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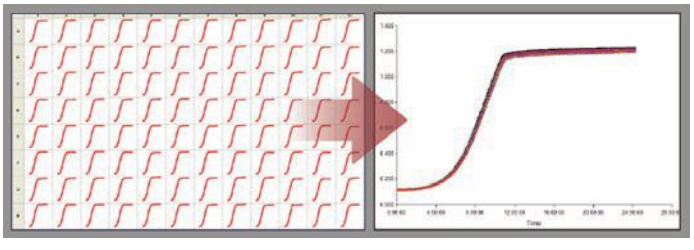

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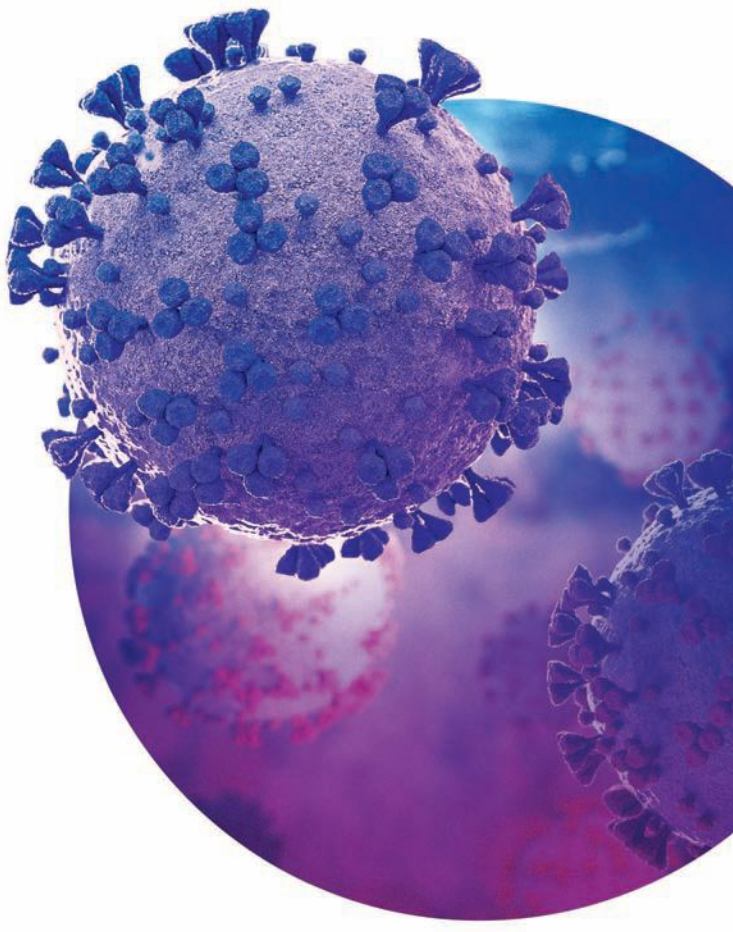

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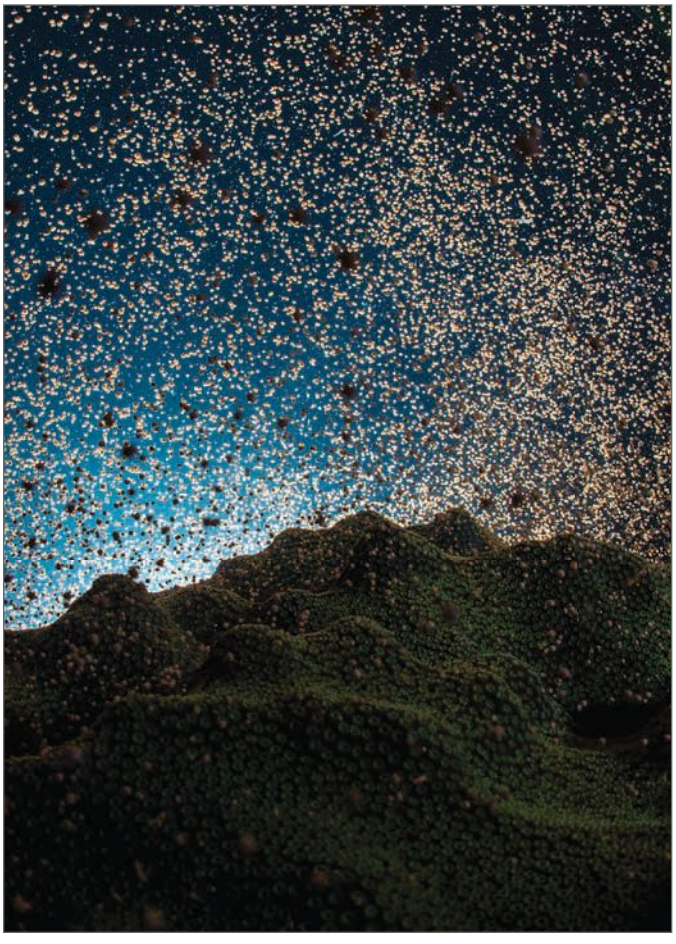

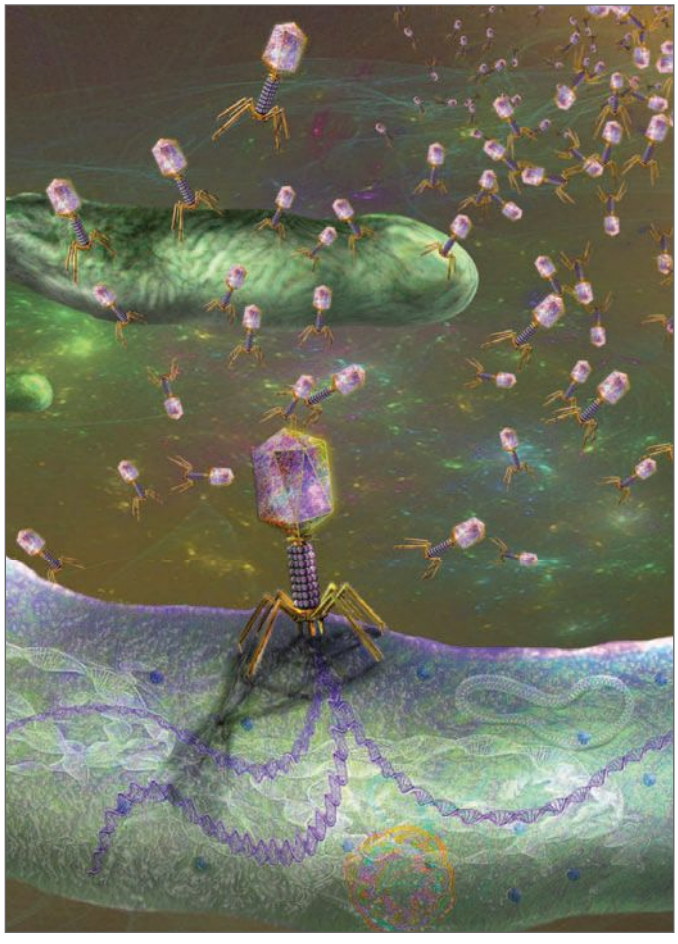

Features

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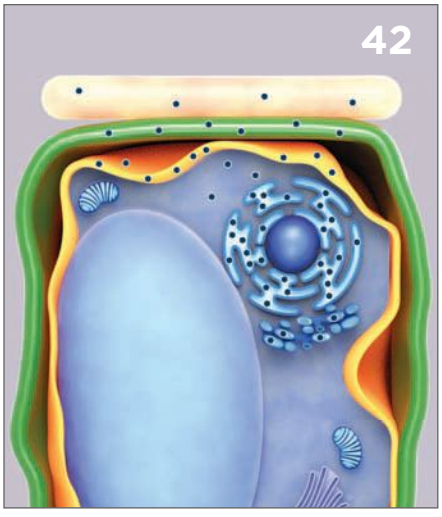

CORRECTIONS:

In "Rewilding with Teeth" from the November 2020 issue, pumas were misidentified as *Felis concolor*, instead of *Puma concolor*, and an elk species described as *Cervus elaphus*, instead of *Cervus canadensis*.

The "Top 10 Innovations of 2020" story from the December 2020 issue stated that GigaGen's Surge platform captured antibodies from samples that came from plasma donors. They were, in fact, blood donors. The story also misstated the title of AbCellara's Maia Smith and the nature of Celium and collaborations surrounding the tool.

The Scientist regrets the errors.

PUZZLE ON PAGE 12

P	I	N	O	A	K	■	W	I	Z	A	R	D
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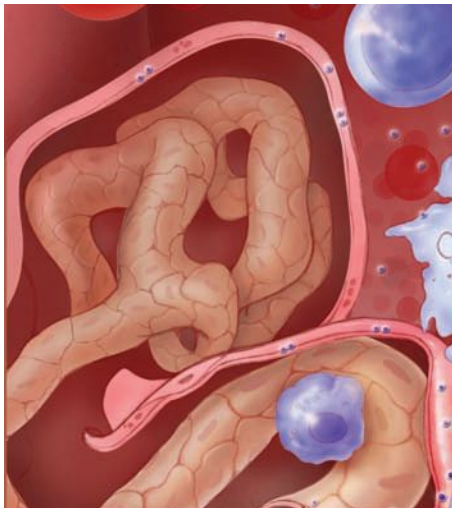

ONLINE AT THE-SCIENTIST.COM:

Human Fetuses Can Contract SARS-CoV-2, but It's Rare
Compared with Zika and cytomegalovirus, the virus that causes COVID-19 appears to have a harder time penetrating the placenta and moving into a woman's unborn baby.

A Dog's View of Optical Illusions
Researchers are using visual tricks to try to better understand canine perception.

Steps to End "Colonial Science" Slowly Take Shape
Scientists from countries with fewer resources are pushing collaborators from higher-income countries to shed biases and behaviors that perpetuate social stratification in the research community.

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Coming next month

- Males and females mount different immune responses to viral infection. It's still unclear if these might explain sex differences seen in COVID-19.
- Researchers get creative to study insects in flight, pulling from video games, sports broadcasting, meteorology, and even missile guidance technology.
- Scientists who work abroad face a patchwork of permits that threaten to slow down or even halt their projects.
- With millions of microbe species waiting to be discovered, scientists consider automating the creation of their binomial Latin or Greek names.

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Contributors

Because both of her parents were biologists, **Angela E. Boag** was essentially "raised on David Attenborough," she says. And she consequently developed a love of nature and ecosystems. At Queen's University in Ontario, Canada, Boag earned a bachelor's degree in biology in 2010, and followed that up with a master's degree in forestry from the University of British Columbia and a doctorate in environmental studies from the University of Colorado Boulder, where she researched climate change effects on forests in and around the US Rocky Mountains. After graduating, she became a policy advisor for climate change, forest management, and energy at the Colorado Department of Natural Resources.

Nathalie Isabelle Chardon has also always been fascinated with biology, but it wasn't until she attended field classes while studying abroad in Chile during her junior year at the University of California, Berkeley, that she began to focus on ecology. She became curious about what drives species' distributional patterns and how climate change influences their performance and distribution. She finished her bachelor's degree in integrative biology in 2010 and worked for the United States Forest Service for a few years before starting a doctoral program in environmental studies at the University of Colorado Boulder, where she studied the consequences of human disturbance on alpine plant distributions. After graduating in 2018, Chardon started a postdoc at the WSL Institute for Snow and Avalanche Research in Switzerland.

Boag and Chardon met and became friends during their doctoral studies at the University of Colorado Boulder, where they realized that something important provided by their jobs outside of academia was missing from their roles as graduate student researchers: clear professional expectations. "Graduate students have really good access to resources for almost everything you can imagine, but there's little proactive management" of students by their mentors, says Chardon. On page 14, Boag and Chardon discuss ways to improve graduate student/advisor relationships.

Michael P. Crosby grew up "at the water's edge" in Key West, Florida. "I remember my father telling me about how beautiful those coral reefs were before I could even really swim," he says. Imbued with this love of the ocean, Crosby pursued a doctorate in marine-estuarine environmental science from the University of Maryland and launched a career in marine and coastal ecology. He held several faculty positions before serving as vice chancellor for research at the University of Hawai'i at Hilo and as associate vice president for research and economic development at George Mason University in Virginia. In 2013, he became the president and CEO of Mote Marine Laboratory and Aquarium, an independent and nonprofit research institution.

Crosby selected **Erinn Muller** as Mote's first postdoc in 2012, and she began her studies of coral health and disease dynamics before becoming a senior scientist and program manager at the laboratory. In 2018, she helped mentor **Hanna R. Koch**, who had joined Mote as a visiting researcher. Koch is now Mote's newest postdoctoral fellow and studies sexual reproduction in corals.

Together, Crosby, Muller, and Koch have been testing new strategies to restore widespread damage to coral reefs caused by climate change and environmental pollutants. "The message that we continually hear . . . is a very real one of alarm with respect to climate change and its devastating impacts on our oceans and coral reefs," says Crosby. On page 24, the three researchers outline the urgency of coral reef loss and describe their research efforts, which Crosby says he thinks will bring hope to the scientific community.

PATRICK CAMPBELL; VERONICA SPARKS; CONOR GOULDING; MOTE MARINE LABORATORY; CONOR GOULDING

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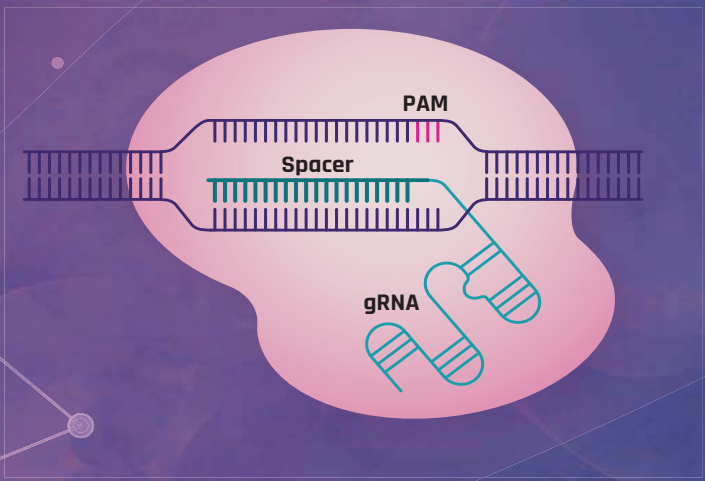
CRISPR-Cas:
The Next Generation

Cas9 (formerly known as Cas5, Csn1, or Csx12)

Class 2 Type II

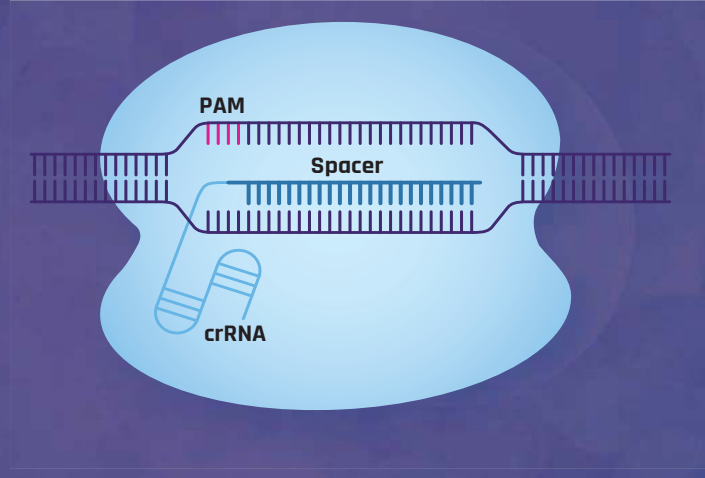


Cas9 was the first CRISPR associated protein researchers used outside of prokaryotic cells, and it is still the most commonly used genome editing tool today.[2,3] It uses a 20-nucleotide spacer and targets the 5'-NGG (where N represents any nucleotide) protospacer adjacent motif (PAM).[3,4] As a type II system, Cas9 generates double-stranded DNA (dsDNA) cuts with blunt ends.

Researchers improved targeting by engineering Cas9 variants. The 5'-NGG PAM limits target site availability to roughly one per eight base pairs.[1] Cas9 variants or orthologues that recognize different or multiple PAMs—such as xCas9, which recognizes 5'-NG, 5'-GAA, and 5'-GAT—overcome this limitation.[5,6] Engineering secondary structures in guide RNA spacer regions also improves targeting specificity, thereby creating a barrier to strand invasion at off-target sites without overly affecting on-target activity.[7]

Cas12 (formerly known as Cpf1)

Class 2 Type V



Cas12a is a type V system, which means that it generates a staggered dsDNA cut with a 5' overhang and does not use a transactivating CRISPR RNA (crRNA). This provides advantages in certain situations, such as integrating DNA sequences in a specific orientation. Cas12 can also generate its own crRNAs by cleaving crRNA arrays, enabling scientists to perform multiplex gene editing using only a single crRNA array.[8]

The first endogenous Cas12a orthologues with activity in mammalian cells recognize the PAM sequence 5'-TTTV. Newer engineered variants not only have higher editing activity for this canonical TTTV sequence, but also recognize and act on other PAMs including 5′-TYCV, 5′-VTTV, 5′-TTTT, 5′-TTCN, and 5′-TATV.[9,10]

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10X GENOMICS

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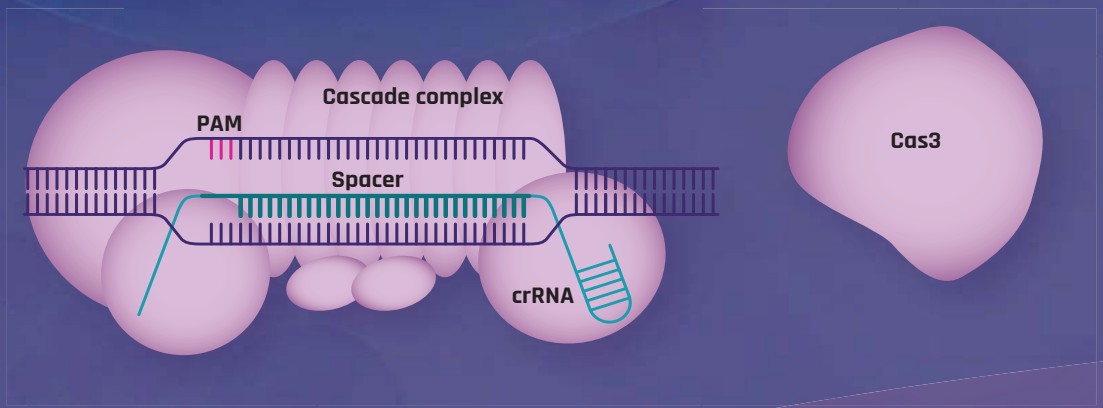
CRISPR-Cas: *The Next Generation*

The development of CRISPR-Cas systems transformed genome engineering. Driven by nucleic acid sequences, CRISPR-Cas targeting made genetic manipulation much more accessible, leading to a wide array of breakthroughs in basic, translational, and medical science.[1]

The CRISPR-Cas success story has inspired scientists to discover and create new CRISPR-Cas systems, including those that can target RNA, epigenetic modifications, or chromatin interactions. The next generation of CRISPR-Cas systems expands the power and potential of CRISPR-Cas, improving biological understanding and inching closer to the ultimate goal of clinical use.[2]

Cascade-Cas3

Class 1 Type 1

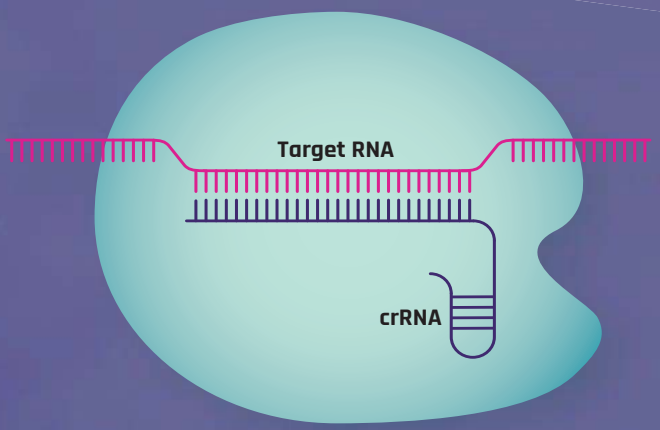


Cascade is a multimeric DNA-targeting complex that binds DNA via PAM and spacer recognition and then recruits Cas3 to generate a single-strand nick, followed by 3′ to 5′ degradation of the targeted DNA.[11,12] Cascade recognizes more PAM sequences than other Cas proteins, giving the Cascade-Cas3 system greater target site flexibility.[13] Researchers are looking to Cas3's unique cutting mechanism as a antimicrobial tool, given that Cas3 is endogenously essential for the degradation of foreign DNA in prokaryotes.[14]

TARGETING RNA

Cas13 (formerly known as C2c2)

Class 2 Type VI



Unlike most other Cas proteins, Cas13a is an RNA-guided RNA-targeting nuclease that activates upon recognition of ssRNA target sequences.[15] After target binding, Cas13a cuts at uracil bases anywhere in the local vicinity, potentially collaterally cleaving nearby, untargeted RNAs. Researchers used this to create a molecular detection platform aptly named SHERLOCK, where collateral RNA cleavage releases a reporter signal.[16] SHERLOCK detects viral and bacterial pathogens, discriminates between single-nucleotide polymorphisms in the human genome, and identifies cell-free, mutated tumor DNA.[16,17]

