





# Trans-generational viral transmission and immune priming are dose-dependent

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## Abstract

1. It is becoming increasingly apparent that trans-generational immune priming (i.e. the transfer of the parental immunological experience to its progeny resulting in offspring protection from pathogens that persist across generations) is a common phenomenon not only in vertebrates, but also invertebrates. Likewise, it is known that covert pathogenic infections may become 'triggered' into an overt infection by various stimuli, including exposure to heterologous infections. Yet, rarely have both phenomena been explored in parallel.
2. Using as a model system the African armyworm *Spodoptera exempta*, an eruptive agricultural pest and its endemic dsDNA virus (*Spodoptera exempta* nucleopolyhedrovirus, SpexNPV), the aim of this study was to explore the impact of parental inoculating-dose on trans-generational pathogen transmission and immune priming (in its broadest sense).
3. Larvae were orally challenged with one of five doses of SpexNPV and survivors from these treatments were mated and their offspring monitored for viral mortality. Offspring from parents challenged with low viral doses showed evidence of 'immune priming' (i.e. enhanced survival following SpexNPV challenge); in contrast, offspring from parents challenged with higher viral doses exhibited greater susceptibility to viral challenge.
4. Most offspring larvae died of the virus they were orally challenged with; in contrast, most offspring from parents that had been challenged with the highest doses were killed by the vertically transmitted virus (90%) and *not* the challenge virus.
5. These results demonstrate that the outcome of a potentially lethal virus challenge is critically dependent on the level of exposure to virus in the parental generation—either increasing *resistance* at very low parental viral doses (consistent with trans-generational immune priming) or increasing *susceptibility* at higher parental doses (consistent with virus triggering).
6. We discuss the implications of these findings for understanding both natural epizootics of baculoviruses and for using them as biological control agents.

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## KEYWORDS

baculovirus, immune priming, outbreak pest, vertical transmission, virus triggering

## 1 | INTRODUCTION

Until recently, it was generally assumed that the innate immune system of invertebrates lack the specificity and memory of the vertebrate adaptive immune response (Fearon, 1997; McFall-Ngai, 2007; Netea et al., 2015). However, it has become increasingly clear in the last two decades that the immune system of invertebrates also possesses elements of immune memory (Kurtz & Franz, 2003). This immune memory is induced by a 'priming' response, in which sublethal exposure to a pathogen or parasite enhances host protection to a future (but not necessarily related) immune challenge (Koch & Schmid-Hempel, 2011; Moret & Schmid-Hempel, 2000; Pham et al., 2007; Tate et al., 2017). There is evidence for this immune priming response in a range of invertebrate species including, for example, honeybees (López et al., 2014), bumblebees (Barribeau et al., 2016; Sadd et al., 2005), tenebrionid beetles (Knorr et al., 2015; Milutinović et al., 2016; Roth et al., 2010), and the Indian meal moth *Plodia interpunctella* (Tidbury et al., 2011). Immune priming may also be transmitted across generations; a phenomenon referred to as trans-generational immune priming (TGIP). For example, in the water flea *Daphnia magna*, offspring from mothers primed with the bacterium *Pasteuria ramosa* suffered less of a reduction in fitness, in terms of reproductive output, when subsequently infected with this same bacterium (Little et al., 2003). Other examples of TGIP are found in Coleoptera, Crustacea, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mollusca, Nematoda and Orthoptera (see Tetreau et al., 2019 for references).

Baculoviruses are almost ubiquitous within lepidopteran species, and it is becoming ever more apparent that they have an extremely close association with their hosts (Williams et al., 2017). Although the phenomenon of trans-generational (vertical) transmission of baculoviruses has long been recognised (Kukan, 1999; Swaine, 1966), more recent molecular studies have highlighted the presence of 'covert' baculovirus infections in Lepidoptera, which do not display any obvious signs of infection or detrimental effects, although some sublethal effects may be observed (Burden et al., 2003; Graham et al., 2015; Kemp et al., 2011; Murillo et al., 2011). Some studies have shown that when larvae are subjected to periods of stress, such as poor diet, crowding or exposure to other pathogens, covert infections may be triggered into lethal overt infections that produce infectious occlusion bodies (OB) that can initiate subsequent horizontal transmission of virus (Virto et al., 2017; Williams et al., 2017). Thus vertical baculovirus transmission can be an important factor impacting the dynamics of lepidopteran populations, especially those of economic and agricultural importance (Graham et al., 2012). One of these is the African armyworm, *Spodoptera exempta*, a major pest in sub-Saharan Africa, attacking cereals such as maize, rice, millet and pasture grasses (Rose et al., 2000). During its larval stage, *S. exempta* is susceptible to an endemic baculovirus,

