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Exploring Host-Virus Interactions in Caenorhabditis Nematodes

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Exploring Host-Virus Interactions in *Caenorhabditis* Nematodes

by
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Kevin Chen

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ABSTRACT OF THE DISSERTATION

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Professor David Wang, Chair

Caenorhabditis elegans is a powerful model organism that has elucidated many biological questions in the fields of genetics, development, and neurobiology. In addition, *C. elegans* has been used in the past decade to investigate host-pathogen interactions with bacteria and fungi. The recent identification of nematode viruses that naturally infect *C. elegans* and *Caenorhabditis briggsae* provides a unique opportunity to define host-virus interactions in these model hosts.

This dissertation first explored the transcriptional response of *C. elegans* and *C. briggsae* to virus infection by RNA-seq. I identified a total of 320 differentially expressed genes (DEGs) in *C. elegans* following Orsay virus infection. The DEGs were mostly genes of unknown function. Interestingly, many DEGs that responded to Orsay virus infection were similar to those induced by *Nematocida parisii* infection, which is a natural microsporidia pathogen of *C. elegans* that like Orsay virus infects intestinal cells. Furthermore, comparison of the Orsay virus DEGs in *C. elegans* to Santeuil virus DEGs in *C. briggsae* identified 58 *C. elegans* genes whose orthologs were likewise differentially expressed in *C. briggsae*, thereby defining an evolutionarily conserved response to viral infection.

A systematic effort that utilized multiple approaches from the available genetic tools was carried out in *C. elegans* to determine if the evolutionarily conserved DEGs impacted Orsay virus replication either positively or negatively. I found two genes that putatively interact with Orsay virus. The first gene was *T27E7.6*, a gene of unknown function, that may play an antiviral role. The second gene, *zip-10*, a nematode transcription factor, may be a proviral gene that facilitates Orsay virus infection. Neither gene was previously implicated in host-virus interactions. The identification of the virus response genes and the discovery of genes that alter Orsay virus infection of *C. elegans* provide a foundation for future studies of host virus interactions in this model system.

Chapter 1: Introduction

Introduction

Background on *C. elegans* as a model organism

Caenorhabditis elegans is an essential model organism for many novel biological discoveries such as apoptosis regulating genes [1], developmental timing [2], RNA interference (RNAi) [3], and the development of green fluorescent protein as a biological marker [4]. One aspect that made *C. elegans* a great model organism is its biological characteristics. *C. elegans* is a small round worm (~1mm in length) that is transparent, making it great for microscopy and using fluorescent markers [5]. A progeny turnover of ~3 days and a fecundity of 300-350 embryos/hermaphrodite allow expansion of animals in a short time [5]. The relative small amount of space needed (petri dishes) to house the animals and the cheap food source (*Eshcerichia coli*) make *C. elegans* an inexpensive model to maintain. Furthermore, strains can be frozen for long-term storage and revived when desired [5]. All these characteristics of *C. elegans* made it suitable for research in the laboratory.

C. elegans is a genetically tractable model organism with its genome annotated. Hermaphrodites can self-fertilize or be crossed with males to establish strains with desired genotypes [5]. In addition, RNAi can be employed to knockdown genes of interest [3,6], ethyl methanesulfonate (EMS) can be administered to create new random mutations [7], and CRISPR/Cas9 homologous recombination techniques can be used to delete, insert, or modify desired genes [8,9].

C. elegans as a model organism for studying host-pathogen interactions

Caenorhabditis elegans is a model organism widely used to interrogate host-pathogen interactions [10,11]. In recent years, studies in *C. elegans* have identified genes that are essential for immunity against bacterial and fungal pathogens. For instance, roles for p38 MAP kinase [12], TGF-β [13], DAF-2/DAF-16 insulin-like receptor signaling [14], and the transcription factor *zip-2* [15] have been established in protection against bacterial or fungal infections in *C. elegans*. In addition, multiple studies have dissected the *C. elegans* transcriptional response to a range of different pathogens including *Bacillus thuringiensis*[16], *Pseudomonas aeruginosa* and *Staphylococcus aureas* [17], *Serratia marcescens*, *Enterococcus faecalis*, *Erwinia carotovora*, and *Photorhabdus luminescens* [18], and fungal pathogens including *Drechmeria coniospora* [19], *Harposporium sp.* [20] and *Nematocida parisii* [21]. There is some overlap in the transcriptional responses to the various bacterial and fungal infections, suggesting that *C. elegans* maintains both "pan-microbial" and "microbe-specific" repertoires of pathogen response genes [22]. From the transcriptionally induced genes, some functional immune response genes have been identified and characterized.

Transcriptional profiling of viral infection in *Caenorhabditis* nematodes

Much less is understood about host responses in *C. elegans* to viral infection, largely due to the lack of, until recently, a natural virus capable of infecting *C. elegans*. Previous studies using artificial viral infection conditions with vaccinia virus [23], nematode cells with vesicular stomatitis virus [24,25] or a transgenic virus replicon system (Flock house virus) [26] have demonstrated antiviral roles for the programmed cell death genes *ced-3* and *ced-4,* and RNA interference (RNAi) pathways in *C. elegans*. With the discovery of Orsay virus, the first known

natural viral pathogen of *C. elegans*, RNAi and ubiquitin-mediated protection against viral infection have been described [21,27–32].

In addition to Orsay virus, two related viruses, Santeuil and Le Blanc, were discovered in wild *Caenorhabditis briggsae* strains. Orsay virus only infects *C. elegans* while Santeuil virus and Le Blanc virus only infect *C. briggsae* [27,33]. All three viruses have a common tissue tropism and specifically infect the intestine [34]. The identification of multiple viruses that infect two host species that diverged ~18 million years ago affords the unique opportunity to define evolutionarily conserved host responses to viral infection [35]. Furthermore, *C. elegans* can also be infected specifically in the intestine by the microsporidia *N. parisii* [36]. Thus, host responses to these various microbial pathogens can be compared and contrasted. We defined the transcriptional response to these natural pathogens, by using high-throughput RNA sequencing (RNA-seq) to quantify the host mRNA levels following different microbial infections. Collectively, these results shed light on the host response to viral infection and provide insight into the larger context of antimicrobial defense in *C. elegans* [37].

Infection characteristics of *Caenorhabditis* nematode viruses

Orsay virus infection in *C. elegans* reference strain N2 neither reduces the lifespan of the host nor causes any obvious physiological change [27]. The virus hyper-permissive strains where the RNAi function was compromised, also did not have shortened lifespan, but disturbance of the worm intestinal cells and increased Orsay virus RNA were observed [27–29]. The limited physiological change in the N2 strain and no lethality by the Orsay virus infection means that the measurement of Orsay virus replication will largely depend on molecular tools. Also, the ability to measure the amount of virus replication for each stage of the virus lifecycle can help dissect

the role of a specific host factor. To that end, the Wang lab had developed assays to measure Orsay virus RNA with real-time quantitative reverse transcription PCR (qRT-PCR) [27], Orsay virus proteins with immunofluorescence assay [34], and Orsay virus titer with an end point dilution assay [37].

The model organism *C. elegans* has many useful genetic tools to facilitate research in the model organism. However, before we can utilize the full suite of genetic tools available to investigate the interaction between *C. elegans* and Orsay virus, it is essential to characterize the infection process better and determine the limitation of the developed assays. We defined the infection characteristics of multiple controls of genetic tools. Collectively, these results elucidated how the controls behave and helped determine the optimal infection conditions and interpret the experimental results.

Discovery of novel host-virus interactions in *C. elegans*

Using *C. elegans* genetics, there are two broad approaches to identify genes in the host-virus interactions. One of the approach is the forward genetic screen that identifies causal mutation from chemical mutagenized animals with phenotype related to Orsay virus replication [39]. The other approach is a reverse genetic screen that investigates a specific set of genes (whole genome or a biological relevant set) and its relationship with Orsay virus replication [40]. The specific approach taken for the current study was a reverse genetic screens of likely host-virus interaction genes. There are multiple ways to select a biological relevant set of genes in the host-virus interaction, including genes under positive selection pressure [40], proteins that directly interacts with virus [40], and genes that responded to virus infection.

The prime candidates for a genetic screen were the differentially expressed genes (DEGs) upon virus infection [37]. More specifically, the evolutionarily conserved DEGs between *C. elegans* and *C. briggsae*. The overlapping response to represent an evolutionarily conserved process that could interacts with virus infection. We performed a comprehensive reverse genetic screen on the evolutionarily conserved DEGs and found two strong candidate genes that were involved in the host-virus interactions.

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Chapter 2: An evolutionarily conserved transcriptional response to viral infection in *Caenorhabditis* nematodes

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Abstract

Background: *Caenorhabditis elegans* is a powerful model organism for probing many biological processes including host-pathogen interactions with bacteria and fungi. The recent identification of nematode viruses that naturally infect *C. elegans* and *Caenorhabditis briggsae* provides a unique opportunity to define host-virus interactions in these model hosts.

Results: We analyzed the transcriptional response of pathogen infected *C. elegans* and *C. briggsae* by RNA-seq. We identified a total of 320 differentially expressed genes (DEGs) in *C. elegans* following Orsay virus infection. The DEGs of known function were enriched for ubiquitin ligase related genes; however, the majority of the genes were of unknown function. Interestingly, many DEGs that responded to Orsay virus infection were similar to those induced by *Nematocida parisii* infection, which is a natural microsporidia pathogen of *C. elegans* that like Orsay virus infects intestinal cells. Furthermore, comparison of the Orsay virus DEGs in *C. elegans* to Santeuil virus DEGs in *C. briggsae* identified 58 *C. elegans* genes whose orthologs were likewise differentially expressed in *C. briggsae*, thereby defining an evolutionarily conserved response to viral infection.

Conclusions: The two different species *C. elegans* and *C. briggsae,* which diverged ~18 million years ago, share a common set of transcriptionally responsive genes to viral infection. Furthermore, a subset of these genes were also differentially expressed following infection by a eukaryotic pathogen, *N. parisii*, suggesting that these genes may constitute a broader pan-microbial response to infection.

Introduction

Caenorhabditis elegans is a model organism widely used to interrogate host-pathogen interactions [1,2]. In recent years, studies in *C. elegans* have identified genes that are essential for immunity against bacterial and fungal pathogens. For instance, roles for p38 MAP kinase [3], TGF-β [4], DAF-2/DAF-16 insulin-like receptor signaling [5], and the transcription factor *zip-2* [6] have been established in protection against bacterial or fungal infections in *C. elegans*. In addition, multiple studies have dissected the *C. elegans* transcriptional response to a range of different pathogens including *Bacillus thuringiensis*[7], *Pseudomonas aeruginosa* and *Staphylococcus aureas* [8], *Serratia marcescens*, *Enterococcus faecalis*, *Erwinia carotovora*, and *Photorhabdus luminescens* [9], and fungal pathogens including *Drechmeria coniospora* [10], *Harposporium sp.* [11] and *Nematocida parisii* [12]. There is some overlap in the transcriptional responses to the various bacterial and fungal infections, suggesting that *C. elegans* maintains both "pan-microbial" and "microbe-specific" repertoires of pathogen response genes [13]. From the transcriptionally induced genes, some functional immune response genes have been identified and characterized.

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natural viral pathogen of *C. elegans*, RNAi and ubiquitin-mediated protection against viral infection have been described [12,18–23].

In addition to Orsay virus, two related viruses, Santeuil and Le Blanc, were discovered in wild *Caenorhabditis briggsae* strains. Orsay virus only infects *C. elegans* while Santeuil virus and Le Blanc virus only infect *C. briggsae* [18,24]. All three viruses have a common tissue tropism and specifically infect the intestine [25]. The identification of multiple viruses that infect two host species that diverged ~18 million years ago affords the unique opportunity to define evolutionarily conserved host responses to viral infection [26]. Furthermore, *C. elegans* can also be infected specifically in the intestine by the microsporidia *N. parisii* [27]. Thus, host responses to these various microbial pathogens can be compared and contrasted. In this study, to define the transcriptional response to these natural pathogens, we used high-throughput RNA sequencing (RNA-seq) to quantify the host mRNA levels following different microbial infections. Collectively, these results shed light on the host response to viral infection and provide insight into the larger context of antimicrobial defense in *C. elegans*.

Results

C. elegans **transcriptional response to Orsay virus infection.**

To define the transcriptional changes in *C. elegans* upon Orsay virus infection, we compared RNA-seq results from infected and non-infected animals. We analyzed both the laboratory reference strain N2 as well as the *rde-1* mutant, which bears a mutation in the Argonaut protein RDE-1 that is part of the RNAi pathway. The *rde-1* mutant sustains higher levels of Orsay virus replication and accumulate ~100-fold more viral RNA compared to N2 [18] enabling us to assess the impact of more robust viral infection levels, as well as a defective RNAi

pathway, on the transcriptional response. Samples were analyzed at 12 hours post infection (hpi), a time by which Orsay virus protein expression is observed in most *rde-1* animals [25]. We used the edgeR package [28] to identify differentially expressed genes (DEGs) in both N2 and *rde-1* strains (n= 3 replicates for each, FDR < 0.05, Table 2.1). The vast majority of the DEGs were up-regulated, while a small subset of DEGs were down-regulated (Table 2.1, Supplementary Figure 2.1). Among the induced genes, up-regulation ranged between 1.8-fold to over 1000-fold compared to mock control (Supplementary Figure 2.1, Supplementary Table 2.1 & 2.2).

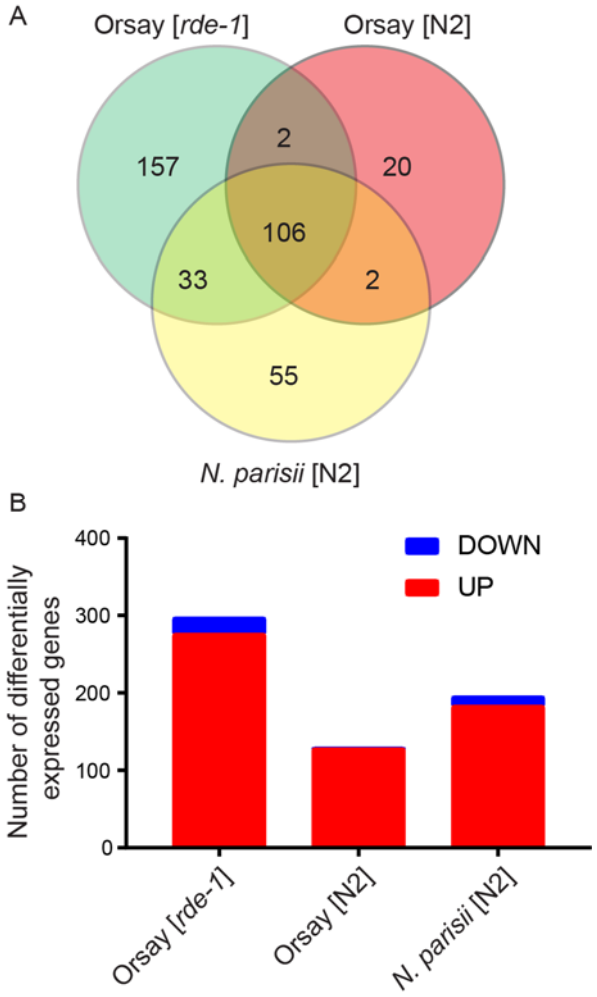

Figure 2.1. ***C. elegans*** **differentially expressed genes upon different pathogen infections.** A) Venn diagrams showed the number of genes as differentially expressed after 12-hour infection

for the three different conditions (Orsay virus [N2], Orsay virus [*rde-1*], and *N. parisii* [N2]) and their relationships. B) Bar graph showed the number of genes as up-regulated or down-regulated upon different infections.

Table 2.1. The number of *C. elegans* differentially expressed genes (DEGs) upon different pathogen infection

	Orsay virus [N2]	Orsay virus [*rde-1*]	*N. parisii* [N2]
DEGs UP	129	277	185
DEGs DOWN	1	21	11
DEGs Shared with Orsay virus [N2]	N/A	108	108
DEGs shared with Orsay virus [*rde-1*]	108	N/A	139

Differentially expressed genes were analyzed using edgeR with 3 replicates and a FDR < 0.05 cutoff. N/A: Not applicable.

Between the two different strains of *C. elegans*, there were 108 DEGs shared, while there were 22 and 190 DEGs specific to N2 and *rde-1,* respectively (Figure 2.1). The majority of the DEGs were of unknown functions. For the subset that had annotations, we identified several enriched gene families and functions using the software package DAVID [29,30], (FDR<0.05, Table 2.2). Both N2 and *rde-1* DEGs were enriched for several gene families including DUF38 domain genes, DUF713 domain genes, MATH (meprin-associated Traf homology) domain genes and a family of paralogs exemplified by *C17H1.3* (*C17H1* family genes hereafter, named after the *C17H1* locus which contains the largest number of genes in this family) (Table 2.2). The *C17H1* family genes have an ortholog in humans, Amyotrophic Lateral Sclerosis 2 Chromosome Region Candidate 12 (ALS2CR12) which encodes a protein of unknown function. Most of the DUF38 domain containing genes also contain an F-BOX domain, which is associated with the ubiquitin ligase pathway. The DUF713 domain genes are specific to the *Caenorhabditis* genus and do not have any associated functions. Of the 22 DEGs that were specific to N2 infected with Orsay virus, no statistically enriched gene families were identified. Of the 190 *rde-1* specific

DEGs, there were several additional enriched gene families including CUB-like domain genes, CUB domain genes, and zinc finger (C6HC-type) domain genes. In addition, innate immune response genes were enriched based on GO annotation (Table 2.2). For the DEGs that were shared between the two strains, *rde-1* DEGs in general were induced to a greater degree (Supplementary Figure 2.1, Supplementary Table 2.1 & 2.2).

To confirm the RNA-seq results, we used quantitative real-time reverse transcription PCR (qRT-PCR) of an independent Orsay virus infection in the N2 strain to evaluate transcript levels of three highly up-regulated genes (*C17H1.3*, *C17H1.8,* and *F26F2.1*) and two genes that did not change following viral infection (*B0024.4* and *tsp-1*). All five genes yielded similar results between transcriptional profiling and qRT-PCR (Supplementary Figure 2.2).

Table 2.2. Gene Ontology (GO), InterPRO term enriched from *C. elegans* genes differentially expressed upon Orsay virus or *N. parisii* infection

Condition	Go term, InterPRO classification	Human ortholog	Functional annotations	Gene count	FDR
Orsay virus [N2]	**IPR026674:ALS2CR12 protein (*C17H1* family)**	Yes	N/A	24	6.9E-32
	IPR002900:DUF38	No	F-box associated	13	6.6E-09
	IPR007883:DUF713	No	N/A	5	1.4E-06
	IPR002083:MATH	Yes	MATH	8	1.3E-06
Orsay virus [*rde-1*]	**IPR026674:ALS2CR12 protein (*C17H1* family)**	Yes	N/A	25	2.3E-25
	GO:0045087:Innate immune response	N/A	Innate immunity	23	6.2E-13
	IPR002083:MATH	Yes	MATH	14	3.1E-09
	IPR007883:DUF713	No	N/A	7	2.9E-08
	IPR002900:DUF38	No	F-box associated	16	3.8E-06
	IPR003366:CUB-like domain	No	N/A	9	4.7E-06
	IPR000859:CUB domain	Yes	CUB	8	6.1E-04
	IPR002867:Zinc finger, C6HC-type	Yes	Ubiquitin related	4	2.1E-02

N. parisii [N2]	**IPR026674:ALS2CR12 protein (*C17H1* family)**	Yes	N/A	25	1.4E-29
	IPR007883:DUF713	No	N/A	9	1.7E-14
	IPR002083:MATH	Yes	MATH	11	3.0E-08
	IPR002900:DUF38	No	F-box associated	13	2.5E-06
	GO:0045087:Innate immune response	N/A	Innate immunity	8	5.2E-03
	IPR001841:Zinc finger, RING-type	Yes	Zinc finger	8	5.9E-03
	IPR016186:C-type lectin-like	Yes	C-type lectin-like	9	1.0E-02
	GO:0005764:lysosome	N/A	lysosome	4	2.9E-02

GO term, InterPRO classification enrichment was analyzed using online DAVID Bioinformatics Resources 6.8. **Bold** denotes conserved terms across the three infection conditions: Orsay virus [N2], Orsay virus [*rde-1*], and *N. parisii* [N2]. N/A: Not applicable.

Orsay virus and *N. parisii* induced a shared transcriptional response.

Because the microsporidia *N. parisii* is also an intracellular pathogen of intestinal cells in *C. elegans*, we performed a parallel transcriptional profiling of *N. parisii* infection in N2. There were 196 DEGs identified in N2 at 12 hpi of *N. parisii* (edgeR, n= 3 replicates, FDR < 0.05, Table 2.1, Supplementary Table 2.3); notably 108 DEGs were shared with Orsay virus infection of N2 (Figure 2.1, Table 2.1). Thus, the majority of the Orsay virus induced DEGs in N2 were also differentially expressed following *N. parisii* infection. Another 33 DEGs were shared between *N. parisii* infection of N2 and Orsay infection of the *rde-1* strain (Figure 2.1). Interestingly, only two genes were down-regulated in both *rde-1* upon Orsay virus infection and N2 upon *N. parisii* infection. The two genes, *pud-1.2,* and *pud-4*, are paralogs known to be regulated by DAF-2, an insulin-like receptor [31,32]. Of the 55 DEGs specific to N2 infected with *N. parisii*, the enriched gene families included zinc finger (RING-type) domain genes and C-type lectin-like genes (Table 2.2). We compared our results with a recently published

expression profile of *N. parisii* infection, which was performed in a different genetic background [12], and found the majority of the up-regulated genes that we identified were also up-regulated at 8 hr post infection in the previous publication (Supplementary Figure 2.3).

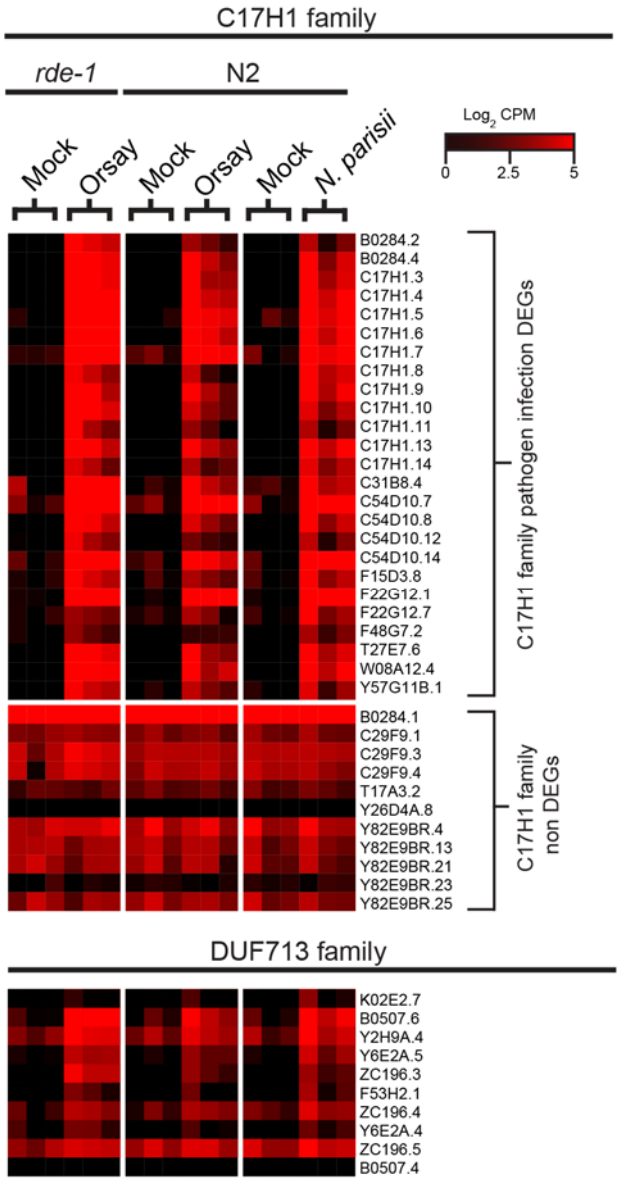

Figure 2.2. **Heatmap of transcription profiles of selected gene families with multiple induced family members.** The heatmap showed the $\log_2$ counts per million (CPM) for each gene in either *C17H1* gene family or DUF713 gene family. Each experimental condition had three replicates and each replicate was represented in a column.

There were four gene families enriched across all infection conditions in *C. elegans*: Orsay virus [N2], Orsay virus [*rde-1*], and *N. parisii* [N2] (Table 2.2). F-box domain genes (DUF38) and MATH domain genes are adapter proteins, which encode a Cullin-binding domain and a substrate-binding domain that target proteins for E3 ubiquitin-ligase mediated proteolysis [33]. There were a total of 35 unique ubiquitin ligase adaptor genes that were highly up-regulated (between 4-fold to 1000-fold), 8 of which were induced in all three conditions. Specifically, F-box proteins act in concert with other proteins that are members of the Skp/Cullin/F-box (SCF) complex to facilitate ubiquitin-ligase mediated proteolysis. Interestingly, *skr-4*, a SCF complex gene was up-regulated in all three infection conditions. Furthermore, in the *rde-1* Orsay virus infected condition, two additional SCF complex genes *skr-5* and *cul-6* were up-regulated.

The *C17H1* family genes and DUF713 domain genes have no known functions. For both the *C17H1* family genes and DUF713 domain genes, more than 50% of the family members were differentially expressed. The *C17H1* gene family has a total of 36 members in *C. elegans,* and of those the same 25 members (except for *F22G12.7* in Orsay virus [N2] condition that was not statistically significant) were up-regulated following both Orsay virus and *N. parisii* infection (Figure 2.2, Table 2.2). The DUF713 domain genes have a total of 10 members in *C. elegans* and have from 5 to 9 members of the gene family up-regulated following pathogen infection (Figure 2.2, Table 2.2). The *C17H1* family had the most DEGs represented in the Orsay virus and *N. parisii* infections (Table 2.2), and some of the genes were among the highest induced with close to 1000-fold increase compared to mock infection. Given the highly distinct nature of Orsay virus from the eukaryotic microsporidium *N. parisii*, this shared transcriptional response may represent a cellular stress pathway in *C. elegans* triggered by intracellular perturbation.

Evolutionarily conserved response to viral infection in *C. elegans* and *C. briggsae*

We next defined the DEGs in *C. briggsae* following Santeuil virus infection. Because the *C. briggsae* laboratory reference strain AF16 does not support Santeuil virus replication in our hands, we used the wild *C. briggsae* isolate JU1264 which we had previously demonstrated to be susceptible to Santeuil virus infection [18]. In *C. briggsae*, there were 258 DEGs following infection by Santeuil virus (edgeR, n = 3, FDR < 0.05, Supplementary Table 2.4). Of the Santeuil virus DEGs, 37 were down-regulated and 221 were up-regulated. On a technical note, JU1264 sequence reads were mapped to the closely related AF16 reference transcriptome; strain specific sequence differences may lead to incomplete mapping to some genes and thus a potential underestimate of the DEGs.

To confirm the RNA-seq results, we used qRT-PCR of an independent Santeuil virus infection of JU1264 to evaluate transcript levels of two up-regulated genes: *CBG03198*, a gene with *C. elegans* orthologs that were also up-regulated, and *CBG06596*, an ortholog of the *C17H1* family in *C. briggsae*. The two genes yielded similar results between transcriptional profiling and qRT-PCR (Supplementary Figure 2.4).

Approximately 60% of all genes in *C. briggsae* have well-defined orthologs in *C. elegans* [34]. We further compared the DEGs in *C. elegans* to their orthologous genes in *C. briggsae*. Of the 320 genes identified as differentially expressed in either N2 or *rde-1* or both (union of N2 and *rde-1* viral infection induced DEGs), 197 have orthologs in *C. briggsae*. 59 of these had *C. briggsae* orthologs that were also differentially expressed following Santeuil infection (Figure 2.3, Supplementary Table 2.5). The majority (57 of 59) of the DEGs were up-regulated in both species. One gene, *hmit-1.1*, was repressed in both the Orsay virus [*rde-1*] and the Santeuil virus [JU1264] conditions while *clec-7* was repressed in Orsay virus [*rde-1*] and induced in Santeuil

virus [JU1264]. In total, there were 58 DEGs in the conserved response to viral infection. 29 *C. elegans* genes were induced in both N2 and *rde-1* and had corresponding *C. briggsae* orthologs induced following Santeuil infection. These, included 14 *C17H1* family genes, four DUF713 domain genes, a gene in the RNAi pathway: *C04F12.1*, and a gene downstream of *daf-16*: *dod-23*. The remaining 9 genes have no known functions. There were two *C. elegans* DEGs in N2 (but not in *rde-1*) whose orthologs in *C. briggsae* were also DEGs. These were *F14F9.3*, a zinc finger (C6HC-type) domain gene and *ZK177.8*, the human ortholog of which is SAMHD1, an antiviral gene against human immunodeficiency virus 1[35]. Finally, there were 27 *C. elegans* DEGs in *rde-1* (but not N2) with corresponding *C. briggsae* DEGs. 6 were immune related genes, four were zinc finger (C6HC-type) domain genes, three were transcription factors (*zip-1*, *zip-5* and *zip-10*), one was an RNAi related gene (*sid*-5) and the remainder had varying annotations (Supplementary Table 2.5).

