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PINPOINTING PATHOGENS

TECHNOLOGIES TO TRACK INFECTIONS FROM THE SKIES AND ON THE GROUND

ERADICATING POLIO AND GUINEA WORM DISEASE

THE CONSEQUENCES OF AN INCOMPLETE MICROBIOME

MAKING CLINICAL STUDIES MORE DIVERSE

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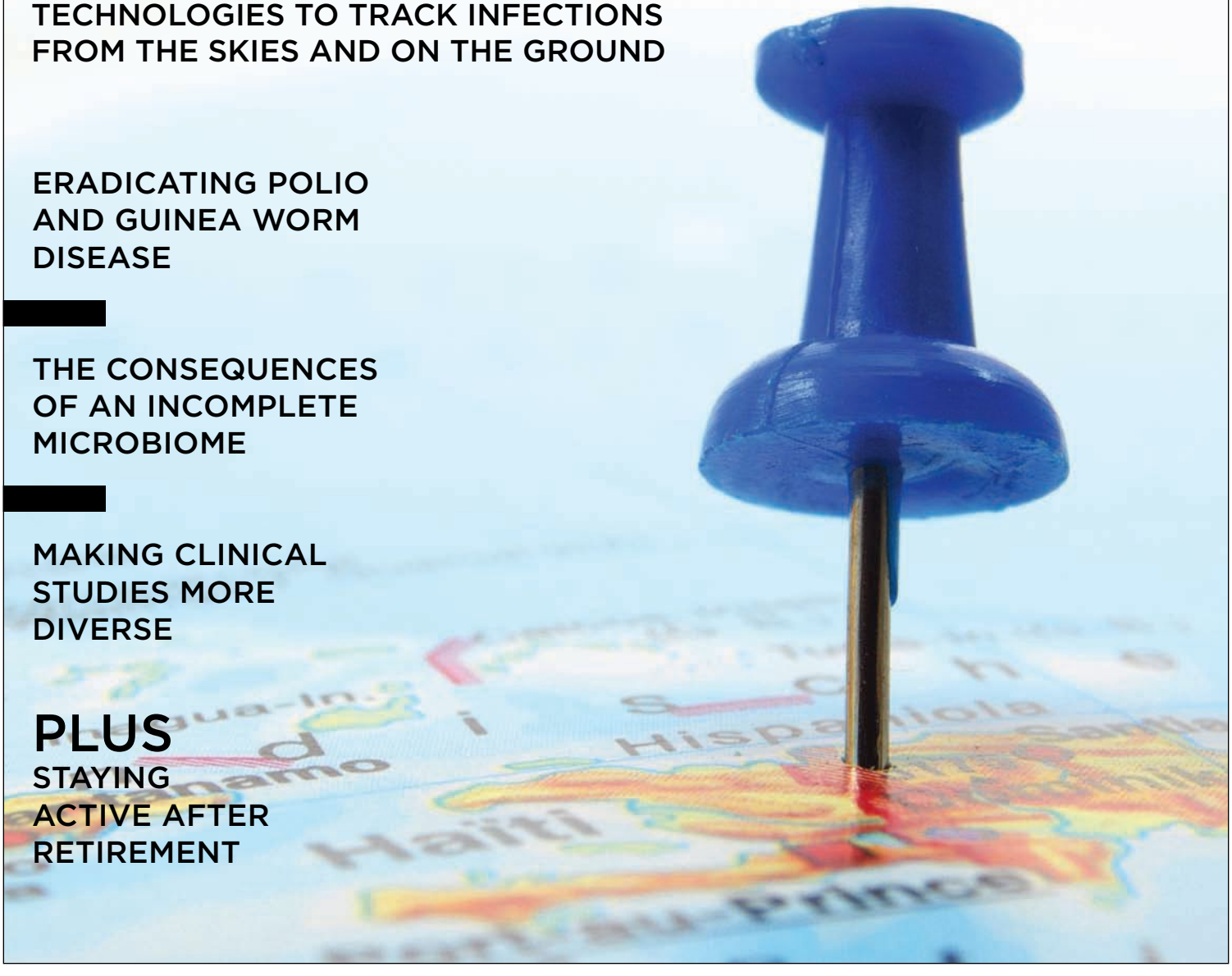
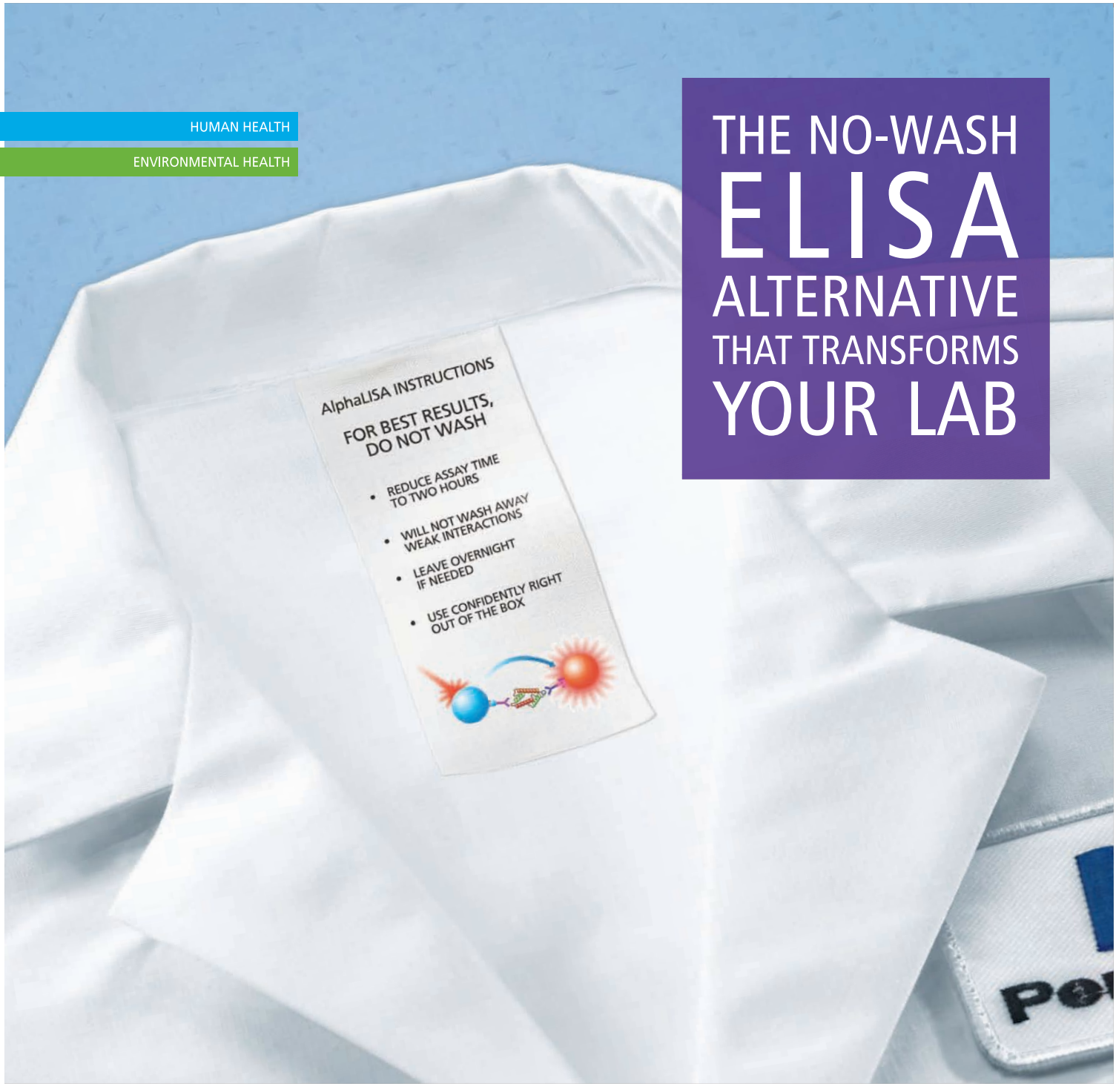


HUMAN HEALTH

ENVIRONMENTAL HEALTH



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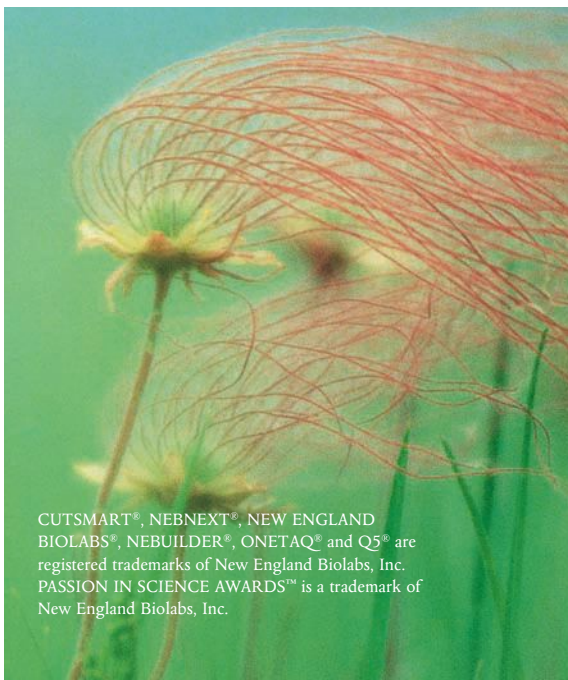

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Features

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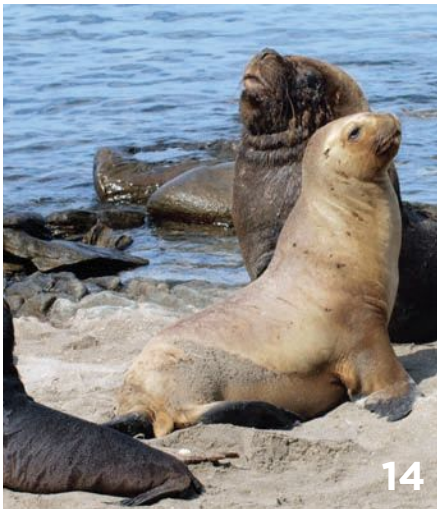

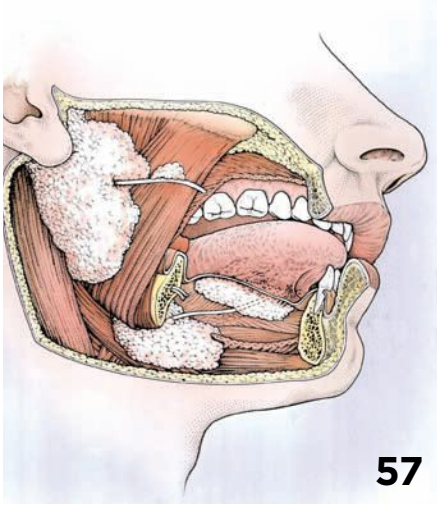

CORRECTION:

Update: Re: "A Plague on Pachyderms" (June 2015). After the issue went to press, the announced death of a five-year-old Asian elephant at the Albuquerque BioPark raised the number of elephants killed by EEHV since 2008 to two.

In "Memorial Research" (June 2015), the fieldwork conducted by Christopher Rodriguez took place in the Davis Mountains near Fort Davis, not the Franklin Mountains near El Paso.

The Scientist regrets the error.

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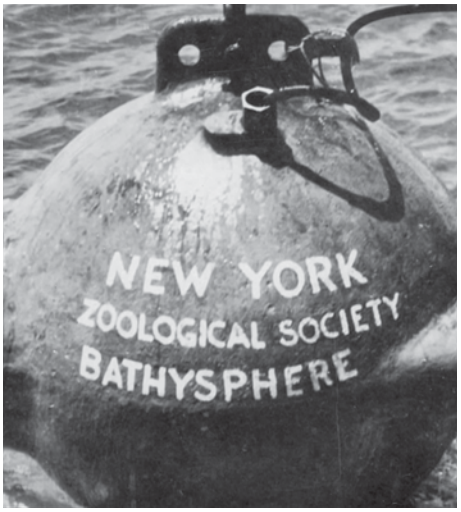

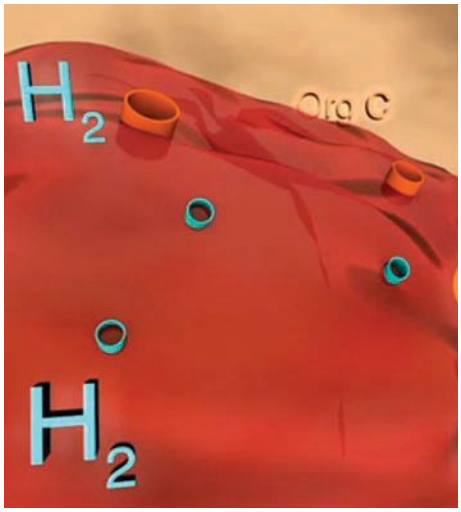

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Orb-iters
See how William Beebe and Otis Barton descended to the ocean's depths in an early submersible designed to allow observation of the mysterious life-forms inhabiting the deep sea.

VIDEO
You Gutless Worm
Meet the digestive tract–lacking oligochaete that has fueled Max Planck researcher Nicole Dubilier's interest in symbiosis and marine science.

SLIDE SHOW
Monkey Business
Travel to Bangladesh to meet the Bedey, a band of river nomads, and their trained macaques, which perform shows and seldom transmit a monkey virus to their handlers.

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

Coming in August

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

- Bio-inspired medical innovations
- The placenta: a vital conduit between mother and fetus
- Deleterious effects of pharmaceuticals in the environment
- Lymphatic system development
- Humanized mouse models

AND MUCH MORE

MONKEY BUSINESS: LYNN JOHNSON; PORCUPINE: © ERIC ISSELEE/SHUTTERSTOCK

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Contributors

When **Rodney Dietert** began his scientific career studying the immune system in animal models as an immunogenetics PhD student at the University of Texas at Austin, he had no idea his path would lead him so deep into public health and human microbiome research. Now a professor at Cornell University, Dietert credits his wife, **Janice Dietert**, a science editor and novelist, for spurring his venture into public-health research. As a former learning-disabilities specialist at the State University of New York at Binghamton, Janice Dietert noted connections between her husband's immunology, toxicology, and microbiome research, and the chronic health and neurological issues plaguing many of the disabled adults she had worked with.

When he was invited to write a journal paper describing what he thought was the key biological sign of a healthy life, Rodney Dietert says the concept of the "completed self" came to him in the middle of the night, ousting his previous immune system–centric focus for the article. To him, the microbiome a baby acquires during a natural birth and early life is vital to the development of an adequately functioning immune system. "We are intended in our healthiest state to be majority microbial," he says. The Dieterts, who live in Lansing, New York, with their two dogs, discuss this concept of the completed self in "The Sum of Our Parts," page 44.

As long as geneticist **Jerry Coyne** has been in science, he's enjoyed writing for nonscientists. He has written more than a hundred book reviews and articles for publications like *The Times Literary Supplement* and *The New Republic*, beginning during his days as a graduate student at Harvard University, then as a postdoc at the University of California, Davis, and finally as a faculty member, first at the University of Maryland and later at the University of Chicago, where he has studied speciation since 1996.

Throughout his career, Coyne has remained active in the lab, mentoring just one student at a time. "My philosophy's always been that if you replace yourself with one good student, you will be a success." He's now replaced himself five times over. "I come from a lineage of scientists who not only worked in the lab with their own hands, but refused to take credit for their students' work," says Coyne. "I'm proud of that lineage."

Now, as Coyne starts winding down his research, he looks forward to focusing on writing. He says that while "science long ago became less challenging, in terms of what skills one must acquire," he is still mastering the craft of writing. Coyne opines on the perpetual conflict between science and religion in "The War Rages On" (page 66), based on his new book, *Faith vs. Fact: Why Science and Religion Are Incompatible*.

Although copy editor **Annie Gottlieb** enjoyed science as a college student, she didn't have the stomach to decapitate mice and was not interested in spending all her time with lab equipment. For more than 35 years, she worked as a freelance critic and writer, contributing reviews to publications such as *The New York Times Book Review* and *The Village Voice*, and authoring several books. But in 2006, the opportunity to edit and blog for *Natural History* rekindled her scientific curiosity. "Here was my love of science flooding back," she says. The job fit well with her other occupation at the time, which was caring for her ill husband, an author, actor, and Gulag escapee who died in 2010.

Gottlieb says that copyediting for *The Scientist* has allowed her to experience the challenge of working with technical material while remaining "on the sidelines" of science. When she's not copyediting, writing, or reviewing books, Gottlieb studies karate—a practice that has taken her to Japan several times over the last 40 years. She also maintains a blog called A Cold Eye, where she explores how science influences culture. As a science communicator, Gottlieb sees herself at the intersection of science and culture, and she wouldn't have it any other way. "It's the place to be," she says.

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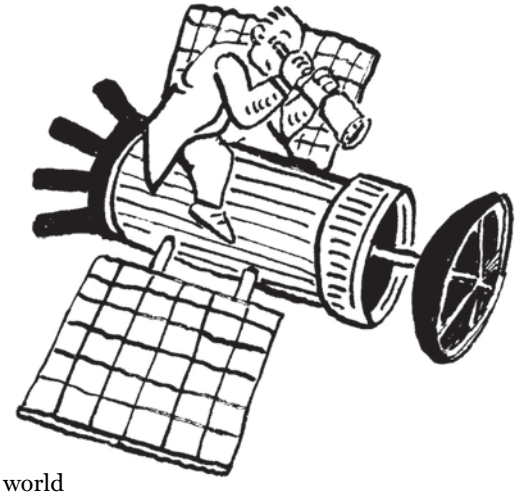
Intelligence Gathering

Disease eradication in the 21st century

BY MARY BETH ABERLIN



ANDRZEJ KRAUZE

To get an idea of the terrible dread that polio used to evoke in your parents or grandparents, read the opening chapter of *Nemesis,* Philip Roth's aptly named 2010 novel set in 1944 in a steamy Newark, New Jersey, neighborhood. Roth's outbreak is fictional, but ever since the first US polio epidemic in June 1916, summer's arrival came with the fear of infection, and parents often limited whom their children could play with and where they were allowed to go.

And that dread was not an overreaction. Although sporadic polio outbreaks occurred every summer, 1952 saw the worst epidemic in the United States, with almost 58,000 reported cases. Of those, 3,145 people died and 21,269 suffered paralysis ranging from mild to disabling. The development of the Salk vaccine three years later, followed by the introduction of an oral vaccine, turned polio from a frightening nemesis to a disease virtually eradicated in industrialized countries.

Polio and guinea worm disease are on the brink of total eradication.

But worldwide, the picture was vastly different. In 1988, when an initiative was launched to stamp out polio worldwide, 1,000 children around the globe were crippled by the infection *every day*. Epidemiologists and public-health workers set out with the firm belief that they could reduce that number to zero. In "Driven to Extinction" (page 28), Senior Editor Jef Akst examines the obstacles, both political and scientific, to the permanent eradication of any infectious disease, using polio and guinea worm disease as examples of two human afflictions that are on the brink of total eradication.

In a companion article ("Outbreak Observatory," page 37), science writer Jyoti Madhusoodanan describes a new kind of epidemiology, one that employs satellites and cell phones to acquire the data necessary to draw increasingly sophisticated disease risk maps. Ecological monitoring of land-cover changes from the air and direct reports phoned in from the field allow public-health officials to fine-tune assessments of where to intervene and how to ration resources, while helping farmers decide where to pasture their animals to avoid disease-carrying pests. Researchers around the world are using the techniques to monitor a number of diseases, including sleeping sickness, cholera, West Nile virus, Lyme disease, and malaria.

This month's third feature, "The Sum of Our Parts" (page 44), by Rodney Dietert and Janice Dietert, switches gears from epidemics caused by infectious disease to those of the noncommunicable sort, examining the intimate relationship between the human microbiome and our environment—from the food we eat to the way we give birth—and the health problems that can result from its disruption and imbalance. Unless an infant "completes" its microbiome in the most efficacious manner, ailments such as allergies, obesity, and inadequate immune responses can result, they argue.

If microorganisms are, after all, not only enemies but allies of our health, they are also proving crucial to the health and species richness of that warm-weather mecca, the ocean. To celebrate summer we have a couple of nods to marine biology: a profile of Max Planck microbiologist Nicole Dubilier, which details her lifelong fascination with the symbiosis between marine worms without a mouth or a gut and the filamentous bacteria that serve as their digestive system (page 52); and a short literature summary of the bacterial symbionts that partner with sponges to cycle phosphorus through coral reef ecosystems (page 51).

If *The Scientist* is your vacation companion this summer, remember that our freedom from the fear of polio—as we enjoy beaches, swimming pools, and family cookouts—is a gift of the ongoing march of translational research. ■

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

Speaking of Science

Cooking reshaped our anatomy, physiology, ecology, life history, psychology, and society. Signals in our bodies indicate that this dependence arose not just some tens of thousands of years ago, or even a few hundred thousand, but right back at the beginning of our time on Earth.

