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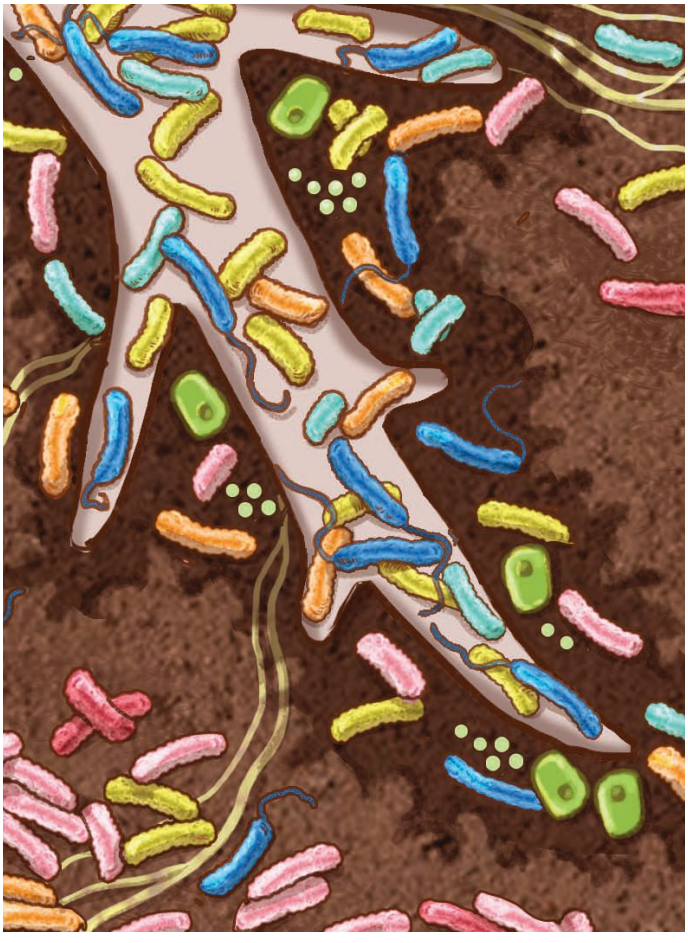

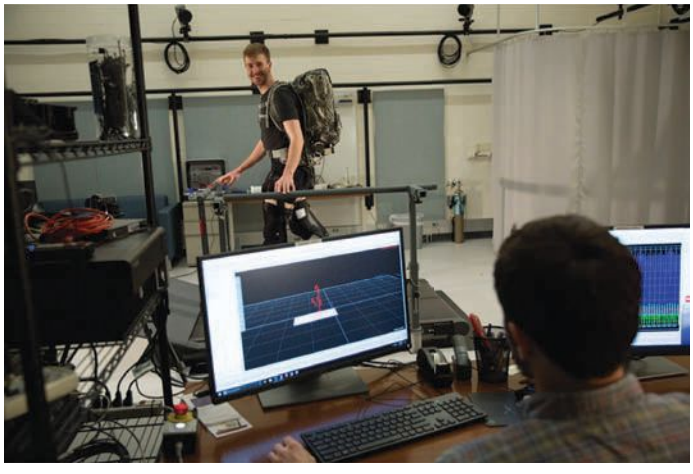

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ON THE COVER: YULIAN ALEXEYEV/UNSPLASH


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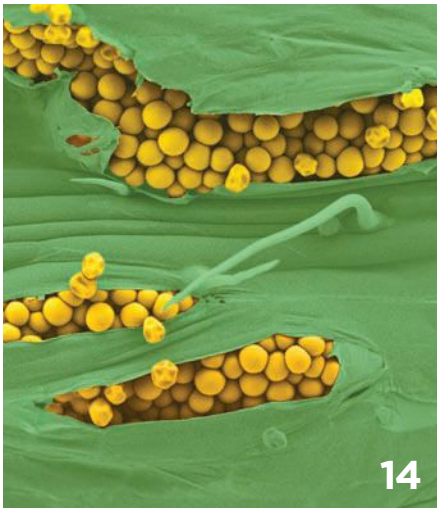

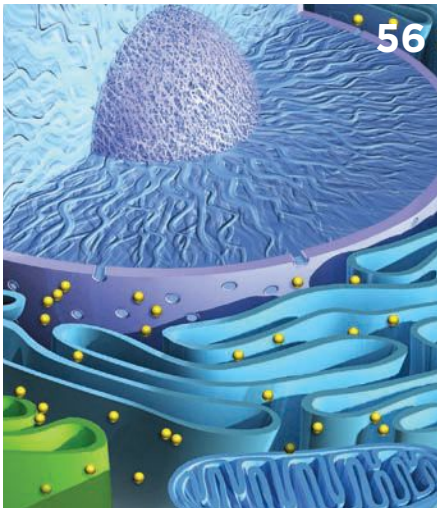

PUZZLE ON PAGE 12

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Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Operation Monkey Rescue
Meet the people trying to save a research colony of rhesus macaques living on a small island off the coast of hurricane-ravaged Puerto Rico.

VIDEO
Growing Awareness
University of California, Riverside, plant biologist Katie Dehesh explains the importance of agricultural research.

VIDEO
Coral Sperm Banker
Mary Hagedorn is racing to save Earth's coral reefs by developing techniques for freezing the colonial animals' gametes.

AS ALWAYS, FIND BREAKING NEWS EVERY DAY, AND LEAVE YOUR COMMENTS ON INDIVIDUAL STORIES ON OUR WEBSITE.

Coming in March

HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Are there biological hallmarks in the brains of transgender people?
- Probing ancient proteomes
- Found in translation: going from cells to sales
- A newly engineered opsin protein and receptor boost optogenetics

AND MUCH MORE

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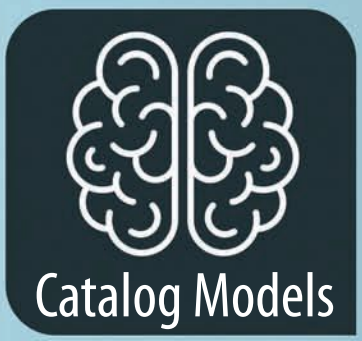

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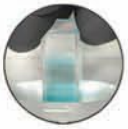

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TheScientist TOP 10 INNOVATIONS

Contributors

Davide Bulgarelli grew up in Northern Italy in one of the key regions for producing Parmesan cheese, among grapevines, livestock, and apple and pear orchards. It's perhaps unsurprising, he says, that this upbringing motivated him to pursue a bachelor's degree in agricultural sciences and a master's in crop production in his hometown in Reggio Emilia at the University of Modena and Reggio Emilia. "I wanted to do something to improve agriculture," Bulgarelli says. After obtaining a doctoral degree at the University of Milan, where he identified a gene in barley that confers resistance to a disease-causing fungal pathogen, Bulgarelli spent five years as a postdoc at the Max Planck Institute for Plant Breeding Research in Cologne, Germany. There his research focused on the microbiome associated with the plant model *Arabidopsis*. But Bulgarelli eventually returned to his roots, studying plants of agricultural importance. "Crops are something that you eat every day, so there is a part of the research that is immediate." In 2013 Bulgarelli joined the School of Life Sciences at Scotland's University of Dundee, where he is now a principal investigator, researching plant interactions with microbes at the root-soil interface. "This field of research is still quite open," he says, "this is what makes me very excited."

Read Bulgarelli's essay on the plant microbiome and agriculture on page 26.

Jessica Eise moved around a lot when she was growing up, and that didn't change when she started her career. Before she turned 18, Eise had lived in four different states between Alaska and Wyoming, and had spent a semester in Spain as an exchange student. After she earned a bachelor's degree in political science and international studies at Saint Louis University and a master's in journalism and international relations from New York University, Eise's curiosity and interest in writing led her across the globe to do various projects in media production, communications, and journalism: in Nicaragua, for instance, she reported on the legacy of US intervention, and in Mauritius she wrote a piece on social tensions. "I think long bucket lists are good for the health," Eise says. Although her parents were both meteorologists, she never really saw herself going into science. She fell into agricultural science "by sheer chance," she says, when she was offered a position as director of communications at the Department of Agricultural Economics at Purdue University in 2014. This sparked an interest in agriculture that led her to write several books on the topic, including *How To Feed The World*, which she coedited with Purdue researcher Ken Foster. Now she is pursuing a PhD at Purdue's Brian Lamb School of Communication, where she is researching strategies to help coffee growers in Colombia adapt to climate change.

Read Eise's essay about her book on page 63.

When **Catherine Offord** was younger, she thought *Jurassic Park* was "the coolest thing" she ever saw. Learning that bioengineering dinosaurs wasn't really feasible in real life didn't deter her from going into science, and she went on to study biology at the University of Oxford. "I was pretty hooked on it after that," she says. Offord then spent half a year happily reeling silk out of golden orb weaver spiders as a laboratory assistant in a biomechanics lab at the university—good practice for later becoming the proud owner of four pet tarantulas. As she became more interested in animal behavior, Offord joined a laboratory studying collective behavior in animals such as ants at Princeton University. "I was kind of fascinated by the idea of swarms and collective decision making," she says. During her time there, she sat in on an undergraduate science journalism course, which she says she enjoyed. "It made me really keen to do more of it," Offord says. After spending months reporting on education and homelessness for two nonprofit newspapers in Philadelphia, she accepted an internship at *The Scientist* in January 2016. After six months, Offord continued to work part-time for the magazine while teaching mathematics at the College of Micronesia in the Pacific, and, she adds, diving with manta rays. When she returned to her home continent, Europe, she was offered a full-time position as assistant editor at *The Scientist*, where she edits the BioBusiness and Career departments of the magazine. "It's a great job. I'm very happy here," Offord says.

JESSICA EISE: BRIAN POWELL

An Enduring Partnership

Humanity would be nothing without plants. It's high time we recognize their crucial role in sustaining life on Earth.

BY BOB GRANT

Plants are far older than most people realize. Although exact dates are hard to pin down, scientists suspect that a single group of green algae colonized terrestrial environments somewhere between 630 million and 510 million years ago. Before that evolutionary leap, photosynthetic microbes in freshwater lakes were likely churning out oxygen as long as 1.2 billion years in the past, a labor that started to make Earth's atmosphere more hospitable to life about 850 million years ago.

Fast forward through the epochs, and plants evolve into a dizzying kaleidoscope of form and function, while some species that look remarkably similar to the ancestors of all plants still survive. As plants did their evolutionary thing, animal life arose and struck up an eons-long love affair with the plants that preceded them. Then, just a hot second ago geologically speaking (about 6 million years ago), a curious creature climbed down from its perch in the canopy and took its first tentative steps on two legs toward an uncertain future.

From our ancestors' departure from their arboreal swinging grounds, to the grassy savannahs where yet more forebears would rise and fall, to the first agriculturalists, whose toil would bend plant life to human will, plants have been humanity's constant companion, sustainer, and savior.

As farming practices spread, writing and culture were born. Again, humans looked to plants as the raw material for the transmission and propagation of knowledge. Reeds and, later, trees would serve dutifully for millennia as vessels that ferried accumulated wisdom from mind to mind. And eventually, wood from centuries-old trees formed the bodies and masts of ships that would spread humanity to every corner of the globe.

But for all that plants have done for *Homo sapiens* and our ancestors, humans still have so much to learn about Kingdom Plantae. For example, researchers are just now determining how plants interact with myriad microbial taxa in, on, and around them. And new insights are emerging every day into the rapidly evolving pathogens that attack plants and how botanical defenses have kept pace, fighting microbial onslaughts through the millennia.

For all that plants have done for *Homo sapiens* and our ancestors, humans still have so much to learn about Kingdom Plantae.

At this point in humanity's relationship with plants, the stakes are high. As the global population mushrooms, researchers and agriculturists are working ever harder to spark yet another agricultural revolution. With a projected human population of 9.7 billion by 2050, we'll need an enhanced form of agriculture to ensure that everyone is fed.

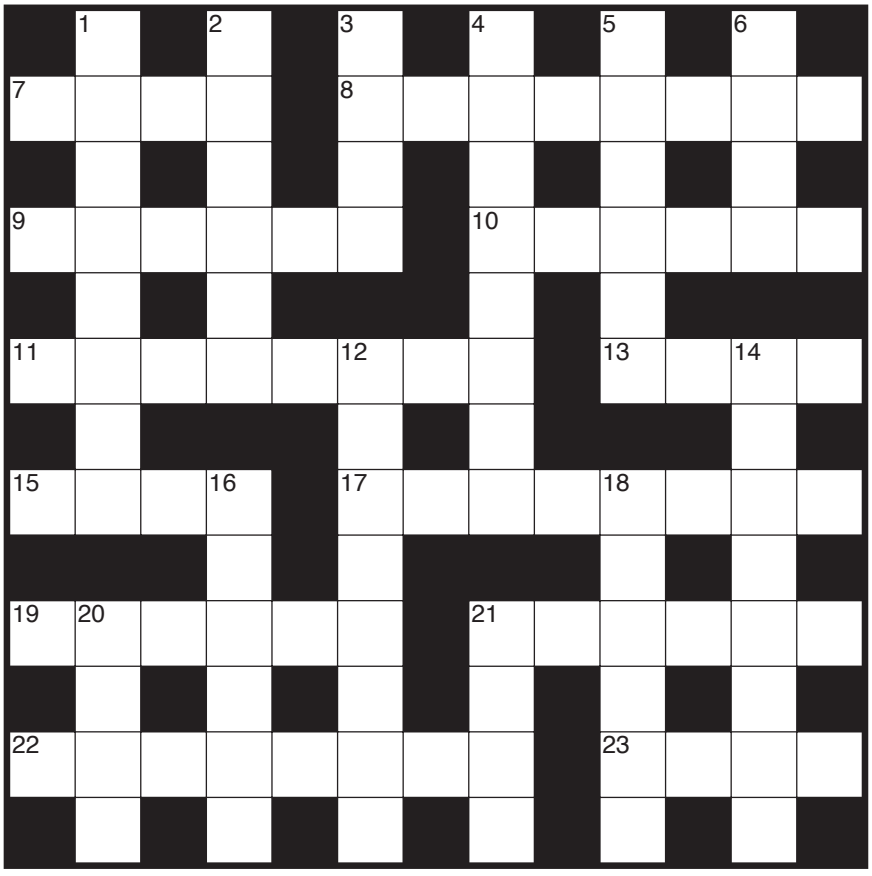
Genetic modification, supercharged with cutting-edge genome editing technologies, represents the latest intertwining of our fates. While the practices still face some popular distrust, scientists and most clear-headed consumers know that using every bit of human knowledge at our disposal (and innovating into the unknown) to provide for ourselves is the only way forward as a global community. Such thinking worked when the first farmer hoed a furrow into the soil and dropped in a line of seeds; it served agriculturists as they carefully considered how to breed advantageous traits into crop plants; and it just may save humanity again as researchers work to engineer disease resistance, drought tolerance, and other valuable qualities into our cultivated fruits, vegetables, and grains.

This issue is a celebration of the work that researchers are doing to further the human-plant marriage so that we might enjoy many more years here on our verdant planet. ■

Editor-in-Chief
eic@the-scientist.com

ANDRZEJ KRAUZE

Speaking of Science



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

BY EMILY COX AND HENRY RATHVON

ACROSS

7. Largest member of the oceanic dolphin family
8. Nitrogenous substance once thought to cause food poisoning
9. An organ's outer layer: Latin for "tree bark"
10. Sound from a chinchilla, mouse, or guinea pig
11. Folkloric shapeshifter or lycanthrope
13. Producer of hips
15. Bioluminescence
17. Allergic rhinitis (2 words)
19. Controlled substance with a morphine-like effect
21. Alloy of tin and copper
22. Barrier breached by osmosis (2 words)
23. Location of the metacarpals

DOWN

1. Living high, like lemurs and sloths
2. Layer between a planet's core and crust
3. Kind of predator 7 Across or 5 Down represents
4. *Musca domestica*
5. Cat spotted in rainforests
6. Andean speaker of Quechua
12. Connected with snakes
14. Symptom of 17 Across
16. Descriptor of an ancient mammoth
18. Geologic spans larger than ages
20. Exfoliation treatment in a spa
21. Having no hair apparent?

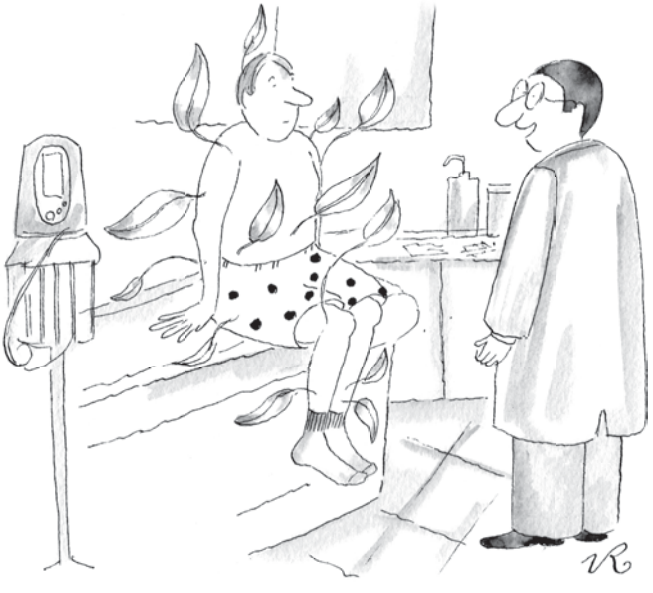
Answer key on page 5

Botany is the art of insulting flowers in Latin and Greek.

—French journalist, novelist, and horticulturist **Alphonse Karr** (1808-1890), for whom several plant species were named, as quoted in the 1960 book *Les noms de plantes*

The climate problem is the most important problem we currently have on this planet. Given all the arguments that people have against genetically engineering plants combined with the urgency of the problem, should we use this engineering?

—Salk Institute plant biologist **Wolfgang Busch,** whose group is researching ways to edit plant genomes to store more carbon and weather drier, hotter climatic conditions, speaking with San Diego State University public broadcaster KPBS (January 3)



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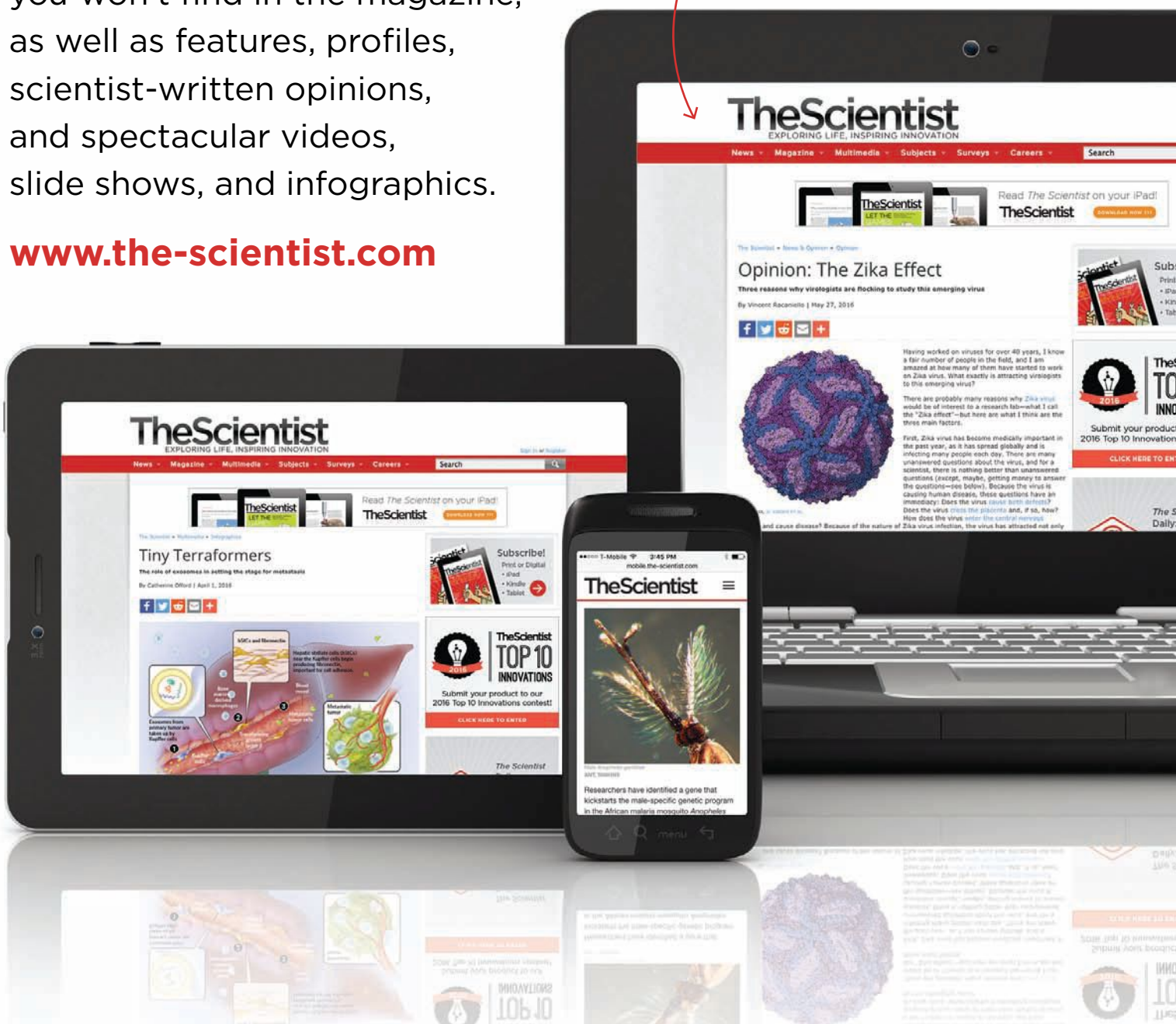
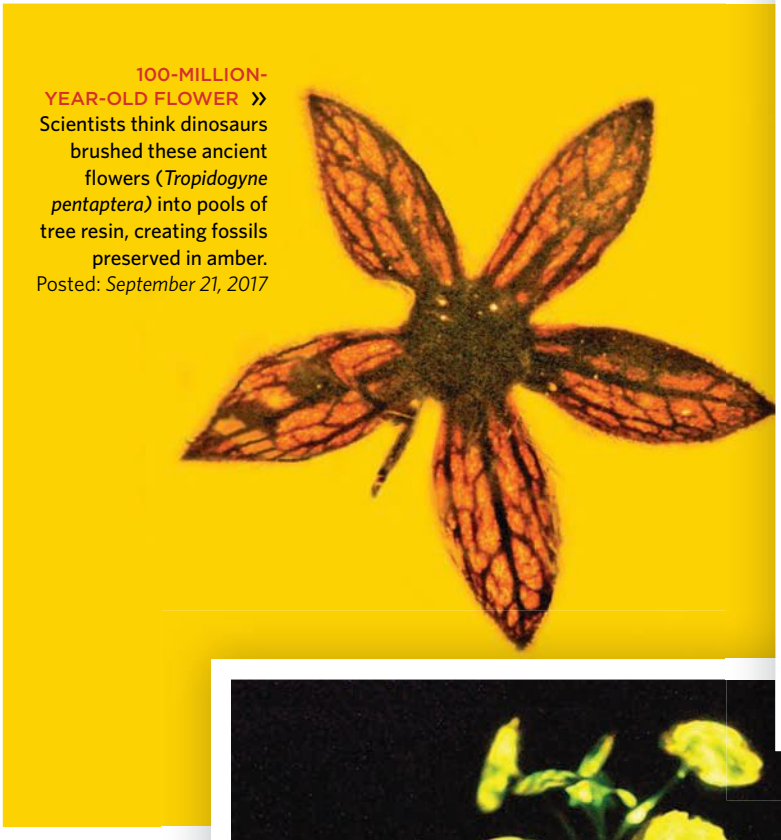
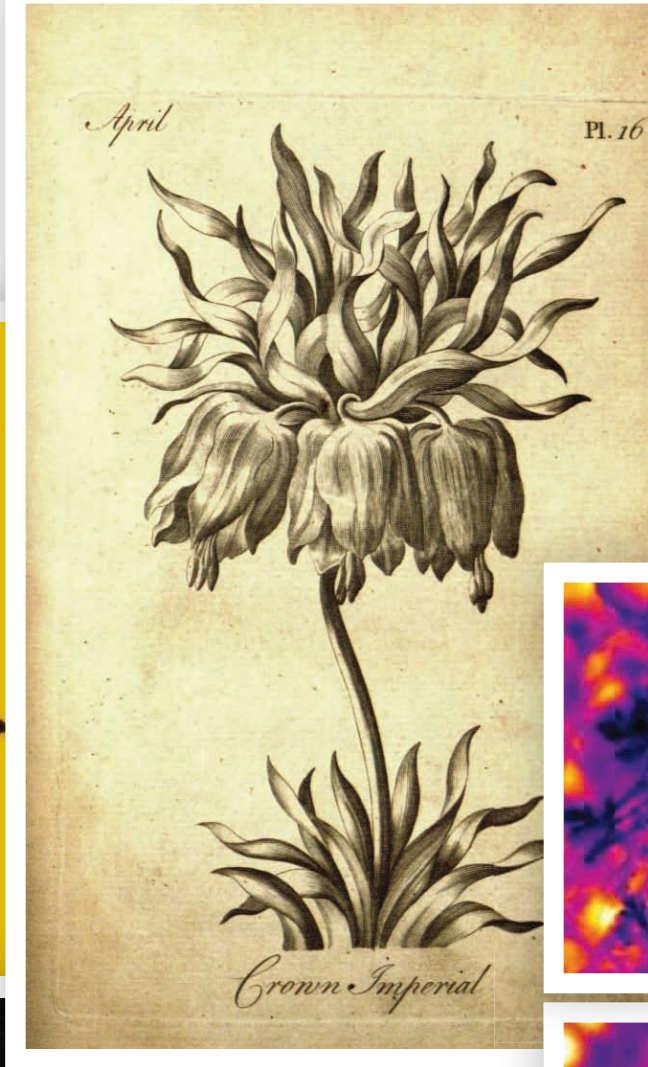


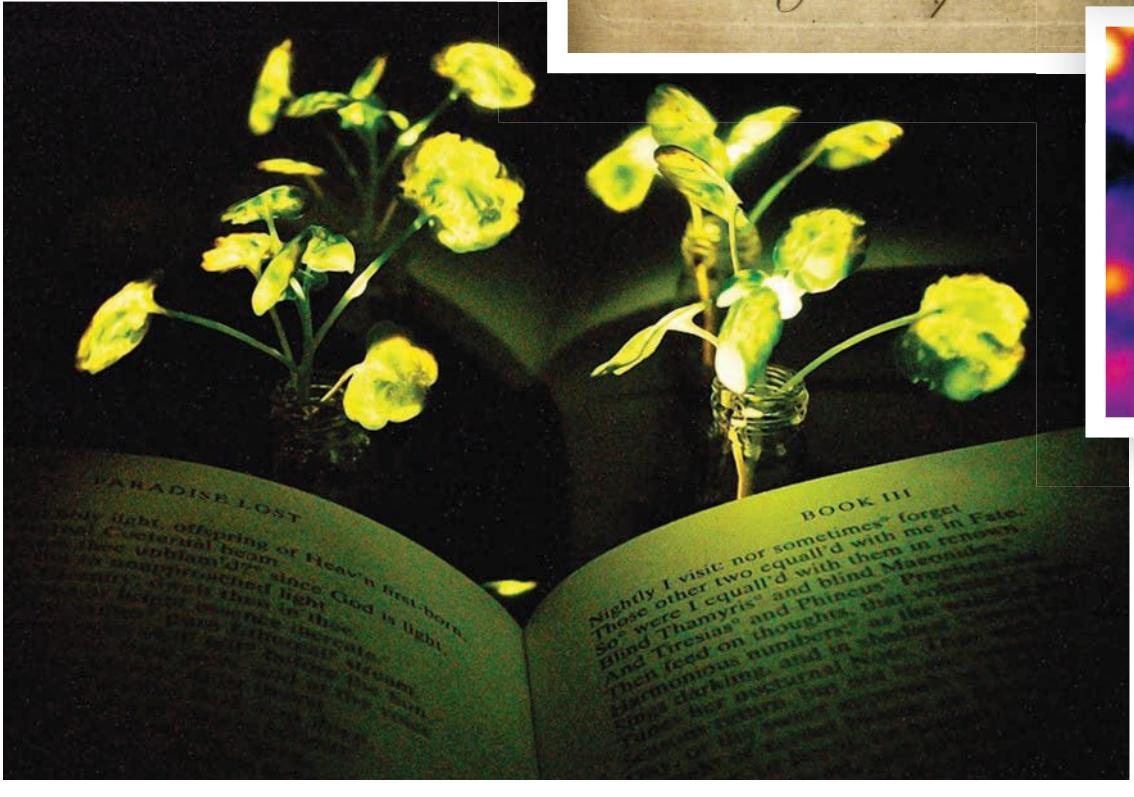
Caught on Camera

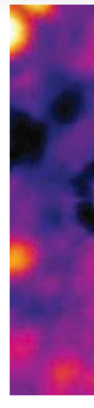
Selected Images of the Day from the-scientist.com



