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On the Border of Life: Bacteriophages and Biodiversity

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Rationale

Horace Howard Furness is a public neighborhood high-school in South Philadelphia with strong administration, high teacher retention, and a long history of celebrating its impressive student diversity. In the 2020 – 2021 school year, more than half of our seven-hundred young people brought knowledge from living in countries outside the United States and shared linguistic resources from twelve different spoken languages. Our program for teaching English to Speakers of Other Languages has a city-wide reputation for excellence, and our end-of-year Multicultural Fair has attracted local dignitaries such as the Mayor of Philadelphia and photographer Zoe Strauss. While our school may be rich in culture and community support, 100% of our students are considered economically disadvantaged, creating very real challenges for emotional wellbeing and educational attainment. By adapting a college-level research course for our ninth grade students, I hope to welcome them to high school with a unit developing science and engineering skills, posing interesting conversations in English, and practicing the joys and urgency of "actually doing science."

Content Objectives: Biodiversity and its Origins

In the first week of ninth grade environmental science, I ask students to come up with as many things that are part of "the environment" as they can think and write in ninety seconds. Although most lists include some mention of the physical environment, for example, water, rocks, and the sun, the focus tends towards naming different plants and animals. Even without a formal definition, most people have an intuitive understanding of Earth's biodiversity. Penguins are different from pandas. A house mouse is different from a potato, and springtime flowers show up in an expressive poetry of shape and color.

The living and nonliving things students write about tend to be easy to see and experience at the level of human senses. However, there are vitally important parts of the environment that are smaller than what humans can see with the naked eye. Bacteria outnumber all plants and animals by an order of several magnitudes,[1] and viruses are "among the most abundant biological particles on earth."[2] Despite their near-ubiquity in Earth's environments, bacteria and viruses have not yet made an appearance on a student list.

An even smaller feature of environmental biodiversity is that it can help us understand variety at the genetic level. Every living thing on Earth has its own unique genetic code found in the molecules that make up its deoxyribonucleic acid, or DNA.[3] Humans might count as one species with many traits in common, but we exhibit a great deal of obvious and not-so-obvious diversity in our genetic heritage. The connection between DNA and biodiversity is the "central dogma" of biology. DNA is transmitted from a parent to its offspring. When required by the daily activities of life or changes in the environment, segments of this DNA, called genes, can be copied, or transcribed, into RNA which a cell then "translates" into proteins to produce observable traits, called phenotypes (Figure 1).[4] Although there are a number of exceptions to this unidirectional flow of information,[5] it is a helpful starting point for ninth grade environmental scientists. Whether plants, animals, or microbiota smaller than human perception, all living things on Earth attribute their unique differences to genetic variation.

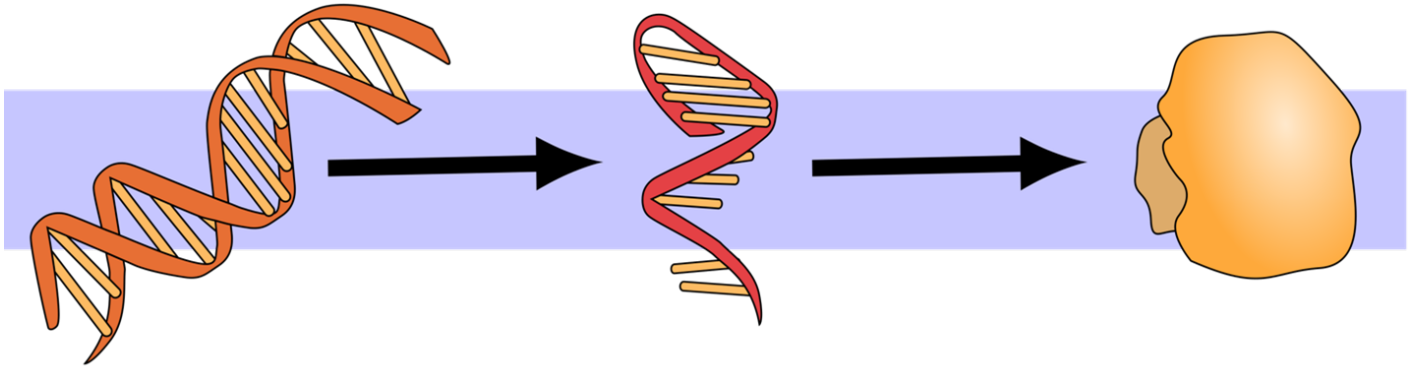

Figure 1. The central dogma of biology is that DNA is copied to RNA is translated to proteins[6]

Human Influence on Global Biodiversity

Although the technology to read and catalog these genetic sequences only developed in the latter half of the 20th century,[7] humans have been influencing genetic diversity on Earth for tens of thousands of years.[8] These practices include many hugely important examples, from agriculture to our favorite house pets, but a critical subset of organisms that humans have changed include those that have developed genetic resistance against human attempts to eradicate them.

The World Health Organization's (WHO) top ten global health issues for 2021 included cancer, insect-borne tropical diseases like malaria, and drug resistant infections.[9] While these might seem like a disparate collection of threats to human health and wellness, they all have one thing in common: certain members of their population are no longer vulnerable to medication or pesticides that were once effective.[10] As summarized by the American Academy for Microbiology,[11]

> Given time and opportunity, the organisms we seek to control will evolve resistance to agents deployed against them.

Recalling the diversity of individual humans, all populations will contain some amount of genetic diversity through random mutation to their DNA.[12] In organisms that reproduce by having sex, additional genetic

diversity is introduced by combining parental chromosomes during fertilization.[13] That resulting offspring will further mix the chromosomes from its parents while making egg or sperm, recombining those genes for the next generation.[14]

The result is that sex and mutations can create many different versions of the same gene. Scientists refer to each of these unique versions as an allele. Two plants might have the same "gene" that controls flower color, but one has an allele that makes a purple pigment while another has an allele that codes for the color white. Different alleles can provide different advantages depending on their environment. For example, in a population of bacteria, a small number of alleles may have randomly evolved such that they confer resistance to a particular antibiotic. When humans then apply that drug in an attempt to treat a bacterial infection, individual bacteria with an allele for resistance will survive the antibiotic while their community members with the non-resistant, "wild-type" allele succumb to human intervention.[15]

Omitting words for clarity, this is "natural selection," a fundamental mechanism for evolution as described in Charles Darwin's *Origin of Life*,[16]

> "...any variation... if it be in any degree profitable to an individual of any species, ...will tend to the preservation of that individual, and will generally be inherited by its offspring. The offspring, also, will thus have a better chance of surviving...".

