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AMP Expression in Energetic Hybrid *D. melanogaster* Infected With *P. rettgeri*

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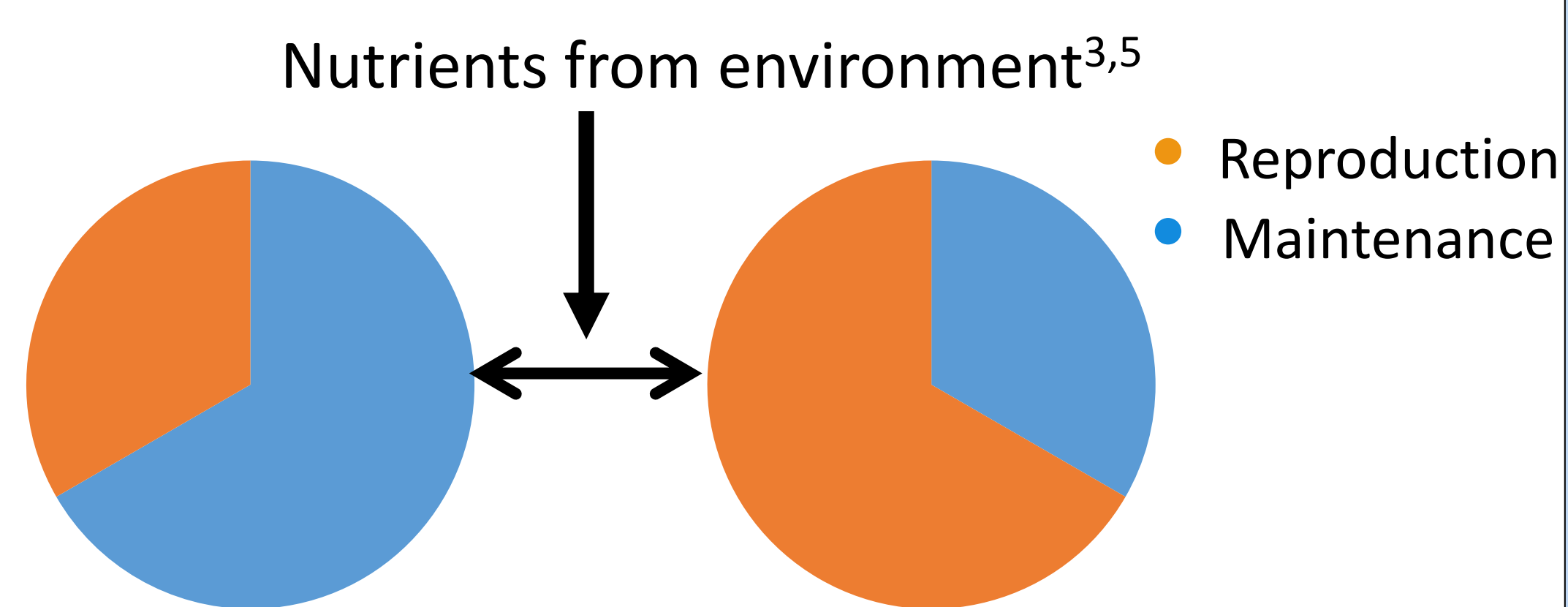


Abstract

The purpose of this experiment was to investigate immune function and energy metabolism, particularly the levels of antimicrobial peptides produced in *Drosophila* energetically compromised genotypes. This will provide the ability to investigate energetics of immunity without changing diet. Flies were infected with the bacteria *P. rettgeri* and the resulting immune response was investigated. Females did not mount as effective an immune response as males. All flies exhibited decreased survivorship from infection. Control flies survived at higher levels and showed no mitochondrial:nuclear interaction.

