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Partiti-like viruses from African armyworm increase larval and pupal mortality of a novel host: the Egyptian cotton leafworm

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Short running title: Host-shift of viruses for pest management

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Abstract

BACKGROUND: The general principle of using microbes from one species to manage a different pest species has a clear precedent in the large-scale release of mosquitoes carry a *Wolbachia* bacterium derived from *Drosophila* flies – new technologies will facilitate the discovery of microbes that can be used in a similar way. Previously, we found three novel partiti-like viruses in the African armyworm (*Spodoptera exempta*). To investigate further the utility and consistency of host-shift of insect viruses as a potential pest management tool, we tested the interaction between the partiti-like viruses and another novel host, the Egyptian cotton leafworm (*S. littoralis*).

RESULT: We found that all three partiti-like viruses appeared to be harmful to the novel host *S. littoralis,* by causing increased larval and pupal mortality. No effect was seen on host fecundity, and partiti-like virus infection did not impact host susceptibility when challenged with another pathogen, the baculovirus SpliNPV. Transcriptome analysis of partiti-like virus-infected and -noninfected *S. littoralis* indicated that the viruses could impact host gene-expression profiles of *S. littoralis,* but impacting different pathways to the two other *Spodoptera* species, e.g. via effects on pathways related to immunity (Jak-STAT/Toll and Imd) and reproduction (Insulin signaling/Insect hormones).

CONCLUSION: Taken together with the previous findings in the novel host *S. frugiperda*, these results indicate a parasitic relationship between the partiti-like viruses and novel insect hosts, suggesting a possible use and novel pest management strategy via the artificial host-shift of novel viruses.

Keywords: Host-shift, partiti-like viruses, *Spodoptera littoralis*, fitness, transcriptome

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1 INTRODUCTION

Viruses are often harmful to the hosts they infect and can be among the most threatening diseases to humanity, either directly (e.g. SARS and dengue viruses can kill humans)[1,2] or indirectly (e.g. plant viruses inflict serious economic losses).[3] Formulated as biopesticides, viruses can also benefit people as part of integrated pest management strategies (e.g. nucleopolyhedroviruses, NPVs).[4,5] The host ranges of viruses are diverse, with some showing high specificity (e.g. *Helicoverpa armigera densovirus*, HaDV2) whilst others have a broad host range (e.g. some plant viruses).[6-8] Naturally, host-range expansion of viruses occurs relatively infrequently due to differences in host immune systems and unsuitable environments for virus replication.[9] However, once a host-jump occurs, there can be harmful effect on the new host, e.g. avian influenza viruses derived from birds threaten human safety.[10-12] Previously, we reported three novel partiti-like viruses by next generation sequencing (NGS) in African armyworm (*Spodoptera exempta*), which can infect African armyworm, Fall armyworm (*S. frugiperda*, FAW) and Egyptian cotton leafworm (*S. littoralis*) by microinjection and can be maternally-transmitted with high efficiency (100%).[13] Interestingly, these partiti-like viruses reduced the growth rate and reproduction of their original host, but enhanced their resistance to a nucleopolyhedrovirus (*S. exempta* nucleopolyhedrovirus, SpexNPV). However, in the FAW, they were deleterious without any detectable benefit to this novel host, suggesting a possible pest management strategy by managed host-shift of insect viruses.[13]

Like African armyworm and FAW, the Egyptian cotton leafworm is another important pest of several agricultural crops in many countries from tropical and subtropical regions. It is a polyphagous pest with a broad range of hostplants that includes more than 80 plant species (e.g. potato) belonging to 40 families.[14,15] The Egyptian cotton leafworm also has an endemic baculovirus, *S. littoralis* nucleopolyhedrovirus (SpliNPV), which has been formulated as a biopesticide.[16-20] Previously, we showed that the partiti-like viruses can infect *S. littoralis* and be maternally-transmitted.[13] However, the impact of infection with these *S.*

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exempta-derived partiti-like viruses on the Egyptian cotton leafworm, including the life table parameters and resistance against SpliNPV, is still unclear.

In this study, we used microinjection to construct partiti-like virus-positive and -negative strains of *S. littoralis* with individuals from single-pair matings, as previously described for African armyworm and FAW, with a view to determining whether the impacts observed in this novel host is similar to that observed in FAW.[13]

2 MATERIAL AND METHODS

2.1 Insect and the three partiti-viruses

The lab colony of *S. littoralis* was established from individuals collected in Egypt in 2011. All larvae were reared using standard artificial diet[21,22] at 27 °C with a 14:10, light:dark photoperiod. Adult moths were provided with 5% sugar water. The three partiti-like viruses were isolated from virus-positive individuals of *S. exempta* and stored at -20 ºC as described previously, which contained 3.8×10^8 copy numbers/μl and 2.7×10^7 copy numbers/μl for *S. exempta* virus 1 (SEIV1) and *S. exempta* virus 2 (SEIV2) respectively.[13]