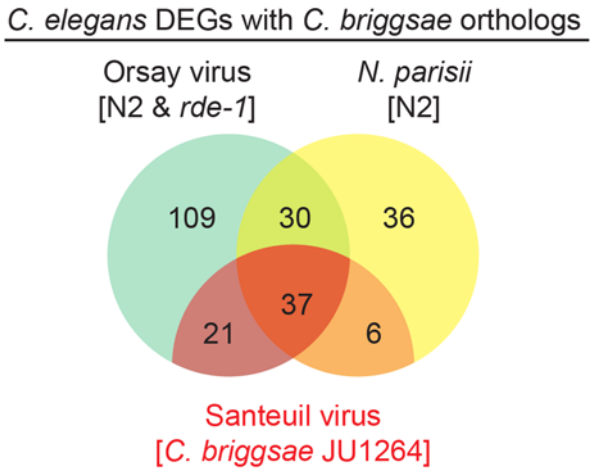

Figure 2.3. Evolutionarily conserved pan-microbial responsive *C. elegans* genes.
C. elegans genes differentially expressed following Orsay and *N. parisii* infection that have orthologs in *C. briggsae* were differentially expressed. Red shading indicates *C. elegans* genes whose orthologs in *C. briggsae* have conserved response to Santeuil infection of *C. briggsae*.

Interestingly, the orthologs in *C. briggsae* of the DUF38 genes and MATH domain genes that responded to both Orsay virus and *N. parisii* infection were not differentially expressed following Santeuil virus infection. This suggests that the induction of F-box and MATH genes may be a *C. elegans* specific transcriptional response. (Supplementary Table 2.5).

Evolutionarily conserved pan-microbial response

In total, 37 of the 58 conserved viral DEGs were also differentially regulated following *N. parisii* infection (Figure 2.3). Strikingly, all of the 29 DEGs that were conserved between the three viral infection conditions, Orsay virus [N2], Orsay virus [*rde-1*], and Santeuil virus [JU1264], were also *N. parisii* induced DEGs. Specifically, members of the *C17H1* and DUF713 gene families appeared to be pan-microbial response genes while the zinc finger (C6HC-type) gene responses were specific to viral infection (Supplementary Table 2.5). To further assess the potential roles of the conserved virus induced DEGs in the context of different pathogens, we compared our current results with previous published studies of pathogen induced host response. A prior microarray study of Orsay virus infection of *C. elegans* identified multiple DEGs [19], of which 24 DEGs were shared between the two studies in N2 background and 40 DEGs were shared in the *rde-1* mutant (Supplementary Figure 2.3). In addition, we identified additional statistically significant viral DEGs, possibly due to the use of synchronized animals. We examined a panel of representative bacterial pathogens including *Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Bacillus thuringiensis, Serratia marcescens, and Photorhabdus luminescens* [7,8,11,36] and fungal pathogens including *Drechmeria coniospora* and *Harposporium sp.* [11]. All up-regulated and down-regulated DEGs identified from previous studies were compared to the up-regulated or down-regulated Orsay virus DEGs. *P. aeruginosa,*

P. luminescens and *D. coniospora* infection each shared a significant number of DEGs with Orsay virus infection (Fisher exact test $p < 0.001$). Notably, *dod-22* was differentially expressed in all four instances. By contrast, there was an inverse association of *E. faecalis* DEGs with Orsay virus DEGs (Fisher exact test $p < 0.001$), with fewer shared DEGs than expected by chance. Finally, the other pathogens did not have a significant relationship with the Orsay virus DEGs (Table 2.3).

We also specifically investigated the transcriptional response of the *C17H1* gene family members following infection by other pathogens. In prior published studies, *P. luminescens* and *D. coniospora* each induced multiple of the virally up-regulated *C17H1* gene family members (Table 2.3). Interestingly, some of the *C17H1* family genes that were not up-regulated following viral infection were differentially expressed following infection by *D. coniospora*, *Harposporium*, *P. luminescens*, and *S. marcescens* (Table 2.3). Thus, there may be pan-microbial responsive *C17H1* family members as well as those that respond to specific pathogens.

Table 2.3. Conserved responses between bacterial or fungal pathogen to Orsay virus DEGs.

	Total DEGs	Bacterial/fungal DEGs shared with Orsay DEGs	Overlap significance	Number of DEGs from *C17H1* family	Number of *C17H1* DEGs shared with Orsay virus infection
B. thuringiensis	246	5	4.4E-01	0	0
D. coniospora	3787	84	6.1E-04	15	11
E. faecalis	3819	40	**3.7E-03**	2	2
Harposporium sp.	3695	60	7.1E-01	6	4
P. aeruginosa	146	10	1.0E-04	0	0
P. luminescens	3797	111	6.4E-12	12	11
S. aureus	386	9	2.1E-01	1	1
S. marcescens	3384	56	6.5E-01	11	7

Pathogen infection expression profile analysis data were obtained from [7,8,11,36]. The DEGs from each pathogen were compared to Orsay virus DEGs. Association significance is calculated using Fisher exact test. **BOLD** indicates a significant negative correlation.

Discussion and Conclusion

We defined the host transcriptional response to viral infection in *C. elegans* and *C. briggsae*. From our statistical analysis of Orsay virus infections in N2 and *rde-1* mutant strains, we identified a total of 320 DEGs in *C. elegans,* of which 108 DEGs were shared. In the *rde-1* Orsay virus infection, there were more DEGs compared to infection of N2. In addition, the magnitude of the transcriptional changes in *rde-1* was generally greater. One possible explanation for this observation is that the higher levels of viral infection in *rde-1* may have created a more significant perturbation from the basal state, leading to a more robust transcriptional response. Alternatively, the lack of competent RNAi in *rde-1* may have resulted in induction of a distinct, compensatory host response. One potential limitation of these studies is that Orsay, Santeuil, and *N. parisii* infection is thought to be limited to at most the 20 intestinal cells present in *Caenorhabditis* nematodes. Because our transcriptional profiling used RNA extracted from populations of entire animals (each *C. elegans* has 959 somatic cells), some transcriptional responses may have been masked by the basal level of transcription in the uninfected cells, and thus our results are likely an underestimate of the transcriptional changes occurring in the intestinal cells.

Strikingly, 108 of the N2 DEGs were also differentially expressed following infection with the microsporidium, *N. parisii* (Figure 2.1). Orsay virus is a small single stranded RNA virus with a bipartite genome of 3.6 Kb and 2.6 Kb that is only known to encode three proteins [18]. By contrast, *N. parisii* has a 4.1 Mb genome and encodes more than 2000 genes [27,37]. Despite the lack of obvious similarity between these two microbes, the fact that a significant

fraction of the transcriptional response to these two pathogens overlapped suggests that *C. elegans* may have some form of a universal "stress response". One clear commonality between the two is that they are both intracellular intestinal pathogens of *C. elegans*; in fact they are the only intracellular pathogens of *C. elegans* described to date. Thus, the conserved transcriptional response may reflect recognition of some shared intracellular perturbation. Interestingly, although some of these shared response genes are potentially involved in the ubiquitin ligase pathway, the majority of the shared response genes are largely unannotated genes of unknown function. These genes could play important roles in immunity against pathogen infection. Alternatively, it is also possible that these genes are important for pathogen infection, and that the pathogen alters the transcriptional response to facilitate infection and replication.

Many of the characterized genes induced by Orsay virus or *N. parisii* infection in *C. elegans* were genes in the ubiquitin ligase pathway. When challenged with either Orsay virus or *N. parisii*, there were 35 unique F-box related or MATH domain genes up-regulated. In addition, SCF complex genes, such as *skr-4*, were up-regulated in all *C. elegans* infections while *skr-5* and *cul-6* were up-regulated in the *rde-1* mutant infected with Orsay virus. Most of the F-box and MATH family members have sites in their substrate binding domains that are under strong positive selection and are greatly expanded in *C. elegans* in comparison to humans [33]. This suggests a possible role of ubiquitin ligase as part of the *C. elegans* host-pathogen interaction to restrict pathogen proliferation. Indeed, SCF ubiquitin ligases are demonstrated as a line of defense against infection by Orsay virus and *N. parisii* in *C. elegans* [12]. Intriguingly, none of the DEGs in *C. briggsae* were known F-box or MATH genes, suggesting that these ubiquitin ligase pathways may be a specific *C. elegans* response.

There are varying degrees of conservation between Orsay virus response genes to other pathogens of *C. elegans*. We analyzed previously published transcriptional profiling studies of infection by 8 bacterial and fungal pathogens and identified three that have a significant fraction of DEGs shared with Orsay virus infection. The three pathogens, *P. aeruginosa, P. luminescens* and *D. coniospora,* all can affect the intestine of the worm, but each does so in unique fashion. *P. aeruginosa* PA14 primarily kills by excreted toxins*, P. luminescens* colonizes the intestinal lumen, which is characterized by the appearance of cytosolic crystalline structures of unknown origin [38], and *D. coniospora* produces threadlike hyphae that penetrate and eventually kill the infected animal [39]. Other pathogens that also target the intestine such as *E. faecalis* and *S. aureus* did not have significant DEGs in common with Orsay virus infection, demonstrating a specificity of the host response. The different responses of *C. elegans* to various pathogens suggest the existence of distinct sensing and regulatory mechanisms. One potential regulatory element in response to virus infection is *drh-1*, a RIG-I like protein in *C. elegans*. Previous studies have determined that *drh-1* both acts directly as a effector in the RNAi pathway to restrict virus replication and as a sensor of virus infection critical for downstream host responses [19,20].

Comparative analysis of the DEGs in virus infected *C. elegans* and *C. briggsae* identified 58 *C. elegans* genes whose *C. briggsae* orthologs were also differentially expressed. Of those, 29 were shared between the three conditions: Orsay virus [N2], Orsay virus [*rde-1*], and Santeuil virus [JU1264]. Strikingly, 14 of the 29 genes were members of a single gene family, the *C17H1* family genes in *C. elegans*. Induction of members of this gene family in response to viral infection was conserved in two divergent *Caenorhabditis* nematode species despite ~18 million years of host evolution. Furthermore, analysis of other published transcriptomes identified induction of *C17H1* family genes by bacterial and fungal pathogens. The upregulation of a

subset of these genes by disparate microbes such as virus, bacteria, and fungi raises the possibility that this gene family may form the core of a pan-microbial stress response. To date, there has been no reported function associated with these family members. The large number of paralogs induced following Orsay virus or *N. parisii* infection suggests the possibility of functional redundancy, which would provide a challenge in experimental testing of the functions of these genes.

Our transcriptional profiling study of both virus and microsporidium infection provides insights into the host response to pathogens. We found that distinct pathogens such as Orsay virus and *N. parisii* elicited a similar set of DEGs in *C. elegans*, suggesting that these DEGs may constitute a broad pan-microbial response to infection. Additionally, within the transcriptional profile of viral infection in the two different nematode species *C. elegans* and *C. briggsae*, we found a shared set of 58 evolutionarily conserved transcriptional responsive genes to viral infection, many of which have no known function. Given the fact that diverse hosts regulate these common genes in response to distinct viral infections suggests that they play important roles. Further studies are needed to define the impact and mechanism of action of these genes on viral infection.

Materials and Methods

Strains

N2 and *rde-1* (WM27) were obtained from the *Caenorhabditis* Genetics Center (CGC). Isolation of wild *C. briggsae* strains JU1264 has been described [18].

Infectious virus filtrate preparation

Orsay virus and Santeuil virus were propagated as previously described [18]. Briefly, *C. elegans rde-1* mutants were subjected to Orsay virus infection. *C. briggsae* JU1264 were subjected to Santeuil virus infection. Infected animals were subsequently collected and homogenized. The homogenate were passed through a 0.22 μm filter to obtain filtered viruses.

Quantification of virus titer

To measure the infectious titer of viruses, we employed a method similar to tissue culture infectious dose 50% ($TCID_{50}$) using live *C. elegans* or *C. briggsae* in wells instead of cultured cells. We were not able to measure killing of nematodes as none of the viruses were lethal. To measure infectivity in a well, we used qRT-PCR to determine whether replication of viral RNA occurred, using a criterion of Ct value of 30 and below as positive infection. Animals were synchronized and plated on 6-well plate seeded with 20 μl OP50 food. Virus filtrates were serially diluted 10-fold to 10^{-8}. 20 μl of each dilution were added to a well containing animals and combined to have four total replicates per condition. Infected animals were incubated at 20°C for three days and collected into Trizol. RNA samples were extracted using Zymo 96-well RNA extraction kit. The stock Orsay virus filtrate had a titer of 2.3×10^6 $TCID_{50}$/ml and Santeuil virus a titer of 8.9×10^6 $TCID_{50}$/ml.

Pathogen inoculation

Three independent infections were conducted for each strain and pathogen. Two methods were used to infect animals. 1) Uninfected *C. elegans* (N2 and *rde-1*) and *C. briggsae* (wild isolate JU1264) were synchronized by standard bleach treatment. 2,000 embryos were seeded per well into 6-well NGM plates containing 20 μl of OP50 food and maintained at 20°C. For each

condition, 18 wells were prepared and collected. 39 hours after bleaching, animals (L3 stage) were inoculated with 20 µl of Orsay virus (4.0 x 10^5 $TCID_{50}$ /ml) for *C. elegans* or Santeuil virus (8.9 x 10^5 $TCID_{50}$/ml) for *C. briggsae*, or M9 buffer as control. 12 hours post infection, animals were rinsed off from the wells with 1 ml M9 buffer, supernatant were removed after centrifugation and 1 ml Trizol were added. 2) Uninfected *C. elegans* (N2 and *rde-1*) were synchronized by standard bleach treatment and 20,000 embryos were added to 10 cm NGM plates seeded with 1.5 ml of OP50. 39 hours after bleaching, animals (L3 stage) were inoculated with either 200 µl Orsay virus (4.0 x 10^5 $TCID_{50}$ /ml), *N. parisii* microsporidia (10,000 spores/animal), or homogenates of uninfected *rde-1* passed through a 0.22 µm pore filter (mock control). *C. briggsae* (JU1264) were treated similarly and infected with 200 µl Santeuil (8.9 x 10^5 $TCID_{50}$/ml) virus. Animals were rinsed off from the plates and harvested in Trizol at 12 hours after exposure to virus, microsporidia, or mock control.

Preparation of RNA-seq sample

Total RNA was extracted using Trizol and mRNA was subsequently enriched using OligoTex mRNA mini (Qiagen) according to the manufacturer's protocol. RNA concentrations were assessed by Qubit fluorimeter (Life Technologies) and 10-100 ng of each sample were sent to Genome Technology Access Center in the Department of Genetics at Washington University School of Medicine (GTAC) for RNA-Seq.

RNA-Seq Analysis

Illumina HiSeq platform-generated single end reads of 50bp were aligned to N2 reference strain and AF16 reference strain transcriptomes (WS250) with TopHat2 [40]. The aligned reads were

counted with HT-Seq [41]. Only uniquely aligned reads were counted and used for downstream analysis. The read counts were processed with R package edgeR [28,42] . Samples were subjected to batch effect adjustment as there were two different methods of preparation and all three replicates were independently conducted on different days. The resulting counts were subjected to standard edgeR differential expressed gene analysis with a statistical cutoff of FDR < 0.05.

Real time quantitative two-step RT-PCR (qRT-PCR) of host genes

1 µg of total RNA from each sample was treated with DNaseI (Fermentus) according to the manufacture's protocol, purified using an RNeasy kit (Invitrogen) and then eluted in 20 µl RNase/DNase free water. cDNA synthesis was performed by using an oligo(dT) primer with thermoscript reverse transcriptase (Thermo Scientific) at 65 °C for 45 minutes. The synthesized cDNA was diluted 1:10 and 5 ul of the diluted cDNA was used for real time-qPCR. Real time qPCR was performed using Taqman qPCR master mix reagents (Applied Biosystem) on a ViiA7 real time PCR system (Applied Biosystem) following the manufacturer's suggested protocol. Each analyzed gene was normalized to an internal control *cdc-42* gene and expressed as fold change of infected samples compared to mocked infected samples.

Association of bacterial or fungal pathogen DEGs with Orsay virus DEGs

The significance of association between bacterial or fungal DEGs from previous studies and Orsay virus DEGs was measured by a Fisher exact test. Briefly, concordant (up-regulated in both or down-regulated in both) DEGs between a selected pathogen and Orsay virus were counted as the overlapped DEGs. Fisher exact test was calculated with the total non-overlapped pathogen

DEGs, the non-overlapped Orsay virus DEGs and all other remaining genes (non-differentially expressed following bacterial, fungal or viral infection). Pair-wise comparison between pathogens DEGs against Orsay RNA were done for all pathogens.

Acknowledgements

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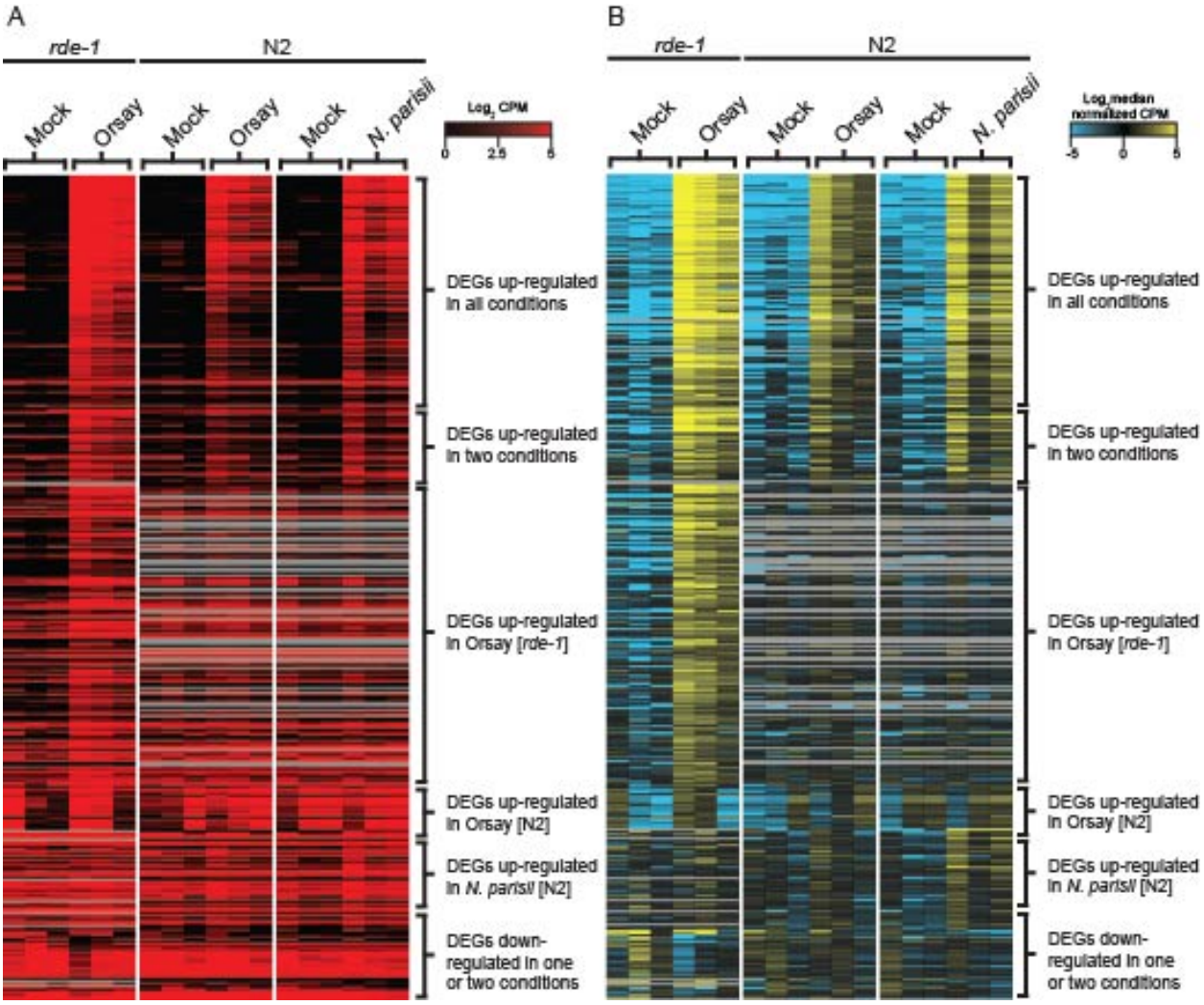
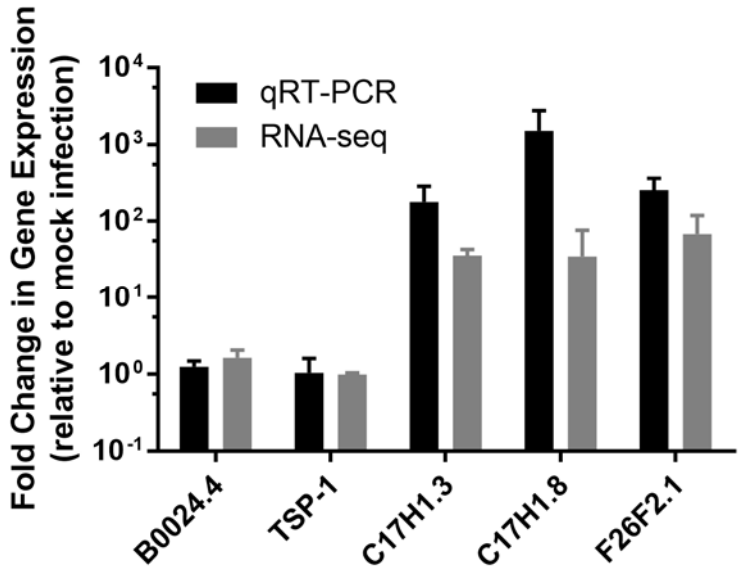
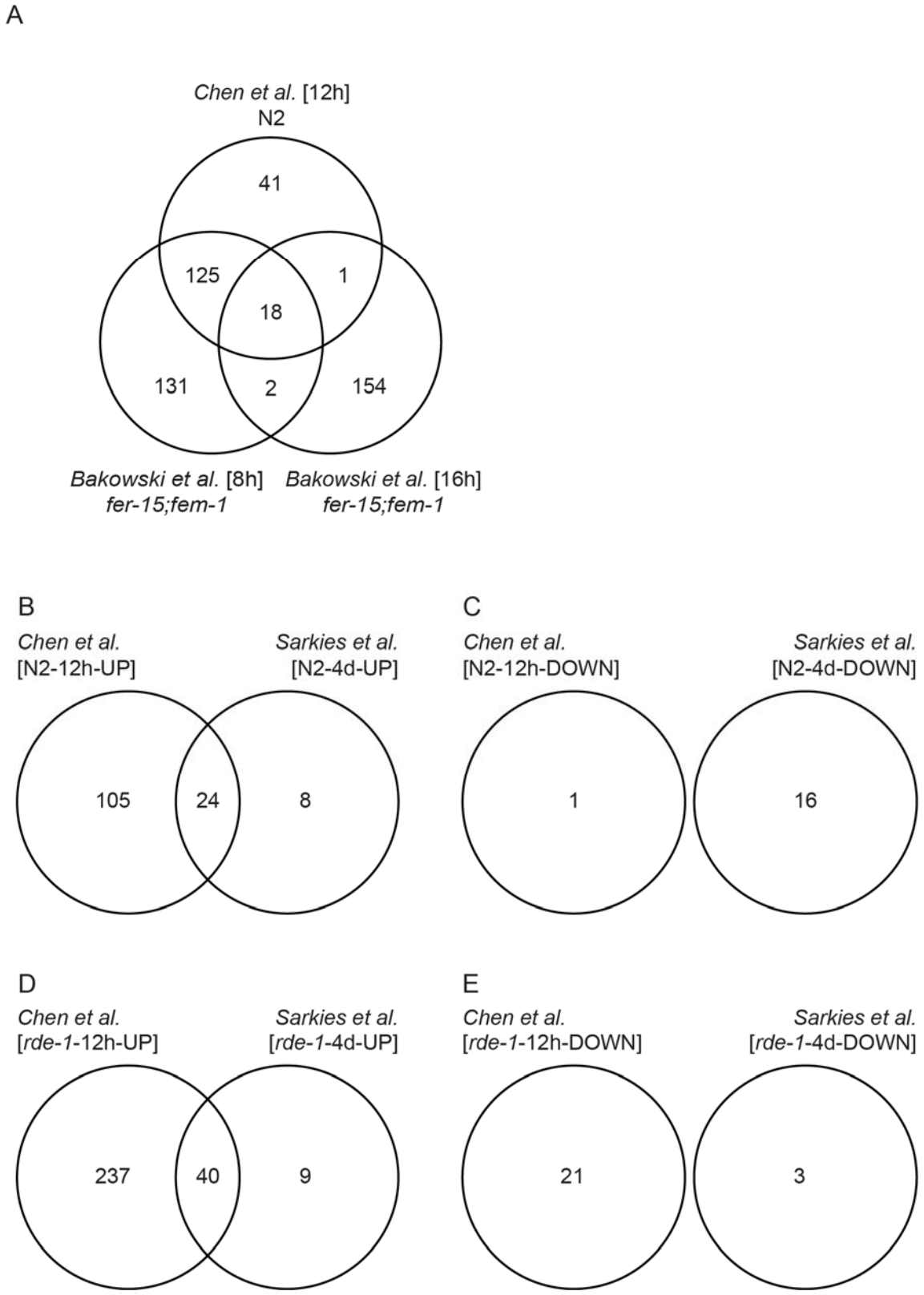
Supplementary Materials



Supplementary Figure 2.1 Heatmap of differentially expressed genes upon pathogen infections. The heatmap showed the expression level for all of the differentially expressed genes in the three infection conditions (Orsay virus [N2], Orsay virus [*rde-1*], and *N. parisii* [N2]). A) Log_2 CPM of each gene presented. B) Median normalized Log_2 CPM of each gene. Each CPM value was normalized to the median CPM for the given gene. Each experimental condition had three replicates and each replicate was represented in a column. Samples that did not have measurable expression were grey.

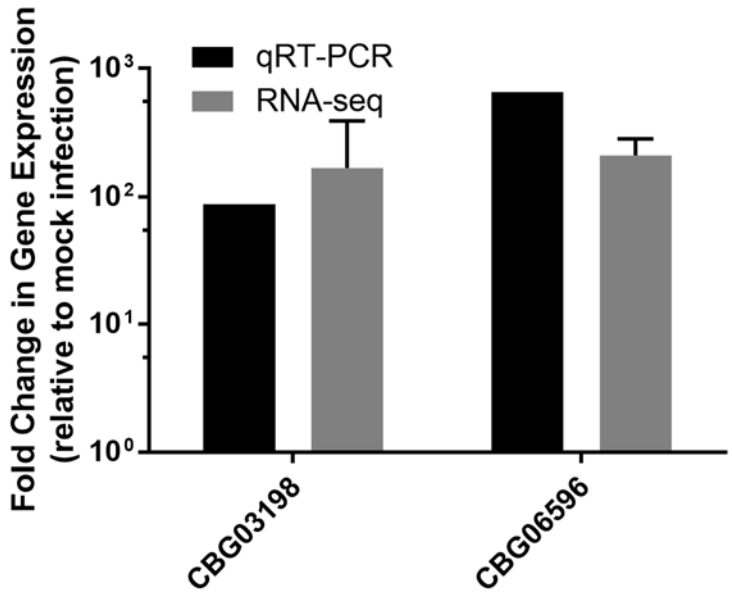


Supplementary Figure 2.2 Confirmation of *C. elegans* RNA-seq with qRT-PCR. Expression of N2 response genes to Orsay virus infection with RNA-seq was confirmed with qRT-PCR. qRT-PCR results were normalized to *cdc-42* before calculating fold-change.



Supplementary Figure 2.3 Comparison of DEGS with previous publications. Venn diagrams showed the comparison of DEGs from our current study to previous published studies. A)

Comparison of up-regulated DEGs from *N. parisii* [N2] infection. The most similar conditions from *Bakowski et al*. were used (8 hours and 16 hours post *N. parisii* infection). B) Comparison of up-regulated genes in N2 after Orsay virus infection between current study and *Sarkies et al*. C) Comparison between down-regulated genes in N2 after Orsay virus infection between current study and *Sarkies et al*. D) Comparison between up-regulated genes in *rde-1* after Orsay virus infection between current study and *Sarkies et al*. E) Comparison between down-regulated genes in *rde-1* after Orsay virus infection between current study and *Sarkies et al*.



Supplementary Figure 2.4 Confirmation of *C. briggsae* RNA-seq with qRT-PCR. Expression level from RNA-seq of JU1264 response genes to Santeuil virus infection was confirmed with qRT-PCR. qRT-PCR results were normalized to *cbr-cdc-42* before calculating fold-change.

Supplementary Table 2.1. List of differentially expressed genes upon pathogen infections (Orsay [N2]) identified by edgeR.