Although the existence of the mutation was random, the bacteria with the resistance allele were "selected" by environmental pressures as better able to survive and reproduce, aka more "fit," than their peers with the wild-type allele. Offspring from the survivors will also carry that pre-existing genetic adaptation and become more common in the face of on-going selection pressure. Over time, this can lead to very big changes in the genetic code and observable traits of a population, sometimes so different as to no longer be considered the same species.

Although high-school students are expected to be familiar with this idea that offspring will be similar to their parents because of heritable genetic material, bacteria and viruses have an additional complication in which genetic information can be exchanged between organisms of the same generation, called horizontal gene transfer.[17] This can lead previously vulnerable populations to gain genetic resistance even without the presence of the selective pressure.[18] There are many molecular mechanisms by which these organisms "outwit" chemical annihilation[19] and the study of how resistance develops and spreads is an on-going field of research.[20]

Antimicrobials: A Case Study in Resistance

Unfortunately for humans, the story of bacteria that do not respond to chemical antibiotics is an increasingly familiar one. Initially discovered by Alexander Fleming in 1928, the ability of Penicillium mold to combat bacterial growth led to a post-World War II "golden age" of pharmaceutical antibiotics that could reliably manage bacterial infection.[21] In 1936, nearly 120,000 Americans died from all forms of pneumonia.[22] By 1956, that number had declined to just over 40,000,[23] cutting the U.S. mortality rate from pneumonia to nearly one-third of the pre-antibiotic level. In 2005, data from the Centers for Disease Control suggested that 200,000 American lives were saved each year due to antibiotic interventions.[24]

However, pharmaceutical antimicrobials are losing efficacy. Bacteria are living systems that reproduce and mutate rapidly. When these chemicals are used against a population that numbers in the billions, a treatment with a 99.999% mortality rate still leaves several thousand survivors. When those survivors contain a trait for antibiotic resistance, that leaves them and their offspring no longer susceptible to the next application of that drug. There are even alleles that provide the ability to survive several different antibiotics, "broad spectrum resistance," in one swift evolutionary step.[25]

This increasing prevalence of antimicrobial resistance (AMR) is a top ten global health threat with grave social and economic consequences for the 21st century. High rates of antimicrobial resistance have been observed in all WHO surveillance regions[26] and, in 2019, *The Lancet* reported that 900,000 to 1.71 million global deaths in the previous year could be attributed to AMR with a 95% confidence interval.[27] In the United States alone, more than 2 million people are sickened each year as a result of antibiotic-resistant infections, resulting in at least 23,000 deaths per year.[28] In 2010, seventy percent of hospital infections had resistance to at least one first-line antibiotic,[29] and there are even microbes that have developed resistance to "all or nearly all" available antibiotics. Called Carbapenem-resistant Enterobacteriaceae because they are resistant to carbapenem, the "treatment of last resort," it is estimated that strains of *Klebsiella* and *E. coli* that have developed this resistance were responsible for 9,300 infections and 600 deaths in the U.S. in 2013.[30] In human terms, these statistics are a daily reality for thousands of parents grieving the death of their child. It means young people losing the love and stability of one or both parents. It means millions of work hours lost to illness and recovery, thousands of missed school days because "the medicine doesn't work anymore."

Biomimicry: A Tale of Two Treatments

While the Western model of pharmaceutical antibiotics developed from Alexander Fleming's observation of Penicillium mold, an additional "bioinspired" means of killing bacteria was adopted elsewhere. In 1915, British scientist Frederick Twort observed antibacterial properties in a "transparent material" that he hypothesized were ultra-microscopic viruses.[31] That this transparent material was indeed full of bacteria-killing viruses was confirmed by French microbiologist Félix d'Hérelle in 1917.[32] Called bacteriophages for "bacteria-eaters," these "phages" infect a bacterium with genetic material and "hijack" the cell's machinery into producing virus particles.[33] Although different viruses can reproduce in different ways, this infection cycle commonly ends when so many viruses have been produced that they "explode" out of the bacterium, killing or "lysing" their host, and spreading into the environment to restart the infection cycle in a new cell (Figure 2).[34]

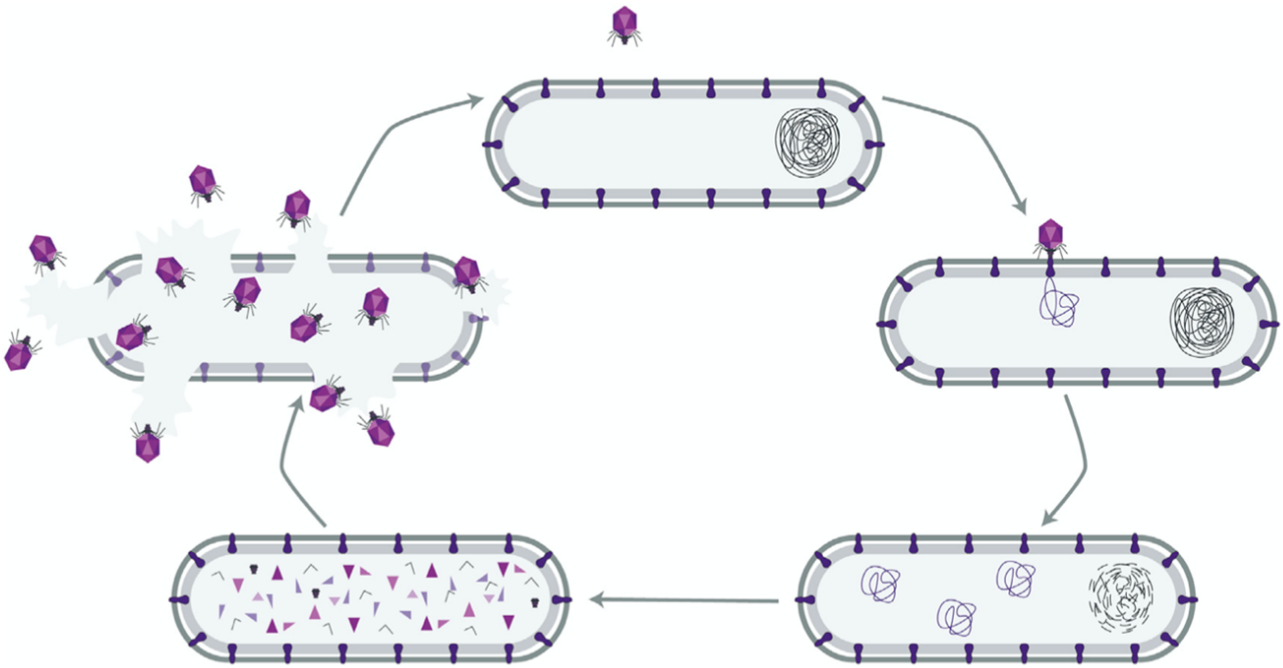

Figure 2. Bacteriophage virus infecting and lysing its host bacterium[35]

