 2010. Paternally derived immune priming for offspring in the red flour beetle, *Tribolium castaneum*. - J. Anim. Ecol. 79: 403–413.

Sadd, B. and Schmid-Hempel, P. 2007. Facultative but persistent trans-generational immunity via the mother’s eggs in bumblebees. - Curr. Biol. 17: 1046–1047.

Sadd, B. M. et al. 2005. Trans-generational immune priming in a social insect. - Biol. Lett. 1: 386–388.

Salinas, S. and Munch, S. B. 2012. Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. - Ecol. Lett. 15: 159–163.

Schmid-Hempel, P. 2003. Variation in immune defence as a question of evolutionary

ecology. - Proc. R. Soc. B Biol. Sci. 270: 357–366.

Shah, P. A. and Pell, J. K. 2003. Entomopathogenic fungi as biological control agents. - Appl. Microbiol. Biotechnol. 61: 413–423.

Shea, N. et al. 2011. Three epigenetic information channels and their different roles in evolution. - J. Evol. Biol. 24: 1178–1187.

Sheldon, B. C. and Verhulst, S. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. - Trends Ecol. Evol. 11: 317–321.

Shikano, I. et al. 2015. Trade-offs between transgenerational transfer of nutritional stress tolerance and immune priming. - Funct. Ecol.: 1–9.

Simpson, S. J. and Raubenheimer, D. 2012. The nature of nutrition; a unifying framework from animal adaptation to human obesity. - Princeton University Press.

Siva-Jothy, M. T. and Thompson, J. J. W. 2002. Short-term nutrient deprivation affects immune function. - Physiol. Entomol. 27: 206–212.

Soberón, M. et al. 2009. Signaling versus punching hole: How do *Bacillus thuringiensis* toxins kill insect midgut cells? - Cell. Mol. Life Sci. 66: 1337–1349.

Sørensen, A. et al. 2010. Dietary ratio of protein to carbohydrate induces plastic responses in the gastrointestinal tract of mice. - J. Comp. Physiol. Biochem. Syst. Environ. Physiol. 180: 259–266.

Stearns, S. C. 1989. Trade-offs in life-history evolution. - Funct. Ecol. 3: 259–268.

Sternberg, E. D. et al. 2012. Food plant-derived disease tolerance and resistance in a natural butterfly-plant-parasite interactions. - Evolution (N. Y). 66: 3367–3376.

Storm, J. J. and Lima, S. L. 2010. Mothers forewarn offspring about predators: a transgenerational maternal effect on behavior. - Am. Nat. 175: 382–390.

Tate, A. T. and Rudolf, V. H. W. 2012. Impact of life stage specific immune priming on

invertebrate disease dynamics. - Oikos 121: 1083–1092.

Tate, A. T. and Graham, A. L. 2015. Trans-generational priming of resistance in wild flour beetles reflects the primed phenotypes of laboratory populations and is inhibited by co-infection with a common parasite. - Funct. Ecol. 29: 1059–1069.

Tidbury, H. J. et al. 2011. Within and transgenerational immune priming in an insect to a DNA virus. - Proc. R. Soc. B Biol. Sci. 278: 871–876.

Trauer, U. and Hilker, M. 2013. Parental legacy in insects: variation of transgenerational immune priming during offspring development. - PLoS One 8: e63392.

Triggs, A. and Knell, R. J. 2011. Interactions between environmental variables determine immunity in the Indian meal moth *Plodia interpunctella*. - J. Anim. Ecol. 81: 386–394.

Uller, T. et al. 2013. Weak evidence for anticipatory parental effects in plants and animals. - J. Evol. Biol. 26: 2161–2170.

Urabe, J. and Sterner, R. W. 2001. Contrasting effects of different types of resource depletion on life-history traits in *Daphnia*. - Funct. Ecol. 15: 165–174.

Van Rie, J. et al. 1990. Mechanism of insect resistance to the microbial insecticide *Bacillus thuringiensis*. - Science. 247: 72–74.

Voordouw, M. J. et al. 2008. No maternal effects after stimulation of the melanization response in the yellow fever mosquito *Aedes aegypti*. - Oikos 117: 1269–1279.

Wilson, K. and Cotter, S. C. 2013. Host-parasite interactions and the evolution of immune defence. - Adv. Study Behav. 45: 81–174.