2.2 Detection of microbes by RNA-seq and PCR

RNA-seq has been widely used to detect DNA viruses and RNA viruses with poly(A) tails and most of known RNA viruses infecting invertebrates contained ploy(A) tails (e.g. iflaviruses, picorna-like viruses). To detect whether the colony of *S. littoralis* harboured DNA viruses (e.g. NPV) as well as RNA viruses with poly(A) tails, we used a transcriptomics method as described previously.[13] Briefly, RNA from whole bodies of first instar larvae (n = 50), fifth instar larvae (n = 20), pupae (n = 20) and adults (n = 20) were sequenced with primers producing two paired-end and 150-nt read lengths, using the Illumina HiSeq™ platform (Majorbio, Beijing, China). Trinity (v2.0.6) software was used to assemble the clean reads and unigenes were generated using contigs longer than 200 bp with default parameters.[23] Then, unigenes were annotated by searching against the NCBI protein non-redundant (NR), SwissProt and clusters of eukaryotic Orthologous Groups (KOG) databases using DIAMOND (v0.8.37) (e-value < 1E-5),[24] the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using KAAS (r140224) (default),[25] the Gene Ontology (GO) database using

Blast2GO (v2.5.0) (default),[26] the Interpro database using InterProScan5 (V5.11-51.0) (default),[27] and the Pfam database using HMMER 3 (v3.1b2) (default).[28] The RNA-Seq data were submitted to the NCBI Sequence Read Archive (SRA) database (Table 1). We also excluded the possibility of *Wolbachia* infection with primers 81F and 691R with PCR program: 30 s at 94 ºC, 30 s at 50/55 ºC, and 30 s at 72 ºC for 40 cycles; and assumed to be free of these microbes if no PCR amplification was detected at 10ng host DNA.[13,29] All of the primers used in this study are shown in Supporting Information 1–Table S1.

2.3 Quantifying the partiti-like viruses impact on development and fecundity of *S. littoralis*

As previously described,[13] the three partiti-like viruses could be transmitted horizontally by microinjection and maternally vertically transmitted with 100%. Herein, microinjection was successfully used to construct partiti-like virus-positive lines using negative *S. littoralis* individuals from single pairs. Briefly, each newly moulted 5th instar larva was injected with 10 μl filtered liquid containing the three partiti-like viruses with a Hamilton Microliter (705N) syringe and Harvard Pump 11 Elite. Then, the larvae were reared as described above until eclosion and the moths were used to detect the viruses with PCR and specific primers (Supporting Information 1–Table S1). To test the impact of the partiti-like viruses infections on the life table parameters of *S. littoralis*, 20 neonate larvae from either positive or negative strains were placed in each pot (diameter of entrance = 4.2 cm; diameter of bottom = 2.8 cm; height = 4 cm) for three days. They were then transferred to a 25-well plate (one individual per well: length = 2 cm; height = 1.8 cm; width = 2 cm) until the start of the 4th larval instar and fresh diets were provided every 3 days; larvae were then individually reared in plastic pots until eclosion. The status of individuals was checked every day at 10:00 am. The weight of pupae on the 1st day was recorded. Individuals dying within 24 hours of the experimental set up were considered handling deaths and excluded from the analysis. In addition, newly-eclosed adults from both the negative and positive strains were mated (F+/M+ and F-/M-) and used to determine longevity and fertility. A single pair of adults was put in each plastic cup

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(diameter = 11 cm; height = 6 cm) with sugar water. The experimental replicates were no less than 20.

2.4 Quantification of the partiti-like viruses in eggs and pupae

To establish the role of vertical transmission in the life-cycle of the partiti-like viruses (SEIV1 and SEIV2, respectively), we quantified the infections in *S. littoralis* eggs, primarily to distinguish between transovarial (within the egg itself) and transovum (due to virus contamination of the eggshell) infection routes. Eggs from positive-strain breeding pairs (F+/M+), were submerged in 5% sodium hypochlorite solution for 10 minutes. They were then filtered through a damp cloth, thoroughly rinsed with distilled water, and allowed to dry. Three groups of hypochlorite-treated eggs (n = 50 eggs per group) were tested against non-treated eggs (control) using qPCR to test for infection status. The copy number of the two partiti-like viruses in virus-positive samples of *S. littoralis*, *S. exempta* and *S. frugiperda* pupae (day one of pupation) was also quantified using qPCR. qPCR was carried out with the TaqMan method in 20 μl reaction agent comprised of 1 μl of template DNA, 2×Premix Ex Taq (Takara, Japan), 0.2 μM of each primer and 0.4 μM probe, using a 7500 Fast Real-time PCR System (Applied Biosystems). Thermal cycling conditions were: 45 cycles of 95°C for 15 s, 60 °C for 34 s. The cDNA sample of each group was replicated three times. The standard curves of SEIV1 (y=-0.9258x+46.029, y=copy bumbers (log2), x=C_T value) and SEIV2 (y=-0.9591x+39.439, y=copy bumbers (log2), x=C_T value) were used to quantify the copy numbers of the two viruses.[13]