In the 1920s, d'Hérelle successfully developed this viral capacity to lyse their hosts into an oral treatment for bacterial dysentery,[36] still a major cause of mortality for children under the age of five.[37] The publication of this success spurred commercial laboratories in the United States, France, and Germany to begin producing clinical applications of phage therapy. While many factors led the Western world to largely abandon phage therapy, republics in the former Soviet Union continued to develop phage therapeutics and employ them in routine medical treatment. Today in Tbilisi, Georgia, home to the Eliava Institute, one of the oldest phage research institutions in the world, "phage cocktails" combining ten or more different viruses can be purchased as an over-the-counter treatment for a variety of ailments.[38]

Both of these bacteria-killing treatments were developed by observing the natural world in action. When humans adapt existing strategies in nature for solving human problems, we call this "biomimicry." Popularized by Janine Benyus in her 1997 book of the same title, these "innovations inspired by nature" include solutions for food systems, healthcare, energy, and a whole host of other existential crises currently facing planet Earth.[39]

This concept is particularly resonant for an environmental science unit grounded in biodiversity. If humans are looking to the diversity of the natural world for inspiration to its problems, it adds urgency to preserve that biodiversity currently under assault. Although not adopted by the International Union of Geological Sciences, the era we currently live in is popularly called the "Anthropocene," named for human beings and the detectable impact our actions have had on the geologic record.[40] One of those impacts is "the sixth mass extinction." As evidenced by fossil records and other measures of historic biodiversity, Earth has gone through five periods of sharp decrease in the number of living species.[41] Since the 1950s, measurable decline of biodiversity due to human action has become so significant as to warrant comparison to a rate of species loss not seen since the extinction of the dinosaurs.[42] If we hope to develop further life-saving solutions inspired by the living world, it is essential that world stays alive.

Viruses: Not Alive But Still Evolving

With a little scientific wordplay, viruses are technically immune to this human-mediated assault against life as we know it; unlike bacteria, viruses are not considered alive under most scientific definitions of life. At the molecular level, virus anatomy is a protein "coat," called a capsid, that is filled with nucleic acids, either DNA or RNA.[43] Although there are many crucially important modifications to this basic blueprint, even the most complex viruses are ultimately very simple compared to the molecular mechanisms of a single cell.[44]

And, at present, having cells is one of the fundamental requirements for life. Another viral failure in the test for life is that viruses do not have a metabolism. They do not obtain energy or "grow" once their protein coats are assembled. Nor, by most measures, do they maintain a stable inner environment relative to external changes, called homeostasis, and they cannot reproduce on their own. In order to make more viruses, they need to infect a host and use its cellular machinery to make more copies of themselves.[45]

Despite these disqualifications from the living, one of the life characteristics they do qualify for is the capacity of their genetic material to undergo change.[46] An important feature of natural selection as a mechanism for evolution is that it happens in the context of a specific environment. How viruses originated is still a major mystery in the history of Earth.[47] However, evidence of bacterial life on Earth exists as far back at 3.77 billion years.[48] At least one team of researchers has hypothesized that viruses may predate the Last Universal Common Ancestor, which would be *older* than the evolution of bacteria.[49] This suggests that viruses and bacteria may have shared an environment and exerted selection pressure on each other's genomes for more than three-billion years. Given a few billion years of coevolution, phage genomes have developed to the point that they typically infect only a narrow range of host bacteria.[50] This means the virus can only reproduce if it encounters the one or two strains of bacteria it "grew up with" and has the complementary genetic code for successful binding, infection, and replication.[51]

This narrow range of host infection presents benefits and challenges to using phage therapy relative to chemical antibiotics.[52] Many pharmaceutical antibiotics are considered "broad spectrum," they are able to kill many different species of bacteria. For a long time, it was not necessary for a medical practitioner to establish exactly which strain of bacteria was infecting a person.[53] While this saves precious time in the fight against infection and was for many years highly effective, the pathogenic, or harmful bacteria, are usually a small minority relative to the beneficial and innocuous bacteria that call the human body home.[54] As broad-spectrum antibiotics move through a human system, they do not distinguish between bacteria that are causing illness and which ones are providing essential health services. The consequences of this indiscriminate "bactericide" are not yet well understood, but early childhood applications of antibiotics have been correlated to gastrointestinal, immunologic, and neurocognitive conditions.[55]

As we continue to learn more about the relationship between the microbiome and human health, phages that infect and kill only one particular species of bacteria could be hugely beneficial. That narrow infection range of phage-derived antibiotics preserves the nonpathogenic microflora, targeting only the undesirable species. However, in the United States, this necessity for "exactly the right phage" leads to delayed approval and application for human use. Due to safety restrictions by the U.S. Food and Drug Administration, extensive time and resources are required to identify which strain of pathogenic bacteria is infecting someone, then perform the necessary experiments to find a safe and effective phage complement that target and lyse the bacteria specifically.[56] This process is likely to improve as more trials are publicized and a more well-characterized

phage library is made available.[57]

But You Said, "Organisms We Seek to Control Will Evolve Resistance"

Yes, the American Society for Microbiology *did* say that resistance will evolve in any organism we try to control. That includes microbes developing resistance to bacteriophages.[58] Observed both in the lab and in clinical treatment, bacteria with phage resistance mutations emerge rapidly and frequently.[59] As of 2018, in the four human trials that monitored for the emergence of phage resistance, three reported successful bacterial resistance. In one of those trials, a multiply-drug resistant strain of *Acinetobacter baumannii* causing pancreatitis was selected for clinical treatment using two, four-phage cocktails that were effective in vitro. Although the infection was significantly reduced, bacteria resistant to all eight phages were isolated after eight days in vivo, and a ninth phage was required to fully clear the infection.[60]

