# Potent Transcriptional Response of Aged *Drosophila melanogaster* Following Infection with an RNA Virus

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## Background

Older individuals tend to exhibit higher mortality rates in comparison with younger individuals following virus infection. Immunosenescence is a contributing factor to increased mortality, however, the exact underlying mechanisms are not fully understood. This phenomenon can be further investigated using the model organism *Drosophila melanogaster.* The fruit fly *Drosophila* mounts potent innate immune defenses against a variety of microorganisms including viruses and serves as an excellent model organism for studying host-pathogen interactions. With its relatively short lifespan, the fruit fly is also an organism of choice for aging studies.

We investigated how aging impacts *Drosophila*'s ability to respond to FHV infection. We demonstrate that wild type 30-days-old *Drosophila* succumb more rapidly to infection in comparison with younger (5-days-old) adults and that the increased mortality is not accompanied by an increase in virus load. These results suggest that mechanisms different from those in control of pathogen burden affect survival to FHV infection of the aged organism. We next used RNA sequencing (RNAseq) to compare transcriptional responses of young and aged *Drosophila* hosts that have been infected with FHV or injected with a control solution. We found that two days post-infection, older hosts exhibited larger transcriptional responses than younger individuals, upregulating ~2 times more genes and downregulating ~2.8 times more genes than young flies. Among differentially regulated genes for both age groups, we found 93% and 57% overlap between upregulated and downregulated genes, respectively. Gene ontology analysis for Biological processes of differentially regulated genes in older FHV-infected flies revealed strongest upregulation of genes encoding for factors involved in neurogenesis and transcription and strongest downregulation for genes involved in organismal reproduction and proteolysis as well as carbohydrate and lipid metabolism. Among genes specifically downregulated in aged FHV-infectd flies, we found an enrichment for mitochondria-related genes. This suggests that FHV infection possibly promotes deregulation of metabolic function in the aged fly leading to more rapid death.

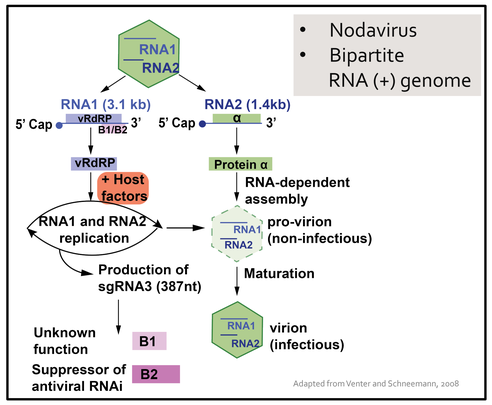
Our findings will set the stage for future analysis of the mechanisms that underlie the increased mortality of older flies and the identification of novel age-specific factors associated with this process.

## Acknowledgements

We thank Dr. Annette Schneemann (Scripps Research Institute, La Jolla, CA for FHV stock and Novogene Co, LTD for RNAseq and Bioinformatic analysis).

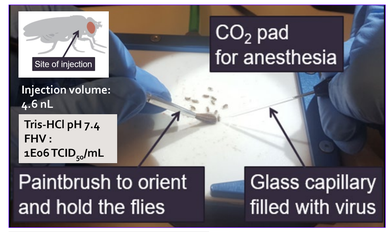
## Methods

### Flock House Virus cycle

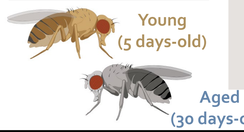



- Nodavirus
- Bipartite RNA (+) genome

### Experimental injection set up

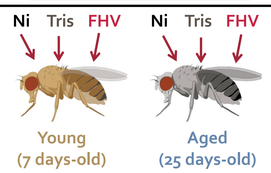



Wild type *Oregon-R* flies




- Survival count
- Virus load determination (qPCR for *FHV RNA1*)

### RNA seq workflow




**3** biological replicates /condition

RNA seq done at **24h** p.i. and **48h** p.i.

