

ASGSR poster abstract for 2015.

Limits: Title not to exceed 15 words; Abstract text not to exceed 350 words.

Font: Times New Roman, font size 12

Keywords:

Drosophila melanogaster, *Serratia marcescens*, space flight, virulence,

Title:

Spaceflight causes increased virulence of *Serratia marcescens* on a *Drosophila melanogaster* host.

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Abstract:

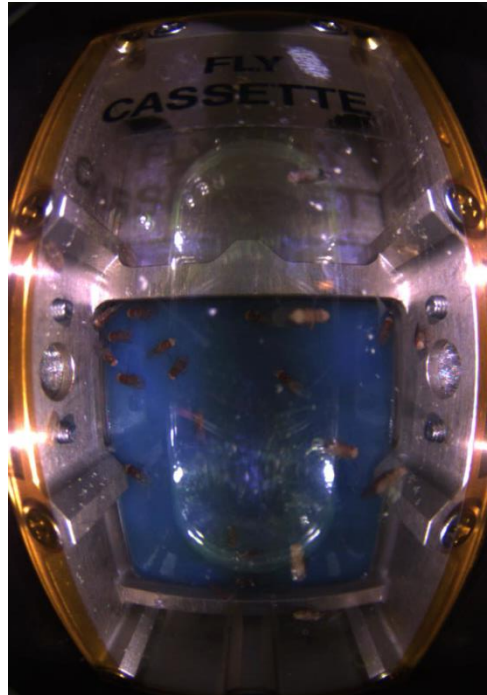
Drosophila melanogaster, or the fruit fly, has long been an important organism for Earth-based research, and is now increasingly utilized as a model system to understand the biological effects of spaceflight. Studies in *Drosophila melanogaster* have shown altered immune responses in 3rd instar larvae and adult males following spaceflight, changes similar to those observed in astronauts. In addition, spaceflight has also been shown to affect bacterial physiology, as evidenced by studies describing altered virulence of *Salmonella typhimurium* following spaceflight and variation in biofilm growth patterns for the opportunistic pathogen *Pseudomonas aeruginosa* during flight.

We recently sent *Serratia marcescens* Db11, a *Drosophila* pathogen and an opportunistic human pathogen, to the ISS on SpaceX-5 (Fruit Fly Lab-01). *S. marcescens* samples were stored at 4°C for 24 days on-orbit and then allowed to grow for 120 hours at ambient station temperature before being returned to Earth. Upon return, bacteria were isolated and preserved in 50% glycerol or RNAlater. Storage, growth, and isolation for ground control samples were performed using the same procedures. Spaceflight and ground samples stored in 50% glycerol were diluted and injected into 5-7-day-old ground-born adult *D. melanogaster*. Lethality was significantly greater in flies injected with the spaceflight samples compared to those injected with ground bacterial samples. These results indicate a shift in the virulence profile of the spaceflight *S. marcescens* Db11 and will be further assessed with molecular biological analyses. Our findings strengthen the conclusion that spaceflight impacts the virulence of bacterial pathogens on model host organisms such as the fruit fly.

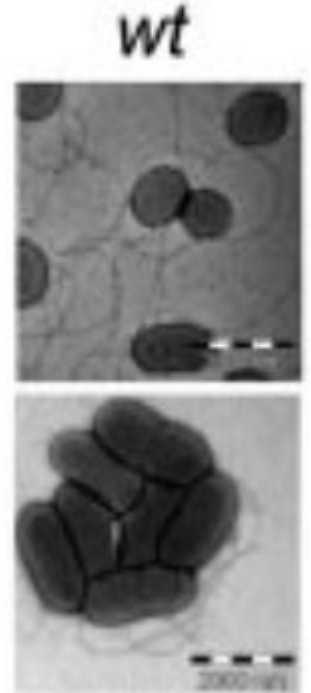
This research was supported by NASA's ISS Program Office (ISSPO) and Space Life and Physical Sciences Research and Applications (SLPSRA).

