Paula Sing

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:

Strength of phage resistance (and changes in strength) when phages are applied simultaneously or in sequence

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The controls in this experiment are:

This figure has multiple experiments, so #1 is a control for one experiment and an experimental group for another experiment

1. Strength of resistance after exposure to a single phage
2. Strength of resistance of ancestral bacteria, PAO1

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

1. Panels A-C
2. Not pictured, but represented as "1.00" on resistance to phage axis (value of 1 means mutant bacteria had same resistance as ancestral bacteria)

The experimental conditions are:

1. Bacteria exposed to phages in sequence
2. Bacteria exposed to phages simultaneously
3. Bacteria exposed to a single phage

They are represented as:

Different colored dots on the graph; the legend on the right specifies which experimental condition the dot represents. Present in panels A-C.

We need to compare the controls in 1A **with the experimentals in** 1A **to find out:**

- When exposed to either PA10P2 or 14/1 (both LPS-targeting phages), bacteria evolved equal resistance to both phages despite only being exposed to one.
- The second step of sequential selection of bacteria with phages PA10P2 and 14/1 (irrespective of order) did not provide any significant increase in resistance in the second phage that was applied. Also, there was minimal trade-off in resistance to the first phage (resistance strength did not decrease much).
- No trade-off in resistance strength occurred when 14/1 was applied first, then PA10P2. Trade-off in resistance strength did occur when PA10P2 was applied first, then 14/1 such that when 14/1 was applied, strength of resistance to PA10P2 decreased.
- Simultaneous selection with phages both targeting LPS resulted in weaker resistance than when the phages were applied sequentially

We need to compare the controls in 1B-C **with the experimentals in** 1B-C **to find out:**

- Mutations that resulted from application of one phage rarely provided cross-resistance to the phage that was applied next.
    - When the first phage applied was a phage targeting LPS, weak cross-resistance was developed for the following type IV pilus-targeting phage.
    - When the first phage applied was PA5P2 (type IV pilus-targeting phage), no cross-resistance against the following LPS-binding phages was developed
- The strength of resistance due to the first phage applied didn't diminish when a second phage was applied after; in some bacteria strength of resistance to the first phage increased when the second phage was applied.
- Simultaneous selection for resistance using phages with different targets resulted in weaker resistance than when the phages were applied sequentially

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

- Multiple resistance mutations were required to protect against multiple phages that targeted different cell surface molecules.
- When phages target different receptors sequential phage selection leads to

additive resistances that don't impose trade-offs in resistance strength.
- Simultaneous exposure of multiple phages reduces the strength of resistance evolved by bacteria.

Was the hypothesis supported? Why or why not?

The hypothesis that bacteria exposed to phages simultaneously would have reduced resistance was supported by the fact that bacteria exposed to phages targeting the same or different surface molecules had lower resistance than bacteria which were exposed to the same phages, but sequentially.
The hypothesis that bacteria exposed to phages sequentially would have reduced resistance was somewhat rejected by the fact that simultaneous application of phages reduced bacterial resistance to phages more than sequential application of phages did.

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):

- One conclusion in the article associated with this figure was that simultaneous exposure of multiple phages can promote reciprocal cross-resistance. I did not understand what was meant by "reciprocal cross-resistance." Does this refer to multiple mutations which both provide resistance to both phages?
- The only test done with phages targeting the same surface molecule was with that of phages targeting LPS since their methods failed in producing bacteria resistant to phage PT7. I would like to see more data on bacteria exposed to phages targeting the same molecule to justify the results found here; it would also be worthwhile to further investigate why resistance to phage PT7 is so difficult to obtain in bacteria.

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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:

Changes in resistance due to application of phages simultaneously or sequentially reveal that fitness cost depends on how phages are applied

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The controls in this experiment are:

This figure has multiple experiments, so #1 is a control for one experiment and an experimental group for another experiment

1. Relative fitness of bacteria after exposure to a single phage
2. Fitness of ancestral bacteria, PAO1

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

1. Panels A-C, as the red and blue dots
2. Not pictured, but represented as "1.00" on fitness relative to ancestor axis (value of 1 means mutant bacteria had same fitness as ancestral bacteria)

The experimental conditions are:

1. Bacteria exposed to phages in sequence
2. Bacteria exposed to phages simultaneously
3. Bacteria exposed to a single phage

They are represented as:

Different colored dots (bullseye dots) on the graph; the legend on the right specifies which experimental condition the dot represents. Present in panels A-C.

We need to compare the controls in 2A-C **with the experimentals in**

2A-C **to find out:**

Sequential resistance caused higher fitness cost than simultaneous resistance in almost half of the samples, but there were also cases where sequential resistance had equal or lower fitness costs compared to simultaneous resistance.

We need to compare the controls in 2B-C **with the experimentals in**

2B-C **to find out:**

In sequential exposure, when the first phage applied was a type IV pilus-targeting phage and the second was a LPS-targeting phage, there were significant reductions in relative fitness after application of the second phage in half of the samples. When the opposite order was applied, there were no changes in fitness cost after the second phage was applied.