Zanchi, C. et al. 2011. Differential expression and costs between maternally and paternally derived immune priming for offspring in an insect. - J. Anim. Ecol. 80: 1174–1183.

Zuk, M. and Stoehr, A. M. 2002. Immune defense and host life history. - Am. Nat. 160: S9–S22.

Zuur, A. F. et al. 2009. Mixed effects models and extensions in ecology with R.

Figure legends

Figure 1: **Experimental design.** The parental generation are dosed with one of six infection or control treatments in the fourth instar (Bb = dip in *B. bassiana* solution, Bt = droplet dose with *B. thuringiensis*, Co = coinfection with both pathogens, Drop = dosed with control droplet of sugar water and food dye, Dip = control dip in distilled water, Na = naïve larvae are handled only). Mothers were mated with males from the stock population, and the eggs from each family were divided between two diet qualities (P = poor diet (20:1:1 wheat bran, brewer's yeast, glycerol), G = good diet (10:1:1)). When the offspring reached fourth instar, they were assigned to one of three pathogen treatments (Bb = dip in *B. bassiana* solution, Bt = droplet dose with *B. thuringiensis*, Co = coinfection with both pathogens).

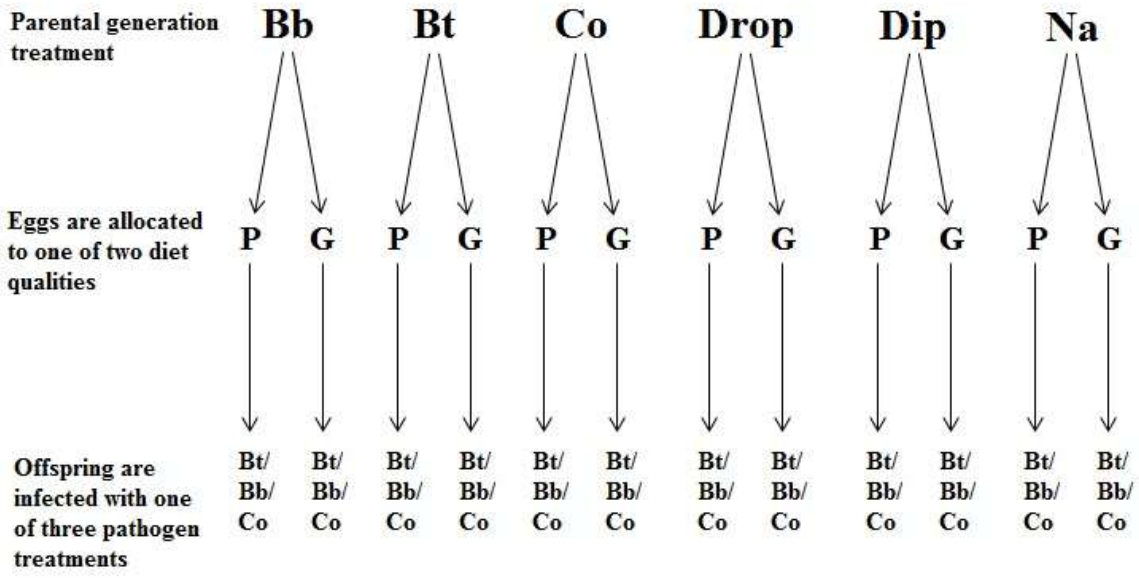

Figure 2: **Diet-dependent survival of Bt-infected offspring:** More offspring survive Bt infection when on the good diet than when nutritionally stressed; error bars are Agresti-Coull intervals calculated in the "binom" R package (Dorai-Raj 2014).