Beyond imaging, Cas13 has also been adapted for single-base RNA editing. Consisting of a catalytically deficient Cas13 (dCas13) fused to adenosine deaminase, the REPAIR system makes directed adenosine-to-inosine edits in eukaryotic cells.[18] dCas13 can also be fused with other RNA editing domains to enable cytidine-to-uridine editing.[2]

Modifying Cas9

Cas9 normally targets dsDNA, but it can also target single-stranded (ss) nucleic acids if PAM-presenting oligonucleotides (PAMmers) are used. PAMmers anneal to single stranded DNA or RNA, thereby directing Cas9 to single-stranded targets.[19] Termed 'RCas9' (RNA-targeting Cas9), this system allows researchers to detect endogenous RNA without genetically encoded tags and to control cellular processes at the transcript level through site-specific cleavage of ssRNA.[19,20]

A number of Cas9 orthologues, such as *Campylobacter jejuni* Cas9, also target RNA. *C. jejuni* Cas9 binds and cleaves endogenous RNAs without PAM guidance, while *Francisella novicida* Cas9 targets bacterial mRNA and alters gene expression.[21,22] Researchers continue to study any potential physiological consequences of Cas9 RNA targeting in eukaryotic cells.[2]

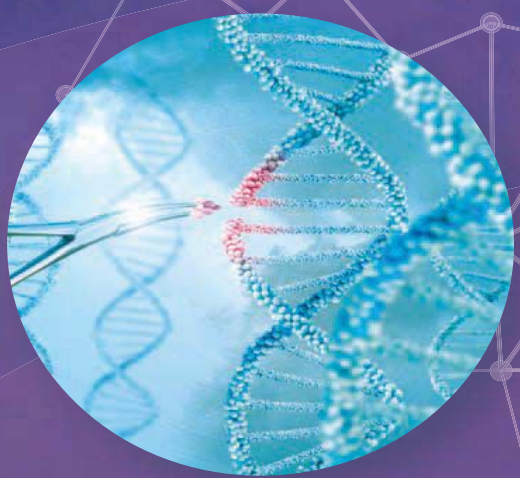
Beyond On/Off: Dynamic Genetic and Epigenetic Regulation

CRISPR-Cas9 regulates gene function by serving as a DNA recognition complex rather than as a targeted nuclease.[23] For example, binding catalytically deficient Cas9 (dCas9) to DNA elements creates gene silencing steric CRISPR interference (CRISPRi) that hinders RNA polymerases.[24] Additionally tethering dCas9 to transcription repressor domains enhances this effect.[25] The reverse is also possible: fusing dCas9 to activator effectors results in programmed transcription activation, or CRISPR activation (CRISPRa).[26] This enables researchers to direct synergistic gene activation by using CRISPRa with synthetic transcription factors or combining different activator domains, an important feature for cellular reprograming.[27-29] dCas9-based tools also enable targeted epigenetic modifications such as the acetylation and methylation of histones and methylation of DNA.[23]

Cas9 function can be dynamically controlled. Chemical compounds or light, for example, can activate Cas9 expression through inducible promoters. Scientists use this approach to generate animal models for research where timed gene knockout is desired or necessary.[30] Inducible Cas9 function gives researchers efficient, tunable, and reversible disease modeling capability and helps shed light on stem cell differentiation and development mechanisms.[31,32]

An Eye on the Clinic

How CRISPR-Cas technology shapes the future of disease research and medicine



Rather than gene insertion/deletion, gene editing is now the main focus for the CRISPR-Cas system.[2] This has obvious implications for genetic diseases caused by mutations, but editing may be a valid strategy for restoring physiological states in more common, complex diseases. For example, CRISPR-Cas9 disruption of the cholesterol homeostasis gene *Pcsk9* in mice reduced levels of low-density lipoprotein cholesterol.[33] CRISPR-Cas also modulates cells ex vivo to create candidates for cell-based therapeutics. Gene editing approaches have enhanced the properties of autologous T cells for immunotherapy and immunoncology.[34,35]

Before CRISPR-Cas can fully transition into the clinic, scientists need to overcome a number of obstacles. The biggest challenge lies in potential off-target effects and immunogenicity. Optimizing guide RNA selection and screening with greater sensitivity can address the former, while identifying and re-engineering immunogenic epitopes may ameliorate the latter.[2] Finally, adeno-associated viruses, the most popular delivery vector for CRISPR-Cas machinery, have limited capacity. Faced with this, researchers are investigating smaller Cas protein orthologues as well as non-viral delivery methods such as lipid nanoparticles.[36]

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Feeling the Foundation

This year has started out in a fashion that is sadly similar to the way 2020 unspooled. But the steady pace of scientific discovery helps maintain our sense of hope.

BY BOB GRANT

Although it appears to be trying its damnedest, 2021 has not yet sapped me of my hope that humanity can turn a corner and put the horrors of 2020 in our rearview mirror. As I've written in previous dispatches, what anchors me to this hope is science.

Since the calendar turned, the COVID-19 pandemic has worsened; new, more-infectious SARS-CoV-2 variants are cropping up around the globe; vaccine rollout has been slower than anticipated; and political division has reached a fever pitch here in the US. But the steady pace of scientific discovery and development churns on. And even with so many aspects of our lives and work bearing the scars of 2020's tumult (some wounds are indeed still fresh), we at *The Scientist*, as well as those in the research community we serve and like-minded members of the general public, continue to look to science, reason, and fact as the keys that will deliver us into a more peaceful existence.

One must keep in mind that I write these editorials weeks before you have the opportunity to read them. For example, I sit to write this piece in the middle of January, but you're reading this on or after it is published on February 1. In normal times, this makes it difficult to encapsulate and comment on the zeitgeist of the current moment. These days, with things happening at such a frenetic pace, this task becomes nigh on impossible.

By contrast, there's something downright comforting about following an enterprise that proceeds at a stable rate and that tends to build slowly, one insight adding to preceding ones to form an ever-clearer picture of reality. That's not to say science can't or doesn't surprise us. To be sure, there have been many great leaps in humanity's understanding of the world facilitated by the research enterprise. And scientists have certainly made ground-shaking discoveries throughout history. But by and large, scientific progress is made by the millimeter, not the kilometer.

Science provides the stability that the world so desperately needs right now. Revealing the truths underlying biology, chemistry, astronomy, physics, and other aspects of our universe must remain unimpeded by the turbulence that may surround us. And those truths can serve as antidotes to the misinformation that has become a regrettable constant in our modern consciousness.

While I cannot predict what might happen in the time between when I pen this editorial and when you read it, I can forecast that the quest for truth, which rests at the foundation of the human experience, will continue to propel our species forward. As long as a sufficient number of us stand up, repeatedly and consistently, to voice the importance of science and fact, we can hope that the infrastructure designed to support research efforts will continue to do so. And although I can't be sure that the divisiveness that marks so much of our social and political discourse these days will ever be reckoned with and healed, I can't think of a better starting point to move forward into an increasingly uncertain future than a shared respect for and trust in science. ■

ANDRZEJ KRAUZE

Revealing the truths underlying biology, chemistry, astronomy, physics, and other aspects of our universe must remain unimpeded by the turbulence that may surround us. And those truths can serve as antidotes to the misinformation that has become a regrettable constant in our modern consciousness.

Editor-in-Chief
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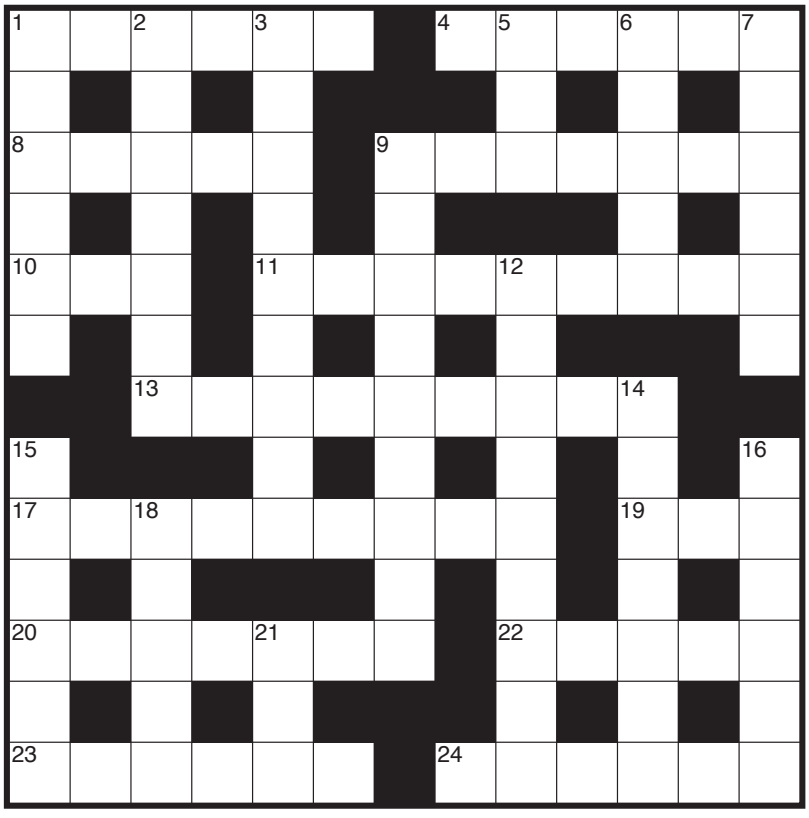
Speaking of Science



Note: The answer grid will include every letter of the alphabet.

BY EMILY COX AND HENRY RATHVON

ACROSS

1. *Quercus palustris* (2 wds.)
4. Edison: "The ___ of Menlo Park"
8. Amber, frankincense, or myrrh
9. Lab activity
10. Masticating malady, for short
11. Seed cone of a gymnosperm
13. Medulla oblongata's location (2 wds.)
17. Change, especially to a higher form
19. "Science Friday" network
20. Tropical grassy plain
22. Talc's place on the Mohs scale
23. Typically anadromous fish
24. Feature of Hale-Bopp?

DOWN

1. Mineral known as fool's gold
2. Rhinoplasty, informally (2 wds.)
3. Some who struggle with information retrieval
5. Measures of brightness?
6. The A in CAT scan
7. What amylase helps us do
9. Arachnid that may have urticating bristles
12. Collectible for Frohawk or Nabokov
14. 12-Down dependent on *Asclepias* plants
15. Period of little evolutionary change
16. Nuclear particle
18. The middle ear's incus
21. Opposite of paleo-

Answer key on page 5

I think it is important that we name these appropriately and we don't call these the South African variant or the UK variant. We need to use the names appropriately because we don't want to stigmatise where these variants have been identified.

—**Maria Van Kerkhove**, an infectious disease epidemiologist and the World Health Organization's COVID-19 technical lead, during a press conference partially focused on emerging, highly infections variants of SARS-CoV-2 (January 5)

This is a health crisis of epic proportions. I am more troubled than ever before, and in part, my concern is rooted in the reality that it will take so much more for us to slow the spread given the high rate of community spread.

—Director of the Los Angeles County Department of Public Health **Barbara Ferrer**, in a statement released amid a steep increase in severe COVID-19 cases in the city (January 6)

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May I Speak to a Manager?

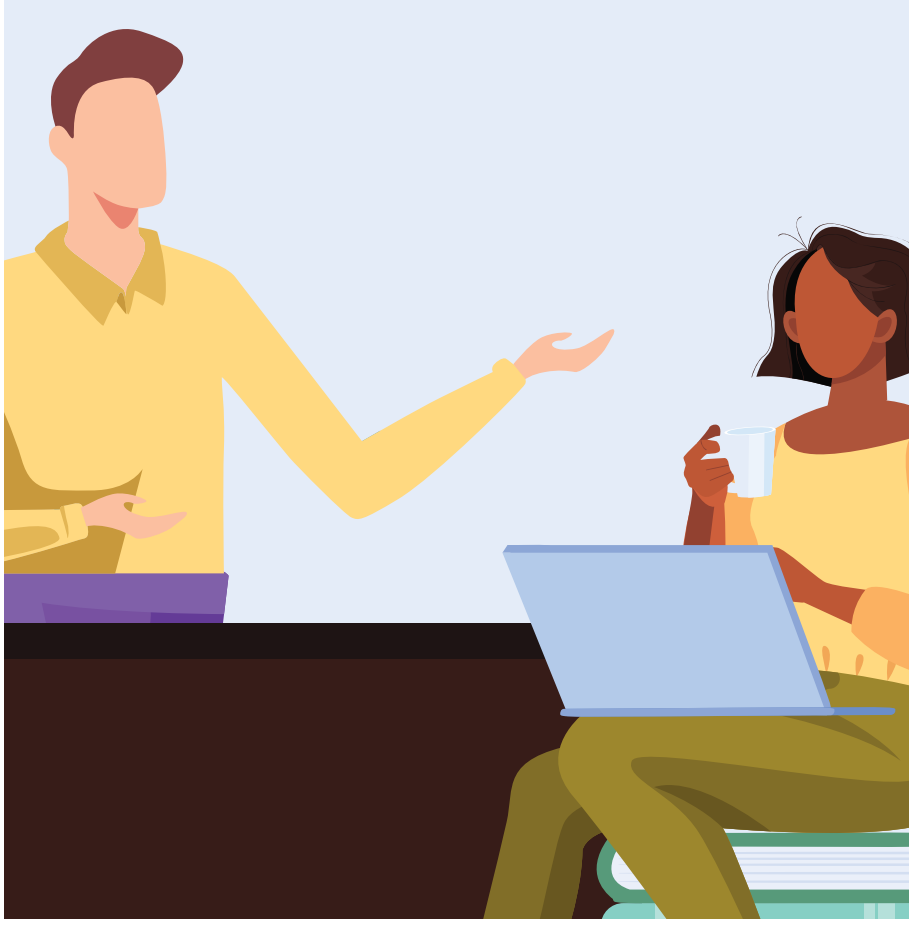
Rethinking professional expectations for professors and graduate students

BY ANGELA E. BOAG & NATHALIE ISABELLE CHARDON

Professors are managers. They manage projects, classes, and often personnel, many of whom are graduate students. Some advisor/advisee relationships are positive and very productive. Others can be downright destructive.

Unsurprisingly, this variability produces some graduates who are confident critical thinkers, while others have their educations and careers derailed by a shockingly common poor professional relationship. Witnessing wildly different advising experiences while in graduate school, we talked with a dozen university colleagues and friends in North America and Europe to identify common problems as well as potential solutions. Through informal conversations, we gathered viewpoints from current and former graduate students of diverse races, ethnicities, and genders across academic disciplines.

Poor graduate student/advisor experiences can largely be chalked up to one thing: there are few specific expectations or standards for professors when it comes to managing their graduate students. This needs to change. Adopting basic principles for good management will enhance productivity, promote higher-impact research, and boost graduate student mental health.



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Some people we spoke with had wonderful advisor/advisee relationships, but others had conflicts, which at best held these students back from making meaningful progress toward their degrees, and at worst caused them to leave their programs. Some students were suffocated by extreme micromanagement. Others had advisors who did not even ask what their research interests were when they started their graduate program, let alone give them any guidance on navigating program requirements. Regardless of the personalities involved, a productive professional relationship can be established by setting clear expectations for both professor and student from the beginning and creating formal opportunities to discuss and revise these expectations.

Our colleagues and friends also broadly agreed that poor management is partly responsible for the mental health crisis plaguing graduate students, who are six times as likely to experience depression and anxiety compared with the general population. Unsurprisingly, feeling valued at work leads to better physical and mental health. Widespread belief by graduate students that they are imposters, undeserving of their achievements, adds to this problem, as it makes some students less willing to reach out to their advisors for help for fear of being perceived as incompetent.

Institutional changes focused on improving advisor/advisee relationships, such as taking graduate students' ratings of their advisor into account during professional reviews, could also help to incentivize faculty to develop their managerial skills. Highly innovative companies, including Google, recognize the benefits of good management. Executives at the internet search giant found that effective managers create a more productive work environment. We combined key managerial behaviors that

Google identified with recommendations from graduate students we spoke with to create a list of five concrete ways to improve advisor/advisee relationships.

Poor graduate student/advisor experiences can largely be chalked up to one thing: there are few specific expectations or standards for professors when it comes to managing their graduate students.

1. **Set expectations:** Communicate research and managerial expectations between students and advisors at the start of the graduate program, and continuously check in to adjust these expectations.

2. **Track performance:** Set a meeting schedule to track project accomplishments and goals.

3. **Coach students:** Graduate students, regardless of work style and personality, will thrive with advisors who coach and challenge them, as well as express interest in their success and personal well-being.

4. **Avoid micromanaging:** Professors must strike a balance between providing advice, showing students they trust them, and empowering them to develop as independent researchers who may soon be managing their own research groups.

5. **Foster a positive environment:** Professors and students should endeavor to create an environment where everyone understands that failures are inevitable and OK. Framing academic challenges in a more positive way allows students to feel comfortable discussing issues early and often, enhancing mental health and research productivity.

Many graduate students inherit the advising style that they experienced, so effective and communicative graduate student/advisor relationships would go a long way in producing successful managers who can go on to propagate future generations of scientists. These skills will also benefit the roughly half of US science and engineering PhDs now employed by the private sector, which tends to value project management skills more than the academic sector traditionally has. Whatever young scientists' futures hold, they will fare better if they are supported by their mentor and trained to be a capable advisor themselves. ■

Angela E. Boag is a policy advisor for climate change, forest health, and energy at the Colorado Department of Natural Resources. Nathalie Isabelle Chardon is a postdoctoral researcher in the Community Ecology Unit at the WSL Institute for Snow and Avalanche Research SLF in Switzerland.

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ONDEMAND | Genetic and Spatial Heterogeneity in Human Papillomavirus-Associated Oropharyngeal Cancer

Head and neck cancers arising from the upper aerodigestive tract are the sixth leading cause of cancer-related mortality, with over 550,000 new cases per year worldwide. Though it continues to be prevalent, the etiology of oropharyngeal cancer (OPC; cancer of the tonsil and base of tongue) has completely changed in the last 30 years. Now, human papillomavirus (HPV) is the leading cause of OPC. While patients with viral OPC tend to be younger and have a superior responses to treatment and better prognoses compared with non-viral-OPC patients, the biological differences between these cancers are not well understood due to the paucity of genomic data in the viral-OPC population. The underlying genetic drivers of diverse cancer cell phenotypes, or "tumoral heterogeneity," affect clinical outcomes but have not been studied in detail.

In this webinar sponsored by 10x Genomics, Joseph Powell discusses how heterogeneous subpopulations of HPV+ head and neck cancer cells drive unique disease states, cell–cell interactions, and microenvironment dynamics, and have implications for cancer behavior, metastasis, and response to treatment.

JOSEPH POWELL, PHD
Associate Professor
Garvan Institute of Medical Research

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- How to study heterogeneous subpopulations of HPV+ cancer cells using single-cell and spatial techniques

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ONDEMAND | How Cancer Evades the Immune System

The body's first line of defense against cancer is the immune system. Yet many tumors evade the immune system and even recruit key immune cells to aid in tumor development. In this webinar, brought to you by *The Scientist* and sponsored by 10x Genomics and Codex DNA, Chuanhui Han discusses how cancer avoids immune system attack after radiation treatment, and Vineet Gupta explores how cancer tricks immune myeloid cells into promoting tumor growth. The speakers also review therapeutic approaches for preventing cancer's manipulation of the immune system.

CHUANHUI HAN, PHD
Postdoctoral Researcher
Laboratory of Yang-Xin Fu, MD, PhD
UT Southwestern Medical Center

VINEET GUPTA, PHD
The Charles Arthur Weaver Chair of Cancer Research
Vice Chair for Innovation, Department of Internal Medicine
Director, Drug Discovery Center
Rush University Medical Center

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TOPICS COVERED

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- Integrin activation as a novel therapeutic strategy against cancer

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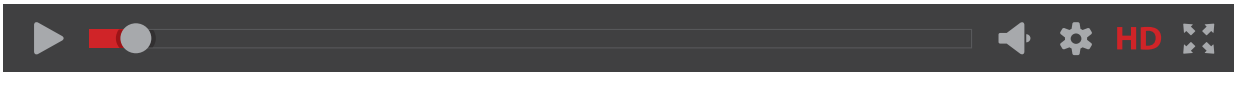

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ONDEMAND | Unpacking the Genetic Contribution of Glia to Parkinson's Disease

Parkinson's disease is the second most common neurodegenerative disorder. It is characterized by misfolded alpha-synuclein deposits and dopaminergic neuron death, which lead to progressive motor impairment and disability. Despite extensive efforts, there are no disease-modifying therapies available for Parkinson's disease or related "alpha-synucleinopathies." Glia may represent a source of untapped therapeutic potential.

In this webinar sponsored by BioLegend, Abby Olsen, Associate Neurologist at Brigham and Women's Hospital, discusses how an innovative *Drosophila* model helps explore the genetic contribution of glia to Parkinson's disease pathogenesis. She reviews how forward genetic screens identify novel glial genes and potential therapeutic targets for downstream investigation in mammalian systems and patients.