SpexNPV (Swaine, 1966) (Graham et al., 2012), which infects larvae through the oral ingestion of viral OBs. When the OBs enter the midgut, their protein coat is dissolved and virions are released into the midgut cavity and enter midgut epithelial cells. Secondary tissue infection occurs once the virus has passed through the midgut and virus proliferation in fat bodies and other tissues leads to cell lysis and tissue destruction. Host death typically occurs within 4–7 days of larval infection; adult moths may carry low-level covert viral loads, and may suffer sublethal effects on fertility, but do not die of infection (Grzywacz et al., 2008). Vertical transmission of SpexNPV is common (Swaine, 1966; Vilaplana et al., 2010) and is believed to contribute to the persistence of SpexNPV in field populations of *S. exempta* that are subject to major annual population fluctuations, driven by the seasonal rains in sub-Saharan Africa (Graham et al., 2012; Vilaplana et al., 2010). In field populations of *S. exempta* larvae, overt viral disease and covert sublethal viral loads tend to increase across successive outbreaks during the rainy season due to intense horizontal transmission of SpexNPV in the high-density populations created by high population growth (Chapman et al., 2015; Graham et al., 2012; Rose et al., 2000), potentially providing a strong selective advantage to TGIP.

While previous studies have independently examined the vertical transmission of pathogens and trans-generational immune priming, as far as we are aware, no previous studies have explored the ecological interaction between these two phenomena. Thus, the aim of this study was to simultaneously study the effects of trans-generational viral transmission and immune priming (in its broadest sense, to include mechanisms not directly associated with the host innate immune defences) in the offspring of virally challenged parents. Specifically, we addressed the following questions: (a) How is the covert viral load of adult moths affected by the magnitude of the viral challenge they receive as larvae? (b) What is the trans-generational impact of parental viral dose on offspring mortality, fitness and covert viral load? (c) What is the trans-generational effect of parental virus challenge (and covert viral load) on offspring susceptibility to subsequent challenge with a heterologous virus strain?

## 2 | MATERIALS AND METHODS

## 2.1 | The insect–virus system

The *Spodoptera exempta* laboratory culture used in this study was collected in central Tanzania in January 2010, and maintained on standard wheatgerm diet (Reeson et al., 1998; Vilaplana et al., 2010) at a constant temperature of 27°C under a 12 hr light/dark cycle. Upon establishing the culture, diagnostic qPCR confirmed the presence of covert SpexNPV infection. High numbers (sufficient for

>100 mating pairs) were maintained at each generation to reduce inbreeding. We used two naturally occurring genetically distinct viral field isolates of SpexNPV (*13Mag9* and *4Mag2*), both of which were previously collected from individual armyworm larvae from high-density outbreaks in Tanzania (Graham et al., 2012). Both isolates are highly pathogenic to *S. exempta* with LD<sub>50</sub>s of 2,852 and 2,488 OB per 4th instar larva, respectively, and lethally infected individuals are easily distinguishable from healthy individuals.

## 2.2 | Experimental design

In brief, the experimental design (Figure 1) involved inoculating larvae of the 'parental' generation with one of six low doses of SpexNPV, isolate *4Mag2*, and quantifying the effects of the virus on larval mortality, development and growth, as well as the viral load of the adult moths once they had reproduced (using qPCR), followed by mating of the surviving moths. In the 'offspring' generation, larvae were dosed with one of five higher doses of SpexNPV using isolate *13Mag9*, quantifying larval mortality and the viral loads of surviving adults (using qPCR) and characterising the identity of the

isolate responsible for causing the death of any non-surviving larvae, *4Mag2* or *13Mag9* (using *EcoRV* digest).

## 2.3 | Viral inoculation of the parental generation

Experimental insects were established from the stock population. Bioassays were conducted following a standard baculovirus diet bioassay protocol (Evans & Shapiro, 1997); small diet-plugs (1 mm<sup>3</sup>) were inoculated with 0, 15, 25, 40, 60 and 150 SpexNPV OBs (estimated to be the control, LD<sub>5</sub>, LD<sub>10</sub>, LD<sub>15</sub>, LD<sub>20</sub> and LD<sub>25</sub> doses respectively). Viral isolate *4Mag2* was used in the parental bioassay. Newly moulted 4th-instar larvae were allowed to ingest the diet-plug ( $n = 25$  larvae per OB dose, except LD<sub>20</sub>, where  $n = 50$ ). After 24 hr, larvae that had eaten all the diet-plug were transferred to individual 30-mL diet pots containing standard wheatgerm diet (those that did not, 2/950, were excluded). Larvae were kept at 27°C and checked every 12 hr thereafter for viral mortality, until death or pupation, excluding larvae that died during the first 24 hr that were due to handling mortality. The following data were recorded for the surviving insects: date of pupation, weight, adult sex and date of emergence.