—Harvard anthropologist **Richard Wrangham**, in *Catching Fire*, his 2009 book about how the control of fire and the cooking of food shaped hominin evolution

Our results indicate that several of the fundamental psychological abilities necessary to engage in cooking may have been shared with the last common ancestor of apes and humans, predating the control of fire.

—Harvard psychologist **Felix Warneken** and Yale postdoc **Alexandra G. Rosati**, in a recent research article showing that chimpanzees understand the process of cooking and prefer the cooked version of certain foods (*Proc R Soc B*, June 3)

Although the routine use of misnomers is more often an annoyance than a critical threat to medical research, this phenomenon can stunt progress and further demonstrates a certain lack of rigor in the scientific process.

—**Vincent Giguère**, associate editor of *Molecular Endocrinology*, in an editorial warning of the dangers of giving misleading names to genes and gene products (June 1)

The Ask Alice article, "Help! My adviser won't stop looking down my shirt," on this website has been removed by *Science* because it did not meet our editorial standards, was inconsistent with our extensive institutional efforts to promote the role of women in science, and had not been reviewed by experts knowledgeable about laws regarding sexual harassment in the workplace. We regret that the article had not undergone proper editorial review prior to posting. Women in science, or any other field, should never be expected to tolerate unwanted sexual attention in the workplace.

—A notice posted by ***Science Careers* staff** about the retraction of a controversial advice column post advising a female postdoc who was uncomfortable with her advisor's inappropriate sexual behavior to "put up with it" (June 1)

As long as your adviser does not move on to other advances, I suggest you put up with it, with good humor if you can. Just make sure that he is listening to you and your ideas, taking in the results you are presenting, and taking your science seriously. His attention on your chest may be unwelcome, but you need his attention on your science and his best advice.

—Caltech virologist **Alice Huang**, in a swiftly retracted *Science Careers* column responding to a female postdoc who sought advice about how to handle her advisor's inappropriate sexual behavior (June 1)

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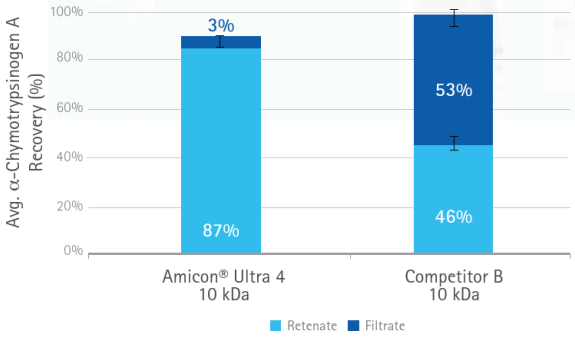

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Notebook

JULY 2015

LYNN JOHNSON

TIGER STYLE: Melia, a trained macaque adorned with stripes of dyed fur, grooms her Bedey owner.

The Lies That Scars Tell

About a decade ago, Lisa Jones-Engel was traveling through rural Bangladesh in a caravan of colleagues she calls "Team Monkey," a band of international researchers comprising a veterinarian, her husband (a physician/epidemiologist), zoologists from Jahangirnagar University, her daughters/field assistants, and others. The group had been collecting macaque feces as part of a project with the Centers for Disease Control and Prevention (CDC) to understand how picornaviruses may be moving between humans and monkeys in the region. As the travelers turned off a paved road to head toward the village of Dhamrai, they drove past a man walking a monkey painted with tiger stripes.

"I thought, 'What the hell was that?'" Jones-Engel, an anthropologist and primatologist at the University of Washington, recalls. The caravan stopped and Jones-Engel approached the monkey. She does this a lot in her work on nonhuman primate–human interactions, often approaching the monkey first before paying any attention to its human companion. It's her way of forging relationships with strangers. Jones-Engel says the reaction is usually, "What is this crazy white blonde woman doing next to this monkey? She's going to get bitten." (She never has been.) "And the next thing you know, I'm grooming their monkey, it's climbing all over me." The handlers recognize her appreciation for the monkey, she says, and on this particular day, her chance encounter launched her on an entirely new research project.

The man was a member of the Bedey, a group of nomadic people, some of whom stage informal shows featuring their macaques. The monkeys are completely integrated into Bedey life—not only do they provide every bit of income the families survive on, they also physically interact with humans all the time. As a result, bites are a frequent occurrence among the monkey trainers, and Jones-Engel recognized that this community could provide an excellent opportunity to study the transmission of simian foamy virus, a common

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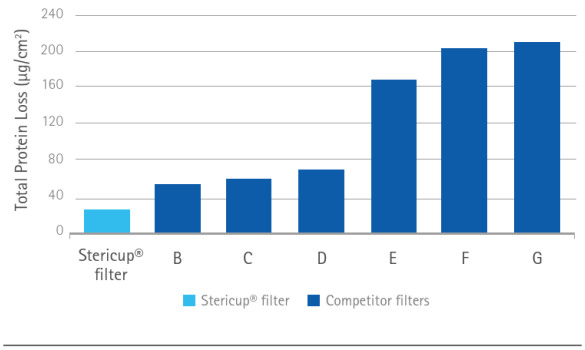

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retrovirus among nonhuman primates that is transmitted through contact with saliva.

Jones-Engel teamed up with Maxine Linial, a virologist at the Fred Hutchinson Cancer Research Center, and colleagues to quantify the prevalence of the retrovirus among 38 performing monkeys and dozens of free-ranging macaques in Bangladesh. Thirty of the performing primates and 119 of the 126 wild macaques tested positive (*Emerging Microbes & Infections*, doi:10.1038/emi.2013.23, 2013).

It stood to reason, then, that Bedey were being exposed to the virus, especially given how often they were bitten. Jones-Engel remembers that when she first started asking Bedey monkey trainers if they could recall specific experiences being bitten, they just showed her their scar-covered arms. The blood work, however, told a different story.

Only 1 of 45 Bedey sampled was seropositive for antibodies against simian foamy virus, while 17 of 269 villagers who don't work with monkeys were positive. At first, Jones-Engel didn't believe the results. "I remember saying to Maxine, 'Is everything OK with the assay?' These folks were never, ever positive, and it's just astonishing given their exposures."

Simian foamy virus is known to infect humans. Interest in the virus picked up steam in the 1980s, when scientists pinpointed apes as the reservoirs of HIV. Concern over zoonotic transmission of other retroviruses led the CDC and other agencies to take a closer look at simian foamy virus. Beginning in the 1990s, Bill Switzer, the non-HIV retrovirus surveillance activity lead in the laboratory branch of the Division of HIV/AIDS Prevention at the CDC, and his colleagues sampled zookeepers and researchers who work with nonhuman primates in the lab. "The rates [of simian foamy virus infection in humans] varied depending on the types of exposure. In research workers it was 1 to 3 percent. In some zoo workers we saw it as high as 11 percent," says Switzer. In Cameroon, a study led by Antoine Gessain of the Institut Pasteur in Paris found that 18 percent of people who reported being bitten or scratched by a nonhuman primate tested positive for simian foamy virus, while only 0.2 percent of the general population was positive (*PLOS Pathog*, 7:e1002306, 2011).

The Bedey, it seemed, should also have a high infection rate. "We were very surprised to find out that the evidence of infection in [the Bedey] is much less than [in] the ordinary village residents," says Linial. Her team has not yet figured out why, but Linial has hunches. She does not think it has to do with the virus or the monkeys (the Bedey capture them from the wild instead of breeding them), but perhaps with the people—specifically, their innate or adaptive immune systems. "The Bedey, even when they're little children, are around monkeys all the time. So it is possible—[though] there's no evidence at all—that perhaps they develop neutralizing antibodies to the virus. This renders them unable to be infected." Another possibility is that the Bedey have different alleles at innate immune genes, such as *APOBEC3G*, which encodes an enzyme that suppresses retrovirus replication.

"This can be possible," says Gessain, adding that it will be important to expand the study to larger numbers of Bedey to confirm the differences between the groups. "It's quite interesting, but this is speculation."

Jones-Engel adds another possible explanation for their results—a long shot, but one to be explored: medicines. The Bedey have a long history as healers and use herbal medicines on themselves and on their monkeys. Perhaps that could account for the low prevalence of the virus, but the group will need to collect more data to say for sure.

Linial is applying for grants to fund further research on the Bedey performers and their monkeys, but she says it can be a hard sell when funders want to focus on diseases with public-health impacts. Although there is no evidence that simian foamy virus is pathogenic to humans, it's clear from the AIDS pandemic that benign retroviruses are capable of evolving into pathogens. "My idea is, we should understand viruses before they become pathogenic," Linial says. "If we had known more about lentiviruses before HIV emerged, maybe things would have happened differently."

—Kerry Grens

PUT HER THERE: A young male rhesus macaque stretches out his hand at a Bangladeshi Bedey encampment, where 20 macaques and nearly 100 people live together.

LYNN JOHNSON

Brrrr-ying the Results

A few years ago, tumor immunologist Elizabeth Repasky realized that she had heard from too many oncologists, colleagues, and friends that cancer patients regularly reported feeling cold and unable to regulate their internal thermometers. At her lab at the Roswell Park Cancer Institute in Buffalo, New York, she decided to build on her experience studying thermal physiology and immunology to see exactly what might be going on with regard to temperature and can-

cer. "I've always gone around telling people it's really important to be warm," she says. "Being warm is a really important part of dealing with diseases like cancer."

Laboratory mice are routinely held at temperatures well below what's called their "thermoneutral zone," or the temperature range in which their metabolism functions most efficiently, without the need to expend excess energy to heat their bodies. The murine thermoneutral zone (TNZ) is variously defined as from about 26–34 °C or, more narrowly, 30–32 °C. But the *Guide for the Care and Use of Laboratory Animals*, a near universally accepted set of recommendations for the housing and use of model organisms, mandates that mice should be held at just 20–26 °C—well below their natural TNZ. This led Repasky and her team to wonder: Are the countless mice used in cancer research too cold? Is there a fundamental physiological shift that occurs in these chilly mice that may be skewing research results?

So Repasky and her Roswell Park colleagues held mice modeling tumor growth at two different temperatures—either at the standard temperature of about 22 °C or at 30 °C, a temperature in the middle of their TNZ. What Repasky and her colleagues found was nothing less than astonishing. Tumors in colder mice grew faster and more aggressively than those in mice housed at the warmer temperature. And this trend held true across a variety of cancer types—breast, skin, colon, and pancreatic. Looking deeper, the researchers found a systemic suppression of the antitumor immune response in the colder mice. Mice held at warmer temperatures had increased numbers of tumor-attacking T cells and reduced numbers of immunosuppressive cells and regulatory T lymphocytes (*PNAS*, 110:20176–81, 2013).

Repasky's results add to the growing body of research that illustrates dramatic physiological differences in mice housed at temperatures below their TNZ: they have twice the heart rate of animals housed in warmer conditions; gain weight faster and become more obese at earlier ages; have higher levels of lipids in their blood; and exhibit a higher respiration rate. "Across biomedical research, we need to pay attention to the model we're increasingly using," Repasky says. "Our control groups are too cold. We're not even modeling the healthy mouse with our current conditions."

And this means that cold-stressed mice could be skewing countless experimental results, past, present, and future. "A lot of these animals may have been more stressed than we could know, and that could have affected the interpretation of whatever effect [the researchers] were looking at," says Chris Gordon, a physiologist at the Environmental Protection Agency who has studied the effects of cold stress in mice and who participated in Repasky's study. John Norton, a Duke University pathologist, agrees. "Any stressors could affect the immune system and other physiological processes to give you different data outcomes," he says.

But solving the problem isn't as simple as cranking up the thermostat in mouse facilities at research institutions. For one thing, keeping mouse rooms at around 30 °C would make the environment nigh on intolerable for the researchers and lab techs who care for the animals. Typically, they wear layers of protective garb, including full body suits, masks, and goggles to prevent contaminating cages or becoming contaminated themselves. Also, the thermal preferences of mice change throughout the day, throughout their life spans, and with changes in behavior.

There may be more subtle ways to provide mice with temperatures that approach their preferred climate, according to Brianna Gaskill, an animal welfare specialist at Purdue University's veterinary college. Increasing the numbers of mice allowed to inhabit a single cage and providing adequate nesting material furnish the animals with ways to increase their ambient temperature, even in a room that is maintained at the colder temperatures comfortable for their human caretakers. Such measures allow mice to "create a microclimate within the cage," Gaskill says. And she has quantified some of the physiological benefits of environmental enhancements that enable this thermal microenvironment change. In a study that Gaskill and colleagues conducted on several strains of mouse models, they found that including 8 grams of nesting materials raised temperatures in the cages to about 32 °C, compared with the 20 °C temperature of the room. This local temperature change reduced circulating thyroid hormones in male mice that had access to nesting material. And in at least one of the strains provided nesting materials, the activation of UCP1—a protein that is pivotal to thermogenesis in brown fat—decreased (*Phys & Behav*, doi: 10.1016/j.physbeh.2012.12.018, 2013).

As the evidence has mounted that temperature affects mouse physiology, the authors of animal care guidelines and the agencies that use those guidelines to accredit facilities for the use of laboratory animals have paid attention. In 2011, the National Research Council committee charged with updating the *Guide for the Care and Use of Laboratory Animals* changed the recommended housing temperatures for mice used in research from 18–26 °C to 20–26 °C. And Chris Newcomer, executive director of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)

ANDRZEJ KRAUZE

International, says that though his organization is concerned primarily with animal welfare rather than research outcomes, it does strongly recommend that the research institutions it works with be aware of the growing body of literature that indicates physiological perturbation in cold-stressed mice. "We tell the institutions and the [Institutional Animal Care and Use Committees] that in the course of reviewing protocols you should be conscious of emerging science and best practices," he says. "We like to convey that to organizations, but we don't use it as the linchpin to whether or not an organization is accredited."

Our control groups are too cold. We're not even modeling the healthy mouse with our current conditions.

— Elizabeth Repasky, Roswell Park Cancer Institute

AAALAC and other accrediting and regulatory bodies, including the National Institutes of Health's Office of Laboratory Animal Welfare, may consider expanding on the recommendations they make regarding the provision of nesting materials, shelter, and other means of regulating the thermal environment. "I do think there's a breaking point," Newcomer says. "This has been discussed already in the circles of laboratory animal science. There is a breaking point, but I just don't think that that breaking point is here yet."

In the meantime, the people at research institutions who are tasked with maintaining colonies of experimental animals are ready to assist scientists who want to test the effects of housing temperatures on their mice. Sandra Sexton, the facility director and attending veterinarian for Roswell Park's Laboratory Animal Resources, says she's prepared to replicate Repasky's experiments in other model organisms under her care. "I will be the first one to assist any of our researchers to explore that and do something similar to what we did for [Repasky]," she says. Norton, director of Duke's Division of Laboratory Animal Resources, agrees. "We could provide rooms with different temperatures with approval of [Duke's Institutional Animal Care and Use Committee] and our clinical veterinary staff," he says. "You've got to have the proper controls in any experiment to properly test your hypotheses."

—Bob Grant

Hunting Off the Hook?

"There is good reason to suppose that the sea lions of the Falklands can be exploited profitably, and that if due precautions are taken a sealing industry can be established on a permanent basis," scientist James Hamilton wrote in 1934. He had traveled to the remote South Atlantic archipelago with a British scientific team in 1929 and spent three years observing the southern sea lion, *Otaria flavescens*, estimating population size. From 1933 to 1937, Hamilton and a small team of assistants recorded sea lion populations at 56 rocky, exposed rookeries by rushing at colonies from the water, causing frightened adults to stampede off while leaving the slower-moving pups behind to be counted. "The bite of the pup, although annoying, is not serious," he noted. The final count of 80,555 pups, which included estimates of sea lion numbers at eight other rookeries in the Falklands, allowed Hamilton to estimate that there were more than 380,000 animals in the full population.

The sea lions were not surveyed again until 1965, when researchers counted only 5,506 pups, revealing a shocking decline. Yet no one sought to explain the drop for another 30 years, until Dave Thompson of the University of St. Andrews in the U.K. and colleagues decided to survey the sea lion populations of the Falklands again in 1995. At that point, the team found only about 2,000 pups. "[The results were] terrifying," Thompson recalls. A second survey in 2002–'03 found a slightly higher number.

Thompson and his colleagues posited that hunting had caused the sharp decline between Hamilton's initial surveys and 1965, both in the Falklands and off the Argentine mainland. (Legal hunting ended in 1966 and thus could not explain the later drop.) Now, a more recent study by another team of scientists suggests that hunting may not have been solely responsible for the decline.

Alastair Baylis of Deakin University in Australia and the South Atlantic Environmental Research Institute of the Falkland Islands and colleagues followed up with another population survey last year. Baylis arrived in the Falklands in 2008 as a fisheries observer, fresh from a PhD spent studying New Zealand fur seals in South Australia. "The Falklands is not only a stunning place in terms of scenery and just the abundance of species here," Baylis says, "but because there's so little research that's been done on things like sea lions, it's an opportunity to live out my childhood dreams" of being both a scientist and an explorer. He soon became interested in the sea lions and spent three weeks surveying roughly 800 kilometers of the Falkland Islands' corrugated coastline to visit 70 scattered colonies. Baylis and his colleagues counted about 4,500 pups.

Next, Baylis's team combed through hunting tallies from 1928 to 1966, which recorded a cull of 60,723 southern sea lions—93 percent of which were killed in the decade when Hamilton conducted his research. A mathematical model based on the new and historical population data suggests that even these staggering numbers could not account for the steep sea lion drop in the middle of the 20th century.

The new research also suggests hunting on the Argentine mainland is likely not to blame. Significant numbers of sea lions would have had to migrate to the mainland over the winter, but it was unknown if this actually occurred. In 2011, the scientists fitted 10 juveniles with GPS tracking devices to map their winter movements and found that the sea lions did not stray as far as Argentina, about 483 kilometers away. Given the new data, "the case for a hunt-related decline is less compelling," Thompson says.

Instead, Baylis and his colleagues suggest that environmental changes could be the cause. The researchers found historical records indicating that sea-surface temperatures rose between the 1930s and the 1950s and between 1965 and 1980. Warmer waters could have disrupted food

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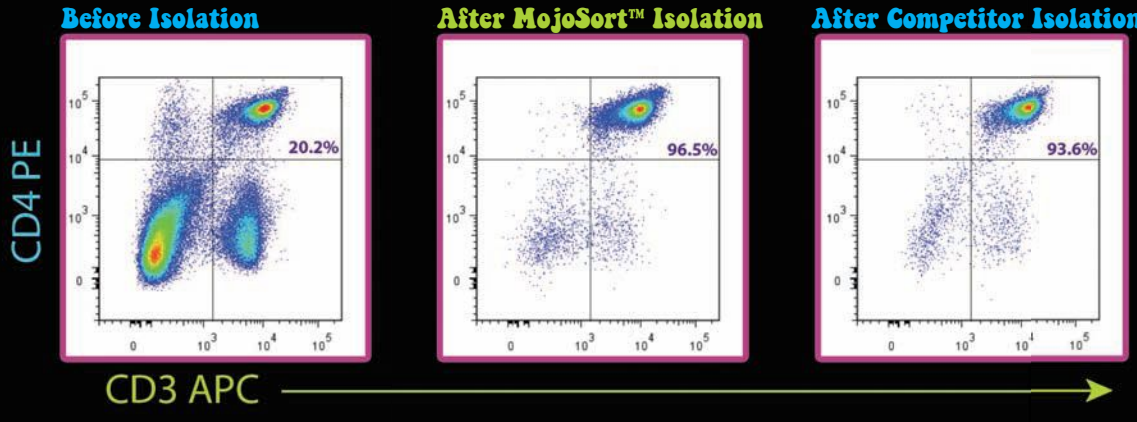

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SEEING SEA LIONS: Southern sea lions (*Otaria flavescens*) breeding at the Falkland Islands, where the population declined from 370,000+ animals in the 1930s to just 30,000 in 1965

webs important to sea lions, possibly providing an explanation for the population drop, although there aren't enough data to prove the hypothesis, Baylis says. "If the population has declined [due to] changes in ocean climate, then that really fundamentally alters how we interpret the resiliency and vulnerability of species," he adds.

The available data on southern sea lions make them a "fabulous model" for other species like the Steller sea lion (*Eumetopias jubatus*) for which there is scant environmental evidence of nutritional stress caused by warmer seas, says Shannon Atkinson of the University of Alaska, Fairbanks. She says that Baylis's research shows the harvest of animals most likely didn't cause the decline, but Atkinson also believes the study didn't find a definitive cause. "We've spent a lot of time looking for a smoking gun," she says. "In reality, most populations don't decline due to a single cause."

