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Phage Predation and Bacterial Motility: A Fitness Tradeoff?

Predator-prey interactions play a central role within any ecosystem, often shaping broader community dynamics. Predation of bacteria by bacteriophages (phages) is widespread in the biosphere and plays important functional roles, including in our microbiome.[1] As understanding of the human microbiome has grown, so too has our interest in intentional microbiome modification to benefit human health. One particular application is the use of phage as an antibacterial agent, often known as phage therapy. While phage therapy offers many advantages over conventional antibiotics, it also has significant drawbacks, such as the evolution of bacterial resistance to phage treatment.[1] Understanding how resistance arises and persists in a population is not only vital to the successful use of phage therapy, it also furthers our understanding of the nature and mechanisms of evolution.

Phage predation begins with surface attachment, and resistance to phage can be acquired by the modification of bacterial surface receptors, which can result in reduced function of other cellular processes.[2] This is known as a fitness tradeoff: improvements in fitness with regard to one trait cause reductions in fitness in a different trait. For instance, when a phage attaches to bacterial flagella, ensuing resistance mutations negatively impact bacterial motility.[3] In *Pseudomonas aeruginosa*, an opportunistic human pathogen, reduced motility reduces fitness.[4] Because tradeoffs restrain the optimization of a single trait, they can limit the presence of resistance in a population, allowing endemic phage predation.[1]

Using bacteria-phage systems, researchers — including my mentor Dr. Michael Travisano — have demonstrated the presence of tradeoffs between resistance to phage and numerous other traits.[5] However, a direct tradeoff between motility and phage resistance has yet to be found.[3] To demonstrate this tradeoff, I followed Dr. Travisano's recommendation to experiment with Habibi, a generalist lytic phage isolated from Lake Michigan.[6] I selected *P. aeruginosa* as the experimental host because it is highly motile,[3] has motility-dependent fitness,[4] and has been a candidate pathogen for phage therapy.[7]

To demonstrate the existence of a tradeoff between motility and resistance to phage, I chose to utilize the two approaches used by Koskella et al.[3] The first demonstrates a fitness tradeoff through correlation: the phenotypes of many individuals under selection for both traits are measured, and the results are compared to the expectation that expression of the two traits is inversely related. I generated isolates through an experimental evolution regime, where *P. aeruginosa* and Habibi were incubated spatially separated in soft-agar plates, and after growth isolates were taken at varying radii from the center of the plate and transferred to a new plate. In previous, yet-unpublished work with Dr. Travisano and another undergraduate, we demonstrated that a soft-agar plate enables bacterial motility while maintaining spatial structure, and that under such conditions motility can evolve. We expected that the resistance of the isolates to phage would decrease with the distance of the sample point from the phage inoculum point, which our results confirmed. Soon, I will measure the motility of these isolates, which are expected to increase with distance from the inoculum point.[8] Once this correlation is established, I will undertake the second approach: demonstrating a fitness tradeoff by selecting for increases in one trait while relaxing selection for the other trait, then re-measuring the phenotypic outcomes. If a tradeoff is present, increases in one trait necessitate a decrease in the other. Selection for motility alone can be performed as in our previous work, by inoculating bacterial isolates into a soft-agar plate without phage and selecting from the cell front for the next generation. Selection for resistance alone can occur by inoculating isolates into shaken broth culture with phage. In these environments differences in the unselected trait have no effect on fitness, and differences in the selected trait strongly affect fitness. If a tradeoff exists we expect any increases in one trait to be offset by decreases in the other.

Once the initial project determining the existence or absence of a fitness tradeoff is completed, I will dig deeper to elucidate the mechanisms of a tradeoff, or lack thereof. By measuring lysis time, the

delay between phage attachment and lysis, and burst size, the number of viral particles released by a single lysed cell, I will define how bacterial resistance affected the timing of the phage life cycle. By measuring the size of clearings created by phage particles in a bacterial lawn, I can determine how the rate of phage attachment and dispersal have changed. Finally, by measuring the growth rate of bacterial isolates, I can learn whether other components of fitness may be changing during selection.

Future research would extend these conclusions to a broader set of conditions. Experimentally, new bacterial members — which would differ from *P. aeruginosa* in motility and in susceptibility to predation, — and/or new bacteriophages — which would differ from Habibi in lysis time, burst size and/or other factors — could be added to the experimental evolution regime. Non-experimentally, a model incorporating spatial structure, bacterial motility and resistance, and phage, could be built. Calibrated on the results of our simple system, this model could be scaled to add additional community members and dynamics. Both methods expand on the original work by identifying the general natural conditions that can create and maintain a tradeoff between motility and resistance to phage predation, and whether the results from the simple system are maintained at higher community complexity.

Evidence of a tradeoff would establish that two fundamental bacterial fitness traits, motility and resistance to phage predation, can be in constant tension with one another under some conditions. Knowledge of this phenomena informs broader questions about the responses of bacteria to phage predation and, more generally, of any mobile prey to predation. In these situations, tradeoffs may prevent competitive exclusion among prey phenotypes or allow endemic predation to persist. This is especially important in considering the application of phage therapy, as tradeoffs may prevent absolute resistance, allowing the therapy to remain effective long after initial application. On the other hand, single-species changes can affect broader ecological connections, making any intentional modification of our microbiome fraught with risk. This work will further our understanding of the interplay between bacterial motility and phage resistance, but many questions, including the effects of phage specificity or abiotic factors, and the dynamics of the onset and persistence of phage resistance, remain to be answered.

References

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