100-MILLION-YEAR-OLD FLOWER »
Scientists think dinosaurs brushed these ancient flowers (*Tropidogyne pentaptera*) into pools of tree resin, creating fossils preserved in amber.
Posted: *September 21, 2017*



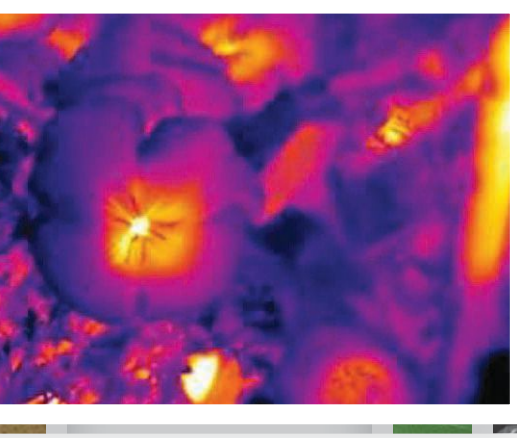


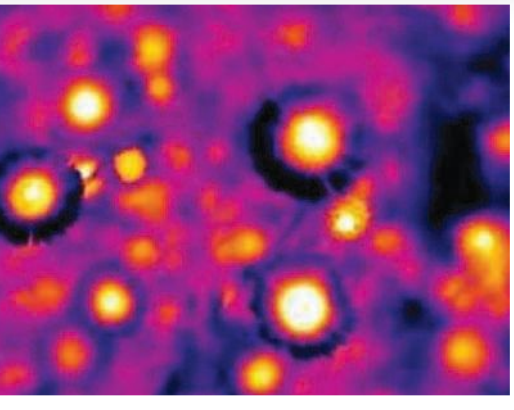


PLANT BULBS »
A "nanobionic" plant, infused with nanoparticles containing light-emitting luciferin.
Posted: *December 14, 2017*

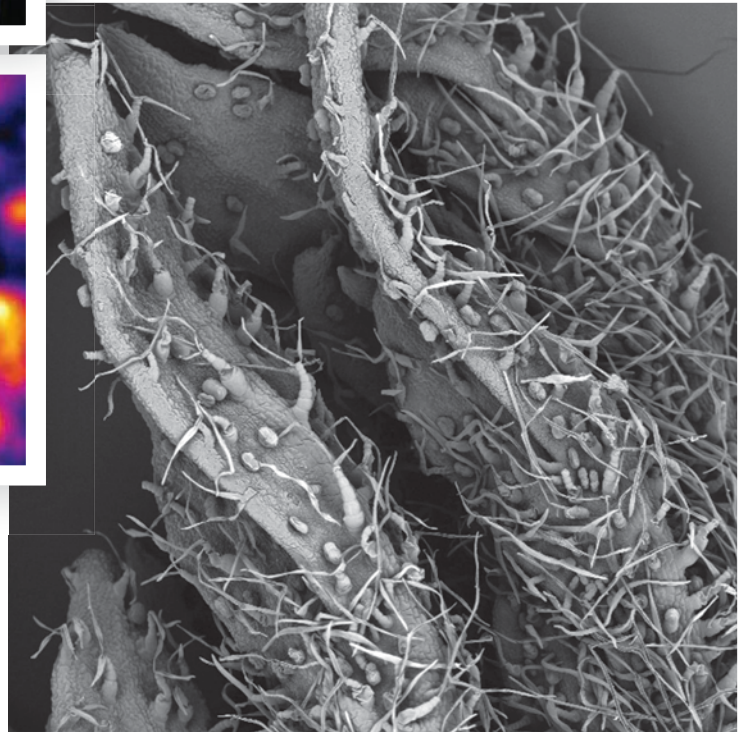
« MID-CENTURY PURSUITS
This image of a crown imperial flower (*Fritillaria imperialis L.*) is one of 60 plant canvasses in an 18th century coloring book.
Posted: *June 27, 2017*

» BREAK OUT
Spores of yellow rust, an agricultural pathogen, rupture the surface of a wheat leaf. A grant from the European Research Council, announced in 2016, is aimed at fighting yellow rust disease.
Posted: *September 9, 2016*





« WHAT DOES A BEE SEE?
Thermograph images show the floral heat patterns of poppy flowers (top) and daisies (bottom).
Posted: *December 21, 2017*

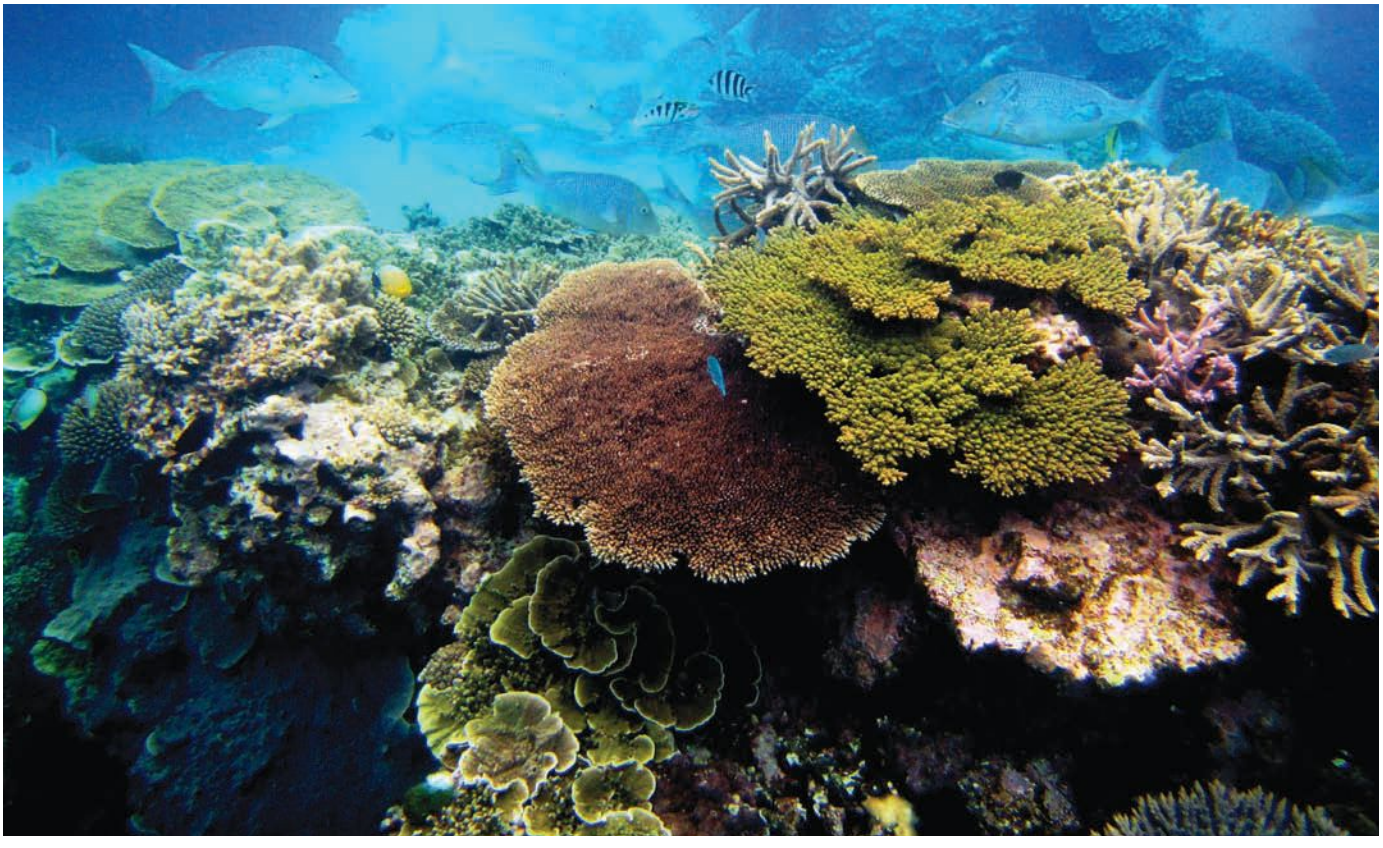


« DOUBLE DOSE
Scientists engineered the *Artemisia annua* plant to produce twice the normal amount of artemisinin, the main component in many malaria treatments.
Posted: *March 29, 2017*

100-Million-Year-Old Flower: George Poinar Jr, Oregon State University; **Mid-Century Pursuits:** Peter H. Raven Library/Missouri Botanical Garden; **Break Out:** The John Innes Centre; **Double Dose:** CRAG; **What Does a Bee See?:** University of Bristol; **Plant Bulbs:** American Chemical Society

Notebook

FEBRUARY 2018



REBECCA SPINDLER/TARONGA ZOO

READY TO FREEZE: Many stony corals such as *Acropora*, pictured here at Lady Elliot Island on Australia's Great Barrier Reef, are vulnerable to the effects of climate change. So researchers are working to cryogenically preserve them for later.

Cryo Corals

Mary Hagedorn is the first to admit she has a somewhat unusual research calendar. "My whole schedule is based on the moon cycle," she says. "I'm like a modern druid." But there's a scientific explanation: Hagedorn works with corals—animals that famously synchronize mass spawning events to nights just after a full moon.

For Hagedorn, a research scientist at the Smithsonian Institution and head of the international Reef Recovery Initiative, such coral spawnings, which occur just once a year for some species, mark the only opportunities to collect the animals' eggs and sperm—key ingredients for one of the latest approaches to coral conservation. As coral communities around the world succumb to climate change—and the attendant increases in water temperature and acidity—researchers such as Hagedorn are shifting their focus from primarily trying to protect corals to banking coral genomes in the form of gametes or other biological material for future generations.

"We're seeing more and more global changes, [and] the protections we're able to do are not enough," explains Hollie Putnam, an integrative biologist at the University of Rhode Island who studies how corals and other marine invertebrates respond to environmental stress brought about by a changing climate.

One approach that Hagedorn's team is exploring is to cryogenically freeze the coral egg and sperm cells to serve as seeds for future species reintroductions to marine environments. "Cryopreservation is an amazing tool for helping wildlife populations," Hagedorn says. "It can reverse extinction and maintain genetic diversity." The method has already been used by other researchers to preserve, and later thaw for artificial insemination, the sperm cells of critically endangered mammals such as the North American black-footed ferret (*Anim Conserv*, 19:102-11, 2016).

A few years ago, Hagedorn and her colleagues embarked on a proof-of-concept project using an important genus of

reef-building coral called *Acropora*. Starting in 2012, she and other members of the Reef Recovery Initiative spent a few years assembling samples of two species—*A. millepora* and *A. tenuis*—from populations at Australia's Great Barrier Reef. Professional collectors "go out and collect for about a week, and bring whole colonies back to the station a day before the full moon," she says. "We help them offload it and get it into tanks."

The researchers then waited for the newly collected animals to release their gametes, parceled up in tiny, buoyant egg-sperm bundles. "You could imagine it as a cluster of grapes," Hagedorn says. "You have several eggs packaged together tightly in this membrane, with a packet of sperm inside. Each little coral polyp produces that." These bundles rose to the surface and separated into eggs and sperm that the researchers then filtered for storage.

To cryopreserve the sperm, the researchers froze the cells using liquid nitrogen—cooling them down to –196 °C. The cells "just go into stasis, or suspended animation," Hagedorn says. Then, in a series of experiments in 2013 and 2014, the team thawed these cells and combined them in glass vials with fresh eggs to trigger fertilization. "Think of it as a human fertility clinic, except for coral."

The researchers found that although cryopreservation reduced the fertilization success of sperm—the frozen-then-thawed gametes were generally less motile than fresh sperm—they could produce viable larvae. Once transferred to larger tanks, these larvae successfully settled and began laying down a calcareous skeleton, just as those produced from fresh sperm did (*Sci Rep*, 7:14432, 2017). Although the team has yet to evaluate how well the larvae would do in a real marine environment—permits to introduce animals to coral reefs are hard to come by, Hagedorn explains—the project marks a first step in making cryopreservation of corals practical.

For now, the team's method falls short of a full restoration, notes Chris Langdon, a coral biologist at the University of Miami. "Preserving the sperm is only half the equation," he says. "A bunch of sperm is not going to do us any good if there aren't any eggs to fertilize." Egg preservation is more challenging, and Hagedorn tells *The Scientist* that for some coral communities, such as the ones she works with in Hawaii, the spawning events aren't always large enough to produce enough cells to work with; her group is now investigating alternatives that include preserving larvae and other parts of coral tissue.

BUNDLES OF JOY: *Acropora* corals release their gametes in the form of egg-sperm bundles during mass spawning events.

ANDREW HEYWARD/AUSTRALIAN INSTITUTE OF MARINE SCIENCE

"I think that's the right course of action," says Ken Nedimyer, president of the Coral Restoration Foundation, a Florida-based nonprofit that creates offshore nurseries for threatened coral species and has agreed to provide coral samples to Hagedorn's team. He adds that there are many other elements of corals, such as their algal symbionts and microbiomes, that would need to be preserved as well. "You can't just have a sperm and an egg, make a larva, and think you're going to restore coral reefs—it's not going to happen."

But Langdon notes that there's a bigger limitation to consider when it comes to Hagedorn's approach to coral restoration: there's no reason to believe that corals dying from oceanic conditions now would be able to survive any better in the likely warmer and more acidic oceans of the future. "That's my criticism of the current-day coral restoration efforts," he says. "It just doesn't make sense to me."

He is not alone in these concerns, and several research groups are investigating other—though not necessarily mutually exclusive—methods to preserve coral populations in a changing climate. Putnam, for example, aims to pin down the factors that make some corals particularly resilient to climate change and other, more specific environmental stressors. "If we made a comprehensive effort to identify [resilient individuals], and then interbred those to create new strains, and have the gametes from those, then we could reseed the environment with climate change–resistant strains," suggests Langdon, who is working on this approach.

Such selective breeding is just one of several tactics included in a conservation strategy known as assisted evolution. Other interventions to aid coral survival could include genetic reprogramming and microbiome manipulation. But first, Putnam says, researchers need a better grip on several aspects of coral biology: much remains unknown about the interactions of individual polyps with their algal symbionts, and the role of the coral microbiome. Then, "we can apply that understanding to these approaches," she says. "I think that's where we're headed right now."

In the meantime, Hagedorn and Nedimyer point out, frozen coral material, which could potentially last decades or even hundreds of years in storage, may help preserve genetic diversity that might otherwise be lost. "I don't want to get to the point where our only corals are frozen," says Nedimyer. "But I think somebody will look at it one day and say, 'I'm glad you guys did that when you did.' I think it's looking into the future, and trying not to lose something really valuable."

—Catherine Offord

Microbial Cartography

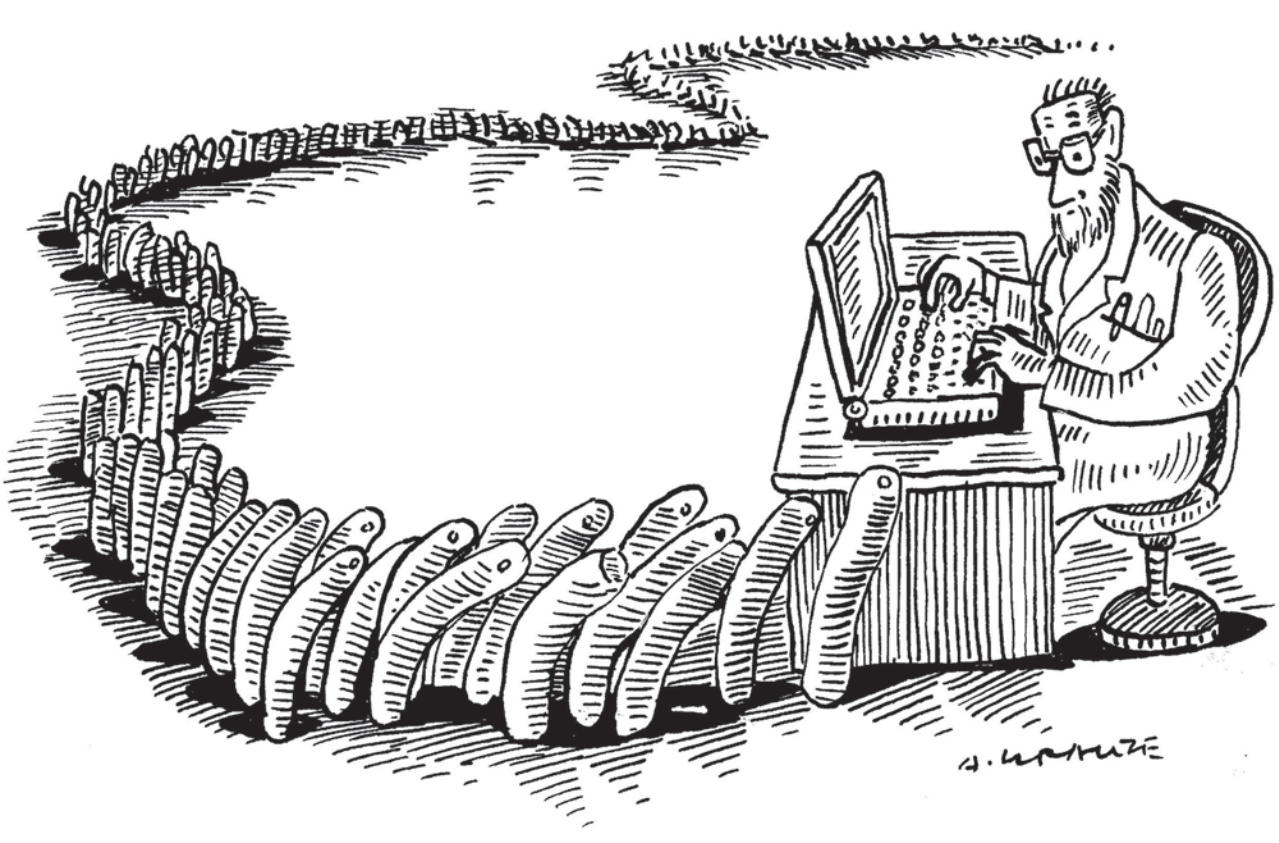
We are surrounded by an invisible world of microorganisms—including many species of bacteria, archaea, and fungi—that play fundamental roles in natural processes, from cycling carbon in soil to fermenting food in the mammalian gut. But until recently, there hasn't been a standardized way of documenting these ubiquitous little organisms, making it difficult to fully understand the extent of their functions on Earth.

In the summer of 2010, 26 leading experts in microbiology and bioinformatics congregated for a workshop in Snowbird, Utah, to discuss the challenges standing in the way of achieving this goal. The trouble, the group concluded, was that while laboratories across the world were rapidly advancing their knowledge of microbes using genetic sequencing, they were going about it in completely different ways, which made it difficult to compare one group's data on microbial samples to another's.

"People who studied particular systems tended to use different protocols, passed down from one person to another in the lab," explains workshop attendee Rob Knight, a microbiologist at University of California, San Diego, School of Medicine and the founding director of the Center for Microbiome Innovation at San Diego. "So a lot of the time it was very difficult to tell what was truly a biological difference" versus what was an artifact of differing methodologies.

Knight was particularly interested in global ecological questions about how microbial communities are distributed, and what factors dictate their distributions. So, together with two colleagues from the workshop, Janet Jansson, chief scientist for biology and laboratory fellow at Pacific Northwest National Laboratory, and Jack Gilbert, a professor of surgery at the University of Chicago and director of the Microbiome Center, Knight founded the Earth Microbiome Project (EMP). Its goal was simple: "Get started with characterizing microbes on a scale that nobody had known before," Knight says.

The trio put out a call to microbiologists around the world to send physical samples to one of their three lab-



ANDRZEJ KRAUZE

oratories, with the promise that the team would sequence the microbes harbored in them according to a standardized protocol and make the resulting genomic data publicly available. These included host-associated samples, such as those from primate guts or the skins of Komodo dragons; aquatic ones, collected from oceans and lakes; and sediment and soil samples, gathered everywhere from the ocean floor to the Alaskan permafrost.

Not everyone thought the project was doable, however. For example, Jonathan Eisen, a professor in evolutionary biology at the University of California, Davis, who also attended the 2010 workshop, told *Nature* in 2012, "Knight and Gilbert literally talk about sequencing the entire planet. It is ludicrous and not feasible—yet they are doing it."

Knight had his own doubts, too: one initial concern was that even with the lure of free sequencing, researchers would want to hang on to their own samples. "But to my delight that turned out to not be true," he says. More than 500 researchers sent in samples, from 43 countries across the world. The team soon had thousands of samples—all neatly packed into about 25 freezers across the three founders' laboratories.

The researchers' meta-analysis, published late last year in *Nature* with 300 coauthors, describes almost 28,000 samples from labs around the world (*Nature*, 551:457-63, 2017).

To catalog the microbes, the team developed a standardized protocol that involved probing for and sequencing the 16S ribosomal RNA (rRNA) gene, which serves as a unique barcode for species of bacteria and archaea. They also used a new method to remove sequencing errors in the data to ensure accuracy. The researchers managed to build a framework to denote where the sequence came from, and which other sequences it was found with—making the addition of any additional sequences to the database easier.

Using this protocol, the team detected a total of 307,572 unique 16S rRNA sequences from the microbial samples. For around 90 percent of these sequences, precise matches could not be identified in reference databases. For Jansson, the database opens up many possibilities: "If we get a sequence and we don't know where it comes from, [we] could have a good probability of finding that it was a soil microbe or one that was associated with a host, or an aquatic microbe, just based on this sequence."

More than 500 researchers sent in samples, from 43 countries across the world.

The researchers also performed a meta-analysis in order to explore ecological principles in microbiology. For instance, they debunked the notion that microbial richness correlates positively with temperature—in fact, data from non-host-associated samples suggest that microbial richness peaks at a narrow and relatively cool temperature range, and then declines, depending on pH and the type of sample.

It's not the only study to take advantage of the EMP's resources. The EMP also undertook the DNA extraction, sequencing, and analysis of the samples involved in about 100 other individual studies. These contributions to the database have caused it to steadily grow since the first entries were made public in 2011.

One global study of the microbes associated with sponges demonstrated that these microorganisms are major contributors to the microbial diversity of the world's oceans (*Nat Commun*, 7:11870, 2016). A different study took a close look at the gut microbes of ant- and termite-eating placental mammals such as aardvarks and pangolins, and found that diet and phylogeny are both important factors in shaping the evolution of mammalian gut microbiota (*Mol Ecol*, doi:10.1111/mec.12501, 2013).

Some researchers went to great lengths to collect the samples that fueled these projects. This includes Jansson herself, who happened to be working on a collaborative research project on microbial communities and oil when the Deepwater Horizon oil rig exploded in the Gulf of Mexico in 2010. Before the wellhead was closed, oil and gas company British Petroleum agreed to send out fleets to collect sediment samples from the 1,500-meter-deep seafloor to find out how microbes in ocean sediments were responding to the oil spill. Jansson was able to show in real time how microbes were helping digest large amounts of oil that would otherwise have reached the shoreline, and she identified new microbes that had genes for oil degradation. (*ISME J*, 8:1464-75, 2014).

"We now have ideas about who they are, even though we've never cultivated them," she tells *The Scientist*. "That was so exciting." She enthusiastically added her samples to the database, where anyone can view them.

Thanks to efforts such as these, the total number of samples collated by the EMP has now reached 100,000, says Knight. He hopes the database will grow even further: the team has made its sequencing protocol publicly available, so that laboratories across the world will be able to contribute their own data directly. And Janssen notes that the scale of the database will allow many researchers to make predictions about what kinds of microorganisms to expect in different environments—or indeed the inverse: to link a microbe to its environment of origin based only on its 16s rRNA sequence.

Being able to make such links could have applications across many scientific disciplines, from microbiology to forensics. For example, in 2001, when at least 22 people contracted anthrax that had been mailed through the US postal service, the FBI was able to use genetic analysis to trace back the spores to the likely source, a single flask in a laboratory in Maryland. A similar approach could also be used to pinpoint the source of food- or water-borne microbial pathogens, or microbes found in specks of dirt at crime scenes.

It may be a while before such evidence routinely reaches the courtroom, says Randall Murch, a former Special Agent and senior executive with the FBI who led the creation of a department

within the agency devoted to combining microbiological and forensic sciences to support bioterrorist and criminal investigations. But databases such as the EMP's could significantly contribute to propelling the field forward, he says. "Anyone in this field understands that repositories—properly constructed repositories of microbes—are crucial."

—Katarina Zimmer

Saving Monkey Island

When Hurricane Maria ripped through the Caribbean last September, the small town of Punta Santiago, Puerto Rico, was devastated. Many homes were destroyed, and people lost reliable access to electricity, clean water, and food. In addition to making sure their own families and neighbors had what they needed to get by, some of Punta Santiago's residents had another pressing concern: the fate of 1,700 rhesus macaques living on an island a kilometer away.

The monkeys inhabit Cayo Santiago, a 38-acre landmass off the east coast of Puerto Rico. The animals are the descendants of about 400 macaques brought to the island from India in 1938 by Clarence Carpenter, a primatologist then working with the School of Tropical Medicine in Puerto Rico. According to Richard Rawlins, a former director of the research site, Carpenter, seeing trouble ahead, established what came to be known as *Isla de los monos* (Monkey Island) as insurance against losing access in wartime to animals needed for vaccine development and other biomedical experiments.

Caretakers who live in Punta Santiago provide the monkeys with food and water—there is no natural source of fresh water on the island apart from rain—but let the animals roam free. The primates have thrived and have served as a resource for generations of animal behaviorists, psychologists, primatologists, and researchers in a variety of other fields.

As they followed the news of Maria and its aftermath, researchers living outside Puerto Rico who had studied the island's monkeys were deeply worried about the staff, the animals, and the residents of the town who'd hosted them. These scientists included Carol Berman, a behavioral ecologist at the University at Buffalo who's studied the Cayo Santiago macaques since 1974.

Cayo Santiago was Berman's second choice as a research site. She'd been attracted to the study of natural behavior patterns in primates after reading a story about Jane Goodall as a teenager, but the famed researcher's site in the Gombe Stream in Tanzania wasn't taking new trainees when she reached graduate school. But Berman soon came to appreciate the Puerto Rican site's unique advantages. Notably, all of the animals on the island have carefully documented histories and are habituated to humans. "It's a great deal easier to study animals that are well-known" than most of those that are truly wild, she says. In contrast to captive monkeys, those on Cayo Santiago are free to form natural social groups. Over the years, Berman and her team have observed the animals to investigate questions such as how the monkeys' parenting styles change as their tribes grow (*Anim Behav*, 53:405-21, 1997), and other ways in which family relationships influence their behavior (*Am J Primatol*, 78:63-77, 2016).

Laurie Santos, a cognitive psychologist at Yale University who's been doing research on Cayo Santiago for 23 years, says she's also gleaned useful scientific insight from the island's resident monkeys. The macaques have enabled her team to answer questions such as whether the monkeys know that other animals have thoughts and can intuit what those might be. This ability, termed theory of mind, had previously only been seen in apes. Santos's group suggested that rhesus macaques also possess this skill (*Am J Primatol*, 78:106-16, 2016).

Rawlins, who has taken part in aiding post-storm recovery, served as the scientist in charge of the site from 1976 to 1981. Over the years, the island has helped researchers answer foundational questions in various fields, he says, adding that the meticulous, multigenerational data on the macaques, as well as their blood and skeletal samples, will likely help uncover links between behavioral traits and genes. From 1956 onwards, scientists have been closely monitoring these animals, tracking their maternal linage and offspring, and collecting blood samples and skeletons, he adds. Since then, "every single animal born on that island is of a known lineage and descent."

> At least for me, going as long as I have, it feels like home.
>
> —Laurie Santos, Yale University

Days after Hurricane Maria ravaged the Caribbean, Angelina Ruiz-Lambides, associate director of the Cayo Santiago Biological Field Station, boarded a helicopter to assess the damage to the area. The hurricane had destroyed Monkey Island's infrastructure and cut a gap through a narrow sand isthmus connecting its halves. The good news was that the macaques themselves seemed to have survived: all six of the island's social groups were spotted, and Ruiz-Lambides and her census team didn't find any dead monkeys on their initial walks around the island, she writes in an email to *The Scientist*.

No humans live on Cayo Santiago—caretakers commute to work by boat each day. With docks destroyed or severed from the land by Maria, Ruiz-Lambides, who was seven months pregnant at the time, and other staff regularly waded into the ocean to climb into a boat for the trip. The team set to work restoring the island, rigging up new water collection and storage systems to replace the ones destroyed by the storm.