Impact of Spaceflight on Virulence
in *Serratia marcescens* Db11 in
Drosophila melanogaster

Introduction

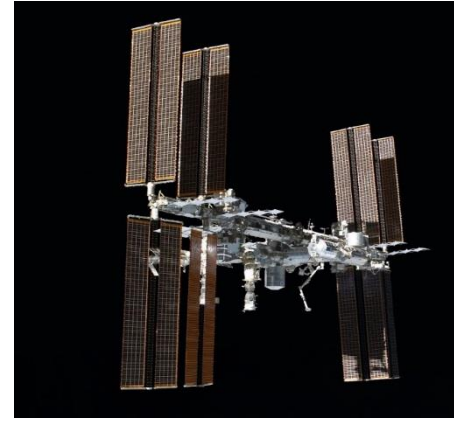
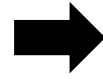
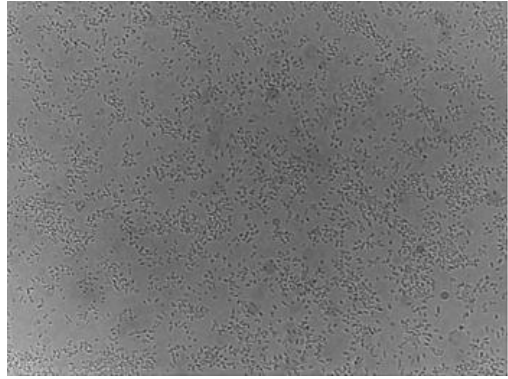


- *Drosophila melanogaster* is an excellent model of the innate immune system and displays an alteration in immune function similar to humans following spaceflight.
- Alterations in bacterial virulence and antibiotic resistance during spaceflight has long been a point of concern for NASA.
- *Serratia marcescens* is a gram-negative opportunistic pathogen to humans typically causing nosocomial infections.
- The *S. marcescens* sub-strain Db11 is a pathogen for *Drosophila melanogaster*.
- We recently flew a sample of *S. marcescens* Db11 as part of separate study and were interested to see if any of the returned samples displayed altered virulence in *Drosophila*.
- In particular we examine the relationships between these samples in mutant fly lines for the humoral pathways in flies (*imd* and *toll*). By examining this we hope to determine how changes in virulence are linked to the host immune system.



Castelli ME. et.al. J.Bac-2008

Methods

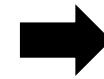
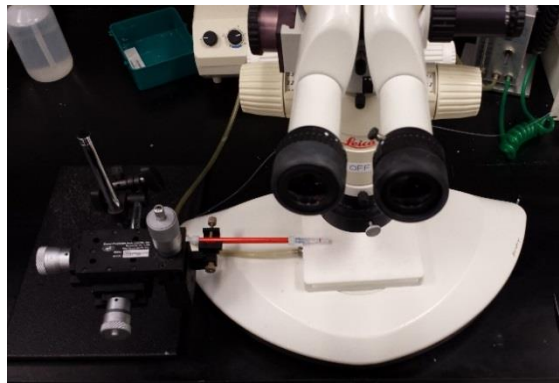


Grow *S. marcescens* Db11 overnight. Then diluted to a set optical density.

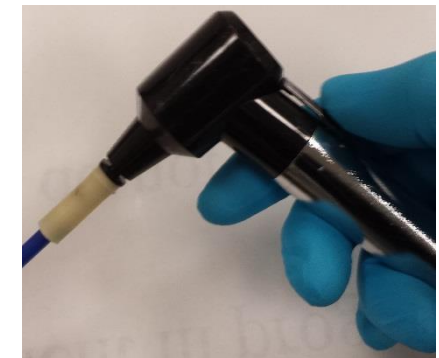
Place 100 μ L onto food tray and store in preparation for spaceflight or parallel ground controls.

Launch to ISS and remain at 4°C for 24 days. Then 120 hours at ambient temperature before and during return to Earth.

Samples were scrapped off of the plate and place into 50% glycerol or RNAlater solutions. Samples were flash frozen and then stored at -80°C.



