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RESEARCH ARTICLE

A dicistrovirus increases pupal mortality in *Spodoptera frugiperda* by suppressing protease activity and inhibiting larval diet consumption

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Abstract
Understanding interactions between viruses and their hosts is conducive to enabling better application of viruses as biocontrol agents. Certain viruses carried by parasitic wasps enhance the parasitic efficiency of wasp-larvae by protecting them against the immune system of their Lepidopteran host. However, the relationship between prey pests and viruses found in predatory natural enemies remains unclear. Herein, we report the interaction between *Arma chinensis virus*-1 (AcV-1), originally isolated from a predatory natural enemy, *Arma chinensis* (Hemiptera: Pentatomidae), and one of its prey species, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). The results showed that the AcV-1 virus appeared harmful to the novel host *S. frugiperda* by inhibiting larval diet consumption and increasing pupal mortality. Meanwhile, sequencing data indicated that the virus altered the gene expression profiles of *S. frugiperda*. KEGG analysis showed that the proteasome and phagosome pathways related to protein degradation and immune response were significantly enriched. Although the expression levels of digestive enzyme genes did not change significantly, the total protease activity of AcV-1 virus-positive individuals was significantly decreased, suggesting that the virus inhibited diet consumption of *S. frugiperda via* the down-regulation of digestive

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enzyme activities. These results indicate that a virus initially isolated in a predatory natural enemy can decrease the fitness of its prey species. The virus was found to impact the host proteasome and phagosome pathways related to protein degradation and immunity, providing a potential mechanism to enhance controlling efficiency.

Keywords: *Arma chinensis virus*-1, diet consumption, fitness, transcriptome, protease activity

1. Introduction

Parasitic and predatory natural enemies play an important role in integrated pest management such as wasps and beetles (Dickman *et al.* 2011; van Lenteren 2012; Yang *et al.* 2014; Dainese *et al.* 2017). Insect-pest hosts have evolved immunity pathways to help protect against parasitoids, e.g., by encapsulating the parasitoids or killing them directly by horizontally-transmitted killing factors (Kim-Jo *et al.* 2019; Gasmi *et al.* 2021). Correspondingly, parasitoids have evolved the capacity to alter the immune response of insect-pest hosts to improve their own fitness, e.g., using polydnaviruses (e.g., bracoviruses) and iflaviruses (e.g., *Dinocampus coccinellae paralysis virus*, DcPV) to overcome the hosts' immune system and improve their parasitic efficiency (Edson *et al.* 1981; Herniou *et al.* 2013; Dheilly *et al.* 2015; Ye *et al.* 2018). Compared to parasitoids, predatory natural enemies typically attack a wide range of pest species. Predatory natural enemies are thought to be able to capture their prey without assistance, because they are generally larger and stronger (Yang *et al.* 2017). Predatory natural enemies cannot avoid being infected by viruses (Shi *et al.* 2016; Li *et al.* 2017; Xu *et al.* 2017). However, the relationship between the viruses from predatory natural enemies and the prey species is unclear.

Arma chinensis (Hemiptera: Pentatomidae) is one of the most important predatory natural enemies of Lepidopterans, feeding on a wide range of pest species (e.g., *Spodoptera frugiperda* and *Mythimna separata*) (Zou *et al.* 2015, 2019; Pan *et al.* 2019; Dong *et al.* 2021). Previously, using RNA-seq, we discovered a novel virus (*Arma chinensis virus*-1, AcV-1) in *A. chinensis*, a new member of the genus *Cripavirus* in the family *Dicistroviridae* (Sun M *et al.* 2021). Most of the members of the genus *Cripavirus* have a broad host range. For example, *Cricket paralysis virus* (CrPV), *Nilaparvata lugens C virus* (NLCV), and *Rondani's wasp virus 1* (RoWV-1) have been shown to infect species from different orders (Scotti *et al.* 1981; Wang *et al.* 2016; Zhang *et al.* 2021). Dicistroviruses and their insect hosts form complex and diverse relationships. For instance, RoWV-1 was found to be conditionally mutualistic in *Drosophila melanogaster* by reducing the eclosion rate and enhancing fecundity. Additionally, CrPV caused increased mortality in *D. melanogaster*, suggesting that it could be used as a potential biopesticide in pest management (Warsaba *et al.* 2020). *Spodoptera frugiperda* is a polyphagous global crop pest, feeding on over 300 host plant species and causing severe damage and economic losses by yield reduction of grains (Hardke *et al.* 2011; Early *et al.* 2018; Montezano *et al.* 2018). *Spodoptera frugiperda* invaded China *via* Myanmar in December 2018, a severe threat to grain production in China (Jing *et al.* 2020; Sun X *et al.* 2021). Previous research has indicated that *A. chinensis* could be used to efficiently control *S. frugiperda* in China (Tang *et al.* 2019; Wang *et al.* 2019).

In this study, we were interested in looking at the potential interactions between the virus (AcV-1), the predator (*A. chinensis*), and the crop pest (*S. frugiperda*). We used microinjection techniques to construct AcV-1 virus-positive and -negative strains of *S. frugiperda*, with a view to determining the interaction between the virus and one of the prey species of *A. chinensis*, namely *S. frugiperda*. Bioassays revealed the transmission mode of the AcV-1 virus and its impact on *S. frugiperda* growth and development, and RNA-seq was used to determine the virus-host interaction mechanisms. The experimental results were further verified by qPCR and total protein activity assay.

2. Materials and methods

2.1. Insect and virus

Spodoptera frugiperda were collected in the field from Jiangcheng, Yunnan Province, China. They were reared on an artificial diet (Liang *et al.* 1999) at 26°C with (60±5)% relative humidity and a photoperiod of 14 h L:10 h D. Adult moths were fed with 5% sugar water.