In the days following Maria, Berman and more than a dozen other Cayo Santiago "alumni" tried to reach friends and colleagues on the island to check on their safety. Some of the concerned researchers started organizing GoFundMe cam-

BONN AURE

ISLAND HOP: Caretakers and research scientists commute to the monkeys' home by boat.

paigns while others began planning a trip to help with cleanup and restoration. Forty people, including some alumni, took a volunteer trip to Punta Santiago and Cayo Santiago in late December and early January, according to one of the organizers, Steven Schapiro, a veterinary professor at MD Anderson Cancer Center.

Although she is doing less fieldwork as her career advances, Santos says she still spends about two weeks a year at the site—2017 marked one of the few Thanksgivings she was not there. "At least for me, going as long as I have, it feels like home," she tells *The Scientist*.

Rawlins has been struck by the actions of the Cayo Santiago staff, who have been caring for the animals, restoring the island, and organizing aid for other Punta Santiago residents, despite losing homes and resources themselves. "If you look at what they've done in the face of the total lack of government support . . . it's really wonderful," he says.

The time since the storm has been extremely challenging, and the staff members "still have a long road ahead," Ruiz-Lambides writes. But she says she hopes that enough funding will be secured to build a new, concrete research facility on the island. "We are all eager to start our 'new normal' and to restart the research," she adds. "You can already see the trees are growing leaves, and we had over 30 [monkey] births post-Maria, which gives us some hope amongst all of the evident destruction."

—Shawna Williams

Flies R Us

When Xiao-Long Lin started a master's/PhD combo program in the College of Life Sciences at Nankai University in Tianjin, China, in 2010, he was just hoping that the degree would help him land a job. But once he got a taste of identifying new insect species, there was no turning back.

He focused on cataloging diversity in nonbiting midges of the chironomid family. "I spent all my time collecting a lot of specimens in summer and in winter in one province," he recalls. "I love it. It's like an adventure to discover more species." All told, he collected thousands of specimens in China's Zhejiang province, comprising more than 300 species—38 of which were new to science.

Given the size of the chironomid family, though, perhaps those numbers should not be all that surprising. More than 6,000 chironomid species have been described so far, and "molecular evidence for so-called cryptic species—that is, look-alikes that actually are genetically separate—suggests at least a doubling or more," Peter Cranston, an honorary professor at Australian National University and emeritus professor at the University of California, Davis, writes in an email to *The Scientist*. Indeed, estimates range from 20,000 to 40,000 species total for this group. "Within the flies (Diptera), the family Chironomidae certainly is amongst the more diverse groups," says Cranston, who coauthored *The Insects*, a popular entomological textbook.

Lin wrapped up his work at Nankai University in 2013, but he wanted more. So he headed off to Norway to work with chironomid researchers Elisabeth Stur and Torbjørn Ekrem at the Norwegian University of Science and Technology's (NTNU) University Museum in Trondheim. Over the past four years, he has continued to collect and characterize chironomid specimens, using a variety of trapping methods in the field. He took trips back to China and also collected around Norway, and he received specimens from other researchers who'd visited different parts of the world, including South America and South Africa. Chironomids are found worldwide, including in Antarctica, where they are the only native insects. "You have them literally everywhere," says Ekrem.

In his earlier work, Lin had characterized the midges he collected based on their physical characteristics. But morphological analyses are not always reliable, he says, as sometimes unexpected variation within a species can mislead even the most trained eyes. So at NTNU, Lin adopted DNA barcoding techniques that use genetic data, exploring established and new markers to more definitively delineate species boundaries in this group.

From the tens of thousands of fresh samples he acquired, Lin prepared more than 2,000 slides for morphological analysis and analyzed the genomes of about 500 of those specimens. In the end, he identified an additional 30 new chironomid species (eight of

ONE AMONG MANY: *Stenochironomus gibbus* (top) and *Manoa xianjuensis* (bottom) are just two members of the hyperdiverse Chironomidae family of flies.

CHUN-TANG CHEN; XIAO-LONG LIN

which he describes in *Insect Syst Evol*, doi:10.1163/1876312X-00002172, 2017) and added about 1,000 DNA barcodes for more than 350 species to the growing reference library database. "Xiao-Long further expanded our reference library on chironomids," says Stur. "His contribution to the barcode reference library has been really good and will help future monitoring of this group using DNA barcoding."

Ekrem adds that the diversity of this group never ceases to amaze him. "When it comes to morphology, you think you've seen most of the different combinations of characters when you have been looking at a few thousand species. But whenever you discover a new species, there's yet another combination of features."

Chironomids' diversity and widespread range can be a valuable tool for monitoring the ecosystems that the insects are a part of. It's possible to "use species compositions in different environments to say something about the health of that ecosystem," says Ekrem. In fact, the European Union has requested that chironomid surveys be incorporated into assessments of water quality, Cranston notes.

In addition to monitoring today's ecosystems, researchers are also using chironomid diversity to recreate past environmental conditions, analyzing the fossil assemblages in core samples taken from various soils and sediments to estimate mean temperatures thousands of years ago, for example. And, of course, as researchers dig deeper into the past, they're only going to add to the impressive diversity of chironomids. "We haven't even mentioned the astonishing preservation of (and abundance of) chironomid adults in ambers, some of great age (100 million years old)," Cranston says.

Although the task of cataloging this hyperdiverse group can seem overwhelming, researchers in the field are not dissuaded, says Ekrem. "Let's sample as much as possible, get barcodes on all these species, without really knowing what they are yet. . . . It's a way to get the genetic data registered, and get it out there so people can see it."

And Lin, who defended his master's/PhD thesis at Nankai in 2015 and officially wrapped up his PhD at NTNU last summer, says he doesn't mind putting the effort in. "It's basic work, but someone has to do this." —Jef Akst

LAB TOOLS
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COMINGSOON | Genetic Variant Detection in Cancer: Using ISH to Track Tumor Evolution

Intratumor heterogeneity (ITH) is a major underlying cause of therapy resistance and disease recurrence and is a read-out of how a tumor has grown. Current methods to analyze genetic ITH rely on the sequencing of "bulk" or flow-sorted populations, in which the spatial context of tumor subclones is not preserved, and rare subclones may not be detected. These shortfalls can be addressed with ACD's BaseScope™ ISH assay—a unique mutation-specific RNA in situ hybridization assay. The BaseScope assay represents a significant technical advance for in situ mutation detection and provides new insight into the mechanisms of tumor evolution with potential ramifications for selecting patients for treatment. Join us to learn more about this new approach to ITH analysis.

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TOPICS TO BE COVERED:

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- How the BaseScope ISH assay enables reliable detection of ITH

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TS WEBINARS

COMINGSOON | Are All Neurodegenerative Diseases Made Equal?

Various neurodegenerative processes result in the development of diseases like Alzheimer's (AD), Parkinson's (PD), amyotrophic lateral sclerosis (ALS), and, arguably, multiple sclerosis (MS). Despite a vast research effort, drug discovery initiatives, and promising clinical trials over the years, these diseases remain incurable. But recent studies have suggested mechanistic links between such diseases. Atypical protein assembly resulting in plaque formation is a common pathological finding in both AD and PD, while neuronal death is a primary (ALS) or secondary (MS) hallmark of disease. For a detailed look at the underlying mechanisms that drive an array of neurodegenerative diseases, *The Scientist* is bringing together a panel of experts to share their research, discuss current therapeutic approaches, and offer their insights. Attendees will have the opportunity to interact with experts, ask questions, and seek advice on topics related to their research.

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- Whether primary and secondary neuro-degeneration distinctions are based on biology

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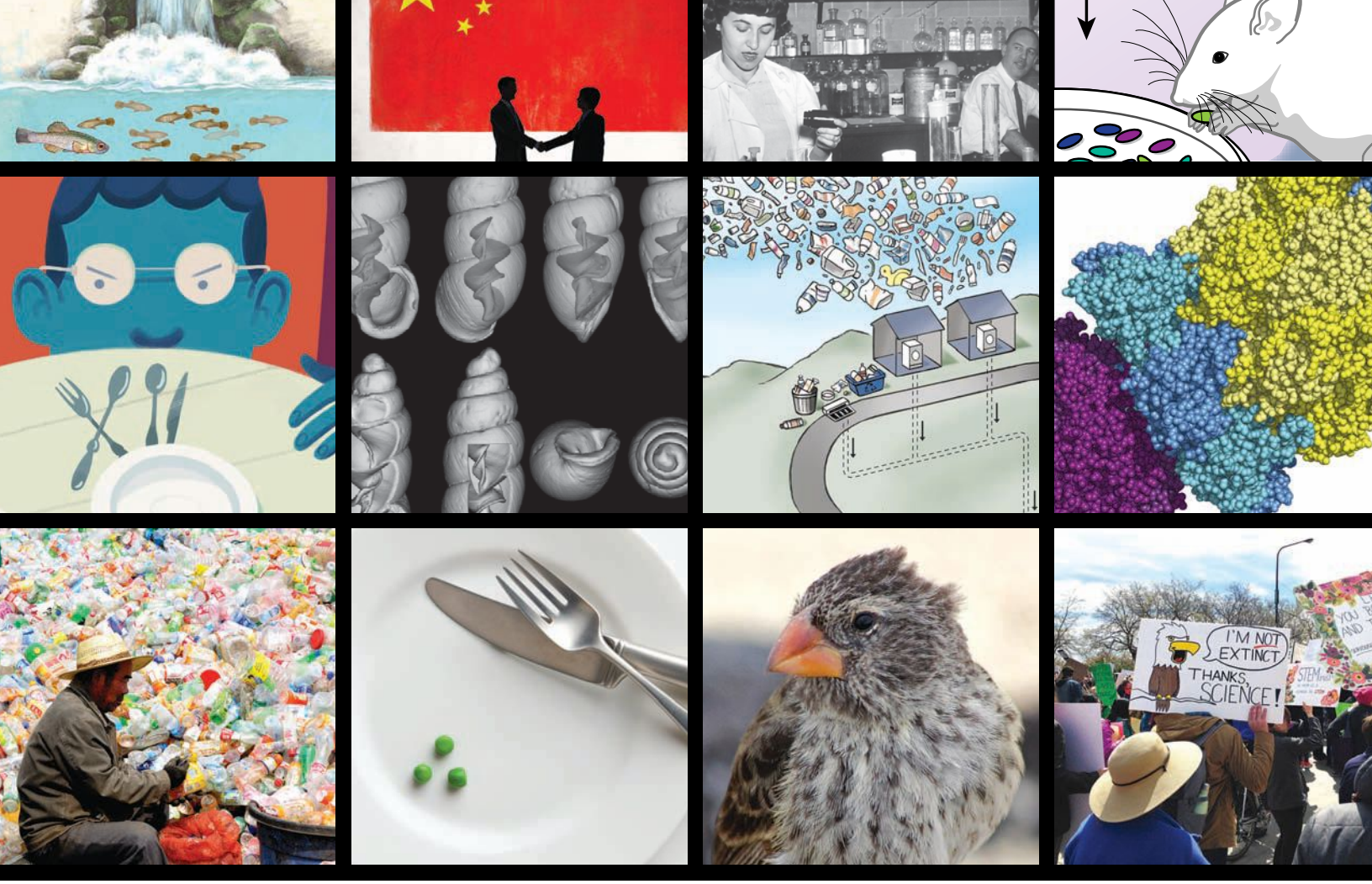

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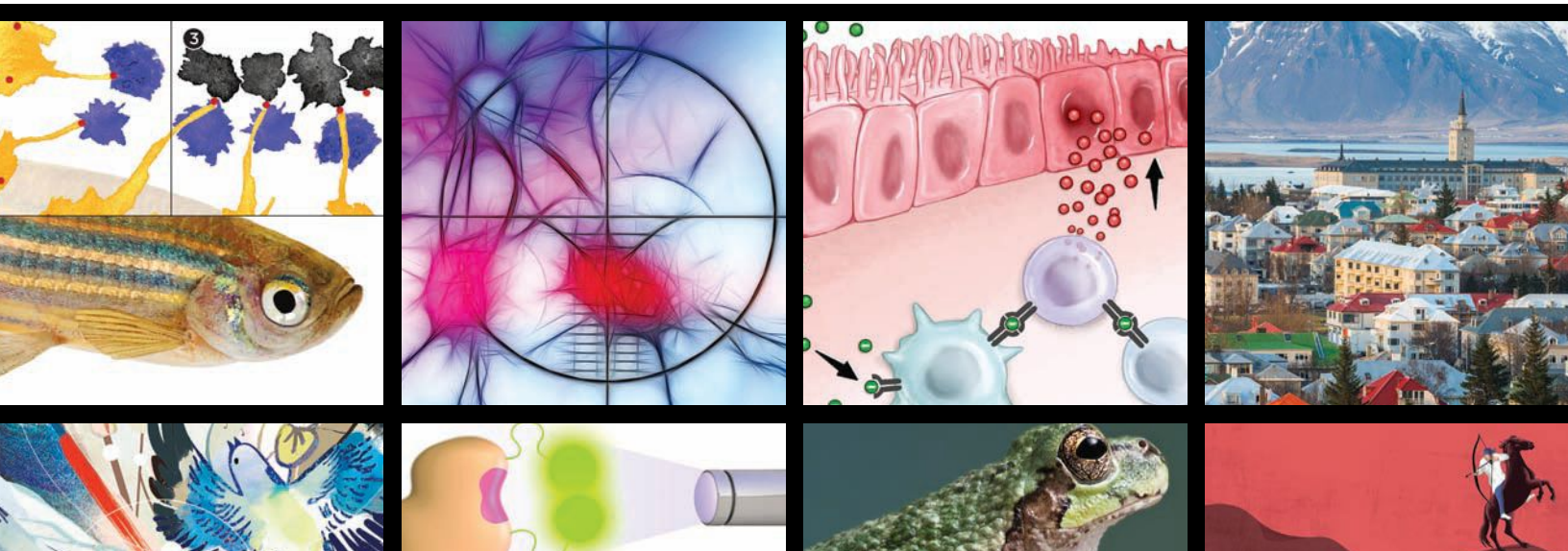
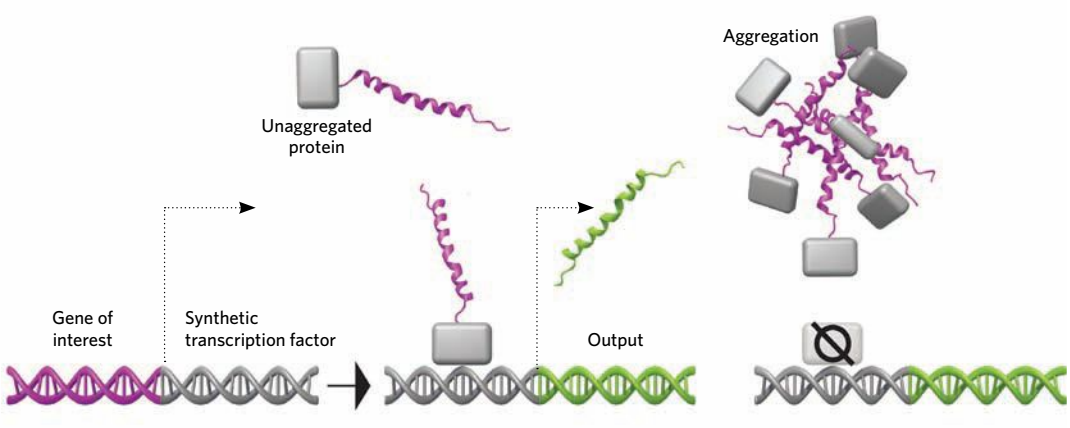


Detecting Protein Clumps

A synthetic genetic tool called yTRAP allows high-throughput detection of protein aggregates in cells.

BY RUTH WILLIAMS



AGGREGATION ASSAY: To detect clumping of a protein of interest, express it together with a synthetic transcriptional activator domain (grey). If the protein remains soluble, the reporter gene (green), which is under the control of a synthetic promoter that corresponds to the activator, will be expressed. If the proteins clump together, it will not.

The aggregation of cellular proteins into insoluble clumps is a hallmark of many diseases, including Alzheimer's, Parkinson's, systemic amyloidosis, prion diseases, and type 2 diabetes. Protein agglomeration can also be a feature of normal cellular functions, such as signal transduction, synapse modification, and the regulation of RNAs during cellular stress.

Tools for studying such physiological and pathological protein aggregations, however, are limited, explains biomedical engineer Ahmad Khalil of Boston University. The principal options for researchers, he says, are either to destroy cells and analyze their innards for protein aggregates, or append a fluorescent tag to the proteins of interest within cells and view the formation of clumps (bright spots) with a microscope. While this second option maintains the protein's normal physiological surroundings, Khalil says, "inherently it is not a very high-throughput way of studying this phenomenon."

Khalil and colleagues' new approach, called yeast transcriptional reporting of aggregating proteins (yTRAP), allows for high-throughput analysis and doesn't destroy cells. An aggregation-prone protein of interest is first fused to a synthetic transcriptional activator, which can drive gene expression from a synthetic promoter only when the protein is not aggregated. Linking the synthetic promoter to a fluorescent reporter allows easy identification and, if desired, sorting of cells with and without aggregates.

The team has used yTRAP to detect accumulations of prions and other proteins in yeast; to perform a high-throughput screen for aggregation-preventing mutants; and to identify cells that "remembered" an environmental stimulus (heat)—using a yeast strain engineered to express a stably aggregating prion under the control of a heat-responsive promoter.

The assay "can inform us about a very important cellular process," says Madan Babu of the MRC Laboratory of Molecular Biology in Cambridge, U.K., who was not involved with the project. But it also "can be applied to a number of different questions," he says. "It's a bit of a tour de force." (*Cell*, 171:966–79, 2017) ■

AT A GLANCE

AGGREGATION DETECTION TECHNIQUE	HOW IT WORKS	ASSAY EQUIPMENT	HIGH THROUGHPUT CAPACITY	CELL TYPES
Fluorescent tagging of aggregation-prone protein	Proteins are fluorescently tagged—either by being engineered as fluorescent fusion proteins, or by applying fluorescent antibodies. Soluble proteins appear as diffuse fluorescence, while aggregates appear as bright spots.	Fluorescence microscope	Poor	Any
yTRAP	Proteins are fused to a synthetic transcriptional activator. The fusion protein is then expressed in cells where corresponding synthetic promoters drive expression of reporters. Soluble proteins activate the reporter, while aggregated proteins do not.	Fluorescence microscope, flow cytometer, fluorescence-activated cell sorter (FACS), or fluorescence microplate reader	Good	Limited to yeast so far, but future versions are planned for other cell types

MODELING THE MICROBIOME: Using synthetic communities of microbes to colonize *Arabidopsis* plants grown in a sterile substrate—the botanical equivalent of germ-free mice—researchers can begin to understand how the microbiome affects plant health.

The Plant Microbiome

It has become increasingly evident that, like animals, plants are not autonomous organisms but rather are populated by a cornucopia of microorganisms.

BY DAVIDE BULGARELLI

A few years ago, as a postdoc in the lab of Paul Schulze-Lefert at the Max Planck Institute for Plant Breeding Research in Cologne, Germany, I used next-generation sequencing to study the bacterial communities that populate roots of the model plant *Arabidopsis thaliana*. Although scientists had known for many years that roots interact with a variety of microorganisms, the composition of these communities was still poorly understood. As our sequencing data began rolling in, I was stunned by the staggering taxonomic diversity of bacteria that a single, tiny root can host. Yet there was an order in this apparent chaos. Almost invariably, members of the phyla Actinobacteria, Bacteroidetes, and Proteobacteria were enriched, differentiating the root specimens from the surrounding environment.

Subsequent studies by other labs supported our findings and posited Firmicutes as an additional dominant member of the plant microbiota. In addition to these bacterial groups, genomic surveys of plants have revealed certain fungal and eukaryotic microbes. And all of these groups of organisms are making themselves at home not just beneath the soil in and around plants' roots, but in other tissues, such as leaves, as well.

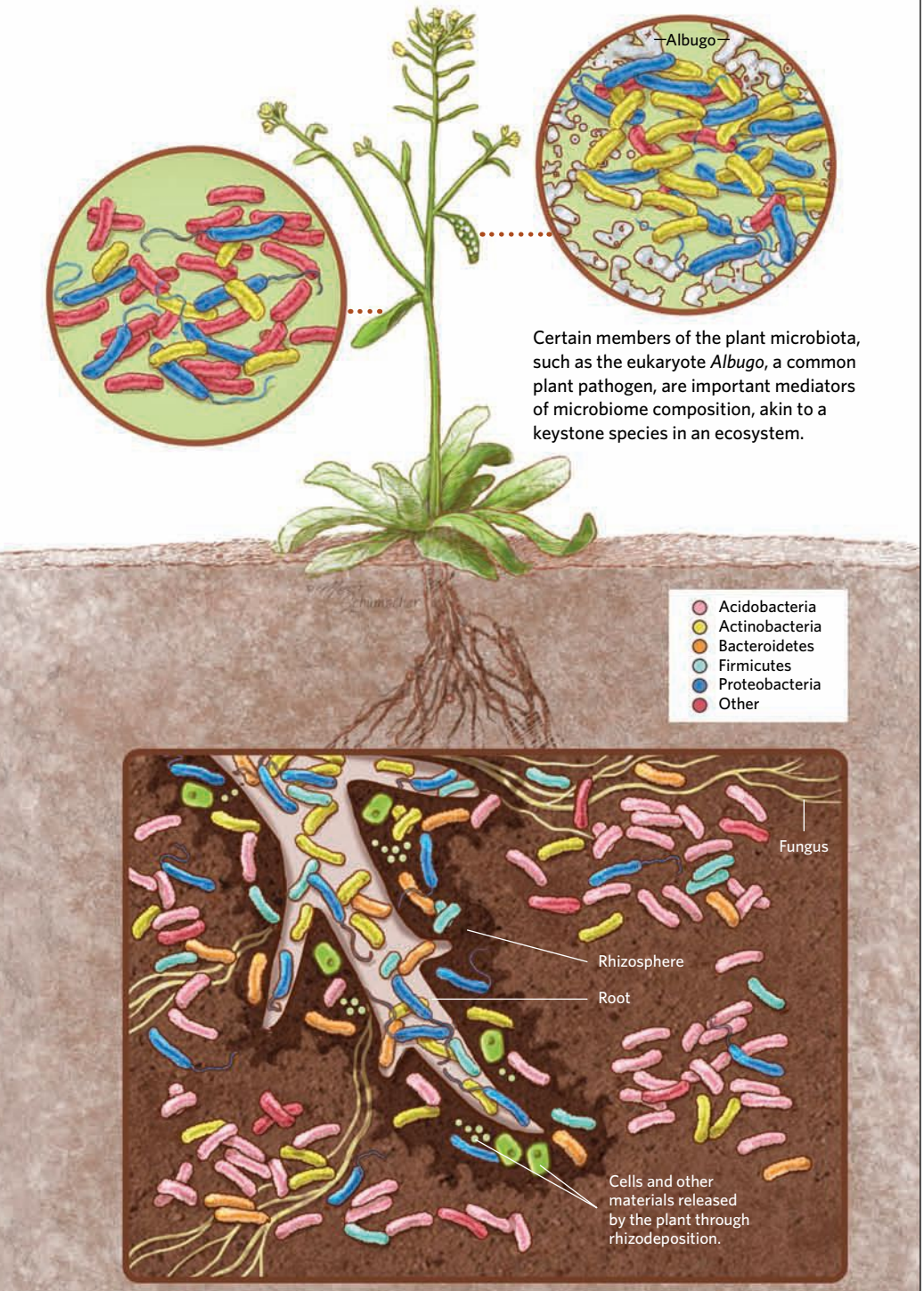
This research immediately raised new questions: Why were certain microbes more abundant in roots and leaves? How did these microbial communities assemble? And most critically, how did they affect plant health?

Recently, in addition to genomic surveys of the microbes present in various plant tissues, researchers have begun to probe the functional consequences of these bacterial, fungal, and eukaryotic symbionts. A better understanding of the molecular dialog between plants and their microbiota could revolutionize agriculture. The world population is expected to reach 9.8 billion in 2050, more than 30 percent larger than at present. This will put enormous pressure on food production globally—pressure that won't be relieved solely by the agrochemicals farmers currently use to increase yield and protect crops from pests and pathogens. To encourage a sustainable food source for humanity, radical changes in the crop production process are needed—changes that could come in the form of microbial manipulation.

The interface between plant roots and soil—a zone called the rhizosphere—and the root itself are sites of colonization for microbes capable of enhancing mineral uptake by the plant, of both actively synthesizing and modulating the plant's synthesis of chemical compounds called phytohormones that modulate plant growth and development, and of protecting plants from soil-derived pests and pathogens. For these reasons, scientists are looking to manipulate the microbes populating this belowground habitat to sustainably increase crop production. And in my lab, we are looking at ancient varieties and wild relatives of crops as a source of insights into beneficial associations between plants and microbes that could be adapted for agricultural settings.

PLANTS' MICROBIAL COMMUNITIES

Like animals, plants host communities of microbes that influence a wide variety of their biological processes. Recent surveys of the plant microbiome have begun to document which species are present—including not just bacteria, but fungi and microscopic eukaryotes as well—and how they affect the plant's health and functioning.



Plant cell material and organic compounds released by roots promote the growth of certain bacteria in the general soil microbial community. The plant's genotype further fine-tunes the bacterial community that grows on, in, and around its roots.

Surveying the plant microbiome

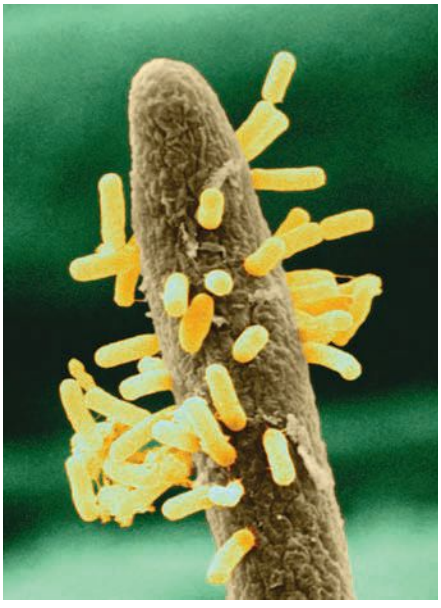
The roots of land plants thrive in soil, one of the richest and most diverse microbial reservoirs on Earth. It has been estimated that a single gram of soil contains thousands of different bacterial species, not to mention other microorganisms such as archaea, fungi, and protists. Perhaps not surprisingly, the establishment of interactions with the soil biota represented a milestone for plants' adaptation to the terrestrial environment. Fossil evidence suggests that the first such interactions with fungal members of the microbiome occurred as early as ~400 million years ago.[1]

Comparative studies indicate that soil characteristics such as nutrient and mineral availability are major determinants of the root microbiome. Just as digestive tract microbes interact with the food consumed by vertebrates, the root microbiome mediates the soil-based diet of plants. Also paralleling host/microbe interactions in the animal kingdom, individual members of the plant microbiome appear to be compartmentalized. I and other researchers working with *Arabidopsis* and with rice have identified at least three distinct microbiomes thriving at the root-soil interface: that in the rhizosphere; another one on the root surface, or rhizoplane; and a third one inside the root, an area known as the endosphere.[2,3] In all three compartments, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria dominate the bacterial communities in multiple plant species. The aboveground portions of plants such as leaves show similarly predictable microbial composition. (See illustration at left.)

While the categories of microbes that make up the plant microbiome are largely conserved, much variation exists in the species compositions of these communities across hosts. One key factor in determining how the microbiome is populated and maintained appears to be the plant's release of organic compounds into the rhizosphere, a process known as rhizodeposition. The amount and composition of these organic deposits vary depending on plant species and developmental stage, but may account for up to 11 percent of net photosynthetically fixed carbon and 10 percent

to 16 percent of total plant nitrogen.[4] This process influences the chemical and physical composition of the rhizosphere and, in turn, provides signaling molecules and organic substrates for microbial growth.

Another factor that likely shapes the composition of the plant microbiome is interaction between microbes. In 2016, Eric Kemen of the Max Planck Institute for Plant Breeding Research and colleagues surveyed the microbes thriving in and on wild *Arabidopsis* leaves at five natural sites in Germany sampled in different seasons. They then plotted correlations between the abundances of more than 90,000 pairs of microbial genera identified in their survey, revealing six "microbial hubs"—nodes with significantly more connections than other nodes within the network. These hubs were represented by the oomycete genus *Albugo*, the fungal genera *Udeniomyces* and *Dioszegia*, the bacterial genus *Caulobacter*, and two distinct members of the bacterial order Burkholderiales.[5] Given the high degree of connectivity within the communities, it is likely that these microbial hubs play a disproportionate role in the microbiome, akin to that of keystone species in an ecosystem.



ROOT BUGS: Plant roots and the interface between the roots and the soil—a zone called the rhizosphere—are home to diverse microbes that can affect mineral uptake by the plant.

> I was stunned by the staggering taxonomic diversity of bacteria that a single, tiny root can host.