2.5 Baculovirus bioassays

To assess the potential of partiti-like viruses as a control method in pest management, we investigated the interaction between partiti-like viruses and the common baculovirus pathogen SpliNPV in *S. littoralis* via a series of laboratory bioassay studies. The strain of SpliNPV was a mixed-genotype isolate originating in Egypt. Twenty neonate larvae were reared in diet pots for three days, and then transferred individually to a 25-well plate and maintained on diet. Third instar larvae (48h post ecdysis) were chosen for the NPV bioassays. Larvae were orally dosed with 4 treatments of SpliNPV (no fewer than 90 larvae per treatment at: 0 (control), 4×10^3,

1×10^4, 2×10^4, and 1×10^5 OBs/larva). Only larvae that ingested all the NPV within a 24 h period were used for the bioassays. Larvae were subsequently monitored daily for NPV mortality until pupation, and all viral deaths stored at -20 °C. PCR with specific primers were used to test for NPV in dead larvae with non-obvious symptoms (Supporting Information 1 – Table S2).

To assess NPV viral load in partiti-like virus-positive and -negative individuals, we performed a separate bioassay with 2×10^4 OBs/larva in *S. littoralis*. Only larvae that ingested all the NPV within a 24 h period were used and collected samples at 72 h after ingesting all the diet. To perform the absolute quantification qPCR methodology for quantifying NPV copy number, firstly we designed primers according to the open reading frame encoding polyhedrin and amplified fragments for constructing plasmids to generate standard curve (764 bp for SpliNPV) as described previously.[13,30] The PCR program was as follows: 30 s at 94 ºC, 30 s at 55 ºC, and 30 s at 72 ºC for 40 cycles. The qPCR was performed as described above.

2.6 Analyzing effects of the partiti-like viruses on *S. littoralis* by transcriptome

To determine the effect of these partiti-like viruses on *S. littoralis* at a transcriptomic level, we collected samples of the partiti-like viruses-negative and -positive individuals from single pairs of *S. littoralis* and performed RNA-seq, using fifth instar larvae (24 h post-ecdysis), pupae, adult males and adult females. There were three groups and nine individuals for each group per stage (Table 1). The cDNA libraries were constructed, sequenced and the data were assembled and annotated as described above. For the gene expression analysis, the number of expressed tags was calculated and then normalized to transcripts per million tags (TPM) using RSEM software packages.[31] Then the R package *edgeR*[32] was used to determine the significantly differentially-expressed unigenes (DEGs) at different comparisons with threshold 'fold change ≥ 1.5 and $P<0.05$. Principal component analysis (PCA) with DEG data were performed with 'prcomp' in R package. The hierarchical clustering method was applied to analyze the expression pattern of significantly differentially expressed unigenes in different samples. The statistical significance of the functional GO enrichment was evaluated using the Fishers exact test with python package

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Goatools (p-values were corrected by the Benjamini-Hochberg, false discovery rate (FDR) < 0.05).[33] Significantly enriched KEGG pathways were also identified using the Fishers exact test (p-value<0.05). Using β-actin and GAPDH as reference genes, qPCR (Sybrgreen method) was performed to confirm the results of RNA-seq in 20 μl reaction agent comprised of 1 μl of template DNA, 2×Premix Ex Taq (Takara), 0.2 μM of each primer, using a 7500 Fast Real-time PCR System (Applied Biosystems). Thermal cycling conditions were: 45 cycles of 95°C for 3 s, 60 °C for 30 s. The samples of each group were biologically replicated three times.

2.7 Statistics

Statistical analyses were conducted using Graphpad InStat 3 and R v3.0.1[34]. Student's t-test or ANOVA with Tukey LSD tests were used to determine the level of significance in the relative levels of the partiti-like virus. Larval/pupal mortality, pupation and eclosion rates, and NPV bioassay data, were analysed using generalized linear models (GLMs) with binomial errors.