Thompson agrees that "you would need to try to do more to tie the population change to the recorded environmental change." To blame sea-surface temperature requires sea lion census numbers prior to the 1930s, Thompson says. Unfortunately, "there's no species anywhere on the planet" for which such data exist.

The 30-year gaps in information about the Falklands populations of sea lions may constrain how definitively researchers can explain the marine mammal's precipitous mid-century decline, but even being able to collect historical data that could potentially exonerate hunting as the primary cause of population decline is serendipitous for scientists. "The whole story . . . is only apparent because, by chance, there was a commercial interest in hunting sea lions," Thompson says. The historical detail available to Baylis and his colleagues is something that many researchers wish they had access to, Atkinson adds. Most importantly, though, the research looks as much to the future as it does to the past. It's "really nice [that] someone has taken on the population monitoring," Thompson says. —Jenny Rood

High-Flying Ducks

A Peruvian man from the floating man-made islands on Lake Titicaca uses a long pole to steer his wooden boat through the reeds. His son crouches on the bow, scanning the water's surface with a flashlight. The father, a member of the Uro people, silently maneuvers the boat towards an unsuspecting blue-billed Puna teal while his son readies a hand net. With a quick swipe, the boy hauls the duck into the boat, and by 3:00 a.m. they deliver six ducks to the researchers waiting back on shore. The Puna teal is one of many resident waterfowl species inhabiting the Andean altiplano. At nearly 4,000 meters, oxygen levels are only about 65 percent of what they are at sea level, but the bird seems oblivious to the effects that altitude imposes on other species. "The big question is," says Bill Milsom, a comparative physiologist from the University of British Columbia, as he watches a Puna teal paddle among the tortora reeds, then fly away: "How do they do it?"

Human visitors to Lake Titicaca are encouraged to gulp down coca tea in an effort to overcome the effects of hypoxia,

COURTESY OF ALASTAIR BAYLIS

or low oxygen levels. A short flight of stairs leaves you breathless, and your body compensates by increasing heart rate and breathing faster and deeper. But these changes are energetically costly to maintain. To thrive at altitude, populations adapt and evolve. And that takes time—tens of thousands to millions of years to acquire increasingly efficient adaptations.

But rising global temperatures will test species' adaptability much sooner. For example, researchers predict that climate change will force species to seek cooler environments at higher altitudes. Yet an average low-altitude bird brought to Lake Titicaca wouldn't fare well. "You'll see about 90 percent hatching mortality in the eggs. The pores in the eggs aren't big enough; they don't have the right hemoglobin gene variants; there's just not enough oxygen," says Kevin McCracken, an evolutionary geneticist from the University of Miami. The 10 percent that survive, he adds, carry traits that make them more robust in the face of hypoxia. Over time, beneficial mutations accumulate along the oxygen cascade, a series of events that moves oxygen from the atmosphere into the mitochondria of cells.

Milsom, McCracken, and colleagues are in Peru to compare how five different duck species cope with reduced oxygen levels. The ducks are all year-round residents, but each species colonized the region at different points in their evolutionary past, and each one independently evolved a set of unique adaptations to deal with hypoxia. Species that colonized the area first, and are isolated from lowland populations, have different adaptations than more-recent arrivals that may still mix with lower-altitude populations.

Air pressure drives oxygen down its cascade: from the atmosphere to the lungs, from the lungs to the blood, and from the blood to the body's cells, where mitochondria use oxygen to make energy and heat. Lower air pressure at high altitude decreases that physical force.

"Strong environmental changes, like altitude, act like an evolutionary bottleneck," says Lucy Hawkes, a physiological ecologist at the University of Exeter who was not involved in the study, "and failing to survive them would mean failing to migrate or breed." She says that insights from studying high-altitude physiology can inform conservation biology by helping scientists understand "how animals can really push the possibilities of their biology to survive in the ever-changing world around us." As climate change forces species to move to higher elevations, understanding the variety of adaptations available can help scientists predict how far animals can stretch their existing physiology to cope in a low-oxygen environment. Milsom says that these predictions depend on our knowing how much plasticity there is within a species and how much time the species has to adapt.

Members of Milsom's Peru team also study creatures in Mongolia that have adapted to extreme hypoxia. They recently published a study on the flight dynamics of bar-headed geese, which migrate from their breeding grounds in Mongolia across the Himalayas to their winter refuge in India (*Science*, 347:250-54, 2015). These geese have a host of adaptations that maximize their ability to fuel their cells with oxygen at extreme altitudes, sometimes upwards of 7,000 meters. But they only fly at such altitudes transiently. In contrast, the ducks in Peru all live at altitude permanently, and each species colonized the region independently. "We get to look at replicate evolutionary experiments," says Graham Scott of McMaster University in Ontario, Canada, "where animals have come up to high altitude and evolved these unique traits many times."

In a makeshift lab, the team preps gas analyzers and tanks, instruments to measure lung volume, and a spectrophotometer designed by team member Peter Frappell of the University of Tasmania that analyzes the oxygen-binding properties of hemoglobin. Each experiment examines a different segment of the oxygen cascade. The researchers compare lung volume and measure the birds' cardiorespiratory responses to experimentally altered oxygen levels; they take heart, lung, and muscle samples from each species to study metabolic enzymes, muscle fibers, and the degree of tissue vascularization.

The team has found that the Puna teal, which colonized the region several million years ago and has speciated from its lowland ancestors, readily copes with extreme hypoxia. Even when the researchers dial down oxygen levels to mimic conditions at the peak of Mount Everest, almost 9,000 meters up, the Puna teal deepens its breathing, but otherwise remains unaffected. In contrast, the yellow-billed pintail colonized Lake Titicaca only a few tens of thousands of years ago and has more contact, and therefore gene flow, with its lowland counterparts. Less time at altitude means less-efficient adaptations: the pintails are far more sensitive to hypoxia and breathe much deeper and faster. Even the teal's hemoglobin has adapted to bind oxygen with greater affinity than that of other species. Individuals can acclimatize to altitude in the short-term, but long-term adaptations spread throughout populations are a function of natural selection. Over time, teals have acquired a variety of adaptations and lost their ancestral acclimatization response. "They are making the best use of every bit of air they bring in," says Scott. —Sarah Hewitt

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SITTING DUCKS: A pair of Puna teals rest on the totora reeds surrounding Lake Titicaca.

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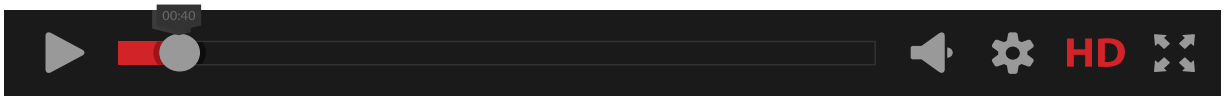

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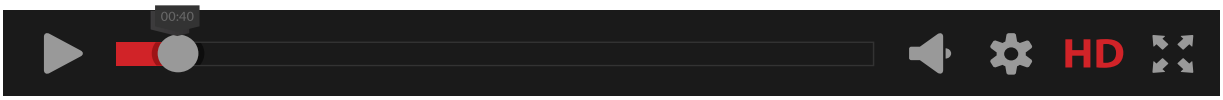

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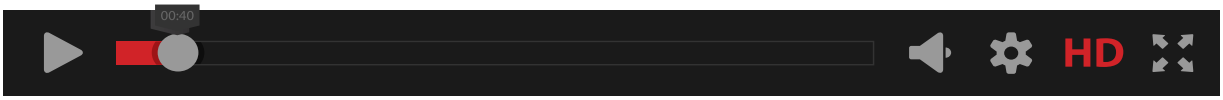

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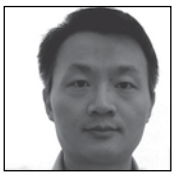

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When Does a Smart Mouse Become Human?

Ethical issues attend the creation of animal-human chimeras.

BY JOHN D. LOIKE

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Late last year, Steve Goldman of the University of Rochester and his colleagues reported that they had transplanted immature glial cells from donated human fetuses into the brains of immunodeficient mouse pups. These human glial cells matured into astrocytes and developed as the primary astrocyte population in the newborn mouse brain. One unexpected outcome of the team's research, published in the *Journal of Neuroscience* (34:16153-61), was that these human-mouse chimeras outperformed normal mice almost fourfold in a variety of cognition tests, underscoring the importance of astrocytes in regulating synaptic plasticity and neural connectivity to enhance learning and memory. But the study also raised important ethical considerations—namely, what biological properties differentiate *Homo sapiens* from other organisms, and when should such "humanized" animals be afforded the rights that people currently enjoy.

Goldman is quick to state that the enhanced memory and learning performance of these human-mouse chimeras did not make the mice more human. "It's still a mouse brain, not a human brain, but all the non-neuronal cells are human," Goldman told *New Scientist* at the time of the publication. "This does not provide the animals with additional capabilities that could in any way be ascribed or perceived as specifically human. Rather, the human cells are simply improving the efficiency of the mouse's own neural networks. It's still a mouse."

At the same time, the team had ethical reservations about repeating these types of experiments on monkeys, presumably following the National Academies' guidelines that no human embryonic stem cells should be introduced into nonhuman primates at any stage of fetal or postnatal development. Is there really an ethical difference in performing these experiments on mice as opposed to monkeys? The scientists have not addressed this question, perhaps because it is a difficult one to answer.

Human intelligence, as difficult as it is to define, is often thought to be one of the most important characteristics that differentiate *Homo sapiens* from all other organisms. However, the capacity to humanize animals has the potential to complicate this assessment of being human. For example, should the definition of human or humanlike intelligence be psychometric, based on a constellation of cognitive processes, or should it be neurophysiologic or neurogenetic because it is inextricably linked to the brain? The question of distinguishing human and nonhuman characteristics has arisen regarding our close primate relatives. Last October, a New York Appeals Court announced that it will consider the issue of whether chimpanzees are entitled to "legal personhood." Similarly, in December, an appeals court in Argentina recognized orangutans as having basic legal rights, stating that these primates deserve living quarters in a sanctuary and not in a zoo.

Reconstituting human glial cells or neurons in animal brains could eventually impart complex cognitive behaviors, self-awareness, and/or other humanlike personality characteristics to these chimeras. Such research highlights the need for scientists and policymakers to resolve the question of how to define humanlike intelligence regarding the genetic or chimeric alteration of animals. While there is no clear answer to these questions, I advocate that intelligence is a valid criterion when considering what is humanlike or animal-like, and that scientists must develop both psychometric and neurophysiologic criteria in the definition of humanlike intelligence. Most importantly, we should not move forward on creating any human-animal brain chimeras before fully exploring the social and ethical implications of endowing nonhuman animals with humanlike personality characteristics.

While scientists and the public value technological development and discovery, such experiments should only be done under conditions where there are clear and important scientific benefits to be gleaned. Indeed, the scientific community must always consider the bioethical ramifications of emerging biotechnologies and remember that research investments in new discoveries should be assessed not by what we can do, but by what we should do. ■

John D. Loike is the director of special programs at the Center for Bioethics, Columbia University College of Physicians and Surgeons.

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How to Make a New Species

Scientists mutate a mating pheromone and its corresponding receptor in yeast to promote speciation.

BY RUTH WILLIAMS

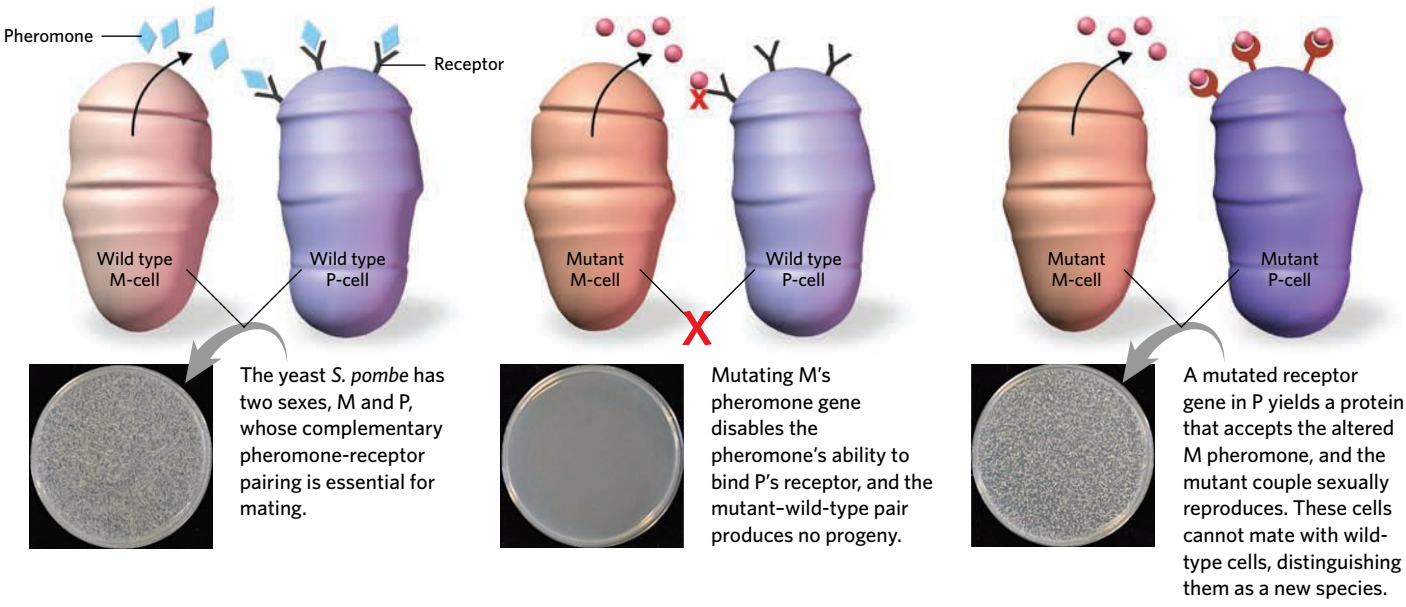
The emergence of one species from another occurs when the two groups can no longer interbreed. Such reproductive isolation is considered a key evolutionary process, and yet our knowledge of the mechanisms and mutations by which it actually occurs has been confined to conjecture. "We can speculate on the history of evolution from various observations," says Masayuki Yamamoto, director general of the National Institute for Basic Biology in Okazaki, Japan. "However, it is virtually impossible to reproduce it experimentally."

Virtually, but not entirely, impossible, it seems. Chikashi Shimoda's team at Osaka City University in Japan has achieved experimental speciation in the yeast *Schizosaccharomyces pombe*.

The two sexes of *S. pombe*—M and P, for "minus" and "plus"—each secrete a pheromone (M factor and P factor), which binds to a corresponding receptor on cells of the opposite sex. This interaction is essential for successful mating. Shimoda's team had previously made mutants of the M-factor gene, *mfm1*, which prevented M cells from mating with wild-type P cells. Now, the team has randomly mutated the gene for the M-factor receptor, *map3*, in P cells to produce individuals with which the *mfm1* mutants can once again reproduce. In total, they've created four *mfm1/map3* mutant pairs that can reproduce with each other but not with their wild-type forebears.

"Although their observation may not reflect the real natural history, it supports the concept that changes in the mechanism to select mating partners can be an initial step for speciation," says Yamamoto, who did not participate in the research.

Pheromone-receptor interactions that drive reproduction have been studied in a variety of life-forms, particularly amphibians and insects, says Shimoda. He therefore suggests pheromone mutagenesis might allow researchers to "extend our achievement to other organisms." (*PNAS*, 112:4405-10, 2015)



The yeast *S. pombe* has two sexes, M and P, whose complementary pheromone-receptor pairing is essential for mating.

Mutating M's pheromone gene disables the pheromone's ability to bind P's receptor, and the mutant–wild-type pair produces no progeny.

A mutated receptor gene in P yields a protein that accepts the altered M pheromone, and the mutant couple sexually reproduces. These cells cannot mate with wild-type cells, distinguishing them as a new species.

AT A GLANCE

SPECIATION TECHNIQUE	EXPERIMENTAL ORGANISM	APPEARANCE CHANGES	METHOD	POTENTIAL OTHER ORGANISMS?
Transgenic synthetic speciation (*PLOS ONE*, doi:10.1371/journal.pone.0039054, 2012)	*Drosophila melanogaster*	Smaller eyes; different wing veination pattern	Mutate five genetic sites to produce flies that can successfully reproduce with each other, but produce nonviable offspring when mated with wild-type flies.	Yes, any organism amenable to transgenesis
Pheromone/receptor mutations	*Schizosaccharomyces pombe*	No obvious differences	Mutate mating pheromone and receptor genes to cause reproductive isolation.	Possibly others that use ligand-receptor interactions for reproduction

EXTENDING THE REACH: A health worker administers polio vaccine to a child while her sisters watch, during a Polio National Immunization Day in Karachi, Pakistan.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Driven to Extinction

The eradication of smallpox set the standard for the global elimination of a devastating infectious disease. Will the ongoing polio and guinea worm campaigns be as successful?

BY JEF AKST

In the spring of 2000, Stephen Cochi, then-director of the Global Immunization Division at the US Centers for Disease Control and Prevention (CDC), stood in the town of Torkham on the border of Pakistan and Afghanistan, watching thousands of Afghans exit their country through the storied Khyber Pass. They were fleeing for their lives from the violence that had become a regular occurrence as Afghanistan entered its fifth year of civil war against the then-ruling Taliban. But Cochi and his colleagues from the World Health Organization (WHO) and Pakistan's Federal Ministry of Health saw another opportunity to save lives. As the families crossed into Pakistan on their way to the city of Peshawar, public-health workers escorted any groups that included children who looked to be less than five years old to a tent set up to the side of the crowd and delivered two drops of an oral polio vaccine into the children's mouths.

"There were people streaming across the border," Cochi recalls. "I would guess that, over the course of a full day, they probably vaccinated in the thousands of children." Cochi found it gratifying to see that so many children were being immunized against the potentially fatal disease that had once killed tens of thousands of people each year and paralyzed hundreds of thousands more. But the scene was also a stark reminder of how far those fighting for polio eradication had to go, he says. "[I could] see how the eradication of polio in Afghanistan is completely linked to the eradication of polio in Pakistan. This was a living, breathing, visible representation of that. We're still dealing with that today."

Indeed, while researchers and public-health officials have made great strides in ridding the world of the virus—the number of polio cases has dropped to just a few hundred from more than 350,000 in 1988, when the eradication campaign was launched—the final steps in extinguishing the disease have not been without setbacks. One major hurdle has been the

Epidemiologists, public-health workers, and researchers involved in eradication campaigns are confident that polio and guinea worm can meet the same fate as smallpox.

migration of people into and out of polio-affected countries. "I think Afghanistan would be a polio-free country were there not so much back-and-forth movement to Pakistan," Cochi says.

Even more threatening to the eradication campaign's success, perhaps, are the challenges in immunizing all vulnerable children, many of whom reside in regions occupied by antigovernment forces such as the Taliban. And beyond the logistical hurdles, eradication efforts must overcome scientific challenges—such as the potential for the attenuated, noninfectious versions of the poliovirus used in the oral vaccine to mutate into an infectious agent.

A better understanding of pathogen transmission may be even more critical in supporting the world's only other ongoing eradication campaign: the abolishment of guinea worm disease. Traditionally, humans have contracted the disease by ingesting water contaminated by parasite-infected copepods, and simply ensuring that affected regions have access to clean drinking water has succeeded in reducing cases of the disease from more than 3.5 million in 1986 to just 126 last year. But recent cases of guinea worm disease in dogs, which likely contract the parasite by eating the scraps of infected fish butchered on the shore by local fishermen, have researchers rethinking the final stages of eradication. "That's a new wrinkle in what Mother Nature has to offer us," says Ernesto Ruiz-Tiben, director of the Carter Center's Guinea Worm Eradication Program.

Despite these setbacks, epidemiologists, public-health workers, and researchers involved in the two eradication campaigns are steadfast. They've done it once before—declaring the world free of smallpox in 1980—and experts are confident that polio and guinea worm can meet the same fate. And the lessons learned from these campaigns could set the stage for other infectious diseases on the chopping block.

"It starts with knowledge and then it carries on to medical practice and then it extends into medical research to get insights into what is going on," says the University of Florida's Grant McFadden. "If everything works right, all those things flow into new ideas for therapies, containment, and, ultimately, eradication."

Putting polio in its place

Not every infectious disease is eradicable. In fact, even with all the resources in the world, most of the pathogens that currently plague humans would be extremely difficult, if not impossible, to banish from the planet. "When you look at the huge array of microorganisms out there, there really is a relatively small number of microbiological agents that would be considered to be good candidates for disease eradication," says Cochi.