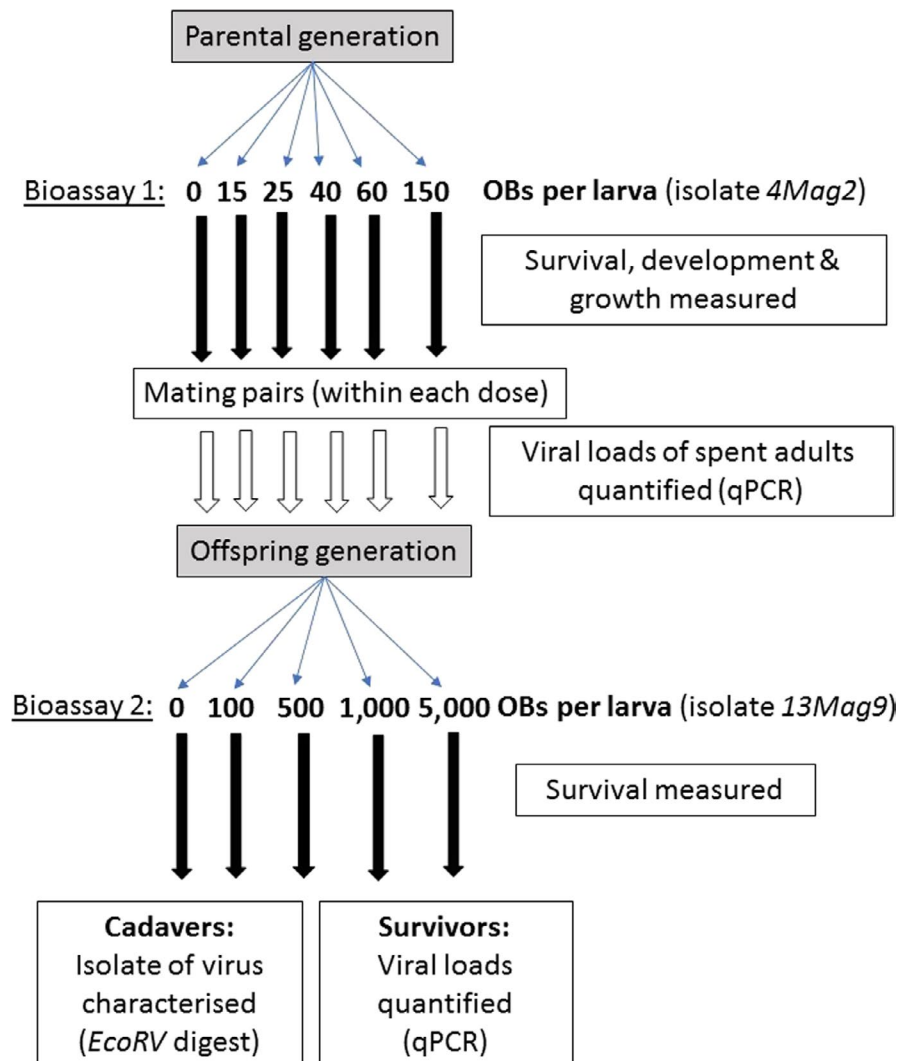


FIGURE 1 Summary of experimental design

## 2.4 | Prevalence of viral vertical transmission

Larvae surviving the viral inoculations were individually paired together with a survivor from the same treatment group. Between 7 and 15 pairs were set up per treatment. The dates of set-up, egg-laying and egg-hatch were recorded, as well as number of eggs laid; none of the eggs were surface-sterilised, so allowing both trans-ovum and trans-ovarial transmission of virus. Neonate larvae hatching from the above pairs (usually the same egg mass) were reared gregariously for 48 hr, and then 60 larvae per pair were separated into individual diet pots. Survival and mortality of larvae was monitored and NPV-deaths were confirmed by identification of OB in larva under phase contrast microscope ( $\times 1,000$ ).

## 2.5 | Bioassay to assess offspring resistance to baculovirus challenge

Offspring from the six parental treatment groups were used to test their susceptibility to dose-dependent oral-challenge with SpexNPV. A genotypically distinct viral isolate, *13Mag9*, was used in this bioassay, to distinguish any mortality from the isolate used in the parental bioassay (isolate *4Mag2*). The diet plug bioassay was performed with 0, 100, 500, 1,000 and 5,000 SpexNPV OBs per 4th instar larva, following the protocol detailed above.

## 2.6 | qPCR to assess intensity of covert virus infection

Taqman qPCR was used to quantify covert NPV infection within individual adult moths (Graham et al., 2015). Briefly, a 62 bp portion of the *polyhedrin* protein gene was amplified, whereby 5  $\mu$ l of genomic DNA was used as template in 25- $\mu$ l reactions containing 12.5  $\mu$ l  $2 \times$  TaqMan Universal PCR Master Mix, 0.4  $\mu$ M each of primers P1 and P2, and probe (Applied Biosystems). This is a highly conserved gene, identical within the two genotypic variants tested in this study. The forward primer CCC GTG TAC GTA GGA AAC AAC A, reverse primer CAA CCG CCG CCC TTC T and probe 6FAM-CGA GTA CCG CAT CAG CCT GGC C-TAMRA. Reactions were run on a Prism 7000 Real-Time Cycler (Applied Biosystems) in triplicate, using a two-step PCR method consisting of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 60 s. For each qPCR assay, a standard curve was constructed and negative controls (water instead of template DNA) were included in all reactions. Ten-fold serial dilutions of viral genomes were made in water, and each dilution was processed in triplicate on the same 96-well PCR plate with the samples. Data were analysed using Sequence Detection Software (1.2.3) 7000 (Applied Biosystems). SpexNPV viral loads are reported as number of viral genomes per  $\mu$ g of total DNA. The most reliable and repeatable limit of detection (LOD) of was equivalent to 5 viral genome copies of SpexNPV (Graham et al., 2015).

