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Host range of a parasitoid wasp is linked to host susceptibility to its mutualistic viral symbiont

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Abstract

Parasitoid wasps are one of the most species-rich groups of animals on Earth, due to their ability to successfully develop as parasites of nearly all types of insects. Unlike most known parasitoid wasps that specialize towards one or a few host species, *Diachasmimorpha longicaudata* is a generalist that can survive within multiple genera of tephritid fruit fly hosts, including many globally important pest species. *Diachasmimorpha longicaudata* has therefore been widely released to suppress pest populations as part of biological control efforts in tropical and subtropical agricultural ecosystems. In this study, we investigated the role of a mutualistic poxvirus in shaping the host range of *D. longicaudata* across three genera of agricultural pest species: two of which are permissive hosts for *D. longicaudata* parasitism and one that is a nonpermissive host. We found that permissive hosts *Ceratitis capitata* and *Bactrocera dorsalis* were highly susceptible to manual virus injection, displaying rapid virus replication and abundant fly mortality. However, the nonpermissive host *Zeugodacus cucurbitae* largely overcame virus infection, exhibiting substantially lower mortality and no virus replication. Investigation of transcriptional dynamics during virus infection demonstrated hindered viral gene expression and limited changes in fly gene expression within the nonpermissive host compared with the permissive species, indicating that the host range of the viral symbiont may influence the host range of *D. longicaudata* wasps. These findings also reveal that viral symbiont activity may be a major contributor to the success of *D. longicaudata* as a generalist parasitoid species and a globally successful biological control agent.

KEYWORDS
Bactrocera dorsalis, *Ceratitis capitata*, *Diachasmimorpha longicaudata*, poxvirus, symbiosis, *Zeugodacus cucurbitae*

1 | INTRODUCTION

Parasitoid wasps, which are obligate parasites of other arthropods, represent one of the most diverse groups of animals known to exist (Forbes et al., 2018; Huber, 2017; LaSalle & Gauld, 1993). The overwhelming success of parasitoid wasps can largely be attributed to their ability to exploit virtually all types of insects as hosts, as well as some noninsect arthropods (LaSalle & Gauld, 1993). Effective

parasitism often involves specialization of a parasitoid species to optimize its developmental strategy towards one or a few closely related host species (Godfray, 1994). Increasing molecular evidence supports that many parasitoid species once thought to have broad host ranges instead represent assemblages of cryptic species, each with a narrower host range (Condon et al., 2014; Forbes et al., 2009; Sheikh et al., 2022; Smith et al., 2008). Parasitoids that develop inside the body of their host, known as endoparasitoids, display more extreme forms of specialization, such as the production of virulence factors, as they must directly combat the host immune system in order to survive (Shaw, 1994; Strand & Pech, 1995). Female wasps inject these factors into hosts during oviposition, which include venoms that can alter host immunity and developmental systems for the benefit of developing wasps (Mrinalini & Werren, 2017). In some cases, additional factors such as virus-derived particles are also produced within female wasps and cause similar detrimental effects when delivered to host insects during parasitism (Asgari & Rivers, 2011).

A growing number of independent viral elements have been documented within parasitoid wasp lineages, and many represent stable, heritable associations that provide substantial benefit to, or are absolutely required by, the wasps that produce them (Burke et al., 2018, 2021; Lawrence, 2005; Pichon et al., 2015; Strand & Burke, 2014). Most examples that have been genetically characterized are known to exist as domesticated endogenous viruses (DEVs) within wasp genomes that are activated towards the end of adult wasp maturation and produce high densities of virus or virus-like particles (VLPs) within the reproductive tissues of the associated parasitoid (Béliveau et al., 2015; Bézier et al., 2009; Burke et al., 2014, 2018; Drezen et al., 2022; Pichon et al., 2015; Volkoff et al., 2010). However, due to viral genome rearrangements that have occurred in all known parasitoid-DEV systems, the virus particles or VLPs cannot replicate outside the wasp (Burke, 2019; Drezen et al., 2017). The virulence caused by these viral elements when injected into the insect hosts of the wasps is primarily due to viral infection and delivery of virulence genes and/or proteins into host cells (Gauthier et al., 2018). Some DEV virulence genes interact directly with the host immune system, and many demonstrate evidence of positive selection (Huguet et al., 2012; Strand, 2012). DEVs are therefore thought to provide additional means of specialization for the associated wasp via coevolution with its host (Branca et al., 2012). Consequently, many DEV-associated parasitoid wasps are specialists with narrow host ranges that encompass a small number of sister host species (Smith et al., 2008; Whitfield, 1994).

Recent genomic sequencing of a heritable poxvirus produced in the venom gland of *Diachasmimorpha longicaudata* wasps, known as Diachasmimorpha longicaudata entomopoxvirus (DIEPV), revealed that this virus is not a DEV but maintains an exogenous double-stranded DNA genome that successfully replicates within both *D. longicaudata* wasps and tephritid fruit fly hosts (Coffman et al., 2020; Coffman & Burke, 2020). DIEPV replication is highly lethal to *Anastrepha suspensa* hosts, while *D. longicaudata* wasps are seemingly unaffected by virus replication (Coffman et al., 2020). Furthermore, we showed that eradication of DIEPV from *D. longicaudata* wasps caused a severe drop in parasitism success, although a small proportion of wasps can survive without the virus (Coffman et al., 2020, 2022). Therefore, DIEPV appears to be a pathogen to *A. suspensa* flies but displays a highly beneficial, if not obligate relationship with *D. longicaudata* wasps.

Native to Southeast Asia, *Diachasmimorpha longicaudata* is a larval–pupal endoparasitoid well-known for its widespread use as a biological control (biocontrol) agent to suppress various tephritid fruit fly pest populations in the tropics and subtropics (Ovruski et al., 2000; Vargas et al., 2012). In 1948, *D. longicaudata* was introduced to Hawai'i along with many other parasitoid species to control invasive populations of the Mediterranean fruit fly, *Ceratitis capitata*, the oriental fruit fly, *Bactrocera dorsalis*, and the melon fly, *Zeugodacus cucurbitae* (Bess et al., 1961). *Diachasmimorpha longicaudata* was one of few parasitoid species to become established on the islands, and together with the species *Fopius arisanus*, led to significant reductions in *B. dorsalis* and *C. capitata* populations, representing one of the most successful fruit fly biocontrol programs worldwide (Haramoto & Bess, 1970; Vargas et al., 2012). Unlike most parasitoids that are specialists, including many of those that harbour DEVs, *D. longicaudata* is a generalist parasitoid that can successfully develop within several genera of tephritid fruit flies, including *Anastrepha*, *Ceratitis* and *Bactrocera* (Ovruski et al., 2000; Vargas et al., 2012). It is therefore of interest to understand how an exogenous viral symbiont, like DIEPV, may influence the host range of a generalist parasitoid wasp compared with the effects of endogenized parasitoid viruses on their associated specialists.

Here, we investigated the role of DIEPV in shaping the host range of *D. longicaudata* wasps by exploring the effects of the virus in three pestiferous fruit fly host species: *C. capitata*, *B. dorsalis* and *Z. cucurbitae*. Our results suggest that DIEPV could be a major contributing factor to the ability of *D. longicaudata* to parasitize a wide range of hosts and indicate that the host range of a viral symbiont may affect the host range of its associated parasitoid wasp. Furthermore, this work shows that a heritable parasitoid virus is highly lethal to a number of major fruit fly pest species and therefore could be used in future agricultural innovations to control fruit fly pests.

2 | MATERIALS AND METHODS

2.1 | Insects

Diachasmimorpha longicaudata parasitoid wasps, as well as *C. capitata*, *B. dorsalis*, and *Z. cucurbitae* fruit flies used in this study were obtained from USDA-ARS laboratory colonies maintained at the Daniel K. Inouye US Pacific Basin Agricultural Research Center in Hilo, Hawai'i, which were reared as previously described (Vargas, 1989; Wong & Ramadan, 1992).

2.2 | Parasitism assays

Female *D. longicaudata* wasps were housed in cages with same-age male wasps upon adult emergence, allowing females to mate prior to oviposition. Fly larvae of a given species were retrieved from within larval diet containers at the second-instar stage and placed between two pieces of organza fabric restricted within a 2.5 in flexible embroidery hoop. Groups of six adult female wasps that were at least 7 days old and naïve (i.e. had not yet laid eggs) were offered fly larvae for 4 h to allow for oviposition. Afterwards, larvae were placed back into fresh diet and kept in standard rearing conditions until they pupated, approximately 48–72 h later. At this time, fly pupae were inspected for those that bore a single oviposition scar on the cuticle, a mark that persists to the pupal stage due to the hardening of the final larval instar cuticle into the pupal case. Each oviposition scar indicated that a single wasp egg had likely been deposited within the fly. Singly scarred flies were kept in standard rearing conditions for 4 weeks, after which the proportions of emerged adult wasps, adult flies and pupal cases from which nothing emerged were recorded. The rate of occurrence for each category was calculated by dividing the number of insects in the category by the total number of singly scarred pupae in the trial. Significant differences in average outcome rate between the three fly species were statistically analysed using one-way ANOVA, and multiple comparison testing was done using Tukey's HSD with JMP Pro 14 software. The details of the assay experimental set-up and one-way ANOVA test results are included in Table S1.