To validate this idea that certain species can drive the composition of the plant microbiome, Kemen's team selected *Albugo* sp. and *Dioszegia* sp. as paradigmatic examples of microbial hubs. *Albugo* oomycetes are eukaryotic pathogens of *Arabidopsis* with an obligate biotrophic lifestyle—meaning that they cannot be cultured outside their host. Consistent with the central role of *Albugo* in the plant's microbial community, *Arabidopsis* that had been artificially infected with *Albugo laibachii* and maintained in potting soil under controlled conditions displayed a bacterial microbiome composition that was less variable across plants than that of uninfected individuals. Conversely, differences between the bacterial microbiomes of three distinct *Arabidopsis* strains were amplified in the presence of *A. laibachii* infection. The fungal microbiome, however, was not significantly affected by the presence of *A. laibachii* and another *Albugo* species.

Kemen's team conducted a parallel set of experiments with *Dioszegia* sp., which—unlike *Albugo* sp.—are culturable under laboratory conditions, and six bacterial isolates from *Arabidopsis* leaves. The results confirmed that the presence of the fungal species can strongly inhibit the growth of *Caulobacter*—plants whose leaves were inoculated with *Dioszegia* sp. showed a 100-fold reduction in the number of colony-forming units of *Caulobacter* sp.—mirroring the significant negative correlation observed between these two groups of microbes in the network analysis.[5]

In 2017, Harvard University's Roberto Kolter and colleagues demonstrated that such microbial interactions are not limited to *Arabidopsis*. The researchers developed a simplified version of the maize root microbiome, consisting of seven bacterial strains previously identified in sequencing surveys. By using a leave-one-out approach to colonizing naive maize plants, they demonstrated that removal of *Enterobacter cloacae* disrupts the composition of the microbial community, which became dominated by *Curtobacterium pusillum*, while the other five species had nearly disappeared. Interestingly, this effect was limited to plant colonization: when the seven strains of bacteria were monitored in a substrate that did not contain maize seedlings, the community's composition was significantly different from the one retrieved from roots, and the regulatory role exerted by *E. cloacae* was not detected.[6]

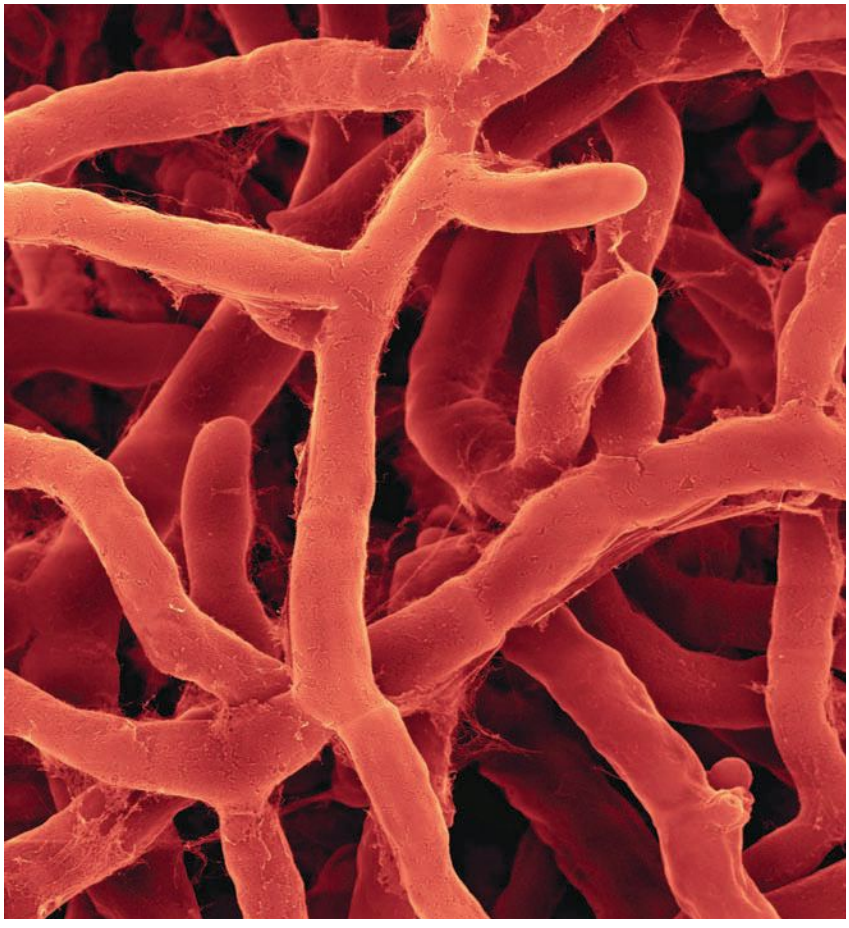
These studies suggest that individual members of the microbiome can have a disproportionate role in assembling and stabilizing the community. Deciphering the interactions within and between the various taxa populating leaves and roots will be required to understand the regulation of the plant microbiome.

From composition to function

For years, researchers have observed that, despite the presence of pathogens and conditions favorable to infection, some regions produce plants that are less susceptible to disease than other areas. The soils in these areas, it turns out, support plant health via the microbiome.

Researchers are making strides in understanding the mechanisms underlying this support. In 2011, for example, a team led by Rodrigo Mendes, then at Wageningen University and Research Centre in the Netherlands, demonstrated that disease suppression was linked to the recruitment of a specific population of Pseudomonadaceae, a family of the phylum Proteobacteria. Using a PCR fingerprinting approach, the researchers discerned that this population could be grouped into ten haplotypes, which the team designated A to J. Of these, haplotypes A, B, and C represented

some 90 percent of the isolated bacteria. When inoculated in soil, a representative strain of haplotype C suppressed the incidence of disease caused by the fungus *Rhizoctonia solani* on sugar beet roots, while, surprisingly, strains from haplotypes A or B did not.[7]



FUNGAL FINGERS: In addition to bacteria, the plant microbiome includes fungal species such as the *Rhizoctonia solani* shown here.

Similarly, in their study published last year, Kolter and colleagues found that maize plants inoculated with the seven selected bacterial strains showed significantly delayed development of *Fusarium verticillioides*, the causal agent of maize blight. This phenomenon was mediated by the specific strains chosen, and not by bacterial colonization per se, as seed treatment with a laboratory strain of *Escherichia coli* did not protect maize seedlings from pathogen development. Likewise, the seven strains together were required for the protective effect: inoculation with individual strains resulted in significantly less protection against *F. verticilloides*.

This method of combining sequencing data with microbial isolation is becoming a powerful tool to formulate testable hypotheses and gain novel insights into the function of the plant microbiome. Like Kolter, researchers are assembling microbial isolates into synthetic communities (SynComs) of known composition and testing their effects on host plants. This approach was once considered a daunting task, as only a very limited fraction—often less than 1 percent—of soil biota was considered culturable under laboratory conditions. But in 2015, Schulze-Lefert's lab teamed up with Julia Vorholt's group at ETH Zurich in Switzerland to investigate the proportion of *Arabidopsis*-associated bacteria that can be cultured, and found the 1 percent statistic to be a vast underestimate.

Comparing the taxonomic relationships among some 8,000 colony-forming microbes from leaves and roots of plants using cultivation-independent sequencing surveys of leaf and root microbiomes, the researchers demonstrated that more than 50 percent of the dominant members of the *Arabidopsis* microbiome can be cultured in vitro.[8] Taking advantage of this finding, the team assembled SynComs representative of the microbiota of the *Arabidopsis* roots and leaves and tested the communities' capacities to colonize these tissues on plants grown in a sterile substrate—the botanical equivalent of germ-free mice. These experiments revealed that, upon plant inoculation, root and leaf isolates form microbial communities resembling the natural microbiomes of those tissues, demonstrating that the SynCom approach accurately recapitulates the effects of a complete microbiota.[8]

Since then, numerous researchers have begun to develop SynComs to further explore the function of the plant microbiome. Earlier this year, for example, Jeff Dangl of the University of North Carolina at Chapel Hill and colleagues used the SynCom approach to explore the role of the root microbiome in phosphate uptake. In nature, less than 5 percent of the phosphorus content of soils is available to plants. To circumvent this limitation, farmers rely on the application of chemical fertilizers, but this approach is not sustainable in the long term. Thus, understanding how plants and their associated microbes can thrive under sufficient and limiting phosphorus supplies is a priority. There is a huge body of literature documenting the contribution of arbuscular mycorrhizal fungi to phosphorus uptake in plants, but the role of the bacterial microbiota remains mysterious.

In experiments with *Arabidopsis*, which does not engage in symbiotic relationships with mycorrhizal fungi, Dangl and his colleagues compared the microbiomes of wild-type plants with those of mutant lines that had impaired phosphate starvation responses (PSRs)—a set

of morphological, physiological, biochemical, and transcriptional activities evolved by plants to cope with phosphorus deficiency. Using a SynCom represented by 35 taxonomically diverse bacterial isolates from *Arabidopsis* and related plants, the researchers demonstrated that wild-type plants and mutants, grown on agar plates, assemble distinct root communities when exposed to both low and high phosphorus concentrations. Remarkably, SynCom inoculation reduced accumulation of phosphorus when plants were grown under limited conditions but not when plants were grown in the presence of abundant phosphate, suggesting that bacteria and plants compete for the element.[9]

By monitoring a core set of 193 marker genes, the team observed that SynCom inoculation greatly enhanced PSR-related transcription in wild-type plants. When the researchers transferred inoculated wild-type plants grown with limited phosphorus to plates with sufficient supplies, they observed a striking result: 20- to 40-fold increases in phosphorus concentration in the plant stem, as compared with mock-inoculated controls. Such a dramatic increase in phosphorus uptake was not detected in inoculated plants initially grown with sufficient phosphorus. Therefore, initial plant-bacteria competition for phosphorus might be part of an adaptive mechanism to maximize PSR in plants.[9]

Further investigation into the binding sites of transcription factors on *Arabidopsis* DNA revealed that PHR1, a master regulator of PSR, and its paralog PHL1 contribute to transcriptional regulation of plant immunity. In particular, *phr1;phl1* mutant plants display enhanced activation of plant immunity genes in response to phosphate starvation and to SynCom inoculation, compared with wild-type plants. Together, these data suggest that the nutritional status of the host is a driver of microbiome composition; through master regulators of mineral starvation, plants can modulate immune responses, which could, in turn, shape microbiome composition. (See "Holding Their Ground," *The Scientist,* February 2016.)

Scientists are looking to manipulate soil microbes to sustainably increase crop production—and novel insights into the plant microbiome are now facilitating the development of such agricultural tactics.

What's next?

Characterizing the plant microbiome and its function could be applied in an agricultural setting, better equipping our crops to grow in resource-poor environments and to fight off dangerous pathogens. Indeed, the private sector has begun to invest in this approach. One strategy many companies are pursuing is a form of plant probiotic, which consists of preparations of beneficial microbes to be mixed with seeds at sowing and again once the seedlings germinate. Another approach is to use plant breeding to select for varieties that have enhanced symbiosis with the microbiota.

Many questions remain about the plant microbiome, however—not least of which is how thousands of years of cultivation have changed crops' relationships with the soil biota. Using a cultivation-independent approach, my colleagues and I recently demonstrated that wild ancestors and modern varieties of barley (*Hordeum vulgare*) host distinct microbiotas.[10] Likewise, Jos Raaijmakers of the Netherlands Institute of Ecology and colleagues last year identified a shift in the structure of the microbiome of modern and ancestral varieties of common bean (*Phaseolus vulgaris*); Bacteroidetes were more abundant in wild relatives, and their contribution to the community was progressively replaced by Actinobacteria and Alphaproteobacteria in the more domesticated plants.[11]

How do these differences translate to altered functionality of the microbiome? Thanks to the experience gained by *Arabidopsis* scientists, we are now in a position to address this question, and developing SynComs from crops will be an important step in the process.

Luckily, the field is motivated to do just that, as well as to define a road map to achieve the translational potential of the plant microbiome. In a few years, the plant microbiome manipulations may have moved from the lab to the field. ■

Davide Bulgarelli is a principal investigator at the University of Dundee in the U.K. His research aims at understanding the structure, function, and host control of the microbiome thriving at the root-soil interface.

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MICROSCOPIC WAR: The leaves of this corn plant redden as a result of infection by maize chlorotic dwarf virus, which caused severe crop losses in the midwest and southern United States in the 1960s and '70s.

Viruses vs. Plants

Plants are locked in an ancient arms race with hostile viruses, but genome editing is giving crops the upper hand.

BY CLAIRE ASHER

In 2011, Noah Phiri was working with local farmers in Kenya to combat the fungal pathogen that causes coffee leaf rust when another virulent plant disease began wiping out maize in the country's southwest corner. Infected plants developed pale streaks on their leaves, then wilted and died. Some farmers lost as much as 90 percent of their crop that year. Phiri, a plant pathologist at the U.K.-based Centre for Agriculture and Biosciences International (CABI), raced to identify the culprit. He and his colleagues collected samples of sick plants and sent them off to the plant clinic at the Food and Environment Research Agency (now Fera Science) in York, U.K. There, researchers sequenced RNA molecules expressed in the infected corn and identified two viruses that were at the root of the epidemic.[1]

The viruses were already familiar to the researchers—in the second half of the 20th century, corn crops in Kansas suffered a similar fate. Known as maize lethal necrosis, the disease is caused by a combination of sugarcane mosaic virus (SMV), a common virus that is not usually harmful to maize, and a strain of maize chlorotic mottle virus (MCMV). MCMV is damaging to maize crops on its own, but in combination with SMV, the effect is exacerbated. While there hasn't been a major outbreak of maize lethal necrosis in Kansas since 1988—thanks to a rotation of disease-tolerant corn varieties—when the viruses struck Kenya in 2011, the local maize had no defense. By the following year, the disease had infected 77,000 hectares of Kenyan farmland, costing an estimated USD $52 million. It has now spread to most east African countries and threatens food security for millions of people.

Unfortunately, maize lethal necrosis is hardly unique; in general, plants are just as susceptible to viral infections as humans and other animals are. And viruses are particularly dangerous because, unlike bacteria and other pathogens, they cannot be killed with antibiotics or pesticides. "At the moment, there's not much to be done with plants that are infected," says Jean-François Laliberté, a virologist at the National Institute of Scientific Research (INRS) in Quebec, Canada. So when viruses strike, farmers are often forced to destroy crops, clean tools and machinery, and then replant with seeds from elsewhere.

In recent years, however, scientists have turned to inventive new ways to protect crops. Genetic modification techniques developed over the last 30 years, for example, can arm plants with defenses against viral invasion, while leaving crop yields and food quality unaffected. Some of these modified plants are now in the food chain. More-recent gene editing techniques are refining this approach, allowing researchers to make precise changes in plants' DNA to engineer a more resistant generation of crops. Several such varieties are now being tested in lab and field trials, while a handful await safety approval from national regulatory bodies.

"With climate change, there will be more new insects appearing, and those insects will be carrying new viruses and new strains," says Laliberté. To secure crop production around the world, "we have to find those means of genome editing."

INSECTS IN CAHOOTS: Most plant viruses are transmitted by insects, such as these black-faced leafhoppers (*Graminella nigrifrons*).

Plants as virus factories

The study of plant viruses has a long history. In fact, it was in plants that viruses were first discovered. In the late 1850s, a devastating disease began spreading across tobacco plantations in the Netherlands. Scientists at the time found that injecting sap from infected plants into healthy ones spread the symptoms—mottling and discoloration of the plants' leaves—leading researchers to assume that the affliction must be caused by a bacterium. However, additional experiments in the 1890s showed that the infectious agent spreading the disease could pass through the tiny pores of a porcelain water filter—far too small to allow the passage of any known bacterium. In 1898, Dutch microbiologist and botanist Martinus Beijerinck coined the term "virus" to describe the mystery contagion, though it would be another few decades before researchers characterized exactly what it was.

Even after scientists identified viruses as protein-encapsulated nucleic acids in the first half of the 20th century, many questions remained about how these particles acted within host cells to cause disease. Again, the study of plants fueled the young field of virology. In the 1950s, scientists began using electron microscopy to view plant-virus interactions in detail, revealing huge cellular rearrangements in infected cells. "[Researchers] noticed that there were a lot of structures that resembled vesicles," says Laliberté. "In [healthy] plant cells we have chloroplasts, a nucleus, and mitochondria, but in infected cells we have novel organelles."

More than 30 years later, researchers discovered that those strange vesicles, ranging in diameter from around 50 to 350 nanometers, are the powerhouses of viral infection. Now known as viroplasms or viral factories, the membrane-bound compartments collect resources from the plant to replicate the viral genome and produce RNAs that will direct the production of proteins and the construction of new viral particles poised to infect new hosts. (See illustration on following page.) The close proximity and high concentrations of the biomolecules made in these factories make for a highly efficient production line, notes Peter Nagy, a virologist at the University of Kentucky. For example, "tomato bushy stunt virus can produce close to one million progeny per cell in 24 hours," he says. "This is an unbelievably powerful process."

By cordoning off viral replication into membrane-bound compartments, the factories also serve to protect the pathogen against the plant immune system. In replicating their genomes, which are commonly single-stranded RNA, plant viruses typically generate a complementary copy to temporarily produce double-stranded RNA, an extremely unusual sight in a plant cell. "This double-stranded RNA does not exist in plant cells," says Nagy, so if not for the protective membrane around the viral factory, "the plant cell would right away know that this was an invading virus."

New viral genomes, sometimes packaged into a new protein capsid, are then carried away to neighboring cells through small channels in cell walls called plasmodesmata. But it takes a little coaxing, as these passageways typically allow the transit of small molecules, but not of proteins and RNA. So viral factories produce what are called movement proteins, which trigger the channels to widen. Some viral particles also manage to make their way into the phloem, where they have a chance of being sucked up by a sap-feeding insect like an aphid and carried away to infect other plants, often decimating entire fields of crops.

> With climate change, there will be more new insects appearing, and those insects will be carrying new viruses and new strains.
>
> —Jean-François Laliberté, Institut National de la Recherche Scientifique

Of course, plants are not passive victims in this relationship, and many have evolved genetic resistance to viral infections. (See "Holding Their Ground," *The Scientist,* February 2016.) Understanding how plants defend themselves against attack has given scientists a head start in the race to protect crops, allowing them to engineer new, resistant varieties.

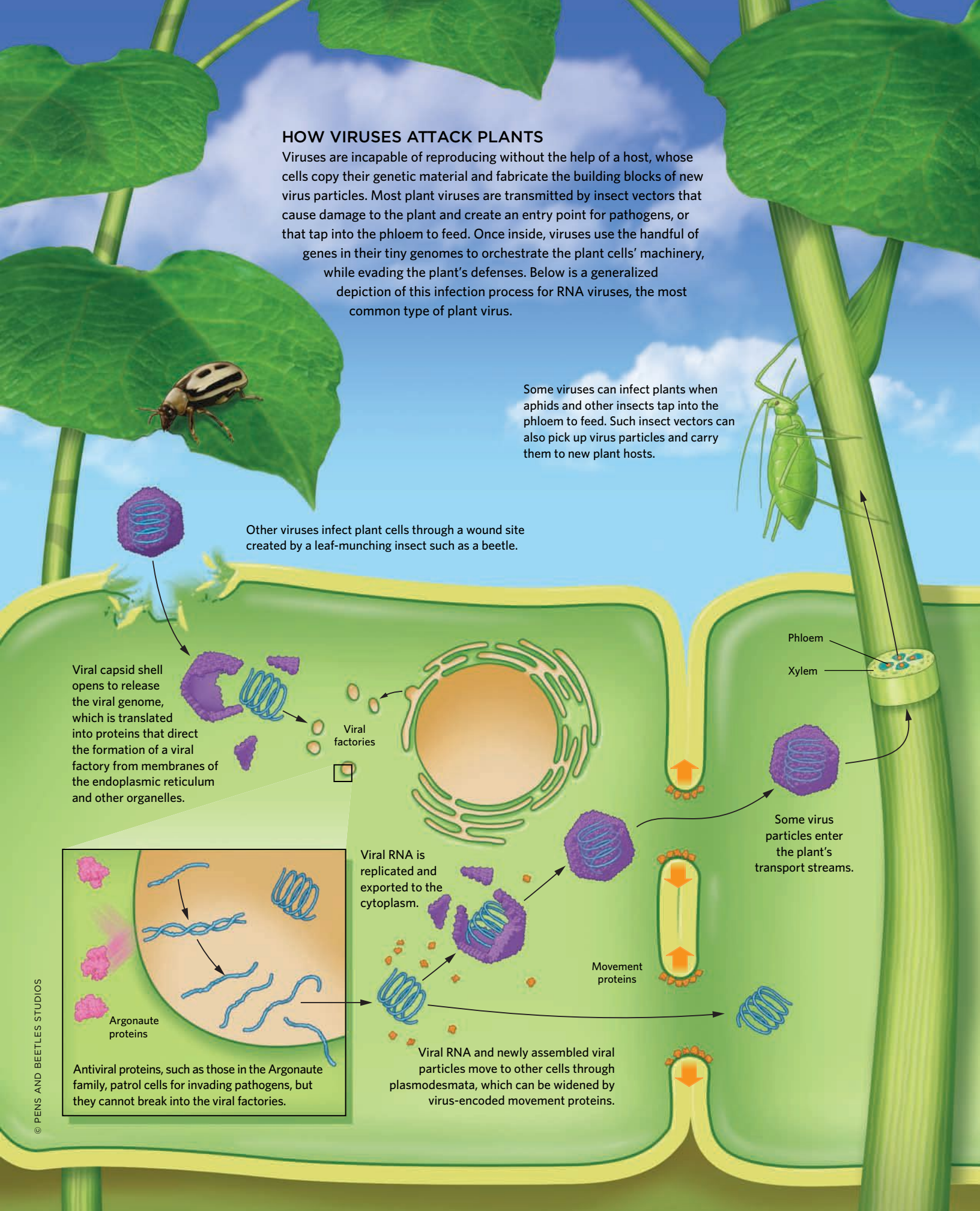
Interfering with viral infection

One of the first lines of plants' natural defense against viruses, deployed when the cell detects double-stranded RNA, is RNA

STEPHEN AUSMUS, USDA/ARS

HOW VIRUSES ATTACK PLANTS

Viruses are incapable of reproducing without the help of a host, whose cells copy their genetic material and fabricate the building blocks of new virus particles. Most plant viruses are transmitted by insect vectors that cause damage to the plant and create an entry point for pathogens, or that tap into the phloem to feed. Once inside, viruses use the handful of genes in their tiny genomes to orchestrate the plant cells' machinery, while evading the plant's defenses. Below is a generalized depiction of this infection process for RNA viruses, the most common type of plant virus.



PAPAYA PEST: Randall Pingel, an entomologist with the US Department of Agriculture, examines papaya fruits for symptoms of papaya ringspot virus, which struck the $11 million papaya industry in Hawaii in the 1990s.

silencing. Plant enzymes called Dicer-like proteins take viral RNAs and turn them into small interfering RNAs (siRNA). These siRNAs bind to a family of proteins called Argonaute as part of the RNA-induced silencing complex (RISC), which tracks down viral RNAs based on their similarity to the siRNA sequence and chops them into tiny fragments. "We now know that this is the major mechanism by which plants defend themselves against viruses," says Hanu Pappu, plant virologist at Washington State University in Pullman.

Researchers have been boosting plants' ability to use this mechanism to fight off viruses for more than 20 years. In Hawaii in the 1990s, the $11 million papaya industry was nearly decimated by papaya ringspot virus (PRSV), which yellows the leaves of the fruit trees and slowly kills them. A team at Cornell University inserted the ringspot virus coat protein gene into a bacterial plasmid, and fired gold particles carrying the plasmid at papaya cell cultures. They then germinated plant embryos that were expressing the foreign RNA, which would trigger RNA silencing against the virus. This new papaya variety, named Rainbow, is primed for PRSV, ready to silence its RNA as soon as it invades a cell. Rainbow papaya has been hugely successful and has come to dominate the Hawaiian papaya market since its commercial release in 1998.

Over the past few decades, plant geneticists have employed similar techniques to combat other damaging crop viruses. For example, regulators in Canada and the U.S. approved a transgenic variety of squash engineered using a bacterial plasmid to carry genes coding for the coat proteins of cucumber mosaic virus, watermelon mosaic virus, and zucchini yellow mosaic virus, in the late 1990s. And in 2001, researchers used a bacterial plasmid to insert the coat protein of the soybean mosaic virus into soybean plants to confer resistance to the virus, although a commercial variety was not developed.[2]

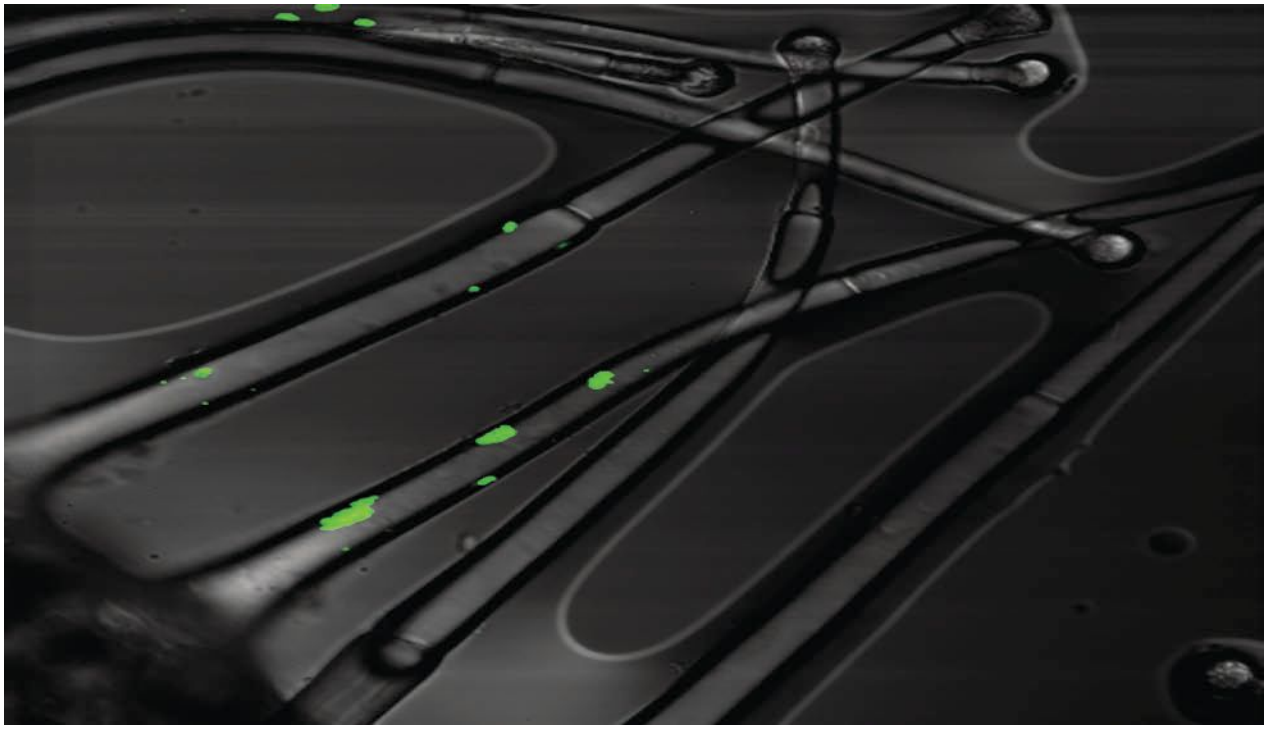
> Tomato bushy stunt virus can produce close to one million progeny per cell in 24 hours. This is an unbelievably powerful process.
>
> —Peter Nagy, University of Kentucky

More recently, scientists have been taking advantage of this natural plant defense system to protect cassava, a starchy root vegetable that's a staple in the diets of hundreds of millions of people in Africa, Asia, the Pacific, and South America. Cassava brown streak disease is caused by two viruses, Ugandan cassava brown streak virus (UCBSV) and cassava brown streak virus (CBSV), which have been infecting cassava crops since the 1980s. The disease only began causing serious problems for farmers in 2004, when the viruses spread out from coastal regions and across Tanzania, Uganda, Rwanda, and the Democratic Republic of Congo. Cassava plants had no natural resistance to the disease.