## 2.7 | Genetic characterisation of progeny virus

DNA from offspring-bioassay cadavers was isolated ( $n = 10$  per treatment) and progeny were then classified as either having died from *4Mag2* (isolate used in parental bioassay) or *13Mag9* (the isolate used in the offspring bioassay). Viral DNA was extracted as described previously (Graham et al., 2004). Briefly, OBs were lysed by addition of 0.5 M  $\text{Na}_2\text{CO}_3$ , 0.1% SDS and incubated for 2 hr at 37°C with proteinase K (200 mg/ml). DNA was purified by phenol/chloroform extractions, dialysed in  $1 \times$  TE buffer, and stored at 4°C until required. Purified viral DNA was digested with the endonucleases *EcoRV* (New England Biolabs) and fractionated in 0.8% agarose gels.

## 2.8 | Statistical analyses

All statistical analyses were performed using the R statistical package, version 4.0.2 (2020-06-22; R Core Team, 2020). Mortality analyses were performed using generalised linear models (GLMs) with binomial errors and logit links. Analyses of most life-history traits (pupal mass, development rates, etc.) were performed using linear models (LMs) or linear mixed-effects models (LMEs, using the *lmer* package) with parental ID as a random term, after transforming, if required, to conform to the Normality assumptions. Fecundity (number of eggs laid over 4 days or on specific days) was analysed using GLMs with quasi-Poisson errors. Viral loads, as determined by qPCR, were analysed using LMs after first log-transforming to conform to Normality. Survivorship was modelled using Cox's proportional hazards model, using the *coxph* function in the *survival* library of R. The predicted values from the model are visualised using thin-plate spline plots created using the *fields* package. These heat maps depict the risk or odds of dying (relative to 1), such that a risk score above 1 is higher risk than the average in the population and below 1 is lower risk than the average in the population.

## 3 | RESULTS

### 3.1 | Viral inoculation of the parental generation

As expected, larval survival declined significantly with the magnitude of the NPV inoculating dose (GLM with binomial errors:  $\log_{10}\text{dose}$ :  $\chi^2_1 = 20.62$ ,  $p < 0.0001$ ; Fig. S1); there were no larval deaths in the control group.

There was also evidence of sub-lethal effects of virus challenge in the survivors, with both pupal mass ( $F_{1,130} = 12.22$ ,  $p = 0.0006$ ) and the time taken to pupate ( $F_{1,130} = 29.22$ ,  $p < 0.0001$ ) declining with increasing NPV dose. The latter effect is probably a consequence of the strong negative relationship between larval development rate and pupal mass ( $F_{1,130} = 35.77$ ,  $p < 0.0001$ ), with viral challenge selecting against slowly developing larvae; viral dose failed to predict

pupal mass if larval development rate was included as a covariate in the model ( $F_{1,129} = 1.81$ ,  $p = 0.18$ ), although we cannot discount the possibility that viral infection acted as a cue to speed up larval development.

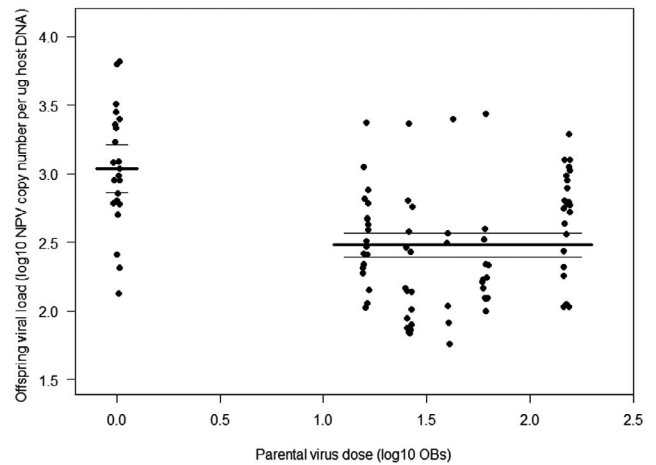
### 3.2 | Parental infection intensity and trans-generational effects

The putative sub-lethal effects observed at the pupal stage did not appear to impact fertility (see also Vilaplana et al., 2008). When survivors from the six virus treatment groups were paired up with mates from the same treatment group, there was no effect of the viral dose they experienced as larvae on the total number of eggs laid by the pair, either overall (GLM with quasi-Poisson errors:  $F_{1,35} = 0.43$ ,  $p = 0.52$ ), or on any individual day ( $p > 0.25$ ).

