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Transgenerational effects modulate density-dependent prophylactic resistance to viral infection in a lepidopteran pest

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ABSTRACT

There is an increasing appreciation of the importance of transgenerational effects on offspring fitness, including in relation to immune function and disease resistance. Here, we assess the impact of parental rearing density on offspring resistance to viral challenge in an insect species expressing density-dependent prophylaxis (DDP); i.e. the adaptive increase in resistance or tolerance to pathogen infection in response to crowding. We quantified survival rates in larvae of the cotton leafworm (*Spodoptera littoralis*) from either gregarious- or solitary-reared parents following challenge with the baculovirus *Spodoptera littoralis* nucleopolyhedrovirus. Larvae from both the parental and offspring generations exhibited DDP, with gregarious-reared larvae having higher survival rates post-challenge than solitary-reared larvae. Within each of these categories, however, survival following infection was lower in those larvae from gregarious-reared parents than those from solitary-reared, consistent with a transgenerational cost of DDP immune up-regulation. This observation demonstrates that crowding influences lepidopteran disease resistance over multiple generations, with potential implications for the dynamics of host-pathogen interactions.

Keywords: *Spodoptera littoralis*, nucleopolyhedrovirus, insect immunity, transgenerational, immune-priming, density-dependent prophylaxis

1. INTRODUCTION

A number of recent studies have shown that invertebrates surviving disease exposure may produce offspring with enhanced disease resistance; a phenomenon known as “transgenerational immune priming” (1-4). This acquired protection against infection in invertebrates may follow an initial exposure to the same parasite, a different parasite or an immune response elicitor. However, other mechanisms enhancing offspring disease resistance, independent of parental pathogen pre-exposure, have not been widely investigated, although some recent studies have examined the impact of parental nutrition and crowding (5-8).

Many erupting insect pest species, including locusts (9) and forest lepidopterans (10), fluctuate between one or more generations at low population density and one or more generations at high density. In phase-polyphenic species (12), individuals at low density express “solitarious” phase characteristics (cryptic coloration, slow growth and sluggish behaviour), whereas at high densities they exhibit “gregarious” characteristics (conspicuous coloration, fast growth and active behaviour). Because the probability of encountering infectious disease agents often increases with population density, due to density-dependent disease transmission (11), increased investment in immune defence is often observed when hosts are crowded; a phenomenon known as “density-dependent prophylaxis” (DDP) (12). As a consequence, insects exhibiting DDP often show reduced susceptibility to pathogen attack with increasing population density (13).

It follows that in many outbreaking pest species, offspring of gregarious parents may be more likely to experience crowded conditions than those from solitarious parents (14), and thus transgenerational transmission of ‘phase’ characteristics could be adaptive (15). However, if DDP or other phase-polyphenic

traits are costly to express, then the offspring of individuals investing in them may suffer reduced fitness. From a fundamental and applied perspective, therefore, it is important to establish whether the offspring from these different parental phenotypes also differ in their susceptibility to pathogens, especially since a number of the pathogens are also used as biopesticides. *Spodoptera littoralis* (Lepidoptera: Noctuidae) is an eruptive agricultural pest that displays extreme phase-polyphenism and density-dependent prophylaxis (16). The aim of this study was to quantify transgenerational DDP effects and to establish whether parents displaying DDP produce offspring that are more or less resistant to baculovirus infection than those from solitary parents that have not prophylactically up-regulated their immune function.

2. MATERIALS AND METHODS

2.1 Insects and pathogen handling

A laboratory culture of *S. littoralis* was maintained on a standard wheatgerm-based semi-artificial diet (16) and kept at a constant temperature of 25°C under a 12h:12h light:dark regime. *S. littoralis* nucleopolyhedrovirus (*Spli*NPV) was amplified in *S. littoralis* larvae and purified following manual homogenization and a low speed spin (400 *g*) to remove larval debris (17). The concentration of virus occlusion bodies (OBs) was estimated using a Neubauer haemocytometer with replicated samples taken at two dilutions.

2.2 Experimental design and pathogen challenge

Upon hatching, neonate larvae were reared in either solitary (1 larva per 30 ml pot) or gregarious (6/pot) conditions (Figure 1). They were maintained in these conditions until 24h post-moult into the 3rd-instar. For the parental bioassay,

gregarious and solitary 3rd-instar larvae were orally-challenged with one of seven doses of SpliNPV on a small amount of artificial diet (30 larvae per treatment of $1x10^4$ OB; $5x10^3$ OB; $1x10^3$ OB; $5x10^2$ OB; $1x10^2$ OB; $5x10^1$ OB; and control dH_2O). Only larvae that ingested all of the virus-loaded diet within 24h were retained. Larvae were subsequently monitored daily for virus-induced mortality until pupation. None of the larvae in the control group died of viral infection. Remaining solitary and gregarious larvae were maintained in culture not exposed to SpliNPV, and emerging moths paired for mating (solitary with solitary; gregarious with gregarious).

