EFFECTS OF SIMULATED MICROGRAVITY ON A HOST-PATHOGEN SYSTEM

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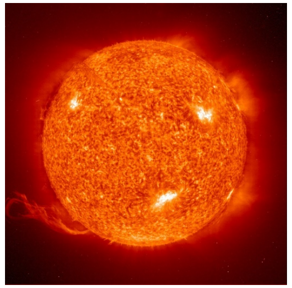
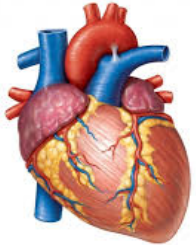
Spaceflight affects human physiology

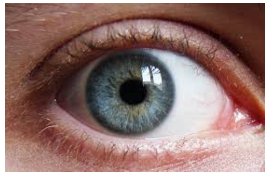


Radiation risk



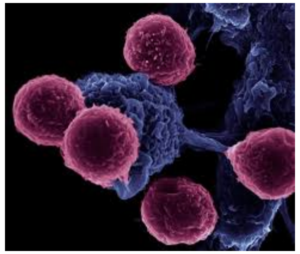
Cardiovascular risk

Bone loss



Visual impairments

Muscle loss

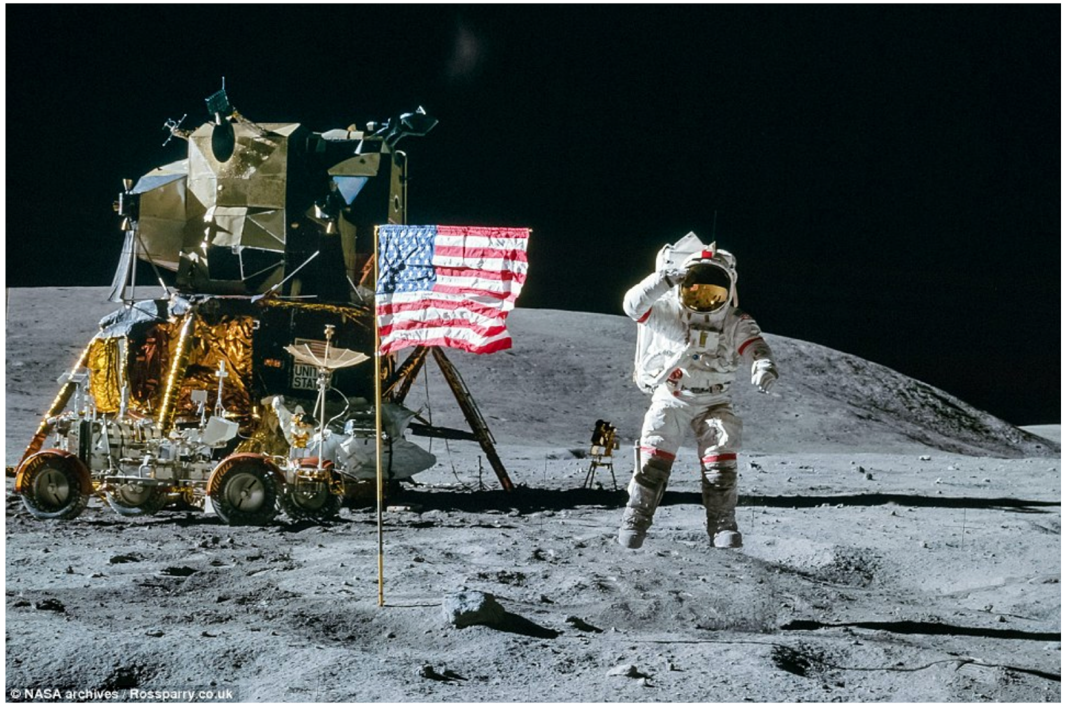


Immune system decrement

Immunity and spaceflight

Studies in humans show:

- Elevated neutrophils (innate immunity)
- Increased and/or decreased T-cell and monocyte counts (depending on mission duration and sample collection methods)
- Reactivation of latent Epstein-Barr virus, varicella-zoster virus, and cytomegalovirus in astronauts during flight
    - Sign of autoimmune disorder



Spaceflight affects bacterial pathogens, too

- Increased antibiotic resistance in *E. coli* (Tixador et al. 1987, Lapchine et al. 1986)
- Increased virulence in *Salmonella typhimurium* (Wilson et al. 2008) and *Pseudomonas aeruginosa* (Crabbé et al. 2011) and others
- Morphological changes to *E. coli*, including higher cell count after growth, thicker cell envelope, and increased cluster formation (Zea et al. 2017)