2.3 | Virus injections

DIEPV was filter-purified from the pooled venom gland tissue of 100 female *D. longicaudata* wasps using a previously performed protocol (Coffman et al., 2020). Resulting purified virus particles were eluted in 400 μL phosphate-buffered saline (PBS), and the inactive virus stock was generated by exposing half of the prepared active virus stock to shortwave (254 nm) UV energy for 10 min using a UVP HL-2000 HybriLinker. Both active and inactive virus stocks were stored at −80°C. A DIEPV dose of 1 oviposition equivalent/μL (approximately 10^7 viral copies/μL) was obtained by making a 1:20 dilution of the virus stock, and successive dilutions were made from this dose to generate the 0.2× and 0.1× oviposition equivalent doses. Third-instar fly larvae were each injected with 1 μL of virus from each treatment and dose, then kept in standard rearing conditions for 4 weeks. Third-instar larvae were used in favour of second-instar larvae for manual virus injections and subsequent experiments, due to their larger size, which aided in consistent viral dosing and reduced initial fly mortality from needle-inflicted wound trauma. The proportions of adult flies that emerged after injection with DIEPV were then calculated by dividing the number of adult flies that had emerged by the total number of larvae injected for each treatment and dose. Normalized fly survival rate was calculated by dividing the active DIEPV fly survival rate by the UV-inactivated fly survival rate for each species and dose combination.

2.4 | DNA isolation and qPCR estimation of viral abundance

DIEPV abundance was estimated over time by collecting fly samples during parasitism by *D. longicaudata* or after manual injection of purified virus. Third-instar fly larvae were used for initial oviposition or injection in both time course analyses. DNA was extracted from whole-body fly samples using the NucleoMag 96 Tissue Kit (Macherey-Nagel) performed on a KingFisher Flex instrument (Thermo). DNA samples were each eluted in 100 μL elution buffer consisting of 5 mM Tris/HCl (pH 8.5). Viral copy number was then determined for each sample with quantitative PCR (qPCR) using specific primers for the DIEPV poly(A) polymerase small subunit gene, as done previously (Coffman et al., 2020). Copy numbers were $\log_{10}$-transformed, then subjected to statistical analysis with JMP Pro 14. One-way ANOVA was used to test for significantly different mean copy numbers across time points during parasitism, and Tukey's HSD was used for multiple comparison tests. For injection data, two-way ANOVA was used to test for differences in means between levels of either timepoint or dosage, as well as the interaction between the two effects. The details of the experimental set-up and ANOVA test results are included in Table S1.

2.5 | RNA isolation and transcriptome sequencing

Transcriptional changes during DIEPV infection were investigated through transcriptome sequencing of 81 whole-body fly samples collected under various treatments and time points after third-instar fly larvae were injected with the virus, as outlined in Table S2. An initial 162 immature flies were collected, consisting of six biological replicates per treatment combination. Each fly was collected in 300 μL TRIsure (Bioline), a guanidine thiocyanate and phenol mixture, and immediately bead homogenized prior to storage at −80°C. Total RNA was isolated from homogenized samples using the Zymo Direct-zol-96 RNA Purification Kit performed on a KingFisher Flex instrument, which included a mid-protocol DNase I (6 U/μL, 5 μL) treatment, as well as a secondary Zymo DNase I treatment following RNA isolation. Final RNA was eluted in 20 μL nuclease-free water. RNA quality was assessed using a 4200 TapeStation System with RNA ScreenTape (Agilent), and three of the six replicates with highest quality for each treatment were selected to constitute the final 81 samples submitted for sequencing. Messenger RNA library preparation and deep sequencing of all samples were performed by Novogene Corporation Inc. Paired end 150 bp sequences were generated on an Illumina Novaseq 6000 platform, generating an average of 88.5 ± 9.5 million reads per sample. Raw read pairs were quality-filtered using Trimmomatic v0.38 with default settings, which

resulted in >99.99% read pairs retained for each sample (Bolger et al., 2014).

2.6 | DIEPV differential expression analysis

DIEPV differential gene expression analysis was conducted on the 54 virus-infected samples by first mapping quality-filtered reads onto the DIEPV reference genome using HISAT2 v2.2.1 (Kim et al., 2015; Pertea et al., 2016). Next, StringTie v2.1.1 was used on each sample with the '-eB' flag to quantify the expression for each DIEPV gene, measured in reads per kilobase per million sequenced reads (RPKMS) (Pertea et al., 2015, 2016). Ballgown v2.26.0 was then used to perform differential expression testing of DIEPV genes among the different fly species for each nonzero time point and dosage combination by using default settings, except the 'libadjust' option was set to 'FALSE' (Frazee et al., 2015; Pertea et al., 2016). Genes with an FDR-adjusted q value <0.1 were considered significantly differentially expressed across fly species. Hierarchical clustering of DIEPV genes based on gene expression patterns across all 54 samples, independent of treatment, was performed using the Ward method with JMP Pro 14 software. Gene expression estimates were $\log_2$-transformed prior to the clustering analysis.

2.7 | RT–qPCR estimation of DIEPV early and late gene expression

Expression of DIEPV early and late genes during virus infection was estimated with reverse transcription–qPCR (RT–qPCR) using gene-specific primers for the DIEPV early core gene DNA polymerase (DNAP) and the late core structural precursor P4b gene (Coffman et al., 2020). A total of 324 fly samples were collected, including 108 of the samples initially collected for RNA-seq analysis. Six biological replicate flies of each species were collected at six time points after injection of third-instar fly larvae with the three original viral doses (Table S1). Each fly was collected and RNA isolated as described before for transcriptome preparation. 500 ng RNA was used to synthesize 8 μL cDNA for each sample using the MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit (Biosearch) with poly-dT primers. RT–qPCR was then performed on a Bio-Rad CFX Opus 96 Real-Time PCR System. Three technical replicate 5 μL qPCR reactions were performed for each sample using the KAPA SYBR FAST qPCR Universal Master Mix, 10 μM primer solutions, and 1 μL diluted cDNA. Absolute copy number for each sample was estimated using separate DNAP and P4b qPCR standards that were run concurrently with unknown samples and included serial DNA concentrations ranging from 10^2–10^7 copies per μL. Copy number per ng of total RNA for each sample was determined from the mean copy number per reaction, multiplied by the cDNA dilution factor and RNA concentration within each cDNA sample. Average copy numbers were $\log_{10}$-transformed and then subjected to the same statistical analyses as was done with prior qPCR viral abundance measurements, indicated in Table S1.

2.8 | Fly differential expression analysis

Differences in fly gene expression throughout initial DIEPV infection compared with mock-infected flies were explored using all 81 RNA-seq samples, outlined in Table S2. Following quality filtering, read pairs from each sample were mapped to its respective fly reference genome using HISAT2. Average read mapping efficiency was 89.5% for *C. capitata*, 87.5% for *B. dorsalis* and 91.6% for *Z. cucurbitae*. Next, transcript abundances were estimated with StringTie '-eB' and measured in fragments per kilobase per million mapped reads (FPKM). Single-copy orthologs (SICOs) shared by *C. capitata*, *B. dorsalis* and *Z. cucurbitae* were identified using OrthoFinder v2.2.7 to directly compare patterns of differential expression between species (Emms & Kelly, 2015). Redundant protein sequences within each reference genome were collapsed into a single sequence for each gene ID using OrthoFinder 'primary_transcript.py' and custom scripts. OrthoFinder was then executed with default settings and identified 8972 SICOs among the three fly species. Significant differences in SICO expression between virus and mock-infected flies for each species were assessed using maSigPro v1.66.0 with the p.vector and get.siggenes functions, including a q value cut-off of <0.01 and a r^2 value cut-off of >0.6 (Nueda et al., 2014). Hierarchical clustering of significant genes for each species was performed with the maSigPro see. genes function using the Ward method with the number of clusters constrained to the default value of 9.

2.9 | Immune signalling pathway gene analysis

Specific immune signalling pathways were investigated for differential fly gene expression in response to DIEPV infection at 12 hpi, including the Toll, Imd, JAK/STAT, RNAi and apoptosis pathways. Gene constituents of the Toll and Imd pathways were obtained from the KEGG Pathway Database map ccat04624, which had been annotated for the *C. capitata* genome (https://www.kegg.jp/kegg/pathway.html). All orthologs of the KEGG gene list were then found within the *B. dorsalis* and *Z. cucurbitae* genomes using OrthoFinder. Genes that comprise the apoptosis pathway were also obtained from the KEGG Pathway database map dme04214, which was annotated for *Drosophila melanogaster*. OrthoFinder was again used to find orthologs between the *D. melanogaster* genome (GCF_000001215.4) and the *C. capitata*, *B. dorsalis*, and *Z. cucurbitae* genomes. The primary JAK/STAT and RNAi pathway gene members were identified through manual searches within *D. melanogaster*, and corresponding orthologs were identified with OrthoFinder (Bang, 2019; Dowling et al., 2016). To assess as many potential immune genes as possible, additional putative pathway genes were identified in all three fly genomes by searching for those with similar gene names (i.e. gene descriptions) to the annotated pathway components and orthologs found in each species. The cumulative groups of genes from these searches were then subjected to two-group differential expression significance F-tests using Ballgown. Genes from each pathway were tested

separately, including one group of tests that compared high-dose virus-infected flies with mock-infected control flies, and a second set of tests that compared low-dose virus-infected flies to mock-infected flies. Differential expression was determined to be significant for all genes with a q value <0.1.