WormBase Gene ID	Gene Name	Sequence Name	Fold-change (log2)	FDR
WBGene00009168		F26F2.3	8.37	6.06E-127
WBGene00009169		F26F2.4	7.79	4.09E-116
WBGene00018345		F42C5.3	6.49	9.12E-90
WBGene00009061		F22G12.1	6.18	2.05E-83
WBGene00045401	eol-1	T26F2.3	6.06	2.32E-79
WBGene00007659		*C17H1*.6	6.72	2.47E-65
WBGene00219309		F26F2.13	5.86	2.52E-62
WBGene00008067		C43D7.4	9.99	1.22E-61
WBGene00044708		*C17H1*.13	7.09	6.47E-61
WBGene00009170		F26F2.5	7.45	1.64E-58
WBGene00007134		B0284.4	8.31	8.32E-55
WBGene00009166		F26F2.1	5.54	2.18E-50
WBGene00195165		F57G4.11	7.10	4.09E-50
WBGene00007660		*C17H1*.7	4.09	9.08E-50
WBGene00008068	sdz-6	C43D7.5	5.63	2.52E-47
WBGene00012091		T27E7.6	6.73	4.99E-41
WBGene00007662		*C17H1*.9	5.51	3.45E-40
WBGene00008301	dct-3	C54D10.7	4.14	7.06E-37
WBGene00007656		*C17H1*.3	5.86	1.90E-36
WBGene00138721		C54D10.14	3.92	8.01E-34
WBGene00008069		C43D7.7	8.85	1.39E-32
WBGene00015225		B0507.8	6.45	4.01E-31
WBGene00008267		C53A5.9	5.29	5.72E-31
WBGene00015834	math-5	C16C4.10	6.19	1.05E-26
WBGene00021081		W08A12.4	5.00	1.02E-24
WBGene00020357		T08E11.1	4.52	2.26E-24
WBGene00044014		F15H10.10	8.09	4.79E-24
WBGene00022476	fbxa-75	Y119D3A.1	8.33	5.88E-23
WBGene00023483		Y75B8A.39	4.11	3.32E-22
WBGene00012726		Y39G8B.5	4.53	1.83E-20
WBGene00007661		*C17H1*.8	5.73	2.07E-20
WBGene00194645		Y105C5A.1269	4.96	1.21E-18
WBGene00008302		C54D10.8	3.61	2.20E-18
WBGene00008269		C53A5.11	4.80	5.62E-18
WBGene00015227		B0507.10	5.80	1.41E-17
WBGene00086568		Y2H9A.6	3.62	2.65E-17
WBGene00007655		*C17H1*.2	6.01	9.95E-17
WBGene00086560		ZK355.8	3.88	1.07E-16
WBGene00044709		*C17H1*.14	5.11	2.38E-16
WBGene00007657		*C17H1*.4	5.53	4.06E-15
WBGene00004149	trpl-5	T16A1.7	4.23	8.72E-15
WBGene00007132		B0284.2	3.82	3.17E-14
WBGene00018353	fbxa-182	F42G2.4	2.32	3.63E-14
WBGene00044237		*C17H1*.10	3.66	5.01E-14
WBGene00009908		F49H6.5	4.45	6.43E-14
WBGene00017214		F07E5.9	6.10	1.28E-13
WBGene00013634		Y105C5A.9	3.22	8.78E-13
WBGene00012910		Y46G5A.20	1.69	1.09E-12
WBGene00020228		T05A8.2	6.97	6.28E-12
WBGene00010821		M01G12.7	4.53	2.23E-11

WBGene00022550	droe-8	ZC196.6	3.45	1.42E-10
WBGene00015835	math-6	C16C4.11	5.13	1.31E-09
WBGene00194646		Y105C5A.1270	2.41	2.25E-09
WBGene00194902		Y39H10B.3	3.23	3.23E-09
WBGene00044711		*C17H1*.11	4.19	5.92E-09
WBGene00013294		Y57G11B.1	2.96	7.22E-09
WBGene00015223		B0507.6	2.74	7.37E-09
WBGene00050906		F20E11.17	6.36	3.06E-08
WBGene00015829	math-15	C16C4.5	2.02	3.25E-08
WBGene00045393		F26D11.13	3.22	4.27E-08
WBGene00010206		F57G4.1	6.18	6.60E-08
WBGene00044416		C08E3.15	6.13	2.81E-07
WBGene00015224		B0507.7	3.22	6.24E-07
WBGene00008507		F01G10.4	4.79	1.01E-06
WBGene00015833	math-17	C16C4.9	5.90	1.44E-06
WBGene00015832	math-16	C16C4.8	5.84	2.77E-06
WBGene00015828	math-14	C16C4.4	2.17	4.27E-06
WBGene00044278		F09C6.14	5.90	4.90E-06
WBGene00020080	math-37	R52.9	2.42	5.51E-06
WBGene00015602	fbxa-158	C08E3.10	3.24	7.10E-06
WBGene00045415		Y43F8B.15	3.02	7.80E-06
WBGene00195181		F15H10.12	4.46	7.80E-06
WBGene00044787		C54D10.12	2.37	9.67E-06
WBGene00012399		Y6E2A.5	2.18	1.12E-05
WBGene00007297		C04F12.1	1.56	1.24E-05
WBGene00020637	fbxa-7	T20H9.1	5.67	1.24E-05
WBGene00008872		F15H10.5	3.94	1.51E-05
WBGene00012961		Y47H10A.5	1.28	3.58E-05
WBGene00015114		B0281.8	3.69	4.44E-05
WBGene00015839	math-10	C16C4.15	2.30	4.57E-05
WBGene00003098	lys-9	C54C8.6	5.62	5.80E-05
WBGene00016281		C31B8.4	3.11	6.94E-05
WBGene00206359		C05E4.15	3.68	0.00011025
WBGene00021179	fbxa-86	Y9C9A.12	2.80	0.0001118
WBGene00007658		*C17H1*.5	5.47	0.00013353
WBGene00009898	dod-23	F49E12.2	2.67	0.000169758
WBGene00020361	fbxc-19	T08E11.5	3.12	0.000294736
WBGene00022375		Y94H6A.2	1.95	0.000318747
WBGene00018614		F48G7.2	2.41	0.000328364
WBGene00022547		ZC196.3	3.00	0.00033229
WBGene00022713		ZK355.3	4.01	0.000439712
WBGene00010508		K02E2.7	3.48	0.001220132
WBGene00004402	rom-3	Y116A8C.14	2.80	0.001304425
WBGene00008858		F15D3.8	2.01	0.001536603
WBGene00022673		ZK177.8	0.87	0.001708763
WBGene00011168		R09D1.12	2.12	0.001751296
WBGene00017112		E03H12.5	1.08	0.001751296
WBGene00020227		T05A8.1	3.70	0.001751296
WBGene00011539	fbxa-135	T06E6.5	2.59	0.002079804
WBGene00004810	skr-4	Y60A3A.18	1.60	0.003822879
WBGene00020364		T08E11.8	1.92	0.003822879
WBGene00022708		ZK354.7	1.03	0.003822879
WBGene00044013		F15H10.9	4.87	0.004133448

WBGene00009835		F47H4.2	1.26	0.005550112
WBGene00012067	sqst-3	T26H2.5	3.27	0.005719825
WBGene00006051	ssq-2	T28H11.5	1.42	0.006265914
WBGene00015108		B0281.1	2.94	0.006853951
WBGene00006053	ssq-4	T28H11.1	1.50	0.011697099
WBGene00006050	ssq-1	K07F5.11	1.29	0.013147644
WBGene00045457		F33H12.7	1.15	0.015304123
WBGene00012400		Y6E2A.7	2.45	0.017681892
WBGene00015780		C14F11.4	1.01	0.022875885
WBGene00050899		T10C6.15	1.66	0.024159858
WBGene00044205		T13F3.8	1.05	0.029367319
WBGene00194713		F19B10.13	2.11	0.030164971
WBGene00015600	fbxa-165	C08E3.8	3.01	0.030910823
WBGene00019410		K05F1.9	1.11	0.030910823
WBGene00012381		Y2H9A.4	1.20	0.032486674
WBGene00020774		T24E12.5	1.17	0.032486674
WBGene00015908		C17F3.3	0.96	0.035953625
WBGene00016351		C33F10.1	1.12	0.037534132
WBGene00017628		F20B6.4	-1.68	0.037534132
WBGene00000596	col-7	C15A11.5	1.23	0.039562478
WBGene00017466		F14F9.3	1.98	0.040997477
WBGene00020713		T23B3.5	0.95	0.040997477
WBGene00044207		Y6G8.5	1.78	0.040997477
WBGene00007159		B0379.7	1.26	0.041200366
WBGene00020116	moa-1	R155.2	1.13	0.041519784
WBGene00010679		K08F4.5	1.07	0.044930017
WBGene00011478		T05D4.5	1.40	0.046327869

Supplementary Table 2.2. List of differentially expressed genes upon pathogen infections (Orsay [rde-1]) identified by edgeR.

WormBase Gene ID	Gene Name	Sequence Name	Fold-change (log2)	FDR
WBGene00009169		F26F2.4	14.14	4.73E-49
WBGene00007657		*C17H1*.4	9.95	3.22E-45
WBGene00008067		C43D7.4	12.63	1.88E-44
WBGene00009168		F26F2.3	10.45	3.66E-44
WBGene00018345		F42C5.3	9.51	1.95E-43
WBGene00219309		F26F2.13	9.66	4.11E-43
WBGene00007659		*C17H1*.6	9.56	5.33E-43
WBGene00008269		C53A5.11	10.55	6.09E-43
WBGene00009061		F22G12.1	8.35	3.94E-41
WBGene00015225		B0507.8	9.07	7.16E-40
WBGene00044708		*C17H1*.13	12.15	1.99E-38
WBGene00007658		*C17H1*.5	8.82	3.72E-38
WBGene00009170		F26F2.5	9.07	2.56E-37
WBGene00195165		F57G4.11	9.04	4.34E-37
WBGene00013634		Y105C5A.9	8.61	1.66E-36
WBGene00007134		B0284.4	8.91	3.68E-36
WBGene00022476	fbxa-75	Y119D3A.1	9.94	3.96E-35
WBGene00045401	eol-1	T26F2.3	8.46	5.85E-35
WBGene00012091		T27E7.6	9.80	1.39E-34
WBGene00010821		M01G12.7	8.54	1.12E-32
WBGene00021081		W08A12.4	7.36	1.48E-32
WBGene00007662		*C17H1*.9	9.16	3.69E-32

WBGene00008068	sdz-6	C43D7.5	8.29	5.79E-31
WBGene00007656		*C17H1*.3	7.88	1.01E-30
WBGene00023483		Y75B8A.39	7.59	1.84E-30
WBGene00044014		F15H10.10	11.30	6.02E-30
WBGene00015227		B0507.10	8.08	8.71E-30
WBGene00020228		T05A8.2	8.79	2.70E-29
WBGene00044237		*C17H1*.10	7.55	3.89E-29
WBGene00004149	trpl-5	T16A1.7	7.05	4.01E-29
WBGene00007660		*C17H1*.7	6.51	6.23E-29
WBGene00020357		T08E11.1	6.86	3.01E-28
WBGene00008301	dct-3	C54D10.7	6.03	7.93E-28
WBGene00009166		F26F2.1	7.92	6.96E-27
WBGene00015834	math-5	C16C4.10	9.21	9.16E-27
WBGene00138721		C54D10.14	5.88	1.11E-26
WBGene00008267		C53A5.9	9.13	1.56E-26
WBGene00086560		ZK355.8	6.19	1.02E-25
WBGene00008069		C43D7.7	9.18	2.26E-24
WBGene00015223		B0507.6	5.07	1.04E-23
WBGene00015602	fbxa-158	C08E3.10	4.72	1.15E-20
WBGene00012726		Y39G8B.5	7.93	1.19E-20
WBGene00017214		F07E5.9	7.76	5.36E-20
WBGene00194645		Y105C5A.1269	6.88	9.35E-19
WBGene00021979		Y58A7A.5	4.68	7.09E-18
WBGene00007132		B0284.2	6.27	1.22E-17
WBGene00015828	math-14	C16C4.4	4.23	3.11E-17
WBGene00016281		C31B8.4	5.24	5.05E-17
WBGene00003092	lys-3	Y22F5A.6	5.30	7.86E-17
WBGene00018353	fbxa-182	F42G2.4	4.08	8.53E-17
WBGene00015833	math-17	C16C4.9	9.81	1.92E-16
WBGene00008302		C54D10.8	5.53	2.28E-16
WBGene00009908		F49H6.5	4.53	2.60E-16
WBGene00009898	dod-23	F49E12.2	5.46	3.40E-16
WBGene00044711		*C17H1*.11	8.44	3.50E-16
WBGene00045393		F26D11.13	7.34	3.83E-16
WBGene00194646		Y105C5A.1270	6.23	4.35E-16
WBGene00022550	droe-8	ZC196.6	6.38	7.91E-16
WBGene00008507		F01G10.4	8.12	8.46E-16
WBGene00044709		*C17H1*.14	9.66	1.78E-15
WBGene00020227		T05A8.1	9.66	5.60E-15
WBGene00021193	math-44	Y16E11A.1	4.12	6.66E-15
WBGene00022547		ZC196.3	6.20	7.74E-15
WBGene00007661		*C17H1*.8	6.61	4.44E-14
WBGene00013294		Y57G11B.1	7.01	9.13E-14
WBGene00007097		B0024.4	5.43	1.14E-13
WBGene00006627	tsp-1	C02F5.8	4.64	2.16E-13
WBGene00050906		F20E11.17	7.47	2.16E-13
WBGene00255420		Y51F10.15	7.52	2.28E-13
WBGene00015832	math-16	C16C4.8	9.26	2.30E-13
WBGene00195181		F15H10.12	9.12	4.34E-13
WBGene00013454	fbxa-126	Y67A10A.5	8.75	5.14E-12
WBGene00014135		ZK896.4	3.56	5.30E-12
WBGene00219750		Y38C1AA.19	6.03	8.65E-12
WBGene00008872		F15H10.5	7.18	1.03E-11

WBGene00044013		F15H10.9	9.07	1.05E-11
WBGene00007655		*C17H1*.2	8.78	1.24E-11
WBGene00045415		Y43F8B.15	4.59	1.26E-11
WBGene00012101	zip-10	T27F2.4	4.33	1.27E-11
WBGene00010206		F57G4.1	8.68	3.17E-11
WBGene00044427		F56A6.5	9.10	4.01E-11
WBGene00015114		B0281.8	8.77	5.10E-11
WBGene00050899		T10C6.15	4.54	6.78E-11
WBGene00202499		B0507.15	8.59	9.21E-11
WBGene00016845		C50F7.5	3.32	1.67E-10
WBGene00015100		B0280.2	4.16	1.74E-10
WBGene00045475		T07A5.7	5.07	3.33E-10
WBGene00015835	math-6	C16C4.11	8.45	3.58E-10
WBGene00022713		ZK355.3	5.76	4.76E-10
WBGene00235133		T26H5.14	3.28	6.19E-10
WBGene00086568		Y2H9A.6	5.26	7.30E-10
WBGene00044787		C54D10.12	4.42	1.09E-09
WBGene00020080	math-37	R52.9	4.53	1.34E-09
WBGene00022189		Y71H2AR.2	8.40	1.48E-09
WBGene00020774		T24E12.5	3.01	1.72E-09
WBGene00015108		B0281.1	8.48	1.89E-09
WBGene00009077		F23B2.10	6.14	1.96E-09
WBGene00194902		Y39H10B.3	5.11	2.00E-09
WBGene00015226		B0507.9	5.38	2.69E-09
WBGene00008858		F15D3.8	3.98	3.67E-09
WBGene00044416		C08E3.15	7.97	3.67E-09
WBGene00020637	fbxa-7	T20H9.1	6.15	3.94E-09
WBGene00044207		Y6G8.5	4.97	4.50E-09
WBGene00021179	fbxa-86	Y9C9A.12	5.56	9.77E-09
WBGene00015829	math-15	C16C4.5	3.99	1.10E-08
WBGene00015216	valv-1	B0496.7	3.10	1.28E-08
WBGene00007694		C23H4.6	3.34	1.67E-08
WBGene00007153	clec-41	B0365.6	2.73	1.69E-08
WBGene00194951		Y105C5A.1274	7.88	2.00E-08
WBGene00206359		C05E4.15	6.50	2.25E-08
WBGene00016164		C27D9.2	3.76	2.46E-08
WBGene00012267		W04G5.7	7.61	2.87E-08
WBGene00044278		F09C6.14	5.26	3.04E-08
WBGene00020364		T08E11.8	4.68	3.90E-08
WBGene00022674		ZK177.9	5.59	7.77E-08
WBGene00044379		F40H7.12	8.41	7.78E-08
WBGene00045457		F33H12.7	5.52	9.13E-08
WBGene00235311		Y57E12B.11	6.45	9.73E-08
WBGene00011957		T23F11.6	3.59	9.82E-08
WBGene00012961		Y47H10A.5	3.39	1.08E-07
WBGene00010764		K10H10.9	7.52	1.15E-07
WBGene00008602	oac-14	F09B9.1	3.13	2.02E-07
WBGene00017422		F13C5.1	3.24	2.41E-07
WBGene00012621		Y38H6C.8	5.52	2.63E-07
WBGene00015839	math-10	C16C4.15	4.01	5.85E-07
WBGene00015224		B0507.7	4.72	6.90E-07
WBGene00022375		Y94H6A.2	3.64	8.25E-07
WBGene00011539	fbxa-135	T06E6.5	4.78	8.77E-07

WBGene00015491		C05E4.12	5.15	1.25E-06
WBGene00007297		C04F12.1	2.83	1.52E-06
WBGene00015537		C06E4.8	4.05	1.76E-06
WBGene00008988		F20G2.5	3.45	3.09E-06
WBGene00004093	ppw-1	C18E3.7	2.08	3.23E-06
WBGene00019314		K02E7.10	3.54	3.26E-06
WBGene00004810	skr-4	Y60A3A.18	2.48	3.27E-06
WBGene00045501		Y6G8.8	6.93	4.01E-06
WBGene00007932	zip-5	C34D1.5	2.95	4.56E-06
WBGene00009675		F44A6.3	6.92	5.31E-06
WBGene00020859	bath-25	T27C10.4	4.73	5.38E-06
WBGene00010686		K08F9.3	3.59	6.06E-06
WBGene00001577	gem-4	T12A7.1	2.64	6.81E-06
WBGene00013637		Y105C5A.13	2.82	6.86E-06
WBGene00008850		F15B9.6	3.25	6.97E-06
WBGene00012399		Y6E2A.5	3.81	7.02E-06
WBGene00044215		ZK228.10	6.95	7.95E-06
WBGene00195240		Y39B6A.69	5.73	8.65E-06
WBGene00015600	fbxa-165	C08E3.8	4.68	8.70E-06
WBGene00022869		ZK1240.4	7.22	8.81E-06
WBGene00020220	clec-140	T05A7.2	4.04	9.06E-06
WBGene00004811	skr-5	F47H4.10	3.86	1.13E-05
WBGene00017839		F26G1.3	4.65	1.31E-05
WBGene00235298		F19B6.10	5.28	1.40E-05
WBGene00009839	fbxa-188	F47H4.8	3.43	1.63E-05
WBGene00017705		F22E5.6	3.87	1.67E-05
WBGene00202514		T09F5.20	5.58	2.22E-05
WBGene00045412		C25F9.12	2.69	2.33E-05
WBGene00012822		Y43F8B.12	3.39	2.42E-05
WBGene00012400		Y6E2A.7	4.68	2.44E-05
WBGene00010125	dod-22	F55G11.5	2.02	2.48E-05
WBGene00000841	cul-6	K08E7.7	2.75	2.67E-05
WBGene00045411		C25F9.11	3.19	3.37E-05
WBGene00194803		C25F9.16	4.39	3.90E-05
WBGene00044687		B0563.9	4.62	4.96E-05
WBGene00011979		T24B8.5	3.18	5.57E-05
WBGene00016300		C32B5.7	4.54	6.34E-05
WBGene00012381		Y2H9A.4	2.40	7.26E-05
WBGene00005657	srr-6	C13D9.1	2.92	8.78E-05
WBGene00020760		T24C4.4	2.72	9.10E-05
WBGene00003734	nhx-6	F58E1.6	4.06	1.01E-04
WBGene00021121		W09G12.7	1.96	1.05E-04
WBGene00022419		Y102A11A.9	2.82	1.20E-04
WBGene00017530		F16H11.1	2.66	1.34E-04
WBGene00006467	magu-2	C01B7.4	2.19	1.43E-04
WBGene00007180		B0457.6	1.94	1.54E-04
WBGene00008843	arrd-9	F15A4.9	3.85	1.71E-04
WBGene00206393		W04G5.16	6.46	1.84E-04
WBGene00016788		C49G7.10	2.69	1.85E-04
WBGene00010658		K08D8.4	2.29	1.92E-04
WBGene00008102		C44H9.7	4.07	1.94E-04
WBGene00019349	trx-5	K02H11.6	4.90	2.29E-04
WBGene00195177		Y43F8B.25	3.74	2.38E-04

WBGene00019057		F58F9.3	2.16	2.53E-04
WBGene00022181	pho-9	Y71H2AM.16	2.10	3.21E-04
WBGene00007506		C10C5.2	2.97	4.13E-04
WBGene00044280		W02A2.9	1.83	4.55E-04
WBGene00016785		C49G7.7	4.86	5.22E-04
WBGene00011168		R09D1.12	3.46	5.23E-04
WBGene00004400	rom-1	F26F4.3	1.96	5.91E-04
WBGene00017467		F14F9.4	2.08	6.00E-04
WBGene00012067	sqst-3	T26H2.5	4.90	7.64E-04
WBGene00044206		T26H5.9	2.07	7.72E-04
WBGene00015048	faah-2	B0218.2	1.88	7.80E-04
WBGene00020221		T05A7.3	3.36	7.99E-04
WBGene00005188	srg-31	T07H8.5	2.74	8.29E-04
WBGene00045515		ZK1037.13	6.04	8.85E-04
WBGene00022013		Y60C6A.1	3.69	8.86E-04
WBGene00045338		M01B2.13	3.41	9.27E-04
WBGene00022546		ZC196.2	3.25	9.67E-04
WBGene00004402	rom-3	Y116A8C.14	3.79	1.13E-03
WBGene00015603	fbxa-159	C08E3.11	4.65	1.14E-03
WBGene00016763		C49A9.9	2.14	1.16E-03
WBGene00018102	math-28	F36H5.3	3.42	1.26E-03
WBGene00077546		C01A2.9	3.95	1.26E-03
WBGene00008584		F08G5.6	1.75	1.33E-03
WBGene00045416		Y37H2A.14	2.29	1.57E-03
WBGene00008847	fbxb-102	F15A4.13	3.90	2.21E-03
WBGene00194713		F19B10.13	4.13	2.21E-03
WBGene00018614		F48G7.2	2.95	2.23E-03
WBGene00017660		F21C10.11	1.57	2.33E-03
WBGene00018209		F39G3.4	3.56	2.44E-03
WBGene00021977		Y58A7A.3	1.68	2.45E-03
WBGene00000503	cht-1	C04F6.3	2.22	2.54E-03
WBGene00008679		F11A5.13	4.52	2.55E-03
WBGene00016678		C45G9.7	2.14	2.59E-03
WBGene00021072		W07B8.4	2.37	3.08E-03
WBGene00020358	math-39	T08E11.2	1.75	3.78E-03
WBGene00013837	fbxa-30	ZC47.4	3.30	3.86E-03
WBGene00008726		F13A7.11	2.67	3.93E-03
WBGene00020579		T19D12.4	2.33	4.18E-03
WBGene00195084		C43C3.4	2.56	4.57E-03
WBGene00008099		C44H9.4	2.41	4.81E-03
WBGene00008617		F09C6.3	3.83	4.87E-03
WBGene00008066	fbxb-65	C43D7.2	4.20	5.22E-03
WBGene00014057		ZK673.1	-2.24	5.30E-03
WBGene00005833	srw-86	C25F9.7	2.32	5.33E-03
WBGene00006894	ver-1	T17A3.1	3.29	5.77E-03
WBGene00008900		F16H6.10	2.22	5.85E-03
WBGene00012910		Y46G5A.20	2.21	6.13E-03
WBGene00021978		Y58A7A.4	2.80	6.19E-03
WBGene00006628	tsp-2	C02F5.11	2.99	6.36E-03
WBGene00017364	clec-7	F10G2.3	-3.75	6.38E-03
WBGene00194926		K08D8.12	2.38	6.38E-03
WBGene00015593		C08E3.1	2.93	6.63E-03
WBGene00011052	arrd-11	R06B9.1	2.57	7.75E-03

WBGene00001399	fat-7	F10D2.9	-2.74	8.44E-03
WBGene00020758		T24C4.2	2.99	8.56E-03
WBGene00003995	pgp-1	K08E7.9	-1.75	8.81E-03
WBGene00022570	sdz-35	ZC239.12	3.76	9.27E-03
WBGene00015605		C08E3.13	1.98	9.28E-03
WBGene00021236	pud-1.2	Y19D10B.7	-1.76	9.62E-03
WBGene00007133		B0284.3	2.21	9.69E-03
WBGene00016156		C27A12.6	1.58	9.87E-03
WBGene00017308		F09F9.3	2.90	1.04E-02
WBGene00044442		T08A9.13	2.56	1.06E-02
WBGene00194708		Y36E3A.2	2.61	1.06E-02
WBGene00014136		ZK896.5	1.48	1.20E-02
WBGene00006649	tth-1	F08F1.8	1.53	1.24E-02
WBGene00009130		F25H5.8	1.77	1.24E-02
WBGene00010508		K02E2.7	3.34	1.28E-02
WBGene00011130	zip-6	R07H5.10	2.64	1.28E-02
WBGene00008593	clec-227	F08H9.5	-2.24	1.30E-02
WBGene00044107		F58G6.9	-1.48	1.30E-02
WBGene00007440		C08E8.4	1.93	1.37E-02
WBGene00009897	skpo-1	F49E12.1	2.52	1.46E-02
WBGene00021654		Y47G7B.2	2.39	1.46E-02
WBGene00000136	amt-4	C05E11.5	-1.80	1.47E-02
WBGene00020613		T20D4.7	2.62	1.47E-02
WBGene00010977		R02D5.3	1.86	1.53E-02
WBGene00016783	irg-2	C49G7.5	2.89	1.58E-02
WBGene00004509	rrf-2	M01G12.12	1.65	1.60E-02
WBGene00007216		C01A2.4	1.66	1.71E-02
WBGene00022479	fbxa-36	Y119D3A.4	3.09	1.71E-02
WBGene00019495	sdz-24	K07E8.3	-1.50	1.82E-02
WBGene00009626		F42A8.1	1.35	1.88E-02
WBGene00008793		F14D7.6	-1.51	1.94E-02
WBGene00235315		Y50D4A.10	1.56	2.09E-02
WBGene00017445	sid-5	F14B8.2	1.53	2.10E-02
WBGene00010004		F53H2.1	2.51	2.12E-02
WBGene00011945	hpo-24	T23D8.7	1.79	2.40E-02
WBGene00013073	hmit-1.1	Y51A2D.4	-1.42	2.50E-02
WBGene00007772	egrh-1	C27C12.2	1.53	2.50E-02
WBGene00019368		K03H6.2	-1.58	2.54E-02
WBGene00018385		F43C11.8	3.37	2.54E-02
WBGene00050939		C05G5.7	-3.03	2.64E-02
WBGene00018643	drd-50	F49F1.1	3.25	2.75E-02
WBGene00019166	tat-2	H06H21.10	1.46	2.84E-02
WBGene00008129		C47A10.12	-2.99	2.84E-02
WBGene00019564		K09D9.1	2.49	2.86E-02
WBGene00000777	cpn-1	F43G9.9	1.40	3.14E-02
WBGene00009488	oac-20	F36G9.12	2.09	3.28E-02
WBGene00010752		K10G4.3	2.02	3.39E-02
WBGene00017398	slc-17.6	F12B6.2	-1.43	3.41E-02
WBGene00008560	pho-13	F07H5.9	-1.53	3.44E-02
WBGene00009146		F26C11.1	-1.89	3.44E-02
WBGene00006987	zmp-1	EGAP1.3	1.54	3.61E-02
WBGene00011850		T20B3.1	-1.68	3.62E-02
WBGene00044707		F22G12.7	2.23	4.00E-02

WBGene00017498	pud-4	F15E11.12	-1.44	4.04E-02
WBGene00018707	oac-31	F52F10.3	1.86	4.12E-02
WBGene00006986	zip-1	Y75B8A.35	1.35	4.18E-02
WBGene00008567		F08A8.4	-1.47	4.18E-02
WBGene00009957		F53B2.8	1.90	4.44E-02
WBGene00010034		F54B11.11	-1.70	4.50E-02
WBGene00012323	oac-54	W07A12.6	2.16	4.60E-02
WBGene00016555	math-20	C40D2.2	1.65	4.69E-02
WBGene00014194		ZK1037.6	2.56	4.71E-02
WBGene00019699	math-34	M01D1.2	2.66	4.75E-02
WBGene00022548		ZC196.4	2.30	4.95E-02

Supplementary Table 2.3. List of differentially expressed genes upon pathogen infections (*N. parisii* [N2]) identified by edgeR.