Introduction

Life-History Tradeoffs

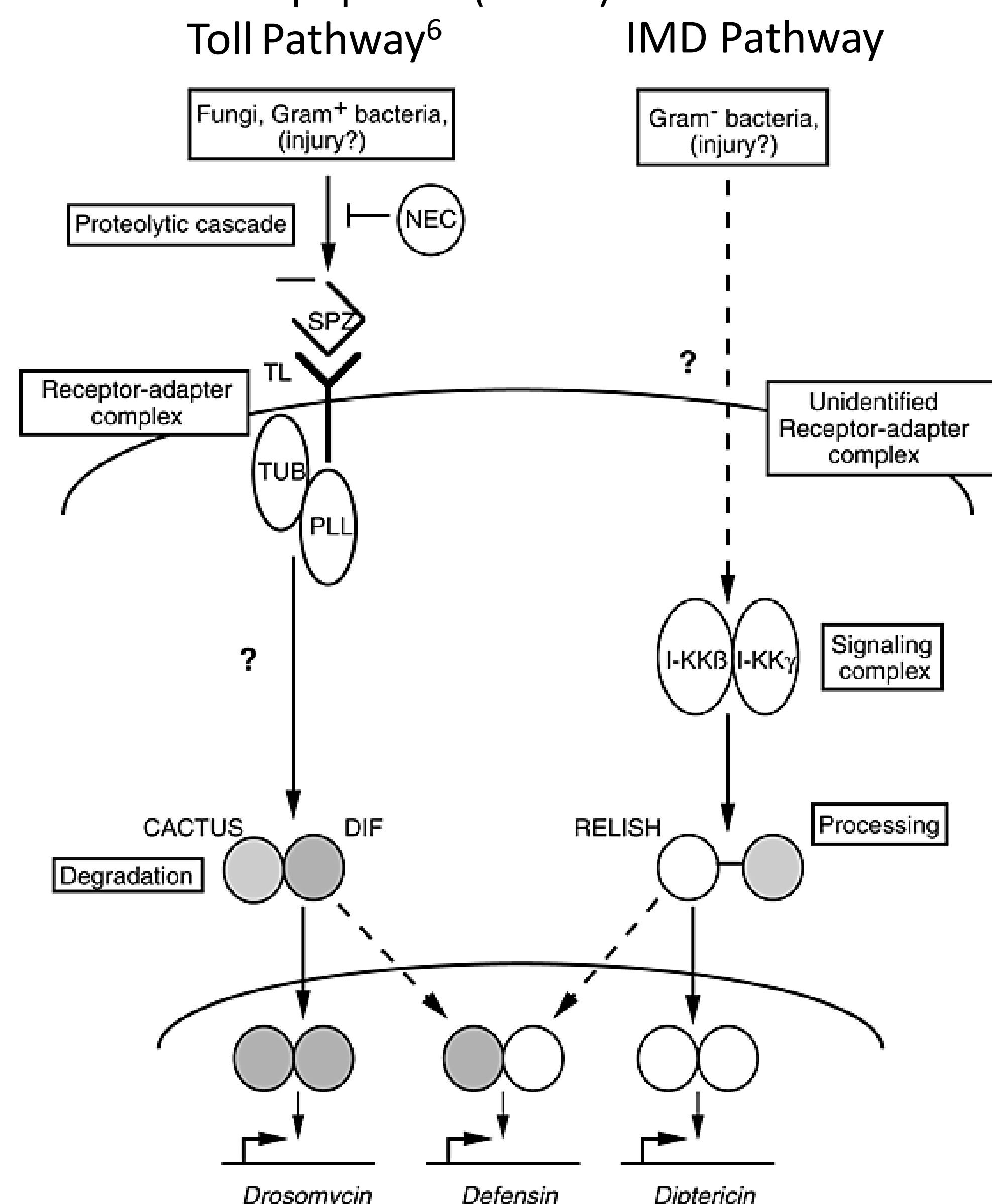


Providencia Rettgeri

- P. rettgeri*, a natural cause of infection for *Drosophila*, is a gram-negative bacteria that is pathogenic to fruit flies.
- The infection multiplies very quickly and causes moderate survival rates, found to be ranging from 40%-80%¹.
- Providencia* has been found in humans as part of natural gut bacteria as well as gastric upset⁷.

Innate Immunity

- While adaptive and innate immunity is present in animals, invertebrates, like *Drosophila*, only possess innate immunity as a defense against pathogens. This form of defense results in production of antimicrobial peptides (AMPs).



Genotypes

- The mitochondrial-nuclear incompatible genotype (*simw*⁵⁰¹);*OreR* decreases the fitness and fecundity of fly larvae.
- This is because the incompatibility causes incorrect synthesis of certain proteins necessary for development, which delays it².
- Five other genotypes were used in this experiment as compatible genotypic controls, with no interactive effect between their mitochondrial and nuclear genotypes, their oxidative phosphorylation functions normally.

Motivation

Figure 1. The incompatible genotype shows decreased survivorship compared to genotypic controls under bacterial infection. Aut nuclear genotype has higher survivorship than Ore. Survivorship of controls matches prior research on infection.