3 RESULTS

3.1 Impact of partiti-like viruses on the development, fecundity and adult longevity of *S. littoralis*

Firstly, we excluded possibility of the infection of DNA viruses, RNA viruses with poly(A) tail as well as *Wolbachia* in our populations of *S. littoralis* using transcriptome analysis and PCR method (Table 1, accession number: SRR8655974) (Supporting Information 1–Table S1). When comparing partiti-like virus-positive and virus-negative strains, larval development (log-transformed) was not affected by infection with the viruses in either females ($t = -0.435$, d.f. = 166, $P = 0.664$) or males ($t = 0.347$, d.f. = 212, $P = 0.729$) (Fig. 1a, 1e). In females ($t = 3.747$, d.f. = 310, $P = 0.0002$), but not males ($t = 0.093$, d.f. = 352, $P = 0.926$), pupal development was extended in virus-infected insects (Fig. 1b, 1f). Pupal weight was heavier for virus-infected insects in both sexes (females: $t = 6.753$, d.f. = 310, $P < 0.0001$, males: $t = 6.147$, d.f. = 352, $P < 0.0001$) (Fig. 1c, 1g). Adult longevity was not affected by virus infection in either sex (females: $t = -0.391$, d.f. = 108, $P = 0.697$, males: t =

-1.044, d.f. = 108, P = 0.299) (Fig. 1d, 1h). When egg production was measured over the entire lifetime of a moth pair, infected pairs were just as likely to produce no eggs than non-infected pairs (3% vs 11%; logistic regression: $\chi^2_1 = 2.054$, P = 0.1518). This translated into similar levels of offspring production for infected and non-infected pairs (t = -0.408, d.f. = 74, P = 0.684; Fig. 1i), even when pairs that produced no eggs were excluded from the analysis (t = -0.787, d.f. = 69, P = 0.434) (Fig. 1j).

Larval mortality rates were significantly higher for virus-infected larvae than non-infected *S. littoralis* larvae (18% vs 6%; logistic regression: $\chi^2_1 = 18.581$, P < 0.0001). Virus-infected larvae also had higher rates of deformity as pupae (deformed pupae, e.g. pupae with larval characters) ($\chi^2_1 = 21.797$, P < 0.0001) and, as adult females (deformed females, e.g. females with deformed wings) ($\chi^2_1 = 11.114$, P = 0.0009) and males (deformed males, e.g. males with deformed wings) ($\chi^2_1 = 15.732$, P < 0.0001), and a higher mortality rate as pupae ($\chi^2_1 = 5.273$, P = 0.0216) (Table 2).

3.2 Quantification of the two partiti-like viruses in eggs and pupae

The virus titres were not significantly different between sodium hypochlorite-treated and non-treated eggs (SEIV1: t = 1.698, d.f. = 4, P = 0.1648, SEIV2: t = 0.2898, d.f. = 4, P = 0.7864) (Fig. 2a, 2b), suggesting that transovarial transmission was occurring. In the first day of pupation, titres of both SEIV1 (anova: F = 16.279, d.f. = 2,9, P = 0.0010, Fig. 2c) and SEIV2 (F = 8.176, d.f. = 2,9, P = 0.0095, Fig. 2d) differed across the three species, with virus titers (as measured by absolute expression levels) being lower in *S. littoralis* than in *S. exempta* (the original host) and *S. frugiperda* (the other novel host).

3.3 Interaction between the partiti-like viruses and SpliNPV

To determine the interaction between the partiti-like viruses and the baculovirus SpliNPV, we first confirmed individuals from the two strains were *Wolbachia*-free and NPV-free, as well as free from other viruses, using PCR with specific primers and RNA-seq. In *S. littorali*s, larvae that harboured the partiti-like viruses were equally susceptible to SpliNPV infection as those that lacked the virus (logistic regression: χ^2_1 = 0.473, P = 0.4915; Fig. 3a); in addition, NPV-induced mortality increased with viral dose and there was no interaction between NPV dose and viral status (log10 NPV

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dose: $\chi^2_1 = 65.414$, $P < 0.0001$; interaction term: $\chi^2_1 = 0.561$, $P = 0.454$). The pupal development periods of survivors of NPV-challenge was not affected by whether or not they harboured the partiti-like virus (linear model: Virus-status: F = 0.121, d.f. = 1,221, P = 0.6799; Fig. 3b), nor the NPV concentration, nor the interaction between the two (Log_{10} NPV concentration: F = 1.04, d.f. = 1,412, P = 0.2593; interaction term: : F = 1.161, d.f. = 1,221, P = 0.2012).

We tested the differences in NPV replication at 72 h post-challenge with NPV between partiti-like virus-positive and -negative individuals by repeating the bioassay with 2×10^4 OBs/larva for SpliNPV. NPV virus titres (as measured by absolute expression levels, Supporting Information 1–Fig. S1) showed no significant difference between partiti-like virus-positive and -negative individuals in *S. littoralis* (F = 0.0038, d.f. = 1,18, P = 0.95) (Fig. 3c).