WormBase Gene ID	Gene Name	Sequence Name	Fold-change (log2)	FDR
WBGene00009169		F26F2.4	10.56	1.18E-240
WBGene00009168		F26F2.3	10.75	4.88E-229
WBGene00219309		F26F2.13	9.18	3.19E-163
WBGene00195165		F57G4.11	10.49	1.92E-147
WBGene00044708		*C17H1*.13	9.75	2.30E-137
WBGene00009170		F26F2.5	10.11	4.67E-132
WBGene00018345		F42C5.3	7.75	2.42E-127
WBGene00007662		*C17H1*.9	8.34	3.40E-106
WBGene00009061		F22G12.1	6.82	8.94E-104
WBGene00009166		F26F2.1	8.09	1.09E-102
WBGene00007659		*C17H1*.6	7.94	2.27E-95
WBGene00045401	eol-1	T26F2.3	6.46	2.53E-92
WBGene00007661		*C17H1*.8	9.87	3.36E-90
WBGene00008067		C43D7.4	11.29	1.07E-88
WBGene00008068	sdz-6	C43D7.5	7.37	1.01E-84
WBGene00138721		C54D10.14	6.20	1.76E-83
WBGene00007134		B0284.4	9.81	1.69E-82
WBGene00012091		T27E7.6	8.91	1.70E-80
WBGene00008301	dct-3	C54D10.7	6.11	1.15E-78
WBGene00007660		*C17H1*.7	5.09	1.10E-75
WBGene00012726		Y39G8B.5	7.54	1.15E-65
WBGene00007656		*C17H1*.3	7.59	7.95E-63
WBGene00044014		F15H10.10	10.85	4.45E-62
WBGene00015834	math-5	C16C4.10	8.37	1.34E-58
WBGene00194646		Y105C5A.1270	5.01	1.29E-48
WBGene00008302		C54D10.8	5.78	2.63E-48
WBGene00020357		T08E11.1	6.24	3.31E-48
WBGene00015225		B0507.8	7.72	4.65E-47
WBGene00086568		Y2H9A.6	5.70	2.32E-46
WBGene00008267		C53A5.9	6.30	8.09E-46
WBGene00008069		C43D7.7	9.83	2.17E-44
WBGene00021081		W08A12.4	6.44	8.29E-43
WBGene00194645		Y105C5A.1269	6.95	2.84E-42
WBGene00012910		Y46G5A.20	2.96	1.38E-40
WBGene00044709		*C17H1*.14	7.36	2.10E-38
WBGene00007655		*C17H1*.2	8.03	1.11E-37
WBGene00005188	srg-31	T07H8.5	4.19	1.18E-37
WBGene00022476	fbxa-75	Y119D3A.1	9.93	3.39E-37

WBGene00023483		Y75B8A.39	5.22	6.49E-36
WBGene00015835	math-6	C16C4.11	7.89	7.33E-34
WBGene00015227		B0507.10	7.38	7.27E-31
WBGene00017214		F07E5.9	8.42	1.56E-30
WBGene00195181		F15H10.12	8.06	2.06E-30
WBGene00044237		*C17H1*.10	5.12	6.46E-30
WBGene00022375		Y94H6A.2	4.85	1.34E-29
WBGene00008269		C53A5.11	6.02	1.37E-29
WBGene00018353	fbxa-182	F42G2.4	3.19	3.22E-29
WBGene00007674		C18D4.4	6.49	4.81E-29
WBGene00022550	droe-8	ZC196.6	5.45	8.77E-29
WBGene00015223		B0507.6	4.60	2.32E-28
WBGene00020228		T05A8.2	9.32	2.51E-28
WBGene00007657		*C17H1*.4	7.09	1.09E-27
WBGene00007132		B0284.2	5.08	9.44E-26
WBGene00010821		M01G12.7	6.32	2.13E-25
WBGene00008872		F15H10.5	7.20	5.34E-25
WBGene00015829	math-15	C16C4.5	3.36	2.06E-23
WBGene00044787		C54D10.12	4.45	7.55E-23
WBGene00015224		B0507.7	5.21	2.86E-22
WBGene00015108		B0281.1	6.22	4.41E-22
WBGene00015833	math-17	C16C4.9	8.60	4.42E-22
WBGene00009908		F49H6.5	5.47	5.79E-22
WBGene00044711		*C17H1*.11	6.04	1.38E-21
WBGene00086560		ZK355.8	4.43	1.77E-21
WBGene00015114		B0281.8	6.44	5.84E-21
WBGene00004149	trpl-5	T16A1.7	5.08	8.84E-21
WBGene00013294		Y57G11B.1	4.47	1.03E-20
WBGene00044427		F56A6.5	6.80	1.52E-20
WBGene00194902		Y39H10B.3	4.43	2.45E-19
WBGene00015828	math-14	C16C4.4	3.62	3.27E-19
WBGene00003098	lys-9	C54C8.6	9.41	1.32E-18
WBGene00012399		Y6E2A.5	3.66	1.85E-18
WBGene00050906		F20E11.17	8.31	2.25E-18
WBGene00044013		F15H10.9	8.76	4.42E-17
WBGene00008858		F15D3.8	3.74	5.88E-16
WBGene00015839	math-10	C16C4.15	3.71	6.25E-16
WBGene00010508		K02E2.7	6.14	6.44E-16
WBGene00010206		F57G4.1	7.90	8.50E-16
WBGene00044707		F22G12.7	3.46	1.36E-15
WBGene00020637	fbxa-7	T20H9.1	7.88	2.39E-15
WBGene00012400		Y6E2A.7	4.87	2.53E-15
WBGene00206359		C05E4.15	5.79	3.52E-15
WBGene00014046	clec-60	ZK666.6	2.17	5.93E-15
WBGene00018614		F48G7.2	4.03	1.03E-14
WBGene00015832	math-16	C16C4.8	7.86	1.20E-14
WBGene00008102		C44H9.7	4.29	5.28E-14
WBGene00011539	fbxa-135	T06E6.5	4.56	8.32E-14
WBGene00012067	sqst-3	T26H2.5	6.02	1.65E-13
WBGene00044207		Y6G8.5	3.87	2.57E-13
WBGene00010004		F53H2.1	4.58	1.49E-12
WBGene00235133		T26H5.14	3.36	2.20E-12
WBGene00020364		T08E11.8	3.39	2.85E-12

WBGene00009895	scl-2	F49E11.10	3.14	3.21E-12
WBGene00009898	dod-23	F49E12.2	3.70	9.84E-12
WBGene00045415		Y43F8B.15	4.08	2.20E-11
WBGene00002055	ifc-1	F37B4.2	1.51	2.37E-11
WBGene00015222		B0507.5	3.98	3.38E-10
WBGene00022869		ZK1240.4	5.22	3.99E-10
WBGene00022713		ZK355.3	5.56	5.53E-10
WBGene00017592		F19C7.2	1.25	5.73E-10
WBGene00020227		T05A8.1	5.50	8.87E-10
WBGene00012381		Y2H9A.4	2.18	1.04E-09
WBGene00004810	skr-4	Y60A3A.18	2.38	1.63E-09
WBGene00011979		T24B8.5	2.54	3.64E-09
WBGene00043062	clec-80	Y54G2A.39	1.06	5.40E-09
WBGene00013634		Y105C5A.9	2.90	6.87E-09
WBGene00016281		C31B8.4	3.52	1.15E-08
WBGene00017199	clec-45	F07C4.2	2.76	1.25E-08
WBGene00020774		T24E12.5	2.03	3.23E-08
WBGene00012267		W04G5.7	7.37	4.09E-08
WBGene00020326	math-38	T07H3.3	2.78	1.10E-07
WBGene00000781	cpr-1	C52E4.1	1.25	1.21E-07
WBGene00045475		T07A5.7	3.03	2.19E-07
WBGene00021193	math-44	Y16E11A.1	2.74	2.38E-07
WBGene00045393		F26D11.13	3.20	3.27E-07
WBGene00007658		*C17H1*.5	5.84	4.13E-07
WBGene00018616		F48G7.5	1.44	4.53E-07
WBGene00004172	pqn-92	Y75B8A.27	1.11	5.65E-07
WBGene00012456		Y17D7C.2	2.61	8.51E-07
WBGene00022547		ZC196.3	3.47	8.51E-07
WBGene00007297		C04F12.1	1.58	1.04E-06
WBGene00020080	math-37	R52.9	2.61	1.35E-06
WBGene00015602	fbxa-158	C08E3.10	3.03	1.42E-06
WBGene00019699	math-34	M01D1.2	2.72	2.08E-06
WBGene00019314		K02E7.10	2.00	2.55E-06
WBGene00012323	oac-54	W07A12.6	2.58	2.78E-06
WBGene00219750		Y38C1AA.19	4.06	5.27E-06
WBGene00021873	clec-82	Y54G2A.8	1.62	7.00E-06
WBGene00195177		Y43F8B.25	2.86	7.27E-06
WBGene00008507		F01G10.4	4.86	8.22E-06
WBGene00012398		Y6E2A.4	2.79	1.06E-05
WBGene00019931		R07C12.1	1.37	1.33E-05
WBGene00050899		T10C6.15	2.26	1.59E-05
WBGene00008850		F15B9.6	1.97	2.10E-05
WBGene00194713		F19B10.13	2.96	2.12E-05
WBGene00001594	glc-4	C27H5.8	1.06	2.25E-05
WBGene00022548		ZC196.4	1.85	2.79E-05
WBGene00020361	fbxc-19	T08E11.5	3.47	3.06E-05
WBGene00219493		C18D4.12	3.00	4.71E-05
WBGene00012583	clec-4	Y38E10A.5	0.89	4.83E-05
WBGene00011330		T01D3.6	1.02	5.97E-05
WBGene00022546		ZC196.2	2.67	7.17E-05
WBGene00021179	fbxa-86	Y9C9A.12	2.98	9.20E-05
WBGene00012822		Y43F8B.12	2.85	9.58E-05
WBGene00015226		B0507.9	2.57	1.46E-04

WBGene00015537		C06E4.8	2.30	1.49E-04
WBGene00016920		C54E4.5	0.92	1.49E-04
WBGene00022870		ZK1240.5	1.54	1.51E-04
WBGene00012093		T27E7.9	3.09	1.73E-04
WBGene00015600	fbxa-165	C08E3.8	3.88	2.54E-04
WBGene00009839	fbxa-188	F47H4.8	1.94	2.74E-04
WBGene00015605		C08E3.13	2.11	2.89E-04
WBGene00022497		Y119D3B.21	0.88	3.08E-04
WBGene00019279		K01A2.4	1.62	3.26E-04
WBGene00015403	clec-10	C03H5.1	0.70	4.43E-04
WBGene00077546		C01A2.9	3.17	5.00E-04
WBGene00219216		K08D10.14	0.97	5.85E-04
WBGene00015052	clec-52	B0218.8	-0.91	6.50E-04
WBGene00004402	rom-3	Y116A8C.14	3.04	6.62E-04
WBGene00009518	clec-166	F38A1.5	1.02	7.32E-04
WBGene00003091	lys-2	Y22F5A.5	0.69	1.24E-03
WBGene00044416		C08E3.15	5.36	1.51E-03
WBGene00006986	zip-1	Y75B8A.35	0.83	1.91E-03
WBGene00022549		ZC196.5	1.07	2.08E-03
WBGene00000391	cdd-1	C47D2.2	0.69	3.18E-03
WBGene00010062	lipl-1	F54F3.3	1.63	3.18E-03
WBGene00000015	abf-4	Y38H6C.22	2.86	3.49E-03
WBGene00011168		R09D1.12	2.05	3.76E-03
WBGene00019590		K09F6.7	0.94	4.20E-03
WBGene00013149		Y53F4B.1	1.22	4.29E-03
WBGene00018724		F53A9.1	0.92	4.34E-03
WBGene00044644		B0205.13	-0.76	4.78E-03
WBGene00021495	sqst-2	Y40C5A.1	0.65	5.03E-03
WBGene00044278		F09C6.14	4.97	5.59E-03
WBGene00022189		Y71H2AR.2	2.95	6.65E-03
WBGene00013608	clec-35	Y102A5B.2	2.34	7.41E-03
WBGene00002260	lbp-8	T22G5.6	-1.47	1.01E-02
WBGene00004093	ppw-1	C18E3.7	0.77	1.06E-02
WBGene00010957	nduo-6	MTCE.3	-1.08	1.24E-02
WBGene00009397	clec-66	F35C5.9	0.67	1.34E-02
WBGene00018354		F42G2.5	1.24	1.72E-02
WBGene00017498	pud-4	F15E11.12	-2.24	1.74E-02
WBGene00044206		T26H5.9	1.28	1.94E-02
WBGene00008099		C44H9.4	1.02	2.07E-02
WBGene00015100		B0280.2	1.17	2.22E-02
WBGene00017501	pud-3	F15E11.15	-1.48	2.72E-02
WBGene00001758	gst-10	Y45G12C.2	-0.63	2.97E-02
WBGene00018645		F49F1.5	1.06	3.02E-02
WBGene00003474	mtl-2	T08G5.10	-0.53	3.17E-02
WBGene00016190		C28G1.5	0.58	3.28E-02
WBGene00018346		F42C5.4	0.89	3.43E-02
WBGene00011162	chil-19	R09D1.6	2.16	3.49E-02
WBGene00010958	ndfl-4	MTCE.4	-1.55	3.65E-02
WBGene00194815		Y43F8B.23	1.52	3.76E-02
WBGene00007393	fbxa-157	C06H5.2	1.32	3.78E-02
WBGene00021236	pud-1.2	Y19D10B.7	-2.83	4.55E-02
WBGene00017660		F21C10.11	-1.09	4.93E-02

Supplementary Table 2.4. List of differentially expressed genes upon pathogen infections (Santeuil [JU1264]) identified by edgeR.

WormBase Gene ID	Gene Name	Sequence Name	Fold-change (log2)	FDR
WBGene00028845		CBG06596	7.86	8.53E-61
WBGene00041842		CBG23492	7.17	5.69E-60
WBGene00089186		CBG27772	6.21	3.00E-57
WBGene00031866		CBG10486	5.91	3.74E-48
WBGene00037928		CBG18528	5.35	9.30E-43
WBGene00029310		CBG07170	7.56	5.63E-38
WBGene00028206		CBG05828	4.64	1.60E-37
WBGene00026106		CBG03198	6.34	2.06E-35
WBGene00041841		CBG23491	5.61	3.61E-35
WBGene00041840		CBG23490	5.47	1.39E-34
WBGene00037931		CBG18531	4.90	2.03E-33
WBGene00037930		CBG18530	3.44	2.71E-31
WBGene00037932		CBG18532	5.33	5.45E-29
WBGene00032649		CBG11544	4.12	3.53E-28
WBGene00087844		CBG26430	5.29	5.45E-28
WBGene00028205		CBG05827	4.72	3.29E-26
WBGene00032650		CBG11545	3.49	1.58E-25
WBGene00037934		CBG18534	9.06	1.82E-25
WBGene00027499		CBG04907	7.01	1.09E-24
WBGene00086653		CBG25239	5.91	1.09E-24
WBGene00035660		CBG15363	4.36	1.18E-24
WBGene00032014		CBG10726	4.84	2.54E-22
WBGene00031174		CBG09614	4.46	2.73E-21
WBGene00028731		CBG06463	3.86	3.62E-21
WBGene00036071		CBG15988	5.57	5.89E-21
WBGene00037927		CBG18525	3.98	1.32E-20
WBGene00042629		CBG24541	3.26	4.35E-20
WBGene00037933		CBG18533	7.17	7.09E-20
WBGene00035828		CBG15672	4.58	9.54E-20
WBGene00027935		CBG05495	2.78	2.36E-18
WBGene00032552		CBG11438	2.46	3.17E-18
WBGene00088570		CBG27156	3.46	6.31E-18
WBGene00033706		CBG12815	2.62	4.59E-17
WBGene00027926		CBG05483	3.75	9.20E-17
WBGene00030380		CBG08621	3.24	1.52E-16
WBGene00088338		CBG26924	5.60	1.87E-16
WBGene00040743		CBG22119	3.01	2.57E-16
WBGene00088730		CBG27316	5.60	2.79E-16
WBGene00041839	Cbr-srh-25	CBG23489	2.83	1.67E-15
WBGene00041311		CBG22848	2.55	2.95E-14
WBGene00088335		CBG26921	3.85	3.51E-14
WBGene00032537		CBG11421	2.57	4.48E-14
WBGene00042683		CBG24613	2.81	5.34E-14
WBGene00041674	Cbr-zip-10	CBG23294	3.00	9.93E-14
WBGene00030420		CBG08668	2.90	7.46E-13
WBGene00026981		CBG04270	4.32	1.01E-12
WBGene00030571		CBG08850	3.10	1.43E-12
WBGene00037926		CBG18524	2.55	3.20E-12
WBGene00032652		CBG11547	3.56	4.76E-12
WBGene00088080		CBG26666	5.02	8.71E-12

WBGene00028187		CBG05803	3.02	1.44E-11
WBGene00087805		CBG26391	5.98	1.74E-11
WBGene00038448		CBG19185	1.73	1.74E-11
WBGene00037923		CBG18520	2.29	3.65E-11
WBGene00038447		CBG19184	1.83	2.10E-10
WBGene00027326		CBG04704	3.02	2.56E-10
WBGene00088195		CBG26781	7.57	3.94E-10
WBGene00032269		CBG11083	3.29	4.05E-10
WBGene00027247		CBG04608	2.82	6.82E-10
WBGene00042151		CBG23914	2.69	1.60E-09
WBGene00042618		CBG24526	2.17	4.17E-09
WBGene00031430		CBG09931	7.34	7.31E-09
WBGene00031885		CBG10508	2.51	9.31E-09
WBGene00027234		CBG04592	3.23	1.15E-08
WBGene00088117		CBG26703	3.13	1.57E-08
WBGene00025146		CBG01998	1.96	2.95E-08
WBGene00087576		CBG26162	2.82	3.51E-08
WBGene00031046		CBG09474	2.14	6.07E-08
WBGene00031199		CBG09641	2.40	1.00E-07
WBGene00037165		CBG17574	2.47	1.07E-07
WBGene00039731		CBG20816	1.72	1.09E-07
WBGene00037935		CBG18535	8.33	1.15E-07
WBGene00026027	Cbr-clec-60	CBG03095	1.73	1.15E-07
WBGene00028943		CBG06703	1.91	1.61E-07
WBGene00037917		CBG18514	2.29	1.62E-07
WBGene00026203	Cbr-thn-1	CBG03322	1.92	1.95E-07
WBGene00031286	Cbr-nhr-6	CBG09761	1.99	2.42E-07
WBGene00031718		CBG10309	2.08	3.03E-07
WBGene00034684	Cbr-crn-4	CBG14049	1.66	3.66E-07
WBGene00038787		CBG19600	4.95	4.70E-07
WBGene00031719		CBG10310	1.87	9.69E-07
WBGene00031134	Cbr-lys-2	CBG09573	2.45	1.18E-06
WBGene00033703		CBG12812	1.86	1.18E-06
WBGene00027239		CBG04600	1.77	1.41E-06
WBGene00029282		CBG07133	4.32	1.64E-06
WBGene00042153		CBG23916	1.71	1.90E-06
WBGene00024645		CBG01398	1.68	2.21E-06
WBGene00037852		CBG18425	2.11	2.82E-06
WBGene00031720		CBG10311	1.88	3.03E-06
WBGene00030189	Cbr-ugt-44	CBG08389	1.77	3.26E-06
WBGene00034025		CBG13234	1.42	3.59E-06
WBGene00038055		CBG18678	2.48	3.65E-06
WBGene00036431		CBG16518	1.60	3.69E-06
WBGene00039962		CBG21098	-1.27	6.34E-06
WBGene00032201	Cbr-clec-86	CBG10980	1.75	6.97E-06
WBGene00031750		CBG10347	2.35	8.27E-06
WBGene00038716		CBG19512	1.89	8.27E-06
WBGene00041843		CBG23493	1.77	8.69E-06
WBGene00027233		CBG04591	2.57	1.35E-05
WBGene00087737		CBG26323	1.80	1.36E-05
WBGene00040234		CBG21474	1.96	2.65E-05
WBGene00038450		CBG19187	1.46	3.11E-05
WBGene00088490		CBG27076	2.33	3.21E-05

WBGene00041343		CBG22882	2.31	3.38E-05
WBGene00032015		CBG10727	2.29	3.40E-05
WBGene00030434		CBG08684	1.69	3.79E-05
WBGene00030568		CBG08847	2.11	4.35E-05
WBGene00032171	Cbr-sid-5	CBG10928	1.47	4.35E-05
WBGene00036824		CBG17057	1.62	4.81E-05
WBGene00088044		CBG26630	4.61	4.81E-05
WBGene00038449		CBG19186	1.55	5.03E-05
WBGene00033498	Cbr-col-56	CBG12567	2.25	5.18E-05
WBGene00042110		CBG23863	2.08	6.01E-05
WBGene00087765		CBG26351	1.89	6.40E-05
WBGene00024109		CBG00772	1.59	7.92E-05
WBGene00087767		CBG26353	1.40	8.11E-05
WBGene00031852		CBG10468	2.09	9.31E-05
WBGene00031865		CBG10483	1.46	9.48E-05
WBGene00035702		CBG15433	2.73	1.06E-04
WBGene00031687		CBG10269	1.47	1.07E-04
WBGene00041151		CBG22628	1.69	1.51E-04
WBGene00038823		CBG19643	1.63	1.61E-04
WBGene00035449		CBG15117	2.71	2.15E-04
WBGene00024304	Cbr-egl-43	CBG01007	1.36	2.49E-04
WBGene00026507		CBG03703	1.25	2.72E-04
WBGene00029346		CBG07223	2.15	2.76E-04
WBGene00031666		CBG10235	1.23	2.76E-04
WBGene00042529		CBG24409	2.54	2.76E-04
WBGene00040475		CBG21784	1.86	3.30E-04
WBGene00042152		CBG23915	2.22	3.30E-04
WBGene00030567		CBG08846	1.82	3.60E-04
WBGene00038786		CBG19599	1.22	3.60E-04
WBGene00038268		CBG18976	1.23	3.70E-04
WBGene00039730		CBG20815	1.28	3.75E-04
WBGene00034762		CBG14185	-1.25	3.90E-04
WBGene00087233		CBG25819	1.37	4.34E-04
WBGene00039801		CBG20896	1.53	4.49E-04
WBGene00087954		CBG26540	1.75	4.93E-04
WBGene00030443	Cbr-ugt-51	CBG08694	-1.62	4.95E-04
WBGene00041345		CBG22884	2.82	5.15E-04
WBGene00029294		CBG07148	2.05	5.32E-04
WBGene00036802		CBG17027	-1.83	5.33E-04
WBGene00040076		CBG21241	3.21	6.34E-04
WBGene00027285		CBG04653	2.72	8.16E-04
WBGene00026814	Cbr-rnt-1	CBG04068	1.27	8.76E-04
WBGene00028142		CBG05751	1.29	9.67E-04
WBGene00034424		CBG13699	1.31	9.83E-04
WBGene00036592		CBG16727	1.36	1.05E-03
WBGene00024012	Cbr-dod-23	CBG00648	1.89	1.06E-03
WBGene00035690		CBG15413	1.33	1.24E-03
WBGene00089048		CBG27634	1.19	1.29E-03
WBGene00040031		CBG21186	-1.19	1.38E-03
WBGene00087810		CBG26396	1.40	1.69E-03
WBGene00024644		CBG01397	1.26	1.75E-03
WBGene00042393		CBG24231	1.13	1.90E-03
WBGene00032390	Cbr-tsp-10	CBG11232	-1.65	1.97E-03

WBGene00038211	Cbr-fil-2	CBG18901	1.70	2.02E-03
WBGene00024424		CBG01149	-1.19	2.04E-03
WBGene00031699		CBG10283	2.79	2.12E-03
WBGene00026980		CBG04269	1.38	2.28E-03
WBGene00030996	Cbr-zip-5	CBG09406	1.20	2.28E-03
WBGene00034760		CBG14183	-1.56	2.34E-03
WBGene00029305		CBG07164	2.32	2.36E-03
WBGene00031014	Cbr-clec-41	CBG09432	1.08	2.36E-03
WBGene00031133	Cbr-lys-1	CBG09572	1.19	2.36E-03
WBGene00027938		CBG05498	1.09	2.39E-03
WBGene00042154		CBG23917	1.13	2.73E-03
WBGene00042557		CBG24449	1.27	2.99E-03
WBGene00033962		CBG13153	1.14	3.11E-03
WBGene00039297		CBG20274	3.43	3.11E-03
WBGene00034396		CBG13659	-1.83	3.38E-03
WBGene00028442	Cbr-lys-10	CBG06112	-1.19	3.39E-03
WBGene00034314		CBG13564	1.20	3.76E-03
WBGene00027936		CBG05496	1.17	4.42E-03
WBGene00031840		CBG10453	-1.22	4.51E-03
WBGene00034325		CBG13577	-2.30	4.65E-03
WBGene00089049		CBG27635	2.38	4.65E-03
WBGene00088644		CBG27230	2.07	5.33E-03
WBGene00038267		CBG18975	1.19	5.74E-03
WBGene00032554		CBG11440	1.41	5.84E-03
WBGene00032734		CBG11640	1.74	6.09E-03
WBGene00034204		CBG13437	1.30	6.12E-03
WBGene00027240		CBG04601	-1.01	6.13E-03
WBGene00023702		CBG00290	1.03	6.45E-03
WBGene00034397		CBG13660	-1.70	6.65E-03
WBGene00040550		CBG21872	1.73	6.65E-03
WBGene00041518		CBG23101	1.38	6.65E-03
WBGene00038677		CBG19468	0.92	7.75E-03
WBGene00034814		CBG14250	1.53	8.40E-03
WBGene00038302	Cbr-srh-30	CBG19015	-1.59	8.63E-03
WBGene00032523	Cbr-aip-1	CBG11407	1.02	8.63E-03
WBGene00023854		CBG00473	0.98	8.74E-03
WBGene00027112	Cbr-fmo-2	CBG04445	-2.96	8.98E-03
WBGene00088337		CBG26923	3.14	9.10E-03
WBGene00038715		CBG19511	1.35	9.16E-03
WBGene00031696		CBG10279	1.28	9.99E-03
WBGene00089523		CBG28109	1.10	1.02E-02
WBGene00089232		CBG27818	-1.15	1.03E-02
WBGene00089257		CBG27843	-1.18	1.03E-02
WBGene00026112		CBG03210	0.95	1.04E-02
WBGene00024899		CBG01704	-2.81	1.07E-02
WBGene00038689		CBG19480	1.74	1.11E-02
WBGene00035681		CBG15402	1.10	1.12E-02
WBGene00041721		CBG23351	-1.13	1.45E-02
WBGene00025479		CBG02420	1.26	1.48E-02
WBGene00029304		CBG07163	1.43	1.55E-02
WBGene00028186		CBG05802	0.98	1.58E-02
WBGene00034719	Cbr-clec-265	CBG14118	0.88	1.58E-02
WBGene00036484	Cbr-odd-1	CBG16590	1.96	1.58E-02

WBGene00033785		CBG12921	-0.90	1.61E-02
WBGene00031068		CBG09500	-0.90	1.69E-02
WBGene00033160		CBG12168	1.54	1.75E-02
WBGene00027937		CBG05497	0.90	1.77E-02
WBGene00038243	Cbr-lec-10	CBG18946	0.97	1.77E-02
WBGene00031839		CBG10452	-1.27	1.79E-02
WBGene00032855		CBG11786	0.88	1.81E-02
WBGene00031139		CBG09578	0.86	2.00E-02
WBGene00027246		CBG04607	2.60	2.01E-02
WBGene00031006		CBG09423	-1.20	2.11E-02
WBGene00032190		CBG10961	1.49	2.19E-02
WBGene00024847	Cbr-trpl-5	CBG01638	1.80	2.31E-02
WBGene00042446	Cbr-srv-36	CBG24302	1.42	2.43E-02
WBGene00042842		CBG24810	1.81	2.43E-02
WBGene00042155		CBG23918	0.81	2.58E-02
WBGene00042510		CBG24384	1.27	2.58E-02
WBGene00028840		CBG06590	1.40	2.62E-02
WBGene00038518		CBG19264	-0.85	2.62E-02
WBGene00026258	Cbr-ech-9	CBG03388	1.40	2.65E-02
WBGene00032570		CBG11458	-0.90	2.77E-02
WBGene00031158	Cbr-ugt-33	CBG09597	-0.93	2.84E-02
WBGene00042997		CBG25024	0.99	2.88E-02
WBGene00024846	Cbr-trpl-2	CBG01637	1.10	2.94E-02
WBGene00023858		CBG00478	1.06	3.12E-02
WBGene00024530	Cbr-cyp-34A4	CBG01268	-1.59	3.18E-02
WBGene00036233		CBG16219	-1.23	3.18E-02
WBGene00035992	Cbr-cyp-37B1	CBG15879	-2.38	3.21E-02
WBGene00042579		CBG24479	1.40	3.26E-02
WBGene00028081	Cbr-ddo-1	CBG05678	-0.93	3.29E-02
WBGene00034022		CBG13231	1.95	3.29E-02
WBGene00037430		CBG17920	1.26	3.39E-02
WBGene00086739		CBG25325	1.38	3.51E-02
WBGene00028101		CBG05701	1.09	3.51E-02
WBGene00037984	Cbr-acs-2	CBG18597	-1.19	3.55E-02
WBGene00036291		CBG16316	-0.93	3.76E-02
WBGene00030656		CBG08978	0.87	4.07E-02
WBGene00040381	Cbr-him-6	CBG21676	1.91	4.07E-02
WBGene00025722	Cbr-chil-11	CBG02717	1.49	4.11E-02
WBGene00039441		CBG20465	-0.77	4.11E-02
WBGene00040925		CBG22335	1.34	4.25E-02
WBGene00038821		CBG19641	1.44	4.29E-02
WBGene00041481		CBG23055	0.91	4.36E-02
WBGene00041829		CBG23478	-1.59	4.36E-02
WBGene00026151		CBG03259	1.26	4.51E-02
WBGene00024292		CBG00992	3.14	4.61E-02
WBGene00042363		CBG24195	1.09	4.63E-02
WBGene00035196	Cbr-ifta-1	CBG14804	1.03	4.71E-02
WBGene00034515		CBG13818	-1.13	4.71E-02
WBGene00032104	Cbr-ilys-5	CBG10836	-0.87	4.96E-02

Supplementary Table 2.5 Summary of Orsay virus DEGs in *C. elegans* that have *C. briggsae* orthologs DEGs by Santeuil virus infection.