In 2011, researchers used a bacterial plasmid to insert the full gene sequence for the coat protein of UCBSV into the genomes of cultured cassava cells, which were then regenerated into whole plants, successfully priming cassava's natural RNA silencing machinery against the virus.[3] Cassava engineered to express the UCBSV coat protein gene showed 100 percent resistance to the virus when infected cuttings were grafted onto them in greenhouse experiments. And researchers at the Virus Resistant Cassava for Africa (VIRCA) initiative found that the best performing transgenic cassava line was more than 98 percent resistant to CBSV in confined field trials.[4]

Meanwhile, to produce a crop with resistance to a greater variety of CBSV strains, VIRCA researchers have combined the full coat protein gene sequences from UCBSV and CBSV into a plasmid and inserted it into the genome of an East African cas-

PEGGY GREB, USDA/ARS



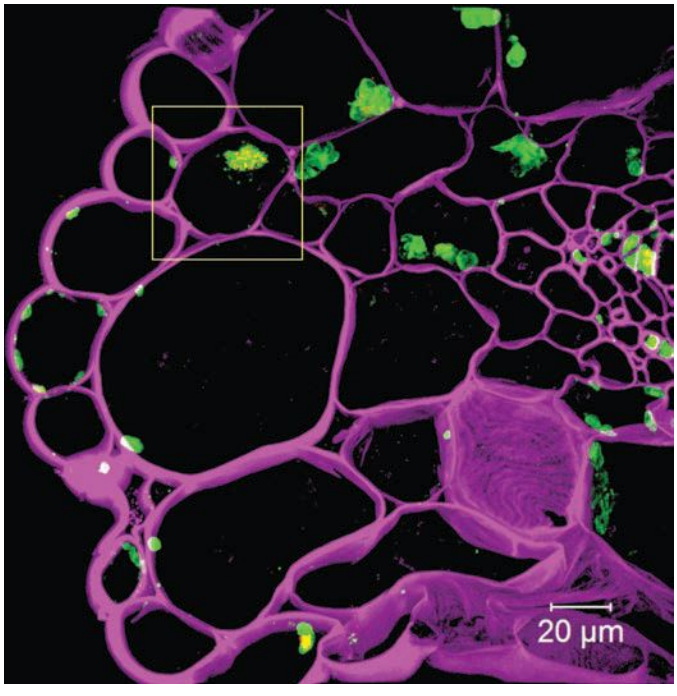
CHURNING OUT VIRUSES: Once inside a plant, viruses direct the production of compartments known as virus replication factories (green) to make copies of their genomes. (Top: leaf trichomes infected by turnip mosaic virus. Bottom: cross-section of stem infected by turnip mosaic virus; cell walls stained in magenta.)

sava variety that is preferred by farmers.[5] This new variety, part of a project dubbed VIRCA Plus, performed well in confined field trials in Kenya and Uganda, with 16 out of 25 transgenic lines remaining symptom-free after 12 months.[6] Field trials with these resistant lines continue, as the team works with national government regulators in Uganda and Kenya to seek approval for the new variety to be released for use by farmers.

"The biggest challenge is still trying to negotiate this regulatory infrastructure," says Becky Bart, a plant geneticist at the Donald Danforth Plant Science Center in St. Louis.

Disrupting viral replication

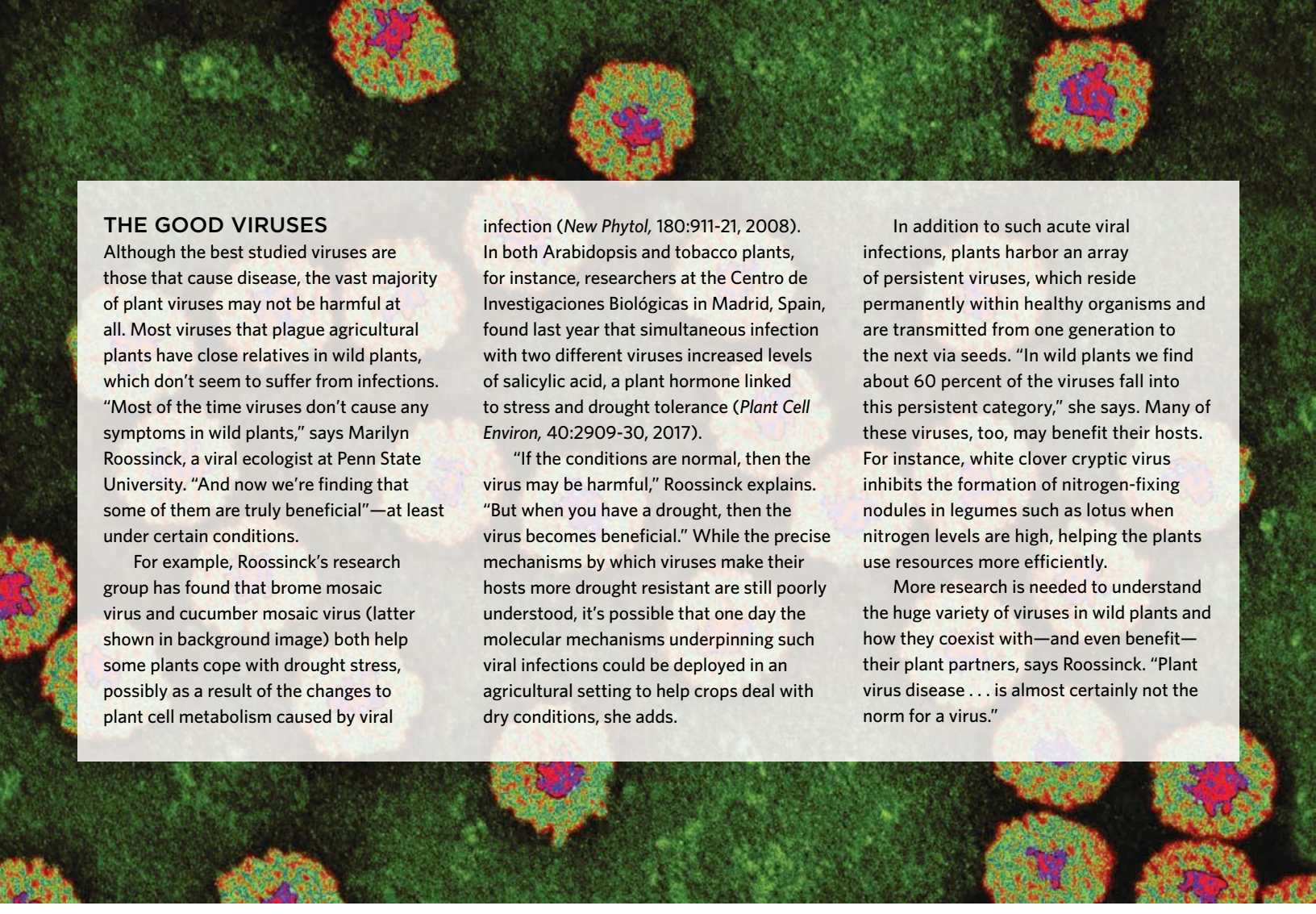
Another way plants can defend themselves against viral infection is through the accumulation of mutations in proteins targeted by viral pathogens. For example, research in the 1990s showed that the viral protein VPg interacts with plant proteins in the eIF4E family of translation initiation factors to produce other proteins critical for viral replication. In 2002, a research team in France showed that naturally occurring resistance to several viruses in peppers (*Capsicum annum*) was caused by a mutation that gave one eIF4E protein a slightly different molecular structure.[7] At the same time, an overlapping group of researchers identified a mutant line of the plant model organism *Arabidopsis thaliana* in which the gene for an isoform of eIF4E was disabled, leaving normal plant growth unaffected but hampering viral replication.[8] More recently, researchers at the University of Tokyo in Japan identified variants of the nCBP protein, part of the eIF4E family, in *Arabidopsis* that prevent the accumulation of certain movement proteins, trapping the



Plantago asiatica mosaic virus in a single plant cell and saving the whole plant from infection.[9]

Plant breeders have long been making use of such naturally occurring genetic resistance, selectively crossing wild varieties to produce more-resistant crops. For example, in the 1980s, work led by scientists at the International Institute of Tropical Agricul-

ROMAIN GRANGEON; JUAN WAN



THE GOOD VIRUSES

Although the best studied viruses are those that cause disease, the vast majority of plant viruses may not be harmful at all. Most viruses that plague agricultural plants have close relatives in wild plants, which don't seem to suffer from infections. "Most of the time viruses don't cause any symptoms in wild plants," says Marilyn Roossinck, a viral ecologist at Penn State University. "And now we're finding that some of them are truly beneficial"—at least under certain conditions.

For example, Roossinck's research group has found that brome mosaic virus and cucumber mosaic virus (latter shown in background image) both help some plants cope with drought stress, possibly as a result of the changes to plant cell metabolism caused by viral infection (*New Phytol,* 180:911-21, 2008). In both Arabidopsis and tobacco plants, for instance, researchers at the Centro de Investigaciones Biológicas in Madrid, Spain, found last year that simultaneous infection with two different viruses increased levels of salicylic acid, a plant hormone linked to stress and drought tolerance (*Plant Cell Environ,* 40:2909-30, 2017).

"If the conditions are normal, then the virus may be harmful," Roossinck explains. "But when you have a drought, then the virus becomes beneficial." While the precise mechanisms by which viruses make their hosts more drought resistant are still poorly understood, it's possible that one day the molecular mechanisms underpinning such viral infections could be deployed in an agricultural setting to help crops deal with dry conditions, she adds.

In addition to such acute viral infections, plants harbor an array of persistent viruses, which reside permanently within healthy organisms and are transmitted from one generation to the next via seeds. "In wild plants we find about 60 percent of the viruses fall into this persistent category," she says. Many of these viruses, too, may benefit their hosts. For instance, white clover cryptic virus inhibits the formation of nitrogen-fixing nodules in legumes such as lotus when nitrogen levels are high, helping the plants use resources more efficiently.

More research is needed to understand the huge variety of viruses in wild plants and how they coexist with—and even benefit—their plant partners, says Roossinck. "Plant virus disease . . . is almost certainly not the norm for a virus."

ture succeeded in breeding partial resistance to the geminivirus-caused cassava mosaic disease—resistance that's found in closely related wild species of the root vegetable—into cultivated varieties across Africa.[10] By cross-breeding cultivated cassava (*Manihot esculenta*) with its wild relative, tree cassava (*M. glaziovii*), the team was able to introduce naturally occurring resistance to the disease, controlled by multiple genes.

Such traditional breeding approaches can take decades, however—a cumbersome prospect when new resistance genes must be introduced for each new viral strain that evolves. More recently, scientists have used genetic engineering techniques to more swiftly and precisely arm crops with such resistance. "Genome editing has just completely revolutionized every part of biology," says Bart.

Last year, doing screens in yeast, Bart and her collaborators identified two eIF4E proteins from cassava that interact with CBSV and UCBSV VPg proteins. Then, using the CRISPR-Cas9 system, they edited the sequence of those genes to prevent their expression, resulting in a cassava variety that showed improved resistance to the viruses in greenhouse trials.[11] The CRISPRed cassava plants weren't fully resistant, however, suggesting that the viruses may also be able to interact with the three remaining unedited eIF4E proteins. The team hopes to fine-tune the system to engineer a fully resistant cassava plant.

> We have a long way to go to develop sustainable and environmentally sound approaches to really control virus diseases.
>
> —Bryce Falk, University of California, Davis

Recent studies have revealed other tricks used by plants to fight off viral infections. For instance, the process designed to recycle damaged or unwanted objects in the cell—autophagy—has been coopted to remove viral particles, too. Working with tomato plants (*Solanum lycopersicum*), Yakupjan Haxim at Tsinghua University in Beijing, China, and colleagues found that the plant's autophagy protein ATG8 binds the viral *βC1* gene, which encodes an essential protein for infection by geminiviruses, transporting it to an autophagosome for degradation.[12] Viruses carrying a mutated version of βC1, which cannot be bound by ATG8, cause more-severe symptoms and replicate more rapidly. Conversely, when the researchers promoted autophagy—by preventing the expression of enzymes that inhibit the process—plants of the model organism *Nicotiana benthamiana*, a close relative of

tobacco, showed more resistance to three geminiviruses: cotton leaf curl Multan virus, tomato yellow leaf curl virus, and tomato yellow leaf curl China virus.

As researchers continue to learn more about the natural defenses plants use to protect themselves against viral pathogens, and as they enlist rapidly advancing genetic engineering techniques to equip plants with such weaponry, the field is on its way to having the tools it needs to develop a new generation of resistant crops. But it will be an uphill battle. Viruses are constantly evolving, many times faster than the plants they infect, and it is only a matter of time before each virus develops a countermeasure to such resistance mechanisms. For example, many viruses manufacture a protein that can mop up siRNAs, binding them before Dicer-like enzymes can form a RISC complex; and potyviruses such as tobacco vein mottling virus have mutated their VPg protein, allowing them to bind to the modified eIF4E proteins that previously offered the plant resistance.

"I think we have a long way to go to develop sustainable and environmentally sound approaches to really control virus diseases," says Bryce Falk, a plant pathologist at the University of California, Davis.

Containing outbreaks

While genetic editing may be paving the way to more-resistant crops, the approach's application to agriculture is still in its infancy. Each new variety requires extensive testing for safety before the engineered plants can be deployed in the field. For now, quick identification of new viral threats and strict hygiene and quarantine regulations remain critical, by containing outbreaks before they can spread and cause widespread crop losses, particularly in developing nations.

The Plantwise project is a global program led by CABI that aims to do just that. Launched in 2011, the project works with farmers in Africa to help diagnose and treat plant health problems. One of their key innovations has been Plant Clinics, where local farmers can meet with trained plant health experts to identify pests and pathogens. These meetings "have been pivotal in the identification of some of these new pests that are coming to different countries," says Phiri. It was at one of the clinics that maize lethal necrosis was first detected in Africa six years ago. "Early detection is crucial, and plant health clinics are playing that role," he says.

But new technology can also help. For example, MinION portable DNA sequencers being used by the Cassava Virus Action Project are helping farmers in developing nations identify new viruses, allowing them to quickly take action to minimize transmission.

Although many challenges remain in the fight against plant viruses, such technological advances are giving researchers the upper hand, says Falk. For now, he adds, it is a fight we are winning. "We're winning it because we're feeding people." ■

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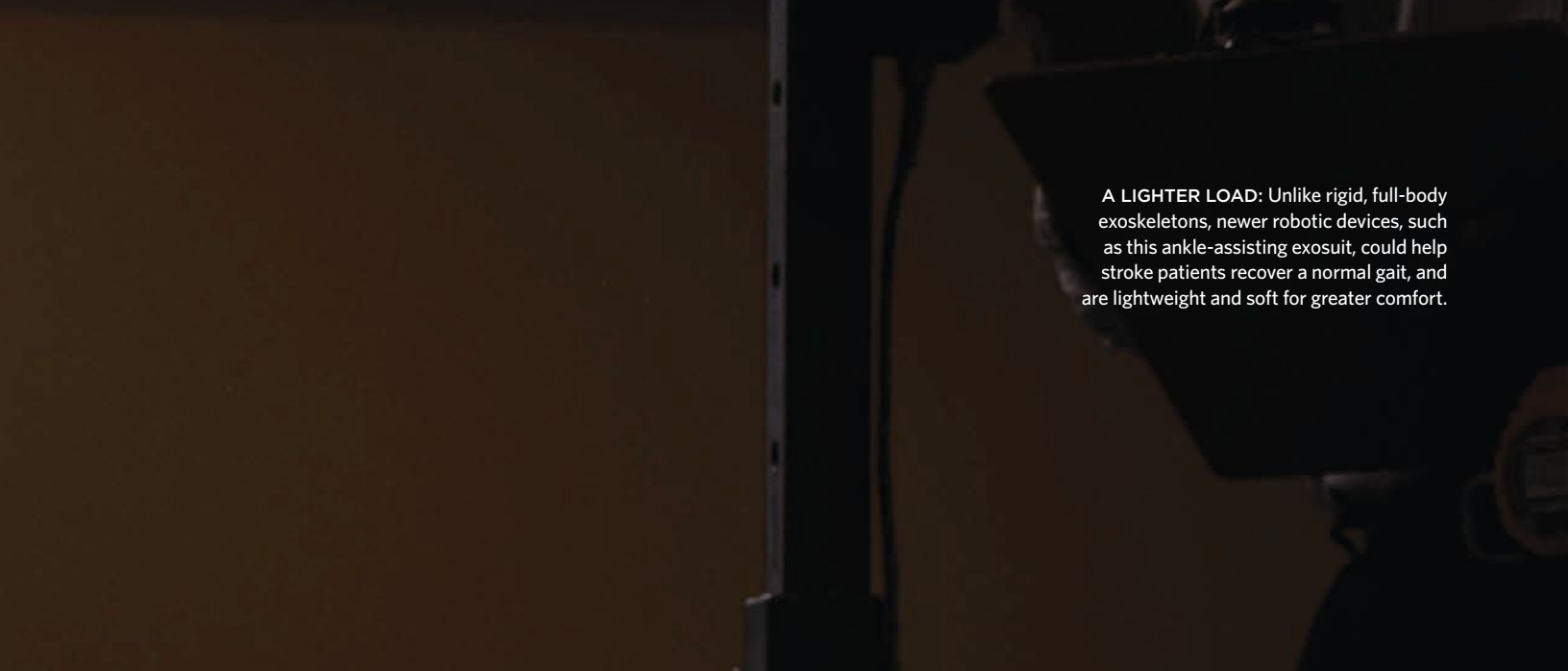
ROLEX AWARDS/
FRED MERZ



A LIGHTER LOAD: Unlike rigid, full-body exoskeletons, newer robotic devices, such as this ankle-assisting exosuit, could help stroke patients recover a normal gait, and are lightweight and soft for greater comfort.

Robotic *Healers*

New exosuits could offer a gentler way to help people with various ailments, from Parkinson's disease to multiple sclerosis, gain movement.

BY KAREN WEINTRAUB

In Conor Walsh's engineering lab at Harvard University, no one looks askance at a staff member wearing a loudly whirring backpack, with wires snaking out and down his leg. A trio of sewing machines have their own workroom. A dozen pairs of identical hiking boots neatly fill a shoe rack on the far side of a treadmill. A disembodied glove clenches and straightens as air fills and drains from its fingers.

All of this equipment is aimed at helping people move faster, more smoothly, while expending less energy. Walsh, also a core faculty member at Harvard's Wyss Institute for Biologically Inspired Engineering, is most excited about the devices his group is designing for stroke patients, who often struggle to regain their strength and fluidity of movement. The team has already shown that "soft exosuits" can provide a robotic assist to movement, enabling soldiers to march for longer.[1] This month, the researchers will begin clinical trials to test the suits' ability to help stroke patients relearn how to walk efficiently.

Walsh belongs to a growing group of researchers worldwide who are using small, lightweight robotics to help people with a range of medical conditions that hinder mobility. Rigid, whole-body "exoskeletons" have made headlines in recent years—perhaps most famously when a suit developed by Duke University neuroscientist Miguel Nicolelis and colleagues enabled a 29-year-old paraplegic Brazilian man to kick a soccer ball at the launch of the 2014 World Cup in São Paulo. Such exoskeletons have helped paralyzed people to walk again, albeit awkwardly, by pushing, pulling, and supporting them to stand up and move one leg followed by the other. But Walsh and other researchers have realized that people with less-disabling conditions need a subtler boost. Now, teams around the world

are developing smaller, lighter devices that help, rather than drive, movement. And sewn into clothes, they can be donned as easily as pulling on pants, a shirt, or gloves.

A group in Italy is designing a suit to reduce falls among the elderly and amputees, for example, while researchers at Stanford University are trying to reduce the energy it takes people such as those recovering from stroke to walk. And a team in New York City is helping children with cerebral palsy get out of the "crouch gait" that makes it difficult and awkward for them to get around.

These advances are supported by a number of technological improvements and cost reductions over the past decade, Walsh says. Motors are smaller, more powerful, and cheaper. Electronics are easier to use. Gyroscopes and accelerometers are now so tiny, inexpensive, and precise that they can give directions on cell phones—and can tell precisely where someone's leg is in space and what direction it's moving in. "The technologies that robotics research groups can pull from have gotten better across the board," Walsh says.

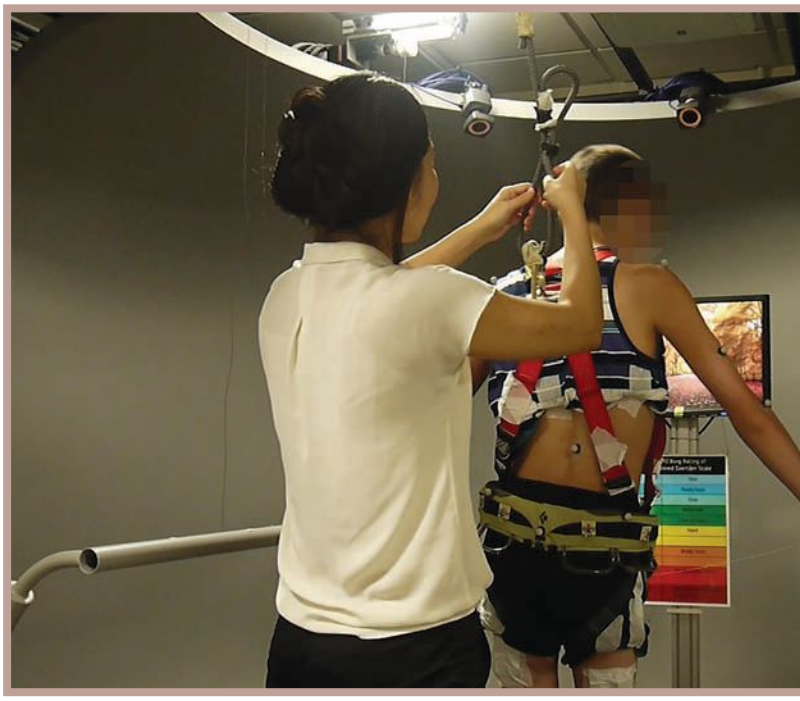
"It's a great time in the field," agrees Columbia University's Sunil Agrawal, who leads the work on cerebral palsy. "There's a general appreciation both within the scientific and clinical community that these robotic devices can make a big difference in people's lives."

Stroke support

Walsh's work on exosuits started nearly six years ago as a collaboration with scientists at the US government's Defense Advanced Research Projects Agency (DARPA), aimed at reducing the energy soldiers have to exert to carry heavy backpacks over long distances. (See "Beyond the Clinic" on page 45.) But a few years into the project, the researchers began to realize the technology's potential for helping patients, too—in particular, people recovering from stroke, which affects nearly 800,000 Americans a year, leaving many with physical disabilities.

If they're both lucky and well-insured, stroke patients get a few weeks of inpatient rehabilitation therapy, says physical therapist Terry Ellis, who collaborates with Walsh and directs the Center for Neurorehabilitation at the Boston University College of Health and Rehabilitation Sciences: Sargent College. But with limited time, rehabilitation specialists focus on getting patients walking again in whatever way possible, often with the use of a walker, a cane, or a hard plastic 90-degree brace that keeps their weaker foot from "dropping" as they lift it off the ground to take a step. Many patients never learn to walk normally again, Ellis says. And because the plastic brace keeps the patient from being able to push off the ground with that foot—an essential part of the biomechanics of walking—the more that person walks, the weaker the ankle gets, and the more the foot drops, she adds. "We're missing out. We're not optimizing on the potential people have to improve."

Stroke patients in wheelchairs fall even further behind: not only do they lack support for working on walking skills, but constant sitting impairs bowel and bladder function, reduces bone mass, and dysregulates blood pressure, notes Paolo Bonato, a researcher at Spaulding Rehabilitation Hospital in Boston who is collaborating with Walsh and Ellis. "Being in a standing, load-bearing position is actually quite important for the body," he says. And a soft exosuit may be just what stroke patients need to get back on their feet.



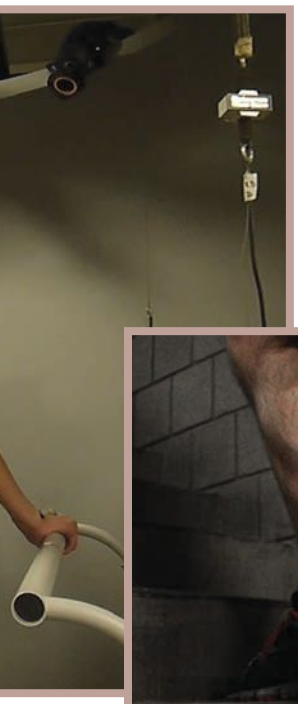
There's a general appreciation both within the scientific and clinical community that these robotic devices can make a big difference in people's lives.

—Sunil Agrawal, Columbia University

In Walsh's lab, graduate student Jaehyun Bae dons a version of the device the group has developed and takes to the treadmill. As he pretends to walk with a dropped foot, a wire from the device wrapped around his calf and ankle pulls up his foot at just the right second to avoid hitting the floor, then quickly lets it go so he can push off. When he picks up his pace, the robotic movements speed up with him. Bae then shifts his gait to swing one leg outward. Again, the device matches his stride to pull the leg back in line.

In preliminary tests conducted by Walsh's collaborators at two Boston clinics, the device seems to be helping stroke patients. Not only does it appropriately correct for the users' aberrant move-

JIYEON KANG AND SUNIL K. AGRAWAL; CARNEGIE MELLON UNIVERSITY COLLEGE OF ENGINEERING



WALK ABOUT: A variety of new devices could help restore impaired locomotion or even reduce the energy it takes for a healthy person to walk. An exosuit developed by researchers at Columbia University could help children with cerebral palsy overcome "crouch gait," a condition in which excessive flexing of the knees, hips, and ankles causes overexertion and pain (left). Meanwhile, researchers at Carnegie Mellon University are working to develop a device that supports the foot's ability to push off the ground to aid in walking (below).

ments, it helps increase their pace. A healthy young adult generally walks about 1.2 meters per second, Walsh says. Someone who walks slower than 0.4 meters per second is considered essentially homebound; those with a pace of 0.4–0.8 meters per second can get out occasionally, and those whose speed exceeds 0.8 can fully integrate into society. "If you could get someone from 0.3 to a 0.6, or a 0.6 to a 0.9, that would be a big deal," Walsh says. Last July, he and his team showed substantial progress toward that goal.[2] "We're not talking about tremendous changes, we're just trying to give enough of a little boost to push people over these thresholds so they can start to be more active."

Early this year, in collaboration with ReWalk Robotics, Walsh and his colleagues will evaluate a commercially viable version of the exosuit for stroke patients. They hope to win US Food and Drug Administration (FDA) approval by the end of 2018. Concurrently, the researchers are developing a system that works on both legs that should be ready within a couple of years to help people with a greater range of ailments, including multiple sclerosis, cerebral palsy, ALS, and Parkinson's disease, Walsh says. Further off but also under development: devices that will address arm issues in the same diseases, he adds. "We've been starting to test and starting to understand how we can best help someone who has an upper extremity impairment."

Robotic help for a range of conditions

A handful of other research teams are also developing soft exosuits for patients with movement disorders. In Pisa, Italy, for example, Vito Monaco at the Scuola Superiore Sant'Anna has developed a pelvic support system for the elderly and amputees that, at least in the lab, can help right someone before they fall.[3] "It's not easy to predict the way a person will fall down," says Monaco. "We as roboticians should combine detecting falls or lack of balance with strategies to assist people to recover their balance." A Belgian group at Ghent University is also aiming to help the elderly get around, and just last year the researchers showed that the exosuit they built to assist with plantarflexion—the "push-off" stage of walking—can reduce the effort it takes for a person over the age of 65 to walk.[4]

Meanwhile, Columbia's Agrawal focuses on in-hospital patient rehabilitation and training, using cable-driven exo-

A VIABLE MARKET?

Larry Jasinski, CEO of ReWalk Robotics, says he's convinced there's a broad market for new exosuits. ReWalk made its name developing rigid exoskeletons for people with spinal cord injury, and now has FDA approval for a device that allows paralyzed people to stand and walk. But he says at least one-third of the calls he gets are from patients with motor neuron diseases asking him when he's going to make something that can help them. "It tells me that we have an audience here that's actually bigger than the spinal cord community," he says.

He says he's hoping that within a few years he will be able to offer even less-expensive suits tailored for other patients: people suffering from multiple sclerosis or Parkinson's disease who need even lighter movement nudges. Jasinski says he expects to round out the offerings with exosuits aimed at people with cerebral palsy and the elderly, once the suit can be redesigned and tested for them. "If we can handle all those, we've got a very large industry," he says.

These newer devices would cost a lot less—on the order of just $19,500—than the whole-body exoskeletons for paraplegics, which run about $80,000, says Jasinski, who notes that rehabilitation hospitals could be financially motivated to buy the suits because fewer staff members will be needed to work with each patient. At-home costs may be harder to justify to an insurance company, at least at first, he says. But a patient might be able to rent the suit as needed, to bring the price down to a manageable sum. "I can make that work from a business model."

suits to train children with cerebral palsy[5] and adult stroke patients to improve the coordination of their limbs. Instead of a single wire or set of wires to help someone who drags a foot, Agrawal says his group's exosuits, which are often attached to the ceiling for stability, have multiple wires that apply forces to manipulate the gait in a much more fine-tuned way appropriate for rehab therapy. He is particularly interested in posture and the curvature of the spine, and has written several recent papers showing that his team's exosuits can train patients to correct for postural weakness.[6]

Other projects around the world include an effort at the University of Michigan to develop a robotic ankle that adapts to the gait of its user, currently being tested on healthy people,[7] and collaborative work at North Carolina State University and the University of North Carolina at Chapel Hill meant to help the mechanics and control of ankle muscles and tendons.[8] Meanwhile, Walsh's postdoctoral advisor, Hugh Herr at MIT, is working with colleagues to develop bionic prosthetics and exosuits meant to help amputees as well as healthy people and other patients hop, run, and walk.[9]

We're at the Toshiba level right now. We have a really long way to go before we get to a MacBook Air type of system.