3.4 Transcriptome analysis in *S. exempta* and *S. frugiperda*

To understand the dynamic interaction between the partiti-like viruses and *S. littoralis*, we determined the differentially-expressed genes (DEGs) between the transcriptomes of partiti-like viruses-positive and -negative individuals at different life stages, including larvae, pupae, and adult males and females (Table 1). We chose several unigenes to validate their expression levels by qPCR (Supporting Information 1–Fig. S2). Using total annotated genes of 58921 (Supporting Information 1–Table S2), gene expression was quantified in the partiti-like positive groups compared to related negative groups with ± 1.5-fold and $P < 0.05$. In *S. littoralis*, the number of DEGs was higher in adult males than that in other life-stages (Supporting Information 1–Fig. S3, Supporting Information 2). The PCA with DEG data clearly distinguished partiti-like viruses-positive from -negative individuals at different stages (Fig. 4a). Taken together with the hierarchical clustering of these DEGs (Supporting Information 1–Fig. S4), these results suggest that the partiti-like viruses have an effect on the gene expression profiles of *S. littoralis*.

As we did in *S. exempta* and *S. frugiperda*,[13] we performed pathway enrichment analysis on the DEGs, focusing particular attention to pathways related to the development, antivirus and reproduction systems (Fig. 4b, Supporting Information 3).

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In contrast to the two *Spodoptera* species, genes in Jak-STAT/Toll and Imd pathways, which are related to some antivirus and immune responses, were not significantly enriched at larval stage (Fig. 4b). Genes in the insect hormone biosynthesis pathway, which are related to the degradation of Juvenile Hormone (JH) (Fig. 4b), and genes in the steroid hormone biosynthesis pathway, which prevent ecdysone functioning and are related to the reproduction of adult females, were significantly enriched and down-regulated in the partiti-like viruses-infected adult female *S. littoralis* (Fig. 4b). Unlike the interactions between the partiti-like viruses and the two other *Spodoptera* species (*S. exempta* and *S. frugiperda*), these results suggest a different interaction between the viruses and *S. littoralis*.

4 DISCUSSION

Numerous insect viruses are considered as potential biological control resources because of their safety to humans and the environment. Traditionally, most attention has been placed upon insect viruses that show pathogenetic symptoms in their hosts or cause lethal infections (e.g. baculoviruses).[4,5] Insect viruses form diverse relationships with their hosts, e.g. parasitic, by killing them directly; mutualistic, by increasing the fitness of their hosts; and conditionally mutualistic, by being both beneficial and harmful to their hosts depending on context.[4,5,7,35-37] It is well established that host-shifts of viruses can result in emerging infectious diseases threatening human health, wildlife and agriculture; but there has been little attention to the possibility that benefits could arise from host-shifts of viruses; for example, utilizing them to develop novel pest management strategies. We have previously reported three novel partiti-like viruses isolated from *S. exempta,* and showed that there was a conditionally-mutualistic relationship between the viruses and *S. exempta*. However, the viruses showed a parasitic relationship with a novel host *S. frugiperda* (by engineering a host-shift).[13] Previously, we showed that the partiti-like viruses could also horizontally-infect *S. littoralis* and be maternally-vertically transmitted with high efficiency (100%).[13] In the study reported here, we were interested in the interactions between the partiti-like viruses and another novel host, *S. littoralis*.

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To exclude the effects of microbes and genetics as comprehensively as possible,[7,13] firstly we determined there were no detectable viruses and *Wolbachia* in our strains of *S. littoralis* by RNA-seq and PCR methods. Then, we constructed virus-positive and virus-negative strains with offspring from a single breeding pair and performed an NPV bioassay. In their original host, *S. exempta*, the partiti-like viruses decreased the growth rate of larvae and reproduction of adults; similarly, in a new host *S. frugiperda*, these viruses decreased the growth rate of pupae and reproduction of adults.[13] However, in *S. littoralis*, there was no effect on larval development and reproduction of adults, and only pupal development of females was extended. Interestingly, the partiti-like viruses marginally increased the larval mortality of *S. littoralis* (~12%) and pupal weight in both sexes. Our results suggest, as was found in a previous new host, *S. frugiperda*,[13] that there was no detectable benefit of the infection in *S. littoralis* with respect to NPV.

Baculoviruses are considered as the best candidates for developing biopesticides.[4,5] Determining the interactions between baculoviruses and other microbes (e.g. *Wolbachia* and HaDV2) in their common pest-hosts has provided interesting insights into how we can best manage pest control strategies accordingly.[7,38] As with SpexNPV in *S. exempta*[38-40] and *S. frugiperda* multiple nucleopolyhedrovirus (SfMNPV) in *S. frugiperda*,[41-43] the Egyptian cotton leafworm also has an endemic baculovirus (SpliNPV), which is being used as a biopesticide.[16-20] As a conditionally mutualistic symbiont, partiti-like viruses were previously found to increase the resistance level of their original host *S. exempta* against SpexNPV. However, they decreased the resistance of *S. frugiperda* to SfMNPV.[13] In this study, we found that infection with partiti-like viruses had no effect on *S. littoralis* against SpliNPV. The partiti-like viruses titre was significantly lower in *S. littoralis* than in the other two host species at the pupal stage, which may explain why these viruses had less impact on the life-history parameters and NPV resistance of *S. littoralis,* than in *S. exempta* and *S. frugiperda*.