Arma chinensis individuals carrying AcV-1 were ground in liquid nitrogen to obtain a powder and stored at –80°C. The virus-positive *A. chinensis* powder was mixed with 0.01 mol L^{-1} PBS (pH=7.2–7.4), centrifuged at

6,000 r min^{-1} for 15 min at 4°C, and the supernatant was filtered through a 0.2 μm filter to obtain the filtrate carrying AcV-1 virus.

2.2. AcV-1 detection, preparation, and quantification

Total RNA was extracted from virus-positive *A*. *chinensis* individuals and stored at –80°C with TRIzol reagent (Invitrogen, Grand Island, USA). cDNA was synthesized using the TransScript® One-step gDNA Removal and cDNA Synthesis Super Mix Kit (TransGen, Beijing, China). The specific detection primers were designed according to the whole genome sequence of AcV-1 (GenBank no. MW846634) (Appendix A), with the amplified fragment of 725 bp in length. The PCR program was as follows: 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C for 35 cycles. The amplicon was cloned into the pEASY-T1 cloning vector (TransGen, Beijing, China) and sent to Shanghai Paisenuo Biotechnology Co., Ltd. for sequence determination. Plasmids were extracted using the EasyPure® Plasmid Miniprep Kit (TransGen, Beijing, China). Plasmids with correct assay results were used for standard curve construction. The standard curve for quantification of AcV-1 was generated with the plasmid above and the TaqMan qPCR method as described previously (Xu *et al*. 2020). Using the above plasmids as templates, the sensitivity of primers AcV-1-F/AcV-1-R was determined by PCR and 10-fold dilutions. All primers used in this study are shown in Appendix A.

2.3. Transmission mode of AcV-1 in *S. frugiperda*

Firstly, we maintained a strain of *S*. *frugiperda* without the AcV-1 infection (N-strain) by confirming virus-negative individuals with specific PCR primers stated above. Horizontal transmission of AcV-1 in *S*. *frugiperda* was investigated by peroral infection and microinjection, as previously reported for partiti-like viruses (Xu *et al.* 2020). Briefly, to establish peroral infection, 20 newly-hatched larvae from the N-strain were put into the Petri dish (35 mm) with the AcV-1 (2.31×10^8 copies μL^{-1}, 150 μL per Petri dish) for 48 hours, and then they were reared in 24-well plates separately for 7 days. To establish infection *via* microinjection, 20 newly-moulted 5th instar larvae were randomly selected from the N-strain culture and injected with 10 μL filtered liquid containing AcV-1 virus (two concentrations of AcV-1 virus were used, 2.31×10^8 and 2.31×10^7 copies μL^{-1}), with a Hamilton Microliter (705N) syringe and Harvard Pump 11 Elite. These were reared individually for a further 7 days to establish a viral infection. The resulting AcV-1 virus-positive *S*. *frugiperda* (injected with 2.31×10^8 copies μL^{-1} AcV-1 virus filtrate) were mated and used to determine if the virus could be vertically transmitted in *S*. *frugiperda*.

2.4. Replication of AcV-1 in *S. frugiperda*

To detect AcV-1 replication in *S*. *frugiperda*, two concentrations of AcV-1 virus were used (2.31×10^8 and 2.31×10^7 copies μL^{-1}) with 10 μL per individual (newly-moulted 5th instar larvae). Individual insects were collected at different time points after microinjection for virus detection, including L-3d (3rd day after injection), L-5d (5th day after injection), P-1d (1st day of pupa), P-3d (3rd day of pupa), P-4d (4th day of pupa) and A (adult). Three biological replicates were sampled at each point. The Ct value of each sample was determined by the absolute fluorescence quantitative probe method, and the copies of AcV-1 in *S*. *frugiperda* were calculated using a standard curve.

2.5. Effects of AcV-1 on the life-table parameters of *S. frugiperda*

The life history parameters of *S*. *frugiperda* were determined with AcV-1 virus-positive and -negative individuals by microinjection, including larval diet consumption, larval weight, pupation rate, pupal weight, eclosion rate, and fecundity. One-day-old 5th instar N-strain larvae were used to perform microinjection with 10 μL filtered liquids generated with AcV-1 virus-negative and -positive individuals, which contained one of two concentrations of the AcV-1 virus filtrate (2.31×10^8 and 2.31×10^7 copies μL^{-1}). Individuals were recorded daily until pupation, including diet consumption, body weight, and survival rate. Diet consumption was calculated by weighing the diet daily. Pupal weight on the 3rd day, the pupation rate, the period of the pupa, and the eclosion rate were recorded. There were four groups and 72 individuals for each group to calculate the pupation rate and eclosion rate. Individuals dying within 24 h after microinjection were excluded from the experiment. Additionally, adult moths from AcV-1 negative and positive individuals were mated in single pairs (F+/M+ and F–/M–) in plastic boxes (diameter=11 cm, height=6 cm) to determine fecundity with no less than 15 replicates.

2.6. Analyzing the effects of AcV-1 on *S. frugiperda* by transcriptome

One-day-old 5th instar N-strain larvae were again used to perform microinjection with filtered liquids.

