Department of Biological Sciences Seminar Blog
Seminar Date: 3/31/17
Speaker: Dr. Graham Hatfull, University of Pittsburgh
Title: *"Microbial Military Strategy: Defense and Counter-Defense"*

From Flicker to Fire: novel research program leads to increase retention in STEM and exciting discoveries in prophage-mediated defense strategies

By: Michelle Valkanas

I have been to a lot of interesting lectures in a variety of scientific fields, but Dr. Graham Halfull's presentation on bacteriophages was the first time that every moment drew me in. The talk by Dr. Graham Hatfull from the University of Pittsburgh, was titled, "Microbial military strategy: defense and counter-defense". Dr. Hatfull's research interests surround mycobacteria, specifically *Mycobacterium smegmatis*. He explained that he chose *Mycobacterium smegmatis* because of its relation to the bacterium causing Tuberculosis (*Mycobacterium tuberculosis)*. Using a related species allows them to study *Mycobacterium tuberculosis* without "his students dying", he jokes. The talk opened with the statement, "there are more phages then every other organism in the world ADDED TOGETHER". Just think about that for a minute. That is an insane number of phages. There are over 10^{31} phage particles in the biosphere (3). Hatfull explained that bacteria are in a constant state of defense because they are constantly attacked by viruses. Yet, bacteria have been able to adapt and survive, primarily due to the aid of bacteriophages.

There are two type of bacteriophages: virulent (lytic) and temperate. Lytic phages infect their host, replicate their DNA, and then lyse the cell, killing the bacteria and releasing their replicated DNA (virions) to go infect another host. There is no chance of

survival for bacteria in this case. In the more common temperate phages, the virus infects the cell and integrates its genetic material into the DNA of the host bacteria. They then often live in harmony, in a lysogenic life cycle. It is the genome of these temperate phages that are integrated into bacterial DNA, referred to as prophage, that provide an "upgraded" line of defense against other bacteriophage attacks. It should be noted that temperate phages can transition into a lytic phage when necessary, essentially your "guard dog" can turn on you.

The Hatfull lab has access to over 1,000 completely sequenced mycobacteriophages and have used them to compose a genetic profile. I noticed some peculiar phage names being mentioned. MichelleMyBell was my personal favorite (obviously), but there were also others such as Charlie and CornDog (a phage that really does look like a corndog!). Where were these cleaver names coming from? The answer is quite remarkable. The majority of phages used in Dr. Hatfull's lab are from undergraduate students across the globe. This is made possible through a course designed by Dr. Hatfull that gives freshman and sophomore college students the opportunity to isolate and name a bacteriophage.

What started as an alternative to the traditional general biology course at the University of Pitt in 2002 (PHIRE- Phage Hunters Integrating Research and Education) has developed into a nationwide program called, Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGE) headed by the Howard Hughes Medical Institute (HHMI). During the first semester, students collect a dirt sample and isolate their novel phage. This is followed up with DNA isolation, purification, and electron microscopy imaging. During the second semester, students perform genome analysis on their phage, and then compare their resulting data to other phage genomes. "You do not need any real prior knowledge, it is designed so that anyone can do it, all you really need is a flicker of curiosity" Hatfull said. His 2015 paper clarifies that techniques ARE important but that they

can be developed and do not have to be a pre-requisite (2). The SEA-PHAGE program is now in over 100 schools representing 4200 students. This cooperation has led 6,000 sequences and 850 complete genomes of mycobacteriophages as of 2015 (1), accounting for 1/3 of all sequenced, double-stranded DNA phage genomes (http://phagesdb.org).

This vast number of sequences and genomes give Hatfull's lab the unique ability to see a bigger picture. This is extremely important in phages as they exhibit mosaicism providing a difficult challenge in studying them. Hatfull found large diversity among phages, even those that attack a similar type of bacteria, like the mycobacteriohages studied here. Due to this mosaicism, looking at metagenomics was not enough. Similarities down to the amino acid level would have to be sought. This revealed things that the DNA alone was unable to provide because genes were sitting in different genomic patterns. This is further discussed in a recent paper entitled "Prophage-mediated defense against viral attack and viral counter-defence" (4).