3 | RESULTS

3.1 | Tropical fruit flies show variation in permissiveness to *D. longicaudata* parasitism

We first investigated the ability of *D. longicaudata* to develop within three tropical fruit fly pest species by conducting parasitism success assays that measured the rates of adult wasp emergence, adult fly emergence and no emergence after fly larvae were subjected to parasitism by *D. longicaudata* (Figure 1a). Parasitism within both *C. capitata* and *B. dorsalis* flies led to substantial average wasp emergence rates of 63.2% and 49.6%, respectively (Figure 1b). In contrast, parasitism within *Z. cucurbitae* flies failed to produce a single adult wasp across all trials, differing significantly from *C. capitata* and *B. dorsalis* (Figure 1b). Average adult fly emergence rates for *C. capitata* and *B. dorsalis* were severely hindered when parasitized by *D. longicaudata*, at 9.9% and 19.0%, respectively (Figure 1c), indicating a forfeit of fly survival over wasp development caused by successful parasitism. *Zeugodacus cucurbitae*, however, displayed a significantly higher average adult emergence rate of 93.2% after parasitism (Figure 1c). Rates of nonemergence between *C. capitata* and *B. dorsalis* were also not significantly different at 26.9% and 31.4%, respectively, although *Z. cucurbitae* showed a significantly lower rate of 6.8% (Figure 1d).

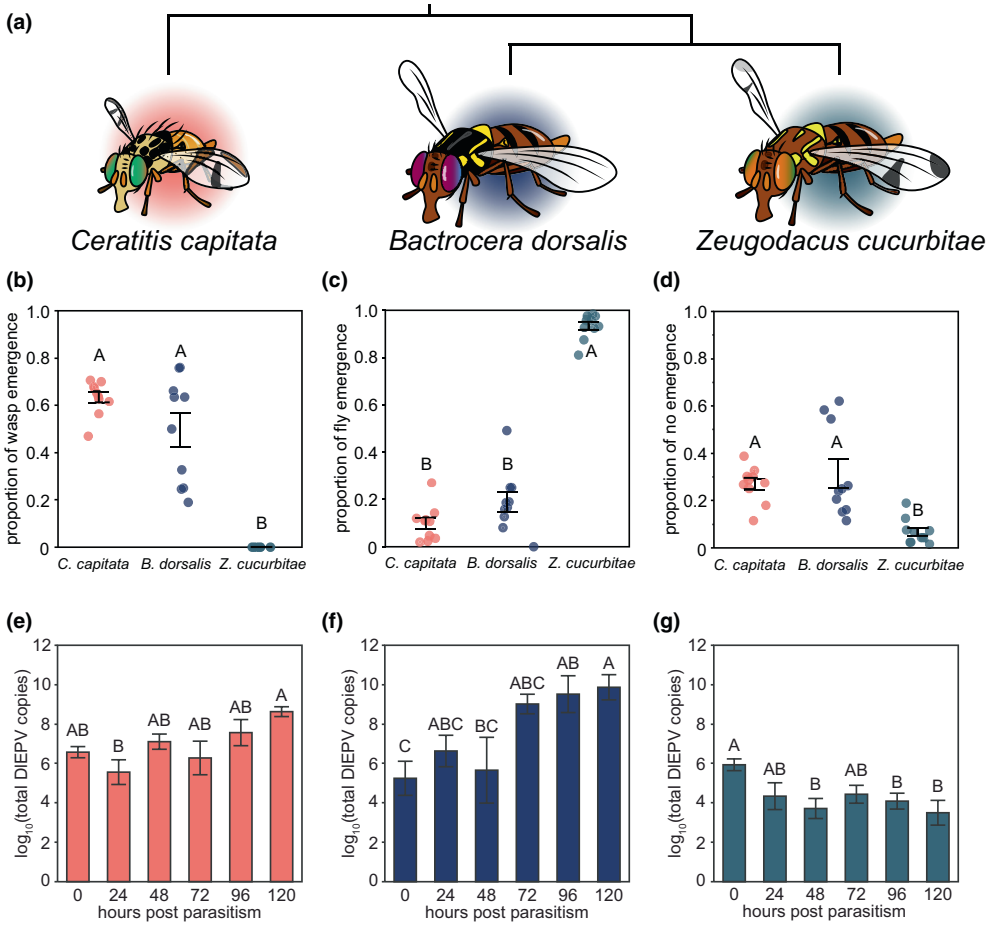


FIGURE 1 Parasitism success of *Diachasmimorpha longicaudata* among tropical fruit fly species is associated with DIEPV abundance. (a) The Mediterranean fruit fly (*Ceratitis capitata*), the oriental fruit fly (*Bactrocera dorsalis*) and the melon fly (*Zeugodacus cucurbitae*) represent three of the most pestiferous fruit fly species around the globe (White & Elson-Harris, 1992). (b–d) Parasitism assays measured the average emergence rates of (b) wasp progeny, (c) parasitized flies, and (d) neither wasp nor fly following oviposition by *D. longicaudata* within the three fruit fly species. Average proportions of wasp, fly, or no emergence were calculated using 10 replicate assay trials per treatment. Each trial is indicated by a dot and represents the fate of >40 singly parasitized flies after oviposition by a group of six female wasps. (e–g) DIEPV abundance was estimated using qPCR measurements of the DIEPV poly(a) polymerase small subunit gene during *D. longicaudata* parasitism within (e) *C. capitata*, (f) *B. dorsalis*, and (g) *Z. cucurbitae* flies. Each bar in panels (e–g) represents the mean $\log_{10}$-transformed viral copy number across 8 biological replicate fly samples per time point. Error bars represent one standard error above and below the mean value. Statistical differences between mean emergence rates in (b-d) and mean copy numbers in (e–g) are indicated by the letter(s) above each bar, as determined by Tukey's HSD tests. [Colour figure can be viewed at wileyonlinelibrary.com]

These results demonstrate that *C. capitata* and *B. dorsalis* are permissive hosts for *D. longicaudata* while *Z. cucurbitae*, despite wasps readily ovipositing in this species (Figure S1), is a nonpermissive host.

3.2 | Permissiveness to parasitism is linked with replication of DIEPV within host flies

We next explored whether the activity of DIEPV within these same fly species during parasitism is connected to the striking difference in host permissiveness observed. We allowed *D. longicaudata* wasps to oviposit within each fly species, and then used qPCR to measure DIEPV copy number during parasitism as a direct estimate of viral abundance within fly tissue. For *C. capitata*, we observed a significant difference in average viral copy number during parasitism, characterized by an initial introduction of nearly 10^7 viral copies at 0 h post parasitism (hpp), a slight drop within 24 hpp, and a significant increase to $>10^8$ copies by 120 hpp (Figure 1e). *Bactrocera dorsalis* flies showed a more dramatic increase of viral copy number throughout parasitism, as viral abundance significantly rose by >4 orders of magnitude from 0 to 120 hpp in a continual trend (Figure 1f). In contrast, viral abundance showed a significant decrease during parasitism within *Z. cucurbitae* flies, as approximately 10^6 viral copies were introduced at 0 hpp, and mean copy number fell to $<10^4$ copies by 120 hpp (Figure 1g). These data support a connection between host compatibility for *D. longicaudata* parasitism and virus replication capability within the fly hosts.

3.3 | Injection of varying DIEPV doses demonstrates a gradation of fly susceptibility to viral infection

We next looked into whether the effects of DIEPV in the absence of parasitism could offer more nuance to the link between viral infection and host permissiveness within the three fruit fly species. We injected nonparasitized fly larvae of all three species with several doses of purified DIEPV and measured whether virus-infected fly larvae survived to adulthood (Figure 2a). We also injected separate larvae with UV-inactivated virus at the same three doses as a control treatment (Table S3). *Ceratitis capitata*, which was highly permissive to parasitism by *D. longicaudata*, displayed the highest susceptibility to DIEPV infection. There was no emergence of *C. capitata* adult flies when inoculated with either 1 oviposition equivalent of DIEPV (1×, approximately 10^7 viral genome copies) or 0.2 oviposition equivalents (0.2×), and only a 10.8% normalized emergence rate at 0.1 oviposition equivalents (0.1×) (Figure 2a). *Bactrocera dorsalis* flies also displayed a complete failure to emerge as adults after injection with the 1× dose of DIEPV as larvae, although 1.4% *B. dorsalis* survival was observed after injection with the 0.2× dose of virus and 42.5% of flies emerged when treated with the 0.1× dose (Figure 2a). *Zeugodacus cucurbitae* flies exhibited the lowest overall susceptibility to DIEPV, with flies surviving virus infection at all three doses: 2.8% adult flies emerged at the 1× dose, 42.6% at the 0.2× dose and 74.2% at the 0.1× dose (Figure 2a). Taken together, these results demonstrate a gradation of fruit fly susceptibility to DIEPV, in which the virus is most virulent within *C. capitata* followed by *B. dorsalis*, and least virulent to *Z. cucurbitae*.