C. elegans WBG name	*C. briggsae* ortholog	Sequence Name	Gene Name	Orsay [*rde-1*] log2 fold change	Orsay [N2] log2 fold change	Santeuil [JU1264] log2 fold change	*N. parisii* [N2] log2 fold change
WBGene00006986	WBGene00087233	Y75B8A.35	zip-1	1.35		1.37	0.83
WBGene00007097	WBGene00031139	B0024.4		5.43		0.86	
WBGene00007132	WBGene00028845	B0284.2		6.27	3.82	7.86	5.08
WBGene00007134	WBGene00028845	B0284.4		8.91	8.31	7.86	9.81
WBGene00007153	WBGene00031014	B0365.6	clec-41	2.73		1.08	
WBGene00007180	WBGene00026112	B0457.6		1.94		0.95	
WBGene00007297	WBGene00026507	C04F12.1		2.83	1.56	1.25	1.58
WBGene00007656	WBGene00028731	C17H1.3		7.88	5.86	3.86	7.59
WBGene00007657	WBGene00028845	C17H1.4		9.95	5.53	7.86	7.09
WBGene00007659	WBGene00028845	C17H1.6		9.56	6.72	7.86	7.94
WBGene00007660	WBGene00028845	C17H1.7		6.51	4.09	7.86	5.09
WBGene00007932	WBGene00030996	C34D1.5	zip-5	2.95		1.20	
WBGene00008301	WBGene00041841	C54D10.7	dct-3	6.03	4.14	5.61	6.11
WBGene00008302	WBGene00041843	C54D10.8		5.53	3.61	1.77	5.78
WBGene00008850	WBGene00031046	F15B9.6		3.25		2.14	1.97
WBGene00008872	WBGene00031174	F15H10.5		7.18	3.94	4.46	7.20
WBGene00009061	WBGene00028731	F22G12.1		8.35	6.18	3.86	6.82
WBGene00009898	WBGene00024012	F49E12.2	dod-23	5.46	2.67	1.89	3.70
WBGene00010004	WBGene00088337	F53H2.1		2.51		3.14	4.58
WBGene00010764	WBGene00024292	K10H10.9		7.52		3.14	
WBGene00010977	WBGene00027239	R02D5.3		1.86		1.77	
WBGene00011979	WBGene00026151	T24B8.5		3.18		1.26	2.54
WBGene00012091	WBGene00041842	T27E7.6		9.80	6.73	7.17	8.91
WBGene00012101	WBGene00041674	T27F2.4	zip-10	4.33		3.00	
WBGene00012381	WBGene00028943	Y2H9A.4		2.40	1.20	1.91	2.18

WBGene0 0012399	WBGene 00032650	Y6E2A .5		3.81	2.18	3.49	3.66
WBGene0 0013073	WBGene 00033785	Y51A2 D.4	hmit-1.1	-1.42		-0.90	
WBGene0 0013294	WBGene 00031866	Y57G1 1B.1		7.01	2.96	5.91	4.47
WBGene0 0015223	WBGene 00088337	B0507. 6		5.07	2.74	3.14	4.60
WBGene0 0015224	WBGene 00037933	B0507. 7		4.72	3.22	7.17	5.21
WBGene0 0015225	WBGene 00037934	B0507. 8		9.07	6.45	9.06	7.72
WBGene0 0015226	WBGene 00037935	B0507. 9		5.38		8.33	2.57
WBGene0 0015227	WBGene 00037931	B0507. 10		8.08	5.80	4.90	7.38
WBGene0 0015537	WBGene 00087767	C06E4. 8		4.05		1.40	2.30
WBGene0 0016281	WBGene 00028731	C31B8. 4		5.24	3.11	3.86	3.52
WBGene0 0016783	WBGene 00024645	C49G7. 5	irg-2	2.89		1.68	
WBGene0 0016788	WBGene 00024645	C49G7. 10		2.69		1.68	
WBGene0 0017445	WBGene 00032171	F14B8. 2	sid-5	1.53		1.47	
WBGene0 0017466	WBGene 00030434	F14F9. 3			1.98	1.69	
WBGene0 0017467	WBGene 00030434	F14F9. 4		2.08		1.69	
WBGene0 0018345	WBGene 00028205	F42C5. 3		9.51	6.49	4.72	7.75
WBGene0 0018643	WBGene 00042151	F49F1. 1	drd-50	3.25		2.69	
WBGene0 0019564	WBGene 00024644	K09D9 .1		2.49		1.26	
WBGene0 0020227	WBGene 00026106	T05A8. 1		9.66	3.70	6.34	5.50
WBGene0 0020760	WBGene 00042363	T24C4. 4		2.72		1.09	
WBGene0 0020774	WBGene 00026106	T24E1 2.5		3.01	1.17	6.34	2.03
WBGene0 0021654	WBGene 00026106	Y47G7 B.2		2.39		6.34	
WBGene0 0021977	WBGene 00030434	Y58A7 A.3		1.68		1.69	
WBGene0 0021978	WBGene 00030434	Y58A7 A.4		2.80		1.69	
WBGene0 0021979	WBGene 00030420	Y58A7 A.5		4.68		2.90	
WBGene0 0022547	WBGene 00037926	ZC196. 3		6.20	3.00	2.55	3.47
WBGene0 0022673	WBGene 00025479	ZK177. 8			0.87	1.26	

WBGene0 0044013	WBGene 00031174	F15H1 0.9		9.07	4.87	4.46	8.76
WBGene0 0044014	WBGene 00031174	F15H1 0.10		11.30	8.09	4.46	10.85
WBGene0 0044427	WBGene 00086653	F56A6. 5		9.10		5.91	6.80
WBGene0 0044787	WBGene 00041840	C54D1 0.12		4.42	2.37	5.47	4.45
WBGene0 0045475	WBGene 00042683	T07A5. 7		5.07		2.81	3.03
WBGene0 0138721	WBGene 00041840	C54D1 0.14		5.88	3.92	5.47	6.20

Chapter 3: Characterization of Orsay virus infection in *C. elegans*

Abstract

C. elegans is an excellent model organism with a variety of genetic tools available to investigate host-pathogen interactions. With the recent discovery of Orsay virus, the first virus that naturally infects *C. elegans*, we are now able to examine how Orsay virus interacts with the host. I have found that when Orsay virus infected the reference strain N2, there were high variations among the replications from the same experiment. This result was consistent with both animals grown on solid medium or in a liquid medium. In contrast, the hyper-permissive strain *rde-1* with compromised RNA interference (RNAi) pathway had less variation and were significantly higher in viral RNA than that of N2.

An important genetic tool for *C. elegans* is the targeted silencing of mRNA with RNAi by feeding the worms homologous double-stranded RNA of that gene. I have discovered that the feeding RNAi may compete with antiviral RNAi as targeting GFP with RNAi in a GFP-expressing strain had increased amount of Orsay virus RNA. With these better understanding of the Orsay virus infection characteristics in *C. elegans*, we can now determine the optimal experimental conditions based on the specific hypotheses we want to test.

Introduction

Orsay virus, the first virus that can naturally infect *C. elegans* was recently described [1]. Orsay virus is a non-enveloped virus that has a positive sense bipartite RNA genome, and it is most closely related to *Nodaviridae*. With this discovery, we can now interrogate the *C. elegans* – Orsay virus model system to understand the host-virus interactions better. There are currently two known cellular pathways utilized by *C. elegans* to antagonize Orsay virus replication. One host pathway is the antiviral RNA interference (RNAi) that directly restricts Orsay virus replication [1,2]. The other host pathway is the ubiquitin-proteasome pathway where the Skp1 - Cul1- F-box protein (SCF) ligase is essential for restricting Orsay virus [3]. However, many of the host factors that are involved in other stages of the virus lifecycle such as entry, translation of viral protein, assembly of virus particles and release/egress of the virus remain unknown in *C. elegans*.

Orsay virus infection in *C. elegans* reference strain N2 neither reduces the lifespan of the host nor causes any obvious physiological change [1]. The virus hyper-permissive strains where the RNAi function was compromised, also did not have shortened lifespan, but disturbance of the worm intestinal cells and increased Orsay virus RNA were observed [1,2,4]. The limited physiological change in the N2 strain and no lethality by the Orsay virus infection means that the measurement of Orsay virus replication will largely depend on molecular tools. Also, the ability to measure the amount of virus replication for each stage of the virus lifecycle can help dissect the role of a specific host factor. To that end, the Wang lab had developed assays to measure Orsay virus RNA with real-time quantitative reverse transcription PCR (qRT-PCR) [1], Orsay virus proteins with immunofluorescence assay [5], and Orsay virus titer with an end point dilution assay [6].

The model organism *C. elegans* has many useful genetic tools to facilitate research in the model organism. Some of the tools included a large collection of knockout animals [7], the ability to knockdown genes by feeding worms with bacteria that expressed dsRNA of the gene of interest [8], and CRISPR/Cas9 system to knockout individual genes [9]. However, before we can utilize the full suite of genetic tools available to investigate the interaction between *C. elegans* and Orsay virus, it is essential to characterize the infection process better and determine the limitation of the developed assays. It is of particular importance to understand how the controls behave to determine the optimal infection conditions and interpret the experimental results properly. Here, I present a systematic approach to characterize Orsay virus infection in *C. elegans* with the reference N2 strain and RNAi feeding.

Results

Virus replication is variable in N2 strain on NGM plates

N2 reference strain is the foundation of most of the genetic tools developed for *C. elegans*. To better understand the amount of Orsay virus that is replicating in the *C. elegans* hosts, we infected the worms at two multiplicity of infection (MOI) of 10 and 0.1 and different length of infection time; two and three days. In addition to the N2 reference strain, I used *rde-1*, an RNAi-deficient strain that is hyper-permissive for Orsay virus infection as a positive control to determine the upper limits of virus replication and an input control to determine how much virus were introduced from the initial virus dose. I first infected both strains with a relatively high MOI of 10 for two days. The total RNA of these infected worms and the input controls were extracted, and the Orsay virus RNA were measured by qRT-PCR. The N2 infected worms have over 2-log higher geometric mean of Orsay RNA compared to input, indicating successful infection, and the hyper-permissive strain *rde-1* have approximately 5-log higher geometric mean of Orsay RNA (Figure 3.1). This observation was consistent with our expectation that the RNAi-deficient strain would have higher Orsay virus replication. One interesting result was the high variation between replications in N2 as the range between the maximum value to the minimum value for Orsay RNA in the N2 strain span approximately 3-log (Figure 3.1). The next condition tested was at a lower MOI of 0.1 and the same 2-day infection time. The amount of input virus measured was lower which is consistent with the lower amount of input virus (Figure 3.1). Orsay RNA in N2 strain was 2-log higher compared to input indicating that infection of Orsay virus was successful. However, the variation of Orsay RNA among N2 replicates was now larger as the range of Orsay RNA in N2 now spans almost 7-log. Some of the samples had a lower Orsay RNA that were comparable to the input suggesting that Orsay virus infection was

not successful in these samples. In contrast, the *rde-1* mutant was approximately 6-log higher in Orsay RNA than input and had a low variation. The last condition tested was at an MOI of 10 and 3-day infection. The N2 strain had comparable geometric means between 2-day and 3-day infection, and the 3-day infection time did not reduce the variation between replications. The *rde-1* mutant had approximately 6-log higher Orsay RNA than input and a low variation, overall comparable to 2-day infection. The lack of difference between 2-day and 3-day infection at MOI of 10 suggest that we may have observed the peak viral replication capacity of Orsay virus in both N2 and *rde-1*.

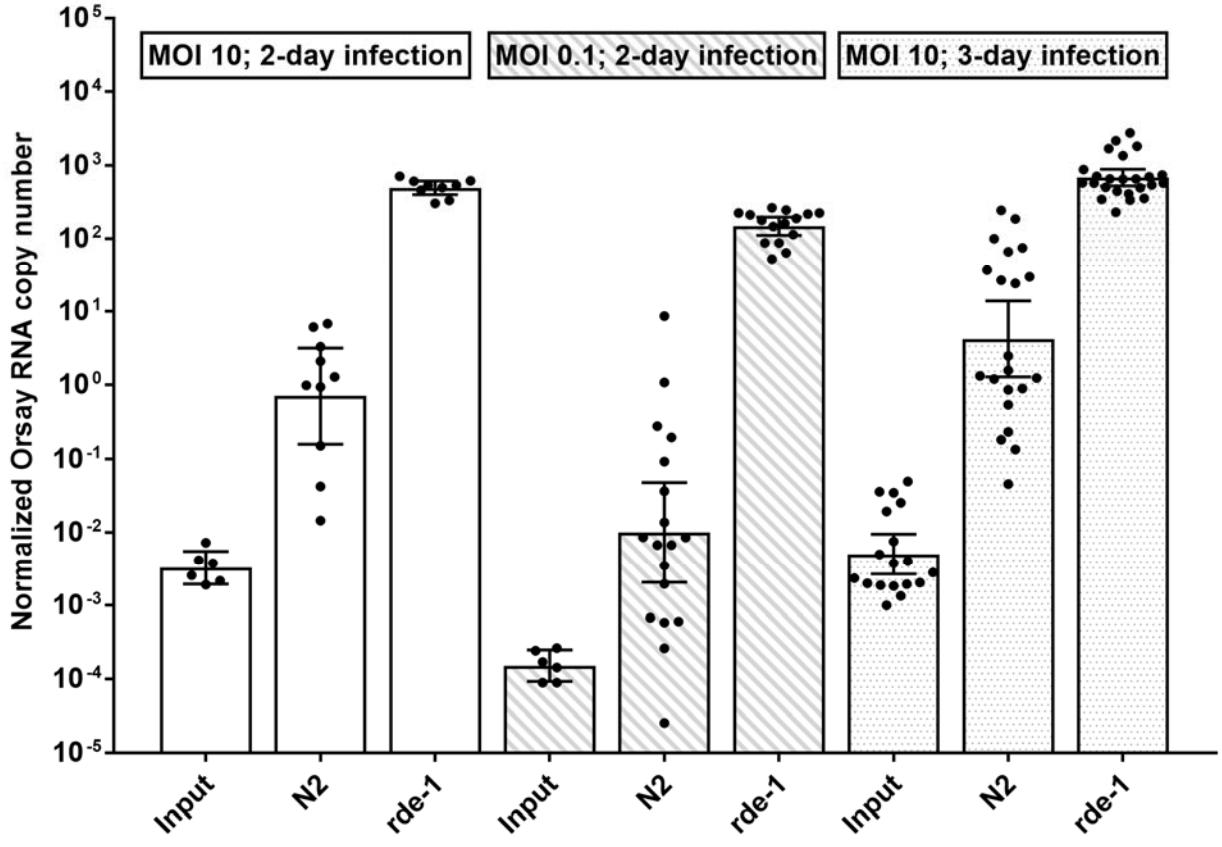


Figure 3.1: Orsay virus replication in N2 and *rde-1* on solid medium/NGM plate. 500 N2 or *rde-1* worms were infected by Orsay virus (MOI 10 or 0.1) for 2 or 3 days. The amount of Orsay virus replication was measured by qRT-PCR and normalized to *cdc-42*. The individual value, the geometric mean, and the 95% CI are shown.

Liquid infection did not reduce variation of infection in N2 strain

The high variation of Orsay virus infection in N2 on NGM plates was less than ideal. To make N2 a more reliable control with reduced variations, I investigated if an alternative culturing condition, liquid culture, could be a better option. The liquid culture had a potential advantage over solid medium in that the liquid culturing condition was more homogenous. This advantage can potentially enable more consistent infections. I tested a stable liquid infection condition where the input virus was incubated with worms for the entire duration of the infection. For this condition, N2 and *rde-1* worms were cultured in liquid culture infected with Orsay virus at MOI of 50, 5, and 0.5 for three days. For the MOI 50 and 5 conditions, infection was successful as both N2 and *rde-1* had increased Orsay RNA compared to input (Figure 3.2). However, the lowest MOI condition of 0.5 had limited increase of Orsay RNA as the geometric means did not change in both N2 and *rde-1* worms when compared to input (Figure 3.2). Some samples in the MOI 0.5 condition were 2-log higher than the input, however, most of the replicates did not. This data suggests that MOI 0.5 is likely too low to consistently achieve infection in liquid culture. For the MOI 50 condition, N2 increased less than 1-fold compared to input and *rde-1* increased less than 2-log. These data suggest that the input virus might mask the real virus replication in infected worms (Figure 3.2). For the MOI 5 condition, the N2 median Orsay virus increased by more than 1-log where *rde-1* increased by more than 2-log (Figure 2.2B). However, the variation in N2 is high and spanned over 2-log, and multiple samples have Orsay RNA amount close to either the input or infected *rde-1*. These data suggest liquid culture infection did not reduce the variation of Orsay virus replication in N2 observed in solid medium culture.

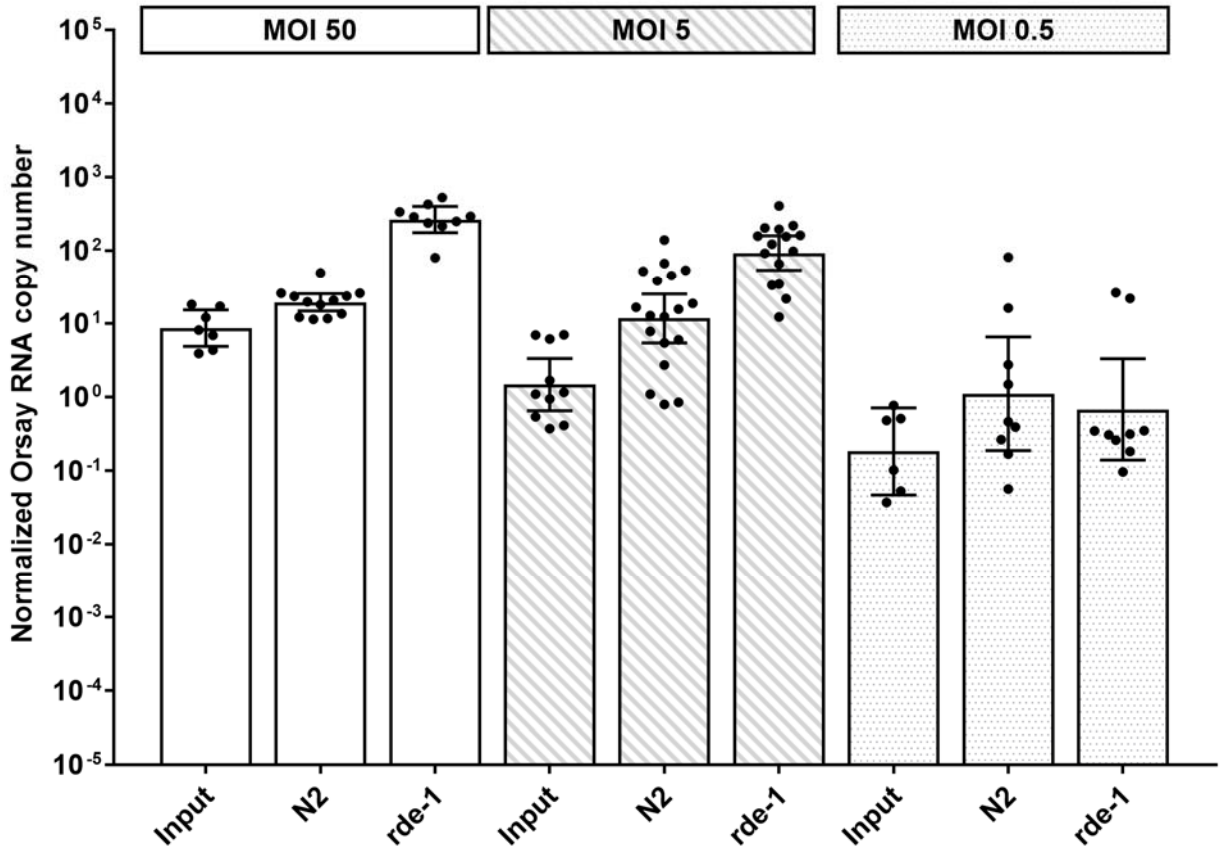


Figure 3.2: Orsay virus replication in N2 and *rde-1* in a liquid medium. 100 N2 or *rde-1* worms were infected by Orsay virus (MOI 50, 5, or 0.5) for 3 days in liquid culture. The amount of Orsay virus replication was measured by qRT-PCR. The individual value, the geometric mean, and the 95% CI are shown.

The process of feeding RNAi may compete with antiviral RNAi

Orsay virus replication is strongly influenced by the antiviral RNAi pathway [1], and both the antiviral RNAi pathway and exo-RNAi pathway have shared components [10]. It is, therefore, possible that both processes might compete and results in reduced RNAi efficiency in one or the other. To test this hypothesis, we used a previously described transcriptional GFP reporter strain that was induced by Orsay virus infection [3]. This strain carried an integrated transgene comprised of the promoter of *C17H1.6*, a gene strongly induced by Orsay infection, fused to GFP. The GFP expressed in the reporter worms provided a transcript target for feeding

RNAi that was not related to the host-virus interaction. This GFP RNAi control would allow the activity of exo-RNAi to occur in the GFP-expressing worms. The amount of Orsay virus RNA in this control can help determine if the exo-RNAi influenced the replication of Orsay virus. In addition to the GFP RNAi, two addition controls were used. The first bacteria clone was an empty vector control that expresses a short dsRNA (~180bp) that does not have sequence homology to worm genome as a control for the bacteria and plasmid. The second target is *rde-1*, which is a member of both exo-RNAi and antiviral RNAi. Targeting of *rde-1* would serve as a control for reduced RNAi capacity. Synchronized worms were fed RNAi clones expressing dsRNA of the controls for two days and infected at an MOI of 10 or 0.1 for another two days. When RNAi knocked down worms were infected with Orsay virus at MOI of 10; the *rde-1* targeted samples had approximately 1-log more Orsay virus RNA compared to empty vector (Figure 3.3) and statistically different. This observation suggests that the *rde-1* RNAi was successful although the degree of difference was not as large when compared to knockout mutants (Figure 3.1). For the GFP RNAi worms, the observed Orsay virus RNA were statistically higher than empty vector and statistically lower than *rde-1* (Figure 3.3). This suggested that the GFP RNAi did compete with antiviral RNA causing the Orsay virus replication to increase. When the worms were infected at a lower MOI of 0.1, the overall amount of Orsay virus RNA compared to MOI 10 were lower in all three controls, and the difference between *rde-1* and empty vector is larger at approximately 3-log (Figure 3.3). However, the variation between replications in the same control also increased from within 2-log for MOI 10 to more than 4-log for MOI 0.1. The GFP target control at this condition was the most varied at more than 5-log. All these data suggested that feeding RNAi to host genes may change the overall Orsay RNA replication even if it is not antiviral-related.

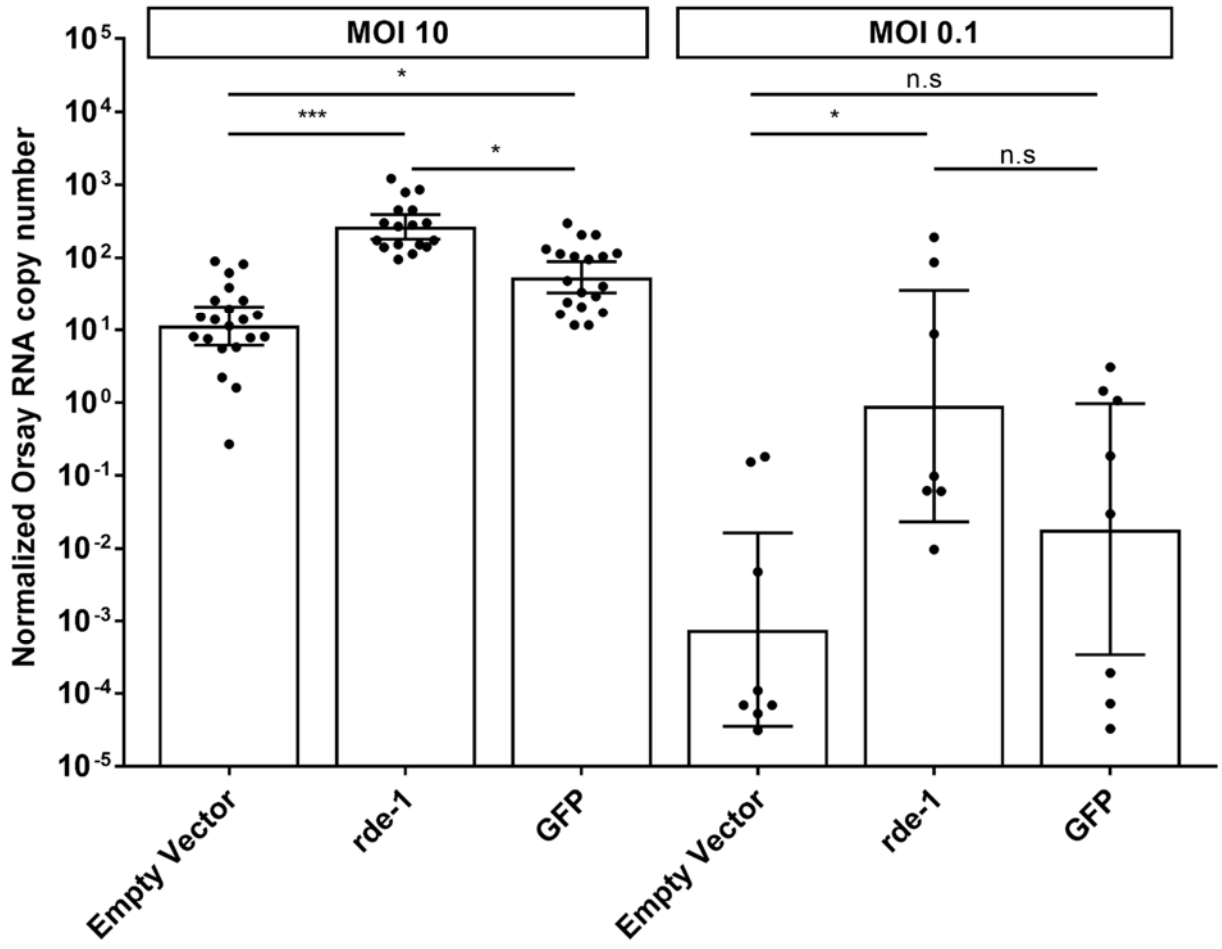


Figure 3.3: RNAi feeding of non-antiviral gene resulted in increased virus replication at MOI of 10. 500 synchronized N2/*jyIs*8 were fed with induced bacterial clone expressing dsRNA of the targeted genes for 2 days. Worms were then infected with Orsay virus at MOI of 10 or 0.1 for 2 days. The amount of Orsay virus replication was measured by qRT-PCR. The bar represents the geometric mean of the measured values. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Benjamini–Hochberg procedure for all pairwise comparison for each MOI. *P<0.05, ***P<0.001, n.s. - not significant.

Discussion and Conclusion

Characterizing how Orsay virus replicates in *C. elegans* is an important precursor to studying host-virus interactions. It will help us better understand the biology of Orsay virus and allow us to determine the suitable conditions to assay the effect of host genes to virus infections. One interesting finding was that the laboratory reference strain N2 was highly variable, especially at the relatively low MOI conditions (MOI < 1). This result contrasts with the low variation in the hyper-permissive strain *rde-1*. In the N2 strain, 2-day or 3-day infection with MOI 10 did not have a significant difference as the median is similar between the two and both have more than 3-log variation between the replicates. The similar amount of Orsay virus RNA between the 2-day and 3-day infection suggest that it may reach the peak capacity of infection for N2 at this stage. The high variation in N2 may indeed indicate that the host-virus interaction relies on some stochastic process that is yet to be defined.

One possible approach to minimize variation changes the infection environment from solid medium to liquid medium. The homogeneous environment of liquid infection could potentially reduce variation. However, we found that the liquid culture infection did not reduce variation in the similar MOI. At the MOI 50 condition, we did found that the variation was reduced, but that was most likely because the input virus has provided a high baseline of Orsay virus RNA and masked the potential variations in N2.

RNAi feeding to knock down host gene transcript from external dsRNA (exo-RNAi) is an important genetic tool for the model organism *C. elegans*. Knocking down host genes with RNAi is efficient, however, can have variable efficiency depending on the specific target. Another limitation is the reduced efficiency when targeting multiple genes for knockdown with RNAi [11]. While the precise reason is not entirely determined, a recent study suggests that

multiple RNAi pathways compete for finite shared components [12]. We found that feeding GFP RNAi alone has increased Orsay virus RNA when compared to empty vector control. Therefore, caution is required when interpreting results of Orsay virus replication from RNAi knockdown experiments. Also, the GFP is a better control for future RNAi experiments as it controlled the effect of exo-RNAi. Additionally, low MOI conditions were not advised as the potential competition between host gene RNAi and anti-viral RNAi produced highly variable results. However, if any of the genes have a significant phenotype, the RNAi should be able to reveal strong candidate host genes that interact with Orsay virus replication.

Together, these results have better defined the characteristics of Orsay virus replication in *C. elegans*. These finding will also help guide the optimal selection of infection conditions to efficiently screen for genes that may have a role in the host-virus interactions.

Materials and Methods

Strains

C. elegans strains N2, and *rde-1* (WM27) along with *C. briggsae* strain AF16 were obtained from the *Caenorhabditis* Genetics Center (CGC). The GFP reporter strain ERT54 *jyIs*8 [*pC17H1.6::GFP; pmyo-2::mCherry*] was kindly provided by Emily Troemel [3].