Drosophila melanogaster – the model organism

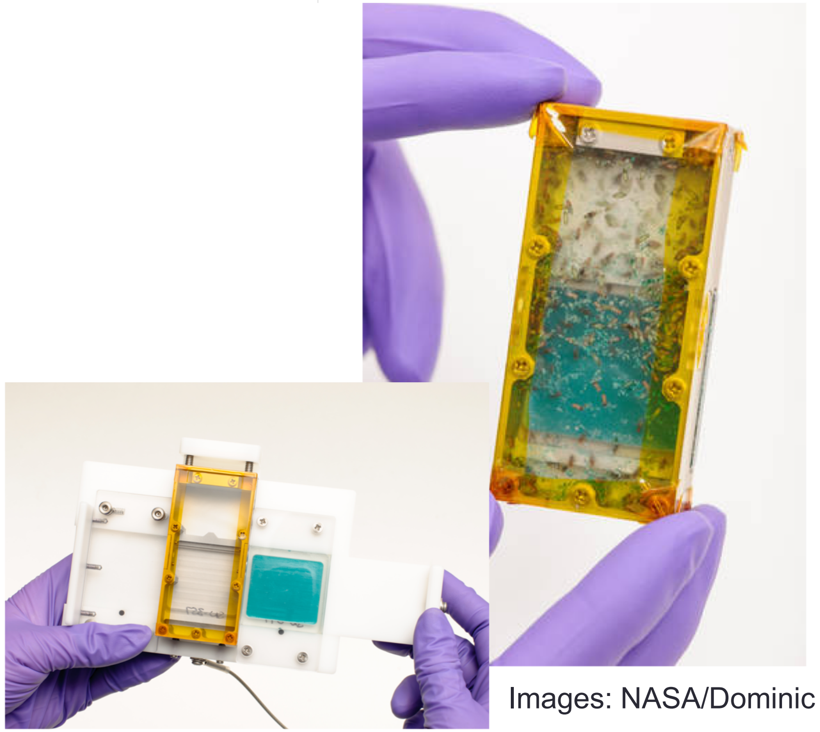
- Low cost
- Easy to maintain in lab
- Amenable to experimental manipulation
  - Readily available genetic tools and lines
  - Well-annotated genome
  - Fast generation turnover
- Small, convenient for ISS experimentation
- ~75% of human disease-causing genes have a functional homolog in the fruit fly
  - Model for human innate immunity

Past experiments

Spx-5, 2015

- Examined the effect of microgravity and other spaceflight factors on the pathogen *Serratia marcescens* (insect strain Db11)

- Stored at 4ºC for 30 days, then grown for 5 days at ambient (23-25ºC) plus 2 days for sample retrieval
    - Stored in 50% glycerol and stored at -80ºC to maintain spaceflight-induced changes