OR



Samples were thawed, diluted, and then injected into *Drosophila*.

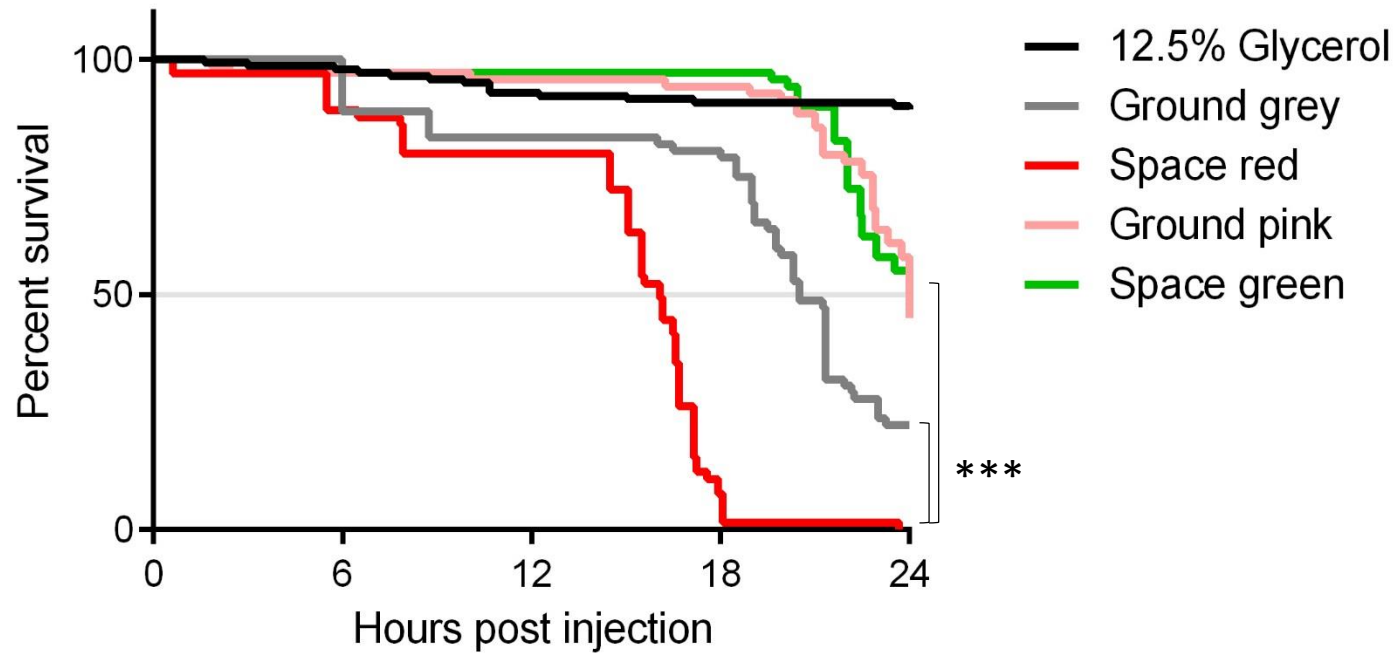
Injections were performed on 6-8 day old female flies in the ventral abdomen (red circle)