Infectious virus filtrate preparation

Orsay virus and Santeuil virus were propagated as previously described in Chapter 1.

Worm infection on solid medium

Uninfected *C. elegans* (N2 and *rde-1*) and uninfected *C. briggsae* (AF16) were synchronized by standard bleach treatment. 500 embryos were seeded per well into 6-well NGM plates containing 20-30 µl of OP50 food and maintained at 20°C. 16 hours after bleaching; worms were inoculated by adding 20 µl of Orsay virus of starting at 2.5 x 10^5 $TCID_{50}$ /ml for an MOI of ~10 directly over the bacterial lawn. Lower MOI infection conditions were obtained by additional 10-fold dilution of the starting Orsay virus stock. After two days or three days post-infection, animals were rinsed off from the wells with 1 ml M9 buffer, the supernatant was removed after centrifugation, and 350 ul Trizol were added to the samples.

Worm infection in liquid medium

Uninfected *C. elegans* (N2 and *rde-1*) were synchronized by standard bleach treatment and arrested at the L1 stage. 100 worms in 20 µl S-media were added to each well on a 96-well plate along with 10 µl of 50x concentrated OP50 and 20 ul of Orsay virus of starting at 2.5 x 10^5 $TCID_{50}$ /ml for an MOI of 50. Lower MOI infection conditions were obtained by additional 10-fold dilution of the starting Orsay virus stock. After 2 or 3 day post infection, all contents in the well were collected into 350 µl Trizol.

RNAi feeding knockdown infection on solid medium

RNAi feeding was used for gene knockdown as described [8]. E. coli strain HT115 carrying double strand RNA expression cassettes for genes of interest were induced using established conditions and were then seeded into 6-well NGM plates. Empty vector, *rde-1* RNAi clones were from the Ahringer RNAi library [13]. 500 arrested GFP reporter animals were seeded into each well of a 6-well plate. After two days of RNAi feeding, Orsay virus was added

to the plates as described with solid medium infection. After 2-day infection, the infected *C. elegans* animals were collected as described with solid medium infection.

Measuring Orsay virus RNA by real-time quantitative one-step RT-PCR (qRT-PCR

Infected worm samples in Trizol were vortexed for 10 minutes at room temperature and centrifuged at 15,000g for 5 minutes. The Trizol supernatants were extracted with Direct-zol RNA miniprep kit (Zymo) and were eluted into 60 µl water. The extracted samples were diluted 1:100 and 5 µl of the diluted RNA were used for one-step real-time RT-PCR (TaqMan® Fast Virus 1-Step Master Mix, Thermo-fisher) on a ViiA7 real-time PCR system (Applied Biosystem) following the manufacturer's protocol. The copy number for each sample was calculated from a standard curve and was normalized to an internal control *cdc-42* gene.

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Chapter 4: Systematic screening of genes that impacted Orsay virus replication from the evolutionarily conserved virus response genes

Abstract

C. elegans is an excellent model organism to investigate the host-virus interaction with the recent discovery of its naturally infecting Orsay virus. To determine if the 58 evolutionarily conserved differentially expressed genes (DEGs) from Chapter 2 impacted Orsay virus replication either positively or negatively, I perturbed the expression these candidate genes with overexpression, RNAi knockdown, readily available knockout mutants, and new knockout mutants generated by CRISPR/Cas9. After measuring the amount of Orsay virus replication phenotype by quantitative real-time PCR and end-point dilutions assays, I have found 1) Overexpression of some viral DEGs may have a protective effect in Orsay virus infection. 2) RNAi knockdown of all these 58 DEGs did not identify any of these genes as functional in host-virus interaction. 3) *T27E7.6,* a gene in the *C17H1* gene family may play an antiviral role. 4) *zip-10*, a nematode bZIP transcription factor, may be a proviral gene that facilitates Orsay virus infection. Both *T27E7.6* and *zip-10* were not known to be associated with virus replication, suggesting novel host-virus interactions may have been identified.

Introduction

With the recent discovery of its naturally infecting Orsay virus, *C. elegans* has became an excellent model organism to investigate the host-virus interactions [1]. Orsay virus is a non-enveloped virus that has a positive sense bipartite RNA genome, and it is most closely related to *Nodaviridae*. With this discovery, we can now interrogate the *C. elegans* – Orsay virus model system to better understand the host-virus interactions. There are currently two known cellular pathways utilized by *C. elegans* to antagonize Orsay virus replication. One host pathway is the antiviral RNA interference (RNAi) that directly restricts Orsay virus replication [1,2]. The other host pathway is the ubiquitin-proteasome pathway where the Skp1 - Cul1- F-box protein (SCF)

ligase is important for restricting Orsay virus [3]. However, many of the host factors that are involved in other stages of the virus life cycle such as entry, translation of viral protein, assembly of virus particles and egress of the virus remain unknown in *C. elegans*.

In *C. elegans* genetics, there are two broad categories of approaches to identify genes in the host-virus interactions. One of the approach is the forward genetic screen that identifies causal mutation from chemical mutagenized animals with phenotype related to Orsay virus replication [4]. The other approach is a reverse genetic screen that investigates a specific set of genes (whole genome or a biological relevant set) and its relationship with Orsay virus replication [5]. The specific approach taken for the current study was a reverse genetic screens of likely host-virus interaction genes. There are multiple ways to select a biological relevant set of genes in the host-virus interaction, including genes under positive selection pressure [5], proteins that directly interacts with virus [5], and genes that responded to virus infection.

The prime candidates for a genetic screen were the differentially expressed genes (DEGs) upon virus infection [6]. More specifically, the evolutionarily conserved DEGs between *C. elegans* and *C. briggsae,* a close relative pair diverged ~18 million years ago and shared 60% of the protein coding genes, were the most interesting set. The overlapping response to its own unique virus in two related species provides a unique set of genes that represent an evolutionarily conserved process that could interact with virus infection.

The model organism *C. elegans* has many useful genetic tools to facilitate research in the model organism. Some of the tools included a large collection of knockout animals [8], the ability to knockdown genes by feeding worms with bacteria that expressed dsRNA of the gene of interest [9], and the CRISPR/Cas9 system to knockout individual genes [10]. Combined with the available assays to measure Orsay virus RNA with real-time quantitative reverse transcription

PCR (qRT-PCR) [1], Orsay virus proteins with immunofluorescence assay [7], and Orsay virus titer with an end point dilution assay [6]. We can fully interrogate host genes that interacts with Orsay virus. Here, I present a systematic screening to identify host genes that influenced Orsay virus infection in *C. elegans*.

Results

Overexpression of virus-induced genes have reduced Orsay virus replication

To determine if differentially expressed genes upon Orsay virus infection were involved in host-virus interactions, I tested two mutants that constitutively overexpressed some of these genes. These two strains, JY1 and JY3, both contain mutation to *C29F9.1*, constitutively overexpressed the three virus induced genes *C17H1.6, F26F2.1, and F26F2.4* by over 100-fold (Personal communication with Emily Troemel). I infected the worms with Orsay virus and measureed the amount of Orsay virus RNA by quantitative real-time reverse-transition PCR (qRT-PCR). When worms were infected at a high MOI of 10, there was no difference between the virus DEGs overexpression strain JY1 and JY3 to their control N2. However, at a lower MOI of 0.1, the two overexpression strains had lower Orsay virus RNA compared to N2. The two independent strains had similar results in both MOI conditions. These results suggested that the *C29F2.1* regulated genes that include several virus DEGs may have antiviral properties.

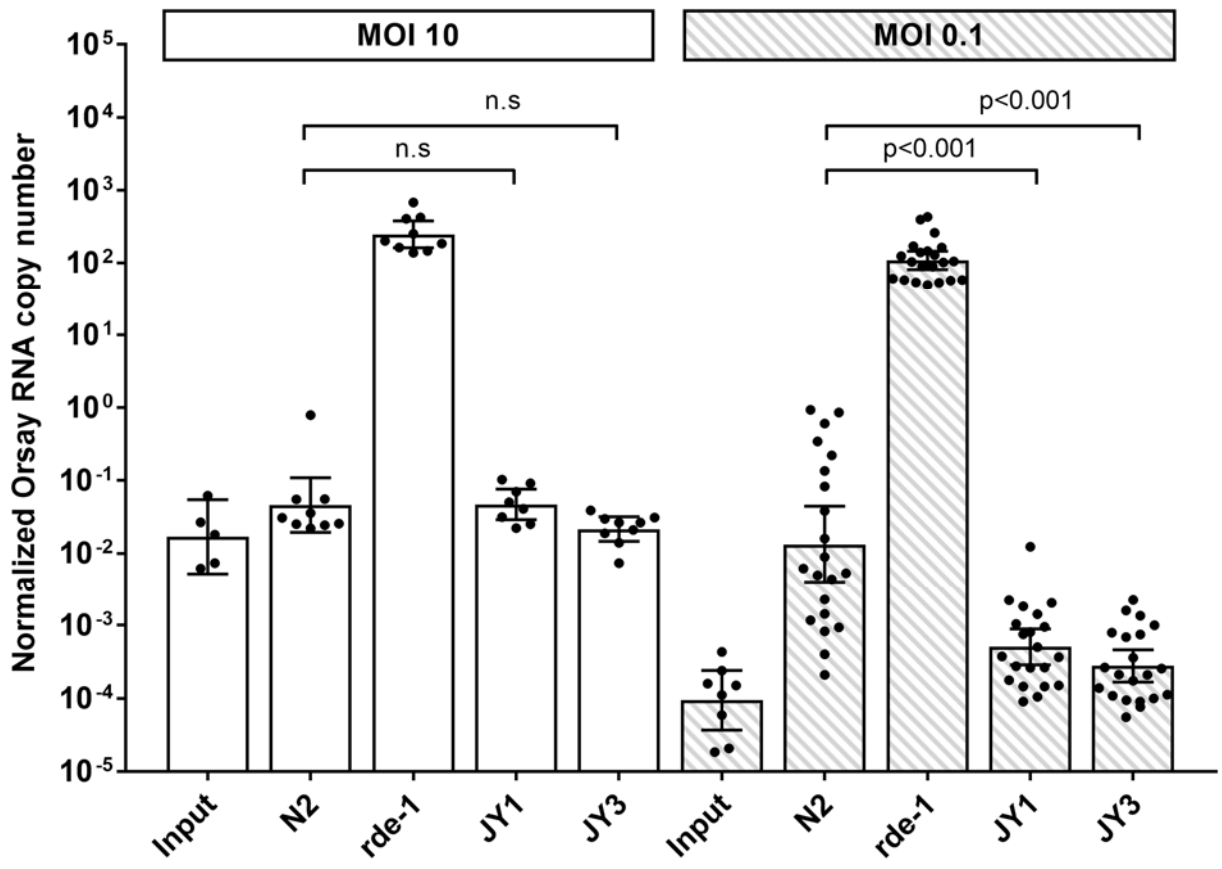


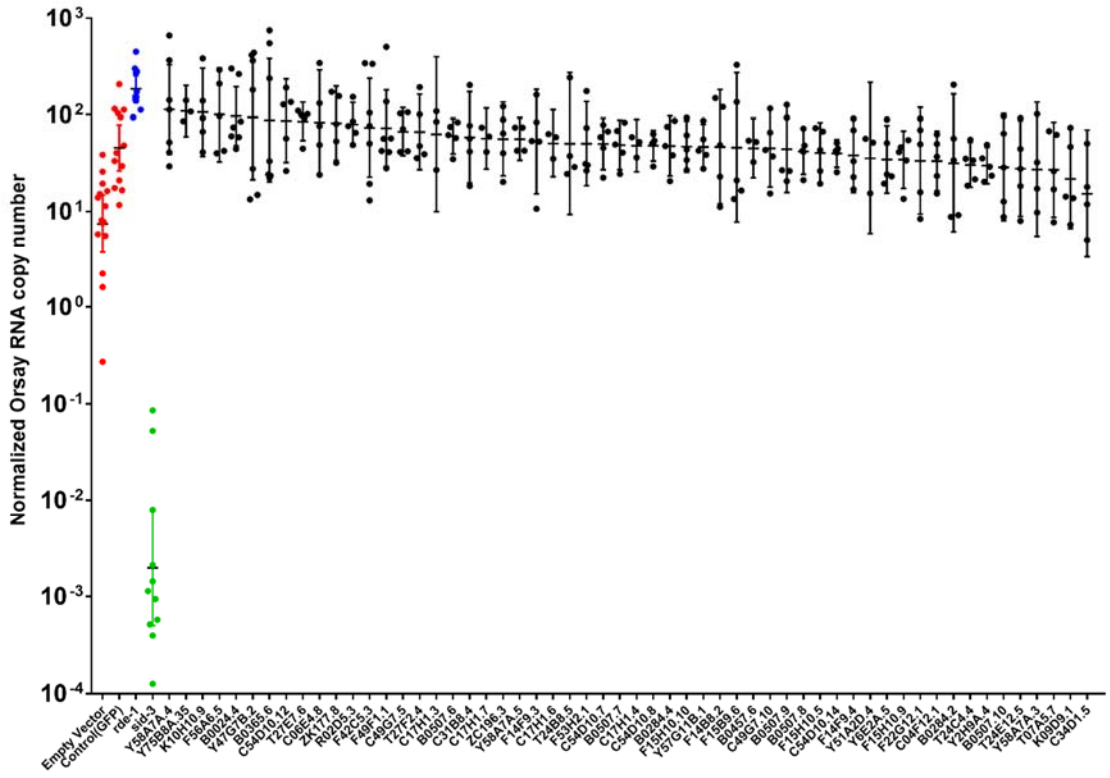
Figure 4.1. Virus DEGs overexpression strains JY1 and JY3 have lower Orsay virus replication at low MOI condition. Mutants JY1 and JY3 which overexpressed multiple virus-induced genes were infected with Orsay virus. The amount of Orsay virus RNA was measured by qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against N2. *P<0.05, ***P<0.0005, n.s. - not significant.

Knockdown of evolutionarily conserved DEGs by RNAi did not influence Orsay virus replication

To screen for genes that were involved in host-virus interactions from the 58 evolutionarily conserved DEGs, I knocked down these genes individually using RNAi feeding [9,11,12]. I conducted the RNAi knockdown experiment in two different genetic backgrounds. The first was the reference strain with a GFP reporter and the second was a *drh-1* mutant which

is competent for feeding RNAi but not anti-viral RNAi [14]. Conducting RNAi in the GFP reporter strain allowed us to use GFP RNAi as a proper control for potential competition between the exo-RNAi and antiviral RNAi. The use of *drh-1* strain was to determine if there were genes that act independently of the antiviral RNAi.

I first fed the GFP reporter strain with the RNAi clones for three days and subsequently infected the animals with Orsay virus at an MOI of 10 for another two days. When compared to GFP control, no RNAi knockdown contributed to a difference in Orsay virus RNA amount (Figure 4.2A). The positive control RNAi targeting *rde-1* was statistically significantly higher than the GFP control, however, the difference between the geometric means were less than 1-log. I next determined if any of the 58 evolutionarily conserved DEGs interacts with Orsay virus independently of the antiviral RNAi pathway. I knocked down the DEGs with feeding RNAi in *drh-1* animals for three days and infected them with Orsay virus at an MOI of 10 for two days. I have found that RNAi knockdown of the DEGs did not change Orsay virus RNA whem compared to *drh-1* feeding control. Given the limitation of RNAi knockdown experiment, it is possible that some these genes may still participate in the host-virus interaction. More experiments are needed to determine if any of these 58 DEGs were indeed involved in host-virus interactions.


Normalized Orsay RNA copy number
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
Empty Vector
Control(GFP)
rde-1
sid-3
Y58A7A.4
Y75B8A.15
K10H10.9
F56A6.5
B0024.4
Y47G7B.2
B0565.6
C54D10.12
T27E7.6
C06E4.8
ZK177.8
R02D5.3
F42C5.3
F49F1.1
C49G7.5
T27F2.4
C17H1.3
B0507.6
C31B8.4
C17H1.7
ZC196.3
Y59A1A.5
F14F9.3
C17H1.6
T24B8.5
F53H2.1
C54D10.7
B0507.7
C17H1.4
C54D10.8
B0284.4
F15H10.10
Y57G11B.1
F14B8.2
F15B9.6
B0457.6
C49G7.10
B0507.9
F15H10.8
F15H10.5
C54D10.14
F14F9.4
Y51A2D.4
Y6E2A.5
F15H10.9
F22G12.1
C04F12.1
B0284.2
T24C4.4
Y2H9A.4
B0507.10
T24E12.5
Y58A7A.3
T07A5.7
K09D9.1
C54D1.5

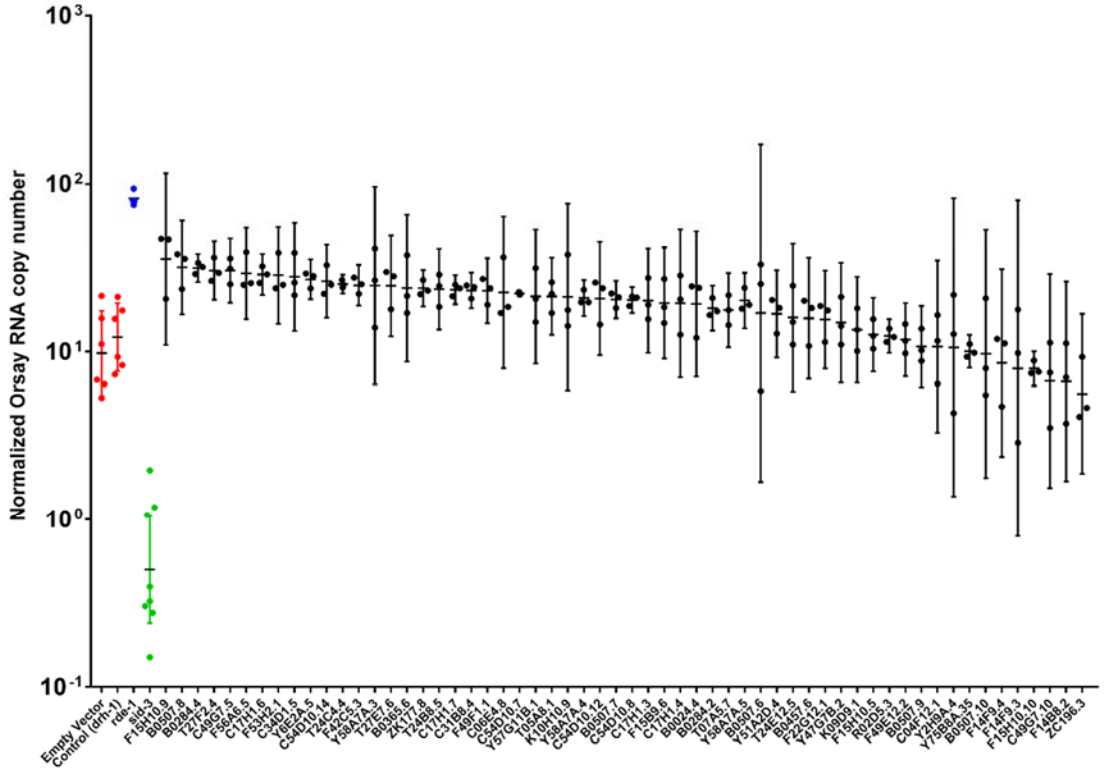

Normalized Orsay RNA copy number
10^3
10^2
10^1
10^0
10^{-1}
Empty Vector
Control (dnh-1)
rde-1
sid-3
F15H10.9
B0507.8
B0284.4
T27F2.4
C49G7.5
F56A6.5
C17H1.6
F53H2.1
C54D1.5
Y6E2A.5
C54D10.14
T24C4.4
F42C5.3
Y58A7A.3
T27E7.6
B0565.6
ZK177.8
T24B8.5
C17H1.7
C31B8.4
F49F1.1
C06E4.8
C54D10.7
Y57G11B.1
K10H10.9
T09A8.1
Y59A1A.4
C54D10.12
B0507.7
C54D10.8
C17H1.3
F15B9.6
C17H1.4
B0284.2
B0024.4
T07A5.7
Y58A7A.5
B0507.6
Y51A2D.4
T24E12.5
B0457.6
F22G12.1
Y47G7B.2
K09D9.1
F15H10.5
R02D5.3
F49E12.2
B0507.9
C04F12.1
Y2H9A.4
Y75B8A.15
B0507.10
F14F9.3
F15H10.10
C49G7.10
F14B8.2
ZC196.3

Figure 4.2. RNAi knockdown of the 58 evolutionarily conserved DEGs individually did not change the Orsay virus RNA amount. The 58 evolutionarily conserved DEGs were individually knocked down by feeding RNAi for three days. Knocked down animals were infected with Orsay virus at MOI 10 for two days, and the amount of Orsay virus RNA was measured by qRT-PCR. A) RNAi feeding in N2 GFP reporter animals. Four controls were used including the empty vector, GFP, *rde-1* and *sid-3* (a proviral gene). Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against Control (GFP). B) RNAi feeding in drh-1 animals. Four controls were used including the empty vector, *drh-1*, *rde-1*, and *sid-3*. Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against Control (*drh-1*). The individual values, the geometric mean, and the 95% CI are shown. The order of genes presented was sorted by geometric means.

The *C17H1* gene family member *T27E7.6* may be an antiviral gene

From the analysis of virus infection DEGs, we identified an interesting gene family, the *C17H1* gene family [6]. The *C17H1* gene family was highly represented in the total list of *C. elegans* DEGs (25/320) and was also the most represented gene family in the evolutionarily conserved response DEGs (14/58). Multiple *C17H1* gene family members had been tested by RNAi knockdown in the previous described RNAi knockdown experiments, and no genes were differentially expressed.

To determine if any members of the C17H1 gene family were involved in Orsay virus replication, the best approach was to conduct the Orsay virus infection experiments in knockout mutants. Compared to RNAi knockdown, we can observe phenotype with a lower magnitude in the knockout mutants, and negative results will be more conclusive. While there were no mutant strains available that targeted *C17H1* gene family members from the *Caenorhabditis* Genetics Center (CGC), there were five strains that contain high-impact mutations such as premature stop codon and splicing-editing dysfunction from the collection of Million Mutation Project [8]. We conducted the Orsay virus infection assay on these five strains and measured the Orsay virus RNA amount by qRT-PCR. We found the strain that contains *T27E7.6* mutation had a

significantly increased Orsay virus RNA compared to N2 control (Figure 4.3). For the remaining four strains, the strain with the *C54D10.8* mutation had lower Orsay virus RNA, but none of the four strains were statistically different from the N2. When these strains were infected at a lower MOI of 0.1, similar phenotype was observed, but none of these five strains were statistically different from N2 (Supplementary Figure 4.1). The mutant strain VC40577 that has *T27E7.6* mutation also had other mutations. One feature of the mutants from the Million Mutation Project is that there are multiple mutations in each strain [8]. Upon consulting the genomic sequencing results available for VC40577, we found there were three high-impact mutations including *T27E7.6* and 87 missense mutations in protein coding genes. None of the mutated genes in the VC40755 strain were in the exo-RNAi pathway (Supplemental Table 4.2). Additionally, RNAi knockdown of *dpy-3* in the VC40577 strain resulted in an animal with strong dumpy phenotype (data not shown) suggesting that the exo-RNAi pathway was intact. All these results indicate that *T27E7.6,* a member of the C17H1 gene family may be antiviral.

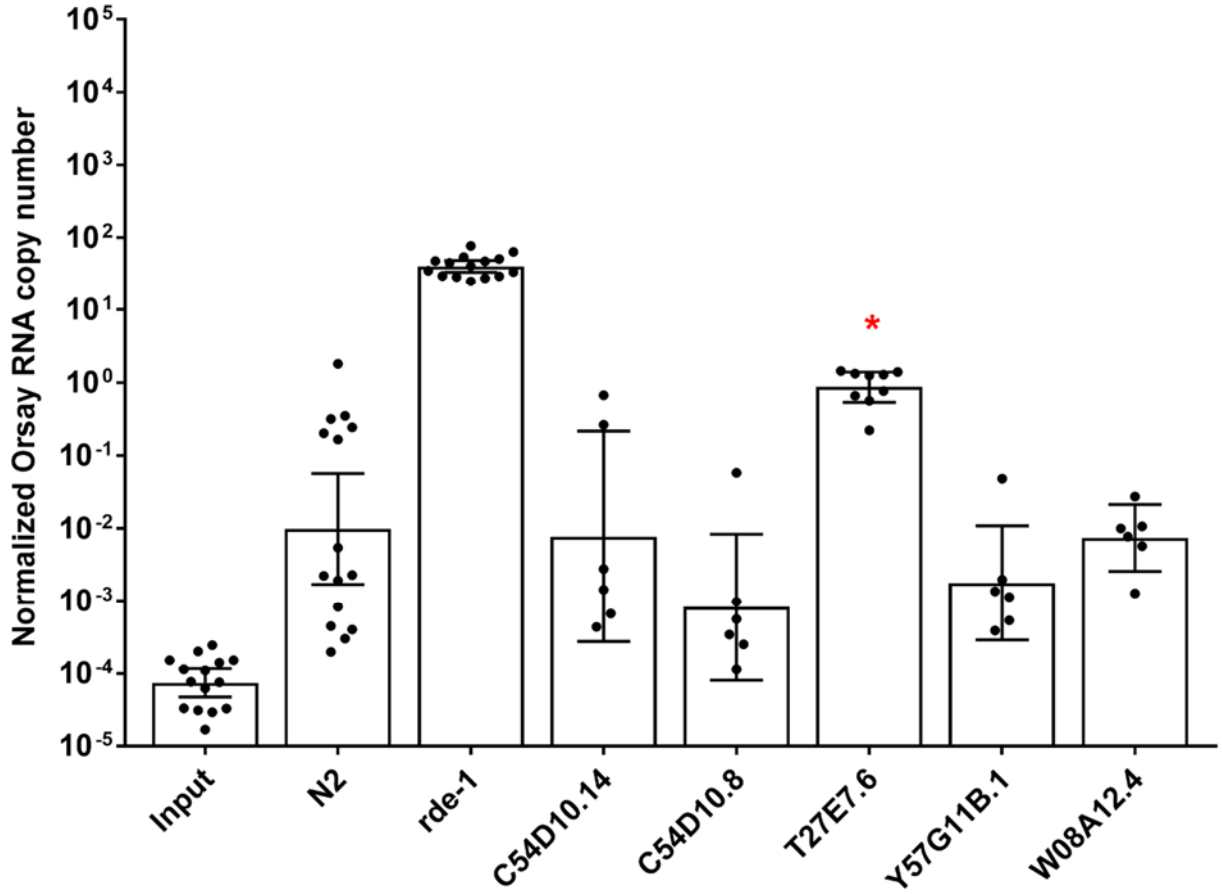


Figure 4.3. The screening of *C17H1* gene family member mutants for Orsay virus replication. Mutants containing *C17H1* gene family members were infected with Orsay virus at an MOI of 10 for two days. The Orsay virus RNA was measured by qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against N2. *P < 0.05.

The *C17H1* locus of the *C17H1* gene family was not part of the host-virus interaction

To further investigate if other members of the *C17H1* gene family plays a role in the host-virus interactions, I generated a strain that knocked out the entire *C17H1* locus with CRISPR/Cas9 system [10,15]. The mutant strain WUM6 has a deletion that spanned 35kb from *C17H1.3* to *C17H1.7* that includes 11 of the 25 upregulated *C17H1* gene family and 4 of the 58

evolutionarily conserved DEGs. I then tested the Orsay virus replication phenotype on WUM6 and found that Orsay virus RNA was not different to N2 (Figure 4.4). This observation was true in both MOI of 10 and MOI of 0.1 conditions. I next crossed the WUM6 with *rde-1* to produce WUM6;*rde-1* to determine if the *C17H1* locus genes act independently of RNAi. We found that there was no difference between the *rde-1* control and the WUM6;*rde-1* strain (Figure 4.5A). One possibility was that the *C17H1* locus genes might act downstream of replication and measure only Orsay virus RNA may not reflect that effect. I measured the Orsay virus titer in these samples with an end-point dilution assay that was similar to $TCID_{50}$. I found that the *C17H1* locus alone did not influence the production of infectious Orsay virus (Figure 4.5B). These results suggest that the *C17H1* locus genes alone were not involved in the host-virus interaction.