AcV-1 virus-positive and -negative individuals were collected, and RNA-seq was performed on the 3rd day after microinjection, as previously described (Xu *et al.* 2020). Briefly, total RNA was extracted as described above and quantified using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system. The cDNA library was constructed with TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions, as described previously (Xu *et al.* 2020). Suitable fragments (200–300 bp) judged by agarose gel electrophoresis were enriched with PCR amplification to prepare the sequencing library, and the library was sequenced with the Illumina HiSeq platform for about 6 gigabases in-depth. Gene annotation was performed using the *S. frugiperda* genome as a reference (https://www.ncbi.nlm.nih.gov/genome/10985). For gene expression analysis, the number of expressed tags was calculated and then normalized to fragments per kilobase million (FPKM) using RSEM software packages (Li and Dewey 2011). Differential expression analysis was performed using the DESeq2 R package (1.20.0). The resulting *P*-values were adjusted using Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted *P*-value (FDR)<0.05 found by DESeq2 were assigned as differentially expressed. Principal component analysis (PCA) with differentially-expressed gene (DEG) data was performed with 'prcomp' in the R package. The hierarchical clustering method was applied to analyze the expression pattern of significantly differentially expressed unigenes in different samples. Significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were identified using the Fishers exact test (FDR<0.05).

2.7. Detection of gene expression levels by qPCR and total protease activity

The RNA-seq results were verified using DEG from the proteasome pathway by qPCR on a 7500 Fast Real-time PCR System (Applied Biosystems, USA), with *β-actin* and GAPDH as reference genes. Three biological replicates were set in each group, and three individuals were taken as a replicate. The reaction system was 20 μL in volume, each consisting of 10 μL of TB Green Premix *Ex Taq* (Tli RNaseH Plus) (2×), 0.4 μL of ROX Reference Dye II, 0.4 μL of upstream and downstream primers, 6.8 μL of sterile water, and 2 μL of template. The reaction conditions for fluorescence quantification were: 95°C for 20 s, 40 cycles of 95°C for 5 s, and 60°C for 30 s. In addition, we also tested the total protease activity of sample individuals (3 days post microinjection of 2.31×10^{8} copies μL^{-1} AcV-1 virus). The total protease activity was measured using a total protease activity kit (microplate method) (Suzhou Grace Biotechnology Co., Ltd., China), and the specific operations were carried out according to the instructions.

2.8. Statistics

Statistical analyses were conducted using GraphPad Prism 8 and SPSS. AcV-1 virus replication in *S. frugiperda* was analyzed using 2-way ANOVA with Tukey's HSD post-hoc tests. Student's *t*-test was used to determine the significance of larval diet consumption, larval weight, pupal weight, and DEGs in qPCR. Pupation rates, eclosion rates, egg production, and total protease activity were analyzed using ANOVA.

3. Results

3.1. Transmission mode of AcV-1 virus in *S. frugiperda*

AcV-1 virus was not found to be transmitted horizontally by oral infection in *S. frugiperda* (Table 1). However, it was found to be transmitted efficiently by microinjection (using a viral filtrate concentration of 2.31×10^{8} or 2.31×10^{7} copies μL^{-1} (100%) (Table 1). AcV-1 was not found to be vertically transmitted from parent to offspring (Table 1).

A standard curve was generated for quantifying the copies of AcV-1 (Appendices B and C), with the minimum detection limit of AcV-1 using primers AcV-1-F/AcV-1-R being 20.5 copies μL^{-1} (Appendix D). AcV-1 copy number

Table 1 Transmission mode of *Arma chinensis virus*-1 (AcV-1)

Transmission mode	Virus filtrate concentration (copies μL^{-1})	Individuals[1)]	Number of test samples (AcV-1+)	Number of test samples (AcV-1–)	Transmission efficiency (%)
Horizontal	2.31×10^{8}	Peroral infection	0	120	0
	2.31×10^{8}	Microinjection	120	0	100
	2.31×10^{7}	Microinjection	120	0	100
Vertical	2.31×10^{8}	Female+/Male+	0	120	0
		Female–/Male–	0	120	0
		Female+/Male–	0	120	0
		Female–/Male+	0	120	0

[1)] Infected individuals indicated as +, uninfected individuals indicated as –.

varied with both the magnitude of the challenge dose (F=168.21, df=1, 29, P<0.0001) and with the life-stage of *S. frugiperda* (F=139.04, df=5, 29, P<0.0001), but the interaction between these two terms was non-significant (F=1.08, df=5, 24, P=0.39). Specifically, at both challenge doses, viral load increased during larval development and was consistently high in the pupal and adult stages. Higher viral challenge doses result in higher viral loads (Fig. 1; Appendix C).

3.2. Effects of AcV-1 on the development, diet consumption, pupation, and eclosion rate of *S. frugiperda*

Initial individual weights of *S. frugiperda* were not significantly different between the control groups and the treatment groups (t=0.48, df=117, P=0.64, virus-negative individuals: n–=49, virus-positive individuals: n+=70). Following inoculation *via* microinjection, the following changes were observed by recording the life-table parameters. When comparing AcV-1 virus-negative and -positive individuals (injected with 2.31×10^9 copies/per individual), the diet consumption was significantly inhibited by infection with AcV-1, including diet consumption (Fig. 2-A–C) and increase of larval body weight (Fig. 2-E–G). Additionally, the larvae with AcV-1 showed symptoms of paralysis. Compared to virus-negative individuals, there was no significant difference in the pupation rate (Appendix E-a), length of the female pupal period (Appendix E-b), or length of the male pupal period (Appendix E-c); however, the pupal weight was significantly lighter in the virus-positive individuals (Fig. 2-D). Additionally, the eclosion

