Publishing student-led discoveries in genetics

Danielle Heller , Viknesh Sivanathan *
Department of Science Education, Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA

*Corresponding author: Department of Science Education, Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA. Email: sivanathanv@hhmi.org

Keywords: undergraduate research; course-based research; SEA-GENES; bacteriophage genetics

In a Mutant Screen Report in this issue, we (Heller et al.) report the findings of a genome-wide screen to identify novel mycobacteriophage gene products capable of inhibiting mycobacterial growth. Overexpression of the 94 genes encoded by phage Waterfowl in the host bacterium Mycobacterium smegmatis revealed 32 gene products capable of hindering mycobacterial growth to varying degrees; half of these gene products harbor no recognizable domains or homology to gene products of known function, offering many novel growth inhibitors for future characterization. This is the first report from the HHMI Science Education Alliance-Gene-function Exploration by a Network of Emerging Scientists (SEA-GENES) project, a new project that engages undergraduate scientists and their faculty instructors in an exploration of bacteriophage genetics within the context of undergraduate science courses.

The bacteriophage population is enormous ($10^{31}$ estimated phage particles) and encodes a remarkably diverse set of genes, the vast majority of which cannot be assigned functions through bioinformatic methods (Hatfull 2015). Given the considerable importance of phages in environmental systems, bacterial pathogenesis, and their promise as therapeutic agents, experimentally investigating the functions of these genes is an important scientific endeavor. The Genetics Society of America (GSA) and G3 are partnering with HHMI to disseminate the student-led discoveries of the SEA-GENES course-based research project to further advance our understanding of this largely uncharted system.

Course-based research as a model for advancing science and science education

It is well-established that engaging undergraduate students in scientific research is a multifaceted and high-impact experience that improves their learning and their likelihood of persisting in the sciences (NASEM 2017). In the past 2 decades, the course-based research model has gained significant traction as a means to scale access to research opportunities for undergraduate students; in this model, authentic research is embedded directly within STEM courses, replacing traditional laboratory instruction with faculty-led research projects (Auchincloss et al. 2014). Such course-based research has the capacity to simultaneously advance science and science education, as exemplified by the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) project.

Supported by HHMI and led in collaboration with scientists at the University of Pittsburgh and James Madison University, SEA-PHAGES is a 2-semester course-based research project that is implemented annually by faculty instructors and cohorts of undergraduate students at >150 colleges and universities. Since its initiation in 2008, over 35,000 undergraduate students have participated in the SEA-PHAGES project, many of them as part of their introductory biology sequence. In the first semester of the SEA-PHAGES project, each student isolates and characterizes a novel actinobacteriophage from their local environment, and in the second semester, class teams produce a high-quality genome annotation for 1 or 2 of their discovered phages. Students emerge from this research experience with a strong foundation in microbiology and bioinformatics, and assessment data show a significant increase in their likelihood of persisting in the sciences as compared to their peers in traditional lab courses (Jordan et al. 2014; Hanauer et al. 2017).

While the rich educational experience of SEA-PHAGES is built through the deep investigation of 1 phage at a time, collectively, SEA-PHAGES researchers have compiled a rich dataset that has significantly broadened our understanding of phage populations and the ways they evolve (Pope et al. 2015, 2017; Hatfull 2020). As of this writing, SEA-PHAGES researchers have isolated over 20,400 novel bacteriophages capable of infecting diverse species of Actinobacteria; genomes for almost 4,000 of these phages have been annotated and submitted to GenBank, revealing thousands of gene families with no known functions. This genomic dataset can be readily explored by the broader community using the Actinobacteriophage Database (phagesdb.org) (Russell and Hatfull 2017) and the comparative genomics tool Phamerator (phamerator.org) (Cresawn et al. 2011), both of which were developed in support of the SEA-PHAGES project; likewise, the vast SEA-PHAGES archive continues to be mined by researchers for further exploration of fundamental questions of phage biology. In recent years, SEA-PHAGES lead scientist Graham Hatfull and his team have deployed many student-discovered phages in the development of therapeutic phage cocktails for the treatment of life-threatening mycobacterial infections (Dedrick et al. 2019; Little et al. 2022; Nick et al. 2022).

The SEA-GENES project is a natural evolution of the SEA-PHAGES project, going beyond genome annotation to the experimental exploration of the functions of phage genes in the bacterial host. Designed to be embedded within mid-level biology...
laboratory courses, the SEA-GENES project engages undergraduate SEA researchers in an additional 1–2 semesters of research, extending their exposure to the culture and process of science and providing experience in molecular cloning and genetics methods. It also leverages the outputs of the SEA-PHAGES project—the vast collection of bacteriophage, high-quality genome annotations, and trained student researchers—to generate a large genetic screening dataset to advance our understanding of the functions of the genes encoded by mycobacteriophages. At each participating school, teams of undergraduate students and their faculty instructors conduct a systematic exploration of one of the >2,000 unique sequenced mycobacteriophages in the SEA-PHAGES archive. Gene by gene, students employ molecular cloning techniques and reagents provided through partnership with New England Biolabs and Integrated DNA Technologies to build an arrayed genomic plasmid library. Then, they work collaboratively to screen this library in simple, accessible plate-based assays to comprehensively evaluate the effects of phage gene overexpression on mycobacterial host phenotypes such as growth and superinfection immunity. Among the thousands of uncharacterized gene families encoded in the mycobacteriophage population, these student-led screens reveal phage genes capable of conferring biologically interesting phenotypes, good candidates for further genetic dissection, protein interaction studies, or other characterization. Together, schools participating in SEA-GENES contribute data for a diverse collection of mycobacteriophages, providing good experimental coverage of the known sequence space; given the highly mosaic nature of these phage genomes, students across schools test many gene homologs as well, allowing for comparative functional analyses and further examination of functional domains or motifs. All generated plasmid libraries are archived with HHMI and available to the research community for further application and study, and the primary screening data will soon be available in an open access database.