3.4 | DIEPV abundance patterns after injection show an added dimension of viral activity within flies

Next, we used qPCR to assess viral abundance patterns in nonparasitized flies after injection of fly larvae with the same three doses of DIEPV used before. DIEPV abundance was also measured after injection with UV-inactivated DIEPV as a control, which resulted in limited viral DNA amplification over time for any dose or species (Figure S2). Within *C. capitata* flies, injection with either 1× or 0.2× active DIEPV resulted in rapid virus replication, as viral copy number significantly rose to $>10^{10}$ copies by 120 h post injection (hpi) (Figure 2b). However, injection of *C. capitata* with 0.1× DIEPV resulted in a diminished viral abundance pattern, in which a significant drop in viral copy number was observed at 24 hpi, and subsequent amplification of the virus only reached approximately 10^6 copies by 120 hpi (Figure 2b). When *B. dorsalis* larvae were injected with a 1× dose of DIEPV, we observed a similar pattern of rapid virus replication to *C. capitata* (Figure 2c). Interestingly, *B. dorsalis* demonstrated a significant drop in viral copy number at 24 hpi when injected with the intermediate dose, 0.2×, followed by a subdued viral abundance curve resembling DIEPV activity in *C. capitata* when injected with the 0.1× dose (Figure 2c). Furthermore, DIEPV was almost entirely abolished by 24 hpi in *B. dorsalis* when injected with the 0.1× dose (Figure 2c). In contrast to the DIEPV replication patterns observed within *C. capitata* and *B. dorsalis*, we observed no virus replication within *Z. cucurbitae* after injection with any of the doses, characterized by >99% decreases in viral copy number throughout infection (Figure 2d). These cumulative results therefore reveal that DIEPV replicative ability within the different fly species is strongly linked to viral susceptibility.

3.5 | Viral gene expression patterns demonstrate DIEPV breakdown in *Z. cucurbitae* flies

Given the patterns of DIEPV replication observed from viral abundance measurements, we investigated the transcriptional dynamics of DIEPV and fly hosts during the first 24 h of viral infection. We deep-sequenced total polyadenylated mRNA from 81 whole-body fly samples: Three biological replicate samples were collected at 0, 12 and 24 hpi for both 1× and 0.1× viral doses, along with a saline-injected mock 'dose' in all three fly species (Table S2).

DIEPV gene expression patterns were first explored using the 54 virus-infected RNA samples. Viral reads from each sample were mapped to the DIEPV genome (GenBank accession KR095315), and viral gene expression for each sample was estimated via normalized

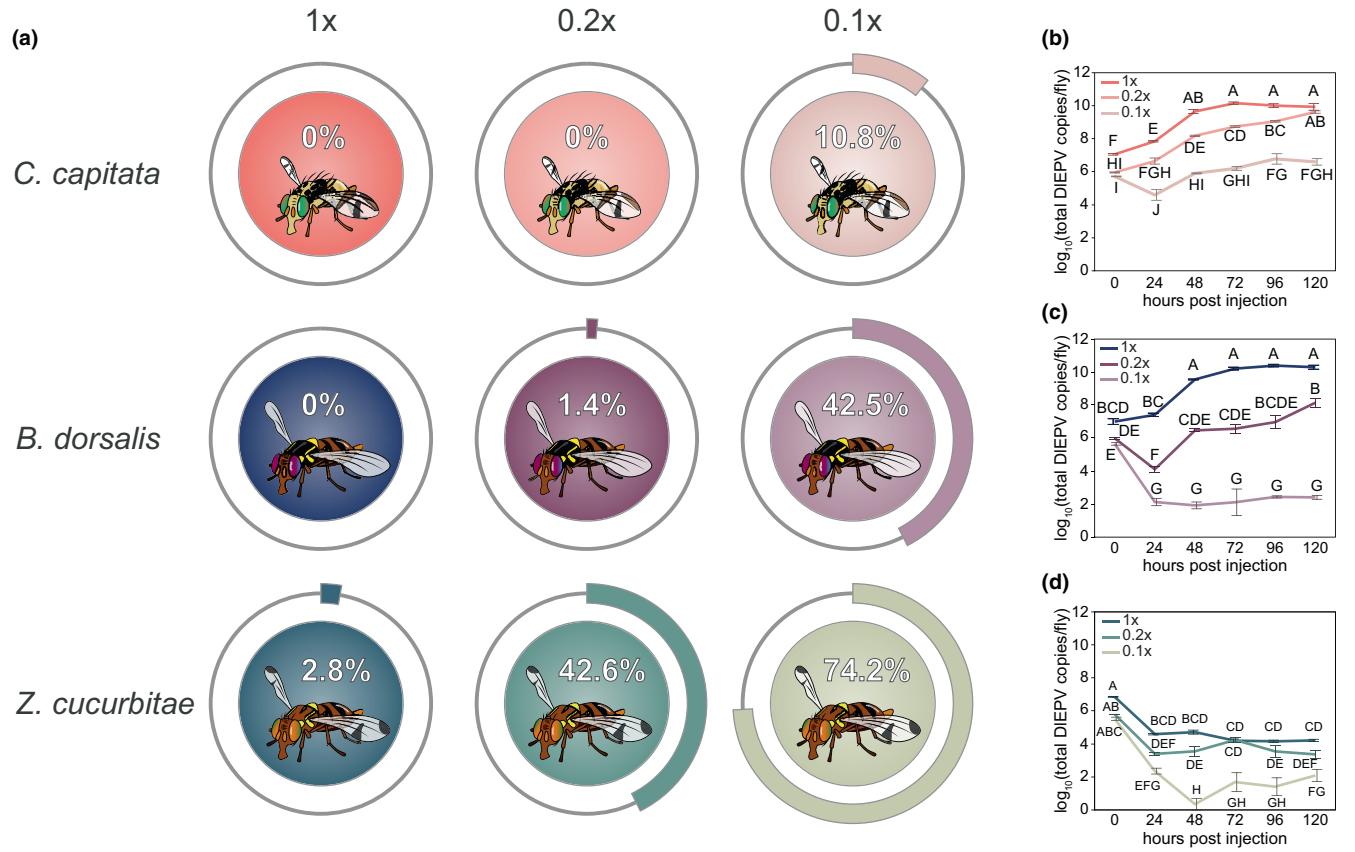


FIGURE 2 DIEPV injection causes differential virulence and virus replication dynamics within fly species. (a) The normalized emergence rates of adult *C. capitata*, *B. dorsalis* and *Z. cucurbitae* flies were measured after larvae were injected with 1 oviposition equivalent (1×), 0.2 oviposition equivalents (0.2×), or 0.1 oviposition equivalents (0.1×) of purified DIEPV. The outer ring length of each pie chart and the numerical label within indicates the percentage of emerged flies after treatment with active virus normalized by the percentage of emerged flies after treatment with inactive virus. Raw emergence data are provided in Table S3. (b–d) qPCR was used to measure DIEPV abundance over time after (b) *C. capitata*, (c) *B. dorsalis* and (d) *Z. cucurbitae* flies were injected with either 1×, 0.2× or 0.1× doses of purified DIEPV. The average $\log_{10}$-transformed DIEPV copy numbers in each graph were determined from four replicate fly samples for each time point and viral dose. Error bars are as indicated in Figure 1, and letter(s) above mean data points indicate significantly distinct values when all means were compared with Tukey's HSD independently of either main effect. [Colour figure can be viewed at wileyonlinelibrary.com]

read counts. We explored differences in viral gene expression between the three fly species for each time and dose combination, except at 0 hpi when minimal viral activity was expected to occur. Using three-group parametric *F*-test comparisons, we found most viral genes exhibited significant differential expression between species regardless of time or dose, which was most often due to decreased expression of viral genes in *Z. cucurbitae* compared with *C. capitata* and *B. dorsalis*. At 12 hpi, 97.4% and 92.1% of viral genes displayed significant differential expression between fly species for 1× and 0.1× doses, respectively. Similarly, 100% and 99.5% of viral genes showed significant differential expression at 24 hpi for 1× and 0.1× doses, respectively (Table S4).

We next performed hierarchical clustering analysis on DIEPV genes to group those with similar expression patterns during infection within the three different fly species across all 54 RNA samples (Figure 3a, Table S5). Overall, DIEPV genes segregated into two large clusters: The first (top) cluster comprised 93 genes, and the second (bottom) cluster included 98 genes (Figure 3a). Notably, the second viral gene cluster was characterized by massive transcriptional die off in *Z. cucurbitae* compared with the other two fly species (Figure 3a). We hypothesized that the two clusters of viral genes correspond to early and late stages of the DIEPV replication cycle. Poxvirus transcription proceeds through sequential stages during the virus replication cycle, and insect poxvirus genes are typically classified by those expressed soon after virus entry and before viral genome replication has begun, known as 'early' genes, and those expressed after genome replication has initiated, known as 'late' genes (Becker & Moyer, 2007; Broyles, 2003). We therefore classified DIEPV core genes into early and late stages based on homology to vaccinia virus genes, the expression timelines of which have been studied extensively (Yang et al., 2010, 2015). We found that most DIEPV core genes were grouped into the two hierarchical gene clusters according to early and late stages: 71.4% (10 of 14) DIEPV early core genes fell within the first gene cluster, and 93.5% (29 of 31) late core genes were found in the second gene cluster (Figure 3a, Table S6). We also classified additional DIEPV early genes as those containing the conserved promoter sequence for insect poxvirus early genes:

TGAAAXXXXA (Afonso et al., 1999; Bawden et al., 2000). We identified 64 DIEPV noncore genes with this conserved promoter motif, and 82.8% (53 of 64) of these additional putative early genes belonged to the first cluster (Table S7). These results show that differences in DIEPV gene activity among the various treatments are connected to early and late virus replication stages. They also imply that while the DIEPV replication cycle can begin within *Z. cucurbitae* due to initial early gene expression, that cycle eventually breaks down and leads to severely reduced expression of viral late genes. (Figure 3a, Table S5).