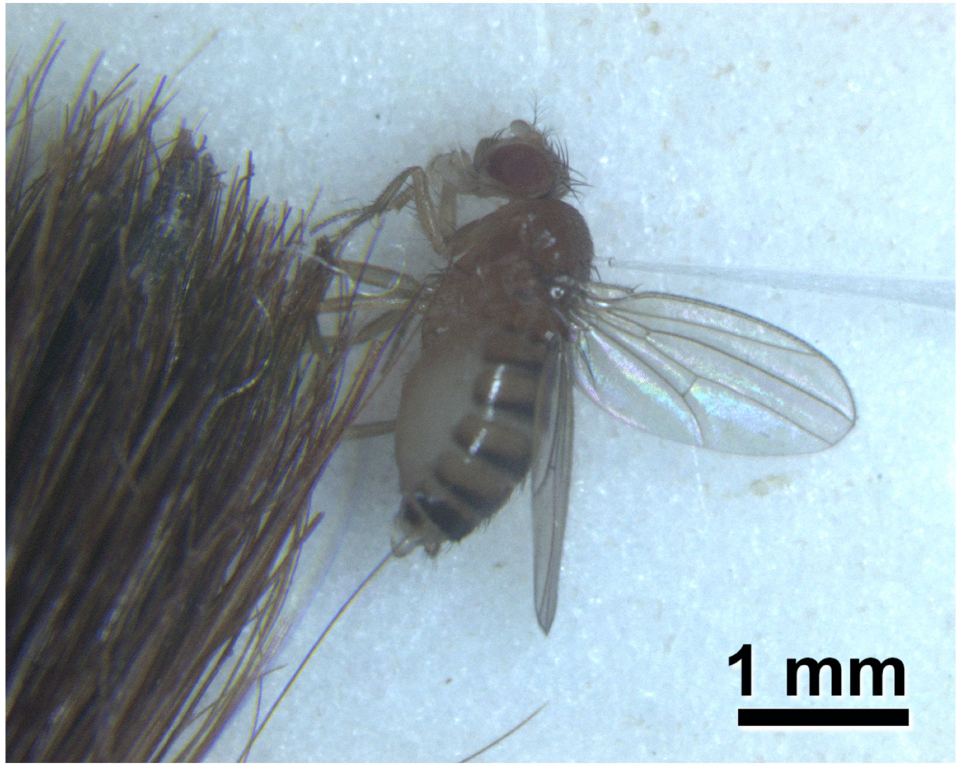


Images: NASA/Dominic Hart

Injections of spaceflight bacteria

Are pathogens that are exposed to spaceflight conditions altered in their virulence or other phenotypic properties?

- Injections of bacteria grown on the ISS into ground-reared flies (directly from glycerol)
- Injections of “second subcultures”, or bacteria that were taken from ISS samples and grown on the ground in culture
- Measured survival, in-host growth of bacteria, and transcriptomic differences of host immune response