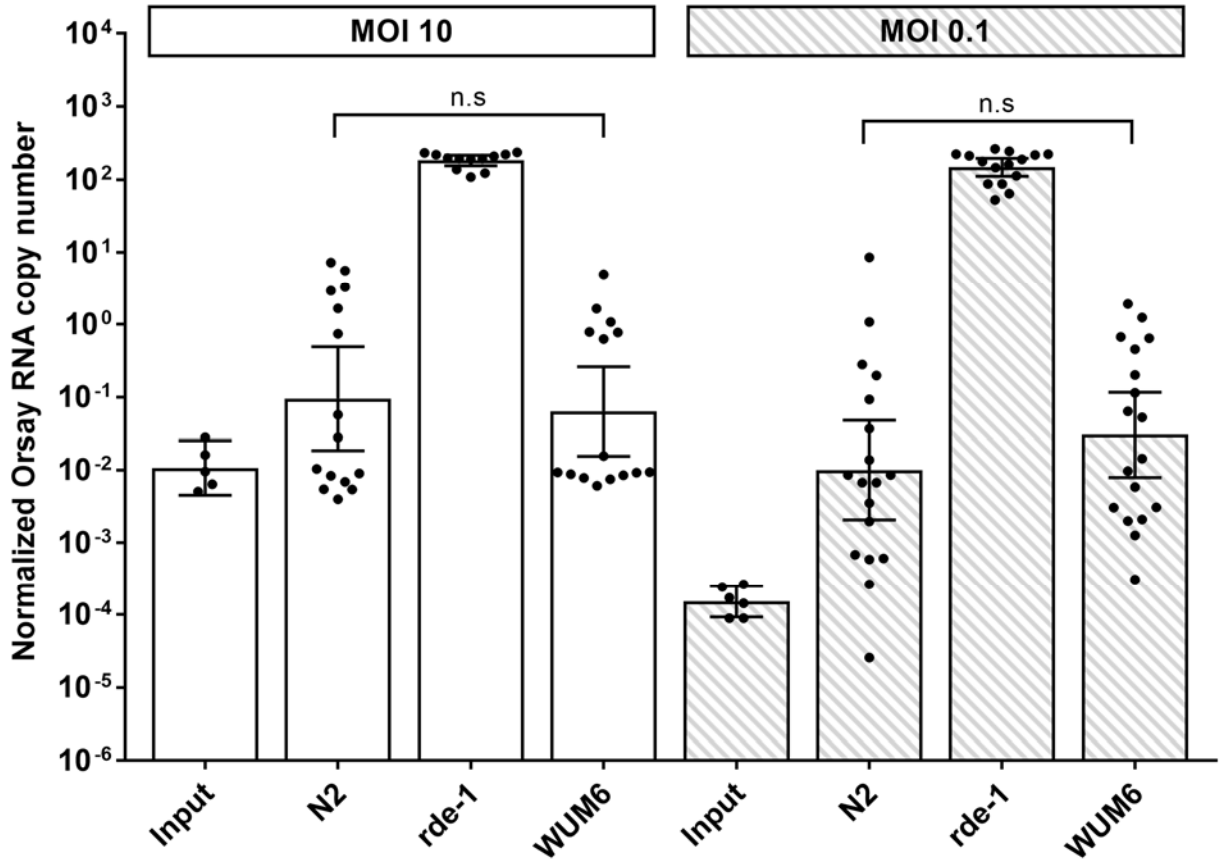


Figure 4.4. The *C17H1* locus deletion mutant WUM6 did not have altered Orsay virus RNA amount. The WUM6 mutant contains a deletion to the *C17H1* locus knocking out 11 of 25 upregulated *C17H1* gene family members. WUM6 was infected with Orsay virus with an MOI of 10 and 0.1 and for two days. The Orsay virus RNA was measured by qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Mann–Whitney U test against N2. n.s. not significant.

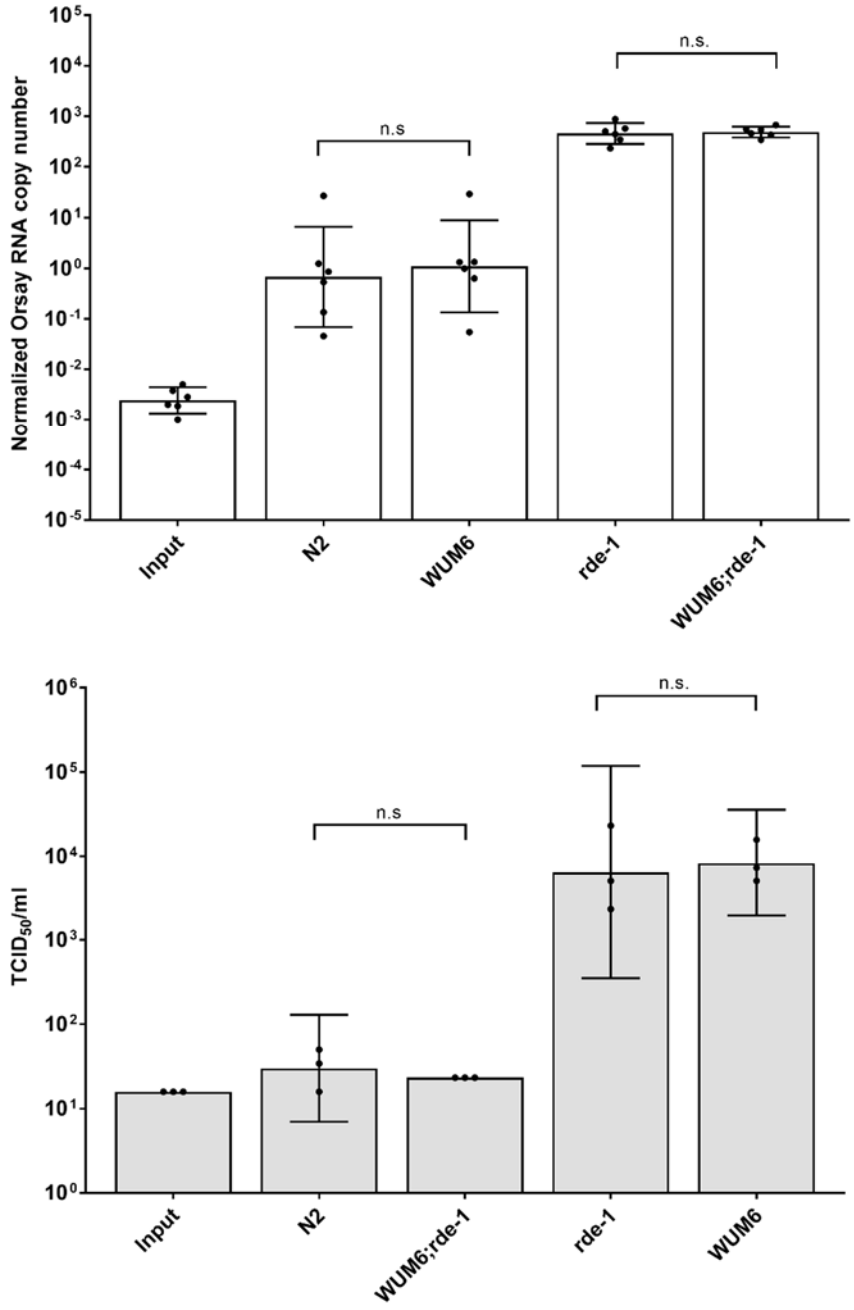


Figure 4.5. The *C17H1* locus alone did not participate in the host-virus interactions. The WUM6 mutant were crossed into an antiviral RNAi defective strain *rde-1.* WUM6 and WUM6;*rde-1*was infected with Orsay virus with an MOI of 10 for three days. A) The amount of Orsay virus RNA was measured by qRT-PCR. B) The amount of infectious Orsay virus was titered with an end-point dilution assay that was similar to $TCID_{50}$. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric

mean, and the 95% CI are shown. Statistical significance was assessed using Mann–Whitney U test against N2. n.s. not significant.

Two genes from the evolutionarily conserved DEGs, *zip-1 and zip-10* may influence Orsay virus infection

From the 58 conserved DEGs, there were 20 strains were available from the million mutation project that had high-impact mutations such as premature stop codon, frameshift, and splicing defects [8]. Additionally, seven genes that have knockout mutants were available from *Caenorhabditis* Genetics Center (CGC). To determine whether any of these mutants were involved in host-virus interaction, I have measured the Orsay virus replication phenotype of these mutants.

First, I tested the 20 mutants from the Million Mutation Project. After the Orsay virus infection assay, I found that the mutant strain VC20199 that have *zip-1* mutation, a gene that belonged to the bZIP transcription factor family, had statistically higher amount of Orsay virus RNA compared to N2 (Figure 4.6) [16]. The VC20199 strain also had higher Orsay virus RNA when infected at a lower MOI of 0.1. However, it was not statistically significant (Supplementary Figure 4.2). This result suggests that *zip-1* may have an antiviral effect. However, mutants from the Million Mutation Project contain additional mutations to the genome [8]. Specifically, the VC20199 contains a missense mutation to the *drh-1* gene which is essential for the sensing of viral RNA for downstream antiviral RNAi pathway (Supplementary Table 3) [2,14]. The specific mutations was an A250T amino acid change on the DRH-1 isoform A. To exclude the possibility that the Orsay virus replication phenotype was due to the exo-RNAi pathway, we conducted a feeding RNAi test with *dpy-3* and found VC20199 was competent in exo-RNAi as the worms displayed a strong dumpy phenotype (Data were not shown). These

results suggest that the VC20199 strain contains mutations that were antiviral in nature, however, the contributing mutations may be *zip-1*, *drh-1*, or other mutations.

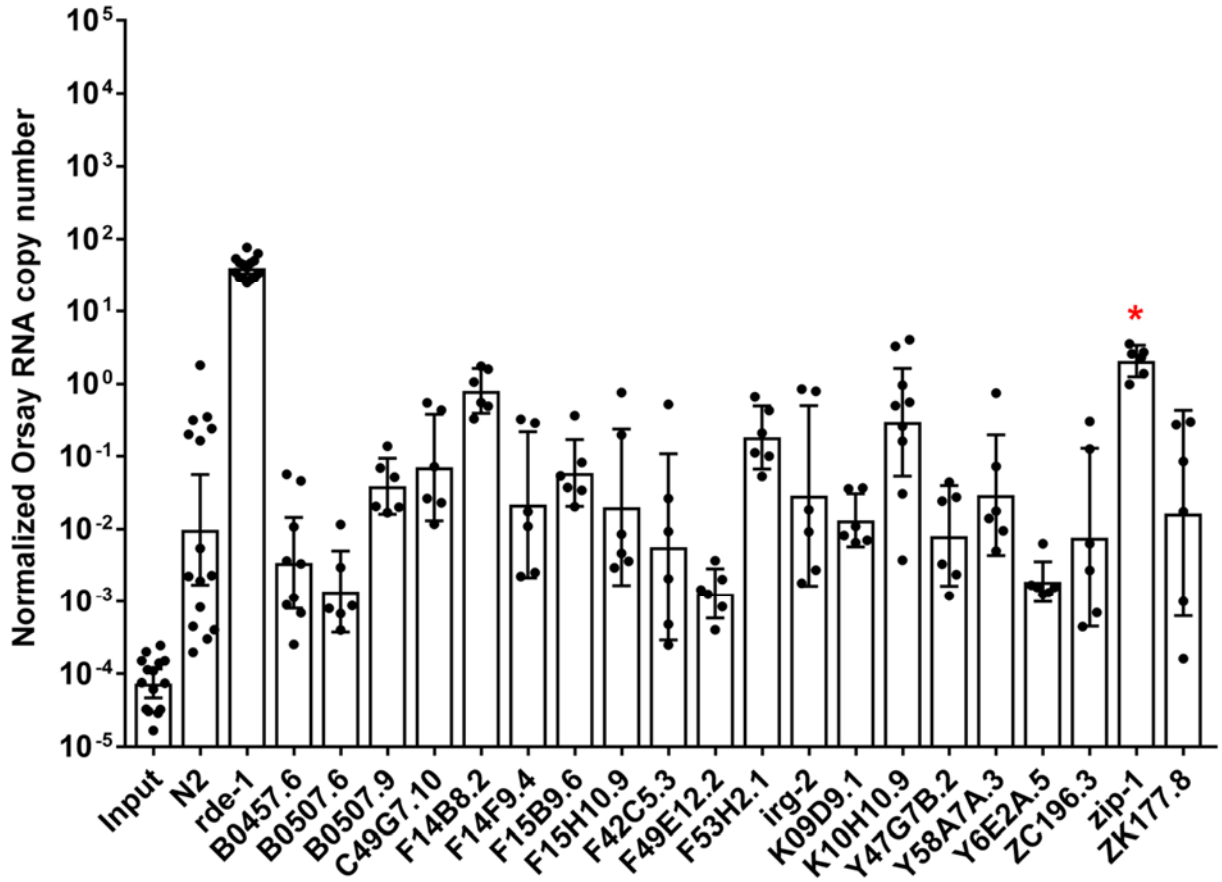


Figure 4.6: Orsay virus replication in knockout mutants from the 58 conserved DEGs. 20 mutants that contain knockout mutations of the 58 conserved virus DEGs were infected by Orsay virus at an MOI of 10 for two days. The Orsay virus RNA was measured by qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against N2. *P < 0.05.

I next tested the seven strains from CGC that had mutations from the list of 58 evolutionarily conserved DEGs. After infection with Orsay virus, two strains had statistically different Orsay virus RNA amount from the N2 control (Figure 4.7A). Interestingly, the two strains with *hmit-1.1* and *zip-10* mutations were lower than N2, suggesting that they were

putative proviral genes. The strain with *sid-5* mutation also had a lower amount of Orsay virus RNA and was statistically lower than N2 when compared individually but not after correction for multiple comparisons. The three genes have annotated functions, *hmit-1.1* is a H+/myo-inositol transporter gene [17], *zip-10* is a bZIP transcription factor [16,18], and *sid-5* is endosome-associated protein required for systemic RNAi [19]. The seven strains were also evaluated for the production of infectious Orsay virus by the end-point dilution assay. I found that while *hmit-1.1*, *zip-10,* and *sid-5* all had lower $TCID_{50}$/ml than N2, only *hmit-1.1* was statistically significant (Figure 4.7B). Since there was ambiguity for the *sid-5* and *zip-10* mutants, I conducted more experiments along with *hmit-1.1* to confirm the phenotype. I found that the *hmit-1.1* mutant did not reproduce the phenotype we initially observed (Figure 4.8A & 4.8B). However, *sid-5* and *zip-10* had the statistically lower amount of Orsay virus RNA than N2 control (Figure 4.8A). *zip-10* mutant also had statistically lower infectious Orsay virus than N2 (Figure 4.8B).

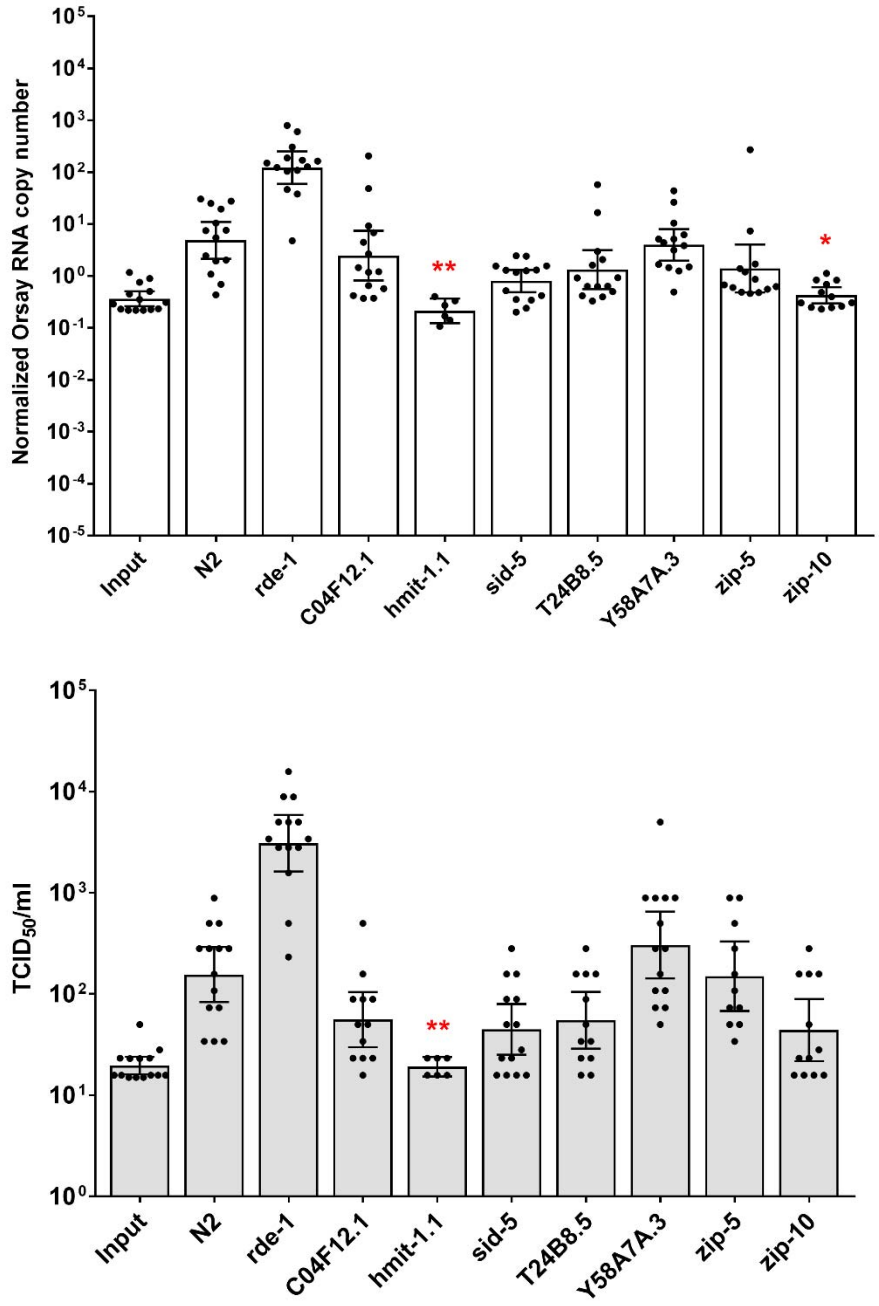


Figure 4.7. Screening of the mutants (CGC) with mutations for the evolutionarily conserved DEGs. Strains with evolutionarily conserved DEG mutants were infected with Orsay virus at an MOI of 10 for five days. A) The Orsay virus RNA was measured by qRT-PCR. B) The amount of infectious Orsay virus was measured by end-point dilution assay. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against N2. *P < 0.05, **P < 0.005.

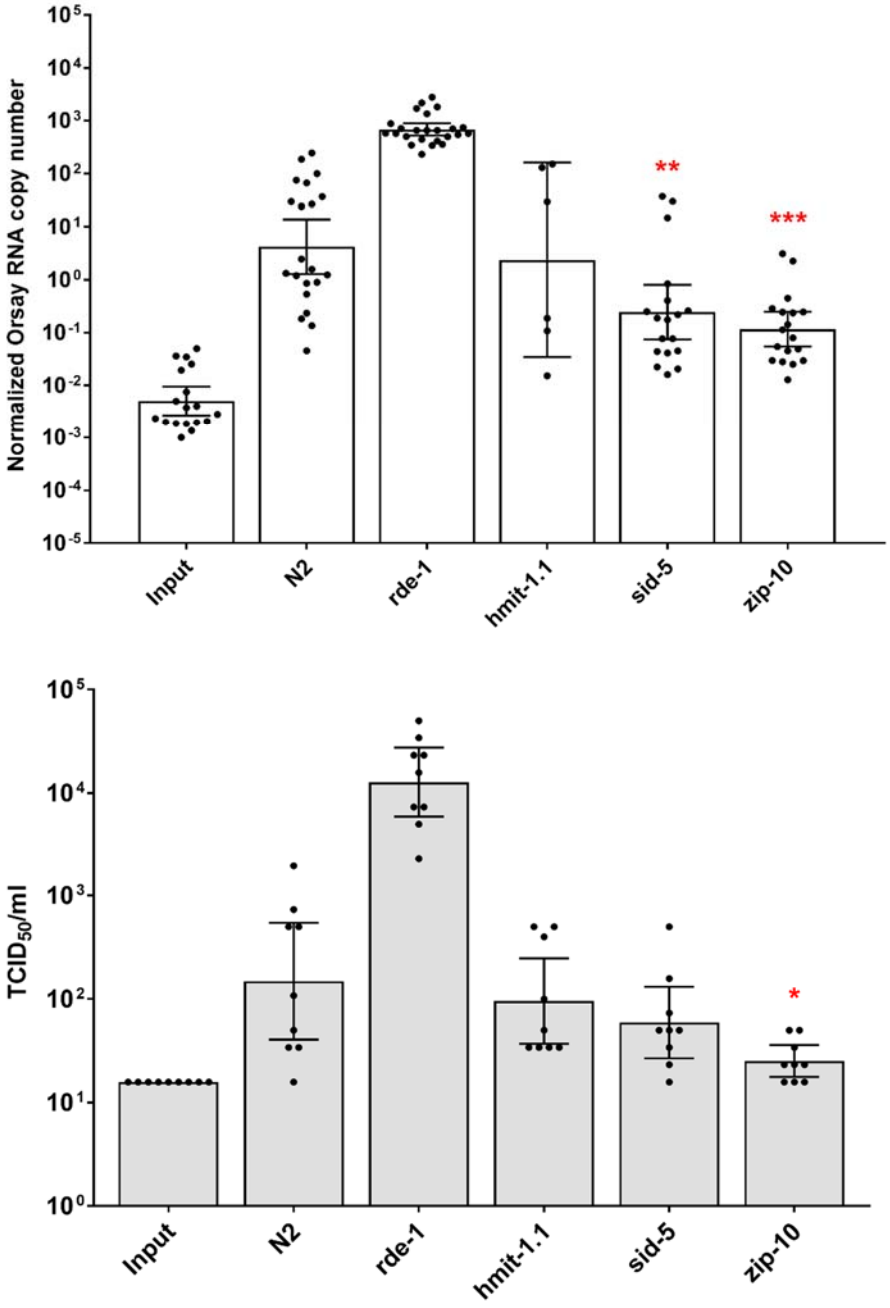


Figure 4.8. Screening of the mutants (CGC) with mutations for the evolutionarily conserved DEGs. 500 animals from each strain with evolutionarily conserved DEG mutants were infected with Orsay virus at MOI 10 for three days. A) The Orsay virus RNA was measured by qRT-PCR. B) The amount of infectious Orsay virus was measured by end-point dilution assay. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance

was assessed using Kruskal-Wallis test with Dunn's post hoc test against N2. *P < 0.05, **P < 0.005. ***P < 0.0005.

I next crossed the three strains into the *rde-1* background to determine if the mutations influenced Orsay virus replication without functional antiviral RNAi. After infecting the mutants with Orsay virus, we found that only *zip-10* mutant in the *rde-1* background was statistically lower than *rde-1* (Figure 4.9).

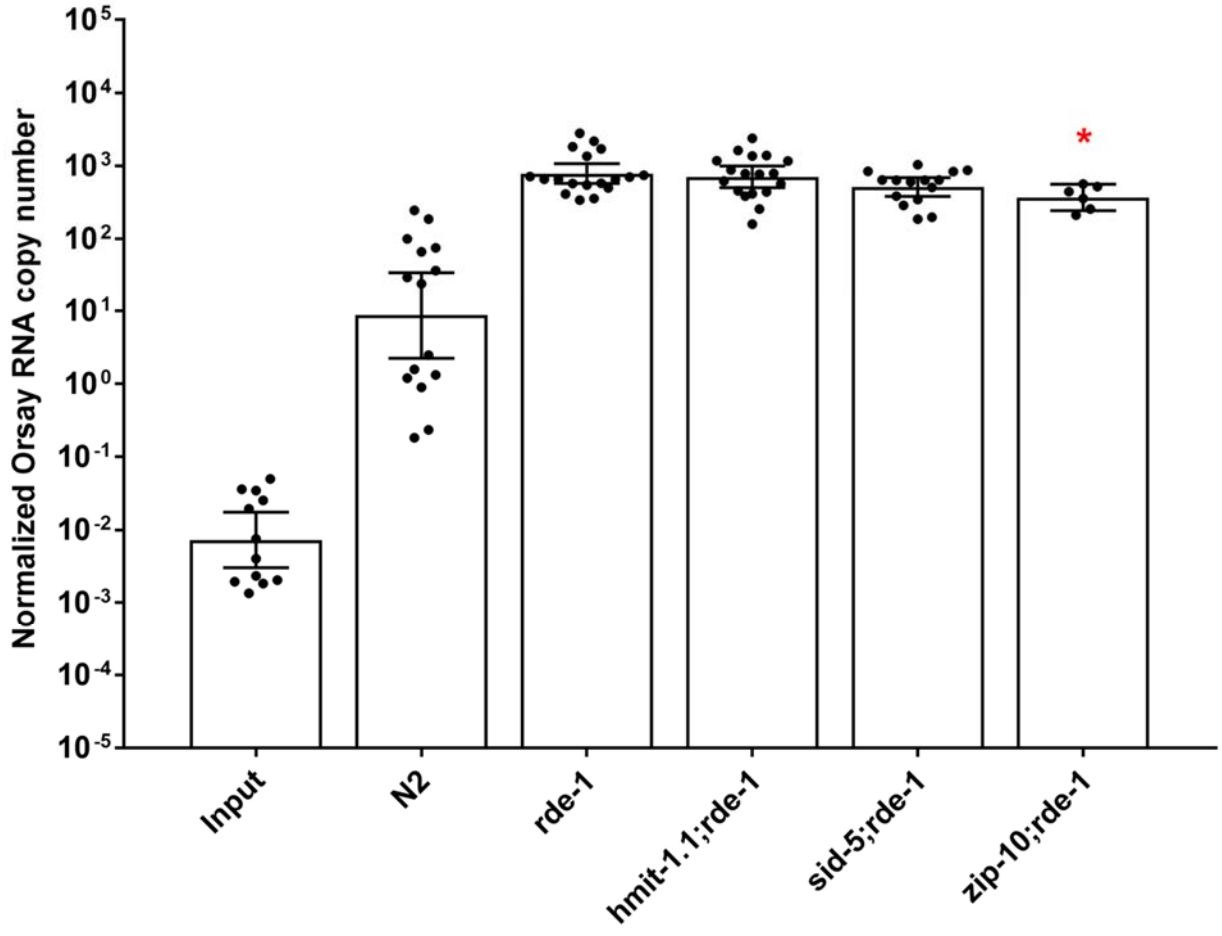


Figure 4.9: Orsay virus replication in mutants crossed into the *rde-1* background. The three mutants *hmit-1.1*, *sid-5*, and *zip-10* were crossed into *rde-1* mutant to make the high virus permissive strains. All strains were infected with Orsay virus and the amount of virus replication measured by both qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown.

Statistical significance was assessed using Kruskal-Wallis test with Dunn's post hoc test against N2. *P < 0.05.

In summary, after screening a total of 27 mutants that contain mutations from the list of 58 evolutionarily conserved DEGs, we found the strain VC20199 with *zip-10* mutation have antiviral properties. I also found *zip-10* mutant that reduced Orsay virus replication and acts in the proviral fashion.

Discussion and Conclusion

With the recent discovery of its naturally infecting Orsay virus, *C. elegans* has became an excellent model organism to investigate host-virus interactions [1]. We can now interrogate the *C. elegans* – Orsay virus model system to better understand the host-virus interactions. With the available genetic tools in *C. elegans* and the assays we developed in the Wang lab, I have systematically screened the evolutionarily conserved DEGs for genes that may influence Orsay virus replications.

The overexpression strains were derived from a forward genetic screen with an integrated GFP reporter strain ERT54 *jyIs*8 [*pC17H1.6::GFP; pmyo-2::mCherry*]; both strains turned on the GFP reporter constitutively. Interestingly, the causal mutation has been mapped to the *C29F2.1*, a member of the *C17H1* gene family that was not up-regulated upon virus infection (personal communication with Emily Troemel). However, one limitation of this approach was that the full set of genes regulated by *C29F2.1* was unclear. Nonetheless, defining the Orsay virus replication phenotype in JY1 and JY3 can help us understand that if the *C29F2.1* regulated genes that include several virus DEGs are involved in host-virus interactions. JY1 and JY3 had significantly lower Orsay virus in the MOI 0.1 condition, suggesting a protective phenotype,

However, the phenotype is not observed in the MOI 10 condition, this suggest that the protective effect is not robust and can be masked by high tier infection. Because it is not possible to rule out the possibility of non-viral DEGs attributing to the observed phenotype, further investigation is needed.

One important genetic tool in *C. elegans* is the sliencing of trasnscript by feeding RNAi. The feeding RNAi is an efficient way to konckdown gene expression, however, there are some challenges to feeding RNAi, such as not 100% knockdown of mRNA and potential competition between exo-RNAi and antiviral RNAi [2,9,13]. The feeding RNAi of GFP in the N2 GFP reporter worms resulted in more Orsay virus RNA. This result suggested that there was indeed competition between the exo-RNAi and antiviral RNAi as GFP should not influence Orsay virus replication. In both the N2 background and *drh-1* background, no RNAi knockdown produced a statistically significant change from the control. It is possible that the limitation of RNAi confounded the results, or it is also possible that indeed none of these genes were not related to the host-virus interactions.

The *C17H1* gene family has a human ortholog, ALSCR12 but both the human orthologs and the *C17H1* gene family have no known functions. Multiple individual genes were examined from the RNAi knockdown screen and none of the genes influenced Orsay virus replication. One possible explanation was that the *C17H1* genes have a weak interaction with Orsay virus replication and that the limitation of RNAi knockdown was not able to provide conclusive results. Another possibility was that there may be redundancy to the *C17H1* gene family, therefore, knocking down individual genes did not change the Orsay virus replication phenotype.

From the stable mutant screen, I have found that *T27E7.6* and *zip-1* appeared to be antiviral genes. However, it is possible that the casual mutations were not the two gene,

especially *zip-1* as the mutant strain also had a mutation important for Orsay virus infection in *drh-1*. There were multiple independent experiments for *hmit-1.1, sid-3*, and *zip-10.* There were conflicting results for the *hmit-1.1* mutant. Since only one out of the three experiments showed that *hmit-1.1* mutant had lower Orsay virus replication and the two other experiments did not, *hmit-1.1* most likely was not involved the host-virus interactions. The *sid-5* mutant had consistent lower Orsay virus replication in all three independent experiments but only have one experiment statistically lower than N2. These results suggest that it is possible that *sid-5* play a minor role in host-virus infection. However, it is also possible that *sid-5* was not involved Orsay virus replication and that the one statistically significant results occurred by chance. Lastly, the *zip-10* mutant also consistently had lower Orsay virus replication phenotype in all three experiments and was statistically significant in all but one assay. These results suggest that *zip-10* played a role in the host-virus interactions and given the mutant had lower Orsay virus replication, *zip-10* most likely acts in a proviral fashion. To confirm that these candidate genes were indeed part of the host-virus interactions, further experiments should be conducted.