To collect more evidence supporting the interaction between the partiti-like viruses and *S. littoralis*, we performed RNA-seq with larvae, pupae, male adults and

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female adults.[13] As previously reported in *S. exempta* and *S. frugiperda*,[13] we focused on pathways related to immunity, development and reproduction.[44-49] Interestingly, there were fewer DEGs in larvae, male-adults and female-adults but more DEGs in *S. littoralis* pupae than in the other two species, suggesting smaller impacts on *S. littoralis* than the two other *Spodoptera* species (except for pupal stage).[13] In *S. exempta* larvae, the antiviral Jak-STAT pathway was significantly enriched by up-regulation in the partiti-like viruses-positive 5th-instar larvae, which is consistent with our NPV bioassay results that partiti-like viruses significantly increased the resistance of *S. exempta* to SpexNPV.[13,46,47] Although no antiviral pathways were enriched in virus-positive or -negative 5th-instar *S. frugiperda*, however, the Toll and Imd immune signaling pathway related to insect immune responses,[47,50] was significantly down-regulated in *S. frugiperda* larvae, which is consistent with the NPV bioassays that larvae infected with partiti-like viruses had significantly greater susceptibility to SfMNPV.[13] Unlike the two other *Spodoptera* species, there was no significant enrichment of Jak-STAT and the Toll and Imd immune signaling pathways. Consistently, our results indicated that the infection of these viruses in *S. littoralis* did not change NPV resistance levels against SpliNPV. However, the NF-kappaB pathway, which also related to innate immune response,[51] was significantly enriched by down-regulation in the partiti-like viruses-positive 5th-instar larvae of *S. littoralis*, sugested a negative effect on development of *S. littoralis*. Genes related to insect hormone pathway were significantly enriched and up-regulated in the partiti-like viruses-infected adult female *S. exempta* and *S. frugiperda*,[13] however, the same ones were significantly enriched and down-regulated in adult female *S. littoralis*, suggesting different effects on reproduction of females. Indeed, unlike decreasing reproduction of females in *S. exempta* and *S. frugiperda*, there were no any detectable changes between viruses+ and viruses- individuals in *S. littoralis*. The infection changed the genes expression pattern at different stages, increased the pupal weight and extended the pupal period of females, supporting that the infection of the partiti-like viruses brought impacts on *S. littoralis*. However, the findings from the transcriptional data could not really be used to explain the results of the bioassay, e.g.

increasing larval and pupal mortality.

5 Conclusion

This study focused on the interactions between insect viruses and a novel host, by engineering a host-shift. As with a previous study using fall armyworm (*S. frugiperda*), partiti-like viruses showed a parasitic relationship with a new host (*S. littoralis*) by increasing larval and pupal mortality and no detectable benefits. However, the infection of these viruses had no effect on resistance against the baculovirus or reproduction. Transcriptome analysis of *S. littoralis* infected (or not) with the partiti-like viruses indicated that the viruses can change the gene expression profiles of *S. littoralis,* but in what appears to be in a different manner to the other two *Spodoptera* species, e.g. effects on pathways related to immunity (Jak-STAT/Toll and Imd) and reproduction (Insulin signaling/Insect hormones). Although the partiti-like viruses could decrease the fitness cost of *S. littoralis* and *S. frugiperda*, it is difficult to use partiti-like viruses for pest management due to the transmission mode (vertical only). However, taken together with the findings in *S. frugiperda*, these results indicate parasitic relationships between the partiti-like viruses and novel insect hosts, suggesting a possible pest management strategy via the artificial host-shift of novel viruses discovered using NGS and easily transmitted by oral or cuticle routes, though these effects are somewhat species-specific.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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AUTHOR CONTRIBUTIONS

P.X. and K.Wi. designed research; P.X. and A.R. performed research; K.Wi., P.X., A.R., J.W., T.L., X.Y., H.Y. and R.I.G. analysed data, P.X., K.Wi., and R.I.G. wrote the paper.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article

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FIGURE LEGENDS

Fig. 1 Life-history parameters of the partiti-like viruses v+ and v- individuals of *S. littoralis*. (**a-d**) Parameters of female, larval period (a) (v+ = 57, v- = 111), pupal period (b), pupal weight (c) (v+ = 136, v- = 176) and adult longevity (d) (v+ = 54, v- = 56). (**e-h**) Parameters of male, larval period (e) (v+ = 69, v- = 145), pupal period (f), pupal weight (g) (v+ = 130, v- = 224) and adult longevity (h) (v+ = 54, v- = 56). (**i-j**) The reproduction of v+ and v- individuals in all samples (i) (v+ = 38, v- = 38) or samples produced no eggs were excluded (j) (v+ = 37, v- = 34). Means ± SD. ***=P<0.001, based on t-tests at each time-point.