The resulting offspring were reared in either solitary (1/pot) or gregarious (6/pot) conditions. For the offspring bioassay, 210 solitary and 210 gregarious 3rd-instar larvae from solitary-reared (non-challenged) parents, and 210 solitary and 210 gregarious 3rd-instar larvae from gregarious-reared (non-challenged) parents were orally-dosed with the same seven doses of SpliNPV as above (30 larvae per treatment). All analyses were undertaken using the *R* statistical package (version 3.0.1). Survival rates were compared using a parametric survival regression model with a lognormal distribution using the *survreg* procedure in *R*.

3. RESULTS

3.1 Parental generation: effect of larval phase on virus-induced mortality

As expected, larval survival post-challenge in the parental generation declined significantly with viral dose and increased with larval size at dosing (Survival model: $\log_{10}$-dose: $z = -9.97$, $P < 0.0001$; larval weight: $z = 2.29$, $P = 0.022$). Moreover, consistent with the DDP hypothesis, after accounting for these effects, larval survival was significantly higher in gregarious than solitary larvae (larval phase: $z = -2.39$, $P =$

0.017; full model likelihood ratio test: χ^2_3 = 151.5, P < 0.0001, n = 359 larvae; Figure 2 inset).

3.2 Offspring generation: effect of larval and parental phase on virus-induced mortality

In the offspring generation, survival again declined significantly with viral dose and increased with larval size at dosing (Survival model: $\log_{10}$-dose: z = -14.78, P < 0.0001; larval weight: z = 4.73, P < 0.0001). Survival was also significantly higher in gregarious than in solitary larvae (offspring phase: z = -2.17, P = 0.030), consistent with DDP. However, after accounting for these effects, larval survival was also significantly higher in larvae from solitarious parents than from gregarious parents (parental phase: z = 3.00, P = 0.0027; full model likelihood ratio test: χ^2_4 = 244.1, P < 0.0001, n = 719 larvae), such that gregarious-offspring from solitarious parents were most resistant to viral infection and solitary-reared offspring from gregarious parents were least resistant (Figure 2); the interaction between larval and parental phase was non-significant (offspring phase * parental phase: z = 0.565, P = 0.57), indicating that these phase effects were additive. The transgenerational effects are unlikely to be explained simply by variation in body condition, since larval weight did not vary with the phase-state (solitary or gregarious) of either the offspring or their parents (Linear model: larval phase: $F_{1,717}$ = 0.70, P = 0.40; parental phase: $F_{1,717}$ = 1.59, P = 0.21).

4. DISCUSSION

The key finding of the present study is that the magnitude of the enhanced resistance gained by *S. littoralis* larvae exhibiting density-dependent prophylaxis is

contingent on the phase-state of their parents. Thus, whilst DDP resistance to viral-challenge was evident in both the parental and offspring generations, larvae in the offspring generation were significantly more likely to succumb to baculovirus infection if their parents had been reared gregariously than if they had been reared solitarily. This could be a result of trangenerational immune suppression incurred by larvae from gregarious parents or transgenerational immune priming of larvae from solitary parents.

Although a number of studies have previously examined disease resistance in (immune-primed) offspring from pathogen-challenged parents e.g. (3, 18), to our knowledge only one previous study has shown that parental density impacts on offspring pathogen resistance. Miller & Simpson (19) challenged day-old desert locusts (*Schistocerca gregaria*) from solitary- or crowd-reared parents with a single dose of the fungal pathogen *Metarhizium acridum*. As here, they found that crowded parents produced offspring that were more susceptible to pathogen challenge than offspring from parents reared solitarily. Thus, both studies show that crowding in the parental generation negatively impacts on the ability of offspring to resist pathogen challenge. The Miller & Simpson study, however, did not quantify DDP in either the parental or offspring generations and so it is unclear whether disease resistance mechanisms were up-regulated in the gregarious parents (but see (20)), and hence whether there was a mismatch between density-effects mediated via the parental and offspring generations.