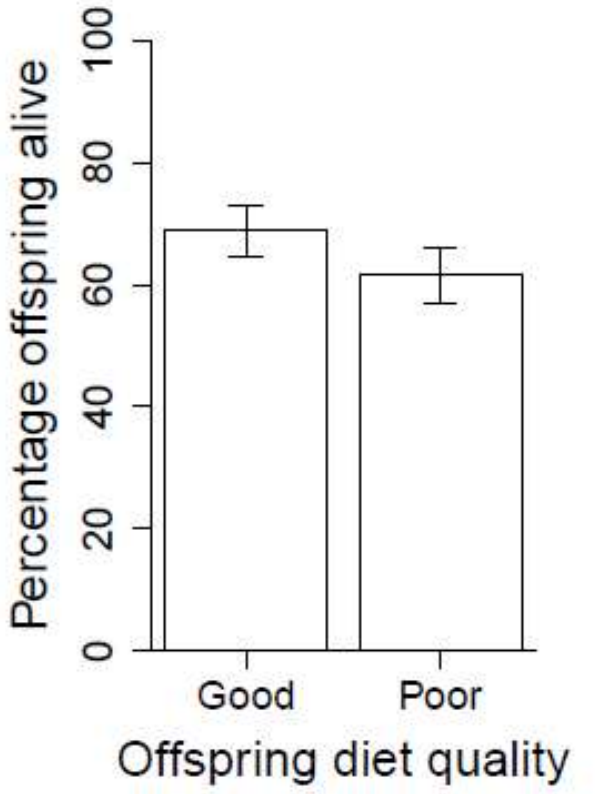

Figure 3: **Interaction between maternal exposure to** Bb**and offspring diet quality**: There was a significant interaction between maternal exposure to Bb and the diet quality available to larvae; error bars are Agresti-Coull intervals; legend refers to offspring diet quality.

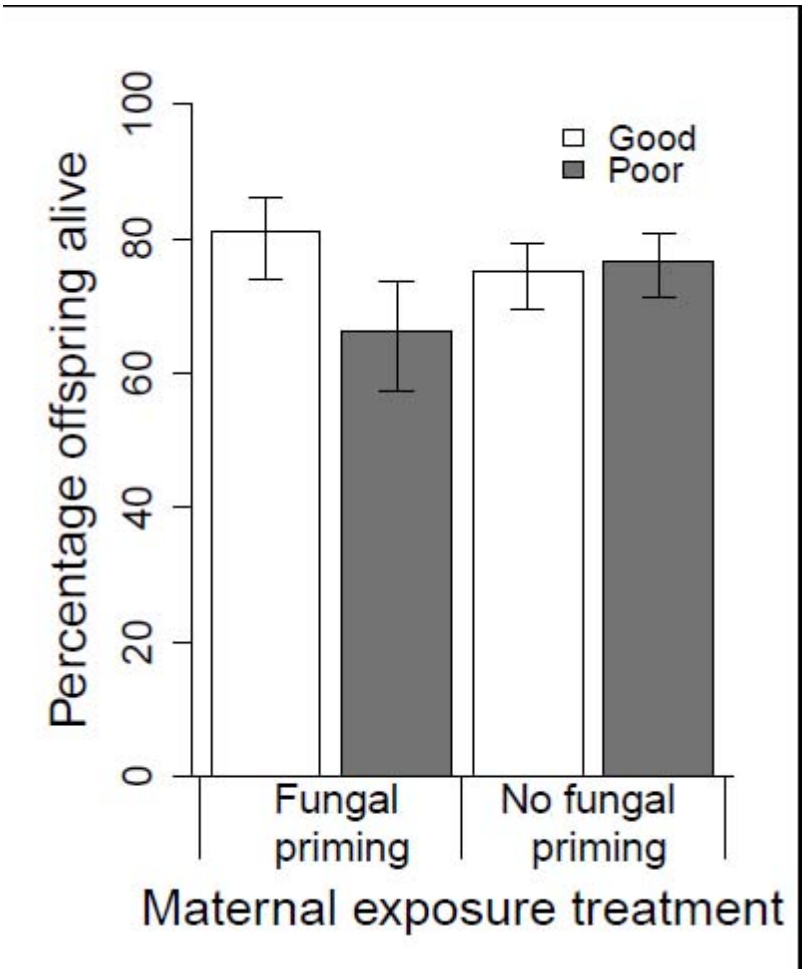

Figure 4: **Effect of maternal infection on offspring**: Maternal infection with Bt, including both the single exposure treatment and coinfection with two pathogens, resulted in reduced numbers of offspring surviving when challenged with both pathogens when compared with offspring from mothers given a fungal or control treatment; error bars are Agresti-Coull intervals; X axis: Bt = droplet dose with *B. thuringiensis*, Coinfection = additive doses of both pathogens, Bb = dip in *B. bassiana* solution, Control.