Following egg-laying, the adults were assayed via qPCR for their viral loads (i.e. intensity of infection). There was no relationship between the viral dose that individuals received as larvae and their subsequent covert viral load as spent adult moths (LM:  $F_{1,66} = 0.868$ ,  $p = 0.355$ ), with insects in the non-challenged control group having similar viral loads to those in the NPV-exposed groups, suggesting an endemic covert infection in the lab population (viral loads were too low to genotype these covert viruses; Graham et al., 2015; Vilaplana et al., 2010). There was, however, some evidence that offspring larval development rate ( $\chi^2_1 = 3.70$ ,  $p = 0.054$ ) and pupal mass ( $\chi^2_1 = 4.07$ ,  $p = 0.044$ ) were negatively correlated with the female parent's covert viral load; neither offspring trait was related to the male parent's covert viral load (larval development rate:  $\chi^2_1 = 0.72$ ,  $p = 0.40$ ; pupal mass:  $\chi^2_1 = 0.95$ ,  $p = 0.33$ ). Thus, females with high viral loads produced offspring that developed slightly slower and pupated at a slightly lower mass.

### 3.3 | Offspring infection intensity

The covert viral loads of the surviving offspring were also assayed by qPCR when they became adult moths. Viral loads were lower in moths with parents that had been dosed with NPV than in those that had not been dosed (LM: parental challenge:  $F_{1,96} = 28.70$ ,  $p < 0.0001$ ; Figure 2); within the group that had virus-challenged parents, however, viral loads did not vary with the magnitude of the parents' viral challenge ( $F_{1,74} = 3.29$ ,  $p = 0.074$ ). After accounting for the virus-challenge status of the parents, offspring viral load did not vary significantly with the viral load of the parents (dam viral load:  $F_{1,88} = 0.31$ ,  $p = 0.58$ ; sire viral load:  $F_{1,95} = 0.81$ ,  $p = 0.37$ ; mean viral load:  $F_{1,88} = 0.93$ ,  $p = 0.34$ ), suggesting that offspring viral load was more dependent on whether or not their parents had been exposed to NPV as larvae rather than on the magnitude of the parental viral challenge or the parents' viral loads as spent adults.



**FIGURE 2** Relationship between parental virus challenge on the viral load of offspring as adult moths. Viral load is measured as the log<sub>10</sub>-transformed number of SpexNPV viral genomes per ug of host DNA. Symbols (jittered for clarity) indicate viral loads of individual moths, the thick lines are the means for non-challenged and challenged parents and the thin lines are the 95% confidence intervals

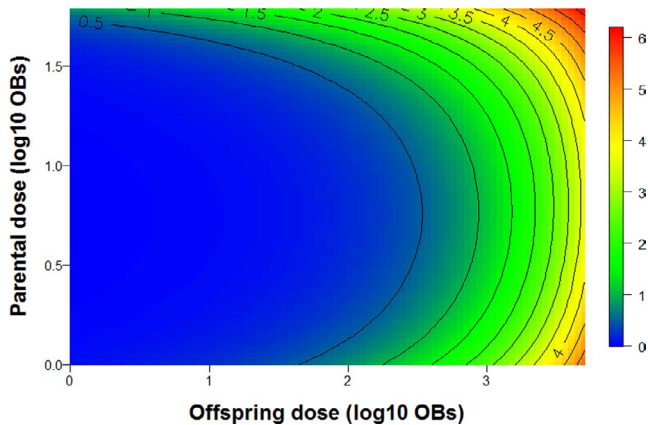
### 3.4 | Offspring bioassay to assess trans-generational resistance

A subset of the offspring generation of larvae was challenged as newly moulted 4th-instar larvae with one of five doses of a genetically distinct SpexNPV isolate (*13Mag9*, as opposed to *4Mag2* used to challenge the parental generation). The effects of parental and offspring virus doses on offspring mortality was explored by conducting a survival analysis on the offspring larvae post viral challenge (using Cox's proportional hazards model); there were just 2/125 (<2%) larval deaths in the control group. This revealed a highly significant non-linear interaction between offspring viral challenge and parental viral challenge (Table 1; model  $\chi^2_5 = 191.5$ ,  $p < 0.00001$ ;  $r^2 = 0.225$ ; Figure 3). Specifically, at zero-low offspring NPV doses, relative mortality risk (*hazard ratio*) was low ( $\leq 1$ ) and more or less independent of parental NPV dose (as reflected in the uniformly blue region to the left of Figure 3), whereas at higher offspring challenge doses mortality risk increased (*hazard ratio*  $> 1$ ), being highest at both low and high parental NPV doses (as reflected in the hotter colours top-right and bottom-right of Figure 3). Thus, at the highest offspring challenge dose (5,000 OB per larva), the risk of NPV-induced mortality was lowest for individuals whose parents had been challenged with a moderate NPV dose (15–25 OBs; *hazard ratio*  $\sim 3.5$ ), whereas it was highest for those that had either not been challenged with NPV (zero OBs; *hazard ratio*  $> 4.5$ ) or challenged with a high NPV dose (40–60 OBs; *hazard ratio*  $> 5.5$ ). Thus, it appears that low parental sub-lethal challenges may result in some protection for their offspring from future NPV challenges, whereas high sub-lethal challenges may increase their offspring's susceptibility to NPV. Of course, we cannot discount the possibility that selection acting on the