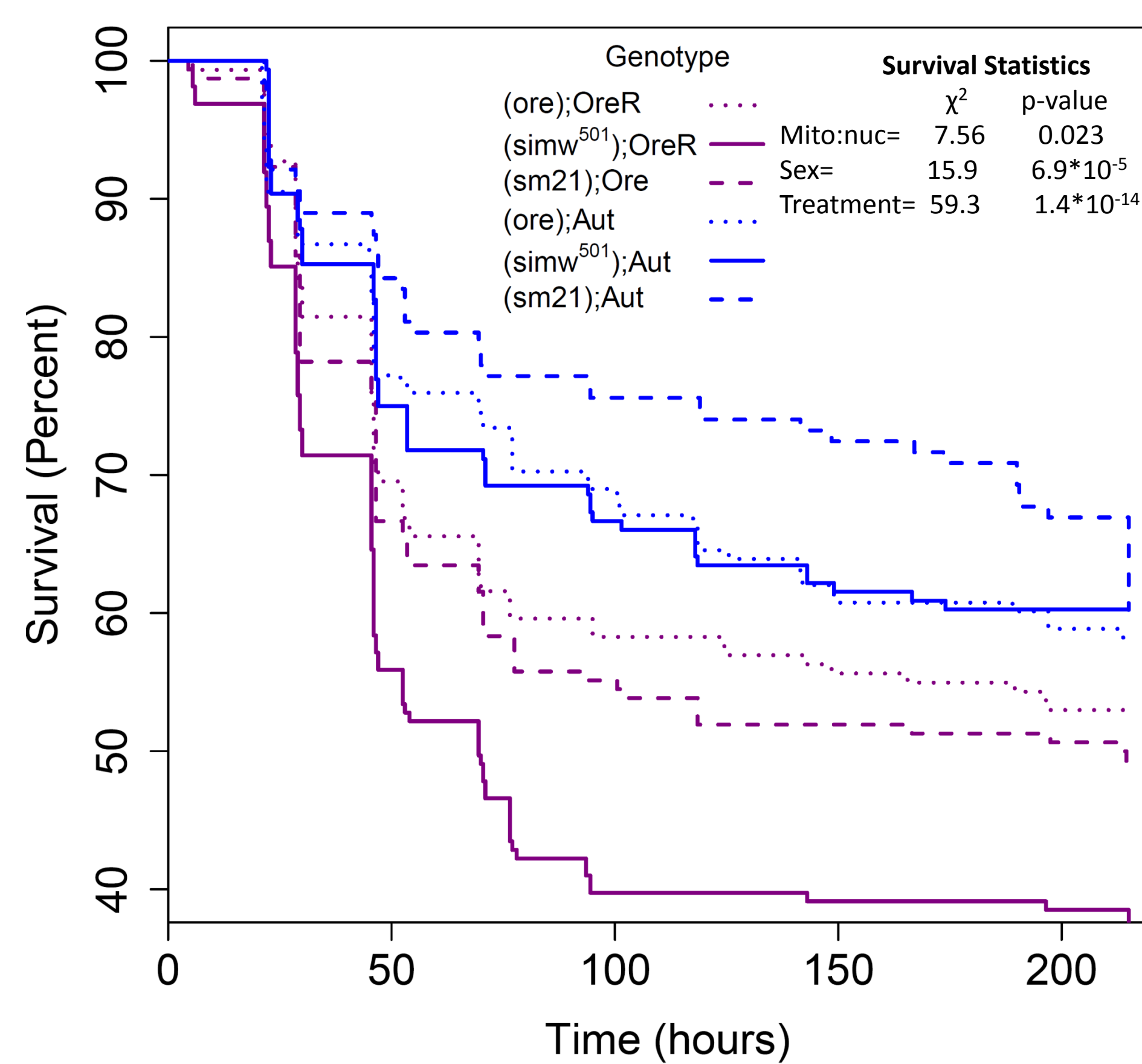


Figure 2. Females have decreased survivorship in infection and under control conditions, with infection resulting in a greater decrease. This may be due to differences in the life-history trade-off s. Virgin females will lay unfertilized eggs.