ABBY OLSEN, MD, PHD
Associate Neurologist
Brigham and Women's Hospital
Instructor in Neurology
Harvard Medical School

ORIGINALLY AIRED THURSDAY, SEPTEMBER 17, 2020

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TOPICS COVERED

- A *Drosophila* model of neurodegenerative alpha-synucleinopathies
- The role of alpha-synuclein in glia
- The unique transcriptional signature of alpha-synuclein in glia in Parkinson's disease
- The pathogenic effects and mechanisms of Parkinson's disease candidate genes when expressed in the glia
- Genetic screens to identify novel glial genes and potential therapeutic targets

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TS WEBINARS

ONDEMAND | T Cells: A New Hope for Lasting Protection Against SARS-CoV-2

Many immunologists are looking at T cells to understand the potential for lasting immunity to SARS-CoV-2. In this multisponsored webinar from *The Scientist*, Alessandro Sette and Shane Crotty will present the latest findings in T cell function following SARS-CoV-2 infection and the implications for vaccine development and lasting immune memory.

ALESSANDRO SETTE, PHD
Professor
Center for Autoimmunity and Inflammation
Center for Infectious Disease and Vaccine Research
La Jolla Institute for Immunology

SHANE CROTTY, PHD
Professor
Center for Infectious Disease and Vaccine Research
La Jolla Institute for Immunology

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TOPICS COVERED

- $CD4^+$ and $CD8^+$ T cell recognition of SARS-CoV-2 sequences in unexposed individuals and potential cross-reactivity with common cold coronaviruses
- Implications of the T cell response against SARS-CoV-2 for vaccine design and viral escape
- How the nature of the acute immune response correlates with COVID-19 severity
- Factors affecting SARS-CoV-2 immune response duration and memory

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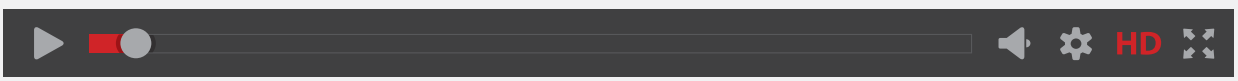


Notebook

Radioactive Bees

A few years ago, on one of her first visits to Chernobyl, Katherine Raines went to the Red Forest, a radioactive cemetery of pine trees scorched by the nuclear accident in 1986. She was curious to see if there were bees living in the area. Research on the effect of chronic exposure to ionizing radiation on insects is limited, and some of the findings are controversial, but most experts support the idea that bees and other invertebrates are relatively resilient to radioactive stress.

Raines, a radioecologist at the University of Stirling in Scotland, didn't spend long in that forest. In one spot there, her personal radiation dosimeter measured an environmental level of ionizing radiation of 200 microsieverts (μSv) per hour; more than a few hours of that exposure could have increased her cancer risk. But even during that brief visit, she did see bees. Whether they were living there or just visiting, Raines says, is hard to tell.

Back in the UK, Raines and colleagues recreated the same levels of radiation in a specialized facility. Boxes each containing a bumble bee colony made up of a queen, workers, and brood were placed at different distances from a radiation source, creating a gradient where bees in each box received a fairly steady dose of between 20 and 3,000 micrograys (μGy) per hour. (The two kinds of units, sieverts and grays, are essentially equivalent measures of the amount of exposure to radiation; sieverts factor in the type of radiation and account for the sensitivity of the exposed tissue. Bees at the site Raines visited in the Red Forest would experience around 200 μGy per hour.) The bees stayed in their artificial homes for four weeks before being moved outdoors into the university gardens for around one month, until the colonies were no longer viable—that is, once the queen had died and only a few workers remained.

The limited lab studies previously carried out by other groups had suggested that bees and other insects should be safe below 400 μGy per hour. So, Raines says, she was

GETTING A BUZZ: Researchers studied how radiation might affect bumble bees like this one at Chernobyl.

COURTESY OF KATHERINE RAINES

shocked when she found that even those colonies exposed to lower rates showed signs of a negative effect of radiation, especially on reproduction. Bumble bee colonies experiencing just 100 µGy per hour, for example, had reduced their production of queens by almost half, dramatically impairing the chances of successfully founding new colonies. According to the study, the overall effect was stronger than the one-fourth reduction observed in colonies exposed to a popular pesticide.

Bumble bee colonies experiencing just 100 µGy per hour had reduced their production of queens by almost half.

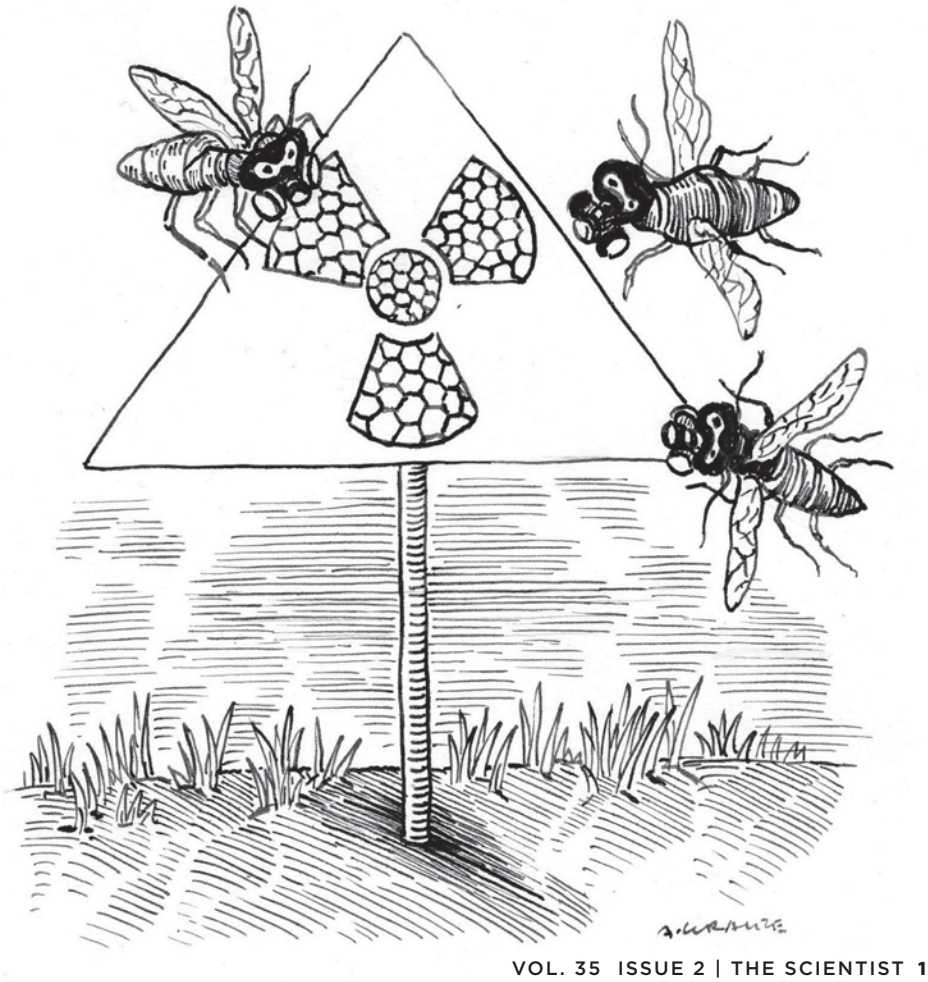
This work "sheds new light on the importance of chronic low-dose radiation exposure in a nonmodel species [with] profound relevance for the natural world," says Timothy Mousseau, an ecological geneticist at the University of South Carolina who was not involved in this research. But he adds that it is hard to determine how some of these results, based on experimental manipulations in an artificial setting, can translate "to what's actually going on in Chernobyl" for these important pollinators.

Mousseau and his colleague Anders Pape Møller (now at CNRS in France) have been doing field studies since 2000 to assess the abundance of wildlife populations living in the Chernobyl Exclusion Zone (CEZ), a 2,600 square-kilometer area surrounding the nuclear power plant. Their results have shown a negative correlation between radiation levels—which vary a great deal within the zone—and wildlife abundance. Insects were no exception: the team observed fewer bumble bees in the most contaminated areas, a relationship that held even within a range of extremely low radiation levels (from 0.01 to 1 µGy per hour).

Those studies have been criticized, partly over the accuracy of their estimations of radiation levels. Mousseau and Møller have collaborated with some of their critics to reanalyze some of their data, and maintain that there has been wildlife reduction in the CEZ due to radiation. But Jim Smith, an environmental scientist at the University of Portsmouth in the UK, is one of several scientists who still has doubts about the studies, telling *The Scientist* that the observations don't align with findings from other surveys in the region. For example, Smith, who has been visiting Chernobyl since the 1990s but is not involved in Raines's or Mousseau's work, failed to find evidence that either the abundance or the diversity of aquatic insects and other macroinvertebrates was reduced by radiation in any of the eight natural lakes that he and his colleagues analyzed in the CEZ in 2011, despite measuring external dose rates of between 0.1 and 30 µGy per hour.

Smith—who, along with Raines, belongs to a UK research program partially funded by the Environment Agency and its safe disposal contractor, Radioactive Waste Management—says that Raines's bee study provides "interesting new data on a species that hasn't really been studied [and] that is potentially more radiosensitive than we thought." But he is skeptical about the wider relevance of studying the effect of radiation levels rarely encountered in nature. Chernobyl's radiation levels are a consequence of "the worst nuclear accident in history," he says. And even within the CEZ, very few spots reach the radiation levels explored in this paper.

Raines says that although she did not detect any negative effect in colonies exposed to less than 50 µGy per hour—close to 200 times what humans experience, on average, from natural radiation sources—she didn't have enough colonies at that level to conclude that bumble bees are unaffected. Her team had not designed its experiment to explore bees' responses to such low levels, having assumed they wouldn't see any



ANDRZEJ KRAUZE

effects below 400 μGy per hour. "I wish I had placed more colonies at lower dose rates," she says. She'd like to understand, for instance, how radioactive discharges from hospitals—which can fall in the realm of 5–10 μGy per hour in some areas in the UK, Raines says—might affect wildlife. "What is happening at those really low, but above-background, levels is definitely important."

Researchers who spoke to *The Scientist* about the study agree that further work is needed to conclusively demonstrate the effects of radiation on bumble bees. "Robust data on effects of radiations on wildlife are scarce, so it is important to perform these kinds of experiments to improve our knowledge in this particular field," Béatrice Gagnaire, an ecotoxicologist at the Institute for Radiological Protection and Nuclear Safety in France who did not participate in this study, writes in an email to *The Scientist*. "To my opinion, this kind of study should be firstly repeated, and if the experts reach a consensus on data robustness, they could be integrated in the revised statements," for instance, by the International Commission on Radiological Protection, an advisory committee whose recommendations for radiation safety inform regulatory authorities worldwide.

Raines is now gathering more data. The next stage of her research, she says, will be to look at the interaction between parasite load, which reduces longevity, and radiation exposure—both in lab-kept bees and in bees she sampled on one of her visits to deserted agricultural land around Chernobyl. "It would be ideal to directly relate lab and field [data]."

—Alejandra Manjarrez

Leaf Blanket

In 2008, a letter arrived at the Center for Ecological Research at Kyoto University in Japan from a volunteer guide at a nature preserve in northern Honshu, asking about a strange phenomenon he had observed in a vine there. Some of the leaves of *Schizopepon bryoniifolius*, a gourd known in Japanese as miyama-nigauri, curved downward, forming a cup around the vine's fruit, wrote the volunteer, Nobuyuki Nagaoka, a retired schoolteacher then around 80 years old, living near the foot of Mount Gassan. Did the scientists at the center know why?

Nobuyuki Nagaoka, now 91, has continued his observations of miyama-nigauri.

One of the center's researchers, Shoko Sakai, was designated to respond to the letter, and as she remembers it, it didn't spark much interest for her. Her focus is on tropical plants, she explains, and she wasn't familiar with the vine, which grows in temperate regions. The center once had a trainee who had studied the vine in the 1990s, but tragically, he had died in a traffic accident while on a research trip. Sakai wrote back to Nagaoka suggesting that the structure he'd observed "might be caused by some pest or insects or pathogens, but we don't know what the cause is." While she doesn't remember the details, Sakai says she probably also suggested he observe how widespread the phenomenon was, which might yield clues about what was going on.

A few years later, Nagaoka wrote back to report that he'd observed the development of the structures on multiple individual miyama-nigauri plants and at different sites, and he'd formed a hypothesis of what they were for: to protect the plant's flowers and fruit. Sakai took notice—"I was very impressed," she remembers. She wrote back suggesting Nagaoka try removing the leaves of some of the leaf cups to see what would happen. In 2016, he did so, and found that removing the leaves appeared to stunt the growth of the enclosed fruit.

The results seemed to confirm Nagaoka's idea, and Sakai decided to join him the following year to conduct more experiments. In summer 2017, Sakai flew from Kyoto to Yamagata prefecture and joined Nagaoka at the foot of Mount Gassan. At two sites, one at the base of the mountain and one on its cooler slopes, they placed thermometers near the flowering vines to monitor the temperature. Nagaoka had previously observed that the enclosures only formed in autumn, so the two returned to the sites in September, where they counted the numbers of immature fruits on a number of plants and then removed the enclosing leaves on some of the vines.

KEEPING WARM: Cupped leaves observed on vines in Japan may help protect the plants' fruit from the cold.

SAKAI SHOKO

SAKAI SHOKO

A RARE FIND: Observations of the new leaf formation were made by Nobuyuki Nagaoka, a retired schoolteacher living near the foot of Mount Gassan in Japan.

They left others with intact leaves as controls, and in a third group, they removed the leaves and instead sheltered the fruit in a paper bag. The following month, one of their coauthors collected the fruits from the experimental plants and sent them to Sakai, who found that the bare fruit was less likely to have matured than the fruit enclosed by leaves or bags (*Proc R Soc B*, 287:20201718, 2020).

In addition, the researchers found that the intact vine structures at the higher, cooler site sported wider leaves than those at the lower site. The team monitored the temperature inside one plant cup for four days and found that it was, on average, higher than the air near a fruit on the same plant that had been stripped of its enclosing leaves. The temperature difference was small but significant, and was greatest—up to 4.6 °C—at midday on sunny days. In their paper on the study, published in October with Nagaoka as first author, the researchers dubbed the leafy enclosure a "green greenhouse."

The observation that miyama-nigauri only produces the enclosures late in the flowering and fruiting season is particularly interesting, notes Atushi Ushimaru, a plant biologist at Kobe University, in an email to *The Scientist*, as it indicates that the vine "can plastically change flowering and seeding strategy along the season to maximize fitness." Ushimaru has collaborated with Sakai in the past but was not involved in the current study.

"I think this is a really cool and interesting study," says Nora Mitchell, who studies plant biology and evolution at the University of Wisconsin-Eau Claire and was not involved in the work. Researchers tend to think of the traits of a plant's reproductive parts and its leaves evolving somewhat separately from one another, she says, but "this paper was really interesting in tying together some of those reproductive and vegetative traits, and how they can interact." That said, it's not the first known instance of leaves assisting in reproduction. Plants such as dogwoods have colored leaves called bracts that, like petals, serve to attract pollinators to flowers.

In her current work, Sakai is investigating whether there is a connection between the leaf enclosures and another miyama-nigauri trait, one observed by Junichi Akimoto, the student who passed away in the 1990s. The vine comes in two sexes, male and hermaphrodite, and Akimoto had found that the proportion of individuals in each category varies with altitude, with fewer males growing at higher sites. Sakai is now working on finding out why. She posits that it may be tied to the fact that the plant cups are thicker at higher altitudes, which might prevent pollen from escaping, making it difficult for males to reproduce.

Nagaoka, now 91, has continued his observations of miyama-nigauri, Sakai says, and still sometimes sends her photos of the plant. "I was impressed very much by his love and enthusiastic attitude."

—Shawna Williams

Making the Most Out of the Ultraviolet Laser:
How BD Horizon Brilliant™ Ultraviolet Dyes Drive Discovery

Flow cytometry is a binary system, where cells are either positive or negative for a specific marker when using an appropriate fluorescent dye-conjugated antibody to detect a specific antigen. The parallel development of instrument hardware and dyes expands the number of overall parameters that can be analyzed in a single experimental run, with each additional parameter translating into an exponential increase in data acquisition capacity and research potential. Thanks to over 45 years of development, flow cytometry is now an integral protein analysis tool that enables scientists to delve progressively deeper into cellular characterization and profiling.

As the new millennium began, flow cytometry had reached a plateau, as scientists could not detect more than 18 parameters simultaneously. Around this time, Sirigen designed and optimized a novel class of dyes derived from the chemistry behind Alan Heeger, Alan MacDiarmid, and Hideki Shirakawa's Nobel Prize-winning discovery of conductive polymers. These new polymer-based dyes were groundbreaking and led initially to a series of direct and energy transfer tandem dyes excited by the violet laser.

Researchers from BD Biosciences recognized the untapped potential of the UV laser and decided to leverage Sirigen polymer dye technology to create a new family of dyes excited by UV wavelength lasers. Over three years, BD Biosciences developed seven BD Horizon Brilliant™ Ultraviolet (BUV) Dyes. These dyes were designed and optimized to work with the 355-nm UV laser rather than the 375-nm

9-Color Panel on a 3-Laser Instrument

Laser	Marker	Fluorochrome
Violet 405 nm	CD24	BV421
	CD27	BV480
	CD19	BV605
	CD20	BV786
Blue 488 nm	IgD	BB515
	CD38	PE
	CD3	PerCP-Cy5.5
	IgG	PE-Cy7
Red 640 nm	IgM	APC

Spread Matrix

Laser	Fluorochrome	BV421	BV480	BV605	BV786	BB515	PE	PerCP-Cy5.5	PE-Cy7	APC
Violet 405 nm	BV421									
	BV480									
	BV605									
	BV786									
Blue 488 nm	BB515									
	PE									
	PerCP-Cy5.5									
	PE-Cy7									
Red 640 nm	APC									

9-Color Panel on a 5-Laser Instrument

Laser	Marker	Fluorochrome
UV 355 nm	CD19	BUV395
	CD3	BUV496
	IgD	BUV615
Violet 405 nm	CD24	BV421
	CD20	BV480
Blue 488 nm	CD27	BB515
Yellow-Green 561 nm	CD38	PE
	IgG	PE-Cy7
Red 640 nm	IgM	APC

Spread Matrix

Laser	Fluorochrome	BUV395	BUV496	BUV615	BV421	BV480	BB515	PE	PE-Cy7	APC
UV 355 nm	BUV395									
	BUV496									
	BUV615									
Violet 405 nm	BV421									
	BV480									
Blue 488 nm	BB515									
Yellow-Green 561 nm	PE									
	PE-Cy7									
Red 640 nm	APC									

Figure 1: As compared to a 3-laser instrument configuration, designing a 9-color panel on a 5-laser instrument is simplified by a more balanced fluorochrome distribution and reduced number of challenging fluorochrome combinations, represented as yellow and red boxes in the spread matrices.

BD Horizon Brilliant™ Blue Reagents **(BB)**
BD Horizon Brilliant™ Ultraviolet Reagents **(BUV)**
BD Horizon Brilliant Violet™ Reagents **(BV)**

laser to minimize potential cross-excitation of the violet dyes. BUV dyes have progressively changed flow cytometry while addressing several unmet needs for the scientific community.

Brilliant dyes help build brilliant panels

Before the development of BUV dyes, many 5-laser instruments came equipped with a 375-nm ultraviolet (UV) laser in addition to the four common violet, blue, yellow-green (YG) and red lasers. However, scientists used the UV laser almost exclusively to detect DNA-binding dyes such as DAPI and Hoechst in cell viability and cell cycle studies or to detect hematopoietic side populations. This configuration enabled detection of 16 phenotypic markers plus two functional dyes, but moving beyond that was difficult.

Scientists who need to design complex multicolor panels are often forcibly guided by reagent availability limitations rather than proper panel design practices. The first two BUV dyes (BUV395 and BUV737) conjugated to monoclonal antibodies increased the choice of available reagents while still enabling the simultaneous detection of 18 phenotypic markers. However, designing 18-color panels remained challenging, especially because of visible spectrum crowding (six violet detectors and five YG detectors) and the use of high spillover fluorochromes such as PE-Cy5 and PE-Cy5.5.

The development of two additional BUV dyes (BUV563 and BUV661) did not increase the number of detectable parameters but rather offered an alternative to PE-Cy5 and PE-Cy5.5, easing visible spectrum crowding, providing less spillover and enabling a more balanced distribution of fluorochromes across the five lasers.

The addition of three more dyes (BUV496, BUV615 and BUV805) to the BUV dye family, together with the development of higher capability flow cytometers able to detect up to 50 parameters, eventually allowed scientists to break the 18-parameter barrier. This opened the way to high-parameter flow cytometry, enabling, for the first time, the detection of up to 28 colors via conventional flow cytometry when BUV dyes were used in combination with other new dyes. The exponential and sudden increase in resolution power enabled cell characterization at an unprecedented depth. Moreover, these three dyes also facilitated a more balanced fluorochrome distribution across the five lasers (Figure 1). The resulting reduction in spillover not only made complex panels possible, it also simplified the process of designing less complex panels.

To further complement the BUV dyes and provide customers with panel design flexibility and expanded flow cytometry capabilities, the scientists at BD created BD OptiBuild™ Reagents, leveraging a groundbreaking technology to produce on-demand conjugations. This resulted in the rapid expansion of BUV dye-conjugated reagents and products to support the community's ever-evolving research needs.