Fig. 2 The quantification of SEIV1 and SEIV2 in eggs and pupae. The copy number of SEIV1 (a) and SEIV2 (b) in eggs of *S. littoralis* (n = 3). W = eggs watched with 5% sodium hypochlorite, CK = control. Copy numbers of SEIV1 (c) and SEIV2 (d) in the 1st day of pupae in different species. Se = *S. exempta* (n = 4), Sf = *S. frugiperda* (n = 4), Sl = *S. littoralis* (n = 4). Significant differences are shown using different letters. Means ± SD.

Fig. 3 Relationship between the baculoviruse (SpliNPV) and the partiti-like viruses in larvae of S. littoralis. (a) Effect of NPV dose (log10-transformed number of occlusion bodies per larva) on larval survival to pupation in S. littoralis (logistic regression: χ^2_1 = 0.473, P = 0.4915). The thick lines are the fitted values and the shaded zones are the standard errors around these fitted values; blue lines and shading are the three partiti-like viruses-negative larvae (V-); red lines and shading are the three partiti-like viruses-positive larvae (V+). The numbers of larvae at different concentrations for SpliNPV (0 (control), 4×103, 1×104, 2×104, and 1×105 OBs/larva) were were 94, 101, 107, 110, 101 for v- individuals and 105, 112, 111, 122, 113 for v+ individuals. (b) The pupal period of survivors of NPV-challenge in S. littoralis (linear model: Virus-status: F = 0.121, d.f. = 1,221, P = 0.6799). (c) NPV copy numbers (log10-transformed) at 72 h post-challenge with SpliNPV (F = 0.0038, d.f. = 1,18, P = 0.95). The concentrations of NPV were 2×104 OBs/larva. V- = the three partiti-like

viruses-negative larvae, V+ = the three partiti-like viruses-positive larvae. Means ± SD, based on t-tests at each time-point.

Fig. 4 Transcriptome analysis using the partiti-like virus-positive individuals compared to related virus-negative individuals in *S. littoralis*. (a) PCA analysis of global gene expression of DEGs in the comparison of partiti-like virus-positive groups and related virus-negative groups in *S. littoralis*. Blue stands for the partiti-like viruses-negative samples and red stands for the partiti-like viruses-positive samples. (b) Heatmaps of –log10 p-values of KEGG pathway representing the up- and down-regulated DEGs in *S. littoralis*. "*" indicate the significantly enriched pathways ($p < 0.05$). Red color shows up-regulation pathways, blue color show down-regulation pathways, gray color shows no value, the redder/bluer the color, the lower P-values.

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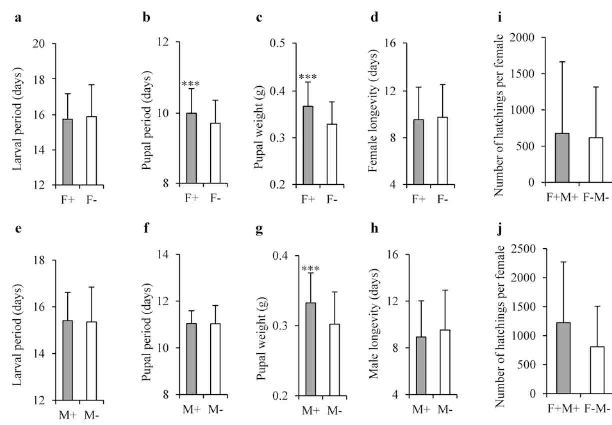

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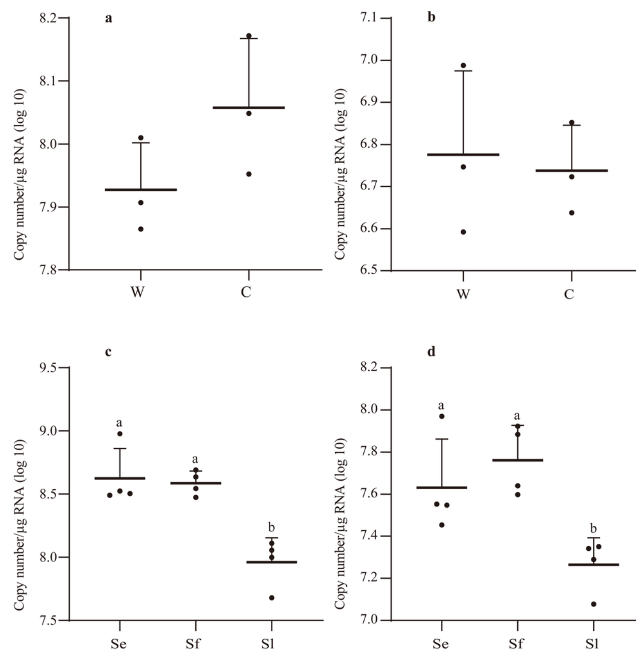