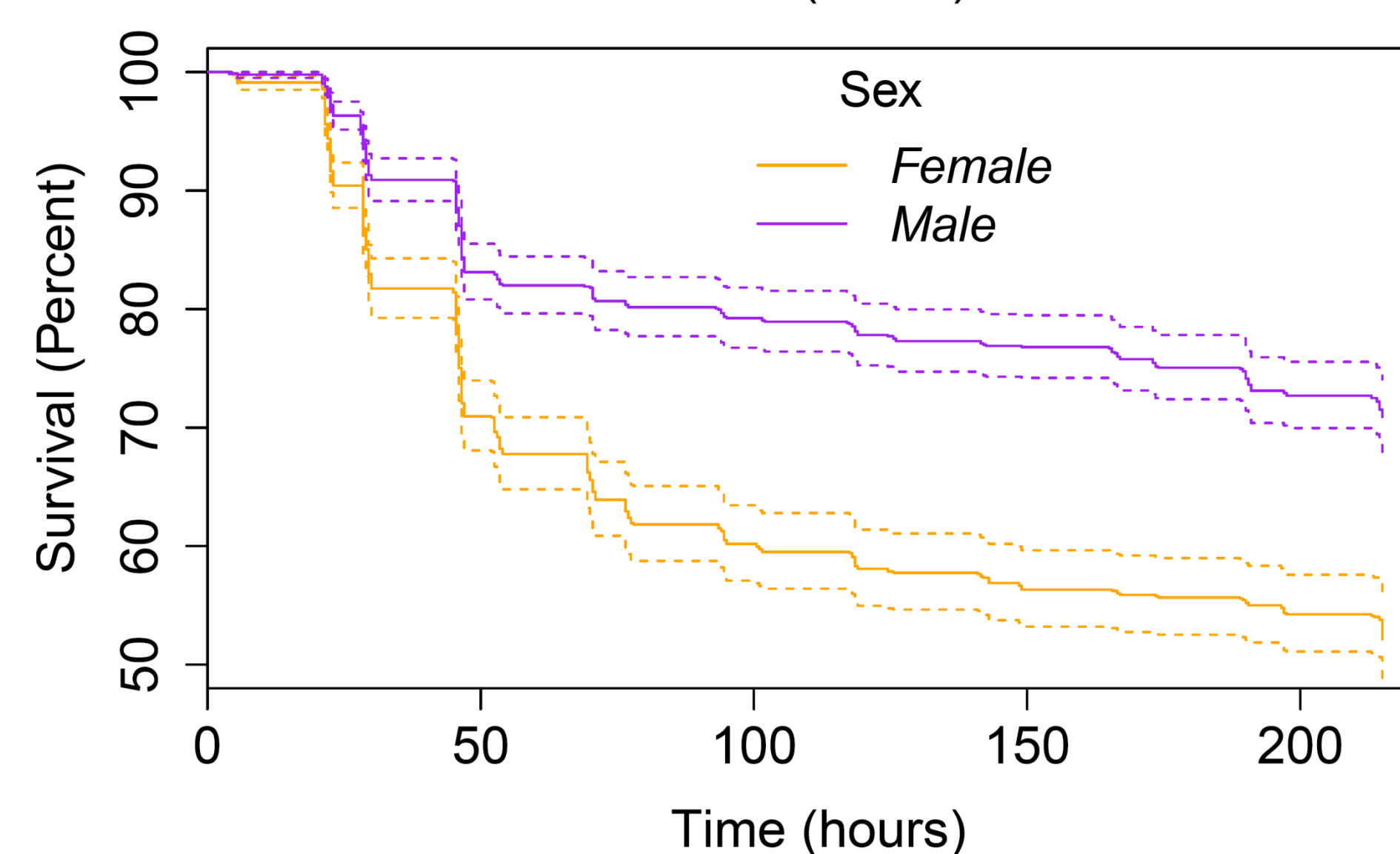


Figure 3. The bacterial treatment reduced survivorship in all genotypes. Control survivorship was lower than expected. This may be due to desiccation as humidity was low during trials.