Making UV mainstream

The rapid expansion of both the BUV fluorochromes and the BUV reagent portfolio established the UV laser as indispensable for high-parameter flow cytometry panels run using instruments such as BD FACSymphony™ A3 and A5 Cell Analyzers and the BD FACSymphony™ S6 Cell Sorter. Today, any high-parameter conventional flow cytometer on the market is equipped with an UV laser and relies on BUV dyes.

The ultraviolet laser and BUV dyes also play a critical role in the development and adoption of spectral flow cytometry today, enabling the simultaneous detection of over 40 parameters. With the evolving advancement of spectral flow cytometry, there are clear innovation opportunities in the BUV dyes (Figure 2) and BD Biosciences is uniquely positioned to continue to lead the dye revolution with its line of BD Horizon Brilliant™ Ultraviolet and BD OptiBuild™ Reagents.

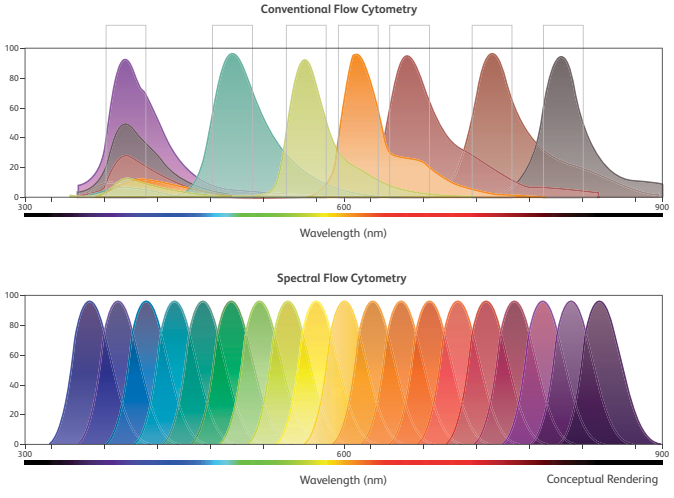


Figure 2: The number of detectable fluorochromes per laser in conventional flow cytometry is limited by the capture of the emission peak. Spectral flow cytometry distinguishes fluorochromes based on full spectrum signatures, thus enabling detection of more fluorochromes per laser.

For more information on the UV laser, BUV dyes, and the increased depth of biology that they provide for both conventional and spectral flow cytometry, please visit **bdbiosciences.com/buv**

REEF RESTORATION ON HYPERDRIVE

Novel technologies establish a new paradigm for global coral reef restoration, with in situ spawning of mature, environmentally resilient corals in five years instead of decades.

BY HANNA R. KOCH, ERINN MULLER, AND MICHAEL P. CROSBY

It was the seventh night after the August full moon, the peak spawning window predicted for the mountainous star coral. We loaded the boat and headed out to a coral reef near Summerland Key, Florida, around sunset. As we donned our scuba gear and jumped in, the sun dipped below the horizon.

We'd been monitoring this site for three years now, watching and waiting for signs that the restored corals we'd planted in 2015 had achieved the next critical developmental milestone in their life cycle—sexual reproduction. In 2018, a storm prevented us from monitoring the corals; in 2019, the site was hit hard by the deadly stony coral tissue loss disease (SCTLD) outbreak, and we did not witness any spawning activity. Last year, however, a preliminary investigation provided reason for hope: we confirmed that the corals were sexually mature in the weeks prior to the predicted spawning window. This was the first indication that this year was going to be momentous, and the countdown began.

We began monitoring the corals around 9:30 PM that August night. We swam in continuous loops to check dozens of the hundreds of corals that in preceding years we had "outplanted," placed onto the dying reef after raising them in our land-based spawning and nursery facility at Mote Marine Laboratory's Elizabeth Moore International Center for Coral Reef Research & Restoration on Summerland Key. Specifically, we were looking to see if any of our outplanted mountainous star corals (*Orbicella faveolata*) had "set"—shifted the pink-orange bundles of eggs and sperm into the mouths of the polyps, where they are visible to the naked eye. Once we saw the gamete bundles in position for release, we'd know that spawning was imminent.

The previous night, our team had observed that a single colony of this mounding coral species had set around 11:00 PM, so that became our target time for expecting setting in subsequent nights. On this night, when the hour came and went, we began to get nervous. We were frantically checking all outplants, over and over. Finally, at 11:05 PM, we heard the signal: *RAP! RAP! RAP!* Someone was smacking their dive light against their tank. We sped through the darkness and converged at the source of the sound. Mote biologist Sarah Hamlyn was hovering over a large coral head and excitedly pointing at three adjacent colonies clearly setting. We all hung there, motionless in the water, with eyes peeled and cameras in hand, for what seemed like an eternity. Then, at 11:15 PM, the polyps erupted, ejecting thousands of tiny pink gamete bundles into the water around us. The colorful spheres hovered momentarily before gracefully floating upwards to the ocean surface in an ascent that resembled reverse snowfall.

Just as quickly as it began, it ended; the whole show lasted less than a minute. We erupted into a quick burst of bubbled screams before peeling off to go check on the other outplants. That night, we observed nine different outplants across three different coral heads spawn within a 10-minute window. Given that there were only five divers with a short window of opportunity to observe spawning, and the fact that we have hundreds of outplants dispersed across numerous coral heads, it's likely that these nine were but a few of the many restored corals that spawned that night. The dive culminated in more underwater cheers, hugs, and dancing in the celebratory, electric-blue glow of bioluminescent plankton.

Apart from being a significant institution-wide achievement for us, these observations provide hope for the future of coral reefs around the world. For several decades, researchers have documented dramatic declines in living coral cover across the globe. Florida's Coral Reef, for example, has lost more than 90 percent of its corals since the 1960s, and Australia's Great Barrier Reef has lost at least 50 percent of its corals just in the last few decades. Numerous issues including disease outbreaks, habitat degradation, and increasing ocean temperature and acidification are to blame. In most cases, there is little evidence of population recovery after major disturbance events. But restoring reefs by outplanting genetically diverse, stress-tolerant corals on a large scale has rarely been attempted. That night last August, we witnessed the first corals of any slow-growing, massive or mounding species to have produced gametes and spawned after being outplanted back to the wild. And thanks to our novel strategy of planting many little coral colonies in proximity on the same coral head, they did so in just five years, far less than the decades it may take a wild colony or a single outplant to mature.

Devastating as it is to witness 50- to 100-year-old corals dying from disease or bleaching, the conclusion that you can't replace a 50-year-old coral in a decade may no longer be true for these massive, slow-growing, reef-building species, providing hope that coral restoration efforts can help save one of the world's most threatened ecosystems.

The colorful spheres hovered momentarily before gracefully floating upwards to the ocean surface in an ascent that resembled reverse snowfall.

Coral restoration comes to the fore

In 1995, founders of the US and International Coral Reef Initiatives highlighted implementation of local and national level coral reef restoration as a high priority to achieve global impacts for coral conservation.[1] Nevertheless, the scientific community has been slow to take up the challenge, and researchers and conservationists continue to debate the efficacy of coral restoration. As recently as 2014, major scientific reports[2] documenting the devastating decline in corals over the past 40 years rarely identified coral restoration as a tool for stemming coral reef decline. Although the US National Oceanic and Atmospheric Administration (NOAA) conducted three major coral reef repairs between 1997 and 2002 in response to large vessel groundings on coral

reefs in the Florida Keys and Puerto Rico,[3] the purpose of the repairs was to stabilize the reef structure and mitigate localized biological damage directly caused by the grounding, not to recover corals lost from decades of local and global threats.

Only in the past decade have researchers begun to develop science-based coral restoration strategies in earnest.[4] Scientists and practitioners around the world have now reported more than 250 coral restoration case studies,[5] involving hundreds of thousands of outplanted corals. The majority of projects occur in the US, but restoration efforts are now increasing in other parts of the world, including the Great Barrier Reef, in response to recent major coral mortality events.

Reef-building corals come in all different shapes, colors, and sizes. These species display wide variation in growth rates, tolerance to thermal stress, and resistance or resilience to different diseases and pollutants, and many are threatened by anthropogenic disturbances, including rapid climate change. To date, most restoration efforts have focused on branching coral species that grow quickly, especially in a nursery setting, because they are relatively easy to propagate. This is done by repeatedly fragmenting the corals into pieces as a form of asexual reproduction to grow up many coral colonies. This process is similar to how one might propagate a plant in their home, where cuttings from one plant are used to create new individuals. Within a few months, branches of coral are snipped off the colony and transported to the reef, where they are secured by a degradable cable to masonry nails hammered into the dead reef or hardbottom substrate. Coral fragments are attached to the reef in arrays of five that all come from the same parent colony (that is, they have the same genotype), and within a year or two, they grow and fuse into a large, reproductively viable adult colony. Arrays are situated on the reef so that adjacent clusters are different genotypes, to maximize the potential for cross-fertilization when sexual reproduction occurs.

However, restoring the backbone of a reef requires massive, slow-growing species such as the boulder and brain corals. These species are much harder to propagate for restoration because they typically grow only a few millimeters a year and usually need to be brought into the lab to be cut, whereas branching corals can be easily fragmented in the field. For the last several years, we have been propagating and outplanting these species using novel techniques that expedite their growth and maturation.[6] We are now implementing these approaches on the reefs of the Florida Keys—and just in the nick of time. The reef system is currently experiencing the largest coral disease outbreak in reported history.[7]

Accelerating growth

The mountainous star coral (*O. faveolata*) is native to the Caribbean and western Atlantic, and is a foundational species that helps build the backbone of Florida's Coral Reef. Owing to severe declines in abundance over recent decades, it was listed as threatened under the Endangered Species Act in 2014. New coral offspring are failing to show up on reefs after annual reproductive events,[8] leading to degraded, low-density populations that cannot sustain themselves via sexual reproduction. This has pushed Florida's coral reefs into a state of functional extinction, no longer providing reef structure and critical habitat. When the devastating stony coral tissue loss disease (SCTLD), a contagious, waterborne disease caused by an unidentified pathogen, began spreading through the Keys in 2016, with mortality rates in excess of 90 percent for the most susceptible coral species,[9] it added insult to injury.

Over the past 10 years, we have outplanted more than 100,000 fast- and slow-growing coral colonies, including some 26,000 in 2020, to reefs throughout the Keys. We are currently propagating 17 species, including *O. faveolata*, while actively incorporating diverse stress-tolerant coral genotypes to impart resilience to disease, ocean warming, and acidification. Although it's possible that scientists could use genetic manipulation tools to design more-resilient corals than currently appear in nature,[10] we focus on using genetic varieties that already exist within the endemic species of the region, with the aim of achieving sexually mature, self-sustaining, species-rich, and genetically diverse coral reefs as quickly as possible.

Importantly, in addition to large-scale asexual propagation, we incorporate new sexually produced genotypes of both branching and boulder coral species into our restoration pipeline each year. This approach ensures that our coral gene pool used for restoration remains diverse. Through assisted sexual reproduction efforts, we produce new generations of coral offspring each year, grow them to six months or one year old, depending on the species, and then use the asexual technique of microfragmentation to more rapidly increase the amount of coral tissue for each genotype.

Only in the past decade have researchers begun to develop science-based coral restoration strategies in earnest.

While fragmentation with fast-growing branching corals is now commonplace, the field remains limited in its ability to regenerate massive, reef-building corals. To tackle this issue, we recently developed and began employing a new approach that combines microfragmentation of reef-building species and outplanting arrays of those microfragments onto dead coral heads. If employed systematically on a large scale, this strategy should accelerate reef recovery.[11,6]

In 2013, we obtained colonies of mountainous star coral previously rescued by the Florida Keys National Marine Sanctuary from a construction site in Key West. On Mote's Summerland Key campus, we fragmented the corals into pieces less than 1 cm in diameter and grew them for several months in our land-based nursery. Once the fragments reached around 3 cm, we outplanted them on patch

reefs—isolated groupings of coral that are in close proximity to each other but are physically separated by sand rings—off Cook Island near Newfound Harbor in the Lower Keys. In 2014, we outplanted dozens of arrays comprising seven genetically identical fragments; from 2015 to 2017, we outplanted dozens more with 20 fragments in each array. Because fragments within an array are clonal, when they grow and eventually come in contact, they recognize one another as "self" and fuse together, essentially "reskinning" the dead coral skeleton with living healthy tissue. Between two and three years after outplanting, all arrays fully fused, creating whole coral colonies ranging from 15 cm to 30 cm in diameter.

This accelerated development breakthrough of up to 50 times natural growth rates is critical because the time required for corals to reach sexual maturity is size-dependent, not age-dependent; colonies of the mountainous star coral have to reach 10–30 cm before they are able to sexually reproduce. Normally, it would take decades for this species to grow to this size in the wild. When we saw our outplants spawn that memorable August night, it demonstrated that these fused colonies had indeed reached sexual maturity in record time.

The importance of sexual reproduction

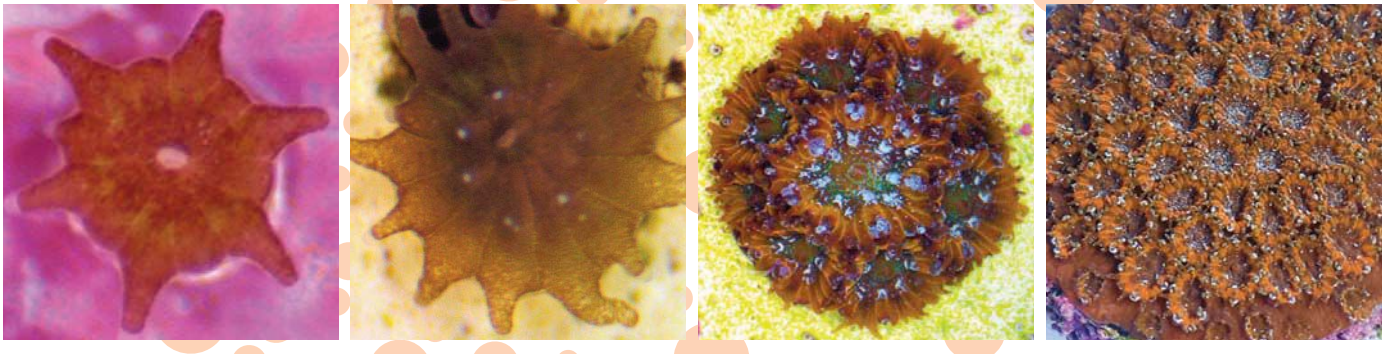
Across the animal kingdom, sexual reproduction is a source of genetic variation that is critical for population survival and long-term persistence, and corals are no exception. For restored coral populations to survive long into the future, they will need sex to withstand rapid environmental change and accelerate adaptive evolution. To achieve sexually reproducing coral populations is a challenge, not only because slow-growing species take a long time to reach sexual maturity, but because once they do, reproduction is a relatively rare event. Most reef-building corals are hermaphrodites, producing both male and female gametes, which are broadcast into the water column for external fertilization with gametes of nearby corals of the same species. Such mass synchronized spawning events typically occur just once a year, and acute stressors such as bleaching events, hurricanes, and disease outbreaks can arrest gamete development and prevent spawning altogether.

Last summer, we confirmed sexual maturity just days before the corals' expected 2020 spawning window, which typically occurs following the first full moon in August. Even before witnessing the spawning event that exciting August night, we knew that the corals were producing gametes. At the end of July, we took small core samples from a subset of outplants looking for the tell-tale pink-orange or "coral" color of the eggs. With the first few samples, we were unsure whether gametes were present, so we placed the cores in tubes to take back to the lab for further inspection. Then, on the second to last core, we found what we were looking for: a bright pink-orange spot. We used an underwater camera to take a macro shot and zoomed in on the image. There, without a doubt, was a string of mature eggs. Next to them—less obvious, but still visible—was a ribbon of sperm. These massive corals were ready to become parents in the wild. A true cause for celebration!

When the time came in August, not only did the corals in our study spawn during the predicted peak spawning window, but they also spawned with high synchrony. Spawning synchrony is critical to fertilization success, and having predictable spawning timing, both in terms of days after the full moon and minutes after sunset, means these outplants have developed reproductive rhythms consistent with wild colonies. Hence, they are capable of breeding with one another and with wild colonies, which should help to increase genetic diversity of coral offspring.

Witnessing our restored corals spawn was even more gratifying because these outplants survived a bleaching event in 2015,[12] a Category 4 hurricane (Irma) in 2017,[13] and the 2019 outbreak of SCTLD.[14] In fact, two of the outplants we saw spawn had multiple SCTLD lesions in the spring of 2019, were treated with antibiotics (administered in a custom medium via syringe on the border of disease lesions), and recovered.[15] This demonstrates that combining acute interventions such as antibiotics, which can help in the short term to prevent complete mortality, with resilience-based restoration strategies will work over the long term to help corals survive stressful environmental conditions.

LAB TO WILD: Baby mountainous star corals (6 weeks to 1 year old shown here, left to right) are grown in the lab at Mote Marine Laboratory and then outplanted to Florida reefs that suffer from disease, acidification, or other stressors.



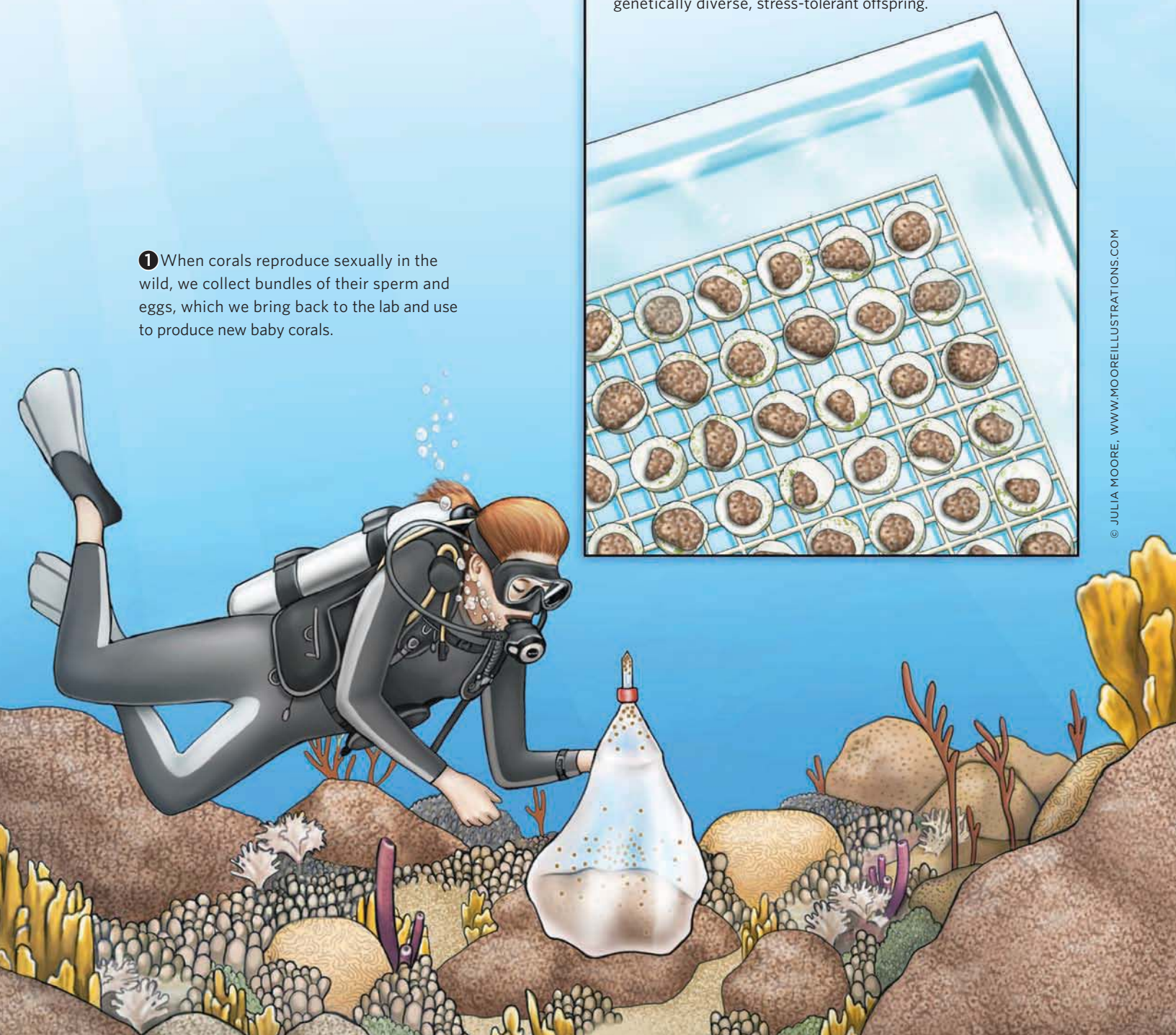
LYDIA WASMER, CODY ENGELSMA, HANNA KOCH, MOTE MARINE LABORATORY

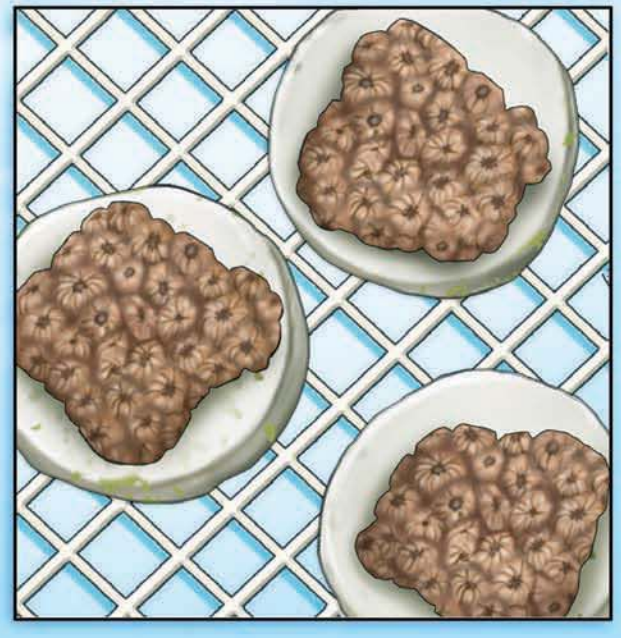
THE MICROFRAGMENTATION PROCESS

At Mote Marine Laboratory, our group has developed a new approach to restoring corals, depicted below. Already, we have seen new coral form across the dead skeleton of massive brain, boulder, star, and mounding coral structures in just one or two years, instead of the hundreds of years it might take a reef to regenerate on its own. Sexual reproduction is vital to the persistence of coral populations, but sexual maturity is size-dependent in reef corals, so expediting the growth of larger corals should support faster population and reef recovery.