Resistance to one phage can also confer resistance to similar phages or even pharmaceuticals that target the same mechanism. In a study by Kortright et al., researchers exposed *E. coli* to two phages and albicidin, a chemical bacterial inhibitor. All three of these antimicrobials interact with Tsx porin, an outer membrane protein channel. In each of the twenty-nine bacterial mutations that conferred resistance to one antibacterial, the mutation conferred cross-resistance to all three. Genetic analysis of the bacterial mutations showed that all of the mutations caused at least one change in the tsx gene, which codes for the structure of the porin.[61] In a laboratory study by Wright et al., *Pseudomonas aeruginosa* was tested against different phages and cross-resistance was again observed due to changes in external structural features, likely phage binding sites including liposaccharides (LPS) and type IV pilus proteins.[62] This type of cross-resistance is receptor specific. Each of the antibacterial mechanisms interact with the same external features.

Generalist cross-resistance has also been observed. In the *P. aeruginosa* study above, phages were selected that bind to either LPS or pilus proteins. Mutations conferring general resistance to both modes of attachment were identified in transcriptional regulation genes rpoN and pilS. PilS is involved in the expression of external pilus proteins by activating rpoN. The gene rpoN (RNA polymerase, nitrogen-limitation N) encodes for a regulatory protein, sigma54 (s54), that is involved in the transcription of over seven-hundred genes. This involvement in a wide variety of bacterial functions makes it more difficult to identify the pathway between mutation and the resistance phenotype; however, this type of general mutation only occurred in 10% of observed strains, suggesting it is rarer, in part because there is only one copy of rpoN in the bacterial genome compared to the several genes required for synthesis of LPS and pilus proteins.[63]

How these resistance mechanisms evolve and disperse is an important research step before wide-spread clinical application of phage therapy.[64] Most resistance mechanisms are the product of random mutations, but they can also arise through adaptive immunity in which bacteria incorporate strings of viral nucleic acids into their genome through the CRISPR-Cas system to recognize the virus during its next infection attempt and initiate a type of bacterial immune response.[65] After a resistance mechanism originates in an individual, the bacteria can then pass on genetic material both to their offspring or to members of the same generation through horizontal gene transfer.

Resistance-Resistant Features of Phage Therapy

Despite the rapid evolution and spread of phage resistance in bacteria, phage therapy offers mechanisms that can delay[66] or limit resistance acquisition,[67] prevent phage resistance from becoming more widespread or

induce other detrimental features into the bacterial population.[68] Although these are promising features of phage therapy that are not available through chemical agents of control, they must be thoroughly researched and used cautiously to prevent a reoccurrence of the widespread resistance currently underway as a result of industrial scale misuse of pharmaceutical antibiotics.

Although viruses are not alive, their genetic material has been coevolving with their bacterial hosts for perhaps billions of years.[69] One proposed mechanism of delaying bacterial resistance takes advantage of this coevolutionary ability to "train" phages to kill successive generations of bacteria in order to preemptively "evolve" them ahead of any resistance mechanism the bacteria might develop.[70] This method is hypothetical and contentious. An alternative hypothesis is that rather than developing phages that are effective longer, the bacteria will evolve several resistance mechanisms at once. When tested in the lab, however, the coevolved phages successfully delayed bacterial resistance by two to four weeks, a significant gain of function if this finding is can be replicated in an in vivo application of phage expected to clear the population within a few days.

One of the most interesting applications is the ability to reintroduce antibiotic vulnerability in bacteria that were previously antimicrobial resistant. Called a fitness trade-off, it means that a mutation conferring one survival benefit may reduce fitness in some other context.[71] There are two prominent ways this has been adapted in the therapeutic context. One method identifies features about the bacterial population that improve its ability to spread, called virulence, or its lethality upon infection, called pathogenicity. An example with demonstrated impact on virulence is to select a phage that adsorbs to LPS. Many bacteria have a polysaccharide "capsule" that can hide them from macrophage digestion during an immune response. If a bacterium contains a mutation that removes or down-regulates the LPS binding site, it can interfere with capsule production and make the bacterium more vulnerable to detection and engulfment by the immune system.[72]

The other method combines phage therapy and pharmaceutical antibiotics. In this strategy, a mechanism of resistance in a drug-resistance bacterial strain is identified. For example, one broad spectrum resistance mechanism is the development of "efflux pumps" that selectively remove chemical antibiotics from bacterial cells and extrude them back into the environment.[73] If a phage is designed to target the efflux pump as a binding cite, the next generation of bacteria that survive the phage may do so by developing a mutation that renders the efflux pump ineffective, restoring its susceptibility to the traditional antibiotic. There are several identified resistance mechanisms that may reintroduce antibiotic vulnerability under selective pressure by well-chosen phages.

Concluding Remarks: Genetic Engineering and DNA as Commodity

One of the factors that limited adoption of phage therapy in the United States was that U.S. patent law is inapplicable to "the natural laws, mechanisms or objects which already exist independently of human beings."[74] Bacteriophages occur naturally. Although it is possible to patent unique applications of phage, called a process patent, the viruses themselves could be used by anyone who discovered them. Although the urgency of antimicrobial resistance is one factor spurring reinvestment in phage therapy,[75] another factor is the advances in biotechnology and genetic engineering.[76]

During the second half of the 19th century, the price of steel decreased from $170 per ton in 1867 to a mere $14 per ton by 1900.[77] In the 20th century, Moore's law described how the processing power of microchips

nearly doubled every year from 1975 to 2012. Now, in the 21st century, we are in the midst of a "Carlson curve." Named for Dr. Rob Carlson at the University of Washington (who does not like the namesake), it observes that the cost of sequencing and synthesizing base pairs of DNA, the biological "blueprint" of life, has decreased exponentially since the ground-breaking and multi-billion dollar Human Genome Project. This means more researchers have the ability to quickly and inexpensively characterize and test phages using whole genome sequencing and more efficient technologies for growing microbes and simultaneously, large-scale screening.[78]

Although not widely discussed in this paper, another consequence of the age of biology is the manipulation of living things, as the final product or themselves as technologies of change. The most effective capacity humans now have for genetic modification is itself derived from the billion-year-old evolutionary struggle between viruses and bacteria.[79] Called CRISPR-Cas9, this is a partnership between a nucleic acid target sequence and an enzyme that cuts the targeted sequence. It was originally discovered in bacteria to incorporate strands of phage DNA into their own genomes as a type of immune response; the enzyme would destroy any viral DNA that matches sequences previously encountered and encoded in the bacterial genome. When intentionally "programmed" by human-designed guide RNA, this system can induce single-point changes into DNA with an accuracy unmatched by earlier technology.