—Paolo Bonato, Spaulding Rehabilitation Hospital

Even big-name corporations have expressed interest in the field. Samsung, for example, is developing full-body and hip-only exosuits designed to support walking in the elderly and disabled, and eventually to improve performance in soldiers. Honda has been developing an assistive exosuit for people with total paralysis. And Toyota announced earlier this year that its rehabilitative exosuit, aimed at people with lower-limb paralysis, would soon be available for rent by medical facilities.

The road ahead

These technologies still face their fair share of challenges. In fact, there are only eight groups around the world "that have demonstrated a device that can improve performance of the user," notes Steven Collins, an associate professor of mechanical engineering at Stanford University who recently moved there from Carnegie Mellon. "And all of those have been demonstrated in the last four years."

One problem is practicality. Monaco, for example, is still struggling to make his exosuits light enough so that they don't further destabilize users. "The last version of our [robotic] pelvis weighs 3 to 4 kilos, which is quite a huge backpack for an elderly person—more than a couple of big bottles of water," he says. Bonato agrees, comparing the exosuits of today to the laptops of 10–15 years ago. Back then, his Toshiba computer was ostensibly portable—but just barely. "We're at the Toshiba level right now. We have a really long way to go before we get to a MacBook Air type of system."

And it's not just the size of the devices, but their function as well, Collins adds, arguing that most of the failures stem from a lack of understanding about how best to help. "It's really easy to accidently make it harder for a person" to walk, he says.

His and Walsh's groups are now employing an iterative approach, in which devices can be changed or "learn" as people interact with them. Too many research groups focus on making a generic device that will move everyone's legs, without addressing the individual motions that make up that movement, Collins argues. With an iterative approach, "you can try lots of designs really quickly without having to build new, specialized hardware" for every person who uses the device, he says. Using an optimization algorithm to efficiently explore the potential movements that might help, Collins and his colleagues showed they could reduce energy expenditure by 24 percent with an exosuit tuned to a healthy individual—an improvement four times greater than they'd ever achieved by making the variations by hand.[10] "We were floored," he says.

Collins says that making a device that will actually help people requires involving them in the development process. The hardware and software are important, and so too is the person inside the suit, he notes. "When we started optimization work, we thought the most important thing was to find the [best] device. [But] just as important as the device learning the person is the person learning the device." ■

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BEYOND THE CLINIC

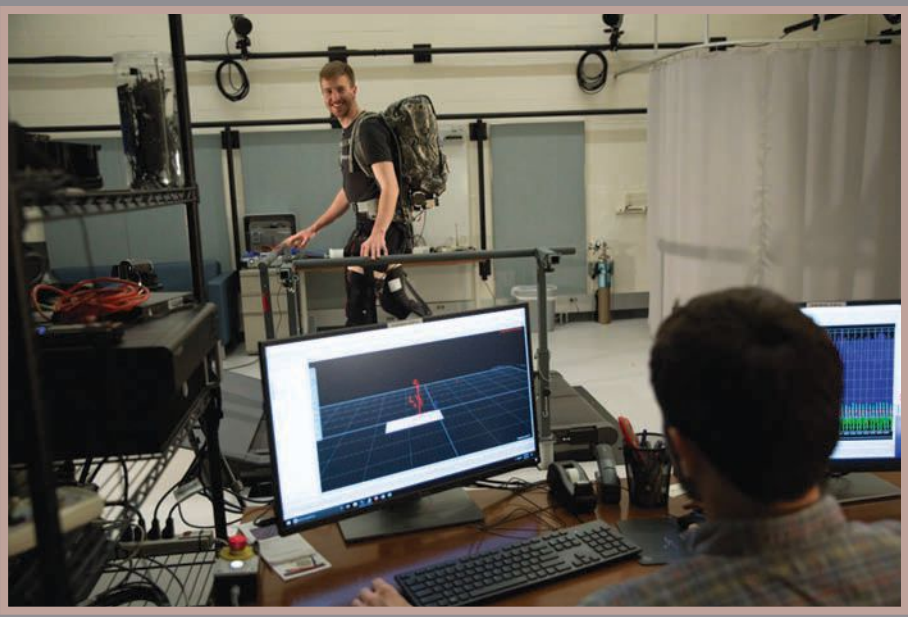
Asa Eckert-Erdheim, a staff engineer in the lab of Conor Walsh at Harvard University, walks slowly and then quickly on a treadmill. Wires emerge from the military-style backpack he's wearing, heading to bands that wrap around his legs and end where his shorts do, just above his knees. The robotic device detects his repetitive marching motions and supports his legs with forces applied in parallel with his muscles. But as he disrupts the rhythm, pretending to climb over a rock in his path, the cords go limp, so they don't get in his way. The device also adapts to his speed as he breaks into a run.

The robotic exosuit is intended to help soldiers march faster and farther while carrying heavy packs, Eckert-Erdheim explains. The latest version of the Harvard suit weighs 4.5 kg and can reduce a soldier's effort by 5 percent to 10 percent in real-world situations. Eventually the goal is to achieve a 25 percent energy reduction, says Walsh, a faculty member at Harvard's Wyss Institute for Biologically Inspired Engineering who in 2014 received a $2.9 million contract from the Defense Advanced Research Projects Agency for the work.

The promise of this technology has attracted military interest for decades. In the early 1960s, for example, researchers at Cornell University teamed up with the Navy to develop the Man Amplifier, a full-body exosuit intended "to augment and amplify [a soldier's] muscular strength and to increase his endurance in the performance of tasks requiring large amounts of physical exertion," according to a 1964 report. More recently, Steven Collins at Stanford University has been working with the Army to design a lower-limb exoskeleton that provides assistance at the hips, knees, and ankles, as well as "human-in-the-loop optimization algorithms" to identify the best patterns of robotic assistance.

Back at Harvard, Walsh and his team are still meeting with Army officials to figure out how they might proceed with the project even though the lab's last military contract has ended. "We don't have a clear idea yet, but we're excited about talking to the Army and the military medical community," says Ignacio Galiana, a staff robotics engineer at the Wyss Institute and former postdoc in Walsh's lab.

First responders such as firefighters could also benefit from devices that reduce their fatigue as they climb flights of stairs or carry limp bodies, for example. Researchers also see a market for these exosuits in athletics, particularly when it comes to training, "to sense how you're doing and give you feedback on how you could improve," says Galiana. "Or you could imagine wearing the device to improve your performance or to recover faster after you do a lot of exercise" or suffer an injury.



MAKING THE JOB EASIER: In partnership with the US military, researchers at Harvard's Wyss Institute are developing an exosuit intended to reduce energy expenditure for soldiers and first responders.

WYSS INSTITUTE AT HARVARD UNIVERSITY

The Literature

PLANT BIOLOGY

Ain't No Sunshine

THE PAPER

S.A. Sinclair et al., "Etiolated seedling development requires repression of photomorphogenesis by a small cell-wall-derived dark signal," *Curr Biol*, 27:3403-18.e7, 2017.

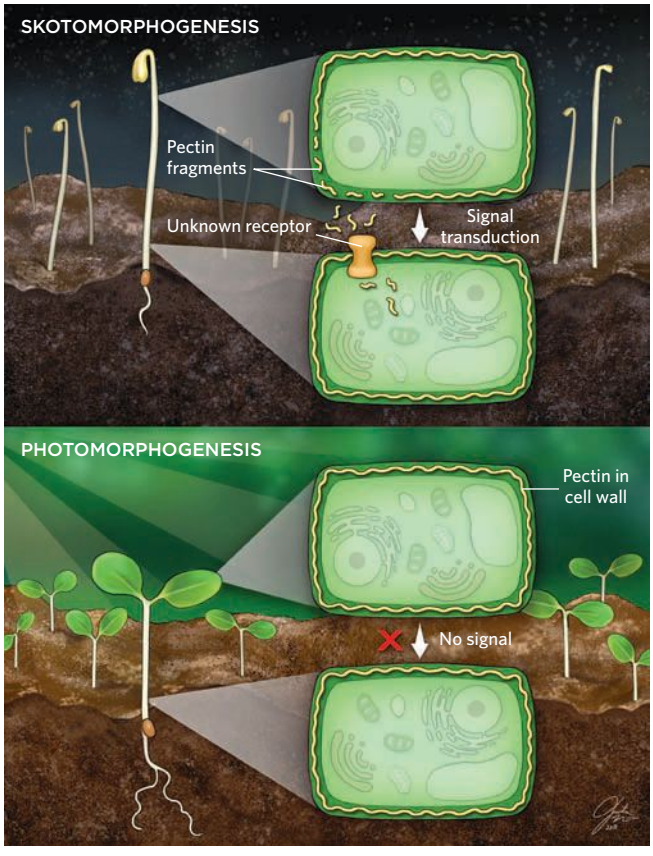
Plants don't always need sunlight to grow. Through a process called skotomorphogenesis, seedlings germinated in the dark—say, too far under the soil surface—will stretch out into long, pale shoots, searching for light. Think of the spindly bean sprouts you might buy at the store, offers Ute Krämer, a plant physiologist at Ruhr-Universität Bochum in Germany. It's an energy-saving tactic to get plants to the light. Once they do get there, they switch irreversibly to light-driven growth called photomorphogenesis—spreading out their roots and developing their leaves.

Krämer says that while the cellular components governing photomorphogenesis have been understood for decades, the cell-to-cell signaling pathways that determine the change in strategy from dark- to light-driven growth remain a mystery. How are light signals transmitted from the top of the seedling further down the plant so the switch from skoto- to photomorphogenesis can commence?

To find out, Krämer's team made use of *Arabidopsis* mutants that use photomorphogenesis even in the dark, ending up with longer roots and fuller, greener leaves than they would through skotomorphogenesis. In these seedlings, the researchers found that pectin, a cell-wall component, had chemical modifications, including more methyl carboxyester groups and less acetylation.

Although the genetics and molecular mechanics of these mutants varied, the unifying theme was pectin alterations, and Krämer reasoned that these were responsible for allowing photomorphogenesis to proceed in the dark. By this logic, normal pectin components might be the signal that wild-type seedlings use to pass information about the absence of light to other cells. To test this idea, the researchers began supplying mutants with normal pectin fragments in their growth medium to see if they could restore skotomorphogenesis.

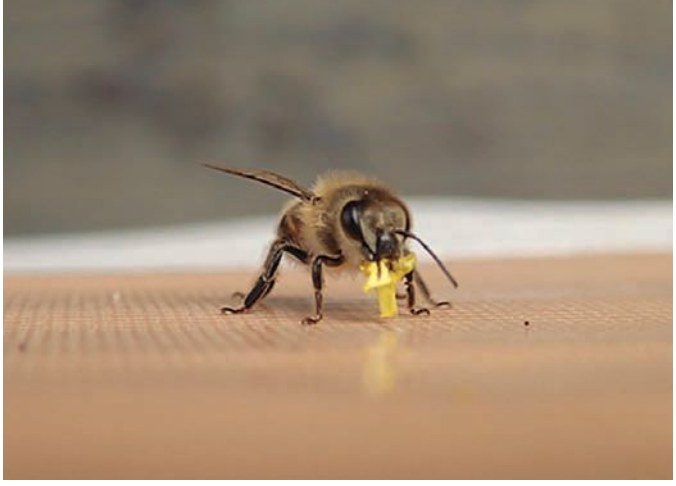
First they tried adding a chunk of pectin backbone called galacturonic acid, initially as a monomer. But nothing happened. So they tried a dimer. Again, no change. But once they gave the mutant seedlings a trimer of galacturonic acid, voilà—they looked just like normal plants that had been grown in the dark. "We believe that, in the dark, the plants generate this compound, and this compound is recognized by a receptor that then acts [through] signal transduction to repress the 'light' type of seedling development," says Krämer, "and therefore the 'dark' type is maintained."



DARK SIDE: Plants use different growth mechanisms in dark and light conditions, called skotomorphogenesis and photomorphogenesis, respectively. A new study suggests pectin fragments in the cell wall signal other cells to maintain skotomorphogenesis in darkness. From these results, a model has emerged in which light somehow interrupts this pectin-based signaling so that photomorphogenesis can commence.

Henrik Scheller, a cell wall biologist at Lawrence Berkeley National Laboratory, developed one of the mutants Krämer experimented with, though he was not involved in her study. He says pectin is known for its role in responding to pathogens, but he wouldn't have predicted that it's also involved in morphogenesis signaling.

"It opens a lot of new questions," Scheller tells *The Scientist*. "And it's not just incremental change in our understanding; it's really a fundamentally new role for cell walls and cell wall–generated fragments that is very exciting." —Kerry Grens



FLOWER POWER: A Japanese *Apis cerana* worker bee brings a petal to the hive.

GREEN MACHINES: One species of *Chlorella* algae uses a photoenzyme to convert fatty acids into fossil fuel–like hydrocarbons.

ENTOMOLOGY

New Bee Boogie

THE PAPER

A. Fujiwara et al., "First report on the emergency dance of *Apis cerana japonica*, which induces odorous plant material collection in response to *Vespa mandarinia japonica* scouting," *Entomol Sci*, doi:10.1111/ens.12285, 2017.

THE WAGGLE DANCE

Honeybees are famous for their waggle dances—figure-eight boogies that foragers use to inform nestmates about the locations of food or water. But entomologists were unclear about whether the dances could also be used to help ensure colony safety.

UNWELCOME GUESTS

Ayumi Fujiwara, a graduate student at the University of Tokyo, and colleagues simulated wasp attacks on hives of the Japanese honeybee (*Apis cerana japonica*) to test the bees' response to danger. "Giant wasps attack the nests of honeybees to feed their brood in autumn. As a result, wasps may sometimes annihilate a whole honeybee colony," she says.

DANCE OFF

The researchers found that the bees did use a waggle dance as a warning signal, but only in response to sightings of one wasp species, *Vespa mandarinia japonica*. "The hive entrance dance informs bees' nestmates of a specific emergency and of the urgent necessity to collect odorous plant materials as a counterattack strategy," Fujiwara says. The bees collect stinky plant materials, such as leaves from Nepalese smartweed (*Persicaria nepalensis*), and smear them at the hive entrance to deter the wasps.

DECODING THE MOVES

The information coded in this new waggle dance is not yet completely clear, notes Margaret Couvillon, a biologist and honeybee specialist at Virginia Tech. "What would be interesting to see is if there are any differences in the conveying of directional information in this defensive context versus the regular foraging context," she says. "Nature tends to be parsimonious in finding solutions, so we might expect that the bees use a similar mechanism in these different situations."

—Karl Gruber

CELL & MOLECULAR BIOLOGY

Sun Fuel

THE PAPER

D. Sorigué et al., "An algal photoenzyme converts fatty acids to hydrocarbons," *Science*, 357:903-907, 2017.

GREEN FUEL

Finding enzymes in nature that convert plant oils into fossil fuel–like hydrocarbons could lead the way toward harnessing new energy sources. After observing that the freshwater alga *Chlorella variabilis* can convert fatty acids into alkanes or alkenes, a team of researchers from France decided to investigate how it accomplished this feat.

FATTY ACID ENGINE

The researchers' assay detected a particularly abundant hydrocarbon-forming enzyme that appears to be located in *C. variabilis*'s chloroplast membrane, says study leader Frédéric Beisson, who researches algae metabolism at the Institute of Biosciences and Biotechnologies at Aix-Marseille University. So they expressed the protein in *E. coli* to test its function, and used mass spectrometry to get a close look at its mechanism of action. The enzyme turned out to be capable of converting a range of fatty acid substrates into hydrocarbon chains, but only under blue light.

A RARE FIND

The researchers were surprised to find that the new enzyme, dubbed fatty acid photodecarboxylase, captures energy directly from light, in contrast to enzymes whose expression is regulated by light. "It wasn't something we were expecting," remarks Beisson. Additionally, unlike enzymes that need just a flash of light to become active, the new enzyme only works under continuous light, making it an addition to a mere handful of known "photoenzymes."

GETTING INTO GEAR

The production of hydrocarbons is a well-studied process in algae, Günther Knör, a chemist at Johannes Kepler University in Austria, writes to *The Scientist* in an email. But he thinks that photoenzymes could be used to more efficiently produce hydrocarbons in light-driven artificial systems in the near future: "This would be a breakthrough for solar fuel generation inspired by nature."

—Katarina Zimmer

AYUMI FUJIWARA; LAURENCE GODART

PROFILE

Planting Independence

After a harrowing escape from Iran, Katayoon Dehesh didn't shy away from difficult choices to pursue a career in plant biology.

BY ANNA AZVOLINSKY

In September 1980, just as the Iran-Iraq War was beginning, Katayoon Dehesh was an assistant professor at National University (now Shahid Beheshti University) in Tehran teaching biology. She had returned home to Iran from the United Kingdom in 1977 after receiving a PhD in plant biology, and aside from lecturing, Dehesh was participating in mandatory military service that barred her from leaving the country. She was also told that she couldn't teach on religiously significant days, but Dehesh disregarded the order, continuing to hold her scheduled classes.

At the same time, Dehesh was making plans to join the lab of a professor in Germany who was working on salt tolerance in plants—the subject of her PhD thesis. All commercial flights were grounded because of the war, so a bus ticket was the only way out of the country. But these were booked up by other people wanting to leave Iran. Then, abruptly, all of the embassies in Iran closed, and no one could exit without a visa.

"Suddenly, there were many available bus tickets," says Dehesh, now the director of the Institute for Integrative Genome Biology at the University of California, Riverside. "I packed a small suitcase and said goodbye to my mother, who was crying, and my father and sister, who thought I was mad to leave. But I thought my life was in danger because I had spoken out about the religious policies at the university and, as such, I could not work there."

Dehesh got on a night bus that drove without lights to avoid the Iraqi bombers. At the Iran-Turkey border, a guard asked to see Dehesh's exit visa, which she didn't have. Her student visa and a letter from the German professor inviting her to work in his lab were of no avail. "'No, where is your exit visa? I cannot let you go without an exit visa, go back on the bus,' he kept saying to me."

As luck would have it, a man behind Dehesh was caught with a secret compartment in his bag that held money and jewelry, diverting the guards' attention. "The border was just this low bar that you could jump over and you would be in Turkey." Dehesh bolted and jumped a barrier, escaping Iran, and hasn't returned since.

Dehesh says that she has not told this story publicly before. "I never talk about it. It saddens me," she says. "But I am getting older and getting over all that. That's life."

A BUDDING PLANT BIOLOGIST

Dehesh was born in Tehran in 1952. Her father was a colonel in the Iranian army, and her mother was a homemaker. Dehesh was the fifth of six children. From her mother, she learned about equality between boys and girls and the value of higher education. "She has a very strong personality and really drilled into her three daughters that marriage was not the goal," Dehesh says of her mom. "She wanted us to strive to be independent and accomplished. Perhaps that is why all of my siblings and I have PhD degrees."

In 1969, Dehesh entered Pahlavi University (now Shiraz University) in southern Iran, an American-style college with classes taught in English. There, she joined the political, anti–royal family movement, giving speeches and marching in demonstrations. Dehesh's political activism came at the expense of her schoolwork—she failed all of her first-semester courses except for Farsi literature.

"My supervisor called me into his office and said, 'Why are you wasting my time and your time and taking a precious space that can be given to a man who will be the breadwinner of a family? Why don't you drop out and learn how to cook and sew?'" Dehesh recalls. "That just electrified me. I gave up all of the political activities and studied, because otherwise I would be thrown out." She earned high marks in all her classes the following semester.

In her second year, Dehesh, inspired by a scientific excursion she took to the salty Lake Maharloo, discovered she wanted to study plants. "I saw these beautiful succulent plants growing around the lake. It was amazing to me that these plants could grow in so much salt," she says. "I suddenly wanted to become a plant biologist."

After graduating in 1973, Dehesh flew with her mother and aunt to London, where members of her family were studying, with the intent of finding a PhD program. Instead of applying by mail, she knocked on professors' doors because she "didn't know how to apply in advance," says Dehesh.

On a drive with relatives, Dehesh passed by the University of Sussex. She was captivated by the red brick buildings and asked to stop so she could look around. Dehesh found the plant biology building and asked a secretary to arrange for her to speak to the department's chair.

The next morning, Dehesh met with the department head, James Sutcliffe, and told him that she wanted to understand the physiology and biochemistry of Salicornia, a succulent plant that grows around the salt lakes of Iran. "I didn't know he was the head of a renowned institute on salt tolerance and salt and iron uptake!"

Dehesh made an impression on Sutcliffe, who told her to bring her undergraduate transcript the next morning. To get

COURTESY OF KATAYOON DEHESH

KATAYOON (KATIE) DEHESH

Director, Institute for Integrative Genome Biology
Ernst and Helen Leibacher Endowed Chair
Professor of Molecular Biochemistry
University of California, Riverside

Greatest Hits

- Demonstrated that plants accumulate compounds, such as quaternary ammonium, for intracellular osmotic potential adjustment and retaining high turgor pressure in high-salt environments
- Identified GT2 as a novel transcription factor that supports maximal expression of phytochrome A
- At Calgene, produced medium chain fatty acids in transgenic plants
- Characterized lipid-derived volatile compounds emitted by plants as a defense mechanism against certain sucking insects such as aphids
- Identified key stress-specific retrograde-signaling molecules used by plastids to regulate selected nuclear stress-response genes in plants

them, Dehesh took a train and bus to London to pick them up and returned the next day to present the documents to Sutcliffe. As a sort of entrance exam, he asked her what she would do first to initiate her research project. "I know that Brighton is on the English Channel and that the water is salt, so there must be some kind of Salicornia here to collect and study. I would first grow them in different salt concentrations," she proposed. Satisfied with Dehesh's answer, Sutcliffe offered her a PhD position in his lab.

THE SALT LIFE

Dehesh applied for and received two PhD scholarships from Iran to study abroad because, she says, "there were not that many women that applied." In Sutcliffe's lab, Dehesh explored the mechanism of salt tolerance in Salicornia plants around England. "We were among the first to show the compounds that plant cells produce in the cytoplasm to retain a high internal osmotic pressure so that the water doesn't leave the plant cell."

After finishing her thesis in 1977, Dehesh went back to Tehran to visit family for a few months. She had planned to write up her paper on the plant compounds while in Iran, but never did because Sutcliffe was diagnosed with lung cancer and died while she was away.

She wanted to go to the U.S. for a postdoc, but in the meantime took a teaching job at National University in Tehran in the fall of 1977. Soon after, she received a letter stating that she and other women with a PhD or equivalent degree were required to do military service and were forbidden to leave the country.

Every day, she reported to a nearby military station for training from 6 AM until 2 PM and then taught classes at the university in the evening between 4 and 9. "Slowly, I saw the changing political face of the country," she says. "There was unrest, and then the revolution started, and I stopped reporting for military duty."

OUT OF IRAN, ON TO GERMANY

In September 1980, Dehesh made her escape. After leaping the border gate and entering Turkey, she got on a bus that was headed for Istanbul. Because of the Turkish coup d'état that month, Turkish soldiers were guarding all public places, checking all the buses, and searching through everyone's belongings, taking the money passengers carried on, including the small sum Dehesh had in her purse. Anticipating thievery, she had sewn extra money into her clothes. The bus journey from the border to Istanbul took three

days, during which Dehesh did not buy food for fear the soldiers would see her money stash. She subsisted on just water.

Once in Istanbul, Dehesh flew to Germany—using the money she had hidden—and was invited to an in-person interview for a research fellowship at a biology institute in Giessen. But when she wouldn't lie and say that she planned on going back to Iran after a year, the interviewer refused to give her the fellowship. "The interviewer kept saying to me, 'You don't understand. You need to tell me when you are going back to Iran. Otherwise, I cannot give you the funding,'" Dehesh recalls. She replied, "'I don't care if you don't give me the money. I am not planning on going back.'"

"After a good cry, I went to Freiburg where I was registered at the Goethe Institute to learn German, and where I met with the head of the plant biology department, Hans Mohr." Mohr arranged for Dehesh to work as a volunteer in Klaus Apel's plant biology lab, where Dehesh says she worked 14 hours a day for three straight months.

The hard work paid off. Dehesh generated enough data for a paper that was eventually published in 1983. In that paper, she reported the role of proteases during photomorphogenesis, the response of plant growth to light. She confirmed prior studies' evidence that proteases are important for organelles called etioplasts, which convert to chloroplasts upon light exposure, to function. After her volunteer stint, Dehesh was offered 50 percent of a postdoctoral salary in 1983, a so-called "habilitation position," which is an educational training requirement for obtaining a professor position in many German-speaking countries.

The same year, Apel's lab moved to Kiel, Germany, a conservative city where no one would rent Dehesh an apartment because she was a single woman and a foreigner. "For three weeks, I slept in Apel's office. The first morning, the cleaning staff found me sleeping there and panicked. It was so terrible." Finally, a professor in the department rented his empty apartment to her.

STRESS RESPONSES

To learn molecular biology, Dehesh took a yearlong sabbatical in 1986 in Peter Quail's botany lab at the University of Wisconsin, Madison. When she asked to stay on longer, not having achieved her scientific goals, Apel refused, so Dehesh resigned from the German lab and became a postdoc in Quail's lab. "I was a bit stubborn and wanted to do things my way, and also realized there was a glass ceiling for me in Germany. I didn't want to be seen as a woman and a foreigner, I wanted to be a scientist," she says.

In 1987, Quail's lab moved to the University of California, Berkeley, and in 1990, Dehesh reported in *Science* that GT2 is a transcription factor that binds to the promoter of phytochrome A, a plant photoreceptor. She revealed that GT2 has a dual DNA-binding and nuclear-localization signal and functions to support maximal expression of phytochrome A, uncovering a new way plants regulate light-induced growth.

In November 1993, Dehesh and Quail, who had married in 1991, welcomed their son. Wanting to start her own independent line of research, in January 1994, Dehesh took a position at Calgene, a Davis, California–based biotechnology company, where she ran the lipid biochemistry program.

Monsanto acquired Calgene in 1997, and in 2002, Dehesh quit her program leadership position, a reaction to the new company's policies, which she considered to veer too much into business at the expense of quality science. That year, she established her first academic lab at UC Davis, starting from scratch on a new topic: stress-response pathways in plants. She homed in on plant lipid-signaling pathways in general, and specifically, on oxylipins. In 2008, her lab identified several volatile plant compounds that function in plant defense responses.

Her lab then focused on identifying the nature and mechanism of action of stress-specific retrograde signals from organelles to the nucleus. These signals have a central and evolutionarily conserved role in organismal integrity and adaptation to environmental conditions. Dehesh focused on the dynamics of perception and transduction of these signals and how they culminate in inter-organelle cooperation. Her lab found that one particular signaling metabolite, methylerythritol cyclodiphosphate (MEcPP), also present in eubacteria and malaria, senses and communicates environmental perturbations, and ultimately alters gene expression to enable the organism to cope with a range of different environmental stresses. Because MEcPP is common between eubacteria and malaria, Dehesh continues to use plants as a surrogate to understand the signaling networks shared among these organisms.

REACHING OUT TO GIRLS

Dehesh moved her lab to the University of California, Riverside (UCR), in 2016, attracted by the opportunity to lead the university's genomics institute. Her research goal now is to identify evolutionarily conserved pathways and metabolites common to a group of parasites called apicomplexa, eubacteria, and plants, but not found in mammals, and to use plants as a platform for drug discovery. Her lab is collaborating with the National Institutes of Health to screen compounds as potential antibiotics and antimalarial drugs.

Dehesh also made the move to Riverside to stretch her influence beyond the lab. As director of UCR's Institute for Integrative Genome Biology, Dehesh plans to start a program to train local students in metabolomics, for which a new facility is being constructed. Dehesh wants to give high school students who may not be inclined to attend university opportunities to train in analytical chemistry and biology techniques. "Metabolomics will be a major approach in the future, and we need individuals to run these machines and do the data analysis."

Her long-term goal is to help women and girls around the world through education initiatives. "I want to stretch my arms to all of the girls across the globe and tell them that being a woman is a pride, not a shame, and that they are capable of achieving what they dream of, and that they just need to believe in themselves. That is my final act in the theater of life. When I achieve that I am good!" ■

Anjali Iyer-Pascuzzi: Root Detective

Assistant Professor, Department of Botany and Plant Pathology, Purdue University. Age: 41

BY SHAWNA WILLIAMS

They may not make the cut for big-budget wildlife documentaries anytime soon, but plant-microbe battles don't lack for drama, as plant biologist Anjali Iyer-Pascuzzi learned in an undergraduate seminar at the University of California, Berkeley. "I loved the idea that the plants and the microbes interact with each other," she explains. "It's this race to see who can win, who can be resistant, and who can be virulent."