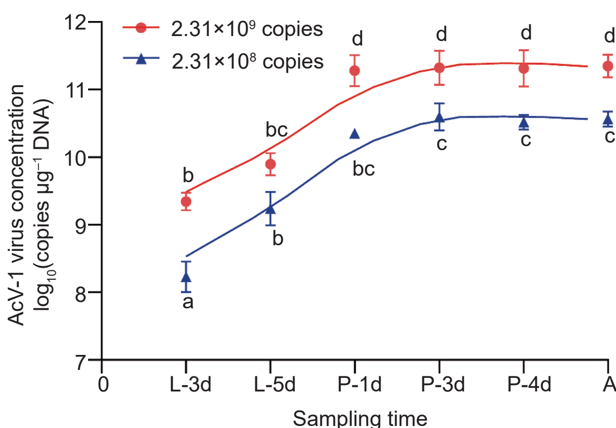

Fig. 1 Viral quantification by *Taq*Man real-time PCR. L-3d, 3rd day after injection; L-5d, 5th day after injection; P-1d, 1st day of pupa; P-3d, 3rd day of pupa; P-4d, 4th day of pupa; A, adult. Bars are SD (n=3). Different letters indicate significant differences among each other at P<0.05, as determined by Tukey's test.

rate decreased significantly (by 29.12%) in virus-positive individuals (Fig. 2-H). The fecundity of individuals surviving to adulthood was not significantly affected by AcV-1 infection (Appendix E-d).

To explore whether the AcV-1 effect on *S. frugiperda* was related to virus concentration, we also injected individuals with 10-fold dilutions of virus inoculant (2.31×10^8 copies per individual). The initial weight of *S. frugiperda* was not significantly different between the control group and the treatment group (t=0.6524, df=89, P=0.5158, n–=49, n+=42). At this dose, there were no significant differences between AcV-1 virus-negative and -positive, in terms of diet consumption, growth of larval body weight, pupal weight, pupation rate (Fig. 2-I–O; Appendix E-e), length of female pupal period (Appendix E-f), length of male pupal period (Appendix E-g) or eclosion rate (Fig. 2-P). These results suggest that AcV-1 could impact *S. frugiperda* only at high doses.

3.3. Transcriptome analysis in *S. frugiperda*

To understand the interaction between AcV-1 and *S. frugiperda* further, we determined the DEGs between the transcriptomes of AcV-1 virus-positive and negative larvae at 3 days post-infection (Appendix F). Using the *S. frugiperda* genome sequence as the reference, gene expression was quantified in the AcV-1 positive groups and compared to related virus-negative groups, with ±2-fold and FDR<0.05. The number of DEGs was higher in larvae injected with 2.31×10^9 copies per individual of AcV-1 virus (up-regulation=264, down-regulation=490) than in ones injected with AcV-1 virus of 2.31×10^8 copies per individual (up-regulation=4, down-regulation=8) (Appendices G and H), suggesting the regulation of gene expression levels on *S. frugiperda* by AcV-1 was related to virus concentration and there were few effects on *S. frugiperda* with 2.31×10^8 copies per individual. Therefore, we only analyzed the data from individuals injected with 2.31×10^9 copies of the virus. The PCA with DEG data clearly distinguished AcV-1 positive from negative individuals (Fig. 3-A; Appendix H), these results suggest that AcV-1 had an effect on the gene expression profiles of *S. frugiperda*. As reported in three *Spodoptera* species previously (Xu *et al.* 2020, 2022), we performed a pathway enrichment analysis with the DEGs. Interestingly, only two pathways (proteasome and phagosome, which were related to protein degradation and immune response) in samples infected with 2.31×10^9 copies viruses were significantly enriched. The majority of the genes in these two pathways were significantly down-regulated (Fig. 3-B and C; Appendix C). These results were consistent with those from the bioassays