However, genetic engineering makes a commodity of the very molecules of life. Humans are discovering naturally existing viruses that combat antimicrobial resistance and genetically modifying them to patent ownership of their useful properties.[80] How is DNA ethically different from steel? What consequences are invited by applying the same structures of ownership and profit margin that so devastated previous centuries? Can ninth grade students come to a consensus about what is fair and just in the world of synthetic biology and can they be agents of change themselves? By exploring the role of viruses and bacteria in their environment, students will have a better vocabulary for what is manipulated during genetic engineering, i.e. change the DNA "code" and you change a protein and its potential functionality. What might make them trust a process of genetic engineering, and how do they perceive balancing "good hearted intentions" with pursuit of the profit margin and who has access to these technologies?

At the level of organisms or DNA base pairs, biodiversity is a way to measure and compare the variety of living things in a particular area. Earth's environments contain magnitudes more diversity when including the microbial world; but, while they often go unnoticed, these microbes have been an essential part of humanity's impressive, if brief, success on planet Earth. They will also prove indispensable for its continued survival. As extractive capitalism and unregulated fossil fuel consumption continue to change Earth's climate with devastating consequences for health and biodiversity, microbial genomes offer a several billion years' evolutionary history of adapting to existential threats. That much time spent refining genetic code has created an Earth spectacularly full of elegant survival systems. However, if human action continues to drive species extinction at a rate unprecedented in last sixty million years of geologic record, that "library of life" will no longer be available for application to human-scale problems. Teaching young people the biomolecular basis for uncovering this "hidden treasure" of genetic diversity is not just an academic framework for the classroom; it has profound implications for how individuals live in and relate to their world.

Teaching Strategies

The anchoring activities of this unit are a lab report quantifying the microbial biodiversity of our school by sampling and culturing communities from different parts of the school, testing different antimicrobial methods using an engineering design protocol, and then developing and proposing public health interventions for our school or community stakeholders based on research. These activities introduce biodiversity, characteristics of life, molecular genetics, cells, and the basic skills for exploring, analyzing, and communicating like scientists and engineers.

Project-Based Learning

Used as the first research experience of ninth grade, sampling our school for microbial biodiversity engages students with hands-on laboratory manipulation. Anecdotally, students report that using the tools of science "makes them feel like they're actually doing science." Developed by the Howard Hughes Medical Institute in 2008, the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) protocol is a research-based investigation where undergraduate students sample, isolate, sequence, and characterize bacteriophages from the environment. In 2017, more than 10,000 bacteriophages had been added to the SEA-PHAGES library, 1800 of which had complete genomic sequences, and at least one successful human trial in phage-therapy had been conducted referencing this student-derived library.[81] At the undergraduate level, the SEA-PHAGES research program has been shown to engage young people in the experiences of a professional scientist, i.e. the thrill of discovery, collaboration within a community, and advancing scientific knowledge relevant to the broader community.[82] These psychosocial elements are correlated to increased persistence in the sciences and benefit all students regardless of their intended area of study.[83] Although very few public high-schools have the time or technology to offer a two-semester course on microbiology, especially the isolation and sequencing steps, the laboratory activities that follow preserve the discovery aspects of the SEA-PHAGES protocol as best as possible while still respecting resource constraints on public high school teachers.

Knowles Engineering and Design for Social Justice

Developed by the Knowles Teaching Initiative for high-impact, culturally relevant lessons, an engineering design process is a professional technique for exploring a problem, assigning constraints and criteria to measure and rank outcomes, then prototyping, testing, and communicating efficacy and trade-offs of proposed solutions. These steps allow significant overlap for the Next Generation Science Standards science and engineering practices. All eight of the identified skills of STEM professionals can be incorporated with intentional design, but these four are always achieved by proper implementation of the process:

- Asking questions and defining problems
- Planning and carrying out investigations
- Analyzing and interpreting data
- Obtaining, evaluating, and communicating information

While this process can be applied to almost any academic material, research shows that self-identifying as an engineer or scientist is more likely when young people are positioned as experts in the problems of their own lives, and the skills of these professions are taught as techniques for empowerment and change.[84] Self-

identifying as a scientist and engineering has been correlated to higher educational attainment and higher persistence in STEM fields.[85] Knowles provides strategies for connecting the engineering protocol to student driven social justice. Framed in four steps from purely engineering design to a fully student-driven project to change their world, the implementers do not encourage educators to aim for a fully student-driven project every time. It is important that young people experience many different kinds of projects as scaffolding for more involved ones.

Place-Based Learning

An essential component of Project-Based Learning is that student work should have real world application. By utilizing a place-based approach to studying biodiversity of our school microbial community, it increases personal investment in the scientific skills and results.[86] "What bacteria and fungi are in the student bathroom?" is potentially more compelling investigation than quantifying fecal contamination from a distant beach students will likely never visit. In the social justice-aligned version of sampling, students read about antibiotic resistant microbes and are given informational cards from the CDC Health Equity group outlining how different communities in the United States are impacted more acutely by certain strains of these microbes. Because my students are members of those communities or know, love, and care for people in those communities, it provides a personal and emotional context to science skills and applies them in a way that could make real change in their lives.

Collaborative Student Choice

Students have many different dreams when they enter the science classroom. Some have a vision of what they might like to pursue professionally, others have interests but are not sure about life after school, and a vast majority of teens are simply dealing with the realities of life as they come. By approaching science conversations that also include avenues for art, emotion, law, philosophy, and religion, I hope to provide many modes of access for discussion, especially for young people who are still developing spoken language proficiency in English. For students who are not intrinsically motivated by the pleasure of hands-on science, producing a "zine," or a small magazine, about genetic engineering invites many different access points for aligning with student interests and expertise. Young people interested in law are invited approach aspects of patent law and bioethics under existing legal structures. Students interested in finance and entrepreneurship are supported to research market demand and the liabilities of producing genetically modified organisms (GMOs). Artists contribute digital or print media examples of before-and-after modification of organisms, potentially utilizing 3D imagining tools. Poets and religiously inclined young people are invited to write additional pieces informing genetics and GMOs. Students proficient in organization or graphic design can manage the presentation of these class materials on a publicly facing website. Although this is ultimately a collaborative project valuing many different skills, students have a great deal of freedom in choosing their mode research and are not penalized for the non-performance of classmates.