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

- Strong resistance against multiple phages can be acquired sequentially without additional fitness cost, whereas weak resistance acquired from simultaneous phage exposure does require additional fitness cost.
- Cost of resistance is mutation specific.

Was the hypothesis supported? Why or why not?

The hypothesis that resistance to multiple phages is associated with a high fitness cost was refuted to an extent since it was shown that strong resistance against multiple phages was able to be obtained sequentially without additions to fitness cost. There was also some evidence supporting this hypothesis in that simultaneous phage exposure did require additional fitness costs.

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 was it necessary to pick three bacterial colonies from the stock to do this experiment? Why not just have replicates of a single colony to make results more uniform?

- Again, more experiments/results with phages targeting different cell-surface molecules
- What was the reason behind the failure of isolating multiple phage-resistant bacteria here - especially for PAO1_FT3?

Paula Sing

CREATES Analysis Template

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:

The types and number of resistance mutations depends on what phages are applied, in what order, and if they are applied simultaneously.

If we compare panel(s)/column(s) 3A-C **and** 3A-C **, we learn about:**

Single-phage resistance results from single mutations while multi-phage resistance results from multiple resistance mutations

If we compare panel(s)/column(s) 3A **and** 3A **, we learn about:**

Application of single phages created phage-resistant mutants with single mutations in receptor-specific genes. Mutations to provide resistance against LPS-binding phage usually occurred in the *wzy* gene. Mutations to provide resistance against type IV pilus-binding phages usually occurred in type IV pilus-associated genes like *pilB, pilN,* and *pilR.*

If we compare panel(s)/column(s) 3B **and** 3B **, we learn about:**

- Resistance to phages applied in sequence required a combination of two mutations: one for each phage applied. When the phage applied second was a type IV pilus-binding phage, the mutation occurred in genes such as *pilT, pilU, pilB, pilY1,* and *pilE.* When the phage applied second was a LPS-binding phage, the mutation occured in gene *wzy* or *galU.*
- For phages targeting the same receptor there was a difference depending on the order of phage exposure: When 14/1 was presented followed by PA10P2, there was only one mutation but if the order was reversed, there were additional mutations after the second phage was presented.

If we compare panel(s)/column(s) 3C **and** 3C **, we learn about:**

- When phage pairs that targeted type IV pilus and/or LPS receptors simultaneously, there was no accumulation of multiple receptor-specific mutations.
    - There were resistant mutants with resistance to LPS-binding phages or type IV pili-binding phages, but not with both.
- Two resistance mutants showed duplication of genes *ssb* and *PA3263*.
-

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

- Trade-offs in resistance strength between phages that have different binding targets did not occur when resistance was acquired sequentially because multiple receptor-specific mutations were acquired which were additive in resistance strength.
- PA10P2 and 14/1 both adsorb to the LPS but probably target different sites

Was the hypothesis supported? Why or why not?

The hypothesis that a single mutation can support resistance to a single phage was supported by the finding of multiple phages which were resistant to single phages and had only one mutation.
The hypothesis that phages with cross-resistance mutations would be resistant to multiple phages was supported by presence of bacteria which only had one mutation, but were resistant to multiple phages.
The hypothesis that phages which obtain resistance to phages through sequential exposure to phages occurs through accumulation of different mutations was supported by the fact that when phages were presented sequentially, the resistant bacteria that resulted showed at least two mutations unless the phages the bacteria was exposed to

both targeted the same surface molecule.

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):

- I would like to know more about the effect of gene duplications on the evolution of resistance in bacteria. Maybe a future experiment could further elucidate this.
- The authors mentioned that a secondary mutation in panel 3A of this figure was a result of "hitch-hiking." What is this?
- Why were LPS associated mutations more common among bacteria which were exposed to simultaneous resistance schedules?

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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:

The fitness costs associated with single mutations and with double mutations

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

Mutation in gene PA0429 did not result in reduction of fitness costs.

If we compare panel(s)/column(s) Fig. 4 **and** Fig. 4 **, we learn about:**

- Fitness costs were usually additive between LPS and type IV pilus mutations
- If the first mutation accumulated was of a type IV pilus gene, then the second mutation in the LPS-associated gene *galU* increased fitness costs. If the second mutation was for the LPS-associated gene *wzy,* then there was no additional fitness cost.
- When the first mutation accumulated was against an LPS gene and the second mutation was also against an LPS gene the fitness cost was zero (or about zero).

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

The fitness costs of sequential resistance against multiple phages likely depends on epistatic interactions between specific resistance mutations.

Was the hypothesis supported? Why or why not?

The hypothesis was generally supported because fitness costs associated with a single mutation tended to be much lower than the fitness costs associated with multiple mutations together.

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):

- One column (for *wbpL* mutation) shows an increase in fitness. How would this occur is this gene is associated with LPS which is important for bacterial growth? What kind of mutation would cause this gene to increase fitness for this bacterial strain?
- Why is the fitness cost associated with a mutation in the gene *rmlA* so big compared to other gene mutations?