The Effect of Endosymbiotic Microbes on the Immune Response of *Drosophila melanogaster* to Nematode Parasites and Their Mutualistic Bacteria

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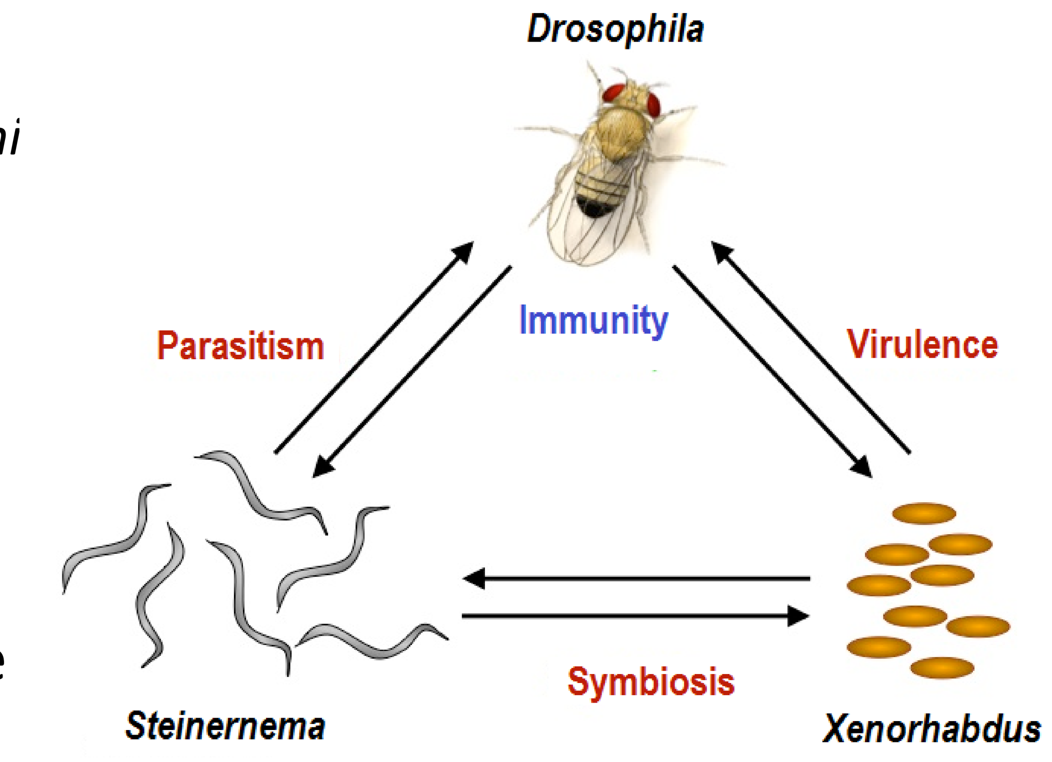
Introduction

The immune response of the model insect *Drosophila melanogaster* consist of a complex multi-layer structure of defensive mechanisms. The *Drosophila* immune response is highly specific, making immune reactions as diverse as the microbes infecting it; such as bacteria and parasitic nematodes (1). *Xenorhabdus nematophi* are enterobacteria that have a mutualistic relationship with the nematodes, *Steinernema carpocapsae*, and are pathogenic towards a variety of insects, *Drosophila melanogaster*. The interaction between *Steinernema* and *Xenorhabdus* with *Drosophila* flies and their endosymbiotic bacteria *Wolbachia* and *Spiroplasma,* represent an excellent model system in which mutualistic and pathogenic processes can be studied in a single host organism (2). To study the pathogenic effects of the nematodes separately from their mutualistic bacteria, 2 strains of nematodes will be used: the *Steinernema-Xenorhabdus* nematode-bacteria complex (symbiotic) and *Steinernema* nematodes without *the* bacteria (axenic). Three strains of *Drosophila melanogaster* will be used: the wild-type W1118 strain that contains *Wolbachia* but not *Spiroplasma* endosymbionts (W+S-), one containing both endosymbionts (W+S+), and the last containing no endosymbiotic bacteria (W-S-).

The main objective of this study will be to investigate the interaction between the symbiotic relationship of *Wolbachia* and *Spiroplasma* with the *Drosophila* immune system and their subsequent response to the nematode parasite *Steinernema* and its mutualistic bacteria *Xenorhabdus*.