In conclusion, I have found 2 strong candidate genes that interacts with Orsay virus infection in *T27E7.6* and *zip-10*. These two genes were not implicated in any virus infections previously and could be novel host pathways that interacts with the Orsay virus.

Materials and Methods

Worm strains

C. elegans strains N2, and *rde-1* (WM27) along with *C. briggsae* strain AF16 were obtained from the *Caenorhabditis* Genetics Center (CGC). The GFP reporter strain ERT54 *jyIs*8

[*pC17H1.6::GFP; pmyo-2::mCherry*] was kindly provided by Emily Troemel [3]. Additional strains used for screening were listed in Supplementary Table 1.

CRISPR knockout in *C elegans*

To make a 35kb *C17H1* region deletion mutant (WUM6), we used a coconversion strategy described by Arribere *et al*. (2014) with a *dpy-10* sgRNA as the selection marker. We first constructed the short guide RNA (sgRNA) plasmids (*C17H1.3*_sg2 and *C17H1.7*_sg2) by designing the sgRNA (5'-gaacagagtgaagcaggaag-3') to target *C17H1.3* and sgRNA (5'-acgggcagatatacagagac-3') to target *C17H1.7*. The short oligonucleotides were synthesized (IDT), annealed and ligated into a modified version of DR274 (Addgene Plasmid #42250) where the sgRNA site was flanked by BsaI and *C. elegans* U6 promoter and terminator from pU6::klp-12_sgRNA (Addgene #42250) (Gift from Michael Nonet). The constructed sgRNA expression plasmids (20 ng/uL each) were co-injected into N2 worms with *C17H1* region single stranded donor DNA (5'-attttgctcttatcacatttatagaaatgacaaaagtcaccgagccctcggtttttctttgcgatagttcagagcttctcaaatctctca-3') (500nM), pDD162 (Addgene Plasmid #47549) that expressed Cas9 with empty sgRNA (50ng/uL), *dpy-10(cn64)* single stranded donor DNA (500nM), and *dpy-10(cn64)* sgRNA (20 ng/uL). Dumpy or roller F1s were selected to ensure that all CRISPR/Cas9 were expressed and *dpy-10* was successfully modified. Worms were subsequently genotyped for the deletion with single worm 3-primer PCR where a primer pair flanking the whole deletion region and a third primer in the proposed deleted region (GW503 (5'-gttagaaatgcgctgtgacgt-3'), GW504, (5'-agctcgctcagcattgttg-3'), GW515 (5'-ggaatggtactaccagtgctg-3')). The *dpy-10* animal was crossed with N2 to obtain the final ~34kb deletion mutant strain without the dumpy phenotype.

Worm infection on solid medium

Uninfected *C. elegans* (N2 and *rde-1*) and uninfected *C. briggsae* (AF16) were synchronized by standard bleach treatment. 500 embryos were seeded per well into 6-well NGM plates containing 20-30 µl of OP50 food and maintained at 20°C. 16 hours after bleaching; worms were inoculated by adding 20 µl of Orsay virus of starting at 2.5 x 10^5 $TCID_{50}$ /ml for an MOI of ~10 directly over the bacterial lawn. Lower MOI infection conditions were obtained by additional 10-fold dilution of the starting Orsay virus stock. After 2 days or 3 days post-infection, animals were rinsed off from the wells with 1 ml M9 buffer, the supernatant was removed after centrifugation, and 350 ul Trizol were added to the samples.

RNAi feeding knockdown infection on solid medium

RNAi feeding was used for gene knockdown as described [9]. E. coli strain HT115 carrying double strand RNA expression cassettes for genes of interest were induced using established conditions and were then seeded into 6-well NGM plates. Empty vector, *rde-1* RNAi clones were from the Ahringer RNAi library [20]. 500 arrested GFP reporter animals were seeded into each well of a 6-well plate. After 2 days of RNAi feeding, Orsay virus was added to the plates as described with solid medium infection. After 2-day infection, the infected *C. elegans* animals were collected as described with solid medium infection.

Measuring Orsay virus RNA by real-time quantitative one-step RT-PCR (qRT-PCR

Infected worm samples in Trizol were vortexed for 10 minutes at room temperature and centrifuged at 15,000g for 5 minutes. The Trizol supernatants were extracted with Direct-zol RNA miniprep kit (Zymo) and eluted into 60 µl water. The extracted samples were diluted 1:100

and 5 µl of the diluted RNA were used for one-step real-time RT-PCR (Fast virus Thermo-fisher). Real-time qPCR was performed using Taqman qPCR master mix reagents (Applied Biosystem) on a ViiA7 real-time PCR system (Applied Biosystem) following the manufacturer's suggested protocol. The copy number for each sample were calculated from a standard curve and were normalized to an internal control *cdc-42* gene.

Measuring Orsay virus titer with reporter infection assay

To measure the infectious titer of viruses, we employed a method similar to tissue culture infectious dose 50% ($TCID_{50}$) using live *C. elegans* or *C. briggsae* in wells instead of cultured cells. We were not able to measure killing of nematodes as none of the viruses were lethal. To measure infectivity in a well, we used the GFP reporter strain ERT54 *jyIs8* to determine whether replication of viral RNA occurred by the GFP signal. Briefly, the GFP reporter worms were synchronized and 50 worms were plated to each well on a 96-well plate seeded with 20 µl 50x concentrated OP50. Virus filtrates were serially diluted 10-fold to 10^{-5}. 20 µl of each dilution were added to each well containing animals and combined to have four total replicates per condition. Infected animals were incubated at 20°C for three days shaking @ 150 RPM. All wells were anesthetized by a final concentration of 20 nM sodium azide, and the GFP fluorescence of the well was scored with Cytation 3 (BioTek). A well has more than two worms positive with GFP was considered a positive infection. $TCID_{50}$ were then calculated using the Spearman and Karber algorithm.

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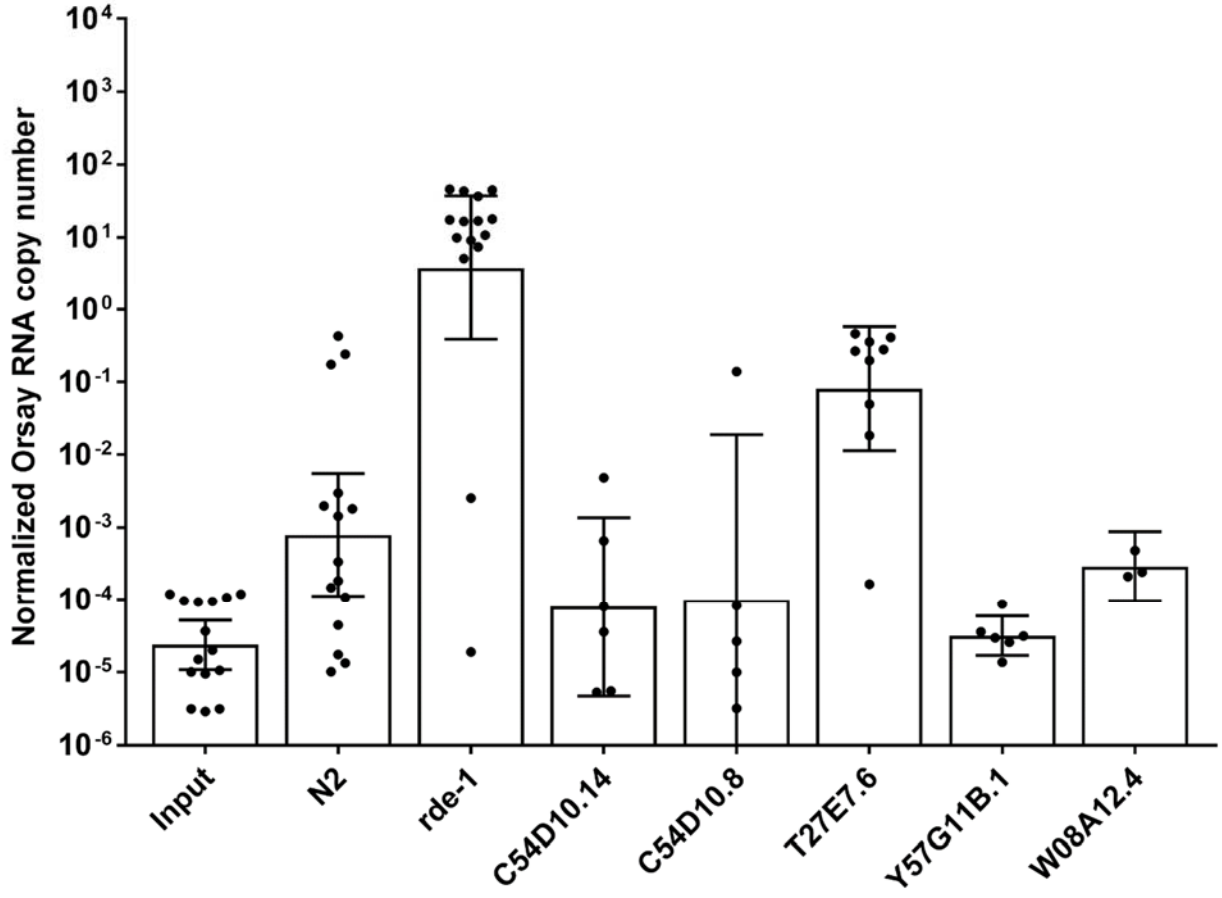
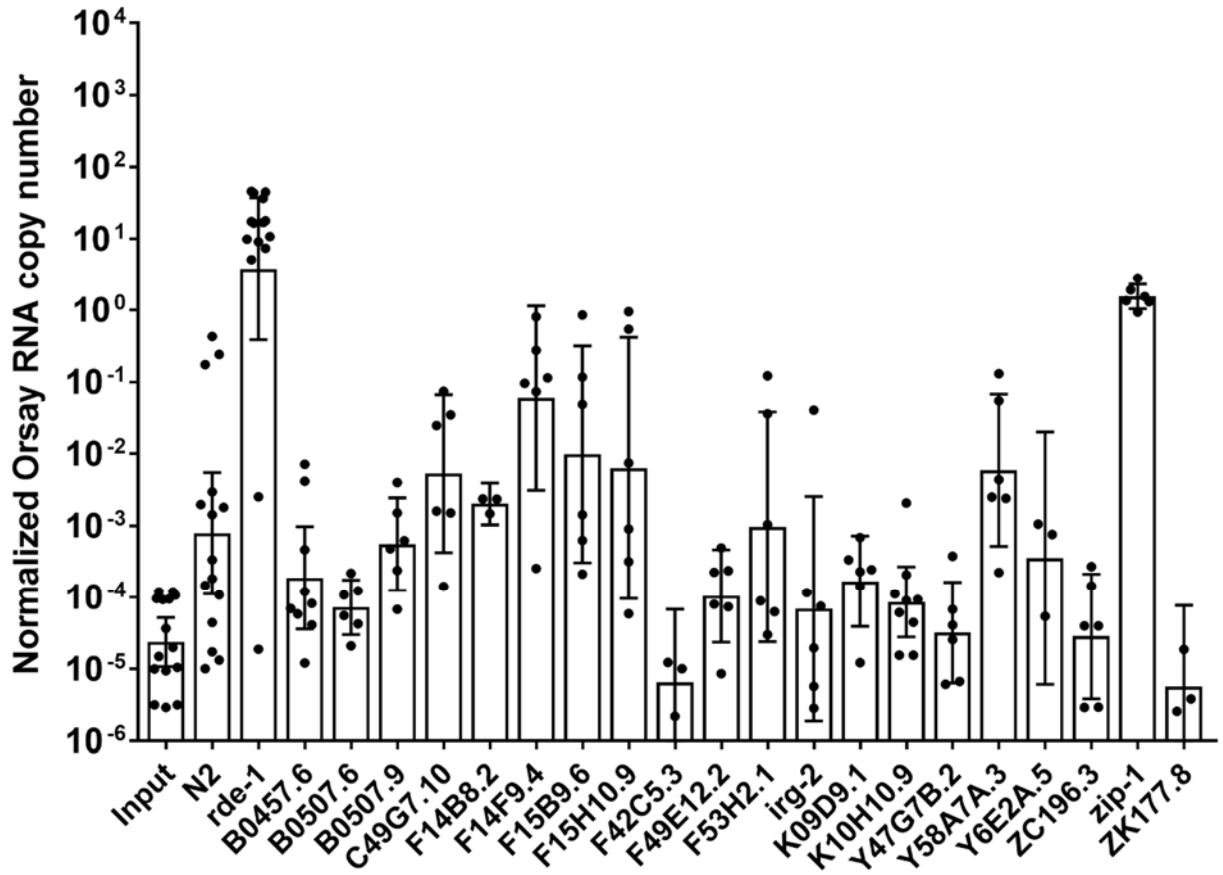
Supplementary Materials



Supplementary Figure 4.1. Screening of *C17H1* gene family member mutants for Orsay virus replication with low MOI infection. Mutants containing *C17H1* gene family members were infected with Orsay virus at an MOI of 0.1 for two days. The Orsay virus RNA was measured by qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Dunnett's Multiple Comparisons Test against N2.



Supplementary Figure 4.2: Orsay virus replication in knockout mutants from the 58 conserved DEGs with lower MOI. 20 mutants that contain knockout mutations of the 58 conserved virus DEGs were infected by Orsay virus at an MOI of 0.1 for 2 days. The Orsay virus RNA was measured by qRT-PCR. The three control conditions used were the input virus, infection of N2 and *rde-1* strains. The individual value, the geometric mean, and the 95% CI are shown. Statistical significance was assessed using Kruskal-Wallis test with Dunnett's Multiple Comparisons Test against N2. No mutants were statistically different from N2.

Supplementary Table 4.1. Mutant strains used.

Sequence	Gene	Strain	Type
C17H1 gene family members			
C54D10.8	C54D10.8	VC20072	nonsense

C54D10.14	C54D10.14	VC20366	intron, splicing
Y57G11B.1	Y57G11B.1	VC40434	intron, splicing
T27E7.6	T27E7.6	VC40577	intron, splicing
W08A12.4	W08A12.4	VC40612	nonsense
Evolutionarily conserved DEGs (Million Mutation Project)			
Y75B8A.35a	zip-1	VC20199	deletion, frame shift
Y58A7A.3	Y58A7A.3	VC20399	intron, splicing
B0507.6	B0507.6	VC20427	intron, splicing
B0457.6	B0457.6	VC40007	deletion, frame shift
F49E12.2	dod-23	VC40063	intron, splicing
C49G7.5	irg-2	VC40096	intron, splicing
K10H10.9	K10H10.9	VC40140	deletion, frame shift
F53H2.1	F53H2.1	VC40146	nonsense
F15H10.9	F15H10.9	VC40209	start ATG
C49G7.10	C49G7.10	VC40358	nonsense
B0507.9	B0507.9	VC40388	intron, splicing
F15B9.6	F15B9.6	VC40517	nonsense
Y47G7B.2	Y47G7B.2	VC40608	intron, splicing
F42C5.3	F42C5.3	VC40701	nonsense
F14B8.2	F14B8.2	VC40799	start ATG
Y6E2A.5	Y6E2A.5	VC40816	nonsense
ZK177.8a	ZK177.8	VC40866	nonsense
F14F9.4	F14F9.4	VC40934	nonsense
ZC196.3	ZC196.3	VC40951	intron, splicing
K09D9.1	K09D9.1	VC40974	nonsense
Evolutionarily conserved DEGs (CGC)			
C04F12.1	C04F12.1	WM153	deletion, frame shift
Y51A2D.4	hmit-1.1	RB2163	deletion, frame shift
F14B8.2	sid-5	RB2536	deletion, frame shift
C34D1.5	zip-5	TM2038	deletion, frame shift
T27F2.4	zip-10	RB2499	deletion, frame shift
T24B8.5	T24B8.5	VC2477	deletion, frame shift
Y58A7A.3	Y58A7A.3	RB2449	deletion, frame shift

Supplementary Table 4.2. Mutations in VC40577 strain.

Gene	Allele	Mutation	Effect	Type
ain-2	gk702316	T->C	affects splicing	intron, splicing
let-363	gk702319	T->A	N1241K	missense

unc-82	gk702515	C->T	A115V	missense
cdc-48.1	gk961350	3741N->		deletion, across exon boundary
str-250	gk702588	C->T	L294F	missense
pro-2	gk702392	T->C	M258V	missense
C08F1.6	gk702357	G->A	A12V	missense
unc-89	gk702311	T->C	F5703L	missense
C16A3.4	gk702450	C->T	A19V	missense
pbrm-1	gk702326	C->T	P668S	missense
syg-2	gk702675	T->A	Y759stop	nonsense
C35C5.6	gk702662	C->T	P1215S	missense
lact-7	gk702663	G->A	G155R	missense
cps-6	gk702314	T->C	D291G	missense
set-5	gk702591	C->T	P865S	missense
C50E3.5	gk702574	C->T	T391I	missense
mpz-1	gk702398	T->C	D1725G	missense
ceh-17	gk702315	G->A	T157I	missense
D1043.1	gk702405	A->T	K886stop	nonsense
D1081.9	gk702332	G->A	S179F	missense
E01G6.2	gk702666	T->A	F74I	missense
din-1	gk702406	G->A	P->L	missense
str-111	gk702597	T->A	E197D	missense
F15E11.15	gk702563	C->T	G157E	missense
srd-46	gk702667	C->T	T148M	missense
F20D6.5	gk702576	C->T	P335L	missense
asp-5	gk702577	T->C	L199P	missense
F21G4.6	gk702656	T->G	M115L	missense
jmjd-3.2	gk702673	T->C	L630P	missense
F25B3.5	gk702580	C->T	E321K	missense
F28C6.1	gk702383	G->A	R174Q	missense
F28C6.5	gk702384	G->A	D89N	missense
lrp-1	gk702328	T->G	V2684G	missense
F32B6.9	gk702522	G->A	W141stop	nonsense
F32D1.3	gk702567	C->T	G692E	missense
nhr-37	gk702573	C->T	P130L	missense
F44F4.1	gk702399	C->T	P34S	missense
F49C5.12	gk702412	G->A	A4V	missense
acl-4	gk702632	C->T	G426E	missense
pqe-1	gk702448	C->T	H317Y	missense
F53A2.7	gk702488	G->A	G168D	missense
mtm-6	gk702489	C->T	P33S	missense

F53H2.1	gk702621	C->A	Q713K	missense
F53H2.1	gk702622	G->A	Q713K	missense
F53H2.1	gk702623	A->T	R738S	missense
rog-1	gk702355	C->T	G479R	missense
F58E6.13	gk702581	C->T	P183L	missense
math-32	gk702358	G->A	G260D	missense
scl-13	gk702566	C->T	A72T	missense
H12D21.6	gk702592	C->T	G94E	missense
K05C4.3	gk702353	C->T	E27K	missense
aptf-1	gk702374	G->A	R35K	missense
K07A1.5	gk702337	C->T	A81V	missense
gpd-3	gk702634	C->T	R329K	missense
smo-1	gk702291	G->A	S54N	missense
K12H6.5	gk702360	G->A	P22S	missense
R119.2	gk702282	G->A	R178C	missense
vet-1	gk702322	A->C	D112E	missense
rps-24	gk702495	G->A	V4I	missense
rmd-5	gk702323	C->T	P239S	missense
T23F1.3	gk702595	C->T	G14E	missense
T27E7.6	gk702541	C->T	affects splicing	intron, splicing
T28C6.7	gk702518	C->T	S1976L	missense
afd-1	gk702300	G->A	V165I	missense
twk-33	gk702605	T->A	I125F	missense
twk-33	gk702606	A->C	Y119stop	nonsense
mig-1	gk702286	C->T	D453N	missense
Y39C12A.1	gk702528	C->T	C123Y	missense
bre-2	gk702486	G->A	A304V	missense
xrn-1	gk702428	G->A	R646Q	missense
snrp-200	gk702414	G->A	P2051S	missense
gcn-1	gk702438	C->T	A162V	missense
Y51F10.10	gk702297	G->A	A163V	missense
Y54E2A.4	gk702430	G->A	E1844K	missense
fbxa-219	gk702440	C->T	S201F	missense
Y55B1BR.2	gk702432	C->T	H561Y	missense
Y57A10A.27	gk702409	G->A	L315F	missense
Y59C2A.3	gk702359	G->A	P327L	missense
ptr-21	gk702284	G->A	V236I	missense
Y69A2AR.7	gk702501	C->T	R181C	missense
aqp-3	gk702524	C->T	E331K	missense
Y71G12B.25	gk702295	G->A	T45I	missense

srv-13	gk702510	C->A	T264N	missense
Y75B8A.13	gk702475	C->A	V449F	missense
Y75B8A.13	gk702476	G->A	A446V	missense
Y87G2A.2	gk702347	C->T	L184F	missense
ZC455.1	gk702585	C->T	P183L	missense
ubq-2	gk702483	C->T	G53R	missense
ZK688.3	gk702455	C->T	R8C	missense
ZK863.4	gk702584	C->T	A612T	missense

Supplementary Table 4.3. Mutations in VC20199 strain.

Gene	Allele	Mutation	Effect	Type
rpia-1	gk177878	G->A	V48I	missense
arrd-25	gk300064	T->C	C174R	missense
srsx-18	gk236191	C->T	P199S	missense
C05B5.17	gk183203	G->A	A97V	missense
C07E3.3	gk152692	T->G	D282A	missense
C17E4.6	gk118735	G->A	R33Q	missense
C27D9.1	gk141511	G->A	V37I	missense
C33A11.1	gk303215	C->T	S513F	missense
C34D10.2	gk286563	T->A	M565K	missense
stam-1	gk111998	G->A	P424L	missense
C48B6.9	gk113945	T->C	T15A	missense
tag-333	gk254472	G->A	T737I	missense
scrt-1	gk105446	G->A	G118D	missense
F07F6.4	gk142911	C->T	A294T	missense
F09C8.1	gk305123	C->T	G134R	missense
F09E5.10	gk315212	C->T	G581R	missense
F09G8.5	gk179828	G->A	G269S	missense
emb-27	gk148369	C->T	A439T	missense
F13A7.1	gk257921	C->T	A330T	missense
drh-1	gk204047	C->T	A250T	missense
him-4	gk290282	C->T	P1577L	missense
srh-92	gk239386	C->T	M159I	missense
F28C10.3	gk269286	C->T	G161E	missense
akt-2	gk300327	C->T	P75S	missense
nac-1	gk302130	G->A	A85T	missense
ags-3	gk280162	C->T	R493Q	missense
F33H2.8	gk130561	G->T	L130I	missense
npr-7	gk289341	C->T	D352N	missense
gst-24	gk159943	C->T	R131Q	missense

nas-39	gk305825	C->T	A790V	missense
F38E9.6	gk952675	CA->C		deletion, frame shift
F40H3.1	gk144430	T->A	C447S	missense
cyp-33C3	gk228633	C->T	A445V	missense
F41C3.5	gk141473	G->A	A344T	missense
tbc-18	gk287318	C->T	R605C	missense
F41G3.2	gk145604	C->T	L74F	missense
clec-119	gk133331	C->T	A47V	missense
F48E3.8	gk285385	C->T	G954E	missense
F53F8.5	gk267729	G->T	P299Q	missense
F55F8.3	gk111542	C->T	V208I	missense
srz-19	gk199039	C->T	L299F	missense
btb-20	gk162064	C->T	D251N	missense
faah-3	gk193118	T->G	H418S	missense
faah-3	gk193119	G->A	H418S	missense
H01M10.1	gk273859	C->T	G147E	missense
H03A11.2	gk302915	C->T	E1212K	missense
pms-2	gk247737	C->T	affects splicing	intron, splicing
ttr-1	gk183053	G->A	D60N	missense
K06A1.2	gk145010	T->A	F292I	missense
K06G5.1	gk300503	G->A	G172D	missense
K10F12.6	gk164534	G->A	A89V	missense
K12H6.12	gk136942	C->T	T261I	missense
M02G9.1	gk152658	C->T	P378S	missense
nhr-99	gk224611	C->T	P71L	missense
R03G8.6	gk297828	C->T	V627M	missense
R04A9.7	gk268952	C->G	S341T	missense
ech-4	gk153777	C->T	A208V	missense
R11A8.5	gk210682	C->T	A340T	missense
atl-1	gk243777	C->T	M1019I	missense
unc-50	gk183862	G->A	T270I	missense
ucr-2.2	gk302795	C->T	A360V	missense
glh-4	gk107809	G->A	G416E	missense
aat-4	gk203428	C->T	affects splicing	intron, splicing
spat-3	gk278486	C->T	E1174K	missense
T19D12.2	gk145401	A->C	N330H	missense
T21B10.4	gk149876	C->T	R265C	missense
T23F11.2	gk173194	T->C	I193V	missense
T26C12.2	gk197673	C->T	A17T	missense
haf-4	gk101015	G->A	S233N	missense

W07G1.1	gk160708	C->T	V222M	missense
W09C5.1	gk127467	G->A	A113V	missense
nkcc-1	gk217690	C->T	V->I	missense
Y37D8A.4	gk189192	G->A	R7C	missense
Y38E10A.22	gk157775	T->A	Y193F	missense
Y39B6A.1	gk264044	G->A	H667Y	missense
Y39E4A.3	gk189510	G->A	L317F	missense
col-96	gk186769	G->A	P160S	missense
Y48E1A.1	gk160133	C->T	D1599N	missense
Y51H1A.2	gk160512	C->T	R768Q	missense
srh-42	gk161601	C->T	E156K	missense
Y56A3A.7	gk187251	T->G	D270A	missense
Y57A10A.4	gk156680	C->T	P416S	missense
csn-1	gk261258	C->T	M464I	missense
Y73C8C.10	gk230096	T->C	D379G	missense
zip-1	gk944976	CA->C		deletion, frame shift
lron-13	gk190679	G->A	V574M	missense
Y8A9A.2	gk139244	C->T	A87V	missense
ZC449.1	gk279542	A->G	K200E	missense
ZC487.1	gk237879	C->T	P69S	missense
ZC84.6	gk181606	G->A	A1405V	missense
ZK1010.2	gk189529	C->T	R249Q	missense
nep-1	gk155586	C->T	D88N	missense
pgl-1	gk204608	C->T	E16K	missense
ZK470.6	gk277524	C->T	C88Y	missense

Chapter 5: Discussion and Conclusion

With the discovery of viruses that naturally infect *Caenorhabditis* nematodes, *C. elegans* has become a great model organism to study host-virus interactions. The transcriptional profiling study of both virus and microsporidium infection provided insights into the host response to pathogens. We found that distinct pathogens such as Orsay virus and *N. parisii* elicited a similar set of DEGs in *C. elegans*, suggesting that these DEGs may constitute a broad pan-microbial response to infection. Additionally, within the transcriptional profile of viral infection in the two different nematode species *C. elegans* and *C. briggsae*, we found a shared set of 58 evolutionarily conserved transcriptional responsive genes to viral infection, many of which have no known function. Given the fact that diverse hosts regulate these common genes in response to distinct viral infections suggests that they play important roles.

Characterizing how Orsay virus replicates in *C. elegans* is an important precursor to studying host-virus interactions. One interesting finding was that the laboratory reference strain N2 was highly variable, especially in the low MOI conditions (MOI < 1). I also found that the liquid culture infection did not reduce variation in the similar MOI. The high variation in N2 may indeed indicate that the host-virus interaction relies on some stochastic process that were yet to be defined. I found that the process of feeding RNAi may compete with antiviral RNAi, therefore, proper control is required when interpreting results of Orsay virus replication from RNAi knockdown experiments.

I used the available genetic tools including feeding RNAi and knockout mutants to determine if the 58 evolutionarily conserved DEGs directly interact with Orsay virus. From the feeding RNAi assays we did not found any of the 58 DEGs to be influencing Orsay virus replication. One limitation of feeding RNAi knockdown is the inability to completely abolish the expression of the targeted genes. Another limitation would be the competition between the

feeding RNAi and the antiviral RNAi. With these limitations, a non-robust phenotype from the feeding RNAi experiments could imply either a weak interaction or no interactions with Orsay virus. However, with genes that have important roles in the host-virus interactions, feeding RNAi still functions as a good method to efficiently screen large number of genes.

From the screening of stable mutants, I had found that *T27E7.6* and *zip-1* appeared to be antiviral genes whereas *zip-10* likely played a proviral role. All three mutants containing the specified mutation have reproducible and strong change to Orsay virus replication. None of the three genes were previously known to be associated with virus replication, suggesting that these genes may represent completely novel host-virus interactions. However, given that these mutants were generated from chemical mutagenesis, other mutations exist in these strains and may be responsible for the observed phenotype. This was especially true for the strain with *zip-1* mutation as it contains another mutation in *drh-1,* which is an important antagonist of Orsay virus infection. Downstream experiments such as generating clean knockout of these genes with CRISPR/Cas9 and transgenic rescue of the worms are needed to determine the true causal mutations.

Collectively, the identification of the virus response genes, the development of assays to efficiently and reliably determine Orsay virus replication phenotypes, and the discovery of genes that alter Orsay virus infection of *C. elegans* provide a foundation for future studies of host virus interactions in this model system.