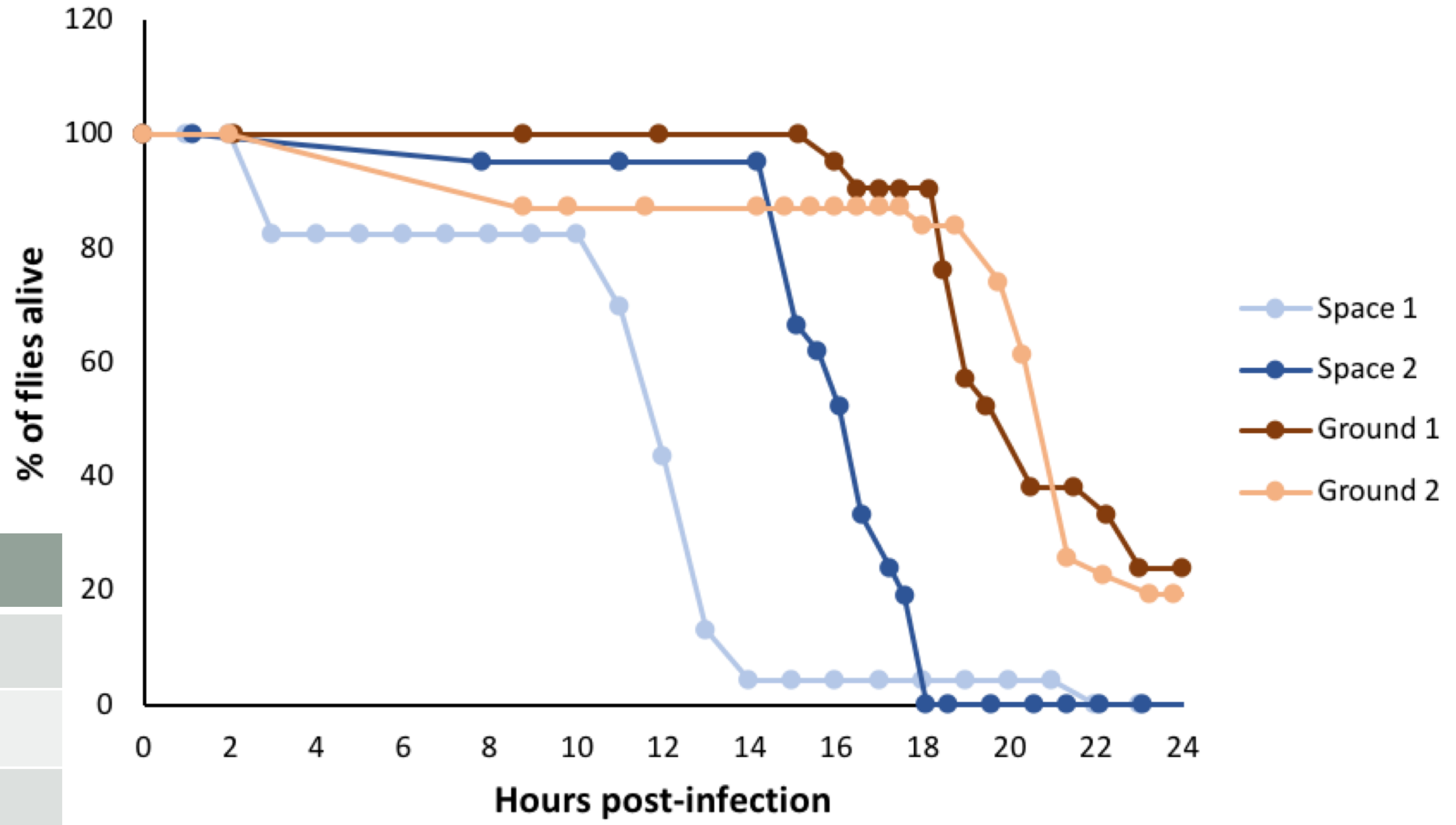


Spaceflight increases virulence of *S. marcescens*

Spaceflight samples overall are 4-6 times more lethal than Ground controls

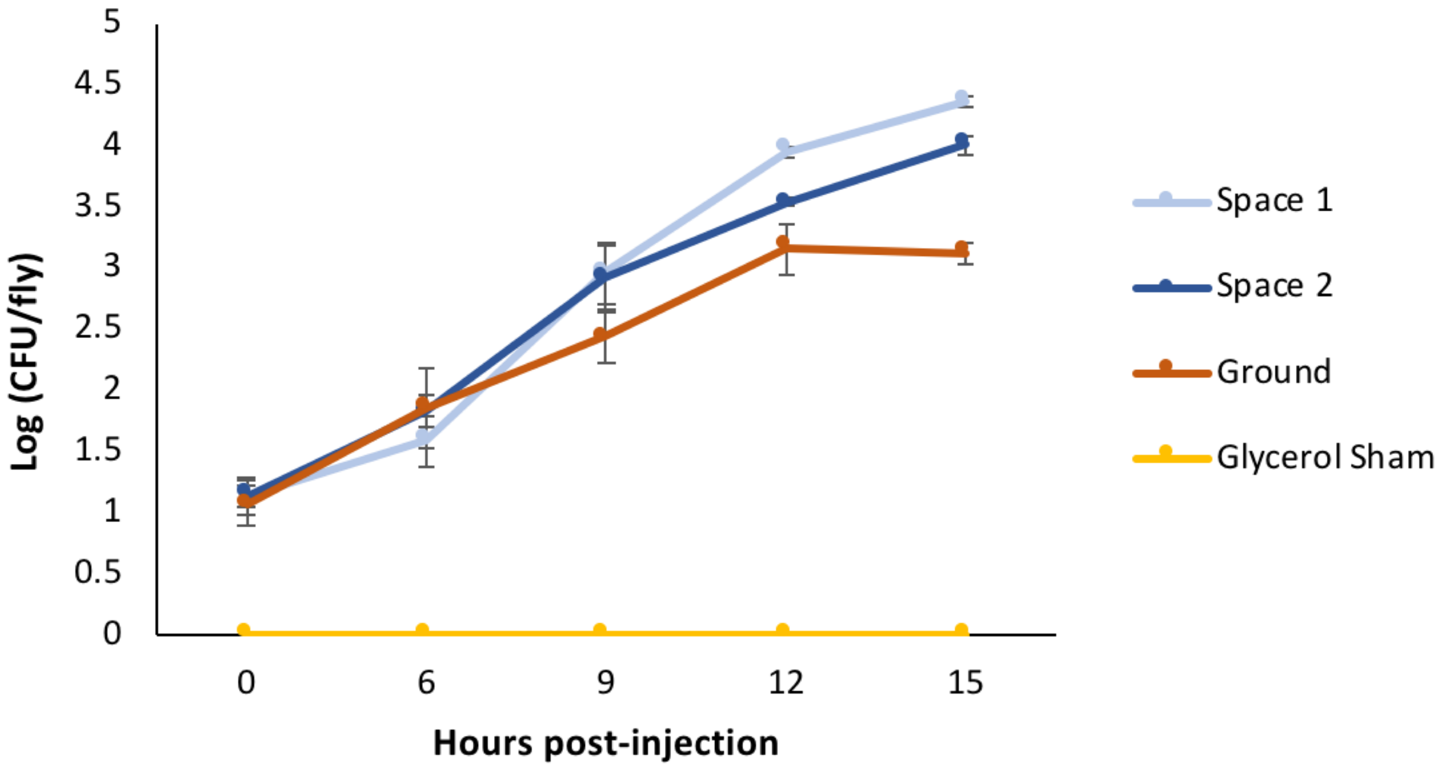
Cox Proportional Hazards test:

Level 1	Level 2	Ratio	P-value
Space 1	Ground 1	6.64	<0.0001
Space 1	Ground 2	5.69	<0.0001
Space 1	Space 2	1.15	0.68
Space 2	Ground 1	5.77	<0.0001
Space 2	Ground 2	4.95	<0.0001



In vivo growth of bacteria increased after spaceflight

Hour post-infection	P-value (compared to ground)
0	NS
6	NS
9	NS
12	0.0009 (Space 1) 0.0042 (Space 2)
15	0.0002 (Space 1) 0.0056 (Space 2)

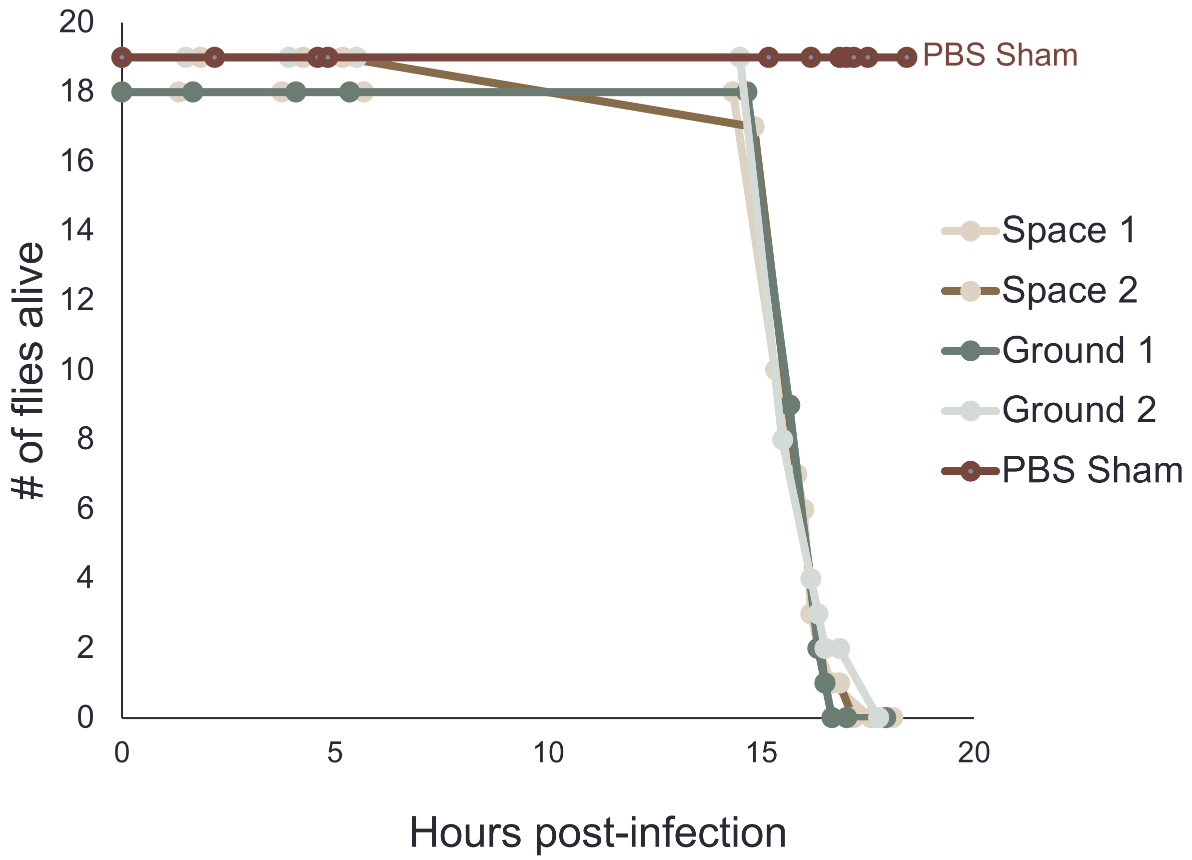


Loss of increased virulence with ground subculture

After culturing spaceflight samples only one time on the ground, they became less virulent, and statistically had the same lethality as ground controls