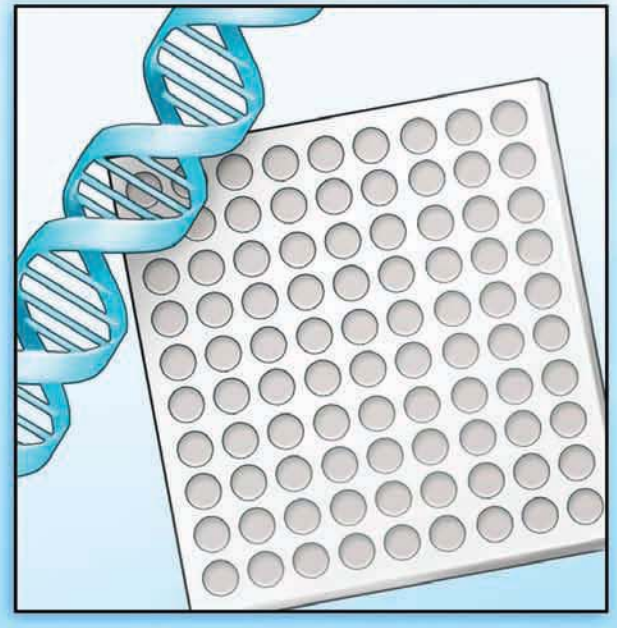
❶ When corals reproduce sexually in the wild, we collect bundles of their sperm and eggs, which we bring back to the lab and use to produce new baby corals.

❷ We also breed nursery-raised corals to produce new, genetically diverse, stress-tolerant offspring.



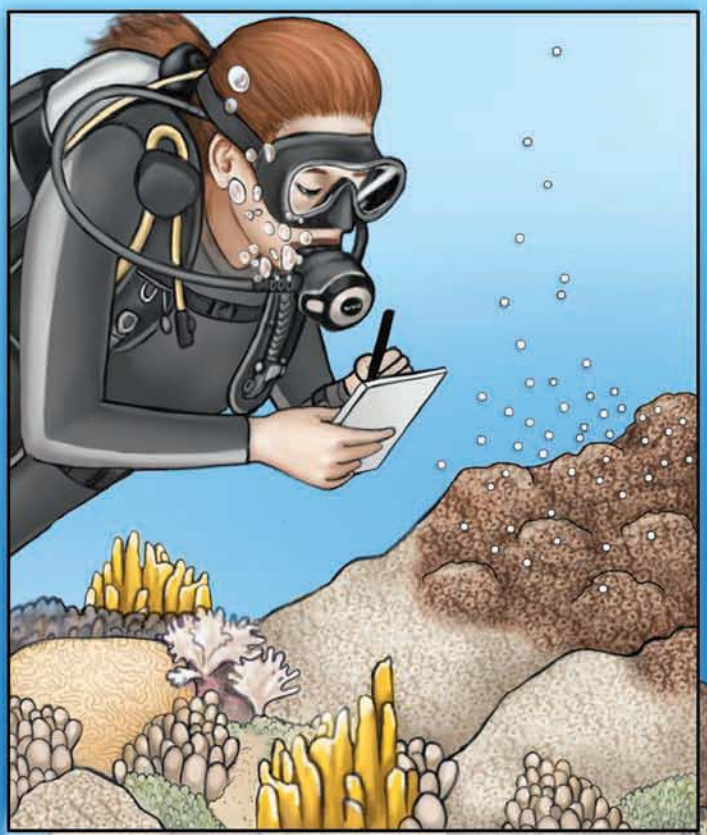


❸ We generate large numbers of corals asexually by microfragmenting the colonies to produce clones.

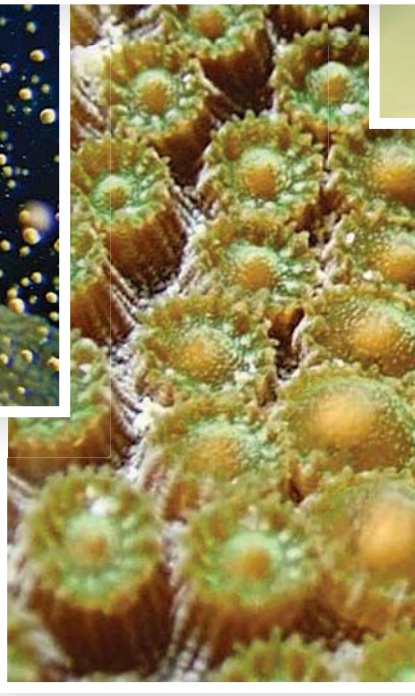


❹ We test coral genetic varieties for resilience to disease, climate change, and related stressors.

❺ We plant coral fragments representing different genetic varieties and species onto damaged reefs to support resilience-based, multi-species restoration.



❻ We monitor the outplanted corals as they grow, fuse together, and reach sexual maturity.



CORAL SPAWNING: We inspected the outplanted *O. faveolata* visually and used an underwater hand drill to extract small core samples (far right), which revealed strings of developed pink-orange eggs and ribbons of sperm. A couple of weeks later, the corals spawned, broadcasting the gametes into the ocean to produce the next generation of corals.

Only a handful of published cases of coral reef restoration projects document corals reaching sexual maturity, and all previous cases pertained to fast-growing corals known as acroporids (genus *Acropora*). This was the first documentation of a slow-growing, massive coral species spawning, and it did so in a similar timeframe as did outplanted acroporids.[16]

Once the outplants reach sexual maturity, their value extends beyond their ability to breed in the wild; the outplants can serve as a source of gametes for assisted sexual reproduction projects as part of continued research and restoration efforts. In some places, populations are so degraded and patchy that attaining access to an effective number of genetically diverse, sexually mature colonies can be challenging. Thus, sexually mature restored populations can become spawning hubs that contribute to both natural population recovery and managed breeding efforts on land.

The road ahead: Questions and hope

Despite our initial success, we still have many questions left to answer. For example, is bigger always better, or can we achieve the same outcome in less than five years and with smaller fused outplants? When it comes to disease susceptibility, research has shown that an increase in size can also mean an increased risk of disease.[17] With our restoration sites now a part of the SCTLD endemic zone, which stretches from north of West Palm Beach down the southeast coast of Florida and through the entirety of the Florida Keys, this is something worth considering. We are also waiting to determine when the four other slow-growing coral species that we've applied this outplanting methodology to will reach sexual maturity and are curious to know how outcomes may differ by species. We've already seen fusion among the replicate fragments for some of the other species and are excited to see when they will spawn in the coming years.

Finally, we will begin investigating the latter stages of the sexual cycle for these outplants, including confirming that new baby corals show up on the reef and contribute to the adult population. Ultimately, these processes are necessary for natural population recovery to take place. But that requires consideration of other mitigating factors such as water quality, suitable habitat availability, and ecosystem

DIVER: SARAH HAMLYN, MOTE MARINE LABORATORY

HANNA KOCH, MOTE MARINE LABORATORY

dynamics, including the presence of certain grazers and the prevalence of competitors.

For the best possible ecological outcomes, coral restoration should be combined with other measures such as habitat protection. Restoration strategies must also take into consideration the genetic consequences of their design and implementation. The methods with which practitioners select, rear, propagate, and manipulate corals for restoring degraded reefs will have consequences for the survivorship of outplants and resilience of restored populations. Understanding the genetic and demographic properties that influence the ability of populations to adapt to rapidly changing selective pressures will help practitioners design and implement optimal strategies. Finally, to mitigate the negative impacts of rapid climate change on coral reef ecosystems, carbon emissions need to be reduced.

Despite the challenges that lie ahead, there is reason for hope. For the first time in a long time, we have research that suggests the world's corals can recover from the devastation they've endured—with some help. Already, hundreds of millions of dollars are being devoted to coral reef restoration around the world. Now, we've demonstrated a way to successfully invest those funds: a microfragmentation-fusion approach that provides the basis for quickly restoring coral populations to a sexually mature, potentially self-sustaining state, fundamentally changing the paradigm for coral restoration science. ■

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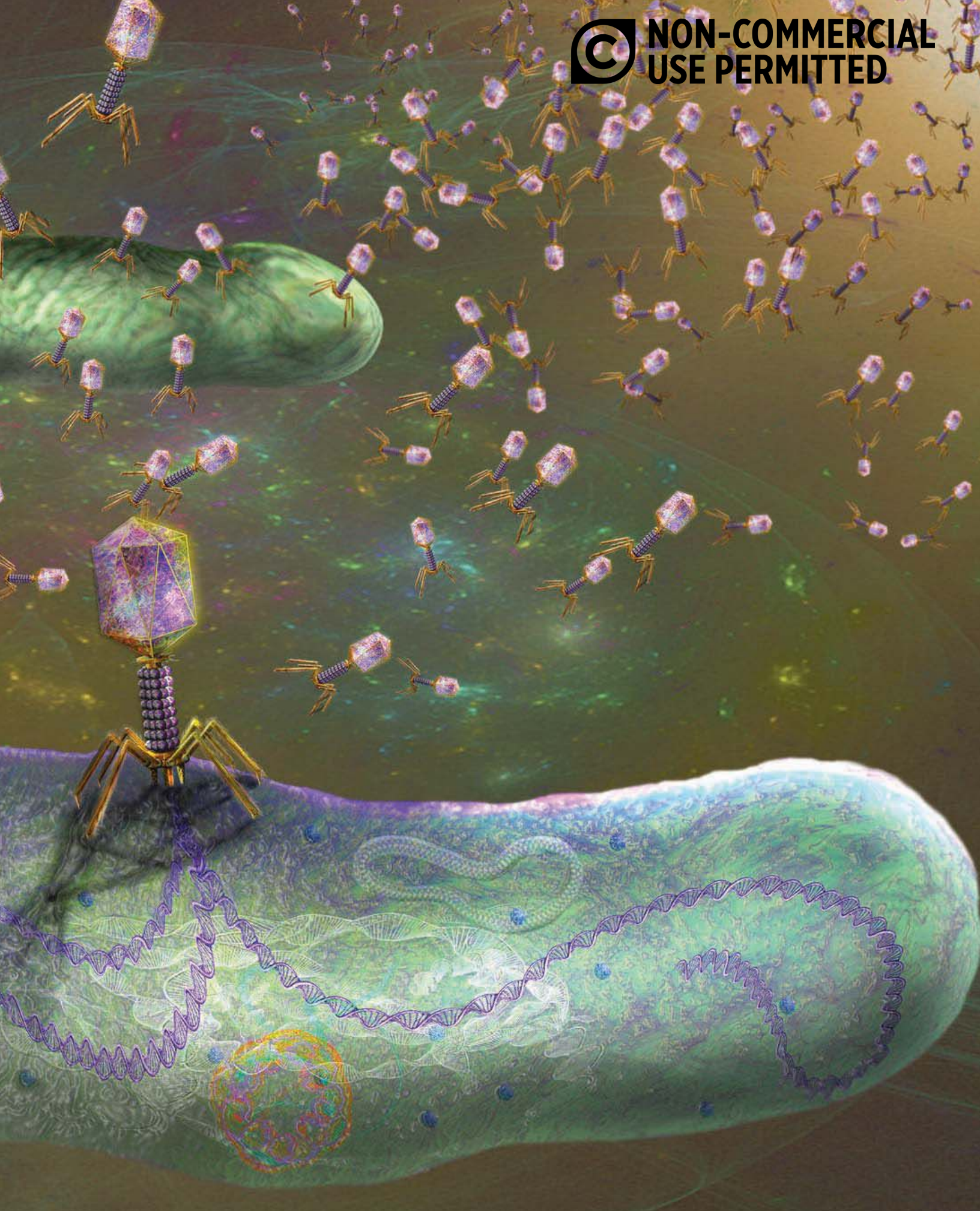
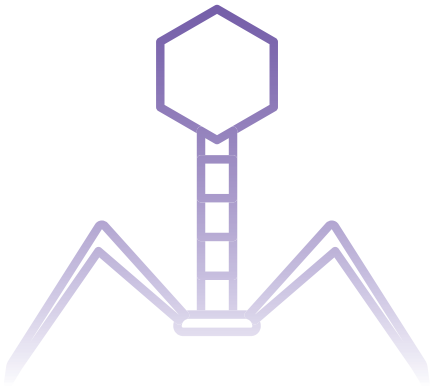
The Phages Within

Bacteria-infecting viruses affect the composition and behavior of microbes in the mammalian gut—and perhaps influence animals' health.

BY CATHERINE OFFORD





When microbiologist Breck Duerkop started his postdoc in 2009, he figured he'd be focusing on bacteria. After all, he'd joined the lab of microbiome researcher Lora Hooper at the University of Texas Southwestern Medical Center in Dallas to study host-pathogen interactions in the mammalian gut and was particularly interested in what causes some strains of normally harmless commensal bacteria, such as *Enterococcus faecalis*, to become dangerous, gut-dominating pathogens. He'd decided to explore the issue by giving germ-free mice a multidrug-resistant strain of *E. faecalis* that sometimes causes life-threatening infections in hospital patients, and analyzing how these bacteria express their genes in the mouse intestine.

Not long into the project, Duerkop noticed something else going on: some of the genes being expressed in *E. faecalis* weren't from the regular bacterial genome. Rather, they were from bacteriophages, bacteria-infecting viruses that, if they don't immediately hijack and kill the cells they infect, can sometimes incorporate their genetic material into the bacterial chromosome. These stowaway viruses, known as prophages while they're in the bacterial chromosome, may lie dormant for multiple bacterial generations, until certain environmental or other factors trigger their reactivation, at which point they begin replicating and behaving like infectious agents once again. (See illustration on opposite page.) Duerkop's data showed that the chromosome of the *E. faecalis* strain he was using contained seven of these prophages and that the bacteria were churning out virus particles with custom combinations of these prophage sequences during colonization of the mouse gut.

The presence of viruses in Duerkop's *E. faecalis* strain wasn't all that surprising. Natural predators of bacteria, bacteriophages are the most abundant biological entities on the planet, and in many fields, researchers take their presence for granted. "Nobody really was thinking about phages in the context of bacterial communities" in animal hosts, Duerkop says. "It would [have been] very easy to just look at it and say, 'Oh, there are some phage genes here. . . . Moving on.'" But he was curious about why *E. faecalis* would be copying and releasing them, rather than leaving the prophages asleep in its chromosome, while it was trying to establish itself in the mouse intestine.

Encouraged by Hooper, he put his original project on hold in order to dig deeper. To his surprise, he discovered that the *E. faecalis* strain, known as V583, seemed to be using its phages to gain a competitive advantage over related strains. Experiments with multiple *E. faecalis* strains in cell culture and in mice showed that the phage particles produced by the bacteria didn't harm other V583 cells, but infected and killed competing strains. Duerkop and his colleagues realized that, far from being background actors in the bacterial community, the phages "are important for colonization behavior" for this opportunistic pathogen.

Predation is just one type of phage-bacteria interaction taking place within the mammalian microbiome; many phages are capable of inserting their genomes into the bacterial chromosome.

The idea that a phage could play such a significant role in the development of the gut bacterial community was relatively novel when the team published its results in 2012.[1] Since then, "it's been pretty well established that phages can shape the assembly of microbial communities in the intestine, and that can influence the outcome on the host—either beneficially or detrimentally," says Duerkop, who now runs his own lab at the University of Colorado School of Medicine in Aurora. There's evidence that phages help bacteria share genetic material with one another, too, and may even interact directly with the mammalian immune system, an idea that Duerkop says would have had you "laughed out of a room" of immunologists just a few years ago.

Tipping the scales

Around the time that Duerkop was working on *E. faecalis* in Dallas, University of Oxford postdoc Pauline Scanlan was studying *Pseudomonas fluorescens*, a bacterial species that is abundant in the natural environment and is generally harmless to humans, although it's in the same genus as the important human pathogen *Pseudomonas aeruginosa*. Bacteria in this genus sometimes evolve what's known as a mucoid phenotype—that is, cells secrete large amounts of a compound called alginate, forming a protective goo around themselves. In *P. aeruginosa*, this goo can help the bacteria evade the mammalian immune system and antibiotics, and "when it crops up, it's not good news" for the patient, Scanlan says. She was curious about what causes a non-mucoid bacterial population to evolve into a mucoid one and had found previous research suggesting that the presence of bacteriophages could play a role. Other studies documented high densities of phages in mucus samples from the lungs of some cystic fibrosis patients with *P. aeruginosa* infections.

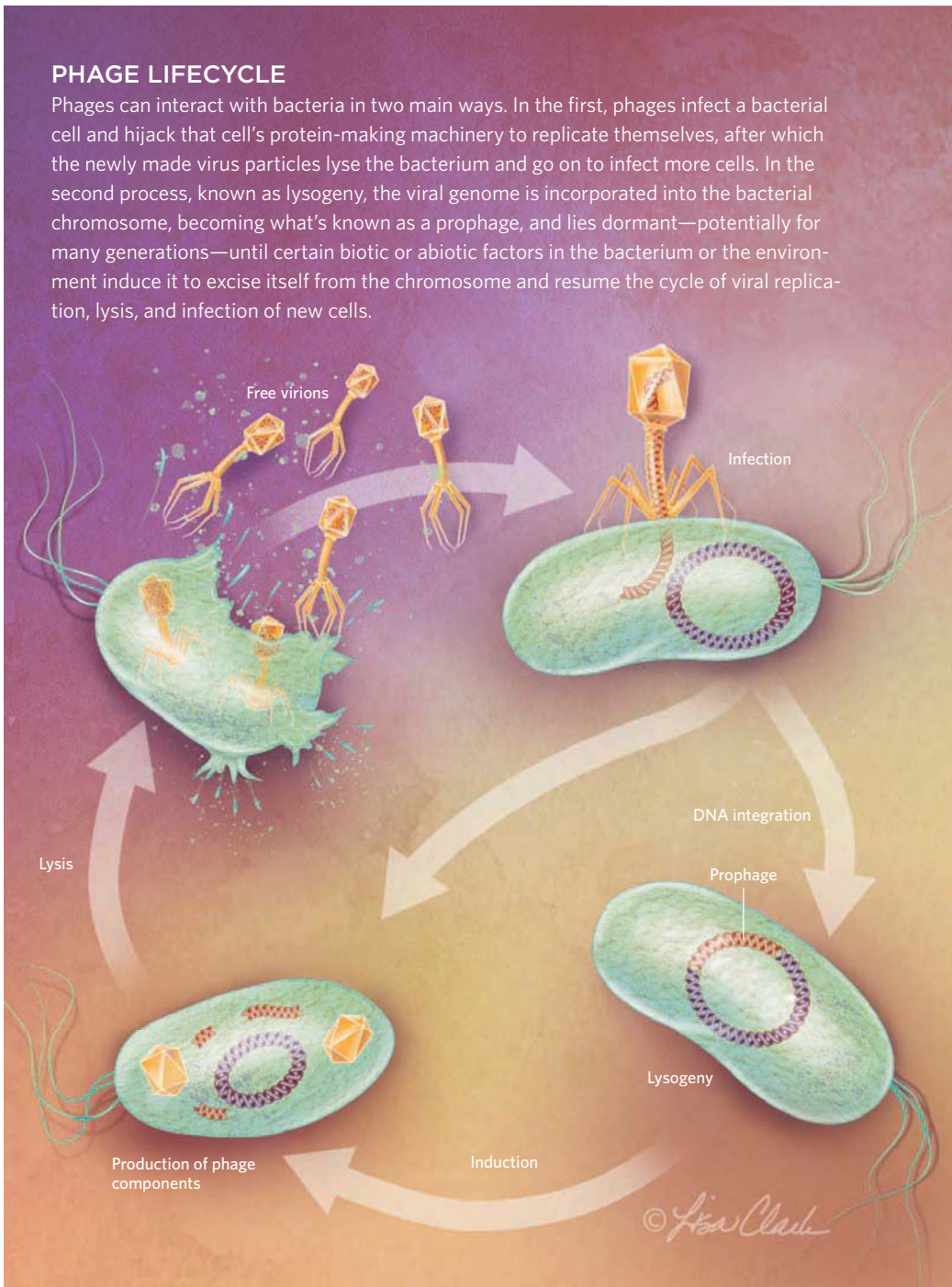
Working in the lab of evolutionary biologist Angus Buckling (now at the University of Exeter), Scanlan grew a strain of *P. fluorescens* with a phage called Phi2