One criterion that makes a pathogen a good target for eradication is the lack of an animal reservoir. Even though outbreaks of SARS and Ebola have been controlled a number of times, for example, the causative pathogens can continue to jump from their animal hosts to kindle new epidemics. Another important feature of an eradicable disease is, typically, that there be an effective treatment or vaccine.

Smallpox fit the bill on both counts, and in 1966, the World Health Assembly, WHO's highest governing body, voted to initiate a worldwide smallpox-eradication campaign. It started out as a mass vaccination program, then converted to a search-and-containment strategy, in which all contacts of smallpox patients and nearby households—depending on the country, sometimes entire apartment buildings—were vaccinated to prevent further transmission of the pathogen. In less than 15 years, case numbers dropped from more than 15 million to zero. "The strategy for eradication was very straightforward and successful in the end," says David Heymann, a medical epidemiologist at the London School of Hygiene and Tropical Medicine who worked for two years on the smallpox-eradication program in India.

But smallpox eradication held some other advantages over the polio campaign. For example, "every infection was clinically expressed in the same way," Heymann says. "There weren't, as is the case with polio, people without symptoms." Indeed, fewer than 1 in 200 cases of polio results in paralysis, making most infections invisible to public-health workers. For this reason, a search-and-containment strategy wouldn't work, as it would often miss infected but asymptomatic individuals who could continue to spread the virus. Instead, the polio campaign must continue to vaccinate every child, and therein lies the principal challenge to clinching the virus's eradication. In Afghanistan and Pakistan, the Taliban has repeatedly interfered with the eradication campaign, prohibiting vaccination and even killing health workers delivering immunizations. "I think there's general agreement that the biggest obstacle is access to children in Pakistan," says John Modlin, deputy director of the polio arm of the Bill & Melinda Gates Foundation.

I consider the Holy Grail to give up using all polio vaccines altogether. We'd like to take those dollars and apply them to other public-health priorities. —John Modlin, Bill & Melinda Gates Foundation

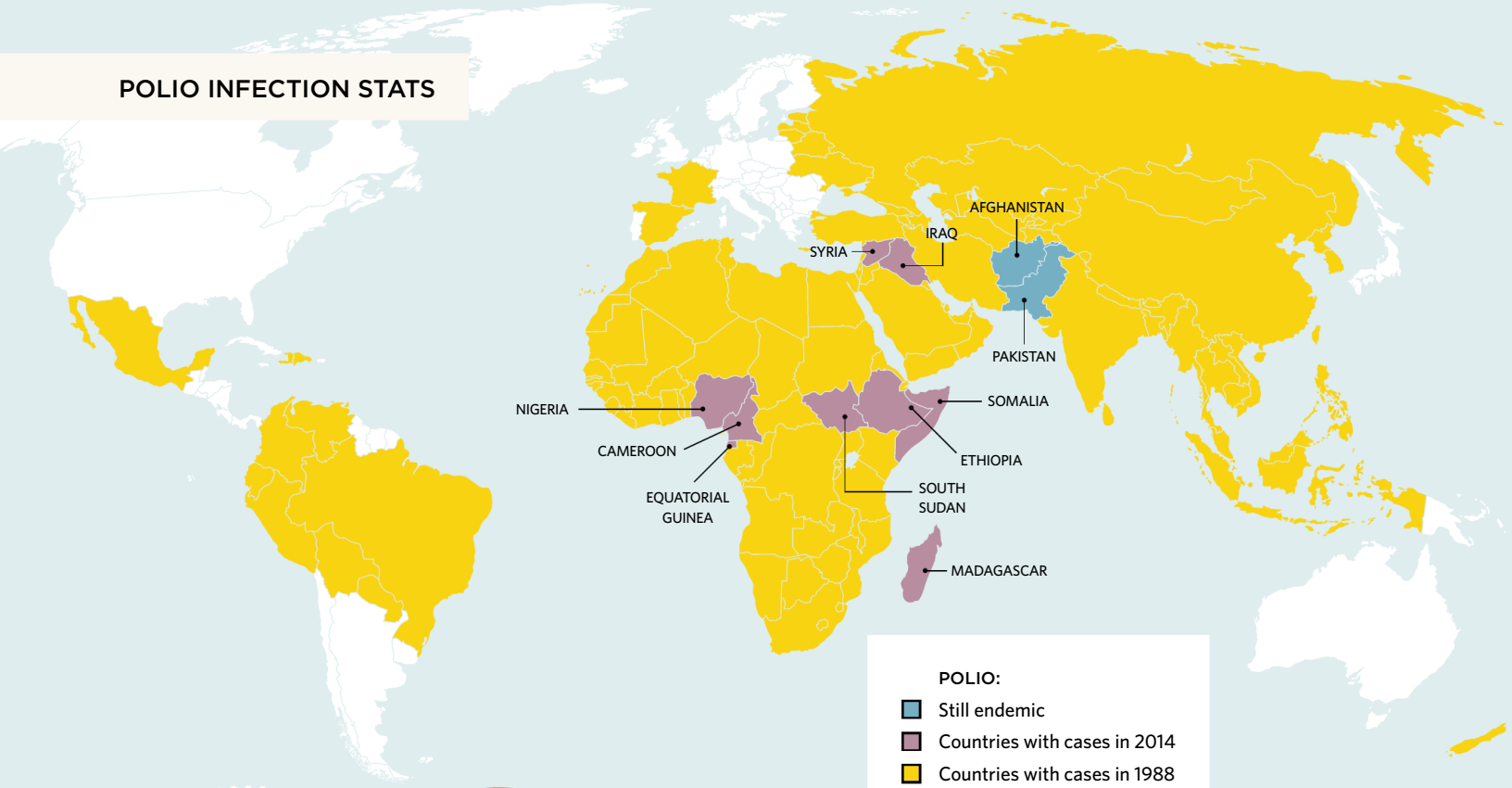
But the campaign has faced, and overcome, such challenges before. In 2009, the northern states of Nigeria—the headquarters of the violent extremist group Boko Haram, which, like the Taliban in Pakistan, has prohibited vaccinations and even killed to enforce this ban—was the scene of a polio outbreak that resulted in more than 350 cases over a period that had seen fewer than 65 infections the year before. Then, in 2013, polio jumped from Nigeria to Somalia, causing an outbreak of 194 infections in a country that had not recorded a case of nonvaccine-related poliovirus since 2007.

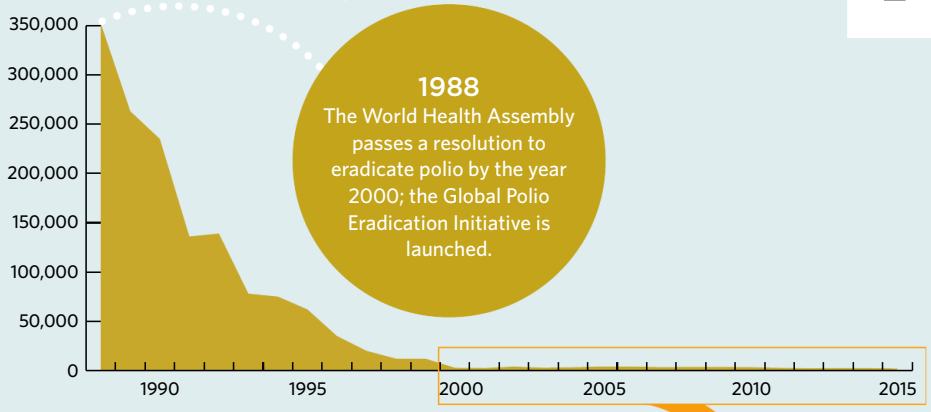
In response to the spike in cases, the Nigerian government and partners set up an emergency operations center and enacted a national emergency-action plan,

TOP ROW: © END POLIO PAKISTAN/2013/ASAD ZAIDI; © END POLIO PAKISTAN/2012/ASAD ZAIDI. WWW.ENDPOLIO.COM.PK
MIDDLE ROW: WORLD HEALTH ORGANIZATION; JASON BEAUBIEN/NPR. BOTTOM ROW: THE CARTER CENTER/L. GUBB; THE CARTER CENTER/E. STAUB

A LOOK AT ERADICATION: (Top row, left) A health worker vaccinates a child against polio during an immunization campaign in Quetta, Pakistan; (right) a polio vaccination team wades through flood water in the Sindh province in Pakistan. (Middle row, left) Cheshire Home for Handicapped Children in Freetown, Sierra Leone; (right) a polio vaccination booth in Rawalpindi. (Bottom row, left) An emerging guinea worm is wound around a moist bandage to prevent it from breaking; (right) a Nigerian woman uses a simple cloth filter to remove the tiny copeopods that harbor the guinea worm parasite.

POLIO INFECTION STATS

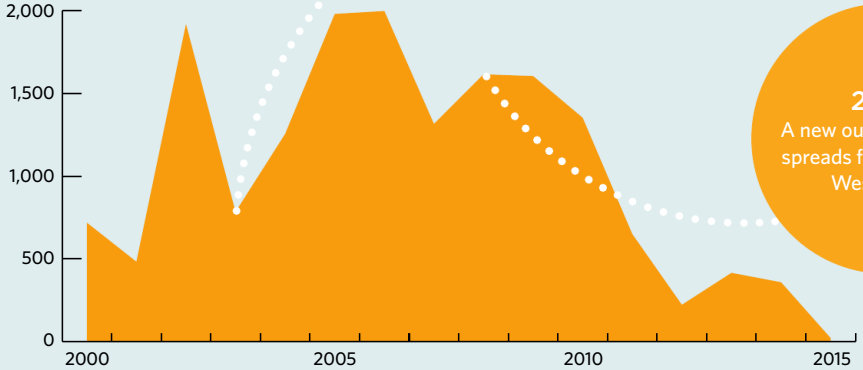




1988
The World Health Assembly passes a resolution to eradicate polio by the year 2000; the Global Polio Eradication Initiative is launched.

NUMBER OF POLIO CASES 1988–2015

2003
In northern Nigeria, polio immunization campaigns are suspended following unfounded rumors regarding the safety of the polio vaccine. Subsequently, a new outbreak occurs.



2008
A new outbreak of polio spreads from Nigeria to West Africa.

NUMBER OF POLIO CASES 2000–2015

Sources: World Health Organization, US Centers for Disease Control and Prevention

including "hit-and-run" immunizations in which security forces protected mobile units of health-care workers who would rush into the war-torn northern regions of the country to vaccinate as many children as possible in just a few hours. In the end, the efforts paid off, with the last case of polio reported in Nigeria in late July 2014. The last case in Somalia was recorded less than a month later. Although it will be another couple of years before polio can be declared eradicated from the continent, "we are hopeful that we now have a polio-free Africa," says Cochi.

Similar emergency actions are now being put into place in Pakistan, including several new emergency operation centers in high-risk areas. "This is a strategy that worked very well in Nigeria and now is being replicated in Pakistan," says Modlin. In addition, the Pakistan Army intervened in Taliban-occupied regions last summer, giving public-health workers access to some half a million children who were previously unvaccinated, Cochi notes.

"To me, that's the story of infectious-disease elimination and eradication attempts as a whole," says the University of East Anglia's Sebastian Taylor, a member of WHO's Technical Advisory Groups for Polio Eradication in Afghanistan and Pakistan. "Biotechnology and finance will only get you so far."

"If polio [eradication] fails it won't be because of technical reasons, it will be lack of political will," agrees Oliver Rosenbauer, a spokesperson for the WHO's Global Polio Eradication Initiative.

Improved targeting

That's not to say that science doesn't have a role to play in finalizing the eradication of polio. Another main challenge is that multiple vaccinations are necessary to ensure complete protection against poliovirus. One reason for this is that the original oral polio vaccine—made from live, attenuated viruses—targets all three serotypes of poliovirus, and type 2 replicates most robustly in the intestinal tract, actually interfering with the replication of types 1 and 3. The type 2 virus is thus the most immunogenic, spurring the production of the most antibodies by immune cells in the gut lining—the primary site for poliovirus replication—as well as in the blood after the first dose of the vaccine. But such antibodies don't do today's children much good, as the last case of polio caused by naturally occurring type 2 virus occurred more than 15 years ago.

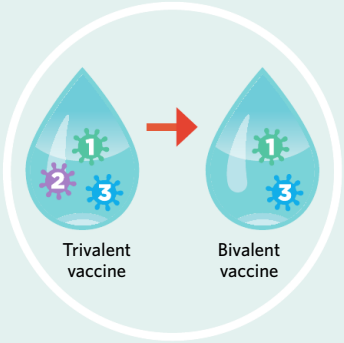
The solution is to remove type 2, going from a trivalent to a bivalent oral vaccine. "As a result, the immune response against types 1 and 3 [is] enhanced, dose for dose," Cochi says. The bivalent vaccine has already been introduced into the polio-eradication campaign across the Middle East, Africa, and India. "It is the vaccine of choice for vaccination campaigns in polio-infected countries and those countries which are considered at high risk of reinfection," Rosenbauer says. Public-health officials are now gearing up to transition from the trivalent to the bivalent form for routine immunization programs. Countries are currently in the process of securing licensing for the bivalent oral vaccine, with the goal of adopting it into routine care by the end of the year.

Although naturally occurring type 2 poliovirus hasn't been seen since before the turn of the century, the type 2 virus isn't completely gone. That's because the live viruses used to make the trivalent oral vaccine can be shed in the stool of vaccinated individuals and, on rare occasion, can mutate to become infectious again. While all three virus types can mutate in this way, the type 2 form is responsible for the vast majority of vaccine-derived polio outbreaks. Last year saw a total of 55 cases of such vaccine-derived polio infections, for example, only one of which was not type 2. By eliminating type 2 poliovirus from the oral vaccine, "the idea is to have all type 2 viruses disappear from the world forever, including the vaccine virus," says Cochi. But until then, vaccinated individuals will still harbor attenuated versions of the virus that could revert to an infectious form, and health officials need a way to continue to protect children against these vaccine-derived type 2 viruses as they make the switch to a bivalent oral vaccine.

The role of bridging that gap falls to the inactivated polio vaccine (IPV), which has been the exclusive vaccine used in the

ERADICATION STRATEGIES

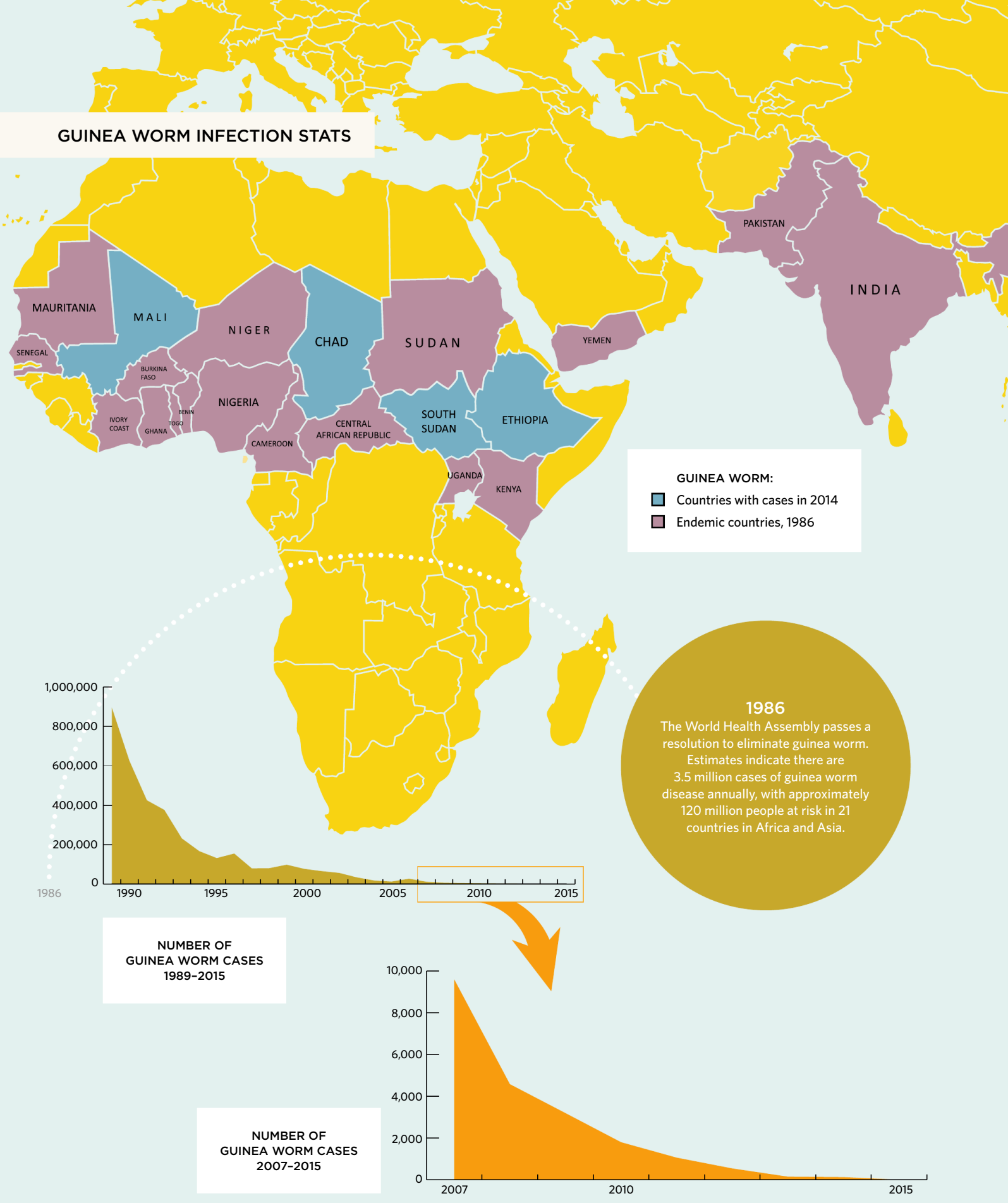
❶ The number one goal of the polio eradication campaign is mass vaccination: to stop the spread of the virus, all children must be immunized.



❷ Transitioning from a trivalent oral vaccine, which contains all three polio virus serotypes, to a bivalent form lacking type 2 will boost effectiveness against types 1 and 3 while preventing continued emergence of type 2 vaccine-derived virus.

❸ At the same time, health officials will begin to introduce the inactivated trivalent polio vaccine, which is administered via intramuscular injection, to continue to provide immunity against the type 2 virus.

GUINEA WORM INFECTION STATS



1986

The World Health Assembly passes a resolution to eliminate guinea worm. Estimates indicate there are 3.5 million cases of guinea worm disease annually, with approximately 120 million people at risk in 21 countries in Africa and Asia.

NUMBER OF GUINEA WORM CASES 1989–2015

NUMBER OF GUINEA WORM CASES 2007–2015

Source: The Carter Center

United States since 2000. IPV is administered via intramuscular injection and provokes an immune response only in the blood, in contrast to gut-based immunity triggered by the oral vaccine. The IPV thus provides personal protection and prevents the virus from reaching the spinal cord where it can cause paralysis, but it doesn't stop naturally occurring polioviruses from entering and replicating in the gut—and spreading the virus. For this reason, as well as the fact that IPV is more expensive and requires a trained professional to administer, this vaccine is not practical for the eradication campaign efforts still ongoing in high-risk countries. But because it affords protection against all three serotypes of the virus, it could serve as the perfect supplement as these regions of the world transition to the bivalent oral vaccine. IPV has been introduced into polio campaigns in affected areas, and "the goal is to have all countries using IPV in [routine immunization programs] by the time of the switch," Modlin says. (See "For Polio, Two Vaccines Work Better Than One," *The Scientist*, August 21, 2014.)

Eventually, health-care officials hope to transition all countries to exclusive use of IPV, eliminating the risk of vaccine-derived viral infections. But IPV's injection mode of delivery remains a challenge. As a possible alternative, researchers are now exploring the use of microneedle patches, as simple to administer as putting on a Band-Aid. An added bonus is that the microneedle vaccines would not require cold storage. Cochi speculates that a microneedle version of IPV, which is currently undergoing testing in early Phase 1 trials, could be available within the next five years, easing the transition from the oral forms of the vaccine.

Even longer-term, if the polio eradication campaign is successful, there is the possibility that polio vaccinations could be stopped altogether, as happened with smallpox. "Speaking for myself, [I] consider the Holy Grail to give up using all polio vaccines altogether," says Modlin. "We'd like to take those dollars and apply them to other public-health priorities, if we can."