Gamification and Reward Structures

Quizlet is a "freemium" software platform that "gamifies" vocabulary memorization. Styled as a timed matching activity that displays a vocabulary word and four possible definitions (or vice versa as a paid option), this interface allows students to review teacher-directed vocabulary terms individually and then compete as random or assigned teams. During individual study time, ranked student performance is projected using animal signifiers to respect anonymity. On the teacher interface, private report are available showing individual student names and scores for participation and proficiency. Although not every student can be the

fastest person in class, the team activity makes it possible for any student in class to earn candy for participation. At least two teens have verbally recognized the correlation between personal studying and team success, and engagement and improvement have been high across all of my classes from 2021 – 2023. In addition to candy rewards for team activities, students know that these vocabulary words are the basis for successive quizzes, first testing five vocabulary words, then ten, then all fifteen words from each three-week unit. The vocabulary from these three successive quizzes also appear on the end-of-quarter and end-of-semester exams. Used as the starting activity at the beginning of nearly every class, repeated exposure to the definitions builds familiarity when seeing them used in context and gives all students a better starting place by sharing a common language for conversation.

Classroom Activities

Prior to all laboratory activities, explicit review of safe laboratory practices is mandatory. Educators should be knowledgeable of risks associated with different tools and materials and clearly convey these risks to students. Discuss what personal protective equipment looks like during lab, including closed toe shoes, tying back long hair, and when gloves or eye coverings are required. In prior years, students in my classes have made artistic interpretations of each lab safety rule to be posted in the lab, which were then reviewed in a gallery walk and sticky notes used to predict possible personal and academic consequences if safety rules were not thoroughly followed. Although there may be education institutions with intentionally designed science spaces, my and many other public high-school classrooms operate using Biosafety Level 1 (BSL-1) standards.[87] Agents used in this research would not pose a risk to healthy human adults and must have a safe disposal protocol prepared in advance. Additional class time may be required before these labs to teach skills such as measuring volume and mass, sterile technique, pipetting, and becoming familiar with the names of different techniques, units, equipment, and materials.

Exploring Microbial Biodiversity in School

For this lab, students work in groups of two to four. While keeping the lid on, teams label the bottom of two Petri dishes with their initials using an indelible marker and draw lines across the diameter of the plate dividing it into four quadrants. Use a graduated cylinder to measure 200mL of filtered water. Pour into a beaker and place on a hot plate with an alcohol-based thermometer under both student and adult supervision. As the water temperature reaches 100°C, students use an analytic balance to measure 5.5 grams of gelatin, 5.5 grams of beef stock powder, and 5.5 grams of sugar which are added to the water after boiling is achieved. Stir for one minute then remove from heat using safety tongs and wait ten minutes for the mixture to cool. When ready to pour, students remove the lid from two Petri dishes pre-labeled with their team initials using an indelible marker and pour half of their solution into each, quickly recapping the lid to avoid further contamination. The dishes will go into a refrigerator overnight.[88]

The next laboratory day, student groups hypothesize where in the school building they expect the greatest biodiversity of microbes. Based on your student population, either lead a discussion or instruct students in the necessity of a “control” condition during experimental design. Have them designate an entire plate or one quadrant of a plate to support that any growth comes from the sampling site rather than contamination during the process. A “sign-up” sheet is projected and students are told that once a site is selected, it may not be sampled again. A whole class demonstration is provided using a document camera showing how to “streak

plate," gently rubbing the sample-covered sterile swab over the surface of the agar in a wave pattern, as well as techniques for sterile handling of swabs and agar plates to avoid as much contamination as possible. When students receive their four sterile swabs and nitrile gloves, they are asked to verbally explain appropriate use of the swabs before they are given a hall pass. They will swab three locations, streak onto three quadrants of one Petri dish, label each quadrant noting where each sample came from and take a photo of each sampling site. Upon student return, Petri dishes are kept in a box with an infrared heating lamp and incubated at ~37°C to accelerate microbial growth. Changes are observed and documented each class period for several days on a laboratory data sheet. Honors classes offer a potential small-group extension activity where they propose design of the data table and critique each other's suggestions until arriving at class consensus.

Engineering and Design: Testing for Efficacy

After students perform the bacterial biodiversity sampling around school, they will then learn about antibiotic resistance and the consequences it has on different members of society based on information from the CDC Health Equity group. Based on the information from the cards and student interest, table groups of three to four members decide who their stakeholders are. They may select the school community or one of the communities disproportionately impacted by antimicrobial resistance. Once their infectious organism and stakeholders are identified, they will develop constraints and criteria to test the efficacy of different antimicrobial interventions on a model bacterial strain that is safer than the AMR strains. This opens the conversation to what is a model and if the model is an effective replacement for the harmful bacteria.

Student groups will select antimicrobial treatments from teacher-provided hand-soap, hand sanitizer, the industrial floor cleaning solution used in the school, "all natural" surface cleaning solutions, and UV light. See the next section if educators would like to include bacteriophage as an antimicrobial intervention. It is advised that more attentive groups of students be given that option because it requires additional considerations to time and safety. Once the treatment is identified, students will devise the testing protocol identifying a control and three different treatments of the independent variable. They will explain what they are applying, what volume and concentration of products they will test, how to measure or prepare those concentrations, and how to apply the correct volume. If a student group selects UV light, they will need to develop additional constraints based on intended application. For example, if students propose the school as the community stakeholders, is UV proposed as an alternative to hand soap or a surface cleaning intervention. These constraints will inform how long they will expose the bacteria as their independent variable. Once a group's testing schema is approved, they can implement their plan and record the results over two class days.