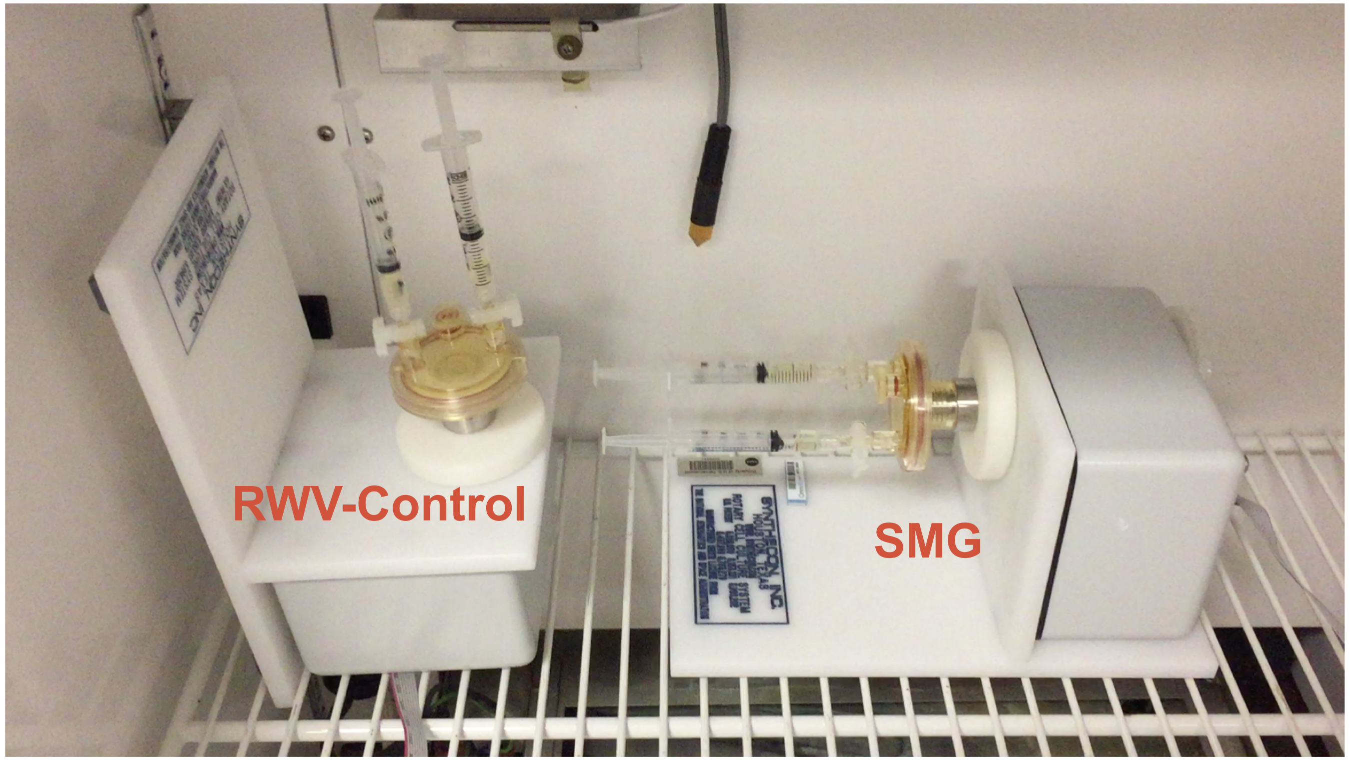
Cox Proportional Hazards test:

Level 1	Level 2	Ratio	P>Chi square
Space 1	Ground 1	0.96	0.938
Space 1	Ground 2	2.29	0.091
Space 2	Ground 1	0.88	0.793
Space 2	Ground 2	2.1	0.106



Rotating wall vessel (RWV) simulates microgravity

- Bacteria prepared overnight in LB+streptomycin subculture, then diluted down to OD_{600}=0.10 in fresh LB+strep medium
- 10 ml placed into each RWV, syringes used to ensure that all bubbles were removed
- Allowed to spin at 37ºC for 24 hours before injections or gene expression analysis