Materials & Methods

Organisms

Insects: *Drosophila melanogaster*, larvae (3-4 days old) wild-type W+S-, W+S+, and W-S-

Bacteria: *Drosophila* endosymbiont bacteria: *Wolbachia pipientis* (Fig 1) and *Spiroplasma pulsonii* (Fig 2)
Steinernema mutualistic bacteria: *Xenorhabdus*

Nematodes: *Steinernema carpocapsae*

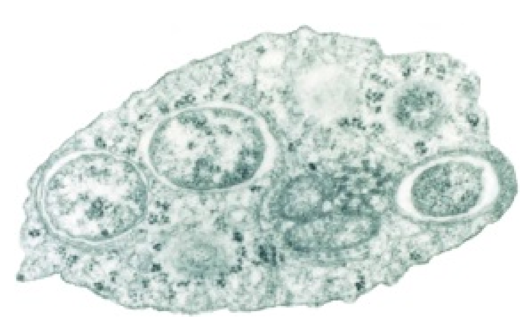


Fig 1. The *Wolbachia* bacteria

Fig 2. The *Spiroplasma* bacteria

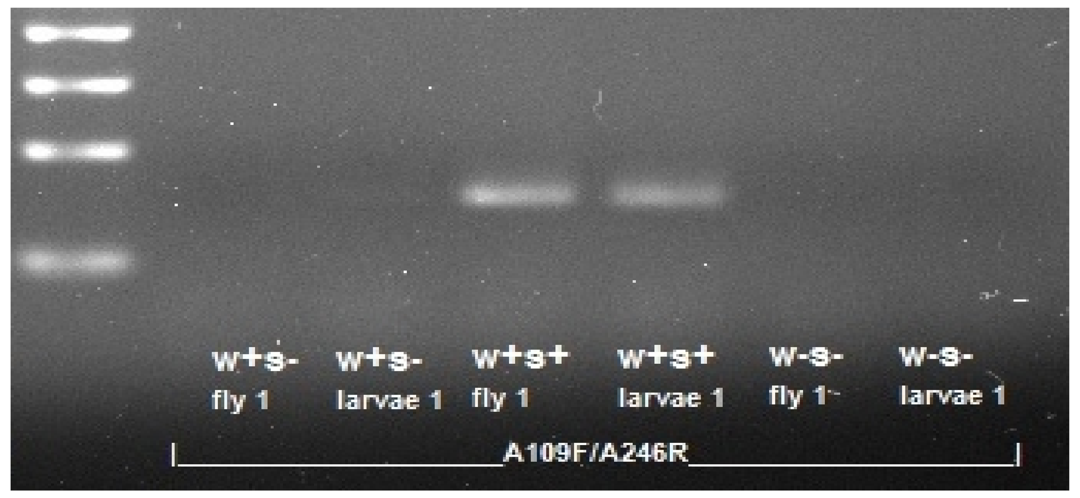


Fig 3. Shows the presence of *Spiroplasma*.

PCR Genotyping: Diagnostic PCR and agarose gel electrophoresis were performed on the 3 strains of *Drosophila* to ensure the presence/absence of *Wolbachia* and *Spiroplasma* (Fig 3) endosymbionts.

Obtaining Axenic Nematodes: A surface-sterilization of *Steinernema carpocapsae* IJ protocol (Shruti Yadav, personal communication) was performed on axenic nematodes to ensure the absence of *Xenorhabdus* bacteria in and on the nematodes.

***Drosophila* Survival Assays:** Individual larvae were placed in each well of an Assay Plate containing 100 µl of 1.25% agarose gel. 20 larvae of each of the 3 *Drosophila* strains underwent a control treatment (10 µl of water) added to their well, while another 20 larvae of each strain underwent an infection treatment (10 µl of water with approx. 100 symbiotic nematodes) added. This was performed again on all 3 strains but for infection approx. 100 axenic nematodes were added.

Results & Discussion

Survival results showed that the control treatments of the *Drosophila* larvae caused minimal mortality. All 3 larvae strains that were infected with *Steinernema-Xenorhabdus* symbiotic nematodes, had similar mortality rates (Fig 4). W-S- and W+S- larvae infected with *Steinernema* axenic nematodes had similar mortality rates but the W+S+ larvae had a much steeper mortality rate in comparison (Fig 5). These findings lead us to consider 1) that because the only difference between the axenic and symbiotic nematodes is the *Xenorhabdus* bacteria, this might be the cause of the higher mortality rates compared to larvae infected with the axenic nematodes and 2) that *Spiroplasma* bacteria may have a detrimental effect on the host's immune system when the host has been infected with axenic *Steinernema* nematodes.