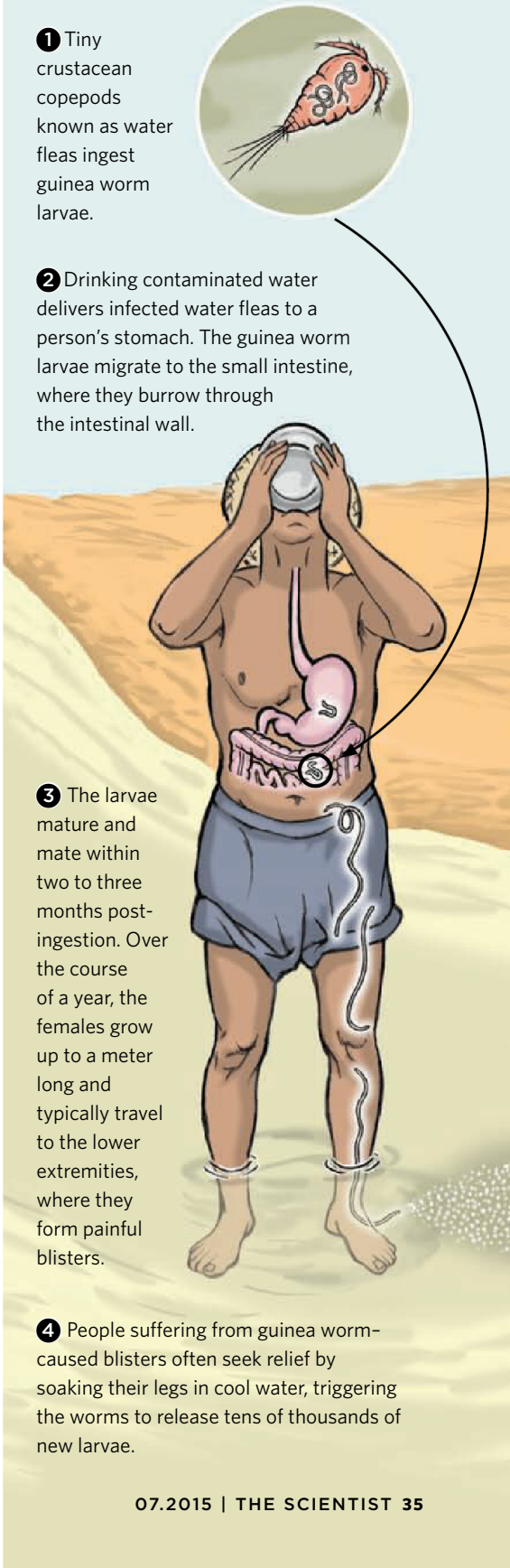
Worldwide deworming

Guinea worm—the only other pathogen currently targeted for eradication—differs dramatically from smallpox and polio in that there is no vaccine. In fact, there isn't even a treatment. Instead, elimination of the disease may be achievable thanks to the extremely predictable life cycle of the parasitic nematode *Dracunculus medinensis*. Guinea worm larvae hiding in infected copepods are ingested by humans, where they mature and mate. Females grow into meter-long worms, migrate to the foot or ankle, and escape via burning blisters, which their human hosts often plunge into water to relieve the pain. Upon sensing the water, the female worms discharge new larvae, starting the cycle over again. Thus, to halt guinea worm disease, the primary focus of the eradication campaign has simply been to provide clean drinking water to affected villages. This strategy has succeeded in reducing cases of guinea worm in Africa and Asia from more than 3.5 million in 1986, when the World Health Assembly passed a resolution to eliminate guinea worm, to just 126 infections last year.

"[There's] no drug, no vaccine, and no real diagnostic," says David Molyneux, a tropical-disease expert at the University of Liverpool. "And yet you can still [eradicate] it by implementing public-health measures."

But in the past couple of years, the parasite has thrown officials a curveball: it began infecting dogs in Chad, one of four remaining countries (all in Africa) where guinea worm is endemic. "This is an entirely new phenomenon as far as human guinea worm—one that is quite concerning," says Molyneux, especially given that lack of an animal reservoir is a commonly cited prerequisite for eradicability. While Chad had only 13 cases of guinea worm disease reported in humans last year, 113 dogs were infected. A similar pattern was observed the year before. A handful of dog infections have also been reported in Ethiopia.

The canine cases suggest that guinea worm may be infecting mammalian hosts, possibly including humans, via a different route than the classic path of contaminated drinking water. The Logone and Chari Riv-



THE CARTER CENTER/GRAPHIC BY AL GRANBERG

SIMPLE SOLUTION: Pipe drinking straws that filter out tiny parasite-carrying water fleas are an important tool in combatting guinea worm disease. The pipe filters are distributed to nomads and people displaced by war in South Sudan, which has become one of the last frontiers on the path to eradicating this debilitating disease.

ers that feed Lake Chad support a thriving fishing industry, in which fish are caught by hand and with baskets in the dry season, when the water levels drop and the rivers become more like large lagoons of stagnant water. "It's quite significant and unique to Africa," Ruiz-Tiben says. "I've never seen this intensity and dependency on fish products for food." While the fishing industry supports the local economy, all signs seem to point to infected fish, which prey on copepods, as a new source of guinea worm infections. As the fishermen bring in their catch, they clean the fish on the river bank, dropping the guts on the ground. The local dogs, of course, are all too happy to clean up the mess. In all likelihood, these fishy meals are the source of the outbreaks of guinea worm in Chad's dogs. And if people do not fully cook the fish themselves, they, too, may become infected.

That only sporadic human cases of guinea worm have been seen in Chad suggests that this is exactly what's going on. Rather than mini-outbreaks, in which one or a couple of infected individuals leads to bouts of dozens of cases in the following year after the worm has completed its cycle and contaminated the village's water source, there have been just a handful of cases in Chad villages, and often no repeat cases the following year. "That was one clue that transmission is not occurring via drinking water," says Ruiz-Tiben. Public-health workers are now striving to educate affected villages about this presumed new mode of guinea worm transmission, encouraging people to cook their fish thoroughly; dispose of the fish entrails in a sanitary way; and keep infected dogs out of the water.

"The change in the number of new [guinea worm] cases in the last 20 years has been spectacular; it's a remarkable public-health achievement," says Molyneux. "But as always, the last few cases are the most expensive and the most difficult."

Next on the chopping block

When polio was selected to be the object of a worldwide eradication campaign in the late-1980s, it wasn't the only pathogen that officials considered. Another potential candidate that made the shortlist was measles, says Cochi. Like polio, it has an effective vaccine—the combination measles, mumps, and rubella (MMR) vaccine—and no animal reservoir. The World Health Assembly's decision to target polio may simply have been a matter of circumstance.

"Beginning around 1980, first Brazil and then an increasing number of countries in Latin America began nationwide polio campaigns," Cochi says, which "knocked polio disease burden way down to low levels. . . . That was one big factor—there was demonstration of success in a large geographic area. The other big factor was [that] Rotary International became interested in polio eradication in the mid-1980s and signed on as the largest private-sector [participant] in the polio eradication effort."

But as polio eradication approaches what may soon be a realistic near-term goal, some epidemiologists are starting to turn their sights back to measles, which, like polio in the 1980s, is now the target of numerous regional campaigns. In fact, each of the WHO's six regions now has an ongoing measles elimination effort. As a result, "there's been a real acceleration in reduction of measles worldwide," Cochi says, with deaths from the disease dropping by 75 percent since 2000.

Despite such progress, measles still kills about 140,000 children each year. And that number means that even regions of the world that were once measles-free, such as the United States, are still at risk, a fact highlighted by the recent outbreak that originated at a Disney theme park in California in late 2014. "This is just a reminder that there's still a lot of measles elsewhere in the world," Cochi says. "These organisms don't respect borders."

Measles eradication does face a few challenges that the polio campaign has largely avoided, however, most notably the fact that the MMR vaccine must be administered by a trained professional. "Because measles is an injectable vaccine, we can't go house-to-house," Cochi says. "[It requires] more of a facility-based measles mass campaign." But, he added, the MMR vaccine does have one advantage over the polio immunization: it needs to be given only twice, instead of the four times recommended for the oral polio vaccine. "The measles campaigns are far less frequent and therefore less disruptive to the health-care system," he says.

For now, however, there is no official talk of a worldwide measles-eradication effort. With polio still circulating, most say it's too soon to think about diverting resources away from the ongoing campaign. "Measles is the next disease that people talk about for eradication," says Heymann. "[But] no one is willing to talk about measles eradication until polio is finished." ■

THE CARTER CENTER/L. GUBB

Outbreak Observatory

Increasingly precise remote-sensing data are helping researchers monitor and predict cases of infectious disease.

BY JYOTI MADHUSOODANAN

Pietro Ceccato vividly remembers his trip to a northeastern Tanzanian Maasai village last July. For more than two hours, the bus he caught in the city of Arusha traversed a flat landscape sprinkled with acacia trees; the summer air was dry and heavy with dust. The village itself, populated by a cattle-herding tribal group dressed in bright red and blue robes, was no more than a small cluster of huts fenced in to keep lions and hyenas out. But there were also hints of modern life in the community. The village chief was frequently on his cell phone, for example, touching base with members of his village to keep track of his cattle, recalls Ceccato, an environmental scientist at Columbia University in New York City.

Ceccato hadn't traveled to the village simply to observe its culture. He was there to count tiny black tsetse flies, which pose a threat more insidious than lions. During the rainy season, grasses and shrubs carpet the land, creating ideal breeding grounds for the flies, which serve as a vector for the parasitic protozoans that cause sleeping sickness. Infection, which begins with fever and fatigue, can cause neurological problems and death, and is a serious threat to both the villagers and their livestock.

Because the fly's life cycle is closely linked to rain and warmth, Ceccato and his collaborators are tracking environmental conditions using remote-sensing satellite data as a way to predict spikes in tsetse fly numbers and, therefore, spikes in sleeping sickness risk.[1] Then, using bright-blue mesh traps baited with the scent of nail polish, the researchers obtain a tsetse fly census to improve how these climate-based models compare to conditions on the ground.

> If you know the conditions are favorable for an epidemic to occur, you can nip it in the bud.
>
> —Rita Colwell, University of Maryland

"The tsetse fly is very particular—it likes nice warm weather, but not too hot; not too wet, but not too dry; and spaces in the vegetation," says Ceccato. "The years when there were less tsetse flies was when there was a drought, and using our climate models we are able to forecast [such] events." He and his colleagues are now in the process of making their models, or risk maps,

NASA IMAGE BY JEFF SCHMALTZ, MODIS RAPID RESPONSE TEAM

DISEASE DETECTOR: Satellites capture phytoplankton blooms (blue-green) in the North Atlantic. Similar blooms have been linked to outbreaks of *Vibrio* infections along European coasts.

available to anyone with a cell-phone connection, such as the Maasai village chief. A quick swipe or few clicks could inform him of specific days when, or areas where, the risk of an infectious insect bite might be particularly high, allowing him to avoid fly-dense patches of vegetation when taking cattle to graze.

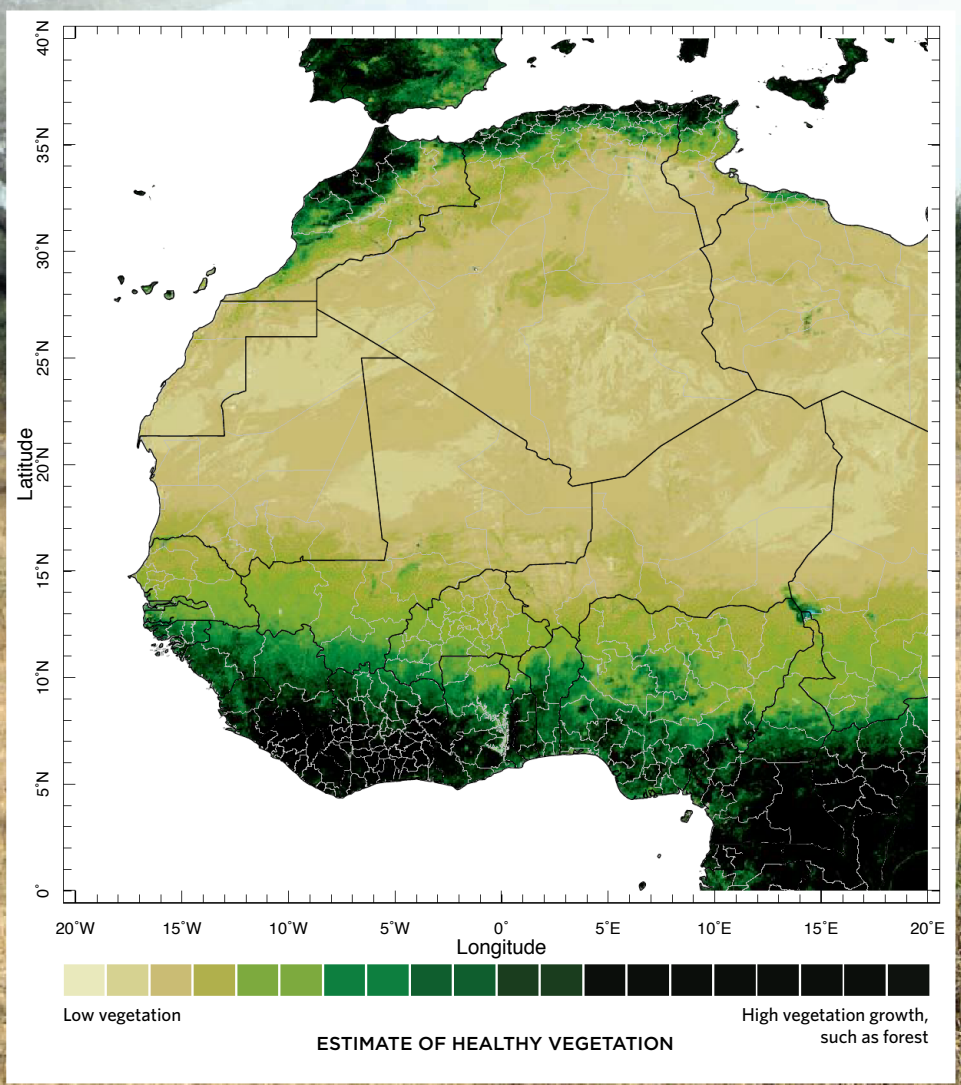
Disease risk maps aren't unique to parasitic infections in eastern Tanzania. With modern geographic information system (GIS) technology providing the capacity to monitor climate conditions and numerous other environmental variables across the globe, researchers are using this information to model outbreaks of diverse diseases, including cholera, malaria, West Nile virus, Lyme disease, and several neglected tropical diseases.[2] Meanwhile, local data collected via cell phones are being used to track the presence of disease carriers—both human and animal—to help researchers estimate and prepare for shifting risk zones.[3] In a few years, these improved simulations could make forecasting an epidemic as precise as predicting the weather.

"It'll be very commonplace that many diseases—particularly those linked to ecological factors—will be monitored and predictable to some extent in the near future," says public-health biologist Kenneth Linthicum, director of the US Department of Agriculture's Agricultural Research Service in Gainesville, Florida.

And knowing when and where outbreaks are likely to occur is key to reducing casualties, says microbiologist Rita Colwell of the University of Maryland. Across Asia and Africa, disease maps have already helped reduce the impact of several diseases, including soil-transmitted helminthic infections, river blindness, trypanosomiasis, dengue, and malaria—primarily by helping authorities identify at-risk regions and direct sanitation and therapy efforts accordingly. "If you know the conditions are favorable for an epidemic to occur, you can nip it in the bud," says Colwell.

Bird's-eye view

Historically, researchers mapped diseases based on the occurrence of reported cases to identify at-risk regions and allocate resources or target preventive action. A century before satellites existed, physician John Snow mapped the spread of cholera as the disease swept through London in the summer of 1853. Snow traced the outbreak's source to a pump dispensing contaminated water. Disabling the pump's



handle contributed to the epidemic's end. (See "John Snow's 'Grand Experiment,' 1855," *The Scientist*, August 2010.)

Today, Colwell continues Snow's tradition of mapping cholera, though she uses much more sophisticated methods and has trained her sights on the oceans, rather than city streets. In the 1960s, during long hours spent sampling the waters of the Chesapeake Bay in Maryland, she discovered that an abundance of *Vibrio* species—including *V. cholerae*, the pathogen that causes cholera—readily attached themselves to the shells of crustacean zooplankton. "I knew *Vibrio parahaemolyticus* was there," she says. "But the distribution of *cholerae* was a surprise, and that led to my understanding that it is an environmentally native bacterium. At the time that was very controversial."

Improved simulations could soon make forecasting an epidemic as precise as predicting the weather.

Colwell began to wonder whether satellites, which had begun to capture images of massive algal blooms in seas across the planet, could also trace oceanic bacteria, including *Vibrio* species. Populations of *V. cholerae*–harboring zooplankton tinted the color of the ocean in the images, and researchers could see that their numbers spiked shortly after algal blooms. Colwell and her colleagues found that peaks and troughs in bacterial abundance correlated with the rise and fall of planktonic populations visible from space.

Since then, several studies have confirmed that pathogenic *Vibrio* species are present in oceans around the world, and planktonic blooms have been linked to sporadic outbreaks of *Vibrio*-caused gastroenteritis, wound infections, and deaths in humans. Following unusually warm seasons in 2003 and 2006, for example, populations of *V. vulnificus* increased along European coasts surrounding the Baltic Sea. During these periods, several studies reported infections and one death (from advanced sepsis) among people who waded or swam in waters off the coasts of Germany or Sweden.[4]

Satellites can also monitor smaller bodies of water: dams, rivers, ponds, and swaths of irrigated agricultural land, any of which can turn into reservoirs for lurking pathogens in the right climatic condi-

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WATCHING THE GRASS GROW: Data from NASA's MODIS sensor (map at left) are used to create estimates of weather-related vegetation changes, which can help forecast malaria outbreaks in some parts of Africa. Similarly, seasonal patches of green in Tarangire National Park, Tanzania, (above) can be monitored by satellite to predict changes in populations of mosquitoes, tsetse flies, and other disease vectors.

tions. In parts of India, Bangladesh, and other developing countries, such water bodies host *Vibrio* species year-round, causing low levels of endemic infection common in people who live in the area. But seasonal changes that grow pathogen populations can trigger outbreaks.

Increasingly detailed maps from space can also yield insights about conditions of terrestrial habitats. (See "Casting a Wide Eye," *The Scientist*, February 2012.) Ceccato and his collaborators use satellite data to map dense, humid patches of vegetation in Tanzania where tsetse flies might breed. And in the northeastern U.S. and Canada, similar imagery of forested patches, heavy with plants and moisture, helps researchers predict the risk of tick-borne Lyme disease. Watching the edges of fragmented forests on satellite images also tells researchers where people are most likely to encounter ticks or other forest-dwelling vectors of human disease.[5] But probably the most commonly used type of satellite data incorporated into disease risk maps is weather.

"Particularly when working in the developing world, it's often very challenging to get meteorological data from local weather stations," says ecologist Michael Wimberly of South Dakota State University. "Satellite remote-sensing is a really rich source of information on the environment—everything from climate change to land cover change."

As the Earth's climate continues to change, incorporating weather patterns into disease risk maps will become even more important. Research has suggested, for example, that when temperatures climb, ticks, mosquitoes, and other disease vectors invade new territories, bringing their pathogens along for the ride. In central Ethiopia and northwest Colombia, malaria was thought to be restricted to low-lying regions; cooler temperatures at high altitudes have been known to deter mosquito breeding. But last year, researchers reported temporary shifts in the spatial distribution of malaria cases: in El Niño years—such as 1997 and 2005—when the world experienced globally higher temperatures, the disease occurred at higher elevations than in cooler years.[6]

TSETSE TRACKERS: Entomologist John Hargrove of the University of Stellenbosch in South Africa shows locals how to set up traps to measure tsetse fly populations (top). Locals clean up after a traditional meal in the Maasai village where Hargrove and other researchers are tracking disease-carrying tsetse flies (bottom).

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In 2009, climate data suggested the possibility of an El Niño–like event similar to that seen in 1997, leading the director of the malaria control program in Eritrea, Ethiopia's northern neighbor, to ask the researchers if a large outbreak of malaria was likely. "We checked on it, and it seemed a little different, so the risk of malaria was not as high," says Ceccato. "But now [the program director] is monitoring the rainfall and vegetation, because that still gives him a heads up two months in advance where there might be a risk."

No model is perfect, however, Ceccato notes; even the best simulations struggle to cope with the complexity of outbreak prediction. In addition to environmental conditions, the accuracy of a model depends on how and when the disease vector breeds, what controls have been put in place, and whether the disease can be transmitted by people traveling to and from endemic regions. "To model every behavior in every region is almost impossible," he says.

As temperatures climb, ticks, mosquitoes, and other disease vectors are invading new territories, bringing their pathogens along for the ride.

Climate data are proving particularly useful for tracking areas where diseases persist at low frequencies but are at risk of spreading to new locales. For example, in some tropical regions that harbor malaria-carrying *Anopheles* mosquitoes year round, such as the Ethiopian lowlands, the mosquitoes breed more quickly—and spread more infections—at the beginning or end of the monsoon season. People who live in such endemic areas may grow immune and suffer less-severe infections, but when the rate of infections rises, pathogens can spill over into new areas, which may trigger an epidemic.