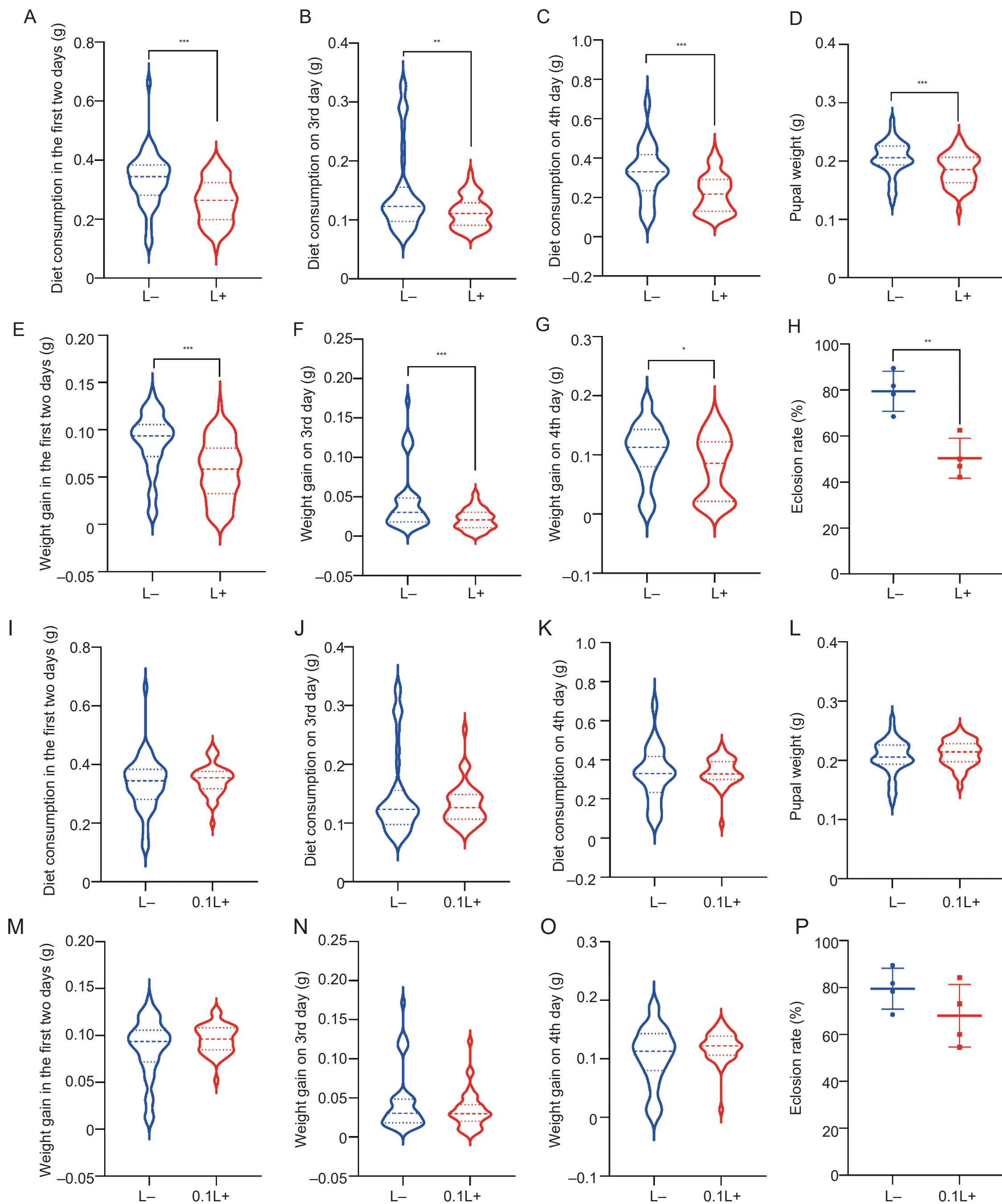

Fig. 2 Life-history parameters of AcV-1 virus-negative (L–) and two concentration AcV-1 virus-positive (L+, 2.31×10^9 copies per individual; 0.1L+, 2.31×10^8 copies per individual) individuals of *Spodoptera frugiperda*. A, diet consumption within 2 days after injection (t=4.853, df=118, P<0.0001, n–=49, n+=70). B, diet consumption on 3rd day (t=3.018, df=90, P=0.0033, n–=41, n+=51). C, diet consumption on 4th day (t=4.355, df=90, P<0.0001, n–=41, n+=51). D, pupal weight on 3rd day (t=3.493, df=90, P=0.0007, n–=41, n+=51). E, growth of larval body weight within 2 days after injection (t=5.072, df=118, P<0.0001, n–=49, n+=70). F, growth of larval body weight on 3rd day (t=3.919, df=90, P=0.0002, n–=41, n+=51). G, growth of larval body weight on 4th day (t=2.421, df=88, P=0.0176, n–=41, n+=51). H, eclosion rate (F=20.389, df=1, 6, P=0.004, n=4 groups, 72 individuals for each group). I, diet consumption within 2 days after injection (t=0.9128, df=89, P=0.3638, n–=49, n+=42). J, diet consumption on 3rd day (t=1.137, df=81, P=0.2590, n–=41, n+=42). K, diet consumption on 4th day (t=0.4306, df=81, P=0.6679, n–=41, n+=42). L, pupal weight on 3rd day (t=3.493, df=80, P=0.2868, n–=41, n+=41). M, growth of larval body weight within 2 days after injection (t=1.790, df=89, P=0.0768, n–=49, n+=42). N, growth of larval body weight on 3rd day (t=1.461, df=81, P=0.1479, n–=41, n+=42). O, growth of larval body weight on 4th day (t=1.749, df=81, P=0.0841, n–=41, n+=42). P, eclosion rate (F=1.133, df=1, 6, P=0.3280, n=4). L–=AcV-1 viruses-negative, L+=AcV-1 viruses-positive (2.31×10^9 copies per individual), 0.1L+=AcV-1 viruses-positive (2.31×10^8 copies per individual). Data are mean±SD. *, P<0.05; **, P<0.01; ***, P<0.001.

in showing that the effect of AcV-1 on *S. frugiperda* was related to virus concentration, i.e., only the higher dose had an effect on gene expression. Pathways related to the digestive system were not significantly enriched. However, genes encoding protease were significantly down-regulated (Appendix H), suggesting protease activities were inhibited in virus-positive individuals.

3.4. Expression levels of genes in the proteasome pathway and enzyme activity of total protease

To confirm the results from RNA-seq, we chose six DEGs in the proteasome pathway to validate their expression levels by qPCR. Consistently, the results showed significant down-regulation of these genes (Fig. 4; Appendix C). Additionally, the total protease activity of AcV-1 virus-positive individuals was significantly (more than 2-folds) lower than that of AcV-1 virus-negative individuals (F=22.279, df=1, 16, P<0.0001, n+=9, n–=9) (Fig. 5; Appendix C). These results suggest that AcV-1 may inhibit the diet consumption of their pest host by reducing protease activity.