Flies survival was monitored for 24hrs

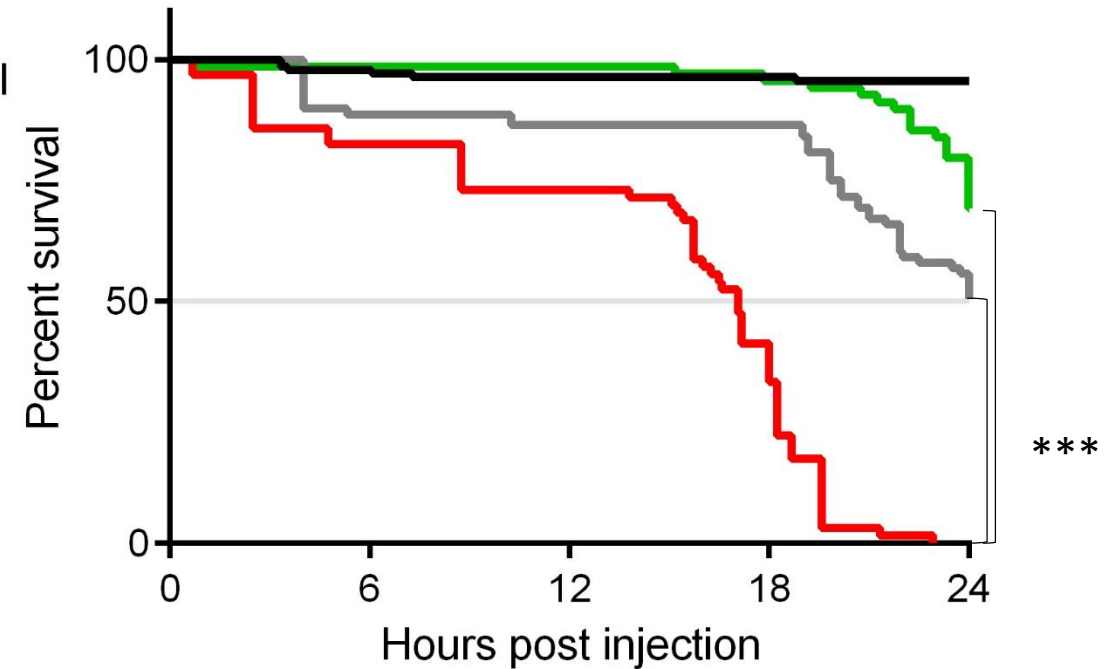
Homogenized at 0, 9, 12, or 15 hr. time points in 100 μ L PBS.