When discussing criteria for success, one option that can be assigned is that more effective treatments have greater ability to kill bacteria as demonstrated by clearing of the bacterial lawn. How students measure this can be open for discussion or can be directed toward lesson in estimated area or, with appropriate computer access, a technology lesson using ImageJ to calculate area of a surface on an image.[89]

In order to test the efficacy of killing bacteria, bacterial plates are required. This is an extension activity that involves live cultures and requires greater attention to materials and laboratory safety. For institutions that have access to a scientific incubator, follow all operation and safety protocols as advised by the device manufacturer. For educators who do not have access to an intentional incubator, use an incandescent light bulb and thermometer to raise the temperature of an enclosed cardboard container to approximately 37°C or consider inoculating the agar plates on a Friday afternoon and allowing the bacteria to grow at room temperature over the weekend. Make sure all Petri dishes are sealed with tape and are clearly labeled to not be disturbed by classroom visitors.

The instructor will select, purchase, and maintain a BSL-1 safe bacterial culture respecting necessary growth conditions specific to that strain. As best as classroom conditions allow, follow all instructions with regards to safe handling, nutrient media, temperature, and humidity. For this lab, students work in groups of two to four. Have them use alcohol or a 10% bleach solution to sterilize the surface area of their work bench. While keeping the lid on, teams label the bottom of three Petri dishes with their initials using an indelible marker and draw lines across the diameter of the plate dividing it into four quadrants. Wearing nitrile gloves, students use a graduated cylinder to measure 75mL of filtered water. Pour the water into a beaker and place on a hot plate with an alcohol-based thermometer under both student and adult supervision. Use an analytic balance to measure 2.1 grams of nutrient agar and stir until all powder is dissolved. Remove from heat using safety tongs and allow the mixture to cool but not solidify. Once cooled, pour the agar solution into three Petri dishes (~25mL each). Allow agar in open Petri dishes to solidify at room temperature on the sterile surface area. If the class is ready to plate the bacterial culture immediately, pipette 100mL of bacterial culture onto the surface of the solidified agar and immediately replace the lid and swirl the plate gently while keeping it level to uniformly distribute the bacteria. Tape shut and store at the appropriate temperature for growth indicated for the particular bacterial strain. Allow at least 24 hours for incubation. If the class will plate bacterial culture on another day, allow agar to solidify, replace the lid, tape shut, and store at 2 - 8°C (~35 - 45°F) until ready for use.[90]

The agar plates may require three to seven days to develop a uniform bacterial lawn, often presenting as a cloudy, yellow-orange or white-ish layer with circular colonies at the edges if the culture was not spread across the entire plate. If plates develop nonuniform growth such as splotchy texture or discoloration, keep them sealed and dispose of properly, as these were contaminated by other microorganisms during preparation. Students should wear nitrile gloves while handling plates.

Engineering and Design: Sampling for Bacteriophage Presence

For educators pursuing the engineering design project, this is an additional antimicrobial treatment option that involves all safety protocols reviewed above and additional consideration that students are cultivating virus which should be noninfectious in eukaryotic cells but utmost attention to safety is still required. Review the necessity of a control condition, streak plating, and sterile technique as necessary. Again, allow student teams to hypothesize locations with the highest viral “biodiversity.” Provide a hall pass, one Petri dish, and sterile swabs to sample the school environment for bacteriophages. They will swab three locations, streak onto three quadrants of one Petri dish, label where each sample was from and take a photo of each sampling site. Plates are then incubated in the necessary conditions for bacterial growth, observed, and documented over a series of days. When students observe small circles of “cleared” bacterial lawn called plaques, they should take a photo of the final plate and use a sterile swab to gently “touch” each differently sized plaque and re-plate onto another Petri dish with bacterial lawn. Again, allowed to grow and monitored for plate contamination, students can expect to observe higher concentrations of plaques where they streak plated across the bacterial lawn.[91]

Engineering and Design: Proposing a Public Health Intervention

Based on the results of their class findings, student groups will propose public health interventions using their data and community stakeholder values. The four-step process proposed by the Knowles Teacher Initiative identified stakeholders when students selected with antimicrobial resistant organism they would study. Keeping those same stakeholders, students will need to reach out to members of that community with adult supervision to develop constraints and criteria for a public health intervention using the most effective bactericide as identified by class tests. Whether groups choose the school community or another group in

Philadelphia, their final presentations will be evaluated and refined in the classroom before scheduling public review, inviting administrators, health officials, and other representatives from community stakeholders to determine if proposals can be implemented.

Resources

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List of Materials for Classroom Use

Petri dishes

Analytic balance

Weighing trays

Graduated cylinders

Hot plates

Nutrient agar

Filtered water

Nitrile gloves

Sterile swabs

Tape

Indelible markers

Non-food refrigerator

UV lights

Industrial surface cleaner

“All natural” surface cleaner

Alcohol-based hand sanitizer

Liquid hand-soap

Bleach

BSL-1 bacterial culture

Erlenmeyer flasks

Incubator or incandescent light bulb

Thermometer

Camera or phone for photography

ImageJ software

Appendix on Implementing District Standards

As of July 2023, Pennsylvania has ratified but not implemented new statewide science standards, moving from the current Keystone Biology Anchors to the NGSS based “Science, Technology & Engineering, Environmental Literacy and Sustainability (STEELS) Standards.” Both state standards are included during this time of transition.

Framed within the central biodiversity question, “what lives in our school?” students will learn about viral infection of bacteria and the process of “hijacking” protein synthesis to make more viruses. In this lab, students observe evidence of bacteria-infecting viruses through the “death” of bacteria, visible as clear circles or “plaques” on a bacterial lawn. Explaining why these circles indicate viral presence in their lab reports, students will need a general understanding of protein synthesis to know how viral infection operates. In the context of genetic engineering, students will have hands-on activities where they manipulate paper models of DNA, RNA, and amino acid sequences, showing that changes in DNA result in changes to the protein. Final presentations will focus on an existing or proposed genetic engineering project that has intended benefit for humanity or the environment, evaluating potential unintended consequences.

Pennsylvania Keystone Biology Anchors

- BIO.B.2.2 Explain the process of protein synthesis (i.e., transcription, translation, and protein modification).
- BIO.B.2.4.1 Explain how genetic engineering has impacted the fields of medicine, forensics, and agriculture (e.g., selective breeding, gene splicing, cloning, genetically modified organisms, gene therapy).

Pennsylvania Science, Technology & Engineering, Environmental Literacy and Sustainability (STEELS) Standards

- 3.1.9-12.A Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.
- 3.1.9-12.N Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.