The present study also demonstrates, for the first time, DDP resistance to baculovirus infection in larval *S. littoralis*. Previous studies on this and similar species suggest that DDP could be due to density-dependent changes in a number of constitutive immune responses, including lysozyme activity, phenoloxidase activity

and encapsulation (16, 21). However, at present, we do not know how the transgenerational DDP response is mediated in this system, nor whether these effects are transmitted by just one or both parents (since both parents experienced the same rearing conditions in this experiment). However, Triggs & Knell (7) found that in *Plodia interpunctella* offspring immune function was down-regulated if one or both parents were restricted to a low-quality diet and argue that this was an adaptive response mediated by epigenetic imprinting.

In order for the transgenerational responses observed here to also be adaptive, it would require the high-density conditions favouring a DDP response in the parental generation to be commonly followed by low-density conditions favouring reduced constitutive immune function in the offspring generation, and for there to be a trade-off between constitutive immune function and some other life-history trait(s) such that individuals expressing reduced immune function at low densities benefit from enhanced fitness. This may well be true for some phase-polyphenic insects, like *Spodoptera* caterpillars, that use density-dependent cues to trigger phenotypic changes that enhance dispersal to lower densities (22) and where trade-offs with constitutive immunity have been identified (13, 16, 23, 24). Alternatively, crowding-induced up-regulation of immune function (or other costly phenotypic changes associated with phase-change) may deplete parents of resources that would otherwise be invested in offspring. However, we found no difference in the larval weights of offspring in relation to parental or offspring rearing density, suggesting that and costs are not reflected in body size.

It is known that strong transgenerational effects have the potential to impact population dynamics and that delayed density-dependent effects, such as those observed here, are generally destabilizing and may lead to complex dynamics such

as stable-limit cycles (25). Indeed, previous models of DDP suggest that the time-delay between changes in population density and the appearance of resistance effects is critical for determining the dynamics of the host-pathogen interaction (26). The inclusion of transgenerational effects is likely to complicate the dynamical outcome still further (6), with potentially important consequences for assessing the long-term success of pathogens as biocontrol agents, or for predicting the severity of natural disease outbreaks.

DATA ACCESSIBILITY

The data supporting this manuscript will be deposited in Dryad.

COMPETING INTERESTS

None.

AUTHORS' CONTRIBUTIONS

RIG carried out the lab work, KW performed the statistical analyses; both authors drafted the manuscript.

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279 **Figure 1.** Diagrammatic representation of the experimental design. G_0, G_1 and G_2
280 are the 3 generations of insects used in the experiment.

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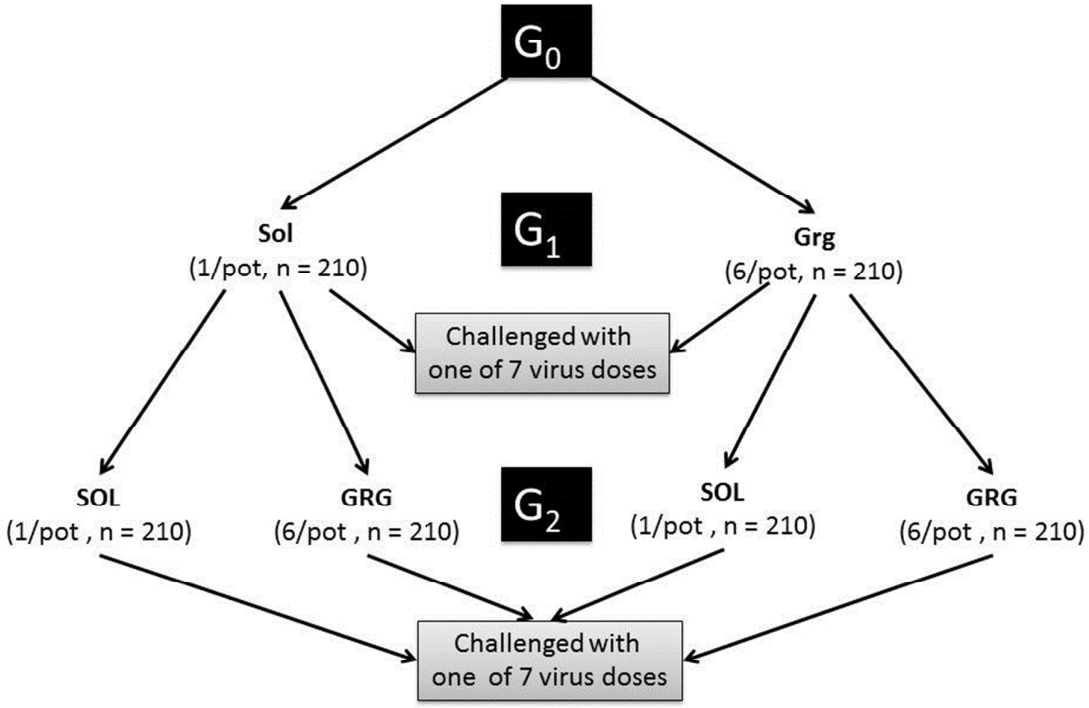

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284 **Figure 2.** Kaplan-Meier survival curves of solitary (SOL) and gregarious (GRG)
285 larvae in the offspring generation (G_2), relative to the rearing density in the parental
286 generation, G_1 (solitary – Sol; gregarious – Grg). Inset = survival curves for parental
287 generation larvae, G_1.