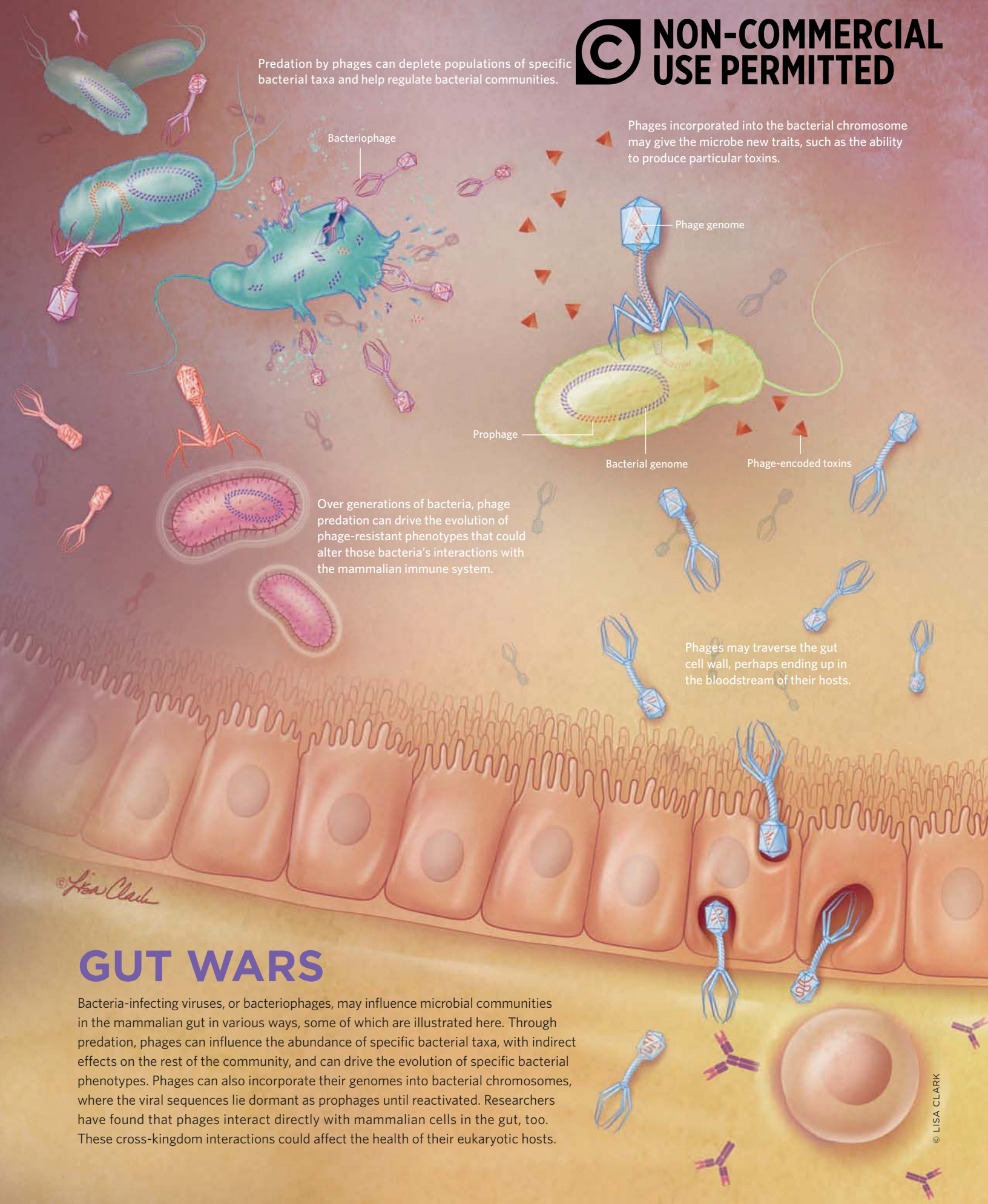
that specifically infects and destroys this bacterium. Cells with the gummy mucoid coating, the researchers noted, were more resistant to phage infection than regular cells were. What's more, over generations, bacterial populations were more likely to evolve the mucoid phenotypes in the presence of Phi2 than they were in its absence, indicating that the phenotype may arise in *Pseudomonas* as an adaptive response to phage attack.[2] Scanlan, now at University College Cork (UCC) in Ireland, notes that more work is needed to extend the findings to a clinical setting, but the results hint that phages could in some cases be responsible for driving bacteria to adopt more virulent phenotypes.

Such a role for viruses in driving bacterial evolution fits well with phages' reputation as "the ultimate predators," says Colin Hill, a molecular microbiologist also at UCC who got his introduction to phages studying bacteria used in making fermented foods such as cheese. Hill notes an estimate commonly cited in the context of marine biology—a field that explored phage-bacteria interactions long before human biology did—that phages kill up to 50 percent of the bacteria in any environment every 48 hours. "The thing that any bacterium has on its mind most, if bacteria had minds, would be phage," Hill says, "because it's the thing most likely to kill them."

Several in vivo animal studies lend support to the idea that predatory phages help shape bacterial evolution and community composition in the mammalian microbiome. In 2019, for example, researchers at Harvard Medical School reported that phages not only directly affect the bacteria they infect in the mouse gut, but also influence the rest of the microbiome community via cascading effects on the chemical and biological composition of the gut.[3] Observational studies hint at similar processes at work in the human gut. A few years ago, researchers at Washington University Medical School in St. Louis observed patterns of phage and bacterial population dynamics that resembled predator-prey cycles in the guts of children younger than two years old: low bacterial densities at birth were followed by decreases in phages, after which the bacteria would rebound, and then the phages would follow suit. The team concluded that these cycles were likely a natural part of healthy microbiome development.[4]

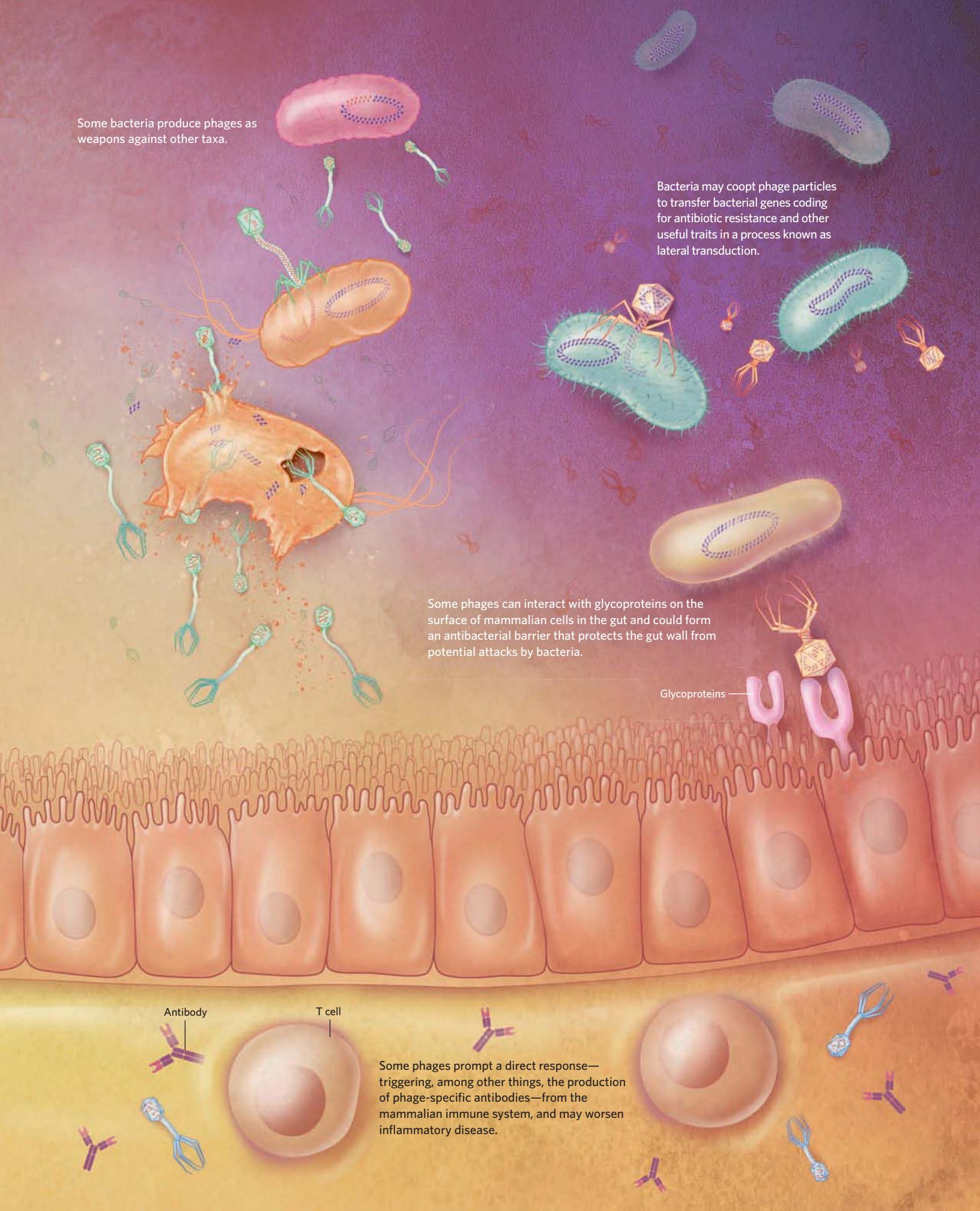
Although researchers are only just beginning to appreciate the importance of phages in microbiome dynamics, they've already begun to explore links to human disease. Authors of one 2015 study reported that Crohn's disease and ulcerative colitis patients showed elevated levels of certain phages, particularly within the viral order Caudovirales. They proposed that an altered virome could contribute to pathogenesis through predator-prey interactions between phages and their bacterial hosts.[5] Other studies have explored possible phage-driven changes in the bacterial community in human diseases such as dia-





GUT WARS

Bacteria-infecting viruses, or bacteriophages, may influence microbial communities in the mammalian gut in various ways, some of which are illustrated here. Through predation, phages can influence the abundance of specific bacterial taxa, with indirect effects on the rest of the community, and can drive the evolution of specific bacterial phenotypes. Phages can also incorporate their genomes into bacterial chromosomes, where the viral sequences lie dormant as prophages until reactivated. Researchers have found that phages interact directly with mammalian cells in the gut, too. These cross-kingdom interactions could affect the health of their eukaryotic hosts.


Some bacteria produce phages as weapons against other taxa.
Bacteria may coopt phage particles to transfer bacterial genes coding for antibiotic resistance and other useful traits in a process known as lateral transduction.
Some phages can interact with glycoproteins on the surface of mammalian cells in the gut and could form an antibacterial barrier that protects the gut wall from potential attacks by bacteria.
Glycoproteins
Antibody
T cell
Some phages prompt a direct response—triggering, among other things, the production of phage-specific antibodies—from the mammalian immune system, and may worsen inflammatory disease.

betes and certain cancers that are known to be associated with a disrupted microbiome. But the observational nature of human microbiome studies prevents conclusions about what drives what—changes in virome composition could themselves be the result of disruptions to the bacterial community, for example.

Currently, researchers are exploring the possibility of using predatory phages as weapons against pathogenic bacteria, particularly those that present a serious threat to public health due to the evolution of resistance to multiple antibiotics. It's the principle that "the enemy of my enemy is my friend," says Yale University virologist and evolutionary biologist Paul Turner. "If we have a pathogen that is in your microbiome, can we go in and remove that bacterial pathogen by introducing a predatory phage, something that is cued to only destroy [that pathogen]?" Although the strategy was first proposed more than a century ago, "we and others are trying to update it," he adds. (See "My Enemy's Enemy" on the opposite page.)

Delivery service

Predation is just one type of phage-bacteria interaction taking place within the mammalian microbiome. Many phages are capable of inserting their genomes into the bacterial chromosome, a trick beyond the bounds of traditional predator-prey relationships in other kingdoms of life that adds complexity to the relationship between phages and bacteria, and consequently, to phages' potential influences on human health.

This role for phages has long been of interest to Imperial College London's José Penadés. Over the last 15 years or so, he and colleagues have described various ways in which many phages help bacteria swap genetic material among cells. He likens phages to cars that bacteria use to transport cargo around and says that, in his opinion, it almost makes sense to view phages as an extension of bacteria rather than as independent entities. "This is part of the bacterium," he says. "Without phages, bacteria cannot really evolve. They are absolutely required."

Some of the biggest recent developments in research on phages in the human gut have turned out not to involve bacteria at all.

In the simplest case, the genetic material being transported consists of viral genes in the genomes of so-called temperate phages, which spend at least part of their lifecycle stashed away in bacterial chromosomes as prophages. These phages are coming to be appreciated by microbiologists as an important driver of bacterial evolution in the human microbiome, notes Hill. The lack of practical and accurate virus detection methods makes it difficult to precisely characterize a lot of the phages resident in mammalian guts, but microbiologists estimate that up to 50 percent are temperate phages, and, more importantly for human health, that many of them may carry genes relevant to bacterial virulence. Researchers have long known, for example, that many toxins produced by bacteria—including Shiga toxin, made by some pathogenic *E. coli* strains, and cholera toxin, secreted by the cholera-causing bacterium *Vibrio cholerae*—are in fact encoded by viral genes contained in the bacterial chromosome, and that infection by temperate phages that carry these genes may be able to turn a harmless bacterial population into one that's pathogenic.

Evidence from other studies points to phages as capable of transporting not just their own genomes, but bits of bacterial DNA as well. In the best-studied examples of this phenomenon, known as bacterial transduction, tiny chunks of the bacterial genome get packed up into viral particles instead of or alongside the phage genome, and are shuttled to other bacterial cells. In 2018, however, Penadés and colleagues presented results showing that very large pieces of bacterial DNA can also be exchanged this way, in a process the team named lateral transduction.[6] Not only does the discovery have implications for how researchers understand viral replication in infected cells, it shines light on a novel way for bacteria to share their genes. "With lateral [transduction] you can move huge parts of the bacterial chromosome," says Penadés. The team first observed the phenomenon in the important human pathogen *Staphylococcus aureus*, and is now looking for it in other taxa, he adds. "Right now, for us, it's important to show that it's a general mechanism, with many bugs involved."

Although the research is still in the nascent stages, this mechanism could help explain findings from University of Barcelona microbiologist Maite Muniesa and others who have been studying whether phages transport antibiotic resistance genes between bacterial cells, and whether they can act as reservoirs for these genes in the natural environment. Early studies on this issue had proposed that, like many toxin genes, antibiotic resistance genes might be encoded in viral sequences and thus transported to bacteria with the rest of the viral genome. But the idea wasn't without controversy—a 2016 analysis of more than 1,100 phage genomes from various environments concluded that phage genomes only rarely include antibiotic resistance genes. That study's authors argued that prior reports of these genes in phage genomes were likely due to contamination, or to the difficulty of distinguishing viral sequences from bacterial ones.[7]

Nevertheless, Muniesa's team has published multiple reports of antibiotic resistance sequences in phage particles, including in samples of meat products from a Barcelonan fresh-food retailer, and more recently in seawater samples—not only from the Mediterranean coastline but even off the coast of Antarctica, far from human populations that use antibiotics.[8,9] "We were pretty surprised that we found these particles in this area with low human influence," Muniesa says. Although her team hasn't determined whether the antibiotic resistance sequences are of phage or bacterial origin,

she suspects they might be bacterial genes that ended up in phage particles during lateral transduction or some process like it. "Bacteria are using these phage particles in a natural way to move [genes] between their brothers and sisters, let's say," she says. "It's happening everywhere."

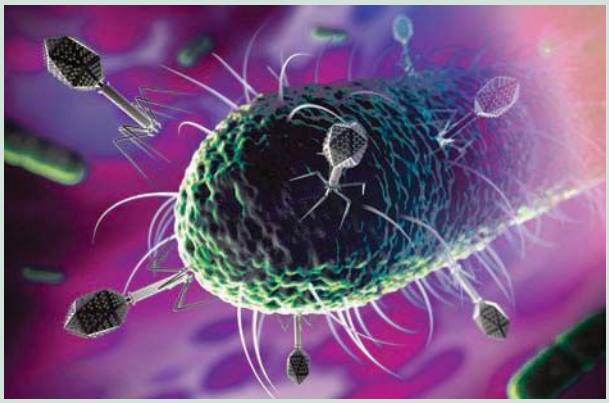
Duerkop cautions that it's not yet clear how often phage-mediated transfer of antibiotic resistance genes occurs or how significant it is in the epidemiology of drug-resistant infections in people. "It's not to say that antibiotic resistance can't be mediated through phage," he says. "I just don't think it's a major driver of antibiotic resistance."

Whatever its natural role, temperate phages' ability to insert themselves into bacterial genomes could have applications in new antibacterial therapies. Viruses that insert pathogenicity-reducing genes or disrupt the normal expression of the bacterial chromosome could be used to hobble dangerous bacteria, for example—an approach that proved successful last year in mouse experiments with *Bordetella bronchiseptica*, a bacterium that often causes respiratory diseases in livestock. Using a phage from the order Siphoviridae, researchers found that infected *B. bronchiseptica* cells were substantially less virulent in mice than control cells were, likely because the viral genome had inserted itself in the middle of a gene that the bacterium needs to infect its host. What's more, injecting mice with the phage before exposing them to *B. bronchiseptica* seemed to completely protect them from infection by the microbe, hinting at the possibility of using temperate phages as vaccines against some bacteria.[10]

Direct contact

Despite growing interest in phages' role in shuttling material among bacteria, some of the biggest recent developments in research on phages in the human gut have turned out not to involve bacteria at all. One of the key

MY ENEMY'S ENEMY



Bacteriophages' ability to selectively target and kill specific bacterial strains has long been recognized as a possible basis for antimicrobial therapies. Proposed by researchers in Europe as early as 1919, phage therapy went on to be widely promoted in Germany, the USSR, and elsewhere before being overtaken worldwide by the soaring popularity of antibiotics in the 1940s. But the strategy has come back into fashion among many microbiologists, thanks to the growing public health problem of antibiotic resistance in bacterial pathogens and to the rapidly improving scientific understanding of phage-bacteria interactions.

Some of the latest approaches aim not only to target specific bacteria with phages, but also to avoid (or exploit) the seemingly inevitable evolution of phage resistance in those bacteria. One way researchers try to do this is by taking advantage of an evolutionary trade-off: bacterial strains that evolve adaptations to one therapy will often suffer reduced fitness when confronted with a second therapy, perhaps one that targets the same or similar pathways in a different way.

Yale University virologist and evolutionary biologist Paul Turner, for example, has studied how phages in the *Myoviridae* (a family in the order Caudovirales) can promote antibiotic sensitivity in the important human pathogen *Pseudomonas aeruginosa*. Turner and colleagues showed a few years ago that one such phage binds to a protein called OprM in the bacterial cell membrane, and that bacterial populations under attack from these phages will often evolve reduced production of OprM proteins as a way of avoiding infection. However, OprM also happens to be important for pumping antibiotics out of the cell, such that abnormal OprM levels can reduce bacteria's ability to survive antibiotic treatment in vitro (*Sci Rep*, 6:26717, 2016).

A handful of groups have published case studies using this kind of approach, known as phage steering, in humans. A couple years ago, for example, Turner and colleagues reported that a post-surgery patient's chronic *P. aeruginosa* infection cleared up after treatment with the OprM-binding phage and the antibiotic ceftazidime (*Evol Med Public Health*, 2018:60–66, 2018). Researchers at the University of California, San Diego, in partnership with California-based biotech AmpliPhi Biosciences (now Armata Pharmaceuticals), reported similar success in a cystic fibrosis patient with a *P. aeruginosa* infection who was treated with a mixture of phages and with antibiotics (*Infection*, 47:665–68, 2019). A Phase 1/2 trial for that therapy was greenlighted by the US Food and Drug Administration last October.

The complexity of the relationship between phages and bacteria, not to mention recently discovered interactions between phages and eukaryotic cells, has many researchers tempering optimism about phage therapy with caution. "There might be off-target effects to this that we hadn't really thought about," says University of Colorado School of Medicine microbiologist Breck Duerkop. That said, thanks to research in the last few years, "the black veil on phage therapy is, I believe, being lifted," he adds, "which I'm really excited about because I think they have a ton of potential to be used in biomedicine."

pieces of this particular puzzle was fitted by University of Utah microbiologist June Round and her colleagues, who as part of a phage therapy study a few years ago fed several types of Caudovirales phages to mice that were genetically predisposed to certain types of cancer and had been infected with a strain of *E. coli* known to increase that risk. "The premise was pretty simplistic," recalls Round. "It was just to identify a cocktail of phage that would target bacteria that we know drive chronic colorectal cancer."

The team was surprised to see that the phages, despite being viewed by most researchers as exclusively bacteria-attacking entities, prompted a substantial response from the mice's immune systems—mammalian defenses that should, according to conventional wisdom, be indifferent to the war between bacteria and phages in the gut. Intrigued, the researchers tried adding their phage cocktail to mice that had had their gut bacteria completely wiped out with antibiotics. Still, they saw an immune response. It was then, Round says, that "we realized that [the phages] were likely interacting with the immune system."

