# Relative CRISPR-phage diversity: experiment ideas

I think there are three key questions to ask regarding relative CRISPR-phage diversity:

1. What is the effect of relative diversity on resistance evolution?
2. What is the role of frequency-dependence in coexistence?
3. What is the potential for the evolution of generalist phage?

These three questions can be explored with at least 3 separate but related experiments, and ideally supported by novel theory.

Throughout I have expressed relative diversity as the ratio of phage:host genotype number.

## Experiment 1: resistance evolution

As the diversity of the phage population increases relative to the host, we might expect that hosts will evolve enhanced CRISPR-based resistance. When the whole host population is susceptible (1:1), CRISPR fitness is reduced and surface mutants (SM) can invade (van Houte *et al.* 2016 Nature) due to a high force of phage infection (Westra *et al*. 2015 Curr Biol). As phage diversity increases, force of infection might decrease as proportionally less of the phage population is infective. This, alongside a higher frequency of unsuccessful phage infections, might favour CRISPR and potentially multi-spacer CRISPR arrays.

These predictions could be tested in an experiment where a monoclonal/susceptible host population is challenged by different levels of phage diversity. The treatments would be:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Phage | | | | |
| Host | 1:1 | 3:1 | 6:1 | 12:1 | 24:1 |
| 1:3 | 3:3 | 6:3 | 12:3 | 24:3 |
| 1:6 | 3:6 | 6:6 | 12:6 | 24:6 |
| 1:12 | 3:12 | 6:12 | 12:12 | 24:12 |
| 1:24 | 3:24 | 6:24 | 12:24 | 24:24 |

This could be done in M9 for 3 days, but potentially also in LB to look for an effect of resource abundance. Include the PA14 ∆*pilA* surface mutant. *N*=24 for the 1:1 treatments, and *N*=8 for the remaining treatments, with a different escape phage in each replicate. Including the *lacZ* labelled BIM here won’t be necessary, as the host is monoclonal.

In terms of statistical tests, the main model will test if CRISPR or SM fitness is explained by relative diversity. Another model can test for an effect of relative diversity on CRISPR spacer number. Further analysis (and figures) of this experiment could also involve:

* Phage dynamics
* Host dynamics
* CRISPR vs. SM competition assay
* PCR and sequencing of CRISPR arrays
* Phage evolution assays (stamps and sequencing)

The last point of the analysis allows for phage evolution to be assessed, either through *de novo* mutation of protospacer sequences or recombination. It is important to note at this point that phage recombination can be evaluated in each experiment suggested here. Currently I’m not sure how to discern recombination from *de novo* mutation, but we can discuss this.

## Experiment 2: frequency-dependent host benefit

Frequency-dependent benefits of host diversity (dilution effects) are widespread in natural host-pathogen systems (Civitello *et al*. 2015 PNAS). Increasing the number of resistant or low-quality hosts decreases the fraction of susceptible hosts, reducing contact rates between free-living pathogens or infectious individuals, which in turn limits the basic reproduction number of the pathogen (Dobson 2004 Am Nat; Gandon 2004 Evol; Lively 2010 Am Nat). Frequency-dependence influences phage dynamics (Dennehy *et al*. 2007 Ecol Lett; Common & Westra 2019 RNA Bio) and evolutionary emergence.

If we assume no cross-infection, then the dilution effect could still operate even if a host population is challenged by a diverse pathogen population. A phage can only ever infect one host genotype, and if that host’s relative frequency depends on host diversity, phage spread could potentially be limited. Consequently, the dilution effect may maintain host protection in the context of challenge by a diverse phage population. Further, accounting for phage diversity may also limit evolutionary emergence at intermediate host diversity (Chábàs *et al*. 2018 PLoS Bio), as the whole host population is potentially infected thereby reducing selection on each phage genotype to evolve.

To test these predictions, I suggest an experiment using the relative diversity treatments shown below. Essentially, the logic is to keep relative diversity constant while changing absolute diversity. Again, this would be performed in M9 for 3 days, and including the *∆pilA* strain.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Phage | | | | |
| Host | 1:1 | 3:1 | 6:1 | 12:1 | 24:1 |
| 1:3 | 3:3 | 6:3 | 12:3 | 24:3 |
| 1:6 | 3:6 | 6:6 | 12:6 | 24:6 |
| 1:12 | 3:12 | 6:12 | 12:12 | 24:12 |
| 1:24 | 3:24 | 6:24 | 12:24 | 24:24 |

*N*=24 for the 1:1 treatment, though it might not even be necessary to do this as it’s included in Experiment 1. *N*=8 for each polyclonal treatment. One model can test how phage titre is affected by diversity treatment; another to test how the proportion of phage that expanded their infectivity range is affected by treatment; another to test how CRISPR and susceptible fitness is affected by treatment. Other statistical questions will likely be needed.

Again, analysis of this experiment could involve:

* Phage dynamics
* Host dynamics
* CRISPR vs. SM competition assay
* Tracking of a labelled strain
* Phage evolution assays (stamps and sequencing)

## Experiment 3: frequency-dependent coexistence

Experiment 1 holds host diversity constant while varying relative diversity, and Experiment 2 holds relative diversity constant while varying absolute diversity. Experiment 3 is intended to explore the effects of intermediate levels of relative diversity. The predictions of what might occur at intermediate relative diversity are a little more vague, and I think this is where we could lean more heavily on theory.

At intermediate relative diversity, we might see complex interactions between the effects observed in Experiments 1 and 2. One main verbal prediction is that host-phage coexistence and genotypic composition might be stabilised under such conditions. This could be a result of a balance between epidemic size and host dilution, leading to a frequency-dependent “kill the winner” dynamic. A given phage genotype is less likely to fix because focal hosts are diluted, and a given host genotype is less likely to fix because increasing its population size beyond a given threshold will benefit its phage. Genotypes are in turn less likely to go extinct because this “kill the winner” dynamic affects the whole population.

An experiment to test for an intermediate relative diversity effect could involve the treatments highlighted below. These are essentially those that span the “intermediate” diversity range that showed strong differences in phage evolutionary emergence in the Ecology Letters manuscript we’re preparing.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Phage | | | | |
| Host | 1:1 | 3:1 | 6:1 | 12:1 | 24:1 |
| 1:3 | 3:3 | 6:3 | 12:3 | 24:3 |
| 1:6 | 3:6 | 6:6 | 12:6 | 24:6 |
| 1:12 | 3:12 | 6:12 | 12:12 | 24:12 |
| 1:24 | 3:24 | 6:24 | 12:24 | 24:24 |

I figure that there’ll need to be *N*=8 for each treatment, because this is the minimum needed to equally represent each labelled BIM.

A potential issue here is that the labelled BIMs would only allow the tracking of a single genotype during the experiment, so it might be a challenge to discern any frequency-dependent kill-the-winner-type dynamics. A complimentary approach might be to do time-shift assays. Additionally, more advanced deep-seq approaches could help too.