Selection on Bacterial Motility and Coevolution with Phage

Background: The question, "why is the biological world so diverse?" has motivated biologists since long before Darwin. One widely-recognized cause of biodiversity is the interaction of selective pressures on multiple traits: the nature of these interactions can constrain organisms' ability to simultaneously optimize those traits, forcing compromises that can both create and maintain biodiversity. Thus, biodiversity can be driven by the interaction of selective pressures. Two nearly-ubiquitous selective pressures faced by organisms are resource competition and biotic antagonism, like that of predators or parasites. These pressures also occur in bacterial communities, where understanding their effects is vital for two reasons. First, bacteria can serve as excellent models of general evolutionary phenomena. Second, bacteria have widespread effects, ranging from global biogeochemical cycling to disease. Thus, it is crucial to study how ubiquitous resource competition and antagonism shape the evolution of bacterial diversity.

Bacteria have evolved in response to these two pressures. For resource competition, they often use motility: the ability to move around their environment, usually coordinated by sensory systems which detect nutrient gradients[1]. Thus, motility enables bacteria to improve fitness by moving to favorable growth conditions. However, bacteria also face antagonism, particularly from bacteriophages (phages): parasitoid viruses that infect bacteria, hijack cell metabolism to replicate, and lyse (kill) their host cell[2]. This strong selective pressure can lead to antagonistic coevolution: bacteria evolve resistance to infection, then phages evolve to circumvent these resistance mechanisms[3]. So, selection on motility and by phage fundamentally affect bacteria, and their interaction could shape bacterial diversity[4,5]. For instance, resistance and motility may tradeoff, facilitating the creation and maintenance of diversity. Despite these possibilities, and the importance of bacteria as a model of the evolution of biodiversity, **it remains unknown whether or how bacteria-phage coevolution and bacterial motility selection can interact**.

System: To study this question, I will use experimental evolution, where populations evolve in real time under imposed conditions. This approach enables direct comparisons of evolutionary change across treatments. Specifically, I will build on past approaches[4], including my own undergraduate work, where phage coevolution was not included. In my novel approach, motile *Pseudomonas fluorescens* bacteria and their lytic phage Φ2 will coevolve on agar plates. Here, phage disperse (diffuse) slowly whereas bacteria spread (swim) quickly, while both microbes also undergo an extended coevolutionary arms race of resistance and infection[3].

Aim 1: Experimental Coevolution. To study how these two selective pressures may interact, I will use treatments that independently vary the strength of phage selection and the strength of motility selection. For phage selection (Fig 1A), *P. fluorescens* will be inoculated with: i) no phage, ii) ancestral phage (weak selection), or iii) phage which have been pre-adapted[3] to the host in liquid culture (strong selection). For motility (Fig 1B), inocula will be i) spotted in the center of the plate only (motility selection), or ii) evenly distributed across the plate (no motility selection)[4]. Thus, when spotted, motility is beneficial by enabling bacteria to swim outwards towards media with unconsumed nutrients and away from phage. After 24 hours, the entire plate

will be sampled and used to inoculate a fresh plate and to create a frozen archive. Each treatment will be performed in five replicates over 30 daily transfers.

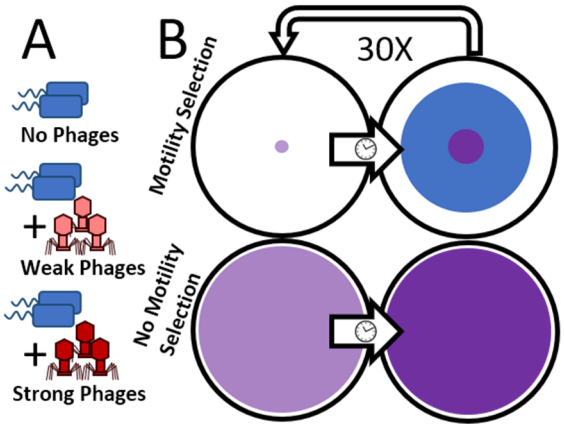

Figure 1. Experimental Treatments. Blue is bacteria alone, purple is bacteria and phage.

Aim 2: Isolate Characterization. I will isolate five bacterial and five viral strains from the archives of transfers 0, 10, 20, and 30. I will then measure bacterial motility and resistance to each viral isolate. I hypothesize that motility selection and stronger phage selection will produce faster evolved motility and stronger coevolution, respectively. Further, because bacterial motility and phage resistance can be costly[1,3], I hypothesize a tradeoff: these traits will be negatively correlated, and the greatest phenotypic diversity will be generated in the treatment with motility and strong phage selection.

Aim 3: Community Sequencing. Using the same four timepoints, I will perform community genomic sequencing to identify bacterial and phage mutations and measure genetic diversity. I hypothesize that bacterial mutations will reside in motility and resistance mechanisms, while phage mutations will be associated with host attachment. I also hypothesize this data will reflect a tradeoff: resistance and motility mutations will co-occur infrequently, and the greatest genetic diversity will be generated in the treatment with motility selection and strong phage selection.

Intellectual Merit: By combining widely-used models of antagonistic coevolution[3] and motility, this project develops a novel system that enables direct experimental tests of evolutionary hypotheses involving dispersal. Here, I will apply this system to determine how selection on bacterial motility and by phage coevolution interact. Through this study of evolutionary responses to combined pressures, we will better understand how bacteria evolve in complex environments and expand our knowledge of how evolution creates and maintains biodiversity.

Broader Impacts: My findings should inform the applied medical use of phage to treat bacterial infections (phage therapy). As with antibiotics, phage therapy is currently limited by the evolution of resistant bacteria. By studying how bacteria evolve in response to phage and other pressures they face, we can better understand how they may respond to phage therapy. This, in turn, can suggest strategies for preventing or limiting the evolution of resistance.

Continuing my history of mentorship, throughout this project I will also work extensively with undergrad mentees. Training in lab work, data analysis and communication skills will help them develop scientific independence. Additionally, this project will translate into my public outreach with the Yale Science Diplomats (YSD). Through our Science in the News public lecture series for high school students, I will use my work to explain microbial evolution and its implications. Furthermore, as an example of the importance of basic science research, this experience will inform my political advocacy with YSD. Throughout, I hope to inspire others about evolution.

1. Harshey, et al. *Annu. Rev. Microbiol.* 57, 2003. **2.** Weinbauer. *FEMS Microbiol. Rev.* 28, 2004. **3.** Brockhurst, et al. *Infect. Genet. Evol.* 7, 2007. **4.** Taylor, et al. *J. Evol. Biol.* 26, 2013. **5.** Koskella, et al. *ISME J.* 5, 2011.