Exploring further, the team found that the phages were activating both innate and adaptive immune responses in mice. In rodents with colitis, the phages exacerbated inflammation. Turning their attention to people, the researchers isolated phages from ulcerative colitis patients with active disease, as well as from patients with disease in remission and from healthy controls, and showed that only viruses collected from patients with active disease stimulated immune cells in vitro. And when the team studied patients who received fecal microbiota transplantation—an experimental treatment for ulcerative colitis that involves giving beneficial gut bacteria to a patient to try to alleviate inflammation and improve symptoms—the researchers found that a lower abundance of Caudovirales in a recipient's intestine at the time of transplant correlated with treatment success.[11]

By the time the team published its results in 2019, a couple of other groups had also documented evidence of direct interactions between phages and host immune systems. Meanwhile, Duerkop, Hooper, and colleagues reported that mice with colitis tended to have specific bacteriophage communities, rich in Caudovirales, that developed in parallel with the disease. Many of the types of phage they identified in the intestines of those diseased mice also turned up in high abundance in samples taken from the guts of people with inflammatory bowel disease, the researchers noted in their paper, supporting a possible role for phages in the development of disease.[12]

Round says that researchers are still unsure about exactly why these trans-kingdom interactions are happening—particularly when it comes to host adaptive immune responses, which tend to be specific to a particular pathogen. She speculates that mammalian hosts might derive a benefit from destroying certain phages if those phages are carrying genes that could aid a bacterium with the potential to cause disease. Exactly how immune cells would detect what genes a phage is carrying isn't yet clear.

Meanwhile, hints of collaboration between eukaryotic cells and phages have cropped up in the work of several other labs. One recent study of a phage therapy against *P. aeruginosa* found that phages and immune cells seem to act in synergy to clear infections in mice.[13] Other work has indicated that phages bind to glycoproteins presented by cells along the mucosal surfaces of the mammalian gut and may provide a protective barrier against bacterial pathogens—a relationship that some microbiologists have argued represents an example of phage-animal symbiosis.[14] Duerkop adds that there's evidence emerging to support the idea that phages in the mammalian intestine not only can be engulfed by certain eukaryotic cells, but also might slip out of the gut and into the bloodstream to make their way to other parts of the body, with as yet undiscovered consequences.

Whether these mechanisms can be exploited for therapeutic purposes remains to be seen, but Round notes that they do raise the possibility of unintended effects in some circumstances if researchers try to use phages to influence human health via the gut microbiome. At least in the type of chronic inflammatory diseases she and her team have been studying, "we might just be making it worse" by using phages to target disease-causing bacteria, she says, adding that all research groups studying such approaches should take into account potential knock-on effects. Considering phages' multiple interactions, with both bacteria and animal cells, she says, "it's a lot more complex than what we'd appreciated." ■

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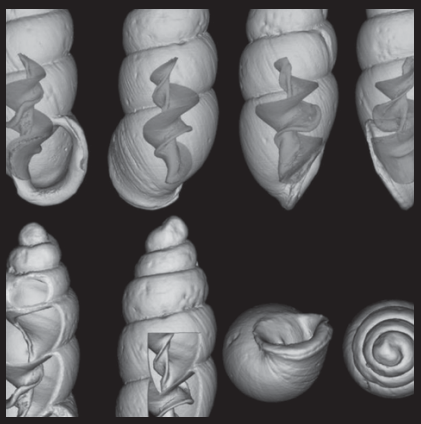
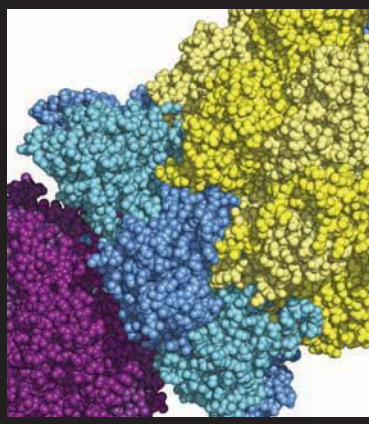
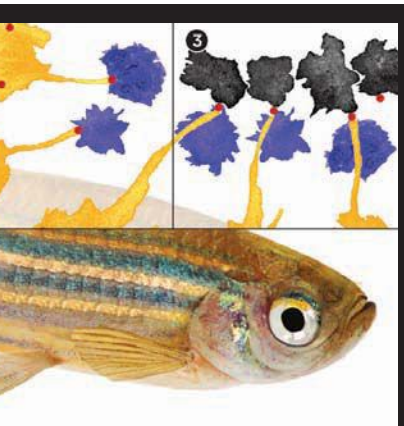
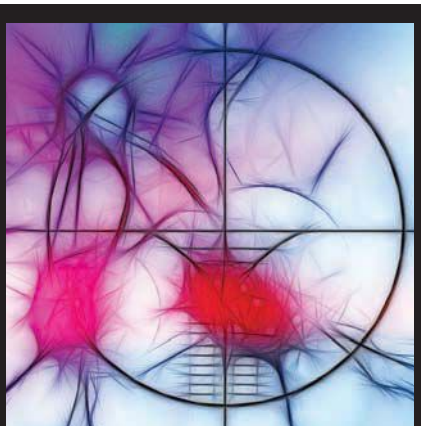
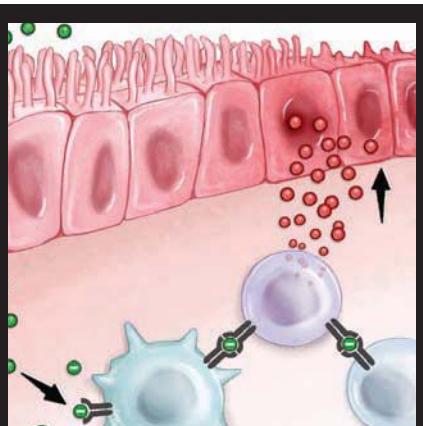









The Literature

CELL & MOLECULAR BIOLOGY

Making Scents

THE PAPER

P. Liao et al., "Cuticle thickness affects dynamics of volatile emission from petunia flowers," *Nat Chem Biol*, doi:10.1038/s41589-020-00670-w, 2020.

Many flowers emit sweet scents to lure pollinators. Those fragrant molecules can, however, cause damage if they begin to collect in the flowers' cells.

To escape into the air, a petunia's scent molecules, called volatile organic compounds (VOCs), have to travel through their cells' cytoplasm, cross an inner membrane and then the cell wall, and finally move through a waxy cuticle. Scientists long thought that diffusion drove the release of the molecules, but in 2015, computer simulations revealed that VOCs can't diffuse out of flower cells quickly enough to prevent internal damage to the plant.

In follow-up experiments to find out how the fragrance molecules might escape the plants, Purdue University biochemist Natalia Dudareva and colleagues found that when the flowers opened and became pungent, levels of a protein called PhABCG1 spiked. Dialing down *PhABCG1* expression cut the emissions of the VOCs, which started to back up in the flower petal cells, causing the plant cell membranes to deteriorate. PhABCG1 was actively transporting the scent compounds across the membrane, Dudareva and colleagues concluded in 2017.

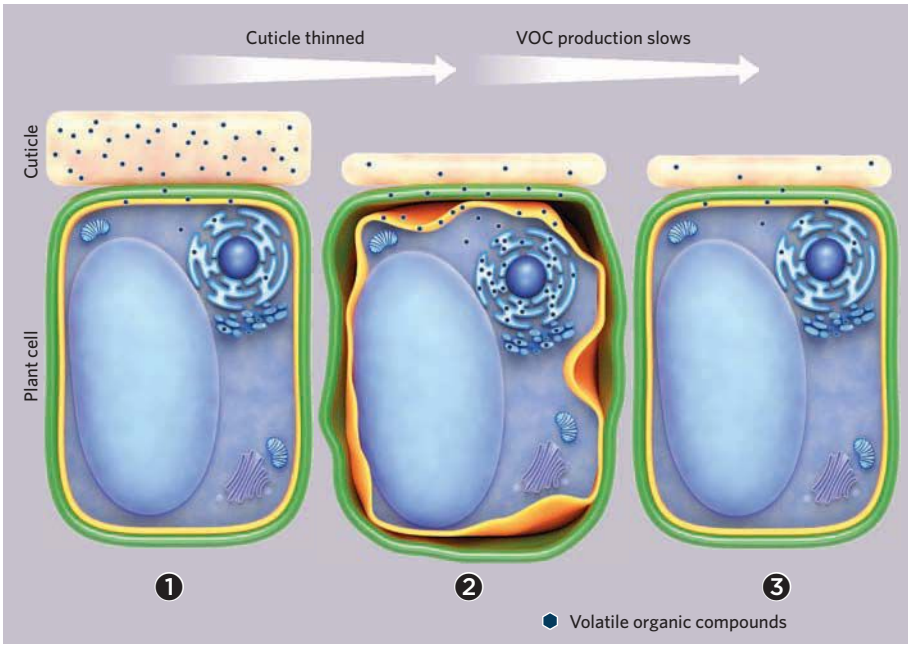
Tracking the location of VOCs in wild-type petunias' flower cells, Dudareva's team noticed that most of them accumulated in the cuticle. This waxy layer that coats the outside of plant cells serves as a sink for roughly 50 percent of a cell's VOCs, the experiment showed. When the researchers used RNA interference to reduce levels of PhABCG12, a wax transport protein, the thickness of the petunia flower cuticle layer dropped, and then VOC emissions, VOC production, and VOC pooling in the cuticle dropped as well. When the researchers repeated the experiment using a chemical to thin the flower's cuticle, they got the same result.

"The idea that when you reduce the cuticle, you actually get less emission—that's totally bizarre," says Jonathan Gershenzon, a biochemist at Max Planck Institute for Chemical Ecology who was not involved in the study.

Dudareva agrees. As she and her colleagues analyzed their data, it became clear that if the cuticle is too thin, VOCs build up within the plant cells, causing damage. Sensing trouble, the cells somehow dial back VOC production. Taken together, the results reveal that the cuticle plays an integral role in regulating petunia's sweet scent, the authors write.

Even with that explanation, the results are flummoxing, Gershenzon says. If researchers find a similar phenomenon in other plants, it could give researchers a way to alter volatile emission, and the messages plants send, just by manipulating cuticle thickness, he notes. In addition, the finding raises questions about the signaling going on between the cuticle and the pathways that control VOC accumulation and production. "We know a lot about metabolism and regulation for so many things," Gershenzon notes, "but for flower volatiles like this, people haven't thought about how that works."

—Ashley Yeager



TOO MUCH OF A GOOD THING: Scented flowers owe their smells to volatile organic compounds (VOCs), but a too-high concentration of VOCs in the cytoplasm can damage cells. Normally, VOCs accumulate in an outer layer known as the cuticle, with a few in the cytoplasm ❶. To examine the cuticle's role in VOC emission, researchers thinned the cuticles of petunia cells, and found that initially, VOCs backed up within the cell membrane and cuticle, causing damage ❷. But hours later, the plants sensed the cell damage and reduced the production of VOCs, leading to lower concentrations in both the cell and the cuticle compared to plants with unaltered cuticles and avoiding further damage ❸.

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PLANT BOX: Experimental ecosystems at the German Centre for Integrative Biodiversity Research allowed researchers to study how invertebrate density influences plant lifecycles and species composition.

VIRUS MONITOR: Genetic elements called retrons help bacteria detect when they've been infected by phages.

ECOLOGY & ENVIRONMENT

Bugging Plants

THE PAPER

J. Ulrich et al., "Invertebrate decline leads to shifts in plant species abundance and phenology," *Front Plant Sci,* 11:542125, 2020.

When Josephine Ulrich and colleagues got the chance to work with the iDiv Ecotron, a system of experimental containers in Germany that lets researchers create and manipulate miniature ecosystems, they decided to investigate the effect of declining invertebrate populations on plant communities. Many studies have explored how projected changes in abiotic factors—rising temperatures, for example—influence plants, says Ulrich, a PhD student at the German Centre for Integrative Biodiversity Research (iDiv) and Friedrich Schiller University, but to look at biotic factors such as invertebrate loss is a new approach.

The team used 24 of the Ecotron units to create tiny grasslands, each with the same 12 herbaceous species but with varying densities of invertebrates collected from a local meadow: 100 percent (the same density as in the meadow), 25 percent, or no invertebrates. Then the researchers observed the ecosystems over the next 18 weeks.

Over that time, units with lower invertebrate densities showed increased abundances of the dominant plant species *Trifolium pratense*, the team found. In addition, some plant species tended to flower later in these units, while others flowered earlier.

An unexpected aphid infestation, primarily in units designated as invertebrate-free, somewhat complicated interpretation of the results, notes Ulrich, although "in the end, it was quite cool that we had it, because this is one of the future scenarios—that there will be more infestations."

The University of Basel's Jürg Stöcklin, who wasn't involved in the work, says the results about flowering time are particularly novel. "It's a proof of concept" study, he adds, noting that the underlying mechanisms aren't yet clear, and that the team's plant communities are less diverse than wild communities. "The question could be asked: How is it in real communities?" he says. But, "if you want to understand what's going on, to disentangle the different effects, we need such [experimental] studies."

—Catherine Offord

GENETICS & GENOMICS

Retro Function

THE PAPER

A. Millman et al., "Bacterial retrons function in anti-phage defense," *Cell*, 183:1551–61.e12, 2020.

Many bacteria contain retrons, DNA sequences which code for enzymes that transcribe RNA into DNA and an unusual molecule made of both DNA and RNA. But microbiologists have puzzled over retrons' function. "People suggested . . . this may be a selfish genetic element, [or] it may be involved in virulence," says the Weizmann Institute of Science's Rotem Sorek. "But nobody actually knew."

Sorek and colleagues recently noticed that retrons often appear in the bacterial genome alongside genes involved in defense against bacteriophages. When the team cloned retrons into *E. coli* strains that normally lack these elements, those populations better resisted viral infection. The effect was due to the retron-equipped cells' tendency to self-destruct if they became infected. "It sounds counter-intuitive," Sorek says—but it's better for the colony to have a few cells die to stop the virus replicating.

The researchers used mutant phages, genome sequencing, and in vitro experiments to show how one retron, Ec48, promotes this self-sacrifice. They found that Ec48 is activated by inhibition of a protein complex called RecBCD, an early responder in a bacterium's anti-phage defenses—and a common target for invading phages. When RecBCD complexes in Ec48-containing bacteria were inhibited, either by a virus or by molecules the researchers added, the bacteria self-destructed within minutes, helping to protect neighboring cells.

"This is another fantastic [study] from Rotem's group," says the University of Exeter's Edze Westra, noting that while other researchers have converged on similar hypotheses, the Weizmann team's study provides mechanistic insight into retrons' role. The study also indicates that not all retrons defend the same cell systems against the same phages. "There's a lot of diversity there, suggesting different retrons are likely to monitor different targets in the cell," Westra says. "Now people can jump on this and try to figure out what all these targets are."

—Catherine Offord

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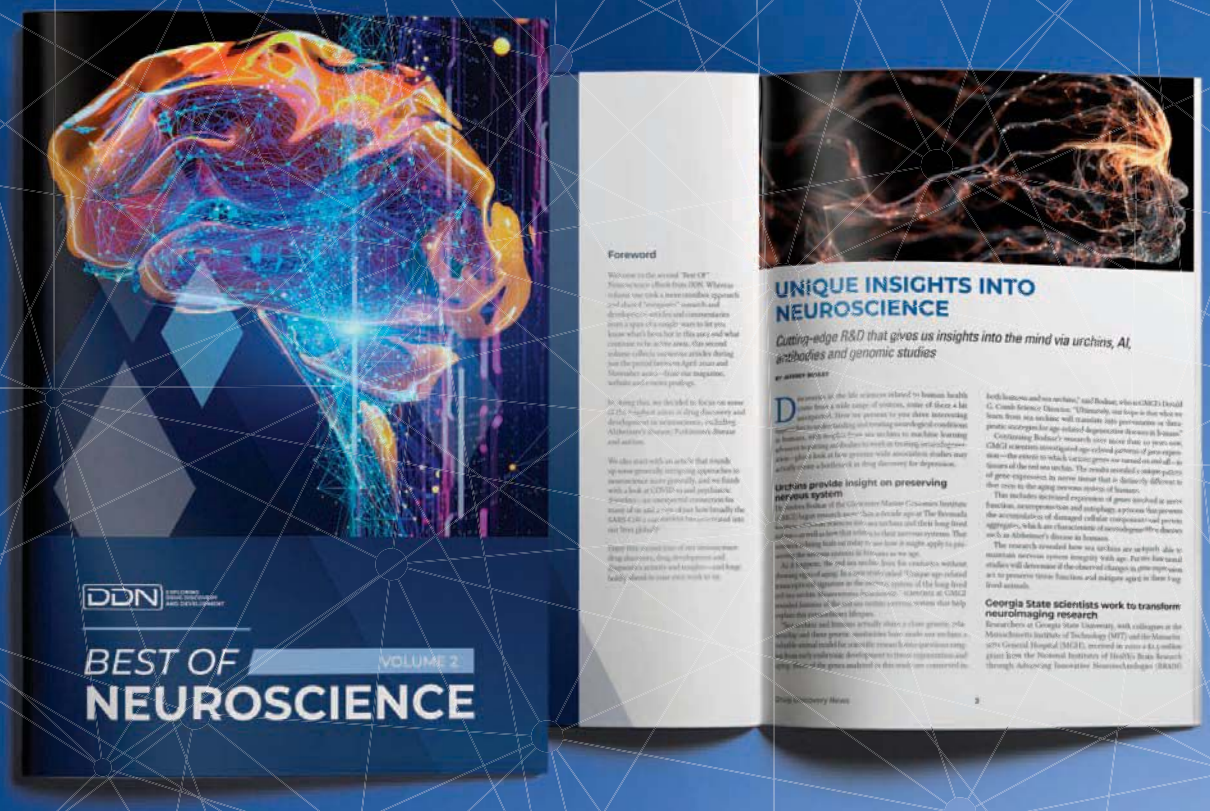

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Siobhán Brady: Root Detective

Professor, Department of Plant Biology and Genome Center, University of California, Davis

BY SHAWNA WILLIAMS

Metal-heavy grasses were what grabbed Siobhán Brady's attention. It was the mid-'90s, she was in her first year at the University of Toronto (U of T), and she was learning about grass varieties that can tolerate taking up normally toxic heavy metals. Having grown up in Canada visiting Lake Erie beaches, some of which had to be closed at times to remove metals originating in nearby steel factories from the sand, Brady "was pretty enamored by the fact that you could use a natural part of the environment to be able to fix what humans had done to destroy it," she says. In this case, the potential solution was growing the grasses in contaminated soil, then harvesting them and disposing of the concentrated contaminants. "I was just totally smitten and decided that this is what I wanted to do for the rest of my life . . . explore plants."

That first year at university was, academically, "a total disaster," she says, but she found her groove the following year, doing research on *Arabidopsis thaliana* in a plant pathology lab headed by Robin Cameron, now at McMaster University. Brady loved the research environment and the process of following the precise steps of a protocol, troubleshooting that protocol, and improving it, she says. "It satisfied something very deep inside of me."

Brady remained at U of T for graduate school, joining the lab of Peter McCourt and initially looking for *Arabidopsis* genes encoding proteins that interact with the plant hormone abscisic acid. While the project yielded some information, including details on other hormone pathways that interact with the abscisic acid pathway, Brady never did find the mutated genes she was looking for (*Plant J*, 34:67–75, 2003). She says she learned a lot from that experience, or example that hard work couldn't compensate for an overly complicated experimental design. Her takeaway: "One should always design relatively straightforward experiments where the answer is going to be very clear." In a second PhD project, Brady built an algorithm to mine transcriptomic data to determine how sequences within the plant's gene promoters related to root development (*Plant J*, 43:153–63, 2005). She earned her doctorate in 2005.

Brady's research interests led her to apply for a postdoc with developmental biologist Philip Benfey at Duke University. Benfey says he recognized at the time that Brady had enormous potential. "She had a way of describing her work and thinking about it that, to me, showed that she had the ability to go beyond what she'd actually accomplished," he explains.

In Benfey's lab, Brady's research involved analyzing the mRNAs present in individual cell types in *Arabidopsis* roots during different stages of development to reveal the patterns of gene expression that enabled their growth and maturation. One of her findings, Benfey notes, is that not only are there genes that are turned on and stay on during development, but also "an oscillating set of genes that would turn on and turn off again, and then turn back on again" (*Science*, 318:801–806, 2007).

Brady was intrigued by how plant cells regulate the types of changes in transcription she saw in her postdoc research. When she started her own lab at the University of California, Davis, in 2009, she had trouble finding funding for *Arabidopsis* research, so she switched to carrying out studies in tomato and sorghum. Her lab recently completed a years-long project to map gene expression and regulation in individual tomato cell types—now under review for publication—that she says she expects will be a valuable resource in researchers' efforts "to breed plants that are going to be more able to tolerate harsh environments."

