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Baculovirus-challenge and poor nutrition inflict within-generation fitness costs without triggering transgenerational immune priming

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ABSTRACT

Invertebrate hosts that survive pathogen challenge can produce offspring that are more resistant to the same pathogen via immune priming, thereby improving the fitness of their offspring in the same pathogen environment. Most evidence for immune priming comes from exposure to bacteria and there are limited data on other groups of pathogens. Poor parental nutrition has also been shown to result in the transgenerational transfer of pathogen resistance and increased immunocompetence. Here, we combine exposure to an insect DNA virus with a change in the parental diet to examine both parental costs and transgenerational immune priming. We challenged the cabbage looper, *Trichoplusia ni*, with a low dose of the baculovirus, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and altered dietary protein to carbohydrate ratio (p:c ratio) after virus exposure. Insects fed a low protein diet had lower haemolymph protein concentrations, and exhibited costs of smaller pupae and slower development, while survivors of virus challenge developed more slowly, irrespective of p:c ratio, and those that were virus-challenged and fed on a low protein diet showed a reduction in haemocyte density. In addition, AcMNPV-challenged parents laid fewer eggs earlier in egg laying although egg size was the same as for unchallenged parents. There was no evidence for increased resistance to AcMNPV (immune priming) or changes in haemocyte number (as proxy for constitutive cellular immunity) in the offspring either as a result of parental AcMNPV-challenge or low dietary p:c ratio. Therefore, although pathogen-challenge and nutritional changes can affect host development and reproduction, this does not necessarily translate into transgenerational immune priming. Our findings contrast with an earlier study on another type of baculovirus, a granulovirus, where immune priming was suggested. This indicates that transgenerational immune priming is not universal in invertebrates and is likely to depend on the host-pathogen system, or the level of pathogen exposure and the type of dietary manipulation. Identifying whether immune priming or transgenerational effects are relevant in field populations, remains a challenge.

1. Introduction

Pathogens are a ubiquitous threat to all organisms. They exploit host resources for their own growth and reproduction, and in doing so inflict significant fitness costs on their hosts by reducing host survival, longevity and reproductive output (Schmid-Hempel, 2011). Therefore, there is likely to be strong pressure to reduce the risk and costs of infection, which should be reflected in investment in disease resistance. While evidence for a specific, long-term immune memory in vertebrates is well established, it is only in recent years that enhanced resistance in invertebrates, as a consequence of surviving pathogen exposure, has been demonstrated (e.g. Sadd and Schmid-Hempel, 2006; Tidbury et al., 2011; McTaggart et al., 2012), although not in all invertebrate-pathogen combinations (Longdon et al., 2013; Reber and Chapuisat, 2012). This response has been termed "immune priming", and can also result from other triggers such as dead pathogens or immune elicitors. In less mobile invertebrates or those species that have multiple generations in a year, there is a high likelihood that future generations will be exposed to the same parasites. Therefore protection from parasites should be extended from parents to offspring. Transgenerational immune priming (TGIP) has been shown in several systems and is assumed to be an adaptive response whereby environmental cues stimulate parents to enhance the fitness of their offspring in a similar environment (Badyaev and Uller, 2009; Mousseau and Fox, 1998). Consequently, if there is a mismatch in the pathogens that parents and offspring are exposed to, additional costs can be incurred by the offspring,

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such as increased susceptibility to an unrelated pathogen or reduced reproductive output (Roth et al., 2010; Sadd and Schmid-Hempel, 2009; Trauer and Hilker, 2013; Zanchi et al., 2011).

Evidence for elevated resistance to pathogens and/or immunocompetence in offspring mostly come from studies that expose the parental generation to injected dead pathogens, immune elicitors or mechanical wounding (e.g. Sadd et al., 2005; Moret, 2006; Sadd and Schmid-Hempel, 2007; Roth et al., 2010; Hernández López et al., 2014). Although these studies remove the effect of pathogen multiplication and possible vertical transmission, injection is an unnatural exposure route and it is not clear how relevant these studies are to actual pathogen infection. There are surprisingly few studies where live pathogens are used and the most convincing evidence for TGIP comes from studies using naturally introduced, live bacteria (Freitak et al., 2014, 2009a, 2009b; Little et al., 2003; Shikano et al., 2015). There is recent evidence, however, that offspring are also more resistant after parental exposure to an insect granulovirus (Tidbury et al., 2011) and that offspring survival time increases after parental exposure to an entomopathogenic fungus, and that this is strain specific (Fisher and Hajek, 2015). How widely TGIP is found and whether it varies with either host or pathogen is not clear.

