Elucidating the Hypothesis

Overall Research Question: How do the evolution, genetic basis, and cost of resistance against different phages targeting the same or different bacterial surface receptors in the Pseudomonas aeruginosa PAO1 strain change depending on the exposure to phages simultaneously or sequentially?

List of Experiments:

Figure 1: Changes in the strength of resistance, regarded as trade-offs, were quantified through spectrometry, measured as a reduction in bacterial growth. There are a total of four phages, PA10P2 and 14/1, targeting LPS cell surface receptors, and PA5P2 and PT7, targeting type IV pilus receptors, that were exposed to different bacterial replicates, PAO1_FT1, PAO1_FT2, and PAO1_FT3, all derived from the same ancestral strain of *Pseudomonas aeruginosa* strain PAO1 in a modified fluctuation test.
Type of experiment: Experimental test
Hypothesis: Since the application of multiple phages either simultaneously or sequentially affects the trajectory of phage resistance evolution, if the change in strength of phage resistance is different based on sequential or simultaneous exposure to multiple phages affecting either the same or different receptors, then there will be different trade-offs displayed for each phage combination.

Figure 2: Changes in fitness relative to the ancestral strain of *P. aeruginosa* were calculated by. This was done for all pairwise combinations and for both sequential (both orders for each pair) and simultaneous exposure. Absorbance values were taken every 30 minutes and a bacterial growth curve was formed for each mutant to determine several characteristic factors of bacterial growth. Max growth rate was divided by mean ancestral growth rate to calculate relative fitness.
Type of experiment: Experimental test
Hypothesis: Since resistance mutations are associated with fitness costs in bacterial hosts and different timing and order of exposure to phages results in different levels of resistance, if different phage combinations, timing, and order of exposure change the levels of resistance mutation-associated fitness costs, then the fitness relative to the ancestral strain will change depending on different selection regimes.

Figure 3: Through Illumina sequencing and alignment of mutants with the ancestral PAO1 reference, the number of mutations in each mutant was identified. Treatment regimes were indicative of the frequency and type of mutation. Types of mutations

included those in Type IV pilus associated genes, LPS associated genes, Other genes, Large deletions (>250 kb) and duplication/insertion events.
Type of experiment: Descriptive study
Hypothesis: Since different phage combinations, timing of exposure, and order of exposure result in different strengths of resistance and associated fitness costs, if the number of mutations in each mutant is also dependent on the selection regime combination, then there will be consistent differences in the number of mutations associated with each regime.

Figure 4: The fitness costs associated with resistance mutations were calculated for single mutations and double mutations. This included mutations in LPS associated genes and type IV pilus associated genes, along with some other genes. Both same-target (LPS + LPS mutation / type IV pilus mutation + type IV pilus mutation) and different-target genes (LPS + type IV pilus mutations) are shown. Fitness was calculated as in Figure 2.
Type of experiment: Descriptive study
Hypothesis: Since selection regimes that combine both LPS and type IV pilus-targeting phages showed more greater changes in resistance, more resistance-associated fitness costs and more mutations overall, if combinations of LPS and non-LPS associated mutations result in additive fitness costs, then there will be significantly greater loss in fitness relative to ancestral strains in LPS+non-LPS mutations compared to single mutations and/or single cell receptor-associated mutations.