As part of her sorghum research, Brady visited Ethiopia in 2016 with her postdoc Sharon Gray. While they were there, anti-government protesters threw rocks at the car they were riding in, killing Gray. Brady, who still has difficulty talking about Gray's death, teamed up with Gray's husband to honor Gray by collecting donations and applying for university funding to provide training opportunities for Ethiopian scientists, particularly women. Brady has hosted several Ethiopian students for short research stints, and so far one has earned a master's degree in her lab. Her colleague Richard Michelmore, the director of UC Davis's Genome Center, says that Brady is "a first-rate scientist, but also she cares very much about the people around her." ■

DAVID SLIPHER, UC DAVIS COLLEGE OF BIOLOGICAL SCIENCES

Going Dutch

Amsterdam is growing into a central hub of life sciences and health R&D, as evidenced by the recent relocation of the European Medicines Agency to the city.

BY JEF AKST

In the fall of 2016, Alnylam Pharmaceuticals was looking to expand. The Cambridge, Massachusetts–based company had therapies based on RNA interference (RNAi) technology in late-stage clinical testing for a handful of rare diseases, and wanted to establish a presence in Europe to better serve patients there. By the end of the following year, the company had opened offices in Maidenhead, UK; Zug, Switzerland; and Amsterdam, the Netherlands, to serve as its three European hubs.

Scouting for new locations in Europe, the company found Amsterdam particularly appealing, says Marco Fossatelli, Alnylam's country manager in the Netherlands. Alnylam already had long-standing partnerships with three large academic medical centers in the Netherlands that had hosted some of the company's Phase 3 trials. And Amsterdam checked all the right boxes: it has a highly skilled workforce and is easily accessible and navigable by public transportation. It also had a blossoming life sciences and health sector. "[The city] has the right biotech spirit," Fossatelli says.

Alnylam is not alone is its assessment of the Netherlands—and Amsterdam in particular—as a good location for biotech and pharmaceutical companies. Nearly two dozen life sciences companies established offices in the country in 2018, according to Charlene Verweij, an international press officer at Amsterdam InBusiness, the city's official foreign investment agency; 42 more joined the following year. But perhaps the greatest endorsement of the city's growing life sciences and health sector was the EU Member States' 2017 decision, following the UK's vote to withdraw from the European Union, to relocate the once London-based European Medicines Agency (EMA) to the Dutch capital.

The presence of the EMA, which officially moved to Amsterdam in March 2019, is an additional lure for the pharmaceutical industry, says Annemiek Verkamman, managing director of Dutch biotechnology industry association HollandBIO. While the agency's presence alone would not necessarily drive a company to open an office in the city, she says, the move drew attention to the country's life sciences and health sector. "Now, the Netherlands is on the map."

A practical choice

Before the COVID-19 pandemic hit early last year, Anant Murthy would regularly leave his home in Geneva, Switzerland, first thing on Monday morning to catch

a 6:40 AM flight to Amsterdam, and be at his office in the center of the city by 9 AM. "That's less time than I know some people commute into New York City," says Murthy, then Alnylam's lead in the Belgium, the Netherlands, and Luxembourg region, although he has since left the company. His rapid commute was made possible thanks to the central location of Schiphol Airport, which is situated 15 kilometers away by highway—or 15 minutes on the train—from the EMA's new location in the heart of the city's business district. Several people who spoke with *The Scientist* praised the ease of travel to and from Amsterdam, with direct flights from Schiphol to many major European cities and overseas destinations.

Good airport access is especially important for Kite Pharmaceuticals, which creates personalized CAR T cell therapies that need to be produced and shipped to patients as quickly as possible. Kite's original US facility in El Segundo, California, is just a few miles from Los Angeles International Airport, and recently the company, which was acquired by Gilead Sciences in 2017, opened a new production facility in Amsterdam a stone's throw from Schiphol. "If you look out the window, you can see the airstrip," says Louis van de Wiel, who heads up Kite's new plant. "We are really close." The Amsterdam facility, which received EMA approval last June, now serves as Kite's European manufacturing hub, churning out a CAR T cell therapy, called Yescarta, that was green-lighted by the EMA in August 2018 for patients with certain types of B cell lymphoma.

Another advantage of Amsterdam for biotechs is its rich labor pool of highly skilled talent, says van de Wiel. Kite's facility there, which opened in 2018 with only a handful of employees, now employs more than 400 people, and it's still growing. In addition to drawing on the Netherlands' own workforce, the company has hired from throughout Europe—something that's easily done because Amsterdam is such a popular destination, van de Wiel says, being close to the ocean with a robust social scene, at least in non-pandemic times. Employees at the Amsterdam facility hail from more than 25 different countries. "Not only from an industry perspective [is Amsterdam] a hotspot, but also [as] an area to live," he says. "People are, I think, attracted a lot of time to the Amsterdam area."

Many international workers also benefit from the Netherlands having the highest English proficiency in the world outside of native English-speaking countries. "Outside of the UK, it's the easiest [European country] for a native English speaker to interact and operate in," says Jason DeGoes, the chief operating officer of the regulatory and compliance services company ProPharma Group, which opened an Amsterdam office in early 2019 to better serve clients in the area.

A couple of years ago, a lot of people were in early phases, maybe clinical Phase 1. And now we see the first Dutch biotechs really deliver a product to the market, which is of course a real milestone.

—Annemiek Verkamman, HollandBIO

But perhaps the primary draw for international biopharma companies to Amsterdam and other Dutch towns with a life science presence is the country's academic and commercial ecosystems. The Netherlands ranks number two in the world for numbers of patent applications in biotechnology. Numerous universities and medical centers coexist with 420 biopharmaceutical companies plus more than 2,500 other life sciences companies throughout the country. Importantly, says Gerard Schouw, general manager of the Dutch Association for Innovative Medicines, Dutch industry and academia have a history of productive collaboration. The country boasts more than 500 public-private partnerships, including several focused on developing a vaccine against COVID-19. And the ongoing clinical development programs foster the growth of supporting sectors, including regulatory affairs consultancies, venture capital firms, and tech companies.

"Talent, science, access to research, access to capital—everything you need to start an enterprise. Amsterdam has that, and it's growing," says Murthy, now head of Europe for the immunology company argenx.

The Netherlands was thus well positioned to host the EMA, not to mention companies looking for new European headquarters to continue serving the European single market after Britain voted to leave the European Union. (See sidebar on page 50.) These shifts helped solidify the reputation of the Dutch life sciences and health sector, and perhaps even accelerated growth of the life sciences hub in Amsterdam.

"The EMA is here because of the healthy biotech industry," says Bas Reichert, founder and CEO of microbial genomics company BaseClear and chairman of the Entrepreneurial Association of the Leiden Bio Science Park where BaseClear is located. "The biotech industry will even be better through the EMA."

A maturing market

The EMA relocated to the Netherlands just as the country's biopharmaceutical pipelines were starting to bear fruit. In August 2018, Genmab earned the agency's approval to market its monoclonal antibody daratumumab (DARZALEX) as part of a combination therapy to treat patients with multiple myeloma. The Denmark-headquartered company has core facilities in the Dutch city of Utrecht, home to one of the country's largest science parks.

Then last fall, the EMA approved filgotinib (Jyseleca), a treatment for rheu-

matoid arthritis developed by Galapagos, a Belgium-headquartered biotech with R&D facilities in Leiden, where much of the work on Jyseleca was carried out. (In the summer of 2019, Galapagos teamed up with Gilead to market the product.) And just last year, Leiden-based Pharming received the green light from the EMA to market its treatment for hereditary angioedema, a chronic disease involving swelling in various parts of the body, to children as young as two years old, in addition to adults and adolescents, who could already access the medication. Several other Dutch companies have products that are now in Phase 3 trials.

NEW HOME: The European Medicines Agency (EMA) made the move to Amsterdam from London in 2019.

ROBERT MEERDING

"The biotech sector in the Netherlands [has] grown up the last few years," says Verkamman. "A couple of years ago, a lot of people were in early phases, maybe clinical Phase 1. And now we see the first Dutch biotechs really deliver a product to the market, which is of course a real milestone."

In October 2019, the Dutch government appointed its first ambassador for the life sciences and health sector. Clémence Ross-van Dorp, former State Secretary for Health, Welfare and Sport, took on the new role at the beginning of last year to lead an action program to capitalize on the EMA's relocation to the country. "The government really knows that the life science and health sector is a game changer for economic growth in the new century," says Schouw. ■

THE BREXIT EFFECT

Although the departure of the European Medicines Agency (EMA) wasn't ideal for the UK biopharmaceutical industry, it hasn't been devastating either. Many UK-based companies invested in opening offices elsewhere in Europe in order to keep operating within the EU. According to data the Netherlands Foreign Investment Agency provided to *The Scientist*, 20 of nearly 100 life sciences and health companies that opened new branches in the Netherlands in the last few years cited Brexit as a motivating factor in moving or expanding there. But that only sometimes meant closing UK branches, says David Jefferys, who leads global strategy and corporate affairs at Eisai, a Japanese pharmaceutical company. Eisai, for example, maintains its facility outside of London, but now has established a presence in Frankfurt, Germany. This allows the company to continue serving both the European single market of nearly 450 million consumers and the UK market of nearly 67 million.

Anant Murthy, Alnylam Pharmaceuticals's former lead for Belgium, the Netherlands, and Luxembourg, agrees that the UK pharma and biotech industry is doing just fine, and says that the company continues to invest in its Maidenhead facility outside of London. "We continue to see the UK as an attractive place for research, and an important investment destination, frankly, for the life sciences sector," he told *The Scientist* before he left Alnylam last year. "We don't see this as an either/or situation."

Jefferys says he sees the direction taken by the UK's Medicines and Healthcare products Regulatory Agency (MHRA) as a potentially positive spin on the loss of the EMA. In October 2020, the regulatory body joined Project Orbis, the US Food and Drug Administration's initiative to support parallel review of cancer drugs internationally, as well as the Access Consortium, formerly the ACSS Consortium, a 2007-founded regulatory collaboration among Australia, Canada, Singapore, and Switzerland. "As [the MHRA] move from being a leading part of the European system," says Jefferys, "I think they're now positioning themselves as being more of an independent, more global agency."

Europe Is Sinking Biotech—Again

Scientifically groundless regulations could undercut the potential of gene-edited crops, much as they have with GMOs.

BY ROBERT PAARLBERG

When it comes to modern agricultural biotechnology, Europe's caution has been slowing progress for more than two decades. It started in the 1990s, when Europe began rejecting crops modified using recombinant DNA, or DNA from other species—crops branded as genetically modified organisms (GMOs). Now it is doing the same for gene-edited crops improved using CRISPR. European scientists have objected to this new blockage, but they are not the only ones paying a price.

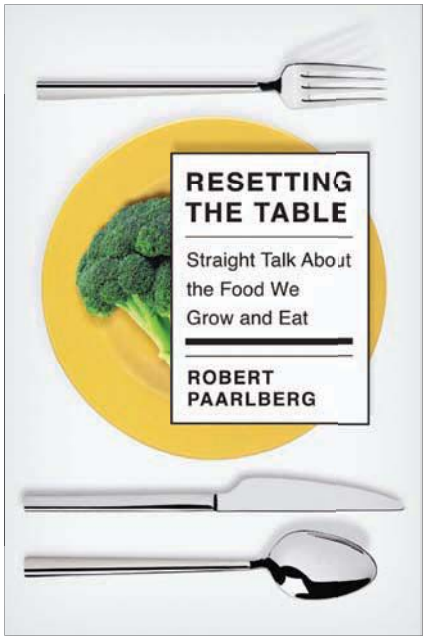
Using CRISPR, researchers are now working to make crop plants that have higher yields, resist disease or stress, or are tastier, more nutritious, or more convenient than cconventionally bred varieties. As farmers seek to adapt to climate change, gene editing could become an even more valuable tool in agriculture—if regulators will allow it.

Decades ago, it was transgenic modification that seemed poised to help increase drought tolerance, disease resistance, and crop yields, and to curtail insecticide use. But consumers in Europe were scared away from the resulting GMO foods by activist organizations, while governments stifled the products with strict regulation; most farmers there have never planted them. GMO consumer foods are also not imported into Europe, due to a burdensome tracing rule that requires all operators in the marketplace to maintain, for five years, a record of every single GMO they handled, where it came from, and where it went. Rather than take on this logistical nightmare, food companies in Europe reformulated their products completely away from GMO ingredients, and those exporting to Europe now do the same or plant no GMOs at all. In the US, where GMO regulations are more permissive, farmers have planted GMO cotton, plus GMO corn and soybeans (mostly for animal feed and auto fuel), but they voluntarily avoid GMO wheat, rice, and potato, partly for fear of encountering commercial rejections in Europe.

As I discuss in my new book, *Resetting the Table*, Europe's policies ignore a consensus among science academies around the world—including in Europe—that GMO crops pose no new risks either to human health or to the environment. Even the European Commission concurs with this view, concluding in a 2010 analysis that "biotechnology, and in particular GMOs, are not per se more risky than e.g. conventional plant breeding technologies."

Now Europe's rejection of new agricultural biotechnology is being repeated for gene-edited crops. First reported in 2012, CRISPR should have been less controversial than transgenic work because it does not rely on bringing in genes from unrelated species, and it closely resembles the natural process of genetic mutation. The EU's own advocate general offered a preliminary nonbinding opinion that CRISPR crops should not fall under the strict regulatory requirements of Europe's GMO Directive, but the European Court of Justice in Luxembourg (the EU equivalent of the US Supreme Court) concluded in 2018 that gene-edited organisms should be regulated like GMOs.

This ruling hit European crop scientists hard. The European Academies Science Advisory Council (EASAC) called the decision a "setback for cutting-edge science and innovation in the EU." In October 2020, the European Federation of Academies of Sciences and Humanities said crops improved through "targeted genome edits, which do not add foreign DNA" were no more dangerous to human health or the environment than crops developed through classical breeding.



Knopf, February 2021

If the EU does not modify its GMO Directive to make more room for gene-edited crops, European regulations will again begin constraining a new farming technology worldwide, especially in developing countries that produce for the European market. EASAC emphasized the potential for damage to developing countries that "stand to benefit most from crops that better withstand the devastating effects of climate change."

At a time when progressive Europeans, alongside Americans, have been telling the world to "follow the science" on climate change, and on COVID-19, it is disappointing to see the same principle not applied to crop biotechnology. ■

Robert Paarlberg is an associate in the Sustainability Science Program at the Harvard Kennedy School of Government. *Read an excerpt of* Resetting the Table: Straight Talk About the Food We Grow and Eat *at the-scientist.com.*

Viral Discoveries, 1929

BY MAX KOZLOV

In 1925, after years of study and research, Helen Purdy Beale seemed to be on track to become the first woman to graduate with a doctorate from Cornell University's plant pathology department. Her final hurdle was to obtain the approval of her adviser, Herbert Whetzel, who, unbeknownst to her, had dissuaded previous female graduate students from obtaining PhDs on the grounds that overqualified women could not get hired at agricultural experimental stations. True to form, Whetzel told Beale that her thesis could not be accepted and returned it, heavily marked up with red ink. Beale hurled the pages into his face, screaming, "You have shown the claws of the devil!" and stormed out, according to an account by virologist Karl Maramorosch. Beale would go on to earn her doctorate from Columbia University in 1929 and change the course of plant virology with her work on tobacco mosaic virus (TMV).

SAY CHEESE: Helen Purdy Beale (front row, in the fur coat) poses for a photo in 1919 with her mycology class at Cornell University, where she began her graduate work in plant pathology.

W.R. FISHER, CORNELL UNIVERSITY

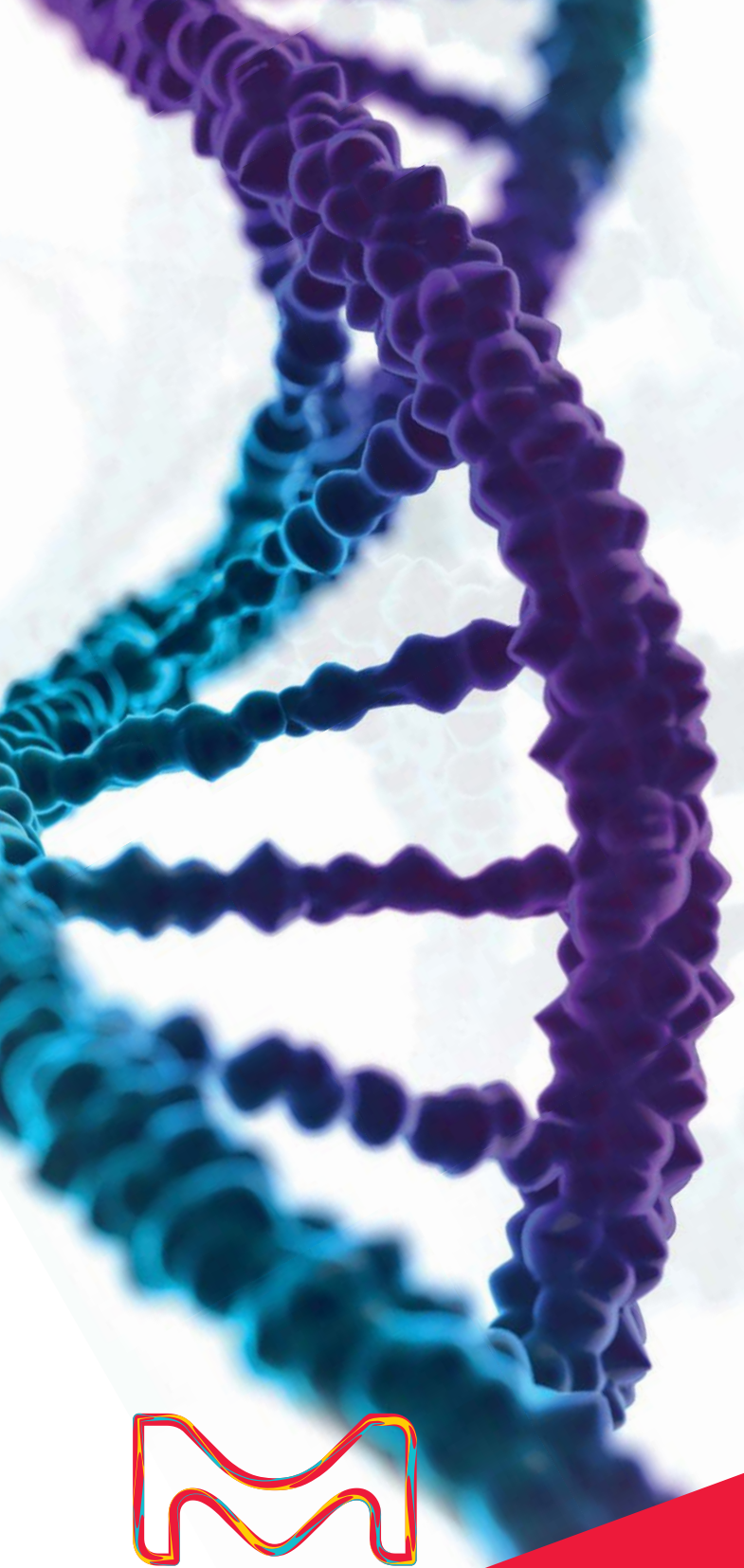
TMV had been discovered only in the late 19th century, when chemist Adolf Mayer noticed that some tobacco plant leaves developed multicolored splotches and eventually shriveled up. Viruses were little-understood at the time, in part because, unlike bacteria, they couldn't be seen with a light microscope. Mayer and other scientists ascribed the condition to parasites, enzymes, or other substances that they were unable to characterize in the plants, and could only diagnose TMV using the rudimentary technique of looking at diseased plants' symptoms. Beale set out to change that.

After graduating from Columbia, Beale returned to the Boyce Thompson Institute (BTI) in Yonkers, New York, where she'd previously worked as a plant pathologist for a few years. She postulated that a substance in animal serum—today known as antibodies—could be used to study plant viruses. Indeed, Beale found that the serum of rabbits that were injected with TMV-infected sap could then be mixed with samples of sap from other plants to test whether they were also infected: only TMV-infected sap would form a heavy precipitate (made of antibody-bound virus) when mixed with the serum. Different plant species infected by the virus yielded similar precipitates, indicating that the disease did not arise from a defect of the plants themselves, but was caused by an infectious agent. Beale subsequently found that the precipitate formation was specific to TMV, and she devised assays to determine viral concentration—methods that were among the first serological techniques in virology.

Yet Beale's work went largely unnoticed for at least 30 years. Texas A&M University virologist Karen-Beth Scholthof, who has written about Beale's contributions to the field and describes her as the "mother of plant virology and serology," notes that plant pathologists were still using the tools and methods of the early 20th century as late as the 1960s before they rediscovered Beale's experiments and began using her assays, the fundamentals of which are still used today. Frederick Charles Bawden, a plant pathologist, wrote in 1970: "I still remain puzzled to understand how it was that so many virus workers long remained reluctant to use these invaluable techniques. With hindsight, it is very evident they were even more valuable than those of us who used them appreciated."

Scholthof says last year's rapid COVID-19 test development owes a debt to Beale's foundational ideas from a century ago. "Then and now, serology is really important for understanding more about the biology of these viruses, where they are localizing in cells, and having rapid diagnostics," she says.

Beale remained at BTI for several decades and, after her retirement, compiled a 1,500-page bibliography with more than 29,000 plant virology references. She died in 1976. Her *Ridgefield Press* obituary described her as "unflappable, witty, and persevering." ■



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