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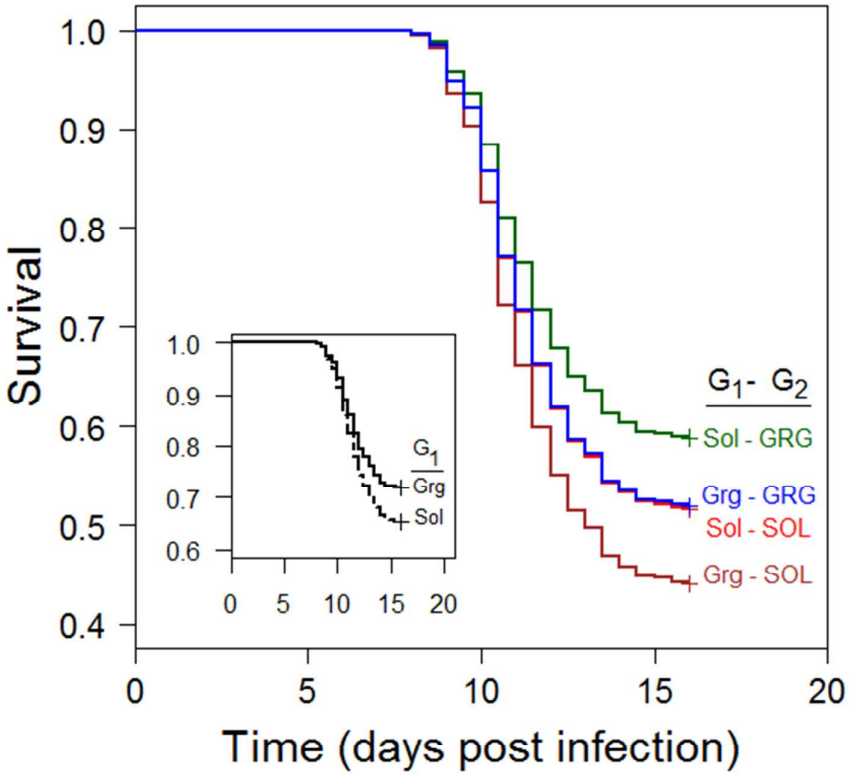

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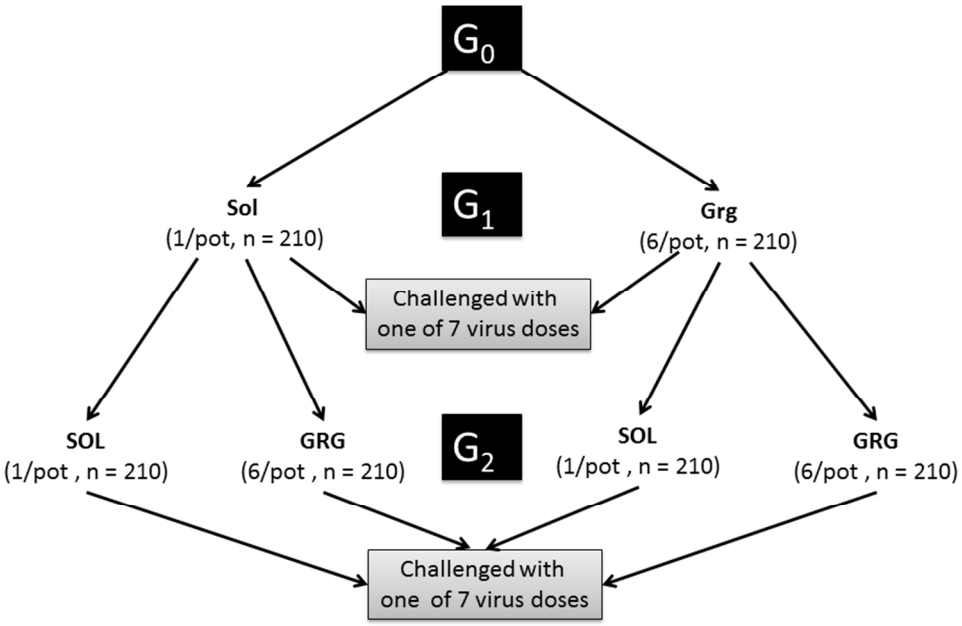