Results

Wild type: *w1118*



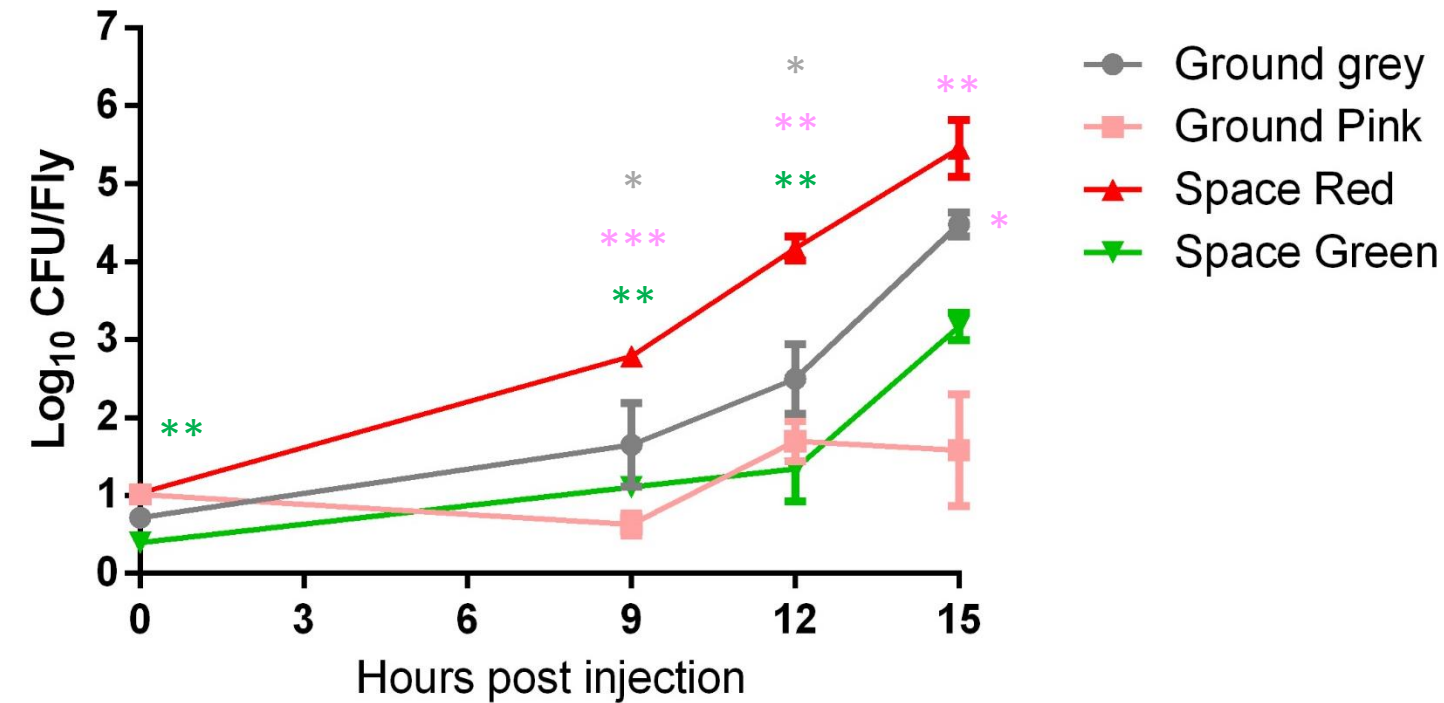
Wild type: OregonR



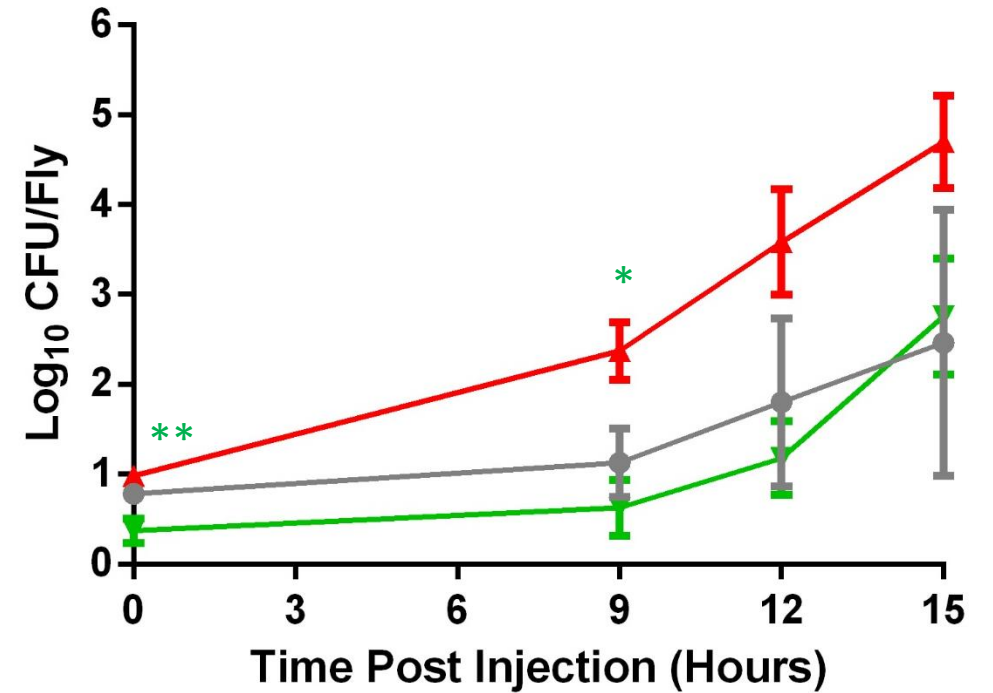
- Log-rank comparisons were performed between each sample.
- Space Red injected flies had a significant increased mortality in both *w1118* and OregonR in comparison to all other injected samples.
- Ground Grey was the second most virulent strain showing clear virulence in both *w1118* and OregonR.
- Ground Pink and Space Green both show similar virulence with ~50% virulence at 24rs for *w1118* and less in OregonR.