NGSS Science and Engineering Practices

During the curriculum unit, students practice the QFT Protocol for asking questions, applying the protocol to teacher supplied images, videos, and then student-generated media prompts and evidence from their laboratory observations on microbial biodiversity in school. The questions and observations will lead students to argue whether or not their findings indicate the presence of viruses and if viruses meet the qualifying characteristics of life to be considered “alive.”

- Asking Questions and Defining Problems
- Engaging in Argument from Evidence

Notes

[1] (Kean 2014)

[2] (Pires et al. 2016)

[3] (“DNA Is a Structure That Encodes Biological Information” 2014)

[4] (National Human Genome Research Institute 2023)

[5] (Learn.Genetics, n.d.)

[6] (Squidonious 2008)

[7] (Gillett and McKergow 2007)

[8] (Eldredge 2001)

[9] (“10 Global Health Issues to Track in 2021” 2020)

[10] (Greene and Reid 2013)

[11] (Greene and Reid 2013)

[12] (Greene and Reid 2013)

[13] (“DNA Is a Structure That Encodes Biological Information” 2014)

[14] (“Replication and Distribution of DNA during Meiosis” 2014)

[15] (Greene and Reid 2013, pg ?)

[16] (Darwin 1859)

[17] (Greene and Reid 2013, pg 6)

[18] (Keeling and Palmer 2008)

[19] (Greene and Reid 2013, pgs 4-5)

[20] (Greene and Reid 2013)

[21] (Kortright et al. 2019)

[22] (Roper 1938)

[23] (Scheele and Dunn 1955)

[24] (CDC 2013)

[25] (Kortright et al. 2019)

[26] (Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022 2022)

[27] (Murray et al. 2022)

[28] (Gottfried 2005; Kortright et al. 2019)

[29] (Weiner-Lastinger et al. 2020)

[30] (Lee Ventola 2015)

[31] (Twort 1915)

[32] (Kutateladze and Adamia 2010)

[33] (Chow 2020)

[34] (Ryu 2017)

[35] (Kortright et al 2019)

[36] (Kutateladze and Adamia 2010)

[37] (“Diarrhoeal Disease” 2017)

[38] (VanderWal 2023)

[39] (Benyus 1997)

[40] (Anthropocene Working Group 2019)

[41] (“UN Report: Nature’s Dangerous Decline ‘Unprecedented’; Species Extinction Rates ‘Accelerating’” 2019)

[42] (“UN Report: Nature’s Dangerous Decline ‘Unprecedented’; Species Extinction Rates ‘Accelerating’” 2019)

[43] (Ryu 2017)

[44] (Ryu 2017)

[45] (Ryu 2017)

[46] (Kortright et al. 2019)

[47] (Koonin, Senkevich, and Dolja 2006)

[48] (Huynh 2017)

[49] (Prangishvili, Forterre, and Garrett 2006)

[50] (Kortright et al. 2019)

[51] (Kortright et al. 2019)

[52] (Kortright et al. 2019)

[53] (Kortright et al. 2019)

[54] (Alberts, Johnson, and Lewis 2002)

[55] (Ramirez et al. 2020)

[56] (Kortright et al. 2019)

[57] (Center for Biological Evaluation and Research 2021)

[58] (Greene and Reid 2013)

[59] (Oechslin 2018)

[60] (Borin et al. 2021)

[61] (Kortright et al. 2021)

[62] (Wright et al. 2018)

[63] (Wright et al. 2018)

[64] (Wright et al. 2018)

[65] (Oechslin 2018)

[66] (Borin et al. 2021)

[67] (Cohan, Zandi, and Turner 2020)

[68] (Kortright et al. 2019)

[69] (Koonin, Senkevich, and Dolja 2006)

[70] Borin et al. 2021b

[71] (Kortright et al. 2019)

[72] (Kortright et al. 2019)

[73] (Kortright et al. 2019)

[74] (Gillett and McKergow 2007)

[75] (Kutateladze and Adamia 2010)

[76] (Pires et al. 2016)

[77] (Myers 2012)

[78] (Kortright et al. 2019)

[79] (Bolt 2019)

[80] (MacLean and Harper 2021)

[81] (Howard Hughes Medical Institute 2023)

[82] (Hanauer et al. 2017)

[83] (Hanauer et al. 2017)

[84] (Wang and Degol 2017)

[85] (Wang and Degol 2017)

[86] (Minero 2019)

[87] ("Biosafety Level (BSL) 1: Hazard Control" 2021; Kutateladze and Adamia 2010)

[88] ("Grow Bacteria On Homemade Agar Plates" 2017)

[89] (Reinking 2007)

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Scheele, Leonard A, and Halbert L Dunn. 1955. “PUBLIC HEALTH SERVICE VITAL STATISTICS OF THE UNITED STATES 1952 VOLUME H MORTALITY DATA PREPARED UNDER THE SUPERVISION OF.” https://www.cdc.gov/nchs/data/vsus/VSUS_1952_2.pdf Another PDF of mortality data in the U.S., this one was searchable.

Squidonius. Mobio-Header.svg. 2008, May 13. Image. Wikimedia Foundation. https://commons.wikimedia.org/wiki/Category:Central_dogma_of_molecular_biology#/media/File:Molbio-Header.svg A Wikimedia foundation image representing DNA being copied to RNA being translated into a protein.

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Weiner-Lastinger, Lindsey M., Sheila Abner, Jonathan R. Edwards, Alexander J. Kallen, Maria Karlsson, Shelley S. Magill, Daniel Pollock, et al. 2020. “Antimicrobial-Resistant Pathogens Associated with Adult Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network, 2015–2017.” *Infection Control & Hospital Epidemiology* 41 (1): 29. https://doi.org/10.1017/ice.2019.296. An awful set of statistic-containing colored rectangles that misused space and were poorly organized.

Wright, Rosanna C. T., Ville-Petri Friman, Margaret C. M. Smith, and Michael A. Brockhurst. 2018. “Cross-Resistance Is Modular in Bacteria–Phage Interactions.” PLOS Biology 16 (10): e2006057. https://doi.org/10.1371/journal.pbio.2006057. Very cool article detailing that bacteria can develop resistance to not just one phage but several at once depending on whether a structural feature or regulatory mechanism is mutated during successive generations of bacterial evolution under selective pressure by phages.