3.6 | Early and late viral gene expression patterns mirror replication dynamics

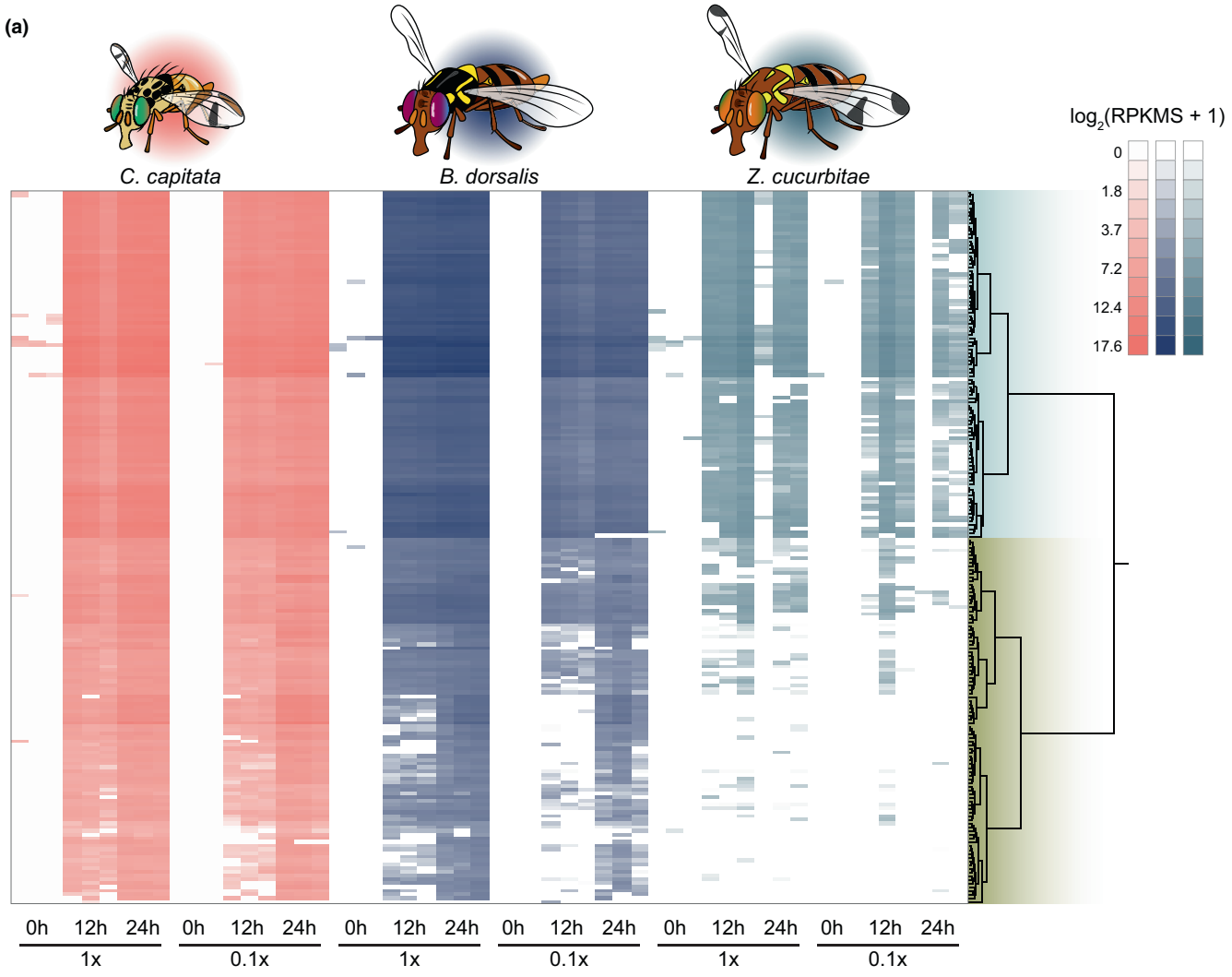
To gain better resolution on early and late DIEPV gene expression patterns within the different fly hosts, we used RT–qPCR to estimate the expression of representative DIEPV early and late genes DNAP and P4b after injection of fly larvae at the same three viral doses used in prior experiments. We observed typical expression patterns for both early and late DIEPV genes within *C. capitata* and *B. dorsalis* flies, in which DNAP expression was quickly initiated by 4 hpi and continued to increase moderately over time in both species (Figure 3b,c). Conversely, P4b expression exhibited minimal gains until 12 hpi, after which point expression rapidly increased for all doses in both fly species (Figure 3b,c). *Zeugodacus cucurbitae* displayed marked differences in expression patterns for DNAP and P4b genes compared with *C. capitata* and *B. dorsalis* (Figure 3d). DNAP mean copy numbers for each dose significantly differed over time but were severely reduced, peaking at 12 hpi and falling to negligible copy numbers by 48 hpi. Furthermore, P4b expression in *Z. cucurbitae* was barely detectable (Figure 3d). Expression profiling of both early and late DIEPV genes therefore shows many parallels with DIEPV replication dynamics within the three fruit fly species that strengthen the link between viral activity and parasitoid permissiveness documented here.

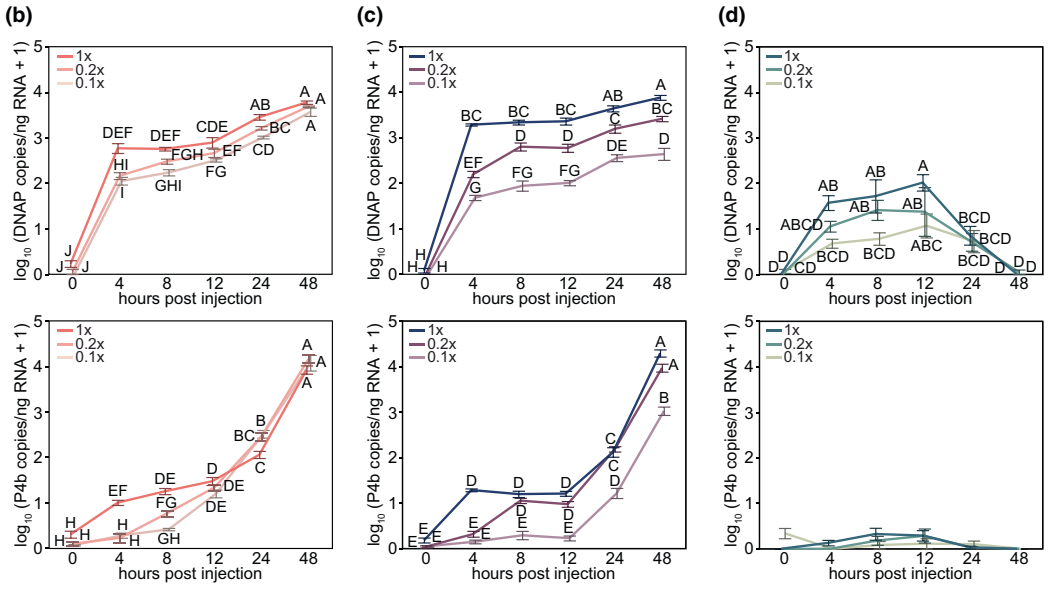
3.7 | Fly transcriptional responses reveal impact of viral infection on *C. capitata* and *B. dorsalis* but limited involvement from *Z. cucurbitae*

We next explored fly transcriptional responses during the initial 24 h of DIEPV infection using the same 81-sample RNA-seq data set previously described, which included flies infected with one of two different DIEPV doses or a mock-infected control treatment (Table S2). Quality-filtered reads were mapped to reference genomes for *C. capitata* (Refseq accession GCF_000347755.3), *B. dorsalis* (GCF_000789215.1) or *Z. cucurbitae* (GCF_000806345.1). Gene expression within each species was estimated using fragments per kilobase per million mapped reads (FPKM) measurements.