In the past, epidemiologists identified such endemic zones based on the occurrence of actual disease cases; mapping these cases to specific regions helped to focus preventive efforts as well as to allocate medicine and other resources. Now, newer technologies enable researchers to proactively use climate and geographic data in these endemic areas to foresee potential outbreaks.

"When there's an outbreak [in an endemic region], it has the potential to move elsewhere," says Uriel Kitron, an environmental scientist at Emory University. "We see that with the Chikungunya virus, which is moving essentially throughout the world."

Adding local color

To build optimally accurate and predictive disease risk maps, researchers must supplement satellite data with information from old-fashioned surveys and case reports gathered on the ground. This local surveillance helps researchers nail down correlations between disease risk and specific environmental circumstances that are critical to accurately predicting an epidemic. "One of the most difficult things is to get good disease data," says Linthicum of the Agricultural Research Service.

One common proxy for disease risk is vector abundance. The bright-blue traps that Ceccato's collaborators set around the Maasai village in Tanzania, for example, allow the team to assess how tsetse fly numbers shift with the changing seasons. To track ticks that carry Lyme disease in the northeastern U.S. and Canada, many researchers trail a large white sheet through the forest and see what sticks. Other groups walk miles along irrigation canals in Kenya to pinpoint how snails that spread disease-causing parasites are distributed near water sources in local villages.

Historical data can also help bolster disease-risk models. To link weather patterns to potential cholera epidemics, Colwell and her colleagues gleaned climate records for the Punjab region in northern India from annual reports made to the Indian government between 1875 and 1900. The researchers also gathered records of cholera fatalities during the same time frame from annual reports of the Sanitary Commissioner to the Government of Punjab. They investigated a period during which few disease interventions were available to better deduce the link between climate and disease. The data revealed that abnormally high temperatures followed by unusually high rainfall usually preceded an epidemic of cholera. Refining environmental models based on these records, Colwell and her colleagues can now predict potential outbreaks of cholera two to four months in advance, based on average temperatures and rainfall in a region.[7]

"When we have an abnormal increase in air temperatures, we start to monitor the region more closely. If there's rainfall shortly after, we're sure there is a chance of cholera outbreaks," says West Virginia University hydrologist Antar Jutla, who collaborates with Colwell. "Then, we use finer-scale data to see which areas may or may not be at risk." The team is now adapting its methods to model the spread of West Nile virus, which last year caused more than 2,000 infections in the U.S. alone.

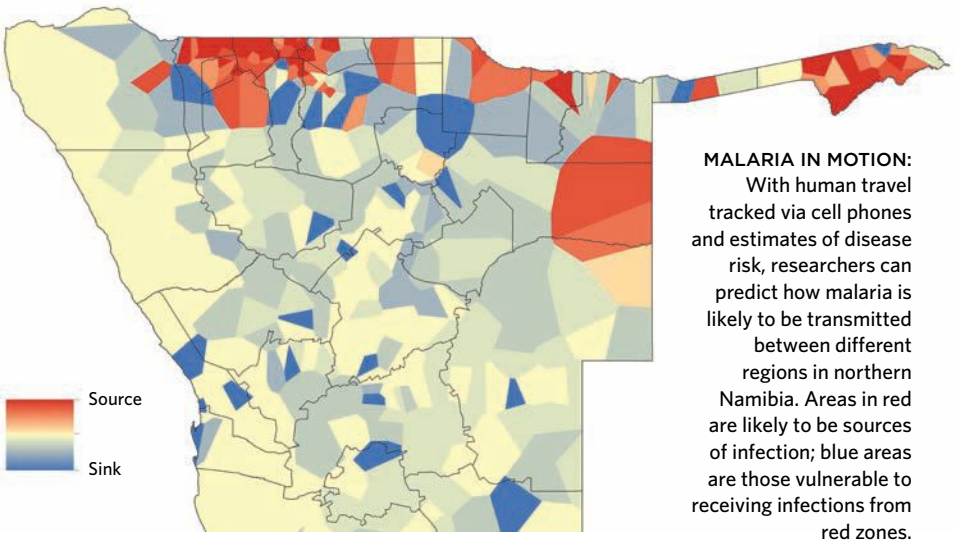
Yet another source of data for improving disease-risk models is the global cellular-communication network, through which local residents can inform researchers of disease cases in real time. Rebecca Flueckiger, an environmental scientist at the Task Force for Global Health in Atlanta uses cell-phone data to build maps of diverse endemic diseases in severely affected regions. Field data gathered by trained local residents or volunteers help Flueckiger and her colleagues pinpoint each occurrence of a disease to a specific locality. Other groups in several parts of Africa rely on residents to monitor malaria cases in their neighborhoods. Mobile-phone networks also clue researchers into the movements of bigger disease carriers: people who travel to or from areas where infections are rampant.

Putting maps into action

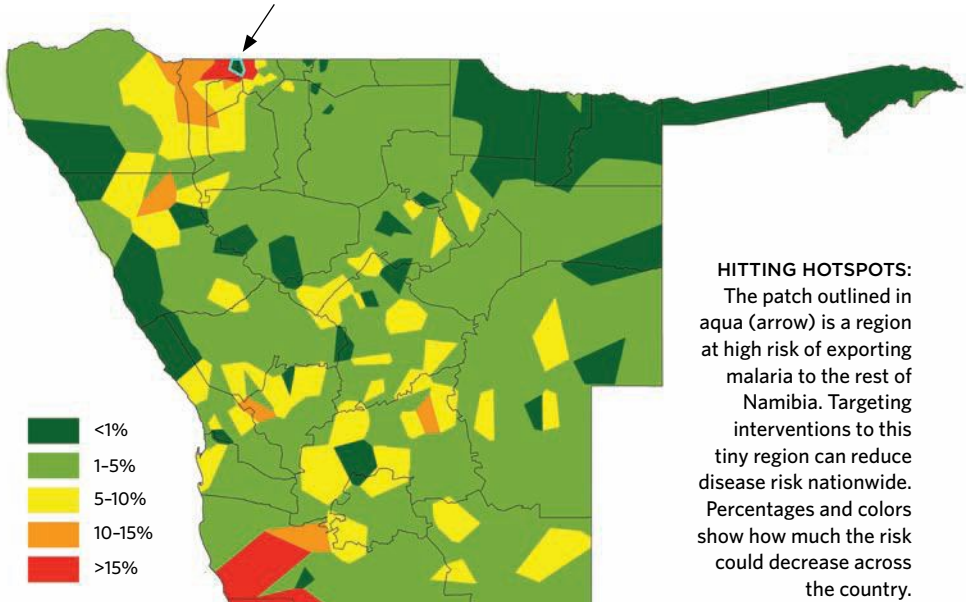
In addition to sending data to the researchers building epidemiological models, mobile phones enable regional public-health agencies to access the risk maps to monitor for impending outbreaks in their districts or villages. "People who don't have a background in remote sensing or climate data are now able to access the information and use it," says Ceccato. "This is really the big change there. This information that was just in the hands of specialists is now available to everybody for their decision-making processes."

Last year, Andrew Tatem of the U.K.'s University of Southampton and his colleagues at the Sweden-based nonprofit Flowminder Foundation integrated satellite imagery of rainfall, temperature, elevation, and other environmental factors with case reports of disease and data on human movement monitored through mobile-phone networks to create high-resolution maps of malaria risk levels in northern Namibia. (See maps on this page.) Putting these diverse data sets together, the group identified specific regions where a high risk of infection converged with increases in human travel. Although current guidelines in Namibia categorize the entire northern part of the country as a high-risk region, this new map highlighted a few areas crucial to transmitting infections (below). The refined estimates reduced the population that required interventions from 1.3 million in the entire northern zone to fewer than 200,000 who live in the predicted areas with higher risk.[8]

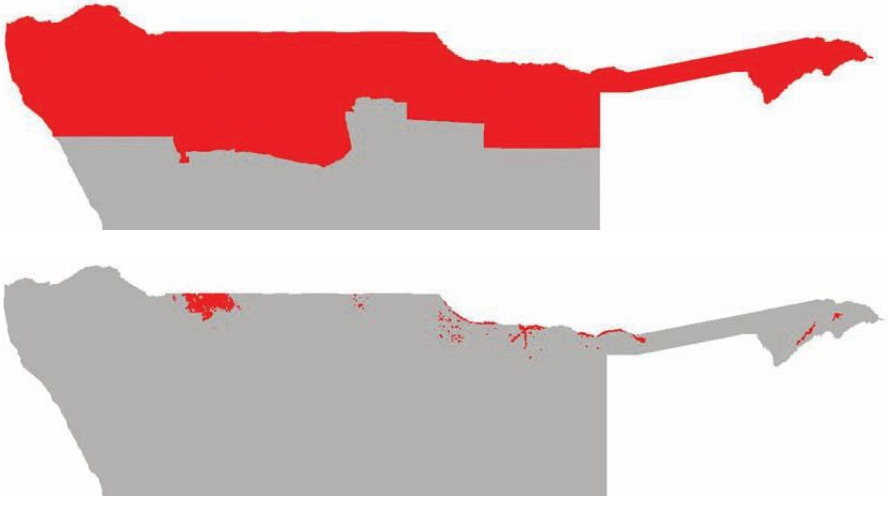
"We went through the whole chain from case reports from the government, combined that with remote-sensing data to create high-resolution risk maps, and then added the mobile-phone data to see what areas are exporting and importing infectious persons," says Flowminder



MALARIA IN MOTION: With human travel tracked via cell phones and estimates of disease risk, researchers can predict how malaria is likely to be transmitted between different regions in northern Namibia. Areas in red are likely to be sources of infection; blue areas are those vulnerable to receiving infections from red zones.



HITTING HOTSPOTS: The patch outlined in aqua (arrow) is a region at high risk of exporting malaria to the rest of Namibia. Targeting interventions to this tiny region can reduce disease risk nationwide. Percentages and colors show how much the risk could decrease across the country.



THEN AND NOW: In the past, preventive actions were targeted to the entire northern region of Namibia, covering a population of 1.29 million in 2011 (top). Risk forecast maps suggest similar protection could be achieved by focusing on a much smaller region, with a 2011 population of just 190,000 (bottom).

TATEM ET AL., *MALARIA JOURNAL*, DOI:10.1186/1475-2875-13-52, 2014

cofounder and executive director Linus Bengtsson. "In the end what we get is a map that tells us where it's most efficient to put in resources. That's what the government of Namibia is now using to steer their allocation of preventive resources." The group is also working on a similar project directed at an ongoing cholera epidemic in Haiti.

As disease risk maps continue to prove their worth in pinpointing potential epidemics, the field is attracting funding to build even better models. Just last year, for example, DARPA announced a challenge, with total awards of more than $150,000, for maps that could help forecast the spread of Chikungunya in the Americas and the Caribbean. Researchers in some parts of the world are now implementing the data not just to predict outbreaks, but to wipe out disease altogether. In Namibia, for example, officials aim to use the improved risk models offered by Flowminder to eradicate malaria in that country. Similar efforts to extinguish malaria using predictive risk maps are underway in other African countries as well as in parts of Asia. In the end, such maps may be key to staying one step ahead of the world's worst pathogens.

"Just because the conditions are optimal doesn't mean there has to be an outbreak," says Colwell. "You can take precautions, and when cases appear, you can react much more effectively instead of waiting to see if there might be an epidemic." ■

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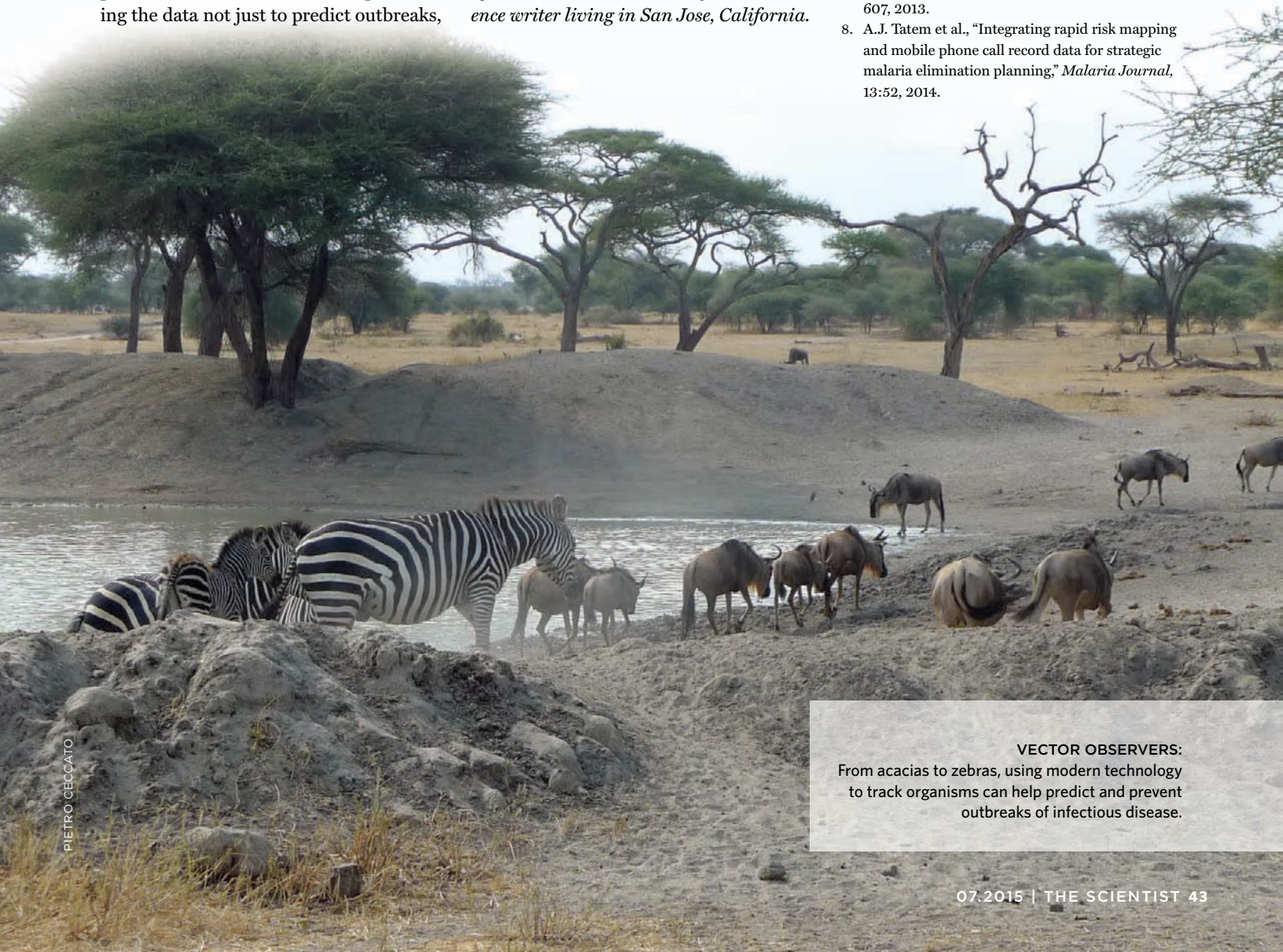
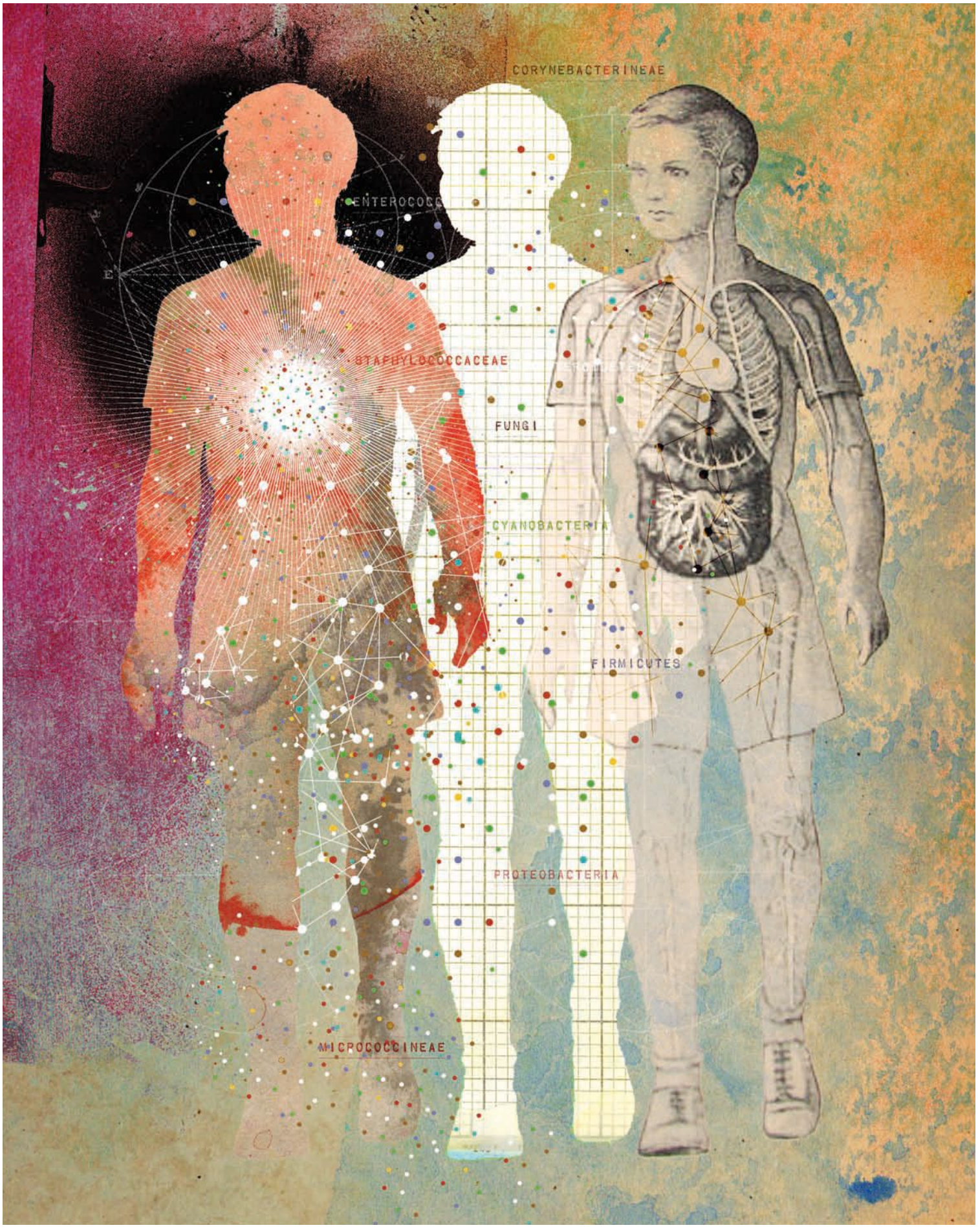


PIETRO CECCATO

VECTOR OBSERVERS:
From acacias to zebras, using modern technology to track organisms can help predict and prevent outbreaks of infectious disease.


CORYNEBACTERINEAE
ENTEROCOCC
STAPHYLOCOCCACEAE
FUNGI
CYANOBACTERIA
FIRMICUTES
PROTEOBACTERIA
MICROCOCCINEAE

The Sum of Our Parts

Putting the microbiome front and center in health care, in preventive strategies, and in health-risk assessments could stem the epidemic of noncommunicable diseases.

BY RODNEY DIETERT AND JANICE DIETERT

Looking across generations at how health concerns have changed over the past century is an enlightening exercise. For your ancestors living in the roaring '20s, fear of infectious diseases—including typhoid fever, cholera, and influenza—far outweighed concerns about heart disease or cancer. Autism, Alzheimer's, attention deficit disorder, and Parkinson's disease were virtually unheard of. Allergies, then called hay fever, were around, but not common. Ratchet ahead through the rock-and-roll and disco generations and on to the '80s and '90s, and the fear of cancer grew enormously, while a number of new diseases began to appear on the radar screen. Asthma, autism, lupus, arthritis, inflammatory bowel disease, attention deficit disorder, celiac disease, multiple sclerosis, obesity, and diabetes, among others, became common concerns. Fast-forward another two decades to present day, and it is not a matter of whether you, your friends, or family members have one of these ailments, but which ones and how many.

In less than 100 years, leading diseases and causes of death have shifted dramatically away from infectious diseases and heavily toward noncommunicable diseases (NCDs), not just in developed countries, but around the globe. NCDs are now the number one killer worldwide, accounting for 63 percent of all mortalities.[1] There is no question that environmental variables, including exposure to cigarette smoke, certain dietary factors, and chemicals such as heavy metals, pesticides, endocrine disruptors, or particular drugs, increase one's risk of developing an NCD. Psychosocial stressors also play a role. But any assumption that the ongoing NCD epidemic is due solely to external factors would be missing a key part of the story: the human microbiome. In reality, the NCD epidemic is as much about the ways we have altered our microbiomes in recent decades as it is about our changing external environment.