**TABLE 1** Cox's proportional hazards model exploring the interaction between parental and offspring NPV challenge on larval survivorship. *Coef* is the regression coefficient, *exp(coef)* is exponentiated coefficient and is the *hazard ratio* associated with a particular term in the model, *SE(coef)* is the standard error; *z* the Wald statistic value corresponding to the ratio of each regression coefficient to its standard error ( $z = \text{coef}/\text{SE}(\text{coef})$ ), *P* is the significance level associated with a given *z* value. Global statistical significance of the model: likelihood ratio test:  $\chi^2_5 = 191.5$ ,  $p < 0.00001$ ;  $r^2 = 0.225$

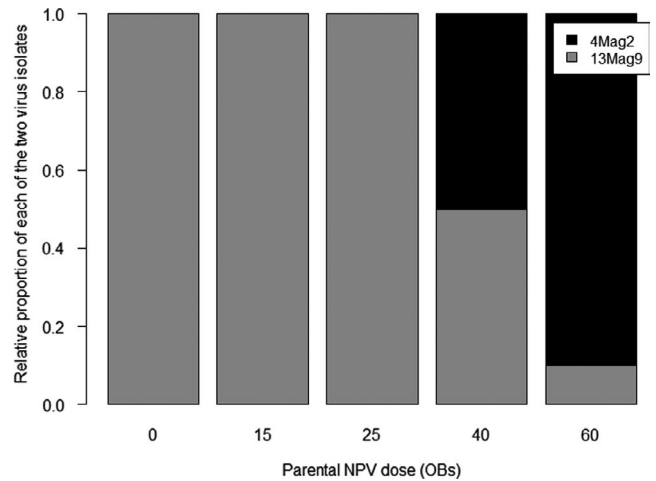
| Term                                          | coef   | exp(coef) | SE(coef) | z      | p       |
|-----------------------------------------------|--------|-----------|----------|--------|---------|
| Parental dose ( $\log_{10}$ OBs)              | -6.521 | 0.0015    | 2.0499   | -3.181 | 0.0015  |
| Parental dose <sup>2</sup> ( $\log_{10}$ OBs) | 4.396  | 81.1298   | 1.1186   | 3.930  | <0.0001 |
| Offspring dose ( $\log_{10}$ OBs)             | 1.150  | 3.1602    | 0.2252   | 5.107  | <0.0001 |
| Parental dose : Offspring dose                | 1.513  | 4.5425    | 0.6487   | 2.333  | 0.0197  |
| Parental dose <sup>2</sup> : Offspring dose   | -1.035 | 0.3551    | 0.3545   | -2.920 | 0.0035  |



**FIGURE 3** Heatmap showing the interactive effects of offspring NPV challenge dose ( $\log_{10}$  OBs per larva) and parental NPV challenge dose ( $\log_{10}$  OBs per larva). The hotter (redder) the colours, the higher the relative mortality risk. The significant interaction is reflected in the nonlinear risk isoclines: at zero-low offspring NPV doses, relative mortality risk is low (<1) and more or less independent of parental NPV dose, whereas at higher offspring challenge doses mortality risk increases (>1) with mortality (risk  $\gg 1$ ) being highest at low and high parental NPV doses (as reflected in the hotter colours top-right and bottom-right)

parental generation contributes to the observed patterns, but this is why relatively low viral challenges were used ( $\leq \text{LD}_{25}$ ) in order to minimise any selection effects.

For those offspring larvae that died following viral challenge, the origin of the expressed (progeny) virus was genetically identified as either that which was used to challenge the offspring (*13Mag9*) or that which was used against their parents (*4Mag2*); based on restriction enzyme digest banding patterns, there was no evidence that larvae died of any virus isolate other than these two or died of mixed infections. Logistic regression revealed that the only determinant of the identity of the progeny virus was the magnitude of the *4Mag2*-dose used to challenge the parents (GLM with binomial errors: parent  $\log_{10}$ dose:  $\chi^2_1 = 36.70$ ,  $p < 0.0001$ ); the magnitude of the *13Mag9*-dose used to challenge the offspring themselves was not a significant predictor of progeny virus ID (offspring  $\log_{10}$ dose:  $\chi^2_1 = 2.16$ ,  $p = 0.13$ ), and there was no significant interaction between the two virus doses (parent-offspring interaction:  $\chi^2_1 = 0.34$ ,  $p = 0.56$ ; Figure 4).