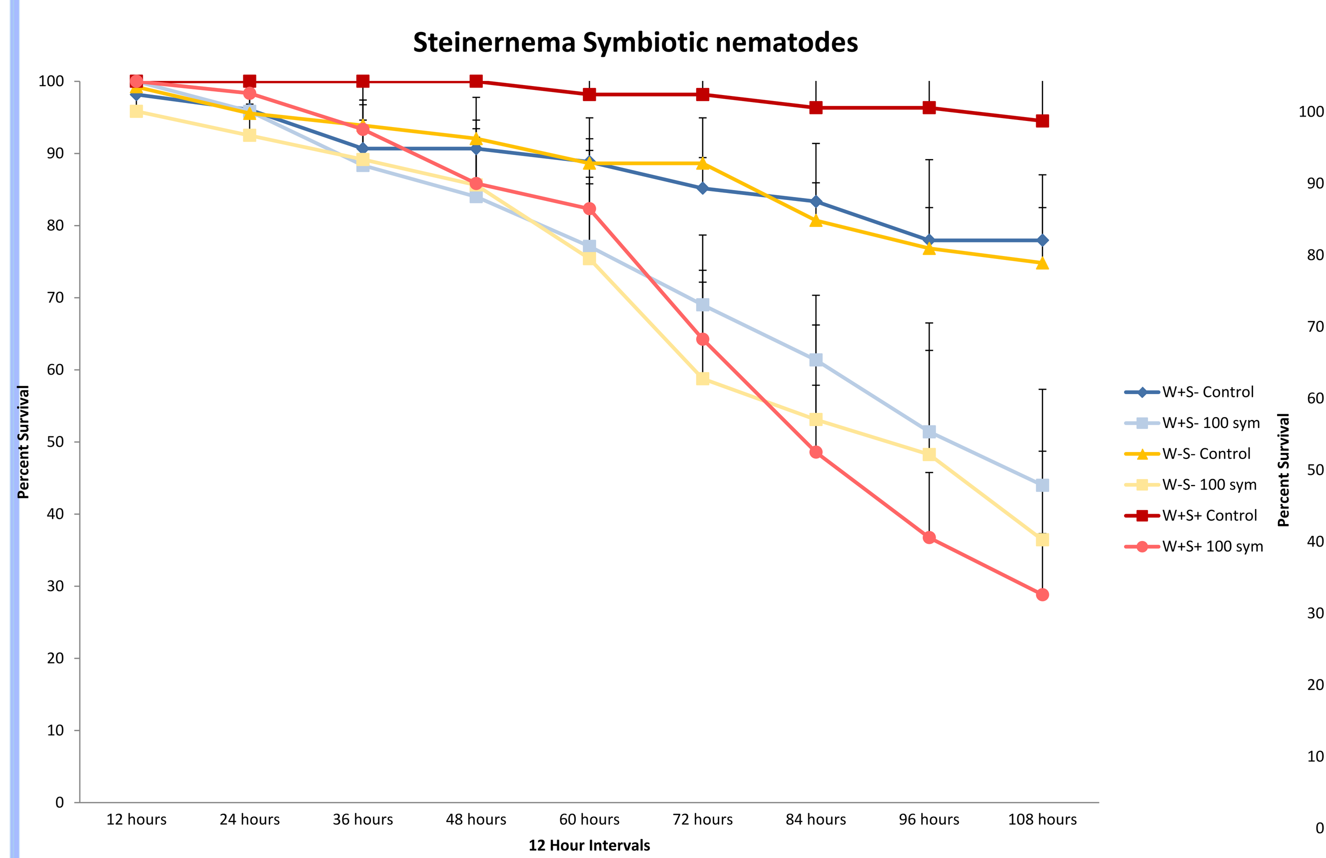


Fig 4. Compares the three strains treated with control and treatment with symbiotic nematodes