4. Discussion

Due to their environmentally friendly nature and host-specificity, insect viruses are considered ideal agents for the biological control of pests (Lacey *et al.* 2015). As well as killing their hosts directly (e.g., baculoviruses) (Haase *et al.* 2015), the complicated interactions between some viruses and insects can also be used in pest management strategies. For example, polydnaviruses are beneficial to parasitoid wasps by helping them combat the immune systems of the wasps' Lepidopteran hosts and increasing the wasps' parasitic efficiency. Additionally, polydnaviruses-derived genes in Lepidopterans increase their resistance levels against baculoviruses (Edson *et al.* 1981; Dheilly *et al.* 2015; Gasmi *et al.* 2015; Li *et al.* 2022). In this study, for the first time, we explored the interaction of a virus isolated from a predatory insect

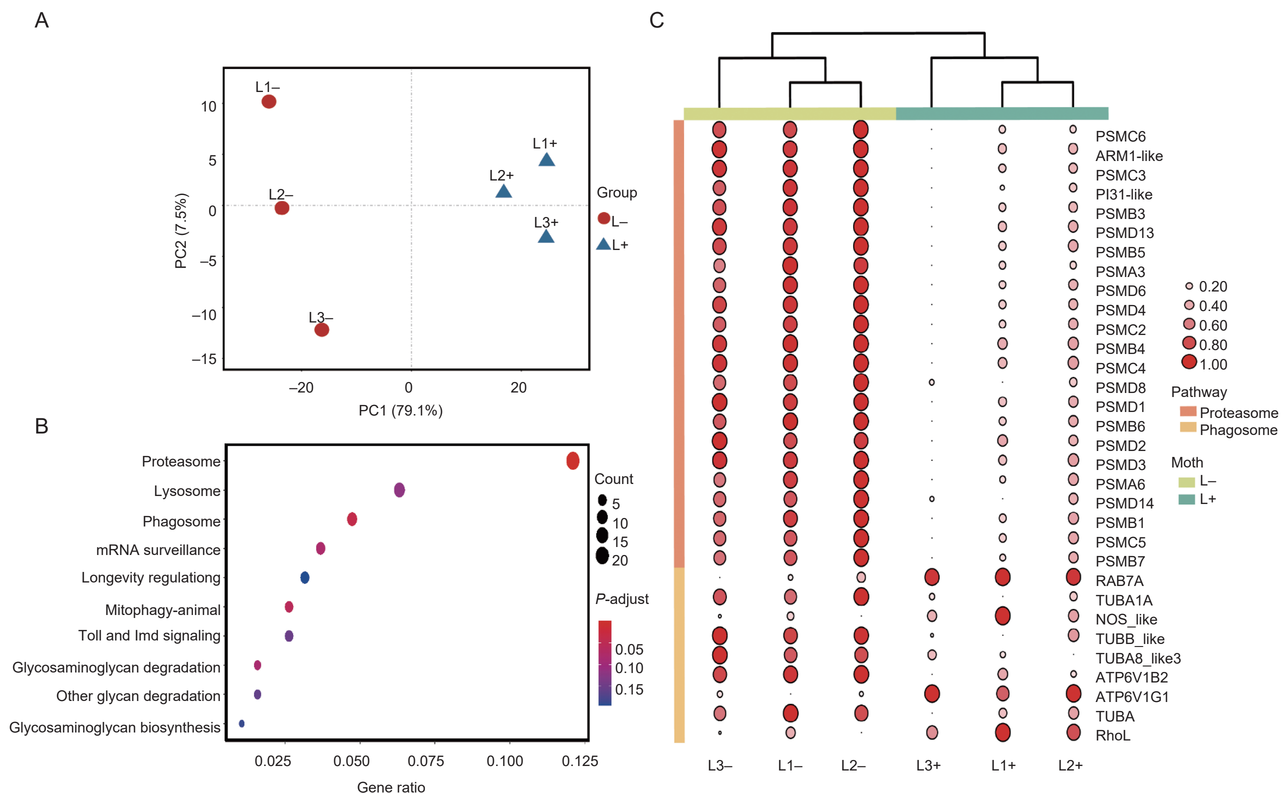

Fig. 3 Transcriptome analysis using *Arma chinensis virus*-1 (AcV-1)-positive individuals compared to related -negative individuals in *Spodoptera frugiperda*. A, principal components analysis (PCA) of global gene expression of differentially-expressed gene (DEG) in the comparison of AcV-1 -positive groups and related -negative groups in *S. frugiperda*. L+, AcV-1 viruses-positive (2.31×10^9 copies per individual); L–, AcV-1 viruses-negative. B, heatmaps of KEGG pathway analysis with DEGs between L– and L+ individuals in *S. frugiperda*. C, expression levels of genes in proteasome and phagosome pathways in *S. frugiperda* based on the values of fragments per kilobase million (FPKM). The FPKM values were normalized by the logarithmic scale with base 2, and then scaled by row. The color and size of the circle indicate the gene expression level. Darker colors and larger circles indicate the genes were highly expressed in the samples. The heatmap was visualized by TBtools.