**FIGURE 4** The relative proportions of the parental challenge virus isolate (*4Mag2*) and the offspring challenge virus isolate (*13Mag9*) in cadavers from the offspring bioassay experiment ( $n = 10$  cadavers per parental virus dose treatment group). None of the cadavers showed any evidence of mixed infections

## 4 | DISCUSSION

We have demonstrated that the impact of offspring viral challenge is dependent on the magnitude of the parental exposure to a viral-challenge, that is, trans-generational effects. Indeed, there is evidence for a threshold exposure level at which a parental viral challenge changes from being beneficial to its offspring (i.e. increased resistance to NPV challenge at  $\leq \text{LD}_{10}$  viral challenge), to detrimental (i.e. increased susceptibility to NPV challenge at  $\geq \text{LD}_{15}$ ). It is likely that the increase in susceptibility at high parental doses is associated with the vertical transmission of virus from parent to offspring, as we were able to demonstrate that the vertically transmitted (parental *4Mag2* isolate) virus was readily 'triggered' when offspring were challenged with a heterologous (*13Mag9*) virus isolate. There are a number of examples of apparent immune priming to viruses in some other insect systems (e.g. Kaltenpoth & Engl, 2014; Tidbury et al., 2011; Williams et al., 2017) but not in others (e.g. Shikano et al., 2016). There are also a number of studies reporting the existence of vertical transmission of baculoviruses (Burden et al., 2003; Murillo et al., 2011; Wang et al., 2015). But, as far as we are aware, this is the

first study to characterise the dose-dependent responses to trans-generational immune priming (TGIP) (in its broadest sense) and vertical transmission of virus in an insect-pathogen system. It remains to be established whether these trans-generational effects are strain-specific and/or symmetrical.

Our experiment was designed to mimic a field situation in which there are successive non-overlapping generations of insects exposed to varying levels of baculovirus, so as to establish whether exposure to virus in one generation enhances or reduces susceptibility to virus in the subsequent generation. This scenario is fairly common among many lepidopteran pests like the armyworms studied here (Graham et al., 2012). Trans-generational immune priming, in which pathogen exposure in one generation enhances resistance to infection in the next generation, has been widely reported in insect systems (Knorr et al., 2015; López et al., 2014; Milutinović et al., 2016), but it is by no means ubiquitous (Tetreau et al., 2019). Indeed, some studies have found that low-dose immune challenges may be detrimental to the subsequent offspring generation. For example, Brown et al. (2003) found that while offspring were more resistant when exposed to the same parasite as their mothers, these same offspring had increased susceptibility to other pathogens. This suggests that priming may be specific and costly in terms of resistance to other parasites, and that there may be trade-offs in the host immune system (Heil & Bostock, 2002). Further study is required into a range of environmental variables that may impact host immunity and trans-generational immune priming. It is well understood that factors such as host density and food quality can impact both immunity (Povey et al., 2014; Reeson et al., 1998; Siva-Jothy & Thompson, 2002; Wilson et al., 2003) and TGIP (Bazazi et al., 2010; Shikano et al., 2015; Triggs & Knell, 2012).

Baculoviruses generally exhibit a 'mixed-mode' transmission strategy (Cory, 2015; Williams et al., 2017), in which both vertical and horizontal transmission modes are possible. In the case of SpexNPV, vertical transmission most likely enables virus survival during periods of low host density, especially during the harsh dry season in sub-Saharan Africa, when horizontal transmission would be a high-risk strategy for the virus and potentially detrimental to its long-term survival (Myers & Cory, 2016; Vilaplana et al., 2010). Vertical transmission is a common strategy in many parasite systems (Burden et al., 2003; Goodacre & Martin, 2012; Williams et al., 2017), and is likely to have co-evolved closely with its host and associated immune system (Busenberg & Cooke, 2012; Jones et al., 2010). Indeed, some baculovirus strains appear to be specifically adapted for vertical transmission (Cabodevilla et al., 2011; Kemp et al., 2011).

Covert infections may persist in many baculovirus systems (Burden et al., 2003; Kemp et al., 2011; Murillo et al., 2011; Williams et al., 2017) (Vilaplana et al., 2010), and this most likely explains why qPCR detected low-intensity virus infections in our control insects. The triggering of covert or sub-lethal virus has been recorded previously (Cooper et al., 2003; Myers & Cory, 2016; Virto et al., 2017) and is believed to be common in lepidopteran species (Williams et al., 2017). By using different genetically distinct isolates of the SpexNPV virus, we were able to show strong evidence of vertical

transmission in this insect-virus system, at least at relatively high parental virus doses ( $\geq LD_{15}$ ), although we cannot totally discount the possibility that the endemic covert virus is identical to that of the parental or offspring isolates, or that this covert virus did not impact our findings in some way.

Surprisingly, offspring from challenged parents had lower viral loads than those from the non-challenged parents. There are a number of possible explanations for this. One is that there was selection imposed by the bioassay experiment, such that those parents with the highest viral loads (or the highest capacity to vertically transmit virus to their offspring) were culled in the virus-treatment groups. Consistent with this, we observed that in the parental generation bioassay, there was relatively strong selection against slowly developing and growing insects; if these insects also harboured higher covert viral loads, then this could explain the higher viral loads of non-challenged insects. Another possibility is that, in the virus-challenged insects, there is competition between the endemic covert virus strain (of unknown genotype) and the challenging parental strain (*4Mag2*), such that when both strains co-occur in the host, the overall viral load of survivors is reduced, perhaps because co-infected host cells have higher mortality rates. A third possibility is that the magnitude of the 'immune priming' response transmitted from parent to offspring increases with the size of parental viral challenge and hence larvae from challenged parents are better able to regulate viral loads, much as is seen with *Plodia interpunctella* inherited resistance to viral infection (Bartlett et al., 2018).