**Differential gene expression analysis (Novogene, Co. LTD):**

**Aging**= Ni 25d-old/Ni 7d-old

**Young FHV**= FHV 7d-old/Tris 7d-old

**Aged FHV**= FHV 25d-old/Tris 25d-old

## Results

### Increased susceptibility to FHV of aged *Drosophila*

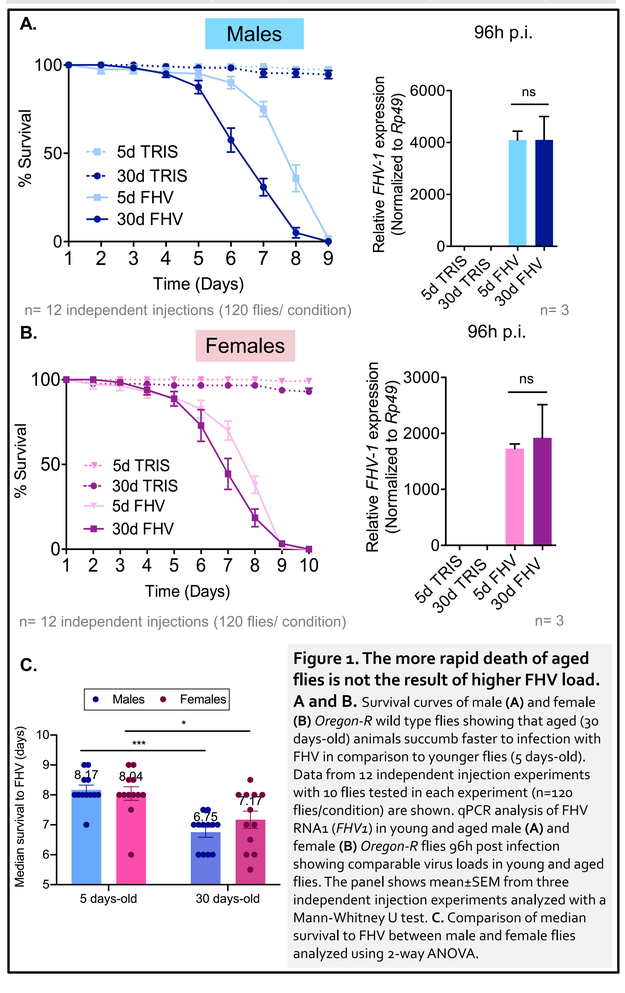



**Figure 1. The more rapid death of aged flies is not the result of higher FHV load. A and B.** Survival curves of male **(A)** and female **(B)** *Oregon-R* wild type flies showing that aged (30 days-old) animals succumb faster to infection with FHV in comparison to younger flies (5 days-old). Data from 12 independent injection experiments with 10 flies tested in each experiment (n=120 flies/condition) are shown. qPCR analysis of FHV RNA1 (*FHV1*) in young and aged male **(A)** and female **(B)** *Oregon-R* flies 96h post infection showing comparable virus loads in young and aged flies. The panel shows mean±SEM from three independent injection experiments analyzed with a Mann-Whitney U test. **C.** Comparison of median survival to FHV between male and female flies analyzed using 2-way ANOVA.

### Strong transcriptional response of aged *Drosophila* following FHV infection

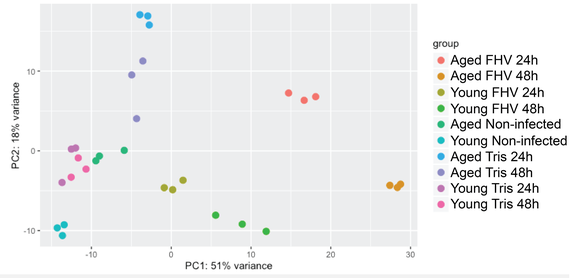



**Figure 2. Principal-component analysis of expression data.** The graph represents estimated variance in counts from alignment to the *Drosophila* reference genome.

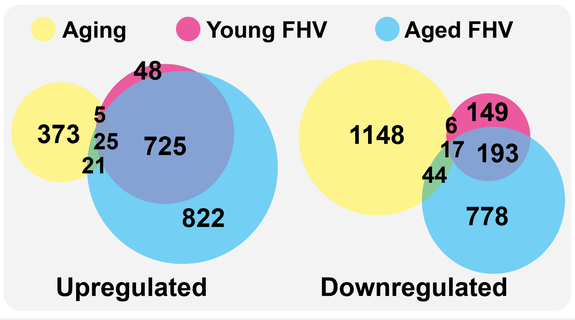



**Figure 3. Transcriptomic profiles of young and aged *Drosophila*, infected or not with FHV.** Venn diagrams showing number of differentially regulated genes (>2 fold, p<0.05) in young and aged male *Oregon-R* flies 48h post FHV infection compared to transcriptional changes occurring during aging alone. We note that response to FHV of aged flies shares a minimal overlap with the transcriptome of aged, non-infected flies. 93% and 57% overlap between up- and downregulated genes, respectively is observed between young and aged FHV-infected flies. 51% and 75% among upregulated and downregulated genes, respectively in aged flies are specific to this age group. Data obtained from RNAseq done on three biological replicates for each condition.

