Name: Romero, Stephanie

CREATES Analysis Template

Experimental Test

Figure or Table Number: 1

"Official" title for this figure or table (from the caption):

Trade-offs in the strength of resistance to each phage combination.

My (simplified, decoded, in regular language) title for this figure or table:

Changes in the strength of resistance depending on different phage combinations and types of exposure (sequential vs simultaneous0

The controls in this experiment are:

First-step phage exposure associated average resistance strength in each panel

They are represented (in which part of the chart or graph, or what figure panels?)

All panels. They are represented by the shaded red and blue regions in the background of each figure.

The experimental conditions are:

- Different phage combinations and timings (sequential vs simultaneous)

They are represented as:

- Red and blue circles in panels A-C (refer to legend on right of each panel)

We need to compare the controls in All of the panels in figure 1 **with the experimentals in**

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All of the panels n figure 1 **to find out:**

The change in resistance (trade off) to certain phages upon second-step exposure to a different phage either with the same or different cell receptor target.

We need to compare the controls in All of the panels in figure 1 **with the experimentals in**

All of the panels in figure 1 **to find out:**

The difference in resistance to phage combinations between simultaneous and sequential exposure.

When we make these comparisons, we conclude from this figure:

In all of the panels, simultaneous exposure results in lower resistance. In phage combinations that target different receptors versus those that target the same receptors, we see large changes in resistance between the first and second steps, meaning there is weak cross-resistance.

Was the hypothesis supported? Why or why not?

Yes, the trade-off values are different depending on the types of phage combinations, timing, and order of exposure, as expected.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

Why is the average resistance in strength in first step phage exposures represented as an entire shaded region as opposed to single value?

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Experimental Test

Figure or Table Number: 2

"Official" title for this figure or table (from the caption):

Relative fitness of resistant mutants is determined by selection regime.

My (simplified, decoded, in regular language) title for this figure or table:

Fitness costs are different depending on phage combination, timing, and order of exposure.

The controls in this experiment are:

Ancestral fitness (set as 1)

They are represented (in which part of the chart or graph, or what figure panels?)

All panels (A-C)

The experimental conditions are:

Selection treatment (phage combination, timing, and order of exposure)

They are represented as:

- Red and blue circles in panels A-C (refer to legend on right of each panel)

We need to compare the controls in Every panel **with the experimentals in** Every panl **to find out:**

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…the average relative change in fitness based on the selection treatment given to each replicate.

We need to compare the controls in Every panel **with the experimentals in** Every panel **to find out:**

The differences in fitness costs between simultaneous and sequential exposure.

When we make these comparisons, we conclude from this figure:

Overall, we see a greater drop in fitness when phages with different receptor targets are exposed to the replicates versus when they have the same receptor target. More specifically, a greater drop in fitness is observed when type IV pilus is targeted before LPS. Also, simultaneous exposure appears to results in, on average, lower associated fitness costs.

Was the hypothesis supported? Why or why not?

Yes, there were notable differences in fitness costs depending on the selection treatment given.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

How are we supposed to explain that extremely low fitness value associated with simultaneous exposure in panel 2A?

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Descriptive Study

Figure or Table Number: 3

"Official" title for this figure or table (from the caption):

Treatment regimes determine the frequency and type of resistance mutations selected.

My (simplified, decoded, in regular language) title for this figure or table:

Different selection treatments result in different numbers and types of mutations.

If we compare panel(s)/column(s) A **and** B+C **, we learn about:**

Single exposures are associated with single, phage specific mutations.

If we compare panel(s)/column(s) B **and** C **, we learn about:**

- There is never more than one LPS-associated mutation in simultaneous exposure, whereas there may be more than one in sequential exposure
- Sequential exposure treatments using phages that target different receptors require at least 2 mutations for each receptor type (LPS and type IV pilus).

When we make these comparisons, we conclude from this figure:

Because more mutations are required for treatments sunig phages that target different receptors and more mutations are likely to occur with sequential exposure versus simultaneous exposure, this may explain why there is a higher associated resistance and

higher associated fitness cost with sequential exposure treatment.

Was the hypothesis supported? Why or why not?

Yes, there were some consistent differences in the amount and types of mutations depending on selection treatment. Single exposure almost always resulted in only one mutation, sequential exposure to phages targeting different receptors always conferred at least two mutations, and in simultaneous exposure there was never more than one LPS associated mutation.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

The second mutation that occurred in one of the single exposure trials was described as "likely due to hitch-hiking". What is hitch-hiking? I looked it up but I'm still not quite sure I understand.

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CREATES Analysis Template

Descriptive Study

Figure or Table Number: 4

"Official" title for this figure or table (from the caption):

Contrasting fitness costs resulting from specific combinations of single and double mutations.

My (simplified, decoded, in regular language) title for this figure or table:

Different fitness costs associated with different phage cocktail combinations.

If we compare panel(s)/column(s) Same receptor type double mutations **and** Different receptor type double mutations **, we learn about:**

Mutations in both LPS and non-LPS associated genes result in additive fitness costs that are significantly greater than changes in fitness costs associated with mutations in only LPS associated genes or only type IV pilus associated genes.

If we compare panel(s)/column(s) wzy **and** PA0429 + wzy **, we learn about:**

Not all mutations that are selected for in first-step phage exposure in sequential regimes confer a significant change in fitness relative to the ancestral strain.

When we make these comparisons, we conclude from this figure:

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These results suggest that fitness costs associated with mutations in LPS and non-LPS targets may depend on specific epistatic interactions between the mutations and thus not all combinations of mutations will result in additive fitness costs.

Was the hypothesis supported? Why or why not?

Yes, the hypothesis was supported. Because LPS + non-LPS double mutations resulted in greater resistance trade-offs and greater fitness costs overall, it was hypothesized that combinations of LPS and non-LPS associated mutations result in additive fitness costs. This was supported due to the fact that greater changes in relative fitness were seen for double mutations with different receptor targets versus those with the same receptor targets.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

What determines a significant change in fitness? This value, as far as I'm concerned, was never established.