Currently, little is known about the mechanisms underpinning trans-generational immune priming in any invertebrate (Mondotte et al., 2020; Tetreau et al., 2019). However, in the current host-pathogen system (*S. exempta* and SpexNPV), it is known that SpexNPV-challenged larvae tend to have lower levels of haemolymph phenoloxidase and higher densities of haemocytes than control insects (Povey et al., 2014), so it is possible that 'signals' of these immune effectors are transmitted to their offspring following virus exposure, though this remains to be tested. Trans-generational effects have also been reported in a closely related species (*S. littoralis*), with the rearing density of parents as larvae affecting the susceptibility of their offspring to a baculovirus (*S. littoralis* nucleopolyhedrovirus, SpliNPV); specifically, resistance to SpliNPV was lower in larvae from gregariously reared parents than from those that were solitary-reared (Wilson & Graham, 2015). Thus, it is possible that the trans-generational effects reported here are, in part at least, due to immune-related effectors transmitted across generations. Another, non-exclusive, explanation for the observed effects is that they are related directly to the vertical transmission of SpexNPV, with covert viral infections gained from their parents enhancing offspring immune responses or establishing a competitive advantage against any incoming low-level horizontal viral challenge, but which may become overwhelmed by larger viral challenges. The precise mechanisms underpinning this switch from protective to deleterious effects of trans-generational infections remains to be established. Many previous studies of TGIP have deliberately used non-infectious immune primers (such as heat-killed pathogens or immune elicitors

such as lipopolysaccharides), specifically to exclude the possibility that active infections could be a mechanism for the observed trans-generational effects. However, vertical transmission is a common phenomenon in invertebrate host-pathogen interactions, and while our results cannot provide new insights into the precise mechanisms of TGIP, it does suggest a strong link between vertical transmission and trans-generational effects on infection outcome. Moreover, it is worth noting that although vertical transmission of baculoviruses is widely observed in nature, and commonly screened for in baculovirus infection experiments, many previous studies of TGIP have failed to look for covert infections, which may have inadvertently affected their studies. Indeed, modern molecular approaches, such as RNA-seq, are revealing new (often beneficial) vertically transmitted symbionts that show no overt effects but may interact with pathogens and the immune system (e.g. Xu et al., 2014, 2020).

Trans-generational effects may have wider implications for altering the dynamics of host and pathogen populations and the interaction between co-infecting pathogens within a population. Using 'Susceptible-Primed-Infectious' models, Tidbury et al. (2011) found that immune priming, while beneficial for the individual host and its offspring, may increase the persistence of a pathogen, resulting in the de-stabilisation of host populations. Strong trans-generational effects of this nature have the potential to be important drivers of population dynamics (Bartlett et al., 2018; Boots & Roberts, 2012; Nystrand et al., 2016). Delayed density-dependent effects, such as immune priming and vertical pathogen transmission, are generally destabilising to populations and are likely to lead to complex behaviour, such as cyclical (Plaistow & Benton, 2009) or chaotic dynamics (Preedy et al., 2010). This observation may also be important when considering the long-term success of using viral pathogens as biopesticides and when predicting the severity of viral disease epizootics. For example, the subsequent persistence of a biopesticide within a population (via vertical transmission) may vary with the magnitude of the inoculating dose used and natural variation in the degree of immune priming (Cabodevilla et al., 2011; Shikano et al., 2015). Theoretical and empirical studies have highlighted the potential importance of nonlinear dose-dependent effects for the stability of host-pathogen interactions (Regoes et al., 2002), sometimes generating Allee effects, as observed here. The present study provides a link between immune priming and these epidemiological processes.

## 5 | CONCLUSIONS

In conclusion, we have shown that both the magnitude and direction of trans-generational effects are strongly dependent on the size of the pathogen challenge experienced by the parents, switching from being protective at low challenge doses to damaging at higher doses. Although there are now a number of studies quantifying the role of immune priming in host resistance and disease dynamics (e.g. Boots & Roberts, 2012), the mechanisms involved in immune priming remain little understood in any system, including this one

(Milutinović & Kurtz, 2016). Only once we have a better understanding of the regulation of baculovirus resistance (Byers et al., 2016; Jiang et al., 2012; Liu et al., 2013; Reeson et al., 1998) will we be able to tease apart the specific mechanisms or pathways that facilitate immune priming.

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## AUTHORS' CONTRIBUTIONS

K.W. and R.I.G. conceived the study and designed the methodology; R.I.G. collected the data; K.W. and R.I.G. analysed the data. All authors contributed critically to the drafts and gave final approval for publication.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.qjq2bvqft> (Wilson et al., 2021).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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