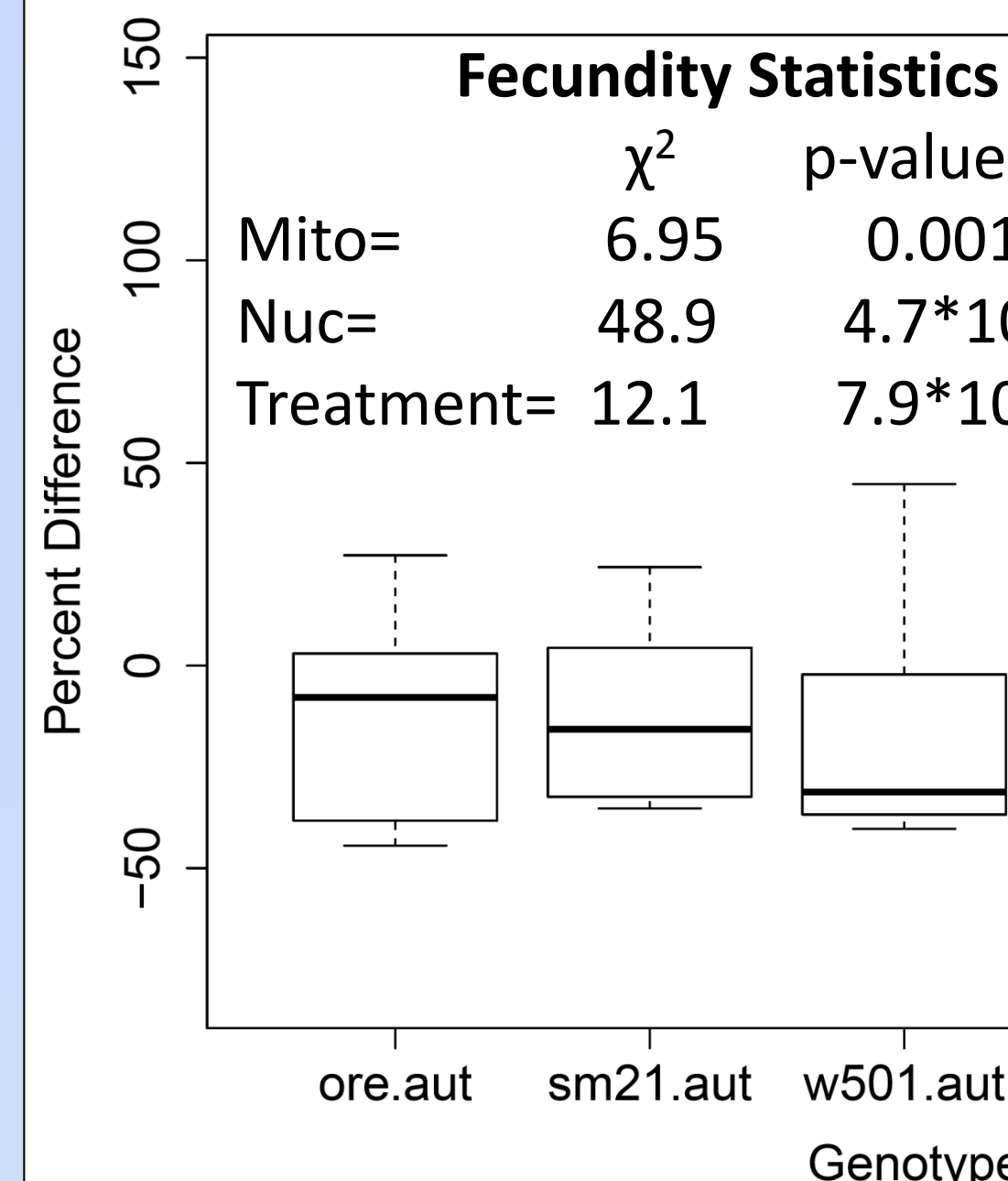
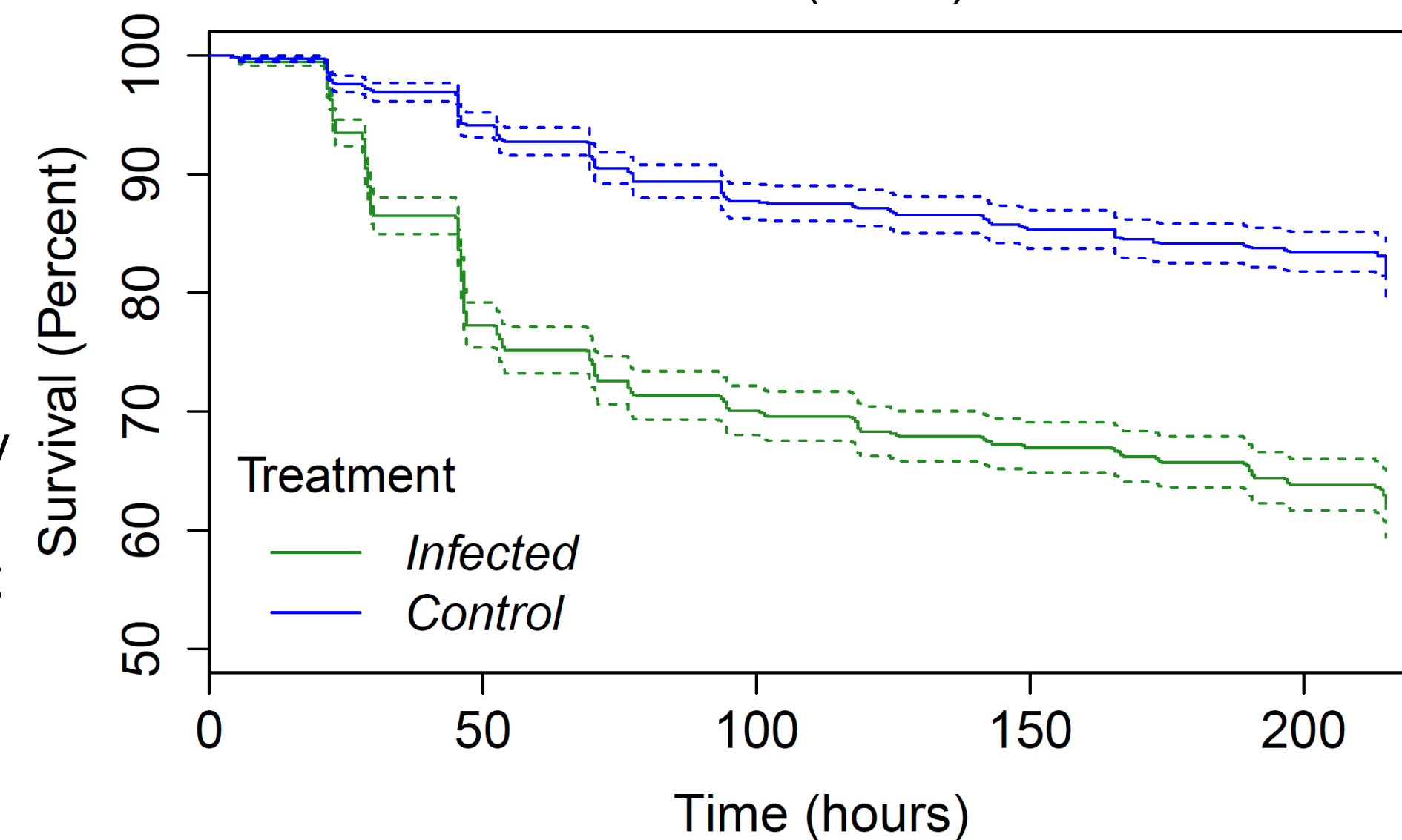