## Results (cont)

### GO term analysis of differentially regulated genes

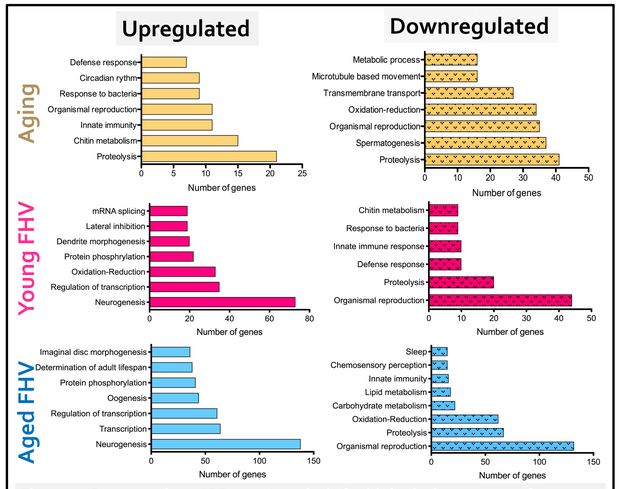



**Figure 4. Gene ontology (GO-term) analysis for biological processes of differentially regulated genes 48h post FHV infection**. Analyses were performed using the The Database for Annotation, Visualization and Integrated Discovery (DAVID) using the list of at least 2-fold differentially expressed genes for each condition.

Aging

Young FHV

Aged FHV

**Upregulated**

Extracellular region (41)
Extracellular space (37)

Lipid particle (5)
Peroxisome (4)
P-granule (2)

Nucleus (149)
Cytoplasm (133)
Plasma membrane (58)
Nucleolus (27)
Polytene chromosome (20)
Nucleoplasm (17)
Centrosome (13)

**Downregulated**

Integral component of membrane (175)
Cytoplasm (119)
Microtubule associated complex (24)
Centrosome (16)
Spindle pole (13)
Microtubule (13)
Spindle (12)
Mitochondrial inner membrane (12)

Extracellular region (13)
Extracellular space (10)
Extracellular matrix (4)

Extracellular space (106)
Extracellular region (65)
**Mitochondrion** (32)
Lipid particle (28)
Endoplasmic reticulum (17)
Endomembrane system (15)
**Mitochondrial inner membrane** (11)
Peroxisome (9)
Ribosome (9)
**Mitochondrial Complex I** (8)
Organelle membrane (8)
**Mitochondrial Complex IV** (5)
**Mitochondrial Complex III** (3)

**Figure 5. Gene ontology (GO-term) analysis for cellular components of differentially regulated genes 48h post FHV infection**. Analyses were performed using the The Database for Annotation, Visualization and Integrated Discovery (DAVID) using the list of at least 2-fold differentially expressed genes specific for each condition. We note higher number of mitochondria-related genes downregulation in aged FHV-infected flies. Number of genes is indicated in parenthesis.

## Conclusions/Future Directions

Our results demonstrate that the RNA (+) virus FHV kills more rapidly older flies than young adults, without the accumulation of higher virus titers. This indicates that survival to FHV infection of the aged organism appears to be controlled by factors possibly involved in infection tolerance. Additional titration assays and evaluation of virus load at other time points in the course of infection will be used to confirm this. We show that the transcriptional response of aged flies to FHV differs from the response observed in younger adults infected with the virus and from the response of aged, non-infected flies. Future experiments will examine the functional role of selected candidate genes for age-specific effects following infection using mutants and/or RNAi knock down. Finally, as increased evidence points to sex differences in immune responses with aging, an important direction will be to address the question why aged male flies succumb at higher rates to FHV infection than aged females.