We identified all SICOs shared among the three fruit fly genomes and used these 8972 genes to directly compare levels of differential expression between the species in response to DIEPV infection. The program maSigPro was employed for differential gene expression testing, which utilizes a two-step regression model approach to identify genes with a significantly different expression profile over time between virus-infected flies and mock-treated control flies (Nueda et al., 2014). Two separate comparisons of fly gene expression profiles were conducted, including the 1× virus-infected treatment compared to the mock-infected control, as well as the 0.1× dose treatment compared with the control. We found 1805 and 1677 differentially expressed SICOs in *C. capitata* for the 1× dose versus control and the 0.1× dose vs. control comparisons, respectively, followed by 1078 and 967 differentially expressed SICOs in *B. dorsalis*, and 1239 and 619 differentially expressed SICOs in *Z. cucurbitae*. These gene sets were then each clustered into nine distinct gene expression profiles for each fly species using the Ward hierarchical clustering method included within maSigPro. Gene clusters were designated by similarity in expression profile trends over time and between the two compared treatments (Nueda et al., 2014). In both *C. capitata* and *B. dorsalis*, several gene clusters exhibited differences in gene expression between one or both virus-infected treatments and the mock-infected control (Figure 4a,b, Figure S3, Table S8). These differences in fly gene expression were often most noticeable at 12 hpi for both species and would then converge to similar expression levels by 24 hpi. Clusters from the 1x dose vs control gene sets that displayed >2-fold difference in median gene FPKM at 12 hpi included *C. capitata* Clusters 2, 3, 4, and 9, and *B. dorsalis* Clusters 1, 2, 4, and 9 (Figure 4a,b). In contrast, the same clustering analysis on orthologous *Z. cucurbitae* genes yielded clusters with relatively similar expression profiles between virus-infected and mock-infected treatments, including no clusters with >2-fold median FPKM differences at 12 hpi (Figure 4c). These results demonstrate that *Z. cucurbitae* has a limited overall transcriptional response to DIEPV infection.

FIGURE 3 DIEPV gene expression patterns demonstrate early and late gene dynamics among fly species. (a) Heatmap shows the expression of all DIEPV genes across the 54 virus-infected fly samples, measured in $\log_2$-transformed reads per kilobase per million sequenced reads (RPKMS). Each row corresponds to a different DIEPV gene clustered by similarity of expression patterns across all samples, and each column represents a different RNA sample manually grouped by species, dose, and hours post injection. Two broad clusters of DIEPV genes likely distinguish between early genes in the top (light blue) cluster and late genes in the bottom (green) cluster. (b–d) Early and late DIEPV gene expression was estimated using RT-qPCR measurements of DNAP (top panels) and P4b (bottom panels) gene expression, respectively within (b) *C. capitata*, (c) *B. dorsalis*, and (d) *Z. cucurbitae* flies after injection with purified DIEPV at 1×, 0.2×, and 0.1× doses. Mean values represent the $\log_{10}$-transformed copy number per ng RNA averaged across six replicate samples for each treatment and dose. Error bars and letter(s) above mean data points are as indicated in Figure 2. [Colour figure can be viewed at wileyonlinelibrary.com]





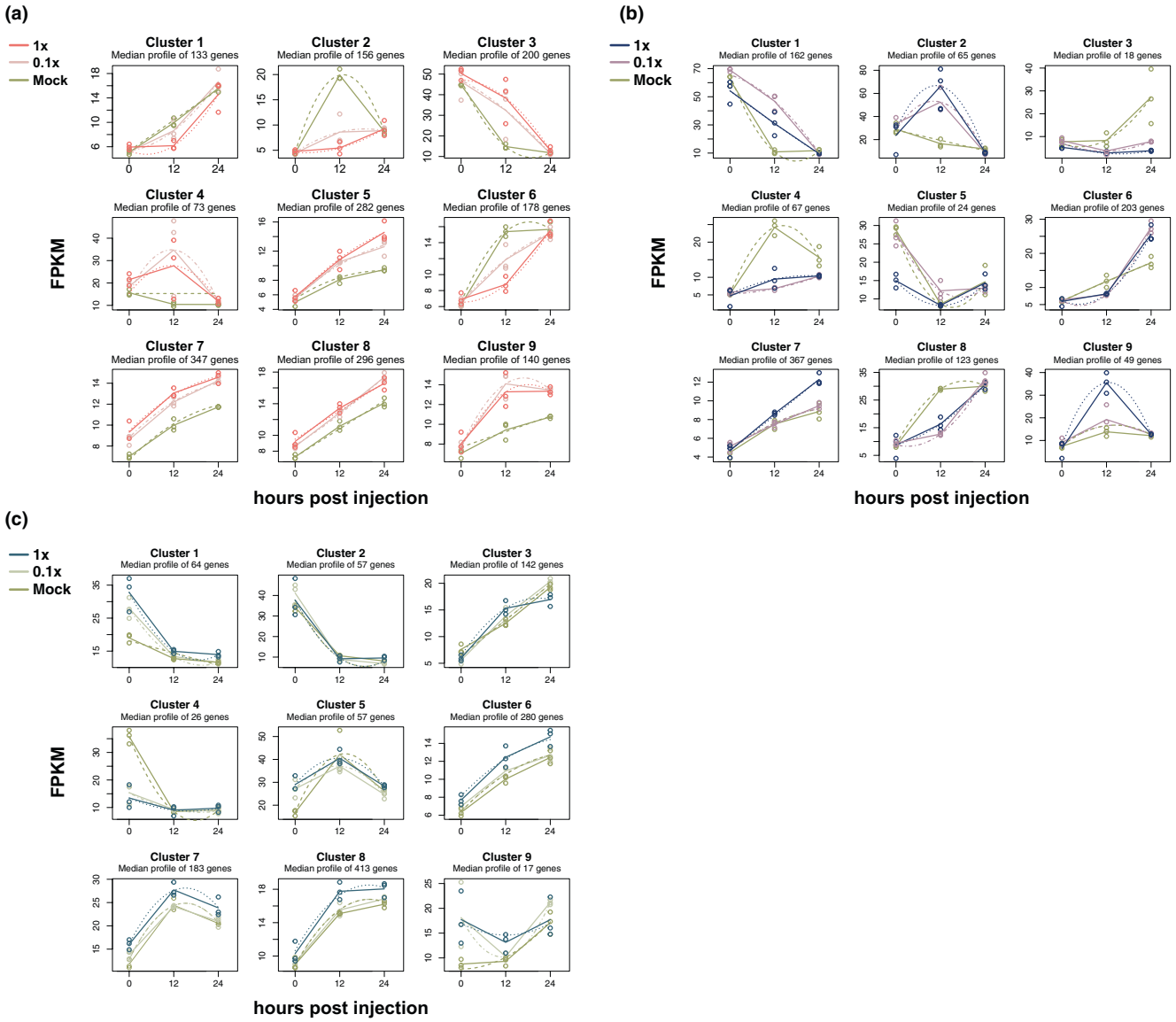


FIGURE 4 Fly hosts show varied gene expression responses to DIEPV infection. (a–c) Fly gene expression, measured in fragments per kilobase per million mapped reads (FPKM) was assessed after injection with 1× and 0.1× doses of DIEPV, each dose separately compared to the mock-infected control treatment. Shown here are nine expression profiles composed of genes from (a) *C. capitata*, (b) *B. dorsalis* and (c) *Z. cucurbitae* genomes that displayed significant differential expression between 1× DIEPV and mock-infected treatments. Representative clustering profiles for flies given the 0.1× dose compared with the mock-infected treatment can be found in Figure S3. Data points on each panel represent the median FPKM profile for each dose, including distinct data points for each of the three biological replicate RNA samples. Dashed lines represent regression curves for each dose. Specific gene composition for each cluster is described in Table S8. [Colour figure can be viewed at wileyonlinelibrary.com]

3.8 | DIEPV virulence may include immune pathway disruption in susceptible hosts

Since little is currently known about how DIEPV manipulates susceptible fly hosts, we explored specific physiological pathways that may be targeted by DIEPV within the different fly species to better understand the mechanisms by which this virus causes virulence. We hypothesized that immune response pathways are likely to be impacted by DIEPV within *C. capitata* and *B. dorsalis*. Antiviral pathways that target insect poxviruses have not been well-studied, but insect viruses are generally targeted by pathways such as JAK/STAT, Toll, Imd RNAi, and the apoptosis pathway (Kingsolver et al., 2013; Marques & Imler, 2016). We therefore focussed on these five pathways to investigate fly immune gene expression patterns in response to DIEPV. We identified a total of 257 immune genes belonging to these pathways in the *C. capitata* genome, 254 in *B. dorsalis*, and 301 in *Z. cucurbitae*. Using the same RNA-seq dataset, we performed two-group *F*-test comparisons of immune gene expression between virus- and mock-treated flies at 12 hpi (Figure 5, Table S9).