Result

Bacterial load of *w1118* flies injected with Db11 samples

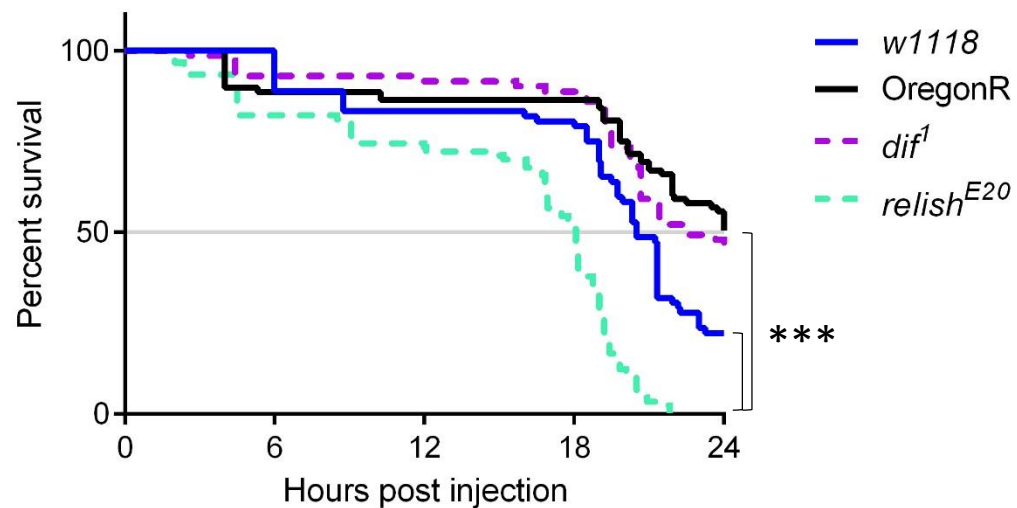


Bacterial load of OregonR flies injected with Db11 samples

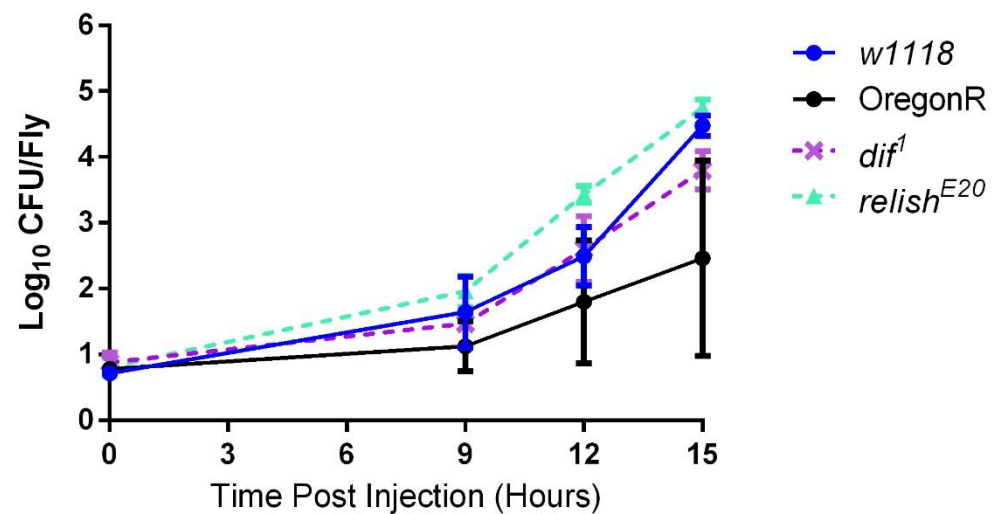


- One-way ANOVA comparisons of the different samples at each time point were performed.
- For *w1118* flies injected with Space Red showed significant difference from other injected samples at 9hrs and 12hrs post injection.
- Alternatively the OregonR injected flies seem to have less significant difference in bacterial growth. There however a clear trend of stronger bacteria growth in the Space Red injected flies at all time points.