Diagrammatic representation of the experimental design. G_0, G_1 and G_2 are the 3 generations of insects used in the experiment.
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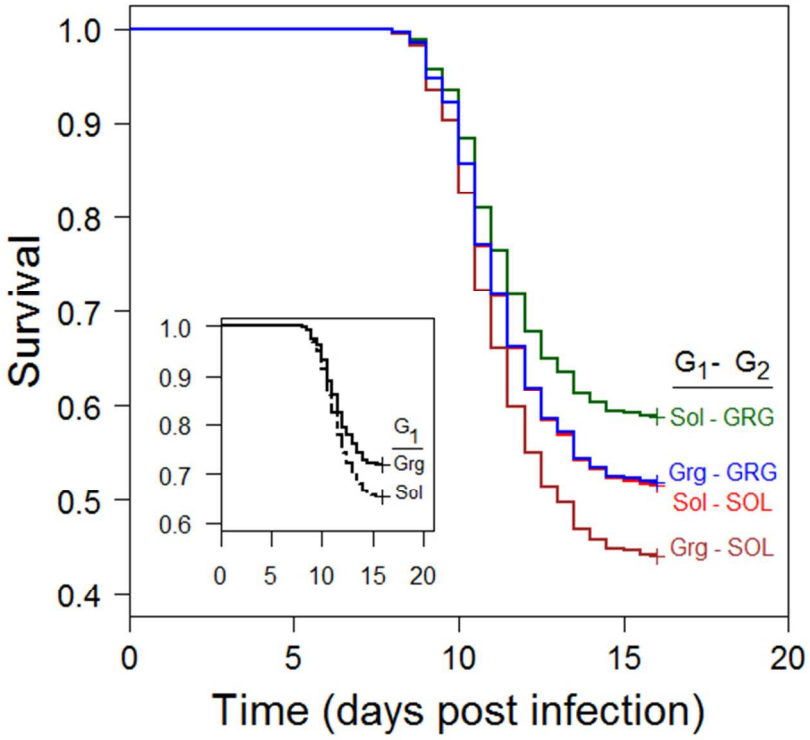

Kaplan-Meier survival curves of solitary (SOL) and gregarious (GRG) larvae in the offspring generation (G_2), relative to the rearing density in the parental generation, G_1 (solitary – Sol; gregarious – Grg). Inset = survival curves for parental generation larvae, G_1.
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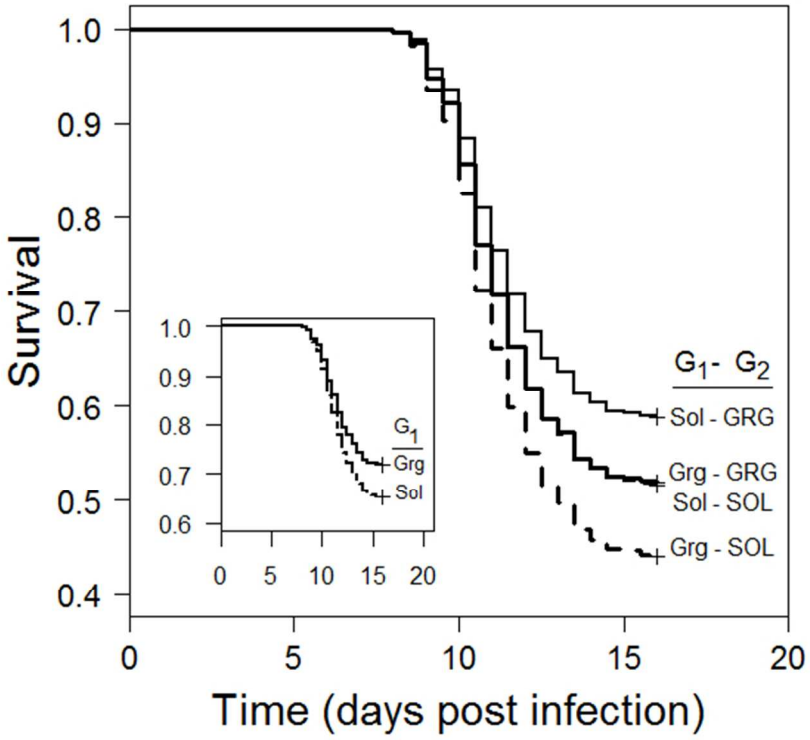

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