For *C. capitata*, we found that several serine protease initiators of the Toll pathway were significantly upregulated in response to DIEPV infection, including Spirit and Spheroide homologues, suggesting that the virus was detected by the immune system upon

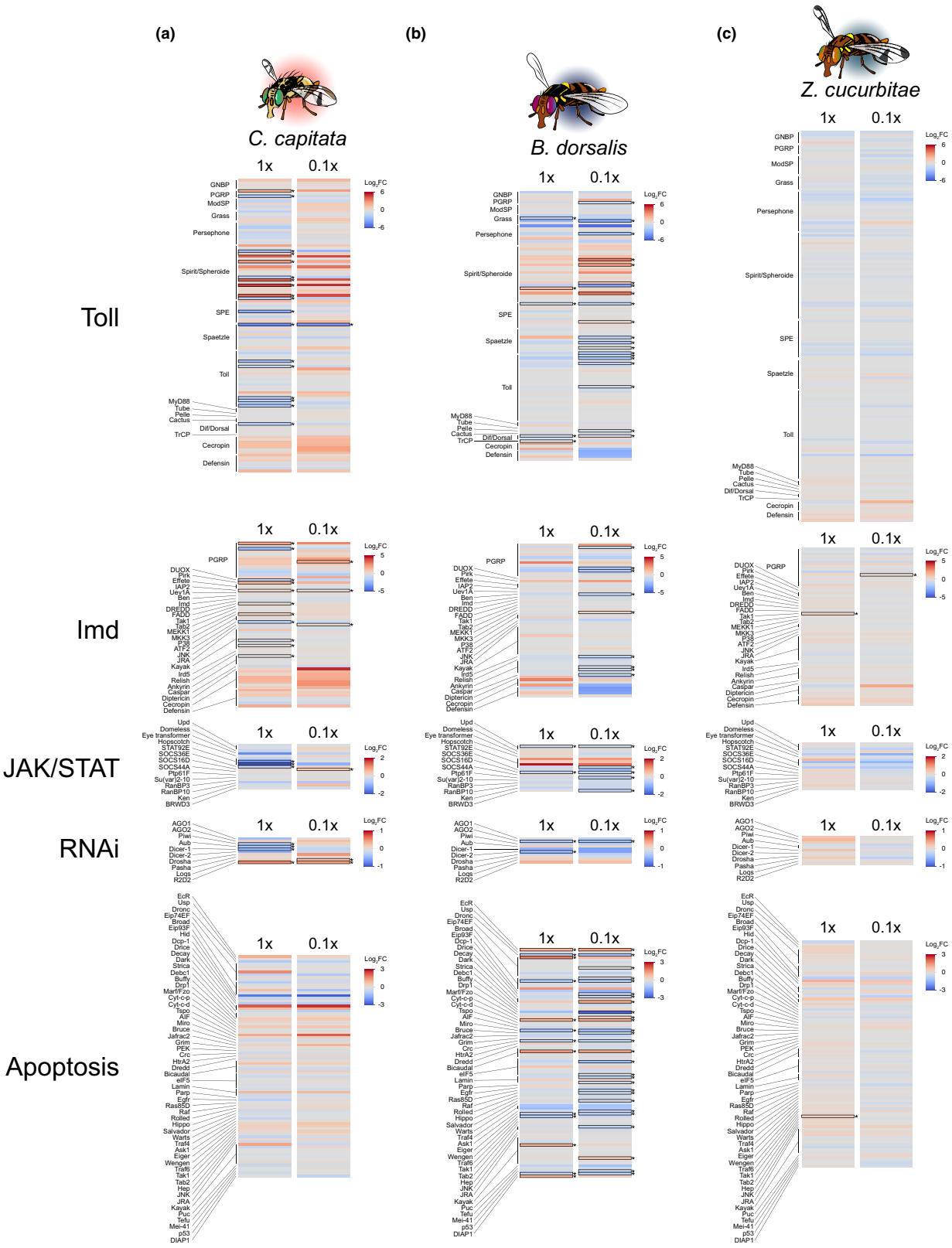


FIGURE 5 Immune signalling pathways are differentially impacted by DIEPV within fly species. Gene components of the Toll, Imd, JAK/STAT, RNAi, and apoptosis signalling pathways were analysed for differential expression when virus-infected flies were compared with mock-infected flies at 12h post injection. (a–c) Heatmaps display the $\log_2$ fold change of pathway gene expression after given each DIEPV dose compared to the control treatment, in which shades of red correspond to genes that were upregulated during viral infection compared to the control and shades of blue correspond to genes that were downregulated during viral infection. Boxes outlined in black denoted with an asterisk (*) indicate genes that displayed significant differential expression in the particular dose comparison. Individual gene $\log_2$ fold change expression values are included in Table S9. [Colour figure can be viewed at wileyonlinelibrary.com]

infection (Figure 5a, Table S9). However, we also found that numerous major activators of the Toll pathway, including four Spirit/Spheroide serine proteases, the peptidoglycan recognition protein SA (PGRP-SA), a Spaetzle ligand, five Toll receptors and the nuclear factor-κB (NF-κB) transcription factor Dorsal were all significantly downregulated in response to DIEPV infection within *C. capitata* (Figure 5a, Table S9). *Ceratitis capitata* flies also exhibited downregulation of important Imd pathway components in response to DIEPV infection, like PGRP-SA, the cyclic AMP-dependent transcription factor ATF-2, and dual oxidase (DUOX). Additionally, known negative regulators of the Imd pathway, such as Poor imd response upon knockin (Pirk), PGRP-SC, and PGRP-LB, exhibited significant upregulation in response to viral infection in *C. capitata*. Within the JAK/STAT pathway, we found significant downregulation of two major Signal transducer and activator of transcription (STAT) transcription factors. Five genes showed significant differential expression within the RNAi pathways including R2D2, which was significantly upregulated during DIEPV infection and is directly involved in the short interfering RNA (siRNA) pathway, the primary antiviral RNAi pathway of insects (Bonning & Saleh, 2021; Obbard et al., 2009). No significant differentially expressed *C. capitata* genes were identified from the apoptosis pathway. Collectively, these results support the hypothesis that DIEPV causes suppression of several fly immunity pathways, which may contribute to its efficient replication and pathogenicity demonstrated in *C. capitata* flies, as well as its beneficial function supplied to *D. longicaudata* wasps.

Bactrocera dorsalis flies also exhibited changes in Toll pathway gene expression in response to DIEPV infection. Like *C. capitata*, several Spirit/Spheroide serine proteases exhibited significant upregulation in response to viral infection, while other serine proteases, including the serine protease Persephone, demonstrated significant downregulation (Figure 5b, Table S9). Furthermore, the PGRP-SA gene, four Spaetzle genes, four Toll genes and Dorsal were also significantly downregulated in *B. dorsalis* flies infected with DIEPV. The *B. dorsalis* Imd pathway displayed overall fewer significantly differentially expressed genes than *C. capitata*, but similar downregulation of important activators, such as PGRP-SA and PGRP-LA, the inhibitor of nuclear factor-κB kinase subunit beta (Ird5), and the major receptor PRGP-LC was observed (Figure 5b, Table S9). JAK/STAT pathway components, such as the Bromo domain-containing protein BRWD3, a STAT transcription factor and an Unpaired (Upd) gene, were modestly differentially expressed, as were negative JAK/STAT regulators, like Suppressor of cytokine signalling 16D (SOCS16D) and protein tyrosine phosphatase 61F (Ptp61F) (Figure 5b, Table S9). Two key components of the siRNA pathway, Argonaute-2 and Dicer-2, showed significant downregulation in response to DIEPV, although the expression fold changes for these genes were limited. Interestingly, the apoptosis pathway yielded the highest number of differentially expressed genes for *B. dorsalis* flies. Twenty-three different apoptosis pathway genes were significantly downregulated in response to DIEPV infection, including the main apoptotic caspases DRONC, DRICE and Dcp-1, along with other pro-apoptotic genes Miro, Eiger, Wengen, p53, Broad and Salvador (Figure 5b, Table S9). Additionally, major anti-apoptotic genes such as ecdysone receptor (EcR) and DIAP1 were significantly upregulated during DIEPV infection. These findings suggest that along with humoral immunity pathways, DIEPV may also target cellular antiviral immunity through the apoptosis pathway within *B. dorsalis* to disrupt other mechanisms of defence in this species.

A total of two immune genes showed significant differential expression in *Z. cucurbitae* flies in response to the virus (Figure 5c, Table S9). The Imd pathway activator PGRP-LE was significantly upregulated with a $\log_2$ fold change of 0.42 during DIEPV infection within *Z. cucurbitae*, as was the TGF-beta activated kinase 1 (Tak1) gene, which had a $\log_2$ fold change value of 0.52 and is part of both Imd and apoptosis pathways. These findings may suggest that a partial immune response was elicited by the virus, although the low magnitude of these expression fold changes and lack of additional differentially expressed genes during viral infection supports an overwhelmingly passive immune response exhibited by *Z. cucurbitae*.

4 | DISCUSSION

4.1 | *Diachasmimorpha longicaudata* displays varying compatibility with tropical fruit flies

Our parasitism assay results demonstrated that *C. capitata* and *B. dorsalis* are compatible hosts for *D. longicaudata*, while *Z. cucurbitae* served as an incompatible host that failed to produce any adult wasps across all replicate trials. These findings agree with previous sampling data from wild fly populations on the Hawaiian Islands that found *C. capitata* and *B. dorsalis* but not *Z. cucurbitae* to be successfully parasitized by *D. longicaudata* within infested fruits (Bess et al., 1961; Nishida, 1955; Nishida & Haramoto, 1953). It is unclear whether *D. longicaudata* did not attack *Z. cucurbitae* flies in these studies, or rather that *D. longicaudata* attempted to parasitize *Z. cucurbitae* in the wild and failed to survive. Regardless, the host range pattern of *D. longicaudata* established in the present study is contrary to what would be predicted by patterns of host fly relatedness and ecology, which are two factors thought to be important for determining parasitoid host range (Godfray, 1994). If phylogenetic relatedness was to dictate the host range of *D. longicaudata*, we would expect that permissive hosts would be more closely related to one another than to nonpermissive hosts. *Ceratitis capitata* is by far the most distantly related of the fly species investigated here, while *B. dorsalis* and *Z. cucurbitae* (formerly *Bactrocera cucurbitae*) belong to sister genera within the Dacini tribe (Virgilio et al., 2015). Furthermore, flies in the genus *Anastrepha* are also compatible hosts for *D. longicaudata*, which constitutes a more distantly related tephritid lineage compared to the three fly species used here (Coffman et al., 2020; Han & McPheron, 1997; Ovruski et al., 2000). Therefore, the

incompatibility of *Z. cucurbitae* as a host for *D. longicaudata* is anomalous with respect to host phylogeny.