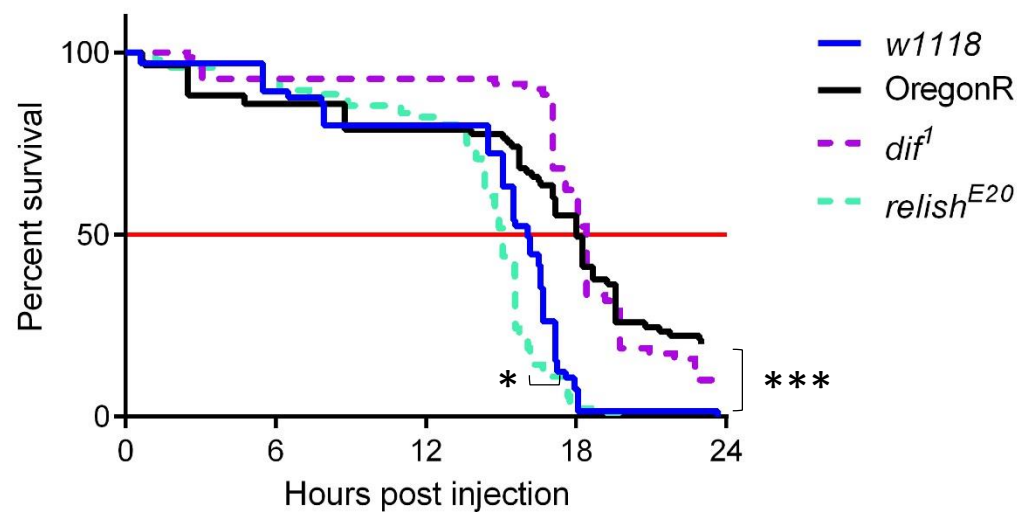
Survival of "Grey" ground
Db11 injected flies



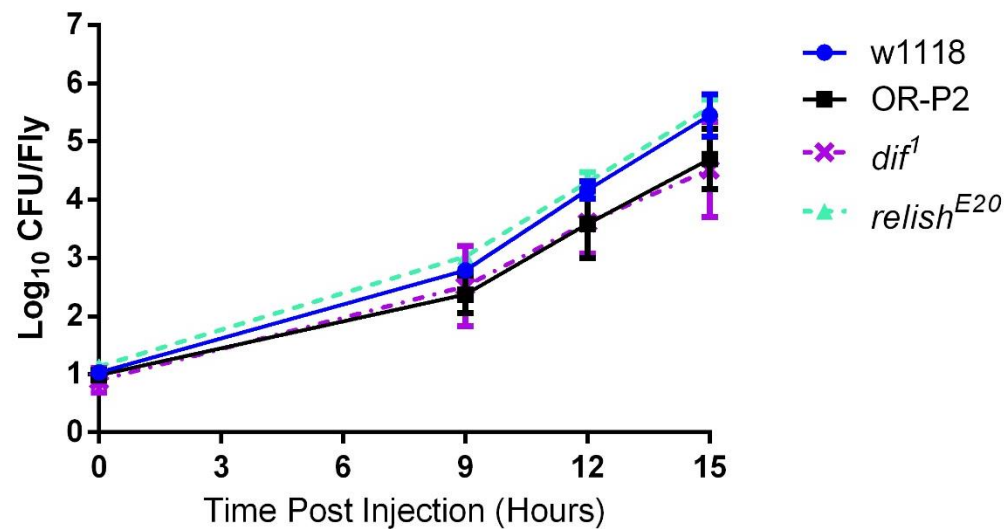
Bacterial Load of "Grey" Ground
Db11 injected flies



Survival of "Red" space
Db11 injected flies



Bacterial Load of "Red" Space
Db11 injected flies



Conclusion

- Space flown samples of *S. marcescens* Db11 display increased virulence.
- This cause a significant increase in bacteria growth in *w1118* flies at 9hr and 12hrs post injection.
- Mutant line data show that altered bacterial growth are the primary cause for altered virulence.
- Survival of pathway mutants suggest that the *imd* pathway, not the toll pathway, is still important in the flies response to Ground Grey and Space Red sample injection.