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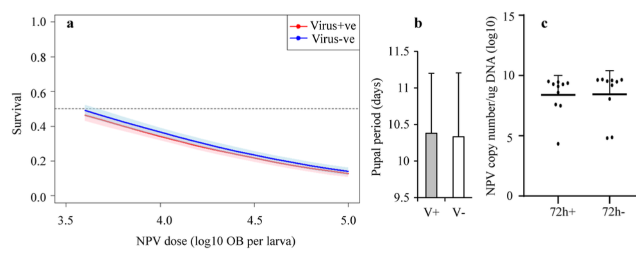

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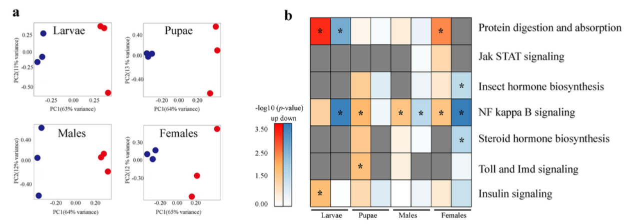

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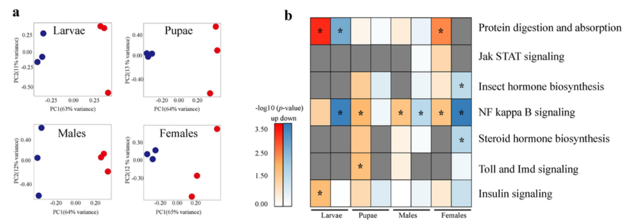

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Engineered host-shift of viruses changed gene expression profiles of *S. littoralis*. (a) PCA analysis distinguished viruses-positive from -negative individuals; (b) Pathways related to immune, hormone and digestion were significantly enriched.

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Table 1. Host information and data output for each sample of *S. littoralis*.

Sample names	Stages	Partiti-like viruses	Total_Reads	Total_Bases	Library accession
SL	Larvae, pupae, adults	/	44682326	6702348900	SRR8655974
SlL1-	Larvae	Negative	56787780	8525244410	SRR8422488
SlL2-			56296428	8447139615	
SlL3-			52963778	7944947585	
SlP1-	Pupae	Negative	54858920	8225343089	SRR8422486
SlP2-			53933268	8085098676	
SlP3-			53854156	8075204052	
SlF1-	Female adults	Negative	54314624	8157094219	SRR8422490
SlF2-			55577000	8342820435	
SlF3-			62667122	9409412854	
SlM1-	Male adults	Negative	56583188	8477398195	SRR8422492
SlM2-			59691668	8941938765	
SlM3-			60789572	9106087243	
SlP1+	Pupae	Positive	52408804	7858144750	SRR8422485
SlP2+			64345234	9648406443	
SlP+3			58598344	8787581084	
SlL1+	Larvae	Positive	59277028	8887277813	SRR8422487
SlL2+			57873534	8681126116	
SlL3+			53273248	7992971169	
SlF1+	Female adults	Positive	62429218	9372125270	SRR8422489
SlF2+			57086798	8568267572	
SlF3+			61245018	9197974779	
SlM1+	Male adults	Positive	58756512	8800877486	SRR8422491
SlM2+			54904056	8221124289	
SlM3+			52189996	7824807434	

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Table 2. The influence of partiti-like viruses on survival rates of *S. littoralis*.

Index	V+ (%)	V- (%)	χ^2_1	n †	P value
Larval mortality	17.81 (± 5.78)	5.85 (± 3.79)	18.581	8	<0.0001***
Pupation rate	70.22 (± 14.34)	88.45 (± 5.20)	21.797	8	<0.0001***
Eclosion rate	87.96 (± 5.83)	93.50 (± 4.32)	5.273	14	0.0216*
Female deformed rate	27.29 (± 16.90)	9.14 (± 8.10)	11.114	14	0.0009***
Male deformed rate	18.20 (± 7.47)	5.90 (± 10.65)	15.732	14	<0.0001***

V+ = partiti-like viruses-infected; V- = non-infected individuals. Larval mortality = proportion of larvae dying before pupation; pupation rate = proportion of surviving larvae that successfully pupated; eclosion rate = proportion of pupae that successfully eclosed; sex rate = proportion of male. † For larval mortality, pupation rate, n = number of batches (71, 73, 49, 72, 86 larvae for V+ and 71, 48, 66, 68 larvae for V-), eclosion rate, female deformed rate and male deformed rate, n = number of batches (71, 73, 49, 72, 86, larvae and 55, 76, 64 pupae for V+ and 71, 48, 66, 68 larvae and 68, 42, 41 pupae for V-).

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