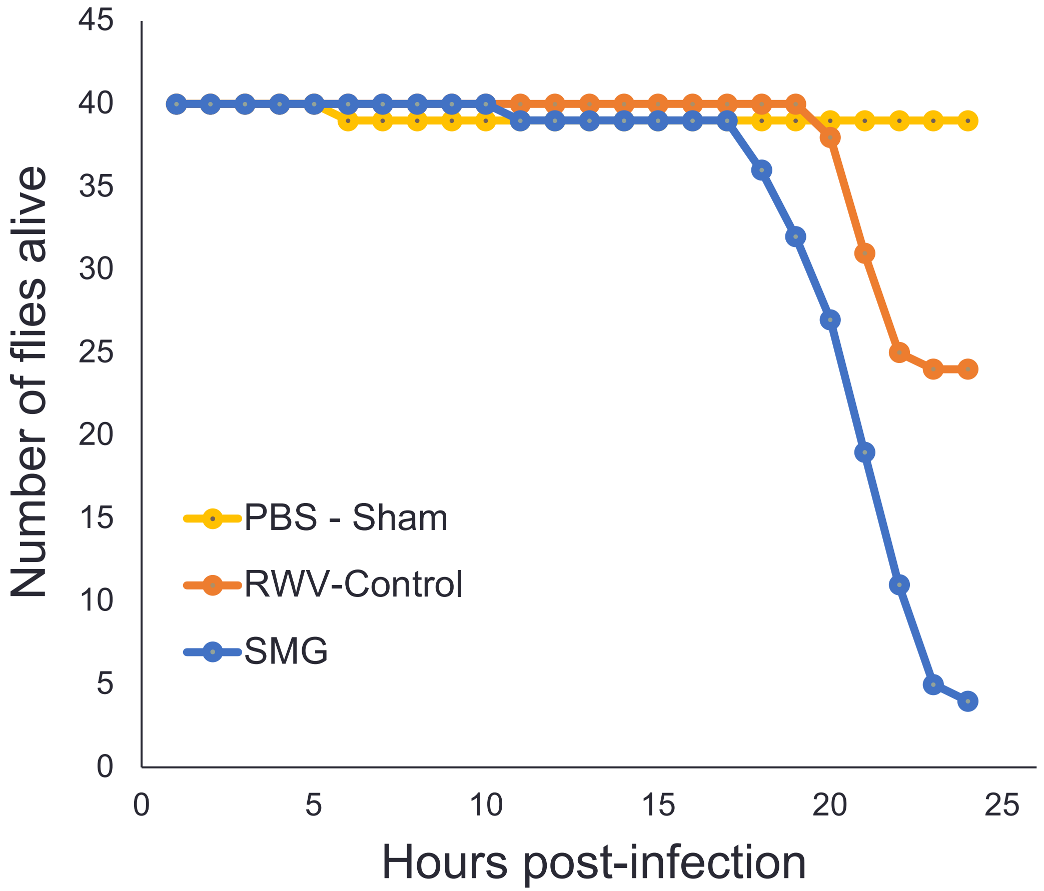
Bacteria injections

- *Serratia marcescens* strain DB11 was injected into 2-3 day old males and females
  - N=40 for each treatment group: simulated microgravity (SMG), RWV control, and PBS sham injections
  - 69nL injection volume using Nanoject II containing 10-15 CFU per fly
  - 2 whole flies from each treatment were homogenized in 200 µl PBS and plated on LB agar overnight in order to confirm CFUs per fly after injection
  - Trypan blue added to suspension to visually confirm injection

Simulated microgravity increases bacteria virulence

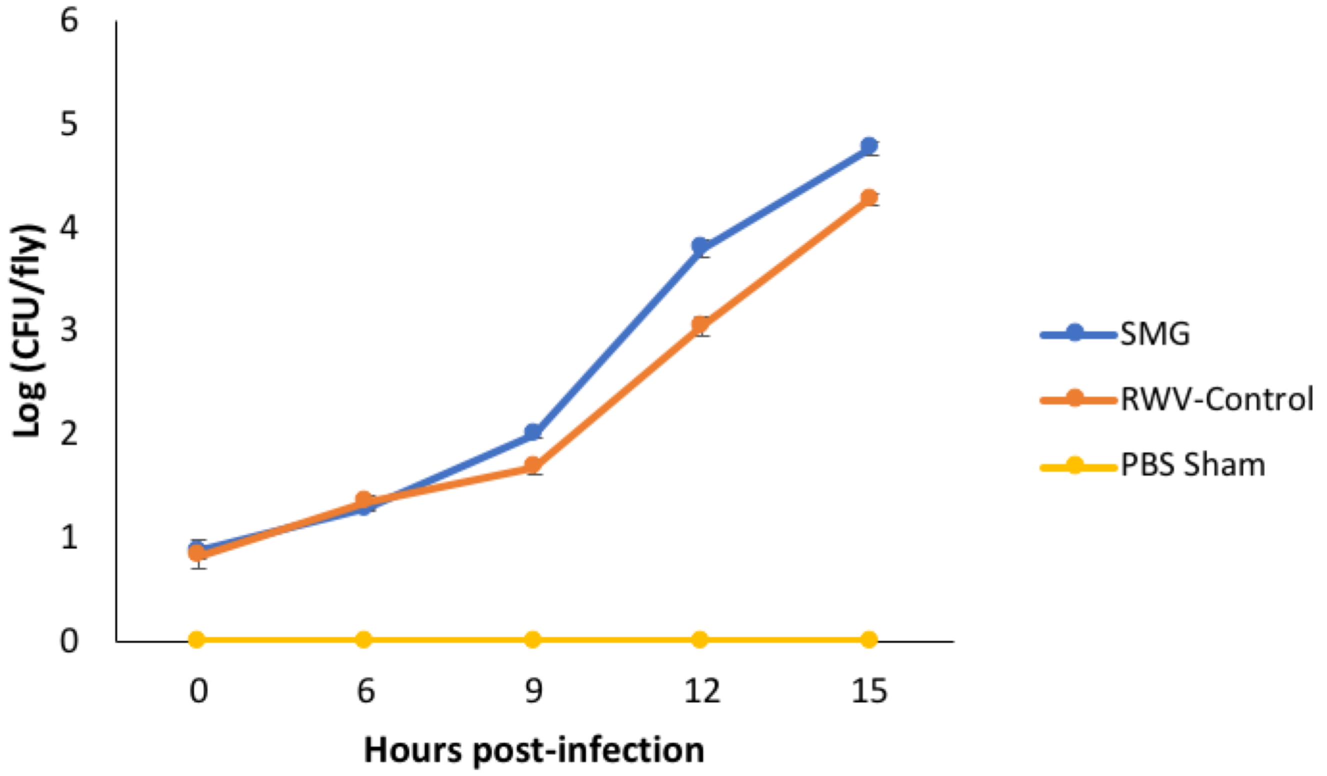
Proportional Hazards: SMG DB11 reduces survival by 3.4 fold (P=0.0002) compared to the RWV control from the same subculture