**Communicating student-led discoveries**

Course-based research is a powerful convergence of science and science education. In course-based research projects like SEA-PHAGES and SEA-GENES, students conduct research using basic techniques well-suited for novice scientists; each cohort contributes a novel research output that en masse form a large, discovery-rich dataset. Communicating these individual student-led discoveries in a timely manner and accessible format is important both for the advancement of science and for the development of the next generation of scientists. In the SEA-PHAGES project, faculty and students have coauthored almost 150 genome announcements published by the American Society of Microbiology in their Microbiology Resource Announcements journal, offering a brief, accessible format for describing student-characterized bacteriophage genomes. Similarly, the Mutant Screen Report by Heller et al. in this issue of G3, represents the first of many SEA-GENES reports that HHMI and GSA hope to share with this community, disseminating the experimental observations and molecular tools generated by undergraduate scientists in their collaborative exploration of the genes encoded by bacteriophages.

**Literature cited**

Auchincloss LC, Laursen SL, Branchaw JL, Eagan K, Graham M, Hanauer DI, Lawrie G, McLinn CM, Pelaez N, Rowland S, et al. Assessment of course-based undergraduate research experiences: a meeting report. CBE Life Sci Educ. 2014;13(1):29–40. https://doi.org/10.1187/cbe.14-01-0004.

Cresawn SG, Bogel M, Day N, Jacobs-Sera D, Hendrix RW, Hatfull GF. Phamerator: a bioinformatic tool for comparative bacteriophage genomics. BMC Bioinformatics. 2011;12:395–395. https://doi.org/10.1186/1471–2105–12–395.

Dedrick RM, Guerrero-Bustamante CA, Garlena RA, Russell DA, Ford K, Harris K, Gilmour KC, Soothill J, Jacobs-Sera D, Schooley RT, et al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat Med. 2019;25(5):730–733. https://doi.org/10.1038/s41591-019-0437-z.

Hanauer DI, Graham MJ, Betancur L, Bobrownicki A, Cresawn SG, Garlena RA, Jacobs-Sera D, Kaufmann N, Pope WH, Russell DA, et al; SEA-PHAGES An inclusive Research Education Community (iREC): impact of the SEA-PHAGES program on research outcomes and student learning. Proc Natl Acad Sci U S A. 2017;114(51):13531–13536. https://doi.org/10.1073/pnas.1718188115.

Hatfull GF. Dark matter of the biosphere: the amazing world of bacteriophage diversity. J Virol. 2015;89(16):8107–8110. https://doi.org/10.1128/jvi.01340-15.

Hatfull GF. Actinobacteriophages: genomics, dynamics, and applications. Annu Rev Virol. 2020;7(1):37–61. https://doi.org/10.1146/annurev-virology-122019-070009.

Heller D, Amaya I, Mohamed A, Ali I, Mavrodi D, Deighan P, Sivanathan V. Systematic overexpression of genes encoded by mycobacteriophage Waterfoul reveals novel inhibitors of mycobacterial growth. G3: Genes|Genomes|Genetics. 2022;12(8):jkc140.

Jordan TC, Burnett SH, Carson S, Caruso SM, Clase K, Dejong RJ, Dennehy JJ, Denver DR, Dunbar D, Elgin SCR, et al. A broadly implementable research course in phage discovery and genomics for first-year undergraduate students. mBio. 2014;5(1):e01051–e01013. https://doi.org/10.1128/mbio.01051-13.

Little JS, Dedrick RM, Freeman KG, Cristinziano M, Smith BE, Benson CA, Jhaveri TA, Baden LR, Solomon DA, Hatfull GF. Bacteriophage treatment of disseminated cutaneous Mycobacterium chelonae infection. Nat Commun. 2022;13(1):2313. https://doi.org/10.1038/s41467-022–29689-4.

Nick JA, Dedrick RM, Gray AL, Vladar EK, Smith BE, Freeman KG, Malcolm KC, Epperson LE, Hasan NA, Hendrix J, et al. Host and pathogen response to bacteriophage engineered against Mycobacterium abscessus lung infection. Cell. 2022;185(11):1860–1874.e12. https://doi.org/10.1016/j.cell.2022.04.024.

Pope WH, Bowman CA, Russell DA, Jacobs-Sera D, Asai DJ, Cresawn SG, Jacobs WR, Hendrix RW, Lawrence JG, Hatfull GF. Mycobacterial Genetics Course. Whole genome comparison of a large collection of mycobacteriophages reveals a continuum of phage genetic diversity. eLife. 2015;4:e06416. https://doi.org/10.7554/eLife.06416.

Pope WH, Mavrich TN, Garlena RA, Guerrero-Bustamante CA, Jacobs-Sera D, Montgomery MT, Russell DA, Warner MH, S-P, ES, Hatfull GF; SEA-PHAGES. Bacteriophages of Gordonia spp. display a spectrum of diversity and genetic relationships. mBio. 2017;8(4):e01069–17. https://doi.org/10.1128/mbio.01069-17.

Russell DA, Hatfull GF. PhagesDB: the actinobacteriophage database. Bioinformatics. 2017;33(5):784–786. https://doi.org/10.1093/bioinformatics/btw711.

National Academies of Science, Engineering, and Medicine. Undergraduate Research Experiences for STEM Students: Successes, Challenges, and Opportunities. Washington, DC: The National Academies Press. 2017. https://doi.org/10.17226/24622.