© BRIAN POWELL

Intrigued, Iyer-Pascuzzi went on to become a graduate student in a plant pathology lab at Cornell University. But she soon found herself more drawn to the work of Cornell rice geneticist Susan McCouch. Due in part to six months spent in India as an undergraduate, Iyer-Pascuzzi had learned to appreciate "the idea that you could make a difference for a huge population by improving crops, and particularly rice," she explains.

After earning her master's, Iyer-Pascuzzi switched to McCouch's lab. For her doctoral work, she cloned a rice gene, *xa5,* that confers resistance to a bacterial blight.[1]

Iyer-Pascuzzi then headed to Duke University for a postdoc, to learn more about molecular biology and plant roots with developmental biologist Philip Benfey. There, she decided to investigate whether different strains of the same plant species produce distinctive root systems, an idea that Benfey says ran contrary to conventional wisdom.

Root architecture had not received much attention from researchers, in part for practical reasons—roots can't be seen through the soil, and pulling them out wrecks their structure. Iyer-Pascuzzi and Benfey devised a system in which plants grew in a transparent, nutrient-laced gel atop a turntable and were automatically photographed as they rotated.[2] When the duo used this method to compare different cultivars of rice plants, they discovered that the roots were, as Benfey puts it, "dramatically different."

During her time in Benfey's lab, Iyer-Pascuzzi also analyzed the responses to various types of stress among different cell types in *Arabidopsis* roots.[3]

When Iyer-Pascuzzi started her own lab at Purdue University in 2013, she wanted to combine her interests in pathology, genetics, and root architecture. In one project, her group is now looking at how a bacterial pathogen, *Ralstonia solanacearum*, moves through the roots of tomato plants. The team found that the microbes reach plants' xylem more quickly in susceptible plants versus those that succeed in warding it off.[4]

Chris Staiger, a plant cell biologist who heads Iyer-Pascuzzi's department, notes that she's an exceptionally well-rounded faculty member, with strong teaching skills and a bent for community outreach in addition to her research chops. "It's often a struggle for many, many faculty to figure out how to get a research program up and running or figure out how to teach," he says. "Anjali does all three facets of this job incredibly well and in a very sincere way." ■

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Going Virtual with Brain Research

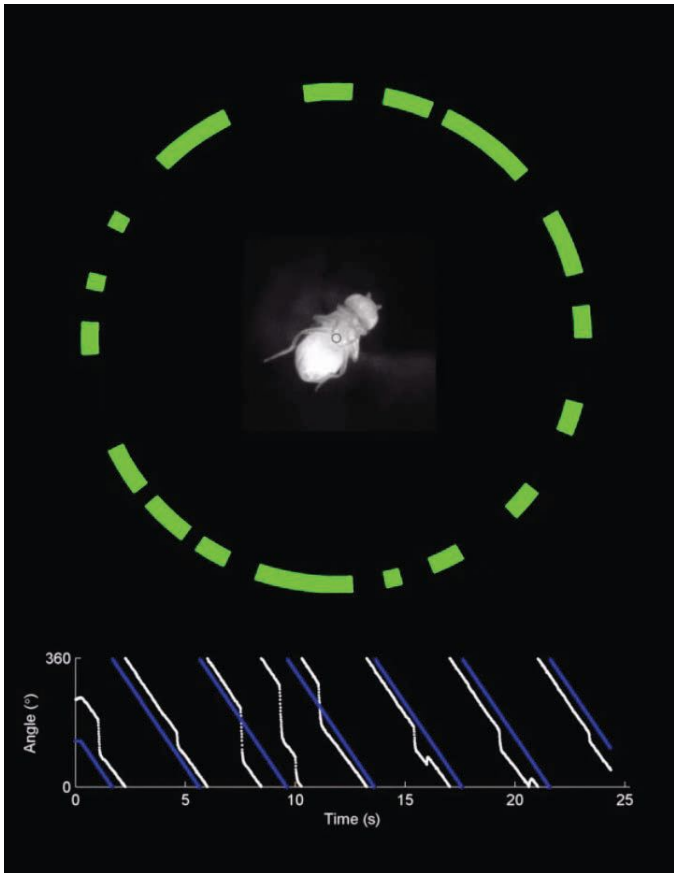
Virtual reality and robots offer an unprecedented view of behavior and the brain, especially in unrestrained animals.

BY ASHLEY YEAGER

Wandering through a maze with striped gray walls, a mouse searches for turns that will take it to a thirst-quenching reward. Although the maze seems real to the mouse, it is, in fact, a virtual world. Virtual reality (VR) has become a valuable tool to study brains and behaviors because researchers can precisely control sensory cues, correlating nerve-cell activity with specific actions. "It allows experiments that are not possible using real-world approaches," neurobiologist Christopher Harvey of Harvard Medical School and colleagues wrote in 2016 in a commentary in *Nature* (533:324–25).

Studies of navigation are perfect examples. Extraneous sounds, smells, tastes, and textures, along with internal information about balance and spatial orientation, combine with visual cues to help a mouse move through a maze. In a virtual environment, researchers can add or remove any of these sensory inputs to see how each affects nerve-cell firing and the neural patterns that underlie exploration and other behaviors.

But there's a catch. Many VR setups severely restrict how animals move, which can change nerve cells' responses to sensory cues. As a result, some researchers have begun to build experimental setups that allow animals to move more freely in their virtual environments, while others have starting using robots to aid animals in navigation or to simulate interactions with others of their kind. Here, *The Scientist* explores recent efforts in both arenas, which aim to develop a more realistic sense of how the brain interprets reality.



SHORT LEASH: Tethering a fly allows researchers to study the eye and body movements of the insect as it sees and responds to a virtual reality scene, which appears as a panorama or a solid bar.

FLY ON A TETHER

RESEARCHER: Mark Frye, neurobiologist, University of California, Los Angeles
VR SET UP: Magnetic tether

Vertical bars, small "boxes," and landscapes of moving vertical lines may seem trivial, but in a fly's world they represent aspects of the landscape such as trees (bars), predators (box), and being blown off course (lines). "We are interested in understanding how visual systems distinguish these sorts of features," says Frye. "Our own brain does the same sorts of things, but we don't have a clear understanding of how, on the molecular and single-cell level."

Frye and colleagues developed a tether system that lets animals take flight in a virtual environment. The researchers glue the dorsal thorax (just behind the head between the two wings) of a fly to a small pin and place the pin and attached fly into a magnetic field, so the insect can move vertically. They then let the fly move about the arena ringed by projectors.

WHAT IT TAKES: A few inexpensive rare-earth magnets ($10 each; see list at Frye lab website, www.fryelab.net/protocols.html), a miniature v-shape pivot bearing, and a steel pin. You also need a video camera and computer to track the fly's body angle and LED panels to generate the visual stimuli that are displayed. "Flies can see faster than humans, detecting the flickering of our standard computer monitors, so we have to use something faster to display movies to them," Frye says. The LEDs

COURTESY OF MARK FRYE

come in small 8x8-pixel panels, connected like Legos ($30 each, total cost ~$1,500, IORodeo). The visual display that the fly sees in full panorama is 96x32 pixels. That seems really low resolution to us, but flies also have poor spatial resolution, so to them, these displays seem like high-definition television, Frye says.

WHAT YOU CAN LEARN: Frye and a colleague recently used the magnetic tether to study flies' saccades—very fast jumps from one eye position to another (*Curr Biol*, 27:2901-14.e2, 2017). Decades of work had shown that when rigidly fixed, flies track a projection of a bar with smooth eye movements. But the new setup showed the opposite. Flies demonstrated sustained bouts of saccades following the bar, with surprisingly little smooth movement. In contrast, the insects' eyes moved smoothly while seeing a projection of a panoramic scene. "What blew my mind was the fact that the bar stimulus is not processed by the smooth panorama system at all," Frye says. "The really interesting implication here is that rigidly fixing a fly in place in virtual reality somehow disrupts visual processing in a systematic way."

FREE-ROLLING RATS

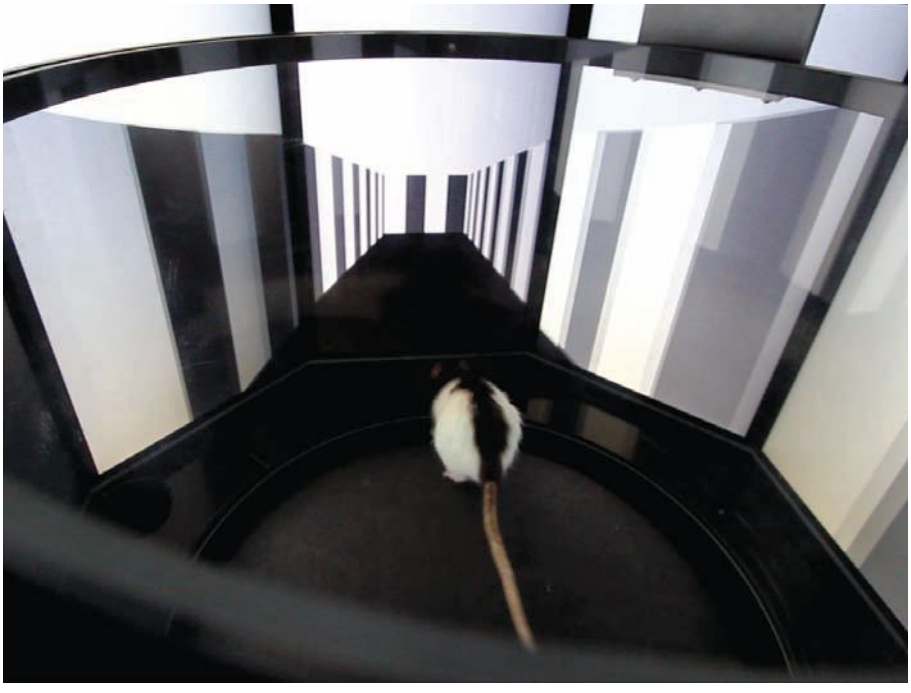
RESEARCHER: York Winter, cognitive neurobiologist, Humboldt University, Berlin
VR SET UP: Virtual Reality ServoBall

The way rodents' heads are fixed in common VR setups dramatically restricts how they act, so complex behaviors such as spatial orientation, which require head movement, are impossible to elicit, according to Winter. Such restriction is especially stressful for rats, and dangerous because rats, compared to mice, are strong enough to hurt someone trying to restrain them. As an alternative to head-fixed VR treadmills, Winter and colleagues developed the Virtual Reality ServoBall (*J Neurophysiol*, 117:1736-48, 2017).

Rats walk from their home cage into the VR environment through a radio-frequency identification (RFID)–controlled gate system. Because the rats can enter the VR arena any time, training them is relatively quick and easy, even for cognitively complex VR experiments, the team notes.

WHAT IT TAKES: A home cage attached to a tunnel into the experimental arena with the ServoBall, a spherical treadmill system ($94, Phenosys). The arena contains a 490-millimeter platform within a transparent cylinder that limits the movement of the animal to the central part of a 600-millimeter-diameter ball. The treadmill is surrounded by a circle of monitors that display the visual environment from the animal's position in the VR scene. Video cameras track animal movement, providing feedback in a closed loop that can alter the movement of the ball, keeping the animal in the center of the arena. There are also eight retractable liquid reward devices located at the periphery, which permit the delivery of a water reinforcement at experimentally predetermined locations.

WHAT YOU CAN LEARN: Because the ServoBall can stop and start to give the animal more autonomy in its exploration, the rat receives touch information from the physical walls of the arena as well as balance and other information when its body rotates. And, because no strength is needed to move the motor-driven ball, it can also be used with mice, certain species of lemurs or birds, and even insects, the team notes. This setup can also be used to study the neuronal activity underlying free exploration by combing the ServoBall with optogenetic techniques or microscope headpieces designed for freely moving animals.



ROLLING WITH IT: A rat navigates a virtual reality maze while walking on a spherical treadmill called a ServoBall.

INTO THE "CAVE"

RESEARCHER: Anton Sirota, neuroscientist, Ludwig Maximilian University of Munich
VR SET UP: ratCAVE-VR

Spherical treadmills are great for presenting precise stimuli to animals, says neuroscientist Andrew Straw at the University of Freiburg in Germany. The downside is that the animal receives

sensory feedback that is unnatural. "This can be particularly problematic when studying spatial awareness and spatial cognition," Straw says. "If the animal doesn't feel like it is moving correctly, it may try to correct the situation rather than behaving as it would in more natural conditions."

To move beyond this limitation, teams of scientists, including Straw, are developing the eCAVE Automatic Virtual Environment setup, in which animals move freely within a cube. Initially developed for flies (*J Exp Biol*, 212:1120-30, 2009), the technology has since been adapted for fish, mice, and, most recently, rats (bioRxiv, doi:10.1101/161232, 2017).

In the ratCAVE-VR experiments, rats gain visual feedback in 3-D space, and, in turn, interact with and follow the virtual walls, explore virtual objects, and avoid virtual cliffs—much more naturalistic behaviors, note Sirota and colleagues in a paper describing the setup.

WHAT IT TAKES: The testing area is a rectangular arena similar to that used for regular open-field experiments. However, in this configuration, the arena is painted white and serves as a projection surface. Sirota used an array of 12 high-speed cameras ($2,499-$3,499 for the array, OptiTrack or NaturalPoint Inc.) to track the position of the rodent's head, which was decorated with reflective dots, in 3-D space. This tracking system enabled the team to update the rodent's head position with very high resolution. To map the virtual environment onto the projection surface, the team used an algorithm identical to those described in other rodent VR setups; in this case, the projection was continuously updated according to the changing 3-D position of the rodent's head.

WHAT YOU CAN LEARN: In the arena, the walls are shifted to appear in a different location from their physical location. The animals are tricked into believing the VR stimulus. After experiencing the shifted VR environment and a normal environment, the animals are no longer fooled by the shift. Straw says the animals probably could feel the walls with their whiskers to discern the mismatch. "I think this demonstrates how powerful physical cues are for knowing where you are," he says.

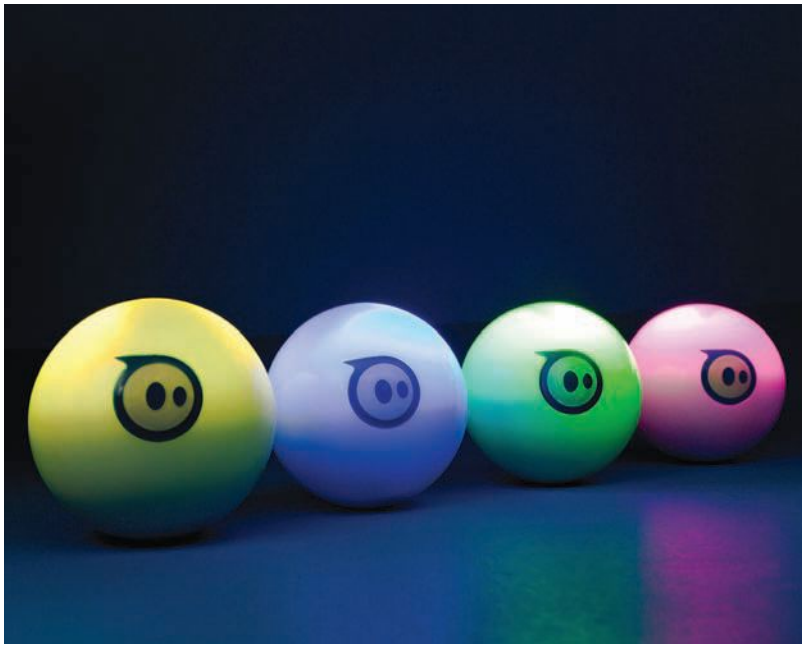
FROM VIRTUAL REALITY TO ROBOTS

RESEARCHER: Jean-Marc Fellous, psychologist, University of Arizona
SET UP: Sphero robot

Even if animals are moving freely, virtual environment constraints may have significant consequences on how the neural circuitry underlying spatial navigation works. As an alternative, Fellous and his colleagues are having rats interact with robots to track how the animals' brain activity correlates with behavior. The team developed a braking algorithm for the robot, so the researchers could precisely control the rodents' direction and speed, and, as a result, used the robot to lead rats through the correct path in a complex maze with nine possible reward sites (*J Neurosci Methods*, 294:40-50, 2018).

WHAT IT TAKES: The robot, called Sphero 2.0 ($130, Sphero) is a small ball, which Fellous harnesses to a chariot-like contraption. Between the wheels of the chariot is a small tray carrying rat treats, which help animals learn to follow the robot. The braking algorithm is used to make the robot stop at precise locations and travel at an exact speed.

WHAT YOU CAN LEARN: Fellous and his colleagues collected electrophysiological recordings from robot-guided rats comparable to those obtained with VR experiments. They showed that place-cell firing in the hippocampus is the same whether rats learn the maze themselves or are taught by the robot, so researchers could use robots instead of VR to study the neural activity underlying spatial navigation.



BUDDY BALL: In addition to virtual reality, researchers are experimenting with robots, such as these called Spheros, that interact with rodents as they navigate without restraint.

COURTESY OF SPHERO

Straw notes, however, that while robots are an exciting addition to the tool chest, they too have drawbacks. "Using a robot to lead animals around or to simulate other animals can be really important for certain experiments, but robots are bound by the laws of physics," he says. "With virtual reality, experimental designs that make use of teleportation and other physically impossible feats become possible." The techniques could complement each other well, he notes. ■

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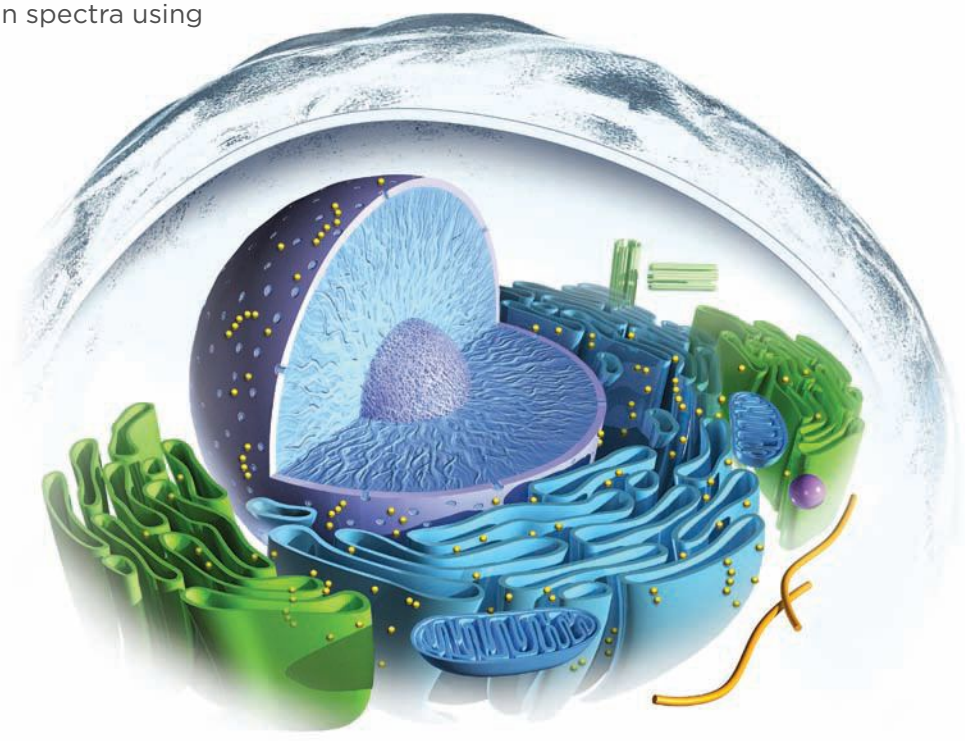
Brain Protein Cartography

Scientists are pinning down protein spectra using subcellular spatial proteomics.

BY DEVIKA G. BANSAL



Cellular factories perform their functions by localizing and trafficking proteins into compartments where they can serve specific purposes. Because of this, a protein's subcellular coordinates offer valuable clues about its activities.

Scientists can visualize protein distribution within cells using super-resolution microscopy—either by tagging proteins with fluorescent probes or by using antibodies. But such methods are typically not scalable and require researchers to restrict their choice of proteins to a known set.

Unbiased mass spectrometry–based proteomic methods offer a broader look, and researchers appreciate the accuracy, specificity, and scale they afford. Recently scientists have adapted the approach to study protein activity at the sub-cellular level. Dubbed spatial proteomics, this new methodology allows researchers to create detailed cellular maps and peek into the hidden life of proteins—where they live, who they interact with, and whether they move around—both in healthy and in diseased cells.

"It's one of the missing pieces in the proteomics toolbox," says Daniel Itzhak, a postdoctoral scholar at the Max Planck Institute of Biochemistry in Planegg, Germany. "You can measure protein abundance and protein half-life, but one of the missing things was spatial proteomics, the answer to 'where is everything located.'"

That's an especially important question for proteins functioning in the brain. Neurons occupy more real estate per cell than other cell types, and their proteins can be widely distributed, from the central cell body, or soma, through extensions called axons, to far-reaching cellular processes called dendrites that terminate at synapses and engage in inter-neuronal communication.

One of the challenges in spatial proteomics is to isolate highly enriched cell fractions, often from tiny amounts of starting material, and then to separate organelles with minimal contamination by other cellular structures. That challenge multiplies when dealing with brain tissues, where cell diversity is high, and so amounts of different cell types are scant. *The Scientist* reports how some researchers are braving those odds to map thousands of proteins within the nooks and crannies of the brain.

VESICLE PILGRIMAGE

RESEARCHER: Giampietro Schiavo, neurologist, University College London

PROJECT: Endosomes are membrane-bound vesicles that move cargo in nearly every cell. In neurons, endosomes traffic proteins between cell bodies and axon terminals. These protein carriers are particularly critical in motor neurons, where they can present an entry point for pathogens. Because motor neuron terminals extend into peripheral body tissues, endosomes can transport viruses from the environment all the way into the central nervous system. In addition, many neurodegenerative conditions, such as amyotrophic lateral sclerosis and Alzheimer's disease, are associated with impaired endosomal transport in neurons. "Some of those endosomes transport stress signals back to the soma, and therefore knowing the exact composition can help," says Schiavo. "Quantitative proteomics allows us to have an unbiased view of organelles."

A few previous reports have mapped the protein content of endosomes in a semi-quantitative manner, Schiavo says. But his team wanted to isolate the signaling endosomes at different stages of their journey from the dendrites to the soma, akin to sampling the population of vehicles at the beginning, middle, and end of a drive down the highway.

CHALLENGE: To create that comparative spatiotemporal map, Schiavo and colleagues considered using mouse primary spinal cord motor neurons. But because those cells are derived from mouse embryos, the amount is limited by the number of animals available, and even with the same genetic background the cells vary widely between animals. In addition, the post-mitotic nature of neurons restricts the use of stable isotope labeling with amino acids in cell culture (SILAC), a method widely used to label dividing cells with "light" or "heavy" forms of amino acids.

SOLUTION: To tackle the issues of homogeneity, quantity, and cell division all at once, the researchers used mouse embryonic stem cells, which they expanded, labeled using SILAC, and then coaxed to differentiate into motor neurons. Next, to label endosomes, the team tagged a cargo protein with magnetic nanobeads, which can be highly purified using a small magnet, Schiavo says. Finally, the team mapped the purified and labeled endosomes to get a roll call of the protein content as the vesicles move from dendrites to the cell body. Schiavo credits this "three-pronged strategy" of magnetic purification, SILAC, and using embryonic stem cell-derived motor neurons for the potency of the approach. (*Mol Cell Proteomics*, 15:542–57, 2016)

FUTURE PLANS: Schiavo now plans to conduct the analysis with murine motor neurons bearing mutations that lead to neurodegeneration in humans and mice, and to study problems with endosomal transport in diseased cells.

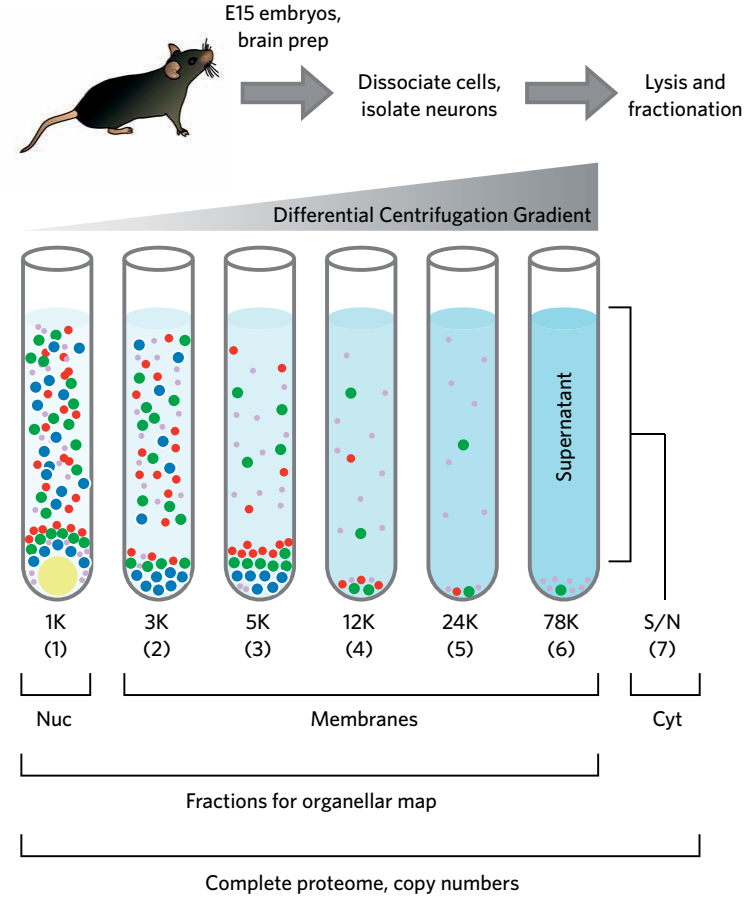
EXPERT TIPS: Schiavo recommends making sure to use high quality stem cells. Unhealthy cells may appear morphologically identical to healthy cells, but they do not transport as efficiently, and the yield of endosomes can be compromised. Also, the magnetic probe should be fresh for efficient transport, he says.

TIME LAPSE

RESEARCHERS: Daniel Itzhak, postdoctoral scholar, and Georg Borner, biochemist, Max Planck Institute of Biochemistry, Germany.

PROJECT: Borner's group wanted to pinpoint the location of all the proteins in a given cell at once and rapidly map out multiple subcellular compartments without purifying each organelle. They also sought to detect changes in the location of proteins under variable conditions, such as before and after drug treatment. In addition, cell signaling processes often lead to proteins relocalizing between cellular compartments, says Itzhak. "We wanted to know what's moving inside the cell."

CHALLENGE: Globally mapping proteins to capture their dynamic movements from one organelle to another is difficult because of the high variability between spatial proteomics experiments. The variability occurs because with every experiment, the contents of the separated subcellular fractions differ, making it impossible to compare the proteome data from one condition to another.



PROTEIN PIN-DOWN: To track protein movements within a cell, researchers at the Max Planck Institute of Biochemistry developed a technique called Dynamic Organellar Maps, which could reliably compare protein maps between two experimental conditions. Here, they isolated cortical neurons from embryonic mice, lysed them, and separated the contents into six fractions based on organelle size and density. Proteins residing in different parts of the cell separated out in these fractions (different colored balls above). By using differential centrifugation and a relatively small number of fractions, the team could control for technical variation between experiments, and thereby track whether a protein moved from one fraction into another after a drug treatment, for example.

CELL REPORTS, 20:2706-18, 2017

SOLUTION: To work around that, Itzhak and colleagues developed an approach they call Dynamic Organellar Mapping, a method that allows researchers to chart protein movements globally. The team tested the technique on HeLa cells and mouse primary neurons. They used either unlabeled cells, cells marked using SILAC before they were lysed, or cells that were chemically labeled after lysis using a mass spec chemical labeling technique called tandem mass tagging (TMT). Borner's team then enriched organelles into distinct fractions, performed mass spectrometry, and analyzed the resulting data. With machine learning, they were able to cluster and localize proteins using markers known to tag the surface of specific organelles. They then repeated the experiments and compared results after treating the cells with epidermal growth factor, or EGF, which is known to initiate a signaling cascade and protein relocalization. The team found that the movement of a select few known proteins clearly stood out after EGF treatment. (*Cell Reports,* 20:2706–18, 2017)

EXPERT TIPS: What makes the technique possible is its repeatability between experiments, Itzhak says. That comes from the ability to keep cellular fractions similar enough between experiments: Itzhak and his collaborators used differential centrifugation, which pellets molecules into multiple tubes based on their size and density, instead of isopycnic separation, which separates molecules into layers inside the same tube and is harder to control, Itzhak says. In addition, the team separated cell organelles into six fractions instead of more, which allowed similarity between replicates. Taking fewer fractions allows proteins of a wider size and density range to group together, whereas taking more fractions makes the ranges too narrow so proteins on the range edges could fall in one or the other fraction—and thus increase variation.