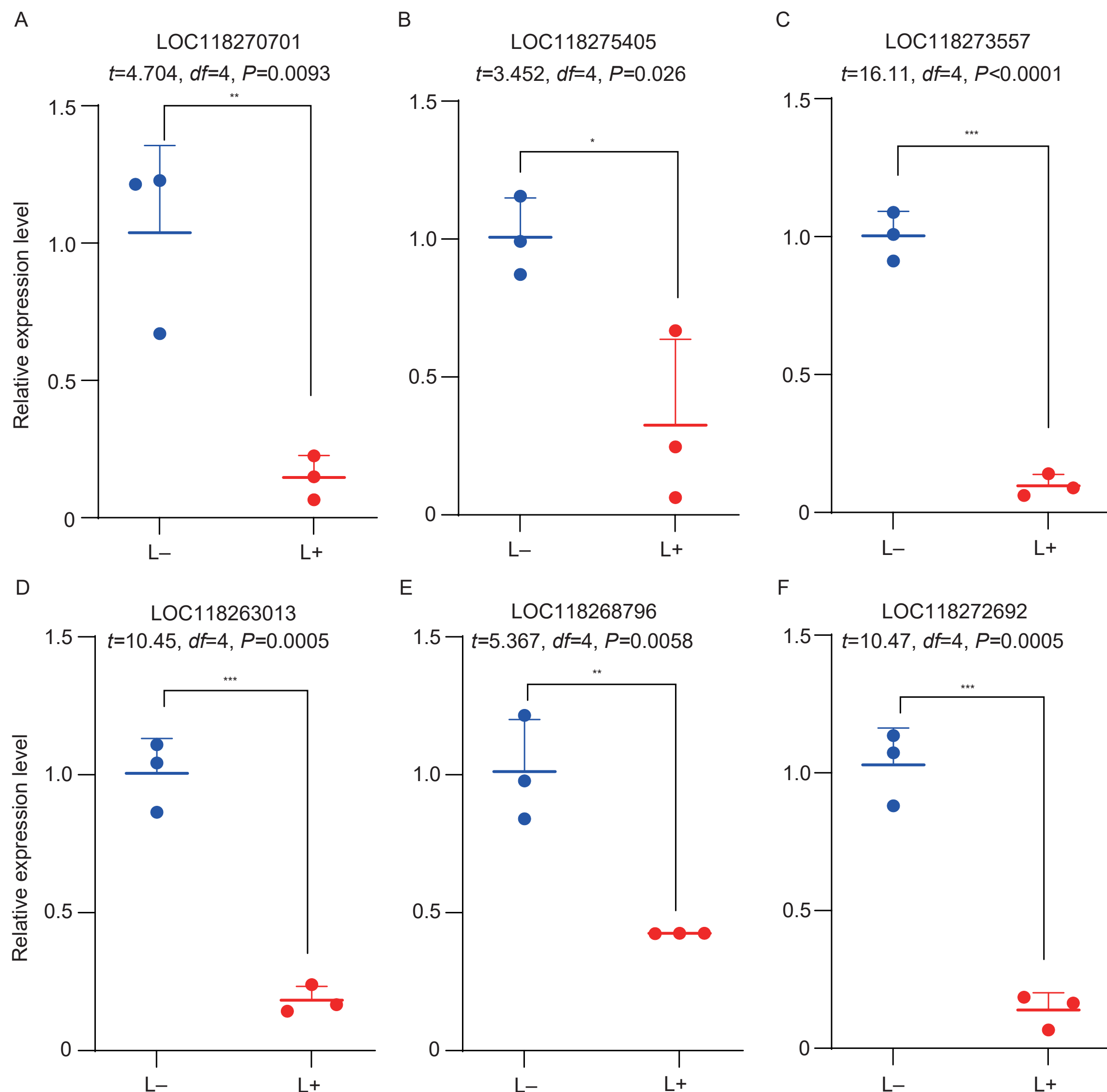

Fig. 4 Expression levels of six genes in the proteasome pathway by TBgreen qPCR using *β-actin* and GAPDH as reference genes. L+, AcV-1 viruses-positive (2.31×10^9 copies per individual); L–, AcV-1 viruses-negative. Data are mean±SD (*n*=3). *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001.

upon a prey insect. Our results indicated that this newly discovered dicistrovirus decreased the fitness of the prey (*S. frugiperda*), suggesting a possible role in the diet consumption of this predator and possible use for the biocontrol of Lepidopterans.

Viruses can be transmitted in a variety of ways, including *via* vertical and horizontal transmission pathways. For example, baculoviruses and *Helicoverpa armigera densovirus 2* (HaDV2) have been found to be transmitted both vertically (transovarial) and horizontally (orally) (Chen *et al.* 2006; Xu *et al.* 2014); partiti-like viruses can be transmitted horizontally by microinjection and vertically by transovarial maternal inheritance (Xu *et al.* 2020), whereas RoWV-1 can only be transmitted horizontally (orally) (Zhang *et al.* 2021). We found that in *S. frugiperda*, AcV-1 was efficiently transmitted only by horizontal microinjection and could not be transmitted horizontally by oral infection or vertically. Using their needle-like ovipositors, parasitoids can act as vectors to transmit some viruses horizontally between insect hosts. For example, *Deformed wing virus* (DWV) is transmitted by *Varroa destructor* in honeybees, and polydnaviruses are transmitted by wasps to Lepidopteran species (de Miranda and Genersch 2010; Strand and Burke 2014). Likewise, *A. chinensis*, with its piercing-sucking mouthparts, could potentially also transmit the AcV-1 to Lepidopteran species naturally during unsuccessful predation attempts.

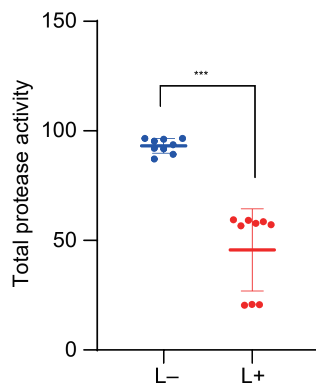

Fig. 5 Determination of total protease activity in *Arma chinensis virus*-1 (AcV-1)-negative and -positive individuals (F=22.279, df=1, 16, P<0.0001). L–, AcV-1 viruses-negative; L+, AcV-1 viruses-positive (2.31×10^9 copies per individual). Data are mean±SD (n=9). ***, P<0.001.