Elevated transgenerational disease resistance or immunity is not limited to the exposure to pathogens or pathogen components; dietary and environmental factors can also affect immunity and disease resistance. Stress in the parental generation, such as reduced food quality or quantity as might occur at high population density, could act as a cue for increased disease risk in the next generation. In addition, in a less than optimal parental environment there may be trade-offs between disease resistance and other life history traits. Parents that experience nutritional stress can produce offspring that are more resistant to pathogens (Ben-Ami et al., 2010; Boots and Roberts, 2012; Shikano et al., 2015; Stjernman and Little, 2011). The complex relationship between nutrient availability and disease resistance has prompted several studies to look at the interaction between parental pathogen exposure and nutrition on offspring immunity and pathogen susceptibility. Cabbage looper moths, *Trichoplusia ni*, exposed to a sublethal concentration of the bacterium, *Bacillus thuringiensis* (*Bt*), produced offspring that were more resistant to *Bt* regardless of parental diet quality but if parents were not exposed to *Bt*, those that consumed diluted diet produced offspring that were more resistant to *Bt* and an additional pathogen (baculovirus) (Shikano et al., 2015). In the water flea *Daphnia magna*, offspring were more resistant to the bacterial pathogen *Pasteuria ramosa* if the maternal environment was poor (a combination of low food, crowding and the build-up of metabolites), whereas the effect of maternal pathogen exposure on offspring resistance depended on host genotype and offspring food quality (Mitchell and Read, 2005). In contrast, parental exposure to a baculovirus and reduced food in the western tent caterpillar had no impact on disease resistance in the next generation (Myers et al., 2011). Thus, the interactive effects of parental pathogen exposure and nutrition are likely to vary with the host-pathogen system and the method of nutrient manipulation.

Costs associated with successfully fighting off parasite challenge are clearly evident in invertebrate host-parasite systems where infections in the host larval stage are cleared before the adult stage, and these often include prolonged development, and reduced adult size and fecundity (Fellowes et al., 1999; Milks et al., 1998; Myers et al., 2000; Shikano et al., 2015). The resource allocation trade-off between immune defense and other life history traits should be alleviated by an increase in the quantity or quality of dietary nutrients (Lochmiller and Deerenberg, 2000; Schmid-Hempel, 2005; Sheldon and Verhulst, 1996), as an influx of nutrients would boost resources needed to mount an effective immune response and repair damaged host tissues, thereby lessening the allocation of resources away from other traits. Interestingly, the cotton leafworm, *Spodoptera littoralis*, and the African armyworm, *Spodoptera exempta*, significantly reduced their chances of dying after challenge by their species-specific baculoviruses by composing a higher dietary protein to carbohydrate ratio (p:c ratio; Lee et al., 2006; Povey et al., 2014). Increasing dietary p:c ratio was associated with heightened immune functioning in these studies.

In a recent study, we found that larvae of *T. ni*, challenged with the baculovirus, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) across a range of dietary p:c ratios and temperatures, showed higher survival with increasing p:c ratio, particularly at moderate and high rearing temperatures (Shikano and Cory, 2015). Here we examined whether TGIP occurs in this system and whether the outcome is altered by parental diet quality post virus challenge. AcMNPV is a particularly interesting pathogen for studying the universality of TGIP in invertebrates, because it has a broad potential host range (Cory and Myers, 2003). AcMNPV is also known to suppress the host immune response (Jakubowska et al., 2013; Nobiron et al., 2003; Ooi and Miller, 1988; Salem et al., 2011), which might guard against the parental transfer of heightened immunity to the next generation. We conducted a comprehensive investigation of the costs associated with AcMNPV-challenge in *T. ni* reared post-challenge on a high or low p:c ratio diet by measuring their survival, development time, pupal weight and condition (i.e. as measured by haemolymph protein concentration), as well as their fecundity, egg size and rate of egg-laying. We also assessed the mortality rate and cellular immune response (haemocyte numbers) of parents challenged with AcMNPV, and the impact of parental exposure to AcMNPV and dietary p:c ratio on offspring immunocompetence (haemocyte numbers) and resistance to AcMNPV. The same *T. ni* line that exhibited strong TGIP in response to *Bt*-challenge and nutrient dilution (Shikano et al., 2015) was used. We hypothesized that: (1) parental virus challenge would result in offspring immune priming; (2) low p:c ratio diet would also result in immune priming; (3) parents challenged by virus on a high p:c ratio diet would transfer immune priming to their offspring while suffering less costs because the high p:c ratio diet will allow parents to compensate for resources used to fight-off virus challenge. (4) Virus-challenge on low p:c ratio diet would induce a resource trade-off such that parents will transfer immune priming to their offspring but suffer higher developmental costs.

2. Materials and methods

2.1. Insects and virus

T. ni (Noctuidae) eggs originated from a colony maintained at Simon Fraser University that were reared at 25 °C and L16:D8 on a wheat germ based artificial diet (colony diet) that has a protein to carbohydrate ratio of approximately 1p:1.1c. The *A. californica* multiple nucleopolyhedrovirus (AcMNPV) strain E2 used to challenge the parental and offspring generations of *T. ni* was obtained from Dr. Martin Erlandson (Agriculture and Agri-Food Canada, Saskatoon Research Centre).

2.2. Treatment diets

Treatment diets were isocaloric, consisting of different ratios of protein (casein) and digestible carbohydrate (sucrose) that made up 60% of the dry weight (Shikano and Cory, 2014a). The protein-rich diet contained 40% protein and 20% carbohydrate, and the carbohydrate-rich diet contained 20% protein and 40% carbohydrate. These two p:c ratios are within the range found in *T. ni* host