If shared ecology of fly hosts contributes to *D. longicaudata* host range, we would expect to see differences between *Z. cucurbitae* ecology that are otherwise shared by *C. capitata* and *B. dorsalis*. All three fly species are highly polyphagous, which has allowed them to become serious pests of hundreds of fleshy fruits and vegetables around the world (Allwood et al., 1999; Liquido et al., 1991; White & Elson-Harris, 1992). While *Z. cucurbitae* appears to have a greater preference for hosts within the Cucurbitaceae family, host overlap between the three species has been documented in places such as Hawai'i and widely throughout South Asia (Harris et al., 1986; Nishida & Haramoto, 1953; Vargas et al., 2015; White & Elson-Harris, 1992). Furthermore, *A. suspensa* is native to the Caribbean Islands and has not spread beyond the Americas, indicating that this fly species maintains a unique distribution and distinct host preferences compared to *B. dorsalis* and *Z. cucurbitae* (White & Elson-Harris, 1992). *Diachasmimorpha longicaudata* therefore attacks flies with a wide range of host preferences and geographical distributions, many of which overlap with *Z. cucurbitae*, suggesting that host ecology does not strongly support the *D. longicaudata* host range pattern observed here.

4.2 | DIEPV activity is strongly associated with *D. longicaudata*-host compatibility

We investigated the symbiotic virus DIEPV as an alternative potential factor that may affect *D. longicaudata* host range given the highly beneficial role that this virus plays for wasps during parasitism (Coffman et al., 2020). Our viral abundance measurements during *D. longicaudata* parasitism within the three fly species showed a remarkable link between host permissiveness and replicative ability of DIEPV, which was corroborated by the virulence, replication, and transcriptional patterns of the virus after manual injection. The connection between permissiveness to *D. longicaudata* parasitization and virulence associated with DIEPV replication uncovered here suggests that viral activity may contribute to maintaining these species as viable hosts for *D. longicaudata*. Similar associations between parasitoid permissiveness and viral activity have been observed in some DEV associations. In the wasp *Campoletis sonorensis*, the ability of its DEV to maintain prolonged virulence gene expression within permissive caterpillar hosts was not observed during parasitism within nonpermissive hosts of the wasp (Cui et al., 2000). Similarly, the DEV inherited by the wasp *Microplitis demolitor* displayed decreased expression of most virulence genes within the nonpermissive host *Trichoplusia ni* compared with the permissive host *Chrysodeixis includens* (Bitra et al., 2016). Viral contributions to parasitoid host range could therefore be a feature of convergent evolution between DEVs and DIEPV.

Many DEVs are likely a means for further specialization of the wasps that inherit them due to their endogenous nature, leading to further speciation within DEV-producing wasp lineages (Branca et al., 2012). In contrast, DIEPV is an exogenous virus and plays a facultative role in *D. longicaudata* survival, suggesting that it may have different impacts on *D. longicaudata* host range compared to DEVs (Coffman et al., 2020; Coffman & Burke, 2020). The unusually broad host range of *D. longicaudata* coupled with its dependence on DIEPV during parasitism challenges the notion that DIEPV is similarly leading to *D. longicaudata* host specialization. Overall, these features indicate that DIEPV is a rare 'true' viral symbiont with a demonstrated link to parasitoid wasp host range.

Further investigation is needed to determine the precise role of DIEPV in *D. longicaudata* host suitability and whether additional factors, such as other microbes, may also contribute to the interactions among DIEPV, wasps and flies observed in this study. For example, some bacterial symbionts protect insect hosts against parasitoid attack, such as the bacteria *Hamiltonella defensa* in aphids and *Spiroplasma poulsonii* in *Drosophila* flies (Oliver et al., 2003; Xie et al., 2010). In addition, the common insect bacterial symbiont *Wolbachia* protects some infected hosts, like *D. melanogaster*, against viral infection by reducing the titre of co-infecting RNA viruses (Hedges et al., 2008; Teixeira et al., 2008). A multitude of RNA viruses have been identified in the three fly species studied here, which may influence *D. longicaudata* host permissiveness or DIEPV activity in their own right (Hernández-Pelegrín et al., 2022; Kumar Pradhan et al., 2024; Llopis-Giménez et al., 2017; Sharpe et al., 2021). Thus, there is still much to discern regarding the effects of various microbes and molecular factors within this multitrophic system.

One unexpected result from our work involved the seemingly disparate survival rates of flies when inoculated with DIEPV through natural parasitism compared with manual injection of DIEPV. Fly survival rates after manual DIEPV injection were often substantially lower than anticipated given the survival rate of each species during *D. longicaudata* parasitism, in which flies were naturally inoculated with comparable doses of DIEPV. This aspect of our results may be due, in part, to variation in the amount of DIEPV administered by each wasp during an oviposition event, as well as the variation in average viral dose wasps gave to each fly species, observed at 0 hpp (Figure 1e–g). While it is currently unclear how flies may be more likely to survive when battling both *D. longicaudata* and DIEPV compared with a sole DIEPV infection, we hypothesize that the process of *D. longicaudata* parasitism comprises a multitude of complex molecular interactions within fly hosts that may impact fly survival in potentially counterintuitive ways.

4.3 | Differential expression of immune signalling pathways hints at possible DIEPV virulence mechanisms

Differential expression analyses of immune signalling pathways support that cellular and humoral immune responses were impacted during DIEPV infection within *C. capitata* and *B. dorsalis* flies. *Ceratitis*

capitata flies exhibited suppression of key components for Imd, Toll and JAK/STAT pathways, while *B. dorsalis* also showed potential siRNA pathway suppression and abundant suppression of apoptosis pathway components. These findings indicate that DIEPV may use a variety of mechanisms to disable the immune systems of susceptible host species. Parasitoid wasp DEVs have numerous strategies for interacting with host insect immune systems. The primary function of DEV virulence gene expression is to inhibit the encapsulation response of the host insect through disruption of both cellular and humoral defences (Strand, 2012). For example, multiple *M. demolitor* DEV genes belonging to the ankyrin family act as inhibitors of host NF-κB regulators, such as Dif, Dorsal and Relish of the Toll and Imd pathways to prevent activation of downstream immune responses like encapsulation and antimicrobial peptide (AMP) production (Thoetkiattikul et al., 2005).

Vertebrate-infecting poxvirus relatives of DIEPV are also known to utilize a diversity of immune evasion tactics, including strategies that target the vertebrate innate immune system, which is conserved in many ways within insects. Two vaccinia virus genes, A46R and A52R, are known to prevent downstream Toll-like receptor signalling cascades in mammalian cells (Bowie et al., 2000). In addition, vertebrate poxviruses contain a multitude of genes that function to inhibit apoptosis in host cells (Seet et al., 2003). Comparatively little is known about insect poxvirus immune evasion strategies, although several insect poxvirus genomes, including DIEPV, contain an inhibitor of apoptosis (Iap) gene. Amsacta moorei entomopoxvirus Iap interacts with Grim and Hid proteins and reduces caspase activity in lepidopteran host cells to suppress apoptotic cell death (Li, Liston, & Moyer, 2005; Li, Liston, Schokman, et al., 2005). Therefore, DIEPV may similarly use Iap to inhibit apoptosis pathway components during infection.

4.4 | Concluding remarks

Our collective results in this study demonstrate that the activity of a facultative viral symbiont is linked to the host range of its associated parasitoid wasp. The inferred function of DIEPV in influencing the wide host range of *D. longicaudata* has novel implications for the history of this parasitoid species as an effective fruit fly biocontrol agent. *Diachasmimorpha longicaudata* remains one of the most important parasitoid species released for the control of tropical fruit flies globally (Ovruski et al., 2000; Vargas et al., 2012). The parasitism behaviour of *D. longicaudata* as a generalist has likely contributed to its consistent ability to become established in numerous introduced locations, such as Hawai'i, the continental United States, South America and various islands throughout the Pacific Basin (Ovruski et al., 2000; Vargas et al., 2012). The findings of this investigation thus insinuate that DIEPV could be responsible for the reliable establishment of new *D. longicaudata* populations, and by extension, the wide-scale success of *D. longicaudata* for pest management programs.

AUTHOR CONTRIBUTIONS

KAC, ANK, GRB and SMG designed the research. KAC, ANK and NEG performed the research. KAC and ANK analysed the data. KAC wrote the initial manuscript draft. All authors read and contributed to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Transcriptomic data were deposited in the Gene Expression Omnibus (GEO) under accession number GSE228918 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE228918).

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