Level 1	Level 2	Ratio	P>Chi square
SMG	RWV-Control	3.37	0.0002
SMG	PBS	4.42	>0.0001
RWV-Control	PBS	1.41	0.0086



Host *in vivo* growth increased after SMG

Hour post-infection	P-value (compared to ground)
0	NS
6	NS
9	0.008
12	0.019
15	0.009



Gene expression analysis using qPCR

- Genes selected from previous spaceflight literature (Nickerson et al., Wilson et al. 2007)
- RNA was extracted from each sample with the Rneasy Mini Kit (Qiagen), and was converted to cDNA using the RevertAid First Strand Synthesis Kit (ThermoScientific).
- Gene expression was determined via qPCR using Quantitech SYBR Green (Qiagen)
- 30 total primers tested so far, 10 differentially expressed

Gene Name	Fold Change	Description
asnB	28.84	asparagine synthetase B - Catalyzes the conversion of aspartate to asparagine
FlgG	2.09	Helps form the filaments of bacterial flagella
secY	2.06	Essential for protein secretion across the cytoplasmic membrane
FliE	2.05	Involved in biogenesis of flagella
nudE	2.00	Enzyme superfamily that helps remove potentially toxic metabolites and stress-induced signaling molecules from the bacterial host
lpxD	2.04	Lipopolysaccharide biosynthesis gene, expression essential for biofilm formation, and decreased expression reduced bacterial attachment to cultured airway epithelial cells
tatB	2.08	Reduced expression results in slowed growth, impaired cytochrome oxidase c activity, and increased susceptibility to intracellular infection.
hslU	2.31	Heat shock protein (ATPase) that is expressed in response to cell stress
groEL	1.21	Heat shock protein, involved with host cell lysis
dnaK	2.00	Involved in the heat shock response, deletion decreases bacteria cell survival

Conclusions and future directions

- Microgravity induces a phenotypic adaptive change in *Serratia marcescens*
- Pathogen exposure to simulated microgravity reduces survival in fruit flies compared to controls
- Future experiments will focus on identifying the genetic pathways involved in the adaptive change that results in increased virulence
- This could be applied in future studies to human cell cultures and other mammalian models

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Host (fruit fly) response to infections with DB11

Genetic (RNA gene expression) comparison of flies infected with space versus ground strains of *S. marcescens*

Gene name	Fold change	Description
Peptidoglycan recognition protein LB	1.42	Gram-negative bacteria defense
vsg (visgun)	5.48	Broadly involved in cell proliferation
Acid phosphatase 1	5.04	Clinical role in human disease, precise function unknown
Longitudinals lacking (lola)	2.96	Involved in axon guidance
Methyltransferase	2.41	Broadly involved in disease and metabolic disorders
Lectin-like gene	2.00	Pattern recognition receptor, immune response
Trypsin (serine protease)	1.02	Melanization response to pathogens/parasites

All values $P<0.05$

No difference of in-host growth after second subculture

Again, no significant difference of in-host growth rate between space and ground bacteria after infection (F=2.129, P=0.152)