Insect viruses and their hosts have evolved diverse relationships. For instance, partiti-like viruses have been found to be harmful to *S. littoralis* by causing increased larval and pupal mortality (Xu *et al.* 2022). Likewise, partiti-like viruses also decrease *S. frugiperda* fitness by reducing their fecundity and increasing their susceptibility to NPV (Xu *et al.* 2020), whereas partiti-like viruses were conditionally mutualistic in *S. exempta* by increasing host resistance to baculoviruses but decreasing the fecundity of the females (Xu *et al.* 2020). HaDV2 was mutualistic in its host by increasing host fitness, manifesting as faster development, higher fecundity, and higher resistance to NPV (Xu *et al.* 2014; Xiao *et al.* 2021), whilst RoWV-1 had no obvious effects on its host (Zhang *et al.* 2021). In this study, we inoculated larvae to produce virus-positive and virus-negative cultures by microinjection and determined the phenotypic effect of the virus on the life-history traits of *S. frugiperda*. As it is believed that many novel infectious diseases emerge *via* host-jump events (Lai *et al.* 2016; Xu *et al.* 2020, 2022), it is important to explore these possible interactions. In the early stage, we explored the effect of AcV-1 on the predator (*A. chinensis*). The results showed that AcV-1 had no significant effect on the nymph development period and the male/female adult weight of *A. chinensis* (Appendix I). Then, we focused on the relationship between the virus (AcV-1) from the predatory natural enemy and the prey (*S. frugiperda*) of the predator, which potentially enhanced the controlling efficiency of the natural enemy. AcV-1 decreased the fitness of *S. frugiperda* significantly, including inhibiting diet consumption and inducing paralysis, and significantly reduced pupal weight and eclosion rate. In this study, we used 5th-instar larvae for virus infection. It showed that inoculation inhibited diet consumption and decreased body weight in both larvae and pupae, as well as reducing the eclosion rate. We cannot completely exclude the possibility that virus infection could increase larval mortality and reduce pupation rate if inoculated at earlier stages (e.g., first instar stage). Viral load was significantly lower in *S. frugiperda* when injected with a 10-fold diluted AcV-1. Interestingly, there were no significant effects on *S. frugiperda* with the low-dose virus, suggesting that the effect of AcV-1 on the host was related to virus concentration. Although we did not detect an obvious effect of AcV-1 on the predatory behavior of *A. chinensis*, we speculate that the paralysis symptom caused by AcV-1 could reduce the effectiveness of *S. frugiperda* defense behavior, which in turn could potentially help to improve the controlling efficiency of *A. chinensis*.

Analysis of DEGs by next-generation sequencing (NGS) is now widely used to explore the interaction between microbes and their hosts (Duneau *et al.* 2017; Park *et al.* 2019; Yan *et al.* 2022). To investigate the interaction between the AcV-1 and *S. frugiperda* at the gene expression and biochemistry levels, we performed transcriptome analysis and enzymatic assays with larvae, as we did previously (Xu *et al.* 2020, 2022; Xiao *et al.* 2021). There were fewer DEGs in larvae infected with the low dose of the virus than the ones with the high dose, suggesting that the effect of AcV-1 on the host was related to virus concentration. Consistent with this, there were significant impacts on diet consumption of *S. frugiperda* with the high dose of the virus but not with the low dose. The KEGG enrichment with DEGs found that the proteasome and phagosome pathways were significantly enriched by down-regulation in larvae infected with the high dose of the virus. The proteasome and phagosome pathways are related to cellular protein degradation and immune response, suggesting that AcV-1 is negative to the fitness of FAW (Zhang *et al.* 2004; Sorokin *et al.* 2009; Levin *et al.* 2016). Proteases were related to the fitness of insects (e.g., *Helicoverpa armigera* and *S. litura*), including body weight, diet consumption, and survival rate (Patankar *et al.* 2001; Bhattacharyya *et al.* 2007; Kanost and Clem 2012). The total protease activity of AcV-1 virus-positive individuals was significantly reduced, indicating that the virus-inhibited diet consumption of *S. frugiperda* was caused by down-regulating digestive enzyme activities. However, related digestive enzyme genes were not clearly defined, and this requires further investigation. Taken together, virus infection appears to have decreased the fitness of *S. frugiperda*, e.g., decreasing body weight and eclosion rate, by decreasing host immunity *via* proteasome and phagosome pathways

and activities of digestive enzymes *via* proteases.

5. Conclusion

Our study focused on the interaction between an insect virus from a predatory natural enemy and the prey of that predator by engineering a host shift. As with a previous study using three *Spodoptera* species and partiti-like viruses, AcV-1 showed a parasitic relationship with its new host (*S. frugiperda*) by inhibiting the diet consumption of larvae and increasing pupal mortality. This could help to enhance predatory efficiency by inducing paralytic symptoms in the prey. Sequencing data comparing infected and non-infected *S. frugiperda* indicated that the virus altered the gene expression profiles of *S. frugiperda*, with the higher titers of viruses inducing more DEGs. The KEGG enrichment showed that the proteasome and phagosome pathways (related to protein degradation and immunity response) were significantly enriched. Additionally, the total protease activity of AcV-1 virus-positive individuals was significantly decreased, suggesting that virus-inhibited diet consumption of *S. frugiperda via* down-regulation of the activities of digestive enzymes. Although AcV-1 could only be transmitted by microinjection in *S. frugiperda*, the piercing-sucking mouthpart of *A. chinensis* could naturally help to transmit the virus theoretically, suggesting a role in the diet consumption of this predator and potential use in pest management. It is well known that viruses increase the parasitic efficiency of parasitoids by preventing an immune response in their prey. For the first time, our results show that a virus isolated from a predator could decrease the fitness of its prey by impacting the proteasome pathway related to protein degradation and immunity. The virus may aid in the efficiency of the predatory insect, providing a potential manner to enhance the management efficiency of predators.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Ethical statement

All applicable international, national and institutional guidelines for the care and use of animals were followed.

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