In less than 100 years, leading diseases and causes of death have shifted dramatically away from infectious diseases and heavily toward noncommunicable diseases.

Commensal microbes that live on and in us are critical for our health. By cell numbers, we are approximately 90 percent microbial, and the vast majority of the genes expressed in our superorganism are not on our mammalian chromosomes but in the bacteria, archaea, and single-celled eukaryotes that call the human body home. Normally, a robust microbiome would be part of our inheritance, a legacy passed, largely maternally, from generation to generation. But recently that chain has been broken, usually more than once. The increase in cesarean deliveries, the reduced prevalence and duration of breastfeeding, overuse of antibiotics both as prescription drugs and in agriculture, modern urban living surrounded by sanitizers, and a general tendency to limit contact with the environment have changed our relationship with the microbes that are an integral part of our biology. In today's world, our best chance of acquiring microbes might be from touching our computer keyboards and cellphones or frequenting shopping malls, hotel rooms, or doctors' offices—and many are not bugs you want in and on your body.

Our microbial gatekeeper

The human microbiome plays a critical role as a filter between us and the world. In fact, it is the microbiome that determines our actual exposure to the environment. Substances such as foods, drugs, and environmental chemicals—collectively termed xenobiotics—must first pass through the layers of microbiota on the skin, in the gut, and in the airways where, depending upon the microbes present, the chemicals will be sequestered, excluded, or metabolized before they ever enter our cells. The common gut actinobacterium *Eggerthella lenta*, for example, can significantly change the potency of the cardiac drug digoxin.[2] Likewise, microbiome composition affects the toxicity of certain environmental chemicals such as arsenic, with some sulfur-reducing gut bacteria able to generate highly toxic, thiolated species of arsenic, thereby increasing health risks following exposure.[3] And, of course, diverse gut microbes are critical components of our gastrointestinal system, helping us process the otherwise hard-to-digest foods we eat.

There is also a flip side to the xenobiotic-microbiome relationship: the external environment affects the composition of our microbial populations. Even some xenobiotics that were previously thought to be safe may need to be reexamined in light of effects on the microbiome. For example, commonly used food emulsifiers such as polysorbate 80 and carboxymethylcellulose have been reported to adversely affect the microbiome of rodents, predisposing them to chronic inflammation and elevated risk of metabolic syndrome. In one study, mice that drank the emulsifiers in water showed reduced overall diversity of the gut microbiota, decreased representation of generally beneficial Bacteroidales species, and higher numbers of some potentially pathogenic bacteria, such as *Ruminococcus gnavus*.[4] In some rodent strains, exposure to the emulsifiers also thinned the mucus barrier, reducing the physical distance between bacteria residing on the surface of the barrier and gut epithelial cells by more than 50 percent. Such alterations can affect the interactions between bacteria and cells of the innate immune system, increasing the risk of inflammation-driven disease. Not coincidentally, microbiomes that have been impoverished or unbalanced by environmental factors often have a skewed bacterial metabolism, affecting their host's energy utilization, hormone status, and control of inflammation.

Thus, it should be no surprise that altered microbiomes and elevated risk of NCDs go hand in hand. Myriad studies have linked specific NCDs to an altered diversity of gut microbiota in early life, with possible risk factors including maternal and infant diet, birth delivery mode, perinatal environmental toxicant exposures, and psychosocial stressors.[5,6] Many disease-associated microbiomes can serve as a type of fingerprint, reflecting the underlying disease condition. In some cases, these skewed, limited-diversity microbial communities may help cause or promote the disease; in others, they may be a consequence.

And if the status of the microbiome appears to affect the outcome of xenobiotic exposures and risk of NCDs, the reverse appears also to be true. Having an NCD appears to influence the composition of the microbiome and the body's susceptibility to some xenobiotics. In recent studies, Yale toxicologist Gary Ginsberg, also of the Connecticut Department of Public Health, and others demonstrated that NCDs, such as cardiovascular disease, obesity, or chronic kidney disease, affect one's vulnerability to certain heavy metals.[7]

The current gold-standard model for assessing environmental health risks was developed in 1987 by the US National Research Council during a time when the role of the microbiome was largely unknown. In effect, toxicologists and risk assessors have been missing the impact of the microbiome for decades. This year, one of us (R.D.) and Ellen Silbergeld of the Johns Hopkins Bloomberg School of Public Health proposed a new health-risk assessment model that places the microbiome as the filter between the external environment and the human body's own cells.[8] The new model relies on biomarkers that correlate with microbiome composition—such as volatile organic compounds (VOCs) and short-chain fatty acids—to help to connect environmental exposures, microbiome status, and risk of NCDs.

Microbial role in immunity

In addition to playing gatekeeper between our mammalian cells and the external environment, the human microbiome is critical to the maturation and function of our immune system, affecting the entire spectrum of immune processes. Commensal microbes have been shown to influence, for example, the body's overall cytokine milieu; the balance among T regulatory cells and inflammation-promoting Th17 cells; T cell–driven adaptive immune responses; macrophage and dendritic cell function; and natural killer T-cell activity, among other immunomodulatory properties. Given this intimate relationship between the human immune system and the microbiome, it is once again not surprising that alterations in our microbial makeup can greatly affect health.

Microbiome-based immune programming largely takes place during a critical window early in postnatal development and extends well beyond the gastrointestinal tract, affecting immune-cell reservoirs in the bone marrow and spleen as well as the functional capacities of resident immune-cell populations in distant organs and tissues. Microbiome-driven immunomodulation occurs via cell surface receptor signaling—involving Toll-like and NOD-like (nucleotide-binding oligomerization domain) receptors, among others—and also through epigenetic regulation, driven by microbe-produced short-chain fatty acids, that can affect the expression of hundreds of genes related to immune function.

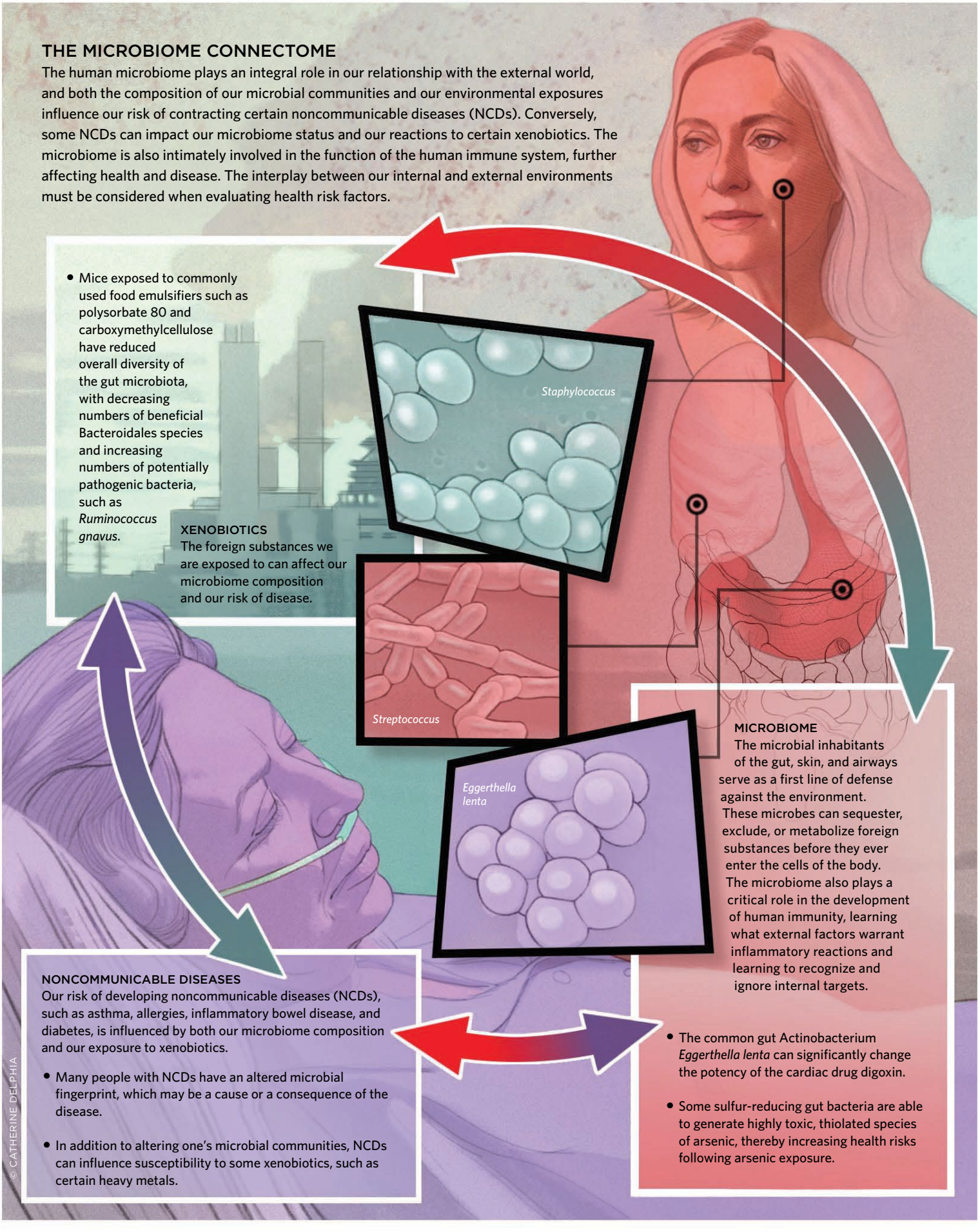
Given the intimate relationship between the human immune system and the microbiome, it is not surprising that alterations in our microbial makeup can greatly affect health.

Germ-free (gnotobiotic) mice provide a sobering model for what happens to a developing human immune system in the absence of microbiome-based training.[9] When microbiota are absent, normal postnatal immune maturation is blocked, and tissue homeostasis is never fully established. Lymphoid deficiencies occur in both the body's mucous membranes and its systemic tissues, such as the lymph nodes and spleen. Germ-free mice also develop imbalances among specialized immune cell populations that result in improper immune responses when challenged with injury or a pathogen. Depending on the nature of the challenge, defective host immune responses may include increased susceptibility to certain infections, reduced vaccine responses, and/or inflammation-induced tissue pathologies, such as asthma or colitis.

Not surprisingly, perinatal treatment with antibiotics can compromise the microbiome, depleting or eliminating the microbial signals needed for a newborn's postnatal immune development. The result can

THE MICROBIOME CONNECTOME

The human microbiome plays an integral role in our relationship with the external world, and both the composition of our microbial communities and our environmental exposures influence our risk of contracting certain noncommunicable diseases (NCDs). Conversely, some NCDs can impact our microbiome status and our reactions to certain xenobiotics. The microbiome is also intimately involved in the function of the human immune system, further affecting health and disease. The interplay between our internal and external environments must be considered when evaluating health risk factors.



- Mice exposed to commonly used food emulsifiers such as polysorbate 80 and carboxymethylcellulose have reduced overall diversity of the gut microbiota, with decreasing numbers of beneficial Bacteroidales species and increasing numbers of potentially pathogenic bacteria, such as *Ruminococcus gnavus*.

XENOBIOTICS
The foreign substances we are exposed to can affect our microbiome composition and our risk of disease.

MICROBIOME
The microbial inhabitants of the gut, skin, and airways serve as a first line of defense against the environment. These microbes can sequester, exclude, or metabolize foreign substances before they ever enter the cells of the body. The microbiome also plays a critical role in the development of human immunity, learning what external factors warrant inflammatory reactions and learning to recognize and ignore internal targets.

NONCOMMUNICABLE DISEASES
Our risk of developing noncommunicable diseases (NCDs), such as asthma, allergies, inflammatory bowel disease, and diabetes, is influenced by both our microbiome composition and our exposure to xenobiotics.

- Many people with NCDs have an altered microbial fingerprint, which may be a cause or a consequence of the disease.
- In addition to altering one's microbial communities, NCDs can influence susceptibility to some xenobiotics, such as certain heavy metals.

- The common gut Actinobacterium *Eggerthella lenta* can significantly change the potency of the cardiac drug digoxin.
- Some sulfur-reducing gut bacteria are able to generate highly toxic, thiolated species of arsenic, thereby increasing health risks following arsenic exposure.

be an immune profile that bears worrying similarities to those of germ-free mice. For example, antibiotic-induced disruption of the neonatal microbiota can result in aberrant immune maturation with altered cytokine production, the creation of a pro-inflammatory state, shifts in both host and microbial metabolism, and altered epigenetic programming.[10] And the results can be long-lasting. Antibiotic administration in infants is associated with higher risk of asthma later in childhood, a risk that scales with the number of rounds administered.[11] Increased use of antibiotics in infants is also associated with a higher risk of childhood obesity,[12] and some investigations have reported an association between antibiotic use and an elevated risk of celiac disease. It is likely only a matter of time before more links between disease and an infant's compromised microbiome are revealed.

Self-completion

Given the undeniable importance of commensal microbes in both training our immune systems and serving as a barrier between ourselves and the outside world, one of us (R.D.) has posited that a complete microbiome, seeded at birth, is absolutely critical for a healthful life, an idea called "the completed self hypothesis."[13] Single-celled organisms from all three domains of life—eukaryotes, archaea, and bacteria—join our mammalian cells to create a superorganism. Inadequate or inappropriate seeding of the microbiome is in many ways the equivalent of being born with a serious birth defect, resulting in inappropriately matured physiological systems.[14] In the absence of effective microbiome-based training, the immune system does not learn what is safe outside of the body, resulting in haphazard, inappropriate reactions to innocuous environmental factors—allergens such as pollen, mold, cat dander, and peanuts. It also fails to properly recognize and ignore internal targets, resulting in autoimmune and inflammatory responses that are misdirected, ineffective, and sometimes never-ending. Such reactions can eventually compromise the function of our own tissues and organs.

A newborn's microbiome is largely inherited from the mother, with birth being the most pivotal step in seeding. During vaginal delivery, the passage of the baby down the birth canal allows exposure not only to the vaginal microbiota but also to a film of maternal intestinal flora. This process is thought to provide direct seeding of the newborn's gut with maternal microbes. Skin-to-skin seeding is also important at birth. When natural childbirth is interrupted—for example, by cesarean delivery—the baby is seeded by default with microbes from the local environment, typically from the largely sterile hospital staff and equipment. Invariably, this results in incomplete and/or inappropriate infant microbial seeding. Indeed, numerous studies have suggested that cesarean-delivered babies typically have altered immune profiles and are at an elevated risk for NCDs such as asthma, type 1 diabetes, and obesity. A recent study of 98 Swedish infants and their mothers, for example, found that cesarean delivery significantly blocked vertical transmission of the maternal microbiome to the infant.[15] Additionally, the microbiome transition toward an adult-type profile was shaped by the infant's feeding pattern after birth, including both breastfeeding and the transition to solid foods.

MANIPULATING THE MICROBIOME

In contrast to our human genome, our microbial genome is more amenable to adjustment by altering the composition of the microbial communities inhabiting our bodies. Some researchers and doctors have already recognized the power of microbiome manipulation—think probiotics and fecal transplants (*Microb Ecol Health Dis*, 26:25877, 2015). Probiotic mixtures can be ingested to shift microbial balance and metabolism in the gut, translating to potentially useful physiological alterations. Recent reports suggest that probiotics can prevent diarrhea in children taking antibiotics, for example, as well as increase the efficacy and reduce the side effects of anti–*Helicobacter pylori* therapies and aid peanut oral immunotherapy for the treatment of peanut allergy. The more radical approach of fecal transplantation, in which microbiota are installed in the gut via a gastric or nasoduodenal tube, an enema, or colonoscope, or orally administered frozen capsules, has proven successful for the treatment of *Clostridium difficile* infection (*Infect Dis Clin North Am,* 29:109-22, 2015), and other potential uses are currently under investigation. Fecal transplants have also been used subsequent to antibiotic administration to reinstate a healthy microbiome. Identification and selection of donor microbes is likely to be an important future consideration for these therapies.

While microbiome manipulation may have benefits at any age, once certain developmental programming of our physiological systems has occurred, it is likely to be much more difficult to correct underlying dysfunctions. Intervention early in life is the most comprehensive technique, as it allows for self-completion in the newborn prior to most postnatal developmental programming. We believe that no baby should go unseeded or be left to haphazardly acquire the daily menu of microbes from a given hospital environment. If elective cesarean delivery is planned, deliberate seeding of the baby should be considered. Maria Gloria Dominguez-Bello of the New York University Langone Medical Center, for example, has promoted the use of vaginal swabs immediately after cesarean birth to simulate the baby's exposure to maternal microbes in the birth canal, and preliminary results are encouraging (*Trends Mol Med*, 21:109-17, 2015). Potential medical complications should be considered in any decision regarding microbial manipulation therapies. These must be balanced against the immune and other long-term health risks that are created if the baby cannot self-complete as a superorganism.

Disruptions to complete microbiome transfer can also occur before birth, as the mother's microbial makeup is influenced by her diet, environmental exposures, and health. Microbiota originating from a mother afflicted with one or more NCDs or from a mother who was treated with antibiotics during pregnancy are likely to differ from the microbiota transferred from a mother who is NCD- and antibiotic-free.

A better understanding of normal microbiome maturation may inform potential microbial-manipulation therapies, in which life stage–specific adjustments to the microbiome can improve health outcomes.

To understand which microbes are critical for the proper development of a baby's immune system, it is first important to know what a healthy microbiome looks like and what happens to it during normal childhood maturation. Merete Eggesbø of the Norwegian Institute of Public Health and his colleagues have provided a useful picture of normal development of a properly seeded microbiome across infancy in the absence of antibiotic administration and overt disease. They reported that the gut microbes present in four-day-old Norwegian newborns were useful in predicting the composition seen at three months of age (in the absence of medical interventions).[16] The recent study of 98 Swedish mother-child pairs provides further documentation of infant microbiota composition during the first year of life.[14] Going forward, it will be important to collect similar data on normal microbiome development across different regions of the world, as geographic differences do exist among microbiomes. This information can help researchers evaluate the risks and benefits of various birthing, infant-feeding, and treatment practices.

A better understanding of normal microbiome maturation may also inform potential microbial-manipulation therapies, in which life stage–specific adjustments to the microbiome can improve health outcomes. (See "Manipulating the Microbiome" on opposite page.) Of course, such therapies should always consider any potential risks, and the ethical implications of microbiome manipulation, as our microbial partners are really a part of our biological identity. (See "Who Are We Really?" *The Scientist*, March 2012.)

For now, we need to rethink the way antibiotic treatments are handled. There has already been a widespread call for priority shifts in the use of antibiotics, designed to slow down the selection of multidrug-resistant bacteria and preserve effective antibiotics for the most serious conditions. But there is another consequence of antibiotic use that has been largely overlooked: a severely altered and/or largely destroyed microbiome. Evidence continues to mount regarding the potential disease outcomes thought to be related to the destruction of the infant microbiome. It's becoming clear that we should not be leaving children deficient in most of their microbiome just to wipe out one pathogenic bacterium. The short-term gain comes at the cost of an increased chance of developing NCDs later in life. That is not to say that antibiotics should not be used, but that antibiotic administration as recently practiced is an incomplete therapy with unacceptable long-term risks. Future treatments with antibiotics should be accompanied by complementary therapies to restore the commensal microbes that were never intended to be killed.

Indeed, the goal of any medical procedure should be to leave patients with the best possible microbiome. The importance of our microbial partners has for too long been overlooked by the medical establishment. A new treatment standard that takes a complete microbiome into consideration could result in sweeping changes in health care, such that more integration and better personalization are likely outcomes. ■

Rodney Dietert is a professor of immunotoxicology at Cornell University in Ithaca, New York. His wife Janice Dietert is a science editor and writer with Performance Plus Consulting.