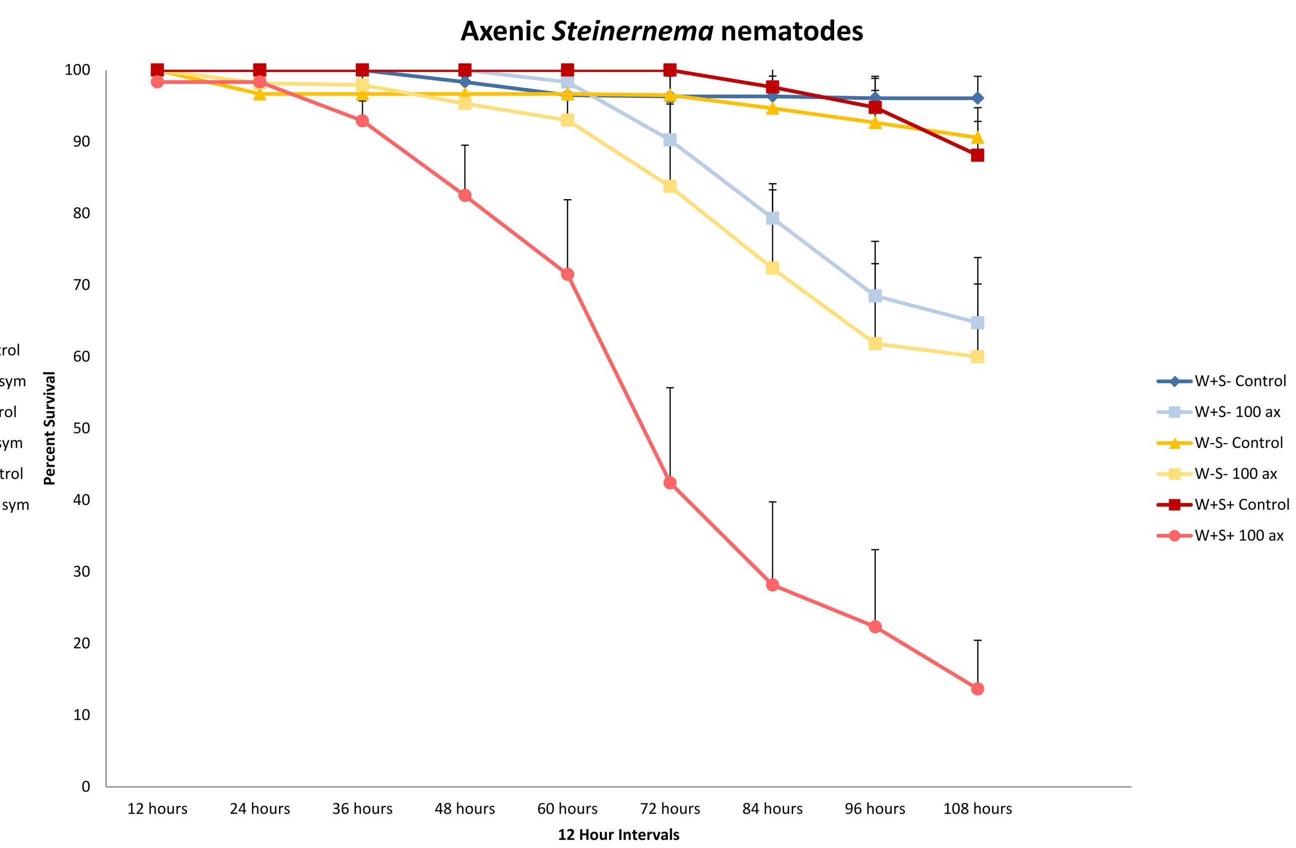


Fig 5. Compares the three strains treated with control and treatment with symbiotic nematodes

Future Work

1) To obtain *Drosophila* carrying *Spiroplasma* endosymbiont only (W-S+) to further study the effect of *Spiroplasma* on *Drosophila* larvae and if in fact it has a negative impact on the host's survival.
2) To examine the *Xenorhabdus* bacterial load inside the larvae post-infection in all 3 strains of *Drosophila.* I expect that the amount of *Xenorhabdus* bacteria can be linked to host survival rates.
3) Because I found the W+S+ strain to be particularly susceptible to infection by axenic nematodes, I suspect that this reflects major changes in the immune function of those larvae. I will investigate the humoral and cellular immune response of the 3 strains of *Drosophila* larvae, upon nematode infection.

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References

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2. Chaston JM, et al. (2011) The Entomopathogenic Bacterial Endosymbionts *Xenorhabdus* and *Photorhabdus*: Convergent Lifestyles from Divergent Genomes. PLoS ONE 6(11): e27909.