FUTURE PLANS: Borner's lab plans to apply the technique to cell or animal models of neurological diseases and cancer. But mass spectrometers must get faster, cheaper, and use smaller amounts of starting material for this assay to become mainstream, says Itzhak.

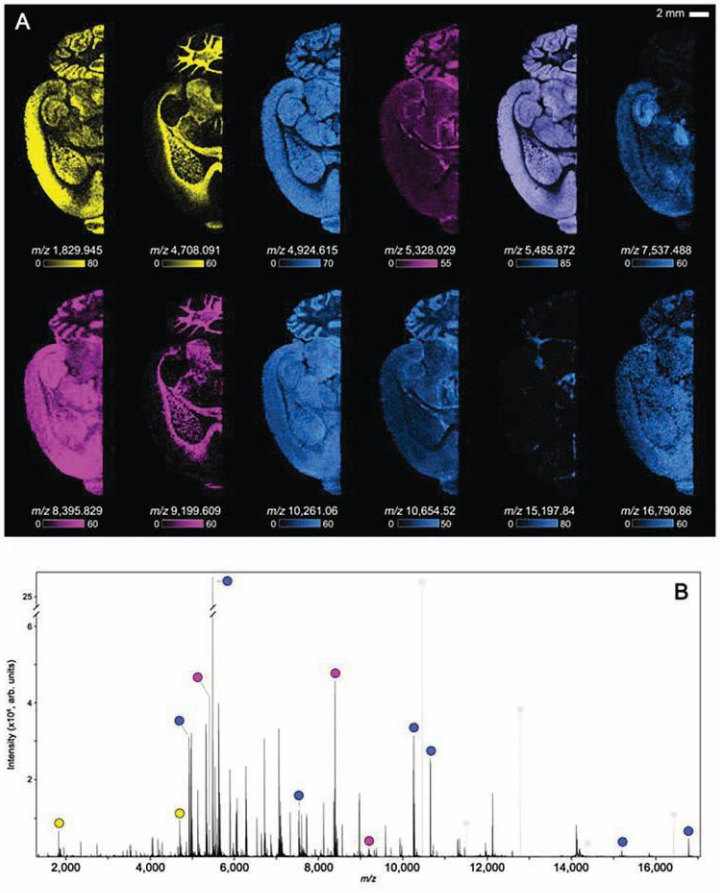
PROTEIN GOGGLES

RESEARCHERS: Marialaura Dilillo, postdoctoral researcher, and Liam McDonnell, chemist, Pisa Science Foundation, Italy.

PROJECT: If probing neurons was not complex enough, tumors in the central nervous system up the ante, because cells in the margins versus the center of the tumor typically have different proteomic profiles. Dilillo and McDonnell wanted to image glioblastoma tissue sections to get a complete picture of where different proteins were located across tumors.

CHALLENGE: Generally, spatial proteomics relies on fractionation to separate out organelles from distinct parts of the cell. But fractionation doesn't work when researchers want to visualize intact biomolecules in tissues of interest. A tweak to mass spectrometry imaging provides a solution; Instead of breaking down proteins into peptides, as mass spec usually does, matrix-assisted laser desorption/ionization, or MALDI, uses a sheet of laser energy–absorbing molecules spread over a particular tissue. When scientists shine a laser on the sheet, it generates heat, which in turn releases ions from the large intact proteins within the sample. The resulting ions can be converted into images by plotting the ion intensity against the relative position of the data in the tissue. However, MALDI coupled with traditional time-of-flight, or TOF, analysis does not clearly distinguish biomolecules

JEFFERY SPRAGGINS, DOI:10.1002/PMIC.201600003



COLOR BY PEAKS: Jeffery Spraggins' lab at the Vanderbilt University School of Medicine, Nashville mapped rat brain proteins using high mass resolution mass spectrometry-based imaging, an effort replicated by Dilillo and colleagues to map intact proteins in mice glial tumor tissues. Shown here is MALDI FTICR imaging data from sectioned rat brain tissue, in which each ion image depicts a protein with a different mass to charge ratio. The image is constructed based on peaks in the overall mass spectrum by plotting the ion intensity against the relative position of the data in the tissue. (Panel B) (*Proteomics*, 16: 1678–89, 2016).

with tiny differences in mass and charge, such as protein isoforms frequently seen in tumor tissues.

SOLUTION: As a workaround, the team employed MALDI-FTICR, or Fourier-Transform Ion Cyclotron Resonance, which determines the mass-to-charge ratio of ions by recording the frequency at which each ion rotates through a magnetic field. Thus, different ions are not detected at different times as with TOF methods, but all at once during the detection period. This improves signal-to-noise ratio and resolution. Dilillo used this method to map mouse glioblastomas, comparing results obtained with TOF versus FTICR. "You can get beautiful images of protein ions using MALDI imaging," Dilillo says. "We could acquire the full distribution of all ions all over the tissue section." (*Scientific Reports*, 7:603, 2017)

Dilillo also complemented her analyses by laser-dissecting specific areas of interest in the glioblastoma, and then running peptides from the dissected cells through a traditional peptide-based mass spectrometer. "You can identify thousands of proteins using [traditional peptide mass spec], and you can get spatial distribution with MALDI imaging," she adds. "The two techniques put together make it really robust."

FUTURE PLAN: Dilillo next wants to perform both MALDI imaging and laser capture microdissection on the same tissue section to minimize variation. The idea, she says, is to first perform imaging and identify regions of interest using the data, then microdissect only those areas to analyze using traditional mass spectrometry.

EXPERT TIPS: Mass spectrometry experiments are extremely specialized, and all the techniques involved in this paper were specifically designed and optimized for the glioblastoma tissue Dilillo's team worked with. "So keep your specific goal in mind," she says. "And standardize the technique for your tissue." ■

Building Better Peer Reviewers

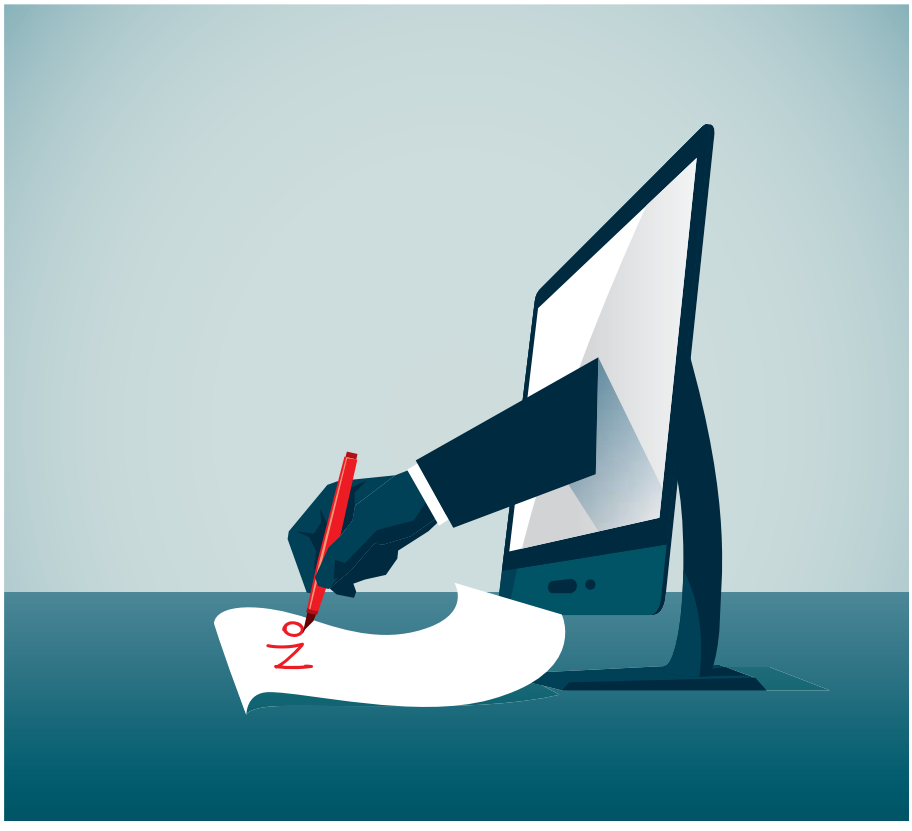
Initiatives to improve scientists' peer reviewing skills are plentiful, but it's too early to tell whether the efforts will bear fruit.

BY ABBY OLENA

California State University, Fresno, biologist Ulrike Müller received her worst peer review when she was a graduate student at the University of Groningen in the Netherlands. In the late 1990s, after submitting a paper about the dynamics of swimming fish to the *Journal of Experimental Biology*, she received an extremely short response—just a few lines long. "The person wrote that this paper was a missed opportunity because we didn't invite him as a coauthor," she says. "No suggestions. Just, 'Sorry, this could've been a wonderful paper if only you'd asked me.'"

Müller's PhD supervisor, John Videler, followed up, and the reviewer, who had hand-signed the review, asked Videler why he, the reviewer, hadn't been invited to sit on Müller's thesis committee. "For me it was just so shocking, making the peer review about professional rivalry when the main author is a junior scientist and [was] caught in this cockfight," Müller says. "'Could we please leave your egos out of this?'" she recalls thinking.

Most researchers remember a bad peer-review experience or two; issues range from reviewers who clearly did not read the manuscript to overly effusive, yet completely unhelpful praise. But there's a growing desire in the scientific community for better, faster peer review. After all, receiving feedback from other researchers in an author's field is one of the defining elements of scientific publication and key to ensuring quality in the scientific literature. "There is nothing like having your scientific argument tested by people who really know what's going on to improve the way that you think about your science," says Sarah Tegen, vice president of global journals development at the American Chemical Society (ACS).



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Although the peer-review process involves multiple players—from authors to journal editors—there is now a range of efforts directed at improving the habits and skills of reviewers themselves, from changing the culture around reviewer anonymity and recognition, to training reviewers to provide better feedback.

Encouraging openness

Traditionally, peer reviewers are anonymous, meaning they are largely shielded from the consequences of writing negative or careless reviews. But in recent years, some journals have introduced alternative procedures that aim to make the whole process more transparent.

In June 2012, the biomedical and life sciences journal *eLife* opened for submissions, with cell biologist Randy Schekman of the University of California, Berkeley, as editor-in-chief. "We wanted to do something different," he explains. "We wanted to take away the sometimes toxic atmosphere that surrounds the submission of anonymous peer reviews, where the reviewers are known to the editor who's handling the paper, but are not known to each other."

Unlike most reviewers, who see their fellow reviewers' comments only after a paper's publication, *eLife*'s reviewers join a private online forum, in which they learn the identities of their counterparts and can read and comment on one another's reviews. At the end of the review process, published papers are accompanied

by the initial decision letter, complete with excerpts of these reviews—individual reviewers are encouraged, but not obliged, to make their names public at this stage—plus responses from the author.

The advantage of this openness is twofold from a reviewing perspective. For a start, the public nature of the reviews throughout the process may help rein in bad behavior. "Because they know their name is going to be associated with it, I think it exerts a little more restraint in the sometimes very negative comments that people make," Schekman says. "You can't hide behind your anonymity here."

What's more, the option of collaboration among reviewers may improve the quality of the review itself. When a reviewer sees what other reviewers have said only after the decision has been rendered, "sometimes you think, 'Well, that's interesting. I hadn't thought of that,' or 'No, he doesn't know what he's talking about, and I wish I'd had a chance to weigh in on this,'" says Schekman.

He acknowledges that breaches in confidentiality or power imbalances when junior and senior scientists are co-reviewers are possible. Nevertheless, the feedback from *eLife* peer reviewers has been positive overall. A 2016 survey of more than 1,000 scientists who served as reviewers for the journal found that 90 percent of respondents felt that reviewer openness in the consultation session is beneficial, and 95 percent said they believed that the process is valuable to authors.

Recognizing reviewers

Another issue influencing reviewer behavior is the lack of recognition of the huge amount of work that goes into peer reviewing, says Müller, who serves as an associate editor at *Proceedings of the Royal Society B*. "Peer reviewer fatigue is a real problem," she explains. "I usually need nine names to get two to three reviews." Without recognition for the work, positive incentives to take time out of busy schedules to serve as a peer reviewer may be minimal.

Publons, a New Zealand–based company focused on reviewer recognition, aims to address this issue. "We really see peer review as at the heart of the research ecosystem," explains Jo Wilkinson, head of communications at Publons. "[We] work with researchers, publishers, and research institutions to turn peer review into a measurable research output."

The company, acquired last summer by Philadelphia-based Clarivate Analytics, allows scientists to create a free online profile where they can maintain a record of their reviewing and editorial activities. Publons automatically verifies that researchers have completed reviews through partnerships with more than 1,400 journals or by contact with editorial staff and review receipts forwarded by users. From their profile, reviewers can download a customized record of their contributions for inclusion in job and funding applications, as well as promotion evaluations.

Publons also attempts to increase the motivation for, and the quality of, peer review through feedback. "Reviewers have actually told us that they want to improve, and that they crave feedback from editors about the quality of their work," says Wilkinson. So the company created a feature where editors can rate the reviews they receive based on timeliness, thoroughness, clarity, and helpfulness. Top scoring reviews receive an "Excellent Review" designation, represented by a gold star on a user's profile.

Many reviewers seem eager for the recognition that Publons offers. More than 240,000 users from all over the world have created profiles and added records for more than 1.3 million reviews. As to whether the company's strategies have actually improved peer review, initial investigations are promising. In a pilot study where Publons collaborated with 15 journals, offering reviewers recognition on Publons led to speedier turnaround on reviews, from 18 days pre-pilot to 15 days during the pilot. And after a collaboration between Publons and the American Society for Microbiology (ASM), reviewers for ASM journals reported that they both appreciated receiving Publons recognition and were subsequently more willing to review for ASM.

"Publons [is] developing pathways that acknowledge the work of peer reviewers, and I think that's very important," says Müller. "We need to make the service that we're doing for our professional community as peer reviewers part of professional recognition."

Providing training

Even with these incentives, some reviewers may simply lack skills needed to produce a constructive review. "Few researchers have received peer-review training, despite being called upon to review hundreds, if not thousands, of papers throughout their career," says Wilkinson.

To address this problem, Publons launched a course in May 2017 called Publons Academy. Composed of 10 online modules, the course covers everything from peer-review ethics to evaluating a manuscript's methodology. Participants also work with a supervisor, such as their graduate or postdoctoral advisor, to write postpublication peer reviews to include on their Publons profile. Upon completion of the course, Publons connects new reviewers with an editor in their field from one of the company's partner journals.

Researchers also have other online options for peer-review training. Since September 2017, Nature Research, part of Springer Nature, has offered a free online master class called Focus on Peer Review. The course covers everything from the role of the peer reviewer to innovations in the peer-review process in lessons that take about three hours to complete. "It's a course designed for anybody," says Victoria Pavry, head of publishing for researcher training at Nature Research. "No matter what type of journal they want to peer review for, we think it would be for them."

ACS is also throwing its hat in the ring. Last August, the organization launched a free four-hour course called ACS Reviewer Lab that is also open to all researchers. The program covers the ethics of peer review, how to assess the significance and quality of the research, and how to write a coherent review. "We don't get into a lot of specifics for chemistry, so just about anyone who is engaged in the peer-review

ecosystem would benefit from this course," explains ACS's Tegen, who oversaw the course's development. Once participants start, they have a month to complete it, and more than 300 researchers have done so already, Tegen says.

Meanwhile, the Genetics Society of America (GSA) just launched a members-only program providing real-world peer-reviewing experience for early-career researchers. Scientists starting out "get very uneven experience and training in peer review," says *Genetics* Editor-in-Chief Mark Johnston of the University of Colorado Denver. "We wanted to provide a training that was more uniform and give them something more concrete."

Last September, course leaders selected 36 participants—most of whom were postdocs—from hundreds of applications. The researchers received seven hours of peer-review training via phone conferences in November and December and, throughout 2018, editors will invite them as reviewers for manuscripts submitted to *Genetics*. Participants will write one review per quarter, receive feedback from the assistant editor overseeing the submission, and read the other referees' responses, as well as the editor's decision letter.

"[Participants] directly interact with the editors at *Genetics*, and they get individualized feedback from the editor on what it is that they did well and where they still have room for growth," says GSA director of engagement and development Sonia Hall, who helped develop the course. "It sends a loud and clear message that the leadership of the journal and the Genetics Society of America respect [these early career scientists] as professionals, and that we're confident in their abilities, and they should be too."

These programs are so new that their effectiveness remains to be assessed. And despite optimism among organizers, it's worth noting that related efforts have had little success in the past, according to University of California, San Francisco, emergency physician Michael Callaham, editor-in-chief of *Annals of Emergency Medicine*. Over the past two decades, he has tried a variety of strategies—from in-person training to direct mentorship from more-senior reviewers—to make new *Annals* reviewers better. After these interventions, he says, there was no difference in the actual review quality as evaluated by the journal's editors.

Moreover, with the lack of data on the effects of current practices, it is still not clear exactly how peer review should be changed, Callaham adds. "We're in such an early, primitive stage of understanding the whole peer-review thought process, which is pretty ironic when you think about the fact that it is the foundation of everything that's done in science," he says. "I totally believe this will be addressed someday, and we will look back on our current practices [and say], 'Wow, how historically quaint.' I think it will happen; I just don't know when." ■

Abby Olena is a freelance science journalist based in Carrboro, North Carolina.

TIPS FOR REVIEWERS

Structure your review: Editors like reviews to begin with a short summary of the paper illustrating what the authors did and what the study contributes to the literature, says Ulrike Müller of California State University, Fresno. "It tells the author and the editor what the reviewer thinks is the purpose of the paper."

Use concrete examples: William Guilford of the University of Virginia suggests asking yourself: "Are you making it clear what is generally right and wrong with the manuscript? [Include] enough specific examples to make it clear to the author and to the editor what the underlying problem really is."

Have the right mindset: Reviewers' comments should be aimed at improving a paper, notes Elisa De Ranieri, head of editorial process and data analytics at Nature Research. "Peer review is a constructive process," she says. "A bad review is when this process fails and, instead of providing constructive criticism, [it] doesn't bring new insight to authors."

Keep it real: Reviewers should make suggestions that can realistically be incorporated. "If someone is insisting that something can't be published until you determine the ultimate answer to life, the universe, and everything, that's just not an acceptable review," says Guilford.

Be a mentor: Good feedback from reviewers can help authors become better scientists, even if their paper doesn't end up being published, says Michael Callaham of the University of California, San Francisco. "Our job is . . . to help improve the literature that we get that's going to be published and to educate and help the people that we don't publish."

Hunger Is the Mother of Invention

Agriculture has been a crucible of innovation since it arose millennia ago. Can a booming human population invent its way out of starvation once again?

BY JESSICA EISE

Some 220 years ago, the somber-faced cleric and scholar Thomas Malthus made a dire prediction: food production could not possibly keep up with population growth in Great Britain. If measures were not taken to limit family size, chaos, starvation, and misery would ensue. And yet, such measures were not taken. The population exploded, but as it turned out, Malthus's dystopian vision never came to pass. Agricultural production rose to the challenge.

Malthus's warnings have a familiar ring today. Once more humanity is staring down the threat of a burgeoning population and concerns that there eventually won't be enough food to go around. By 2050, we will have almost 10 billion mouths to feed in a world profoundly altered by environmental change.

Will history repeat itself, and again refute Malthusian doomsaying? Or will we and our food production capacity succumb to the pressures of unsustainable population growth?

In *How to Feed the World*, a diverse group of experts breaks down these crucial questions by tackling issues surrounding food security.

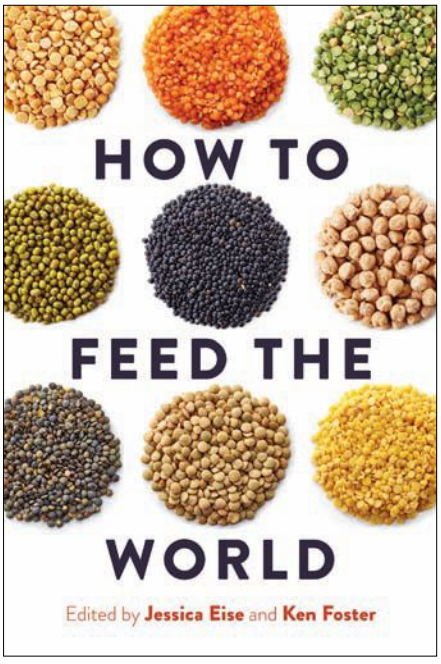
One critical factor that Malthus left out of calculations of population growth and sustainability was the effect of agricultural revolutions. Humans have experienced three such revolutions, each fueled by technological advances, throughout history: the first, about 12,000 years ago, as our ancestors transitioned from hunting and gathering to settled agriculture; the second as 18th- and 19th-century British farmers drastically increased production, proving Malthus wrong; and the third as commercial-scale agriculture bloomed in the 20th century.

None of humanity's past successes, however, indicate that our modern concerns aren't warranted. Environmental pollution, unsustainable water use, and large-scale land use changes raise doubts about our current food production systems. Ironically, many of the same technological innovations that have prevented starvation also wreak havoc on the environment.

But just because elements of past technologies harm the environment, we need not cast aside the concept of innovating our way out of a food crisis. On the contrary, returning to the crucible of technological innovation will help us find modern solutions.

As Purdue University agricultural economist Uris Baldos explains in his chapter on technology, although genetically engineered (GE) crops are extremely controversial in public dialog, all indications are that they are here to stay. Since the technology's development in 1973, several GE crops have been created and commercialized. For example, crops containing a gene from the bacterium *Bacillus thuringiensis* were developed to prevent crop damage from insects, and farmers have adopted them worldwide. There are ongoing efforts to roll out GE versions of fruits, oilseeds, and root crops. Aside from pest and herbicide resistance, plant breeders are also looking to incorporate other useful agronomic traits, such as drought and cold tolerance, virus resistance, and enhanced nutrient content. Some plant breeding programs aim for even-more-ambitious goals. There is an effort to supercharge the photosynthetic process of rice to overcome its current yield limit, for example.

The technology undergirding genetic engineering is expanding at an extraordinary rate, and we are able to do things today that we hadn't imagined possible mere years ago, such as precision genome editing. With the advent of more-efficient and more-precise genetic editing techniques, it is likely that any successful plans to feed the world will involve the use of GE crops.



Island Press, March 2018

Accomplishing that goal entails a range of challenges, as illustrated in *How to Feed the World*. Technological innovation can, once more, provide us with the means to overcome many of these seemingly insurmountable odds. But the technologies that saved us before definitely won't save us again. Therefore, we face one central challenge. Before it is too late, can we innovate, invest in, and accept the technologies we will need to feed the world sustainably? ■

Jessica Eise is an author and Ross Fellow in the Purdue University Brian Lamb School of Communication doctoral program. She coedited How to Feed the World *with Ken Foster, former head of the Department of Agricultural Economics at Purdue.* *Read an excerpt of* How to Feed the World *at the-scientist.com.*

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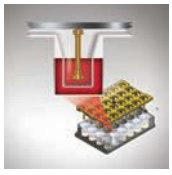

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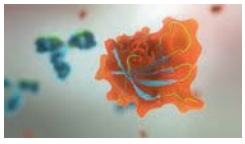

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A Brush with Inheritance, 1878

BY CATHERINE OFFORD

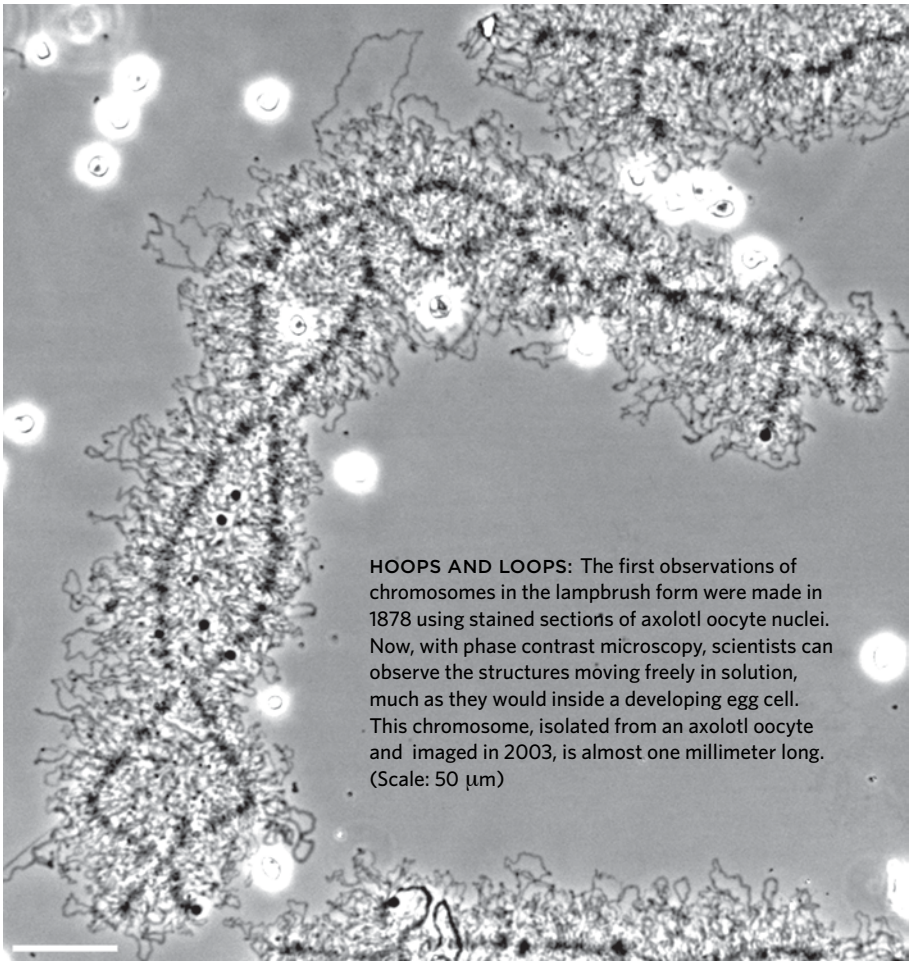
In a laboratory in Kiel, Germany, in 1878, cytologist Walther Flemming saw something extraordinary through his microscope. He and a student were studying oocyte development using stained sections of nuclei from an axolotl, a type of salamander. In one of those sections, Flemming could make out long, thin objects with fiber-like protrusions that formed loops, giving the cellular structures a fuzzy appearance.

In 1882, he published his observations of these "*merkwürdige und zierliche Anordnungen*"—strange and delicate structures. As for what they were, however, Flemming was stumped. "He thought they might be artifacts," says Garry Morgan, a geneticist at the University of Nottingham in the U.K. "He wasn't convinced that they were truly cellular structures. . . . He was struggling to recognize what they were—which isn't surprising given the context."

At the time, researchers hadn't yet identified the physiological unit of heredity. Although Flemming had described and named chromatin in 1879, it wasn't until 1888 that Wilhelm Waldeyer coined the term "chromosome," and scientists began making connections between the structures and genetic inheritance.

German anatomist Johannes Rückert had been observing the same structures that Flemming saw, but in oocytes from a catshark. "Rückert was able to recognize that what he was looking at was a genetic structure, rather than simply an unusual organelle in an unusual cell type," Morgan says.

They were chromosomes, Rückert concluded, but in a peculiar state. For starters, they were big—up to 120 µm, he noted (they extend up to a millimeter in some species). And the tangle of side loops differed from the smoother appearance of somatic cell chromosomes. In a paper published in 1892, Rückert named the structures "lampbrush chromosomes," for their resemblance to wiry brushes used at the time to clean oil lamps.



HOOPS AND LOOPS: The first observations of chromosomes in the lampbrush form were made in 1878 using stained sections of axolotl oocyte nuclei. Now, with phase contrast microscopy, scientists can observe the structures moving freely in solution, much as they would inside a developing egg cell. This chromosome, isolated from an axolotl oocyte and imaged in 2003, is almost one millimeter long. (Scale: 50 µm)

GARRY MORGAN

Since then, scientists have explored the structures in more detail. It's now known that the lampbrush form is adopted by oocyte chromosomes in almost all animals, mammals excepted—perhaps owing to the spatially constrained development of mammalian embryos, Morgan says. Because the loops are sites of intense transcriptional activity, the structures are thought to play a role in oocytes' synthesis of large quantities of protein prior to the rapid cell division associated with embryonic development.

With the genome of the axolotl to be published later this year, Morgan and other researchers are hoping to learn more about how the loops on lampbrush chromosomes might regulate gene expression. But even for geneticists not studying oocyte development, the lampbrush chromosome still holds appeal 140 years after its discovery, Morgan says.

Often in genetics, "you don't really get a feeling for the physical reality of what's going on," he says. But with modern microscopy, researchers can observe these enormous chromosomes swishing around in 3-D space. "You can see that genes aren't static structures—they have a mobility. It's a fascinating thing simply to be able to look at them." ■

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