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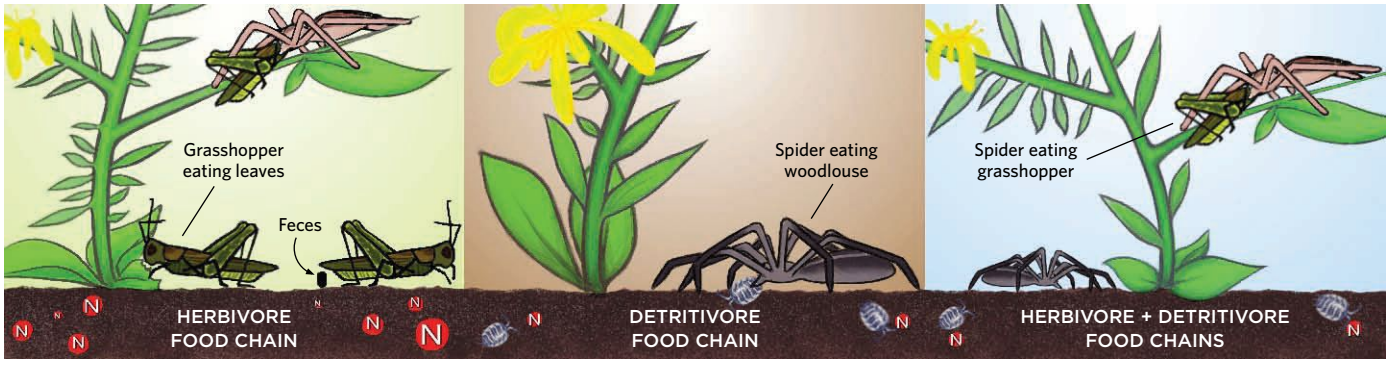
The Literature

ECOLOGY

1 + 1 = 1



NOT ADDING UP: Three experimental ecosystems demonstrate the effects of herbivore (left), detritivore (middle), and combination (right) food chains on soil nitrogen levels (N). In the herbivore food chain, grasshoppers' feces elevate nitrogen levels over those in a plant-only control ecosystem (not shown). In the detritivore and combination food chains, nitrogen levels are the same as the control, suggesting the food chains interact to dampen the nitrogen-elevating effects of the herbivores.

PHEBE LI FOR *THE SCIENTIST*

THE PAPER

R.W. Buchkowski, O.J. Schmitz, "Detritivores ameliorate the enhancing effect of plant-based trophic cascades on N cycling in an old-field system," *Biology Letters*, doi:10.1098/rsbl.2014.1048, 2015.

Life on Earth may be carbon-based, but it wouldn't exist without nitrogen. Soil microbes transform nitrogen from the air and from decaying organic matter into forms of the element available to plants and, in turn, the animals that eat them.

Within their respective food chains, detritivores—dirt-dwelling invertebrates that feed on decaying matter—and herbivores have been shown to raise soil nitrogen levels. And although it stands to reason that interactions between these food webs might act synergistically on nitrogen levels, it was unknown what their combined impact might be.

To examine this question, graduate student Robert Buchkowski and his advisor Oswald Schmitz at Yale University set up 45 mesocosms: cage-covered pots one foot wide by three and a half feet tall that hold self-contained ecosystems. Each mesocosm contained soil and one of nine different permutations of herbivore and detritivore food chains: a plant-only control; two herbivore-based food chains including plants, plant-eating grasshoppers, and grasshopper-eating spiders; two detritus-based food chains of plants, detritus-eating woodlice, and woodlice-eating spiders; and four combinations of herbivore and detritivore food chains. The researchers left their simplified ecosystems untouched for 46 summer days, then measured the soil's nitrogen content.

At the end of the experiment, the herbivore food chain of spider, grasshopper, and plants increased the nitrogen content of the soil. The detritus-based food chains alone, on the other hand, did not appear to impact nitrogen levels (although the researchers suspect this could have been due to the experimental conditions). But what was striking was that the combinations of detritus and plant food chains also yielded no increases in soil nitrogen. The researchers concluded that the presence of the detritus-based food chain must have erased the nitrogen-adding impact of the plant-based food chain.

Buchkowski speculates that the ground-dwelling woodlice might be aggressively competing with the grasshoppers for space, forcing the grasshoppers to spend more time above the ground level and are thus more vulnerable to being eaten by spiders. The increased predation would therefore cut into the grasshoppers' impact on soil nitrogen through their herbivory. In the future, Buchkowski hopes to test whether this is the case. "We need to consider the interaction between species that are in these two different food chains," he says.

The real questions, says Richard Bardgett of the University of Manchester in the U.K., are whether these effects on nutrient cycling happen in different and more-complicated ecosystems in the wild and how the interactions among food chains alter nitrogen content on the ecosystem level.

"People very rarely look at these two food webs in unison," says Bardgett, who studies interactions between aboveground and belowground organisms. "Nutrient turnover in ecosystems is a very complex issue that's driven by not just decomposer organisms but also their interactions with the food webs aboveground." —Jenny Rood

DNA INTERFERER: Just a few millimeters long, this marine tunicate, *Oikopleura dioica*, has provided the first glimpse into DNA interference by an animal.

SUCK UP: Bacterial symbionts living in tropical sponges, like this giant barrel sponge, produce polyphosphate granules.

GENETICS & GENOMICS

Metazoans in the DNAi Club

THE PAPER

T. Omotezako et al., "DNA interference: DNA-induced gene silencing in the appendicularian *Oikopleura dioica*," *Proc R Soc B*, 282:20150435, 2015.

ACCIDENTAL DISCOVERY

Scientists often exploit the natural phenomenon of RNA interference (RNAi) to knock down specific genes in model organisms. Although much less common than RNAi, DNAi has been described in plants, ciliates, bacteria, and archaea. And now, thanks to an accidental finding by Tatsuya Omotezako of Osaka University, it appears that DNAi can also silence genes in a metazoan, specifically, the tiny tunicate *Oikopleura dioica*. "I introduced DNA fragments for another purpose," Omotezako explained in an e-mail, but instead he found a surprising phenotype—one he would have expected from RNAi.

AS GOOD AS RNAI

To validate the hunch that DNAi was responsible for the effect, Omotezako and his colleagues microinjected fragments of the widely conserved developmental gene *brachyury* into *O. dioica* oocytes. They found tail defects in developing larvae that were indistinguishable from those induced by RNAi. Injected double-stranded DNA also reduced levels of targeted mRNA transcripts and proteins, indicating that DNAi was operating in the animal.

FINDING THE MECHANISM

The chordate has nine homologs of the protein Argonaute, which mediates RNAi in multiple plant and animal species and DNAi in a bacterium. Edze Westra, who studies Argonaute proteins at the University of Exeter, says the next clear step is to knock down those homologs, if possible, to validate the biological relevance of DNAi in *O. dioica*.

A HANDY TOOL

"Preparing DNA fragments is much easier, faster, and less expensive than preparing [double-stranded] RNA for RNAi," says Omotezako. His lab is already taking advantage of DNAi to study *O. dioica* development while working to figure out the mechanism behind it. —Amanda Keener

MARINE BIOLOGY

Sponging Up Phosphorus

THE PAPER

F. Zhang et al., "Phosphorus sequestration in the form of polyphosphate by microbial symbionts in marine sponges," *PNAS*, 112:4381-86, 2015.

DYED SURPRISE

Fan Zhang, a graduate student in Russell Hill's lab at the University of Maryland Center for Environmental Science, was using microscopy to study how Caribbean coral reef sponges process nitrogen. But the sponges autofluoresced so brightly that their nitrogen-fixing bacterial symbionts were difficult to see. To detect the bacteria, Zhang applied a blue fluorescent stain called DAPI, but to his surprise, he saw something else: bright yellow dots.

BACTERIAL ORIGINS

An Internet search suggested that polyphosphate—chains of phosphate molecules—could be the cause, and indeed, with specific extraction methods and scanning electron microscopy, Zhang's team observed polyphosphate granules that accounted for up to 40 percent of the phosphorus in three sponge species. To find the source, the researchers cultured the symbiotic cyanobacteria, finding that they contained not only polyphosphate granules but the genes necessary to make them.

NUTRIENT NETWORK

Sponges were already known to provide carbon and nitrogen to the reef community. The symbiont-synthesized polyphosphate granules sequestered in the sponges now made it clear "that sponges are right at the center of cycling of phosphorus in coral reef ecosystems," Hill says.

SPONGE SINKS?

Sponges may serve as sinks that remove phosphorus from the ecosystem, says Fleur van Duyl of the Royal Netherlands Institute for Sea Research. This could explain why phosphorus is considered the limiting nutrient on some reefs, she adds. Filling in the remaining details of the sponge phosphorus cycle could help researchers predict what might happen to the nutrient balance on reefs as the climate changes and sponges become more prevalent there, Hill says. —Jenny Rood

HIROKI NISHIDA; IMAGE COURTESY OF ANDIA CHAVES-FONNEGRA

PROFILE

Sold on Symbiosis

A love of the ocean lured Nicole Dubilier into science; gutless sea worms and their nurturing bacterial symbionts keep her at the leading edge of marine microbiology.

BY ANNA AZVOLINSKY

Nicole Dubilier doesn't have fond memories of her high school science classes. "Unlike many scientists who say they loved to dissect frogs and insects, I was not interested in science when I was young," says Dubilier, director of the Symbiosis Department at the Max Planck Institute for Marine Microbiology in Bremen, Germany.

Dubilier grew up in Manhattan, where her exposure to nature was limited to Central Park. But, vacationing on Fire Island in the summer, she fell in love with the ocean and decided to become a marine biologist. "It wasn't so much the biology," she says. "There was absolutely nothing I found inspiring or interesting about biology class. It was my worst subject in school; it was about learning without understanding."

> "I am just really interested in how two species come together: Why are they associated? What is the benefit?"

Dubilier is unapologetic about her early science experience and emphasizes that an early and vivid interest in chemistry, physics, or biology is not a necessary prelude to a successful science career. "I actually don't think it's that important," she says.

Her love of the ocean and its marine inhabitants led Dubilier to pursue a PhD in marine biology at the University of Hamburg. After completing her doctoral studies, she was still not sure she had the passion and stamina required to be an independent researcher. Dubilier says she was jealous of colleagues who said they thought of project ideas in the shower. "I thought of everything else but my research!" But a postdoc year spent in the lab of Harvard professor Colleen Cavanaugh studying symbiosis in gutless marine oligochaetes—a type of worm—cemented her love for research. "It was the first time I started to work in depth on marine symbiosis, and this topic evoked a deep, deep interest that is emotionally right next to marine biology for me. I am just really interested in how two species come together: Why are they associated? What is the benefit? And why these two species and not another two? Simple questions, really."

Here, Dubilier talks about the research cruises that add elements of beauty and adventure to her work, how diving in Bermuda beats wading in freezing German waters, and how sheer persistence first landed her a coveted position at the Max Planck Institute.

DUBILIER DISCOVERS

Romantic notions. Growing up, ballet and the ocean were Dubilier's two loves. At age 14, she chose not to continue with ballet because it would have meant quitting formal schooling. But the sea continued to draw her. "I had this unrealistic concept that I would spend half of my day diving, one-third doing research, and then the rest with the beautiful men I imagined on Jacques Cousteau's ships! That was the concept of marine biology I had in my head."

Back and forth. After her parents divorced when she was 13, Dubilier and her siblings were moved by her mother, a native of Berlin, to Wiesbaden, Germany. Every summer, she came back to New York to visit her father and developed what she calls "trans-Atlantic schizophrenia." "A more positive way to put it is we had the best of both sides of the Atlantic," she says.

Starting from the bottom. After graduating high school, Dubilier worked at a marine station on Helgoland, Germany's only deep-sea island. "This was pivotal in my decision to pursue marine biology. Even though I had a menial job of cleaning fish tanks, there was something about the physical closeness of the ocean around me and working with marine organisms that inspired me. I got a basic, emotional satisfaction from it," she says, and she immersed herself in learning the Latin names of marine species and understanding their taxonomy.

Butt bacteria. Dubilier received a bachelor's degree in zoology from the University of Hamburg and then—always seeking travel opportunities—took a summer course in tropical marine ecology at a biological station in Bermuda. She went on to pursue a master's degree in the University of Hamburg laboratory of Olav Giere, who studied the biology and ecology of marine oligochaete worms. Giere suggested that Dubilier study how a marine oligochaete from mud flats off the coast of Germany lives at low oxygen and high sulfide concentrations. Then Dubilier discovered that long, filamentous bacteria grew on the tail end of these worms, and became really excited. "For no reason, really," she says, laughing. The observation turned into her first (single-author) publication. "Twenty years later I went back and looked at these funny bacteria [the worms] had on their tail ends, and we made fun of this and called it their butt bacteria." Those bacteria are similar to the ones she studies now, found on and in invertebrates living in hydrothermal vents.

Researchers discovered deep-sea hydrothermal vents in 1977 in the Pacific Ocean off the coast of the Galapagos Islands, just a few years before Dubilier became interested in chemosynthetic sym-

COURTESY OF NICOLE DUBILIER

NICOLE DUBILIER

Professor, Marine Biology, University of Bremen
Director, Symbiosis Department
Max Planck Institute for Marine Microbiology
Bremen, Germany

Greatest Hits

- Identified the first sulfate-reducing bacterial species as an obligate animal endosymbiont, as well as the first example of a symbiotic, syntrophic relationship that includes multiple symbionts within a marine host—two species, both living in *O. algarvensis*
- Conducted the first detailed metagenomic analysis of an endosymbiotic microbial community in a eukaryotic marine host—a gutless worm (*O. algarvensis*) associated with four bacterial species that provide the animal with energy and waste clearance
- Identified hydrogen as a third, previously unknown energy source for bacterium–animal symbioses, providing energy for mussels within deep-sea hydrothermal vents—the first new symbiotic chemical energy source discovered in 25 years
- Provided the first combined metaproteomic and metabolomic analysis examining a host with multiple symbionts—the gutless marine worm *O. algarvensis* and four of its bacterial symbionts. The study also identified carbon monoxide as a previously unknown energy source for marine invertebrate symbiosis.
- Organized the first Gordon conference on animal-microbe symbioses, which took place last month

biosis. "Suddenly there was a lot of interest in bacteria associated with invertebrates, and particularly those bacteria that use reduced sulfur compounds as an energy source, because the hydrothermal vent worms didn't have a mouth or a gut and were being fed by their symbiotic bacteria that use hydrogen sulfide as a source of energy."

Mucking around. For her graduate work at Hamburg, Dubilier spent a lot of time on the German coast, "freezing to death" she says. The silt and sand sediments off the coast exuded a rotten-egg smell from hydrogen sulfide, used by the bacteria living on the tail end of *Tubificoides benedii*, the inch-long marine oligochaete she studied for her PhD. Wading into the muddy water, Dubilier used a sieve to collect worms to bring back to the lab for analysis. "This was not the glorious image of diving I had envisioned. The sediments were so muddy that you could lose your rubber boots if you were not careful. The mud would end up in almost every opening in your body. That was where I swore that, after finishing my PhD, the next group of animals I would work on would live in warmer climates!"

DUBILIER DIVES IN

Molecular biology for dummies. After completing her PhD in 1992, Dubilier was still not convinced research was for her. "It was clear to me that if you are not absolutely dedicated to your research, you are going to be very miserable. I had realized that I could not continue in this profession without being dedicated and excited about it." To help her decide, Dubilier took a molecular biology course for marine biologists offered by the University of Southern California on Catalina Island. "It could have been molecular biology for dummies for all I knew about molecular biology! But it was taught by some of the best marine microbiologists who were just starting to use the newly developed molecular biology methods, including PCR."

Warmer climes. When Dubilier was still a grad student, she met Colleen Cavanaugh at a Woods Hole course, and the Harvard professor, who had been among the first to characterize hydrothermal vent symbioses, suggested postdoctoral work in her lab. There Dubilier sequenced the 16S RNA genes—used as phylogenetic markers—of bacteria found on gutless marine worms, concluding that the worms harbored two symbionts. The two-to-three-centimeter-long worms Dubilier characterized are found in coral reef sediments in tropical environments such as Belize, Bermuda, and Australia. "The fieldwork was much better than during my PhD. These trips alone were magnificent." With better tools, Dubilier's lab later identified an additional three symbionts in these worms.

Back to Germany. After her postdoc, Dubilier returned to Germany along with her husband, an orthopedic surgeon. In 1995, she had funding from both Harvard and the University of Hamburg and began to knock on the door of the Max Planck Institute. In 1996, the Molecular Ecology Group welcomed Dubilier as a postdoctoral fellow. "They finally broke down and gave me a contract," she says. "I was persistent to the point that they later told me they were worried I was going to be this super-annoying person once I arrived." Dubilier decided to continue to work on the gutless oligochaetes because no one else in the world of marine symbiosis was working on their molecular biology and ecology. "They were small and difficult to work with because everything was complicated, including that they had more than one symbiont. So it was great not to have any competition, although for the first 5 to 10 years, almost no one cited my papers."

Her own cheerleader. By 2001, Dubilier transitioned to a research associate position at the institute. That year, her laboratory published a paper describing the first example of two symbiotic bacteria that, rather than competing, provide each other with a growth advantage. In the gutless oligochaete *Olavius algarvensis,* the primary bacterial symbiont is a sulfur oxidizer that uses hydrogen sulfide as an energy source to fix carbon dioxide and provide organic carbon compounds to its host. Surprisingly, Dubilier could never measure hydrogen sulfide in the worm's environment. This puzzle was solved when she discovered a second symbiont in the worms, a sulfate reducer. The sulfate reducer produces the hydrogen sulfide used by the primary symbiont, which in turn produces oxidized sulfur compounds for the sulfate reducer.

To prove that the second symbiont was producing hydrogen sulfide, Dubilier collaborated with Max Planck colleagues Dirk de Beer and Tim Ferdelman to design a laboratory experiment in which the bacteria were incubated with radiolabeled sulfate, which was then converted to radiolabeled sulfide by the bacteria. Because sulfide precipitates on silver, the team stuck silver needles into live worms and then observed if radiolabeled sulfide had precipitated on the needles by exposing the needles to autoradiography film. *The New York Times* covered the work because Dubilier had written to one of paper's science writers. "I told him that my dad is a businessman and a golfer and does not understand my work and how cool would it be for him to read about it in *The New York Times?* And he said that had to be the best plug he had ever read."

Evolution driver. In 2006, Dubilier's lab produced the first detailed metagenomic analysis of a marine animal–microbe symbiotic community. The analysis demonstrated that four bacterial symbionts of *O. algarvensis* act as the energy source and excretory system for the invertebrate—the first example of such an adaptation among free-living marine animals. The work provided evidence of the worm's evolution to a gutless animal that relies solely on its symbiotic relationships for both digestion and waste functions.

An unexpected finding. On a 2005 research cruise to the Mid-Atlantic Ridge to study hydrothermal vents that emitted high concentrations of hydrogen, Dubilier thought that the bacteria living on mussels in these vents might be using hydrogen as an energy source. Up until that point, there were only two known energy sources for chemosynthetic symbioses in hydrothermal vents: hydrogen sulfide and methane. Both gases, as well as hydrogen, are produced by geochemical processes in the hot hydrothermal vents. "Naively, I was not aware of the negative results on this. Thomas Pape from the MARUM in Bremen, also on the cruise, conducted a 24-hour experiment to measure hydrogen concentrations of the symbiosis community that suggested that hydrogen indeed was being consumed by these symbiotic bacteria. It took another five years to really piece the data together," says Dubilier. The study, published in 2011, made the cover of *Nature*, and showed that hydrogen provides energy for symbioses in mussels and other hydrothermal vent animals. "Our work is at the crossroads of environmental microbiology and molecular microbiology. We use omics methods to form hypotheses but then validate them using physiology and imaging methods." Using metaproteomics, the lab has also found evidence that carbon monoxide is used as an energy molecule in marine invertebrate symbioses. The lab has since uncovered physiological evidence that this is the case, and this study has just been accepted for publication.

DUBILIER DIVULGES

Career-life balance. Dubilier says that she rarely mixes her work with her family. Her son and husband have come with her on several research expeditions to collect gutless worms in the Bahamas, Belize, and Australia, but research cruises—typically six weeks long—are restricted to scientists only. "I am not sure it's a good thing for meetings or excursions to bring your kids along. If you're there with a partner and kids at a meeting, you're usually rushing home to take care of kids, and you don't have time for some of the most important parts of a conference—the socializing. For myself, I question the integration of family with work, and whether it benefits your family or your work."

Improv. "I loved and still love fieldwork. It's completely independent work where you have to adjust to a situation immediately and to improvise. You need to be clear about what you want and to be able to deal with going out three times when things don't work, and then if you're lucky, on the fourth try, it might work out. I was also on the ocean, which was immensely satisfying. That mix of feeling that with a little forethought, afterthought, and engagement, that I could bring my work to a productive level, I always enjoyed."

An American at heart. After her postdoc at Harvard, Dubilier and her husband, who had completed a research fellowship in orthopedic surgery during their stay in Boston, made a two-body decision to return to Germany. "I would like to have stayed, and that was when I really realized that I am an American at heart. But my husband is a German at heart, and he made the point, which I agreed with, that he would rather live in Europe and vacation in the U.S. than the other way around." ■

Shawn Douglas: DNA Programmer

Assistant professor, Cellular and Molecular Pharmacology, University of California, San Francisco. Age: 34

BY ANDY EXTANCE

Shawn Douglas traces the origins of his current work fashioning nanoscale DNA robots to his boyhood backyard workshop. From age five on, he spent countless hours with his father building