Figure 4. Treatment decreases number of offspring ~15%. Ore nuclear background has decreased fecundity. (w501);Ore has further decreased fecundity.

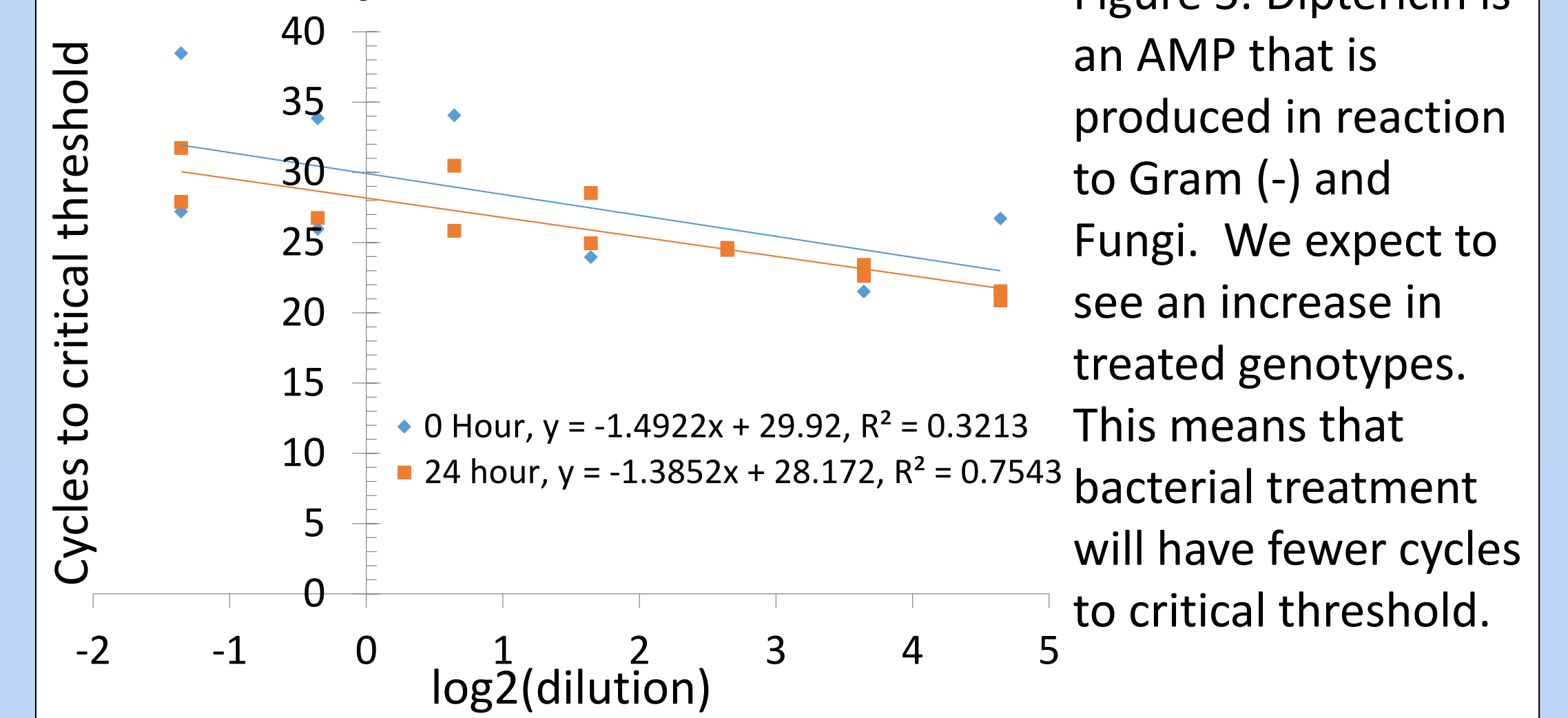
Predictions

- The mitochondrial-nuclear incompatible genotype (*simw*⁵⁰¹);*OreR* mount a less effective AMP response than the other genotypes due to its lower level of energy. This will correspond with lower immune activation and decreased survival.
- Genotypes mount similar immune responses, but the incompatible genotype will have lower survival due to energy limitations.

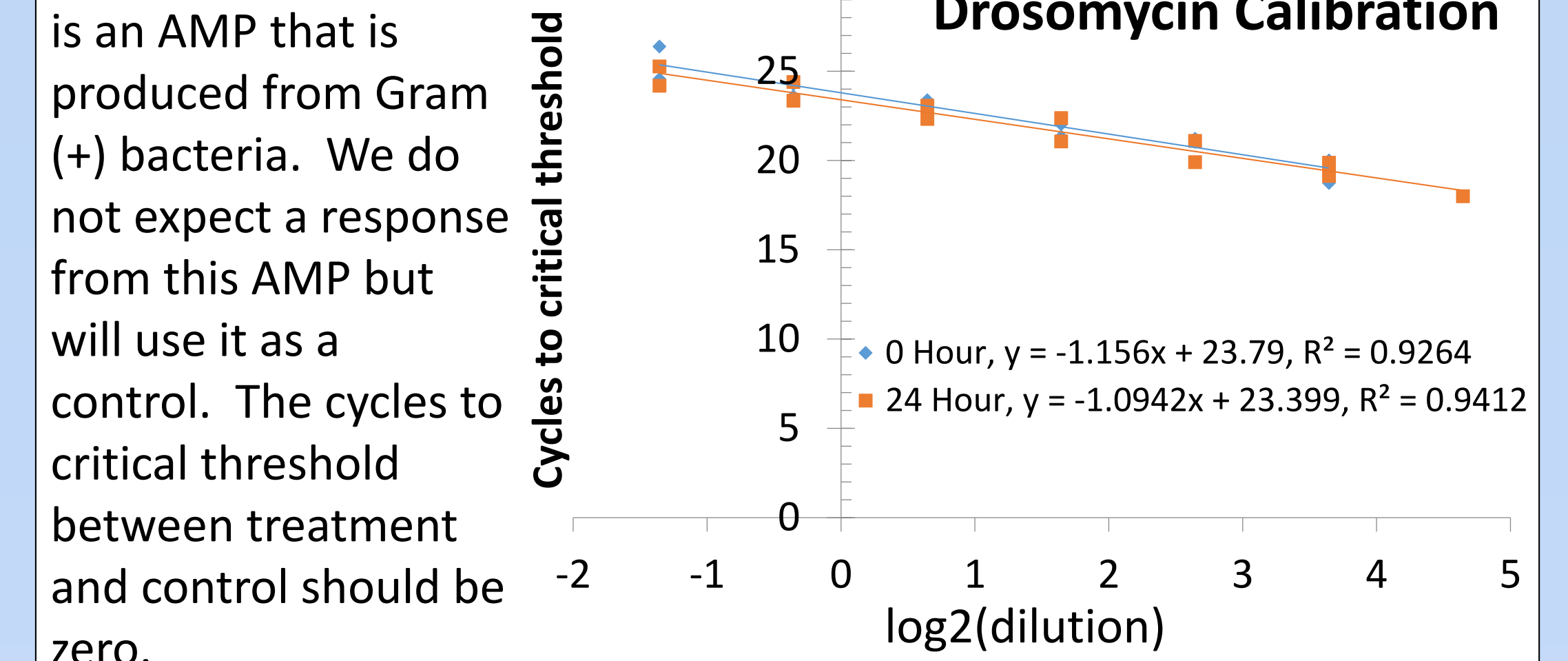
Results

The slopes in these plots are the efficiency of the qPCR and R² measure reproducibility between samples ideally, which should be one. A slope of one means that the efficiency of the primers predict the concentration. Slopes under or over one will result in decreased ability to determine unknown concentrations.

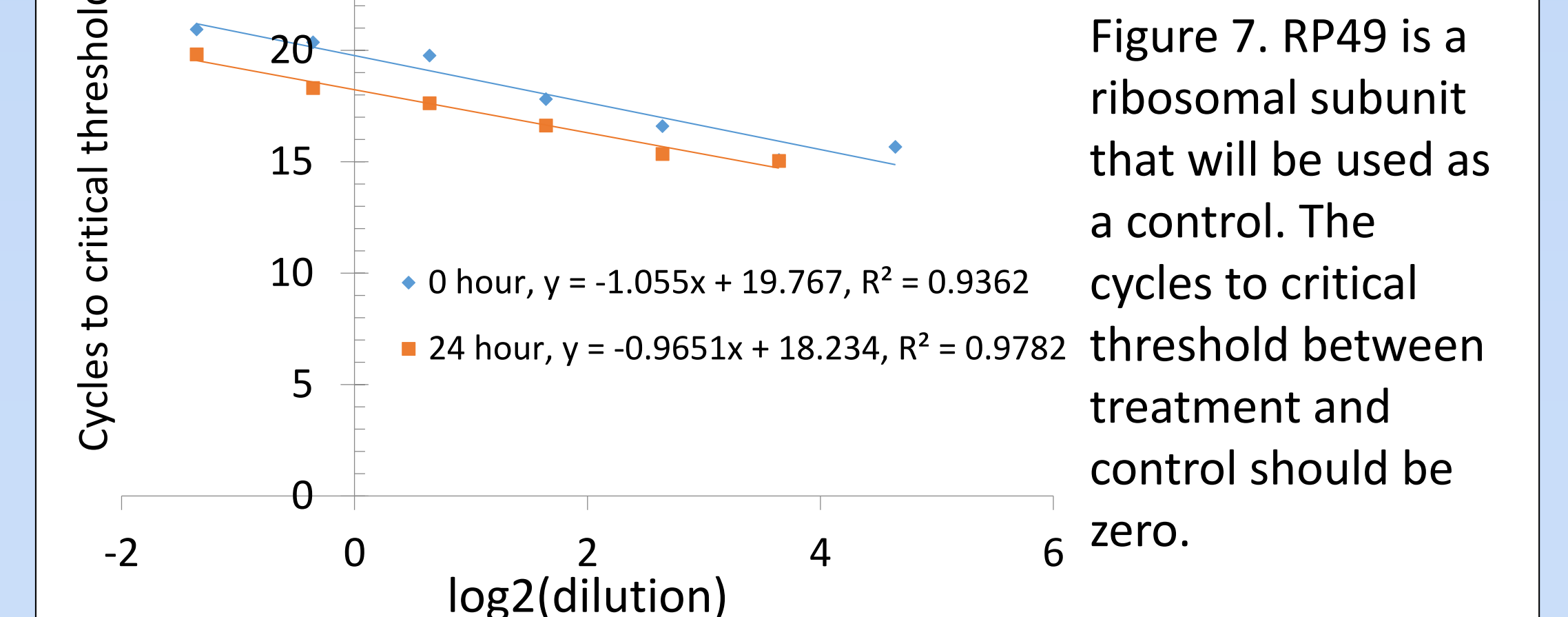
Diptericin Calibration



Drosomycin Calibration



RP49 Calibration



Continued Research

- Two of four treatment blocks have been collected and are in the stages of mRNA extraction and cDNA processing. qPCR primers will continue to be optimized by changing temperature of the reaction.
- Future experimentation on larvae will be done to investigate the differences in energy spent, since larvae don't need an energy allotment for reproduction, just for growth. Bacterial load in adults will also be performed to test effectiveness of pathogen clearance.

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Acknowledgements

Thanks to Justin Buchanan, Kristi Montooth, Katie, and Montooth Lab for help and guidance, Brian Lazzaro for *P. rettgeri* and research, and the UCARE Program for funding.

Methods and Materials

(w501);Ore, (w501);Aut, (ore);Ore, (ore);Aut

Virgin males and females collected in Blocks⁴

Flies infected by poking with 0.1 mm needle with *P. rettgeri* and sterile needle as control

Samples flash frozen at 0 h, 5 h, 10 h, and 10 days

RNA extraction

EtOH precipitation to RNA purification

RNA reverse transcribed to cDNA

cDNA used for Quantitative PCR

AMPs: diptericin (IMD) RP49 (ribosome)
 drosomycin(Toll) Actin5c(cytoskeleton)

WO:OA:OO
 OA:OO:WA
 WA:OO:WO
 WA:OA:WO