Phages themselves have defense mechanisms against other viruses. Once integrated in the bacterial genome, phages defend the cell to assure their own survival. Hatfull's laboratory assembled genomes and saw that during lysogeny more than just repressor genes were being expressed. At least some of the genes were being used for defense against other phages. Further studies revealed that defense was selective and variable (4). For example, Panchino was capable of defending against 1/3 of all phages tested (80 types) while Pipsqueaks had very little capability. Additionally, Panchino exhibited a prophage-encoded restriction system, while Charlie gp32 (a membrane protein believed to be used in phage defense) prevented DNA insertion, characteristic to superinfection exclusion (4). A superinfection exclusion occurs when a pre-existing virus prevents infection by a similar, secondary virus infection. This is just a small glimpse of phage defense and with the continue increase in sample size there is enormous potential to uncover the

defense systems involved in sustaining bacteriophage and bacterial survival. Having student power behind such a large and growing sample size is an added bonus.

Getting students excited about science is great, but does it stick? Dr. Hatfull was discussing this with graduate students during a STEM Journal Club meeting at Duquesne University, a club that meets monthly to discuss different teaching techniques and programs in science pedagogy. The SEA-PHAGE program not only provide advances in student learning, but encourages retention in STEM related fields (1). He went on to say that this increased interest in STEM occurred in both students that were and were not previously interested in STEM. This is incredibly important, as 40% of students in the United states are not exposed to research-based learning. Specifically, 2 year colleges and the 4 year nonresearch-based institutions could greatly benefit from a program like SEA-PHAGE. One phenomenal aspect of the programs is its flexibility, making it useful across various academic establishments. Hatfull commented that undergraduate research is limited to upperclassmen and those with extraordinary grades, partially due to limited space. Yet, many great researchers do not fit the "cookie cutter" perfect student image. In fact, Hatfull himself didn't. On his HHMI profile he reflects on his time as a student, "I was the kind of kid who was average academically, to put it nicely." That's not to say that that grades are not important, but having one standard criteria excludes many talented students. The SEA-PHAGE program is a shining example of an alternative avenue to give the same research opportunities to every caliber of student.

When asked what his favorite phage name was, Hatfull could not pinpoint just one. He did note that he can follow pop culture trends as there were at least six recent phages named after Game of Thrones characters. He assured us that there is a screening process for names, with urban dictionary as a go to source. Hatfull is not exempt from this process, and he himself was flagged during a routine screening. Hatfull submitted an application for a custom

license plate that read "PHAGES". After a few weeks, he got a call from the Pennsylvania Department of Transportation that went something like this:

PennDot: Hello I am calling about the license plate request you submitted.

Hatfull: Yes.

PennDot: Well, sir, what exactly does it mean? My boss wanted me to call and see what exactly you mean by this.

Hatfull: They are viruses that attack bacteria. Google it.

PennDot: I was told not to google it because my boss was afraid of what might come up

Hatfull: It is ok, let's google it together…

--- 30 Minutes later ---

PennDot: Wow! Can I isolate my own phage?

Though this story had us all laughing, it shows the general lack of knowledge about phages. This is not just with the general public, but in the science community as well. Thankfully, Dr. Hatfull is assembling an army to try to get through uncharted territory. "I believe that every student should isolate a phage" Dr. Graham Hatfull said over lunch. He compared his passion for everyone to isolate a phage to Bill Gates' passion for everyone to own a computer. With the rapid growth in bioinformatics coupled with the affordable cost of sequencing, who is to say that phages are not the next "frog dissection" in general biology laboratories?

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2. Hatfull GF. 2015. Innovations in Undergraduate Science Education: Going Viral. Journal of Virology 89:8111-8113.

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4. Dedrick RM, Jacobs-Sera D, Bustamante CAG, Garlena RA, Mavrich TN, Pope WH, Reyes JCC, Russell DA, Adair T, Alvey R, Bonilla JA, Bricker JS, Brown BR, Byrnes D, Cresawn SG, Davis WB, Dickson LA, Edgington NP, Findley AM, Golebiewska U, Grose JH, Hayes CF, Hughes LE, Hutchison KW, Isern S, Johnson AA, Kenna MA, Klyczek KK, Mageeney CM, Michael SF, Molloy SD, Montgomery MT, Neitzel J, Page ST, Pizzorno MC, Poxleitner MK, Rinehart CA, Robinson CJ, Rubin MR, Teyim JN, Vazquez E, Ware VC, Washington J, Hatfull GF. 2017. Prophage-mediated defence against viral attack and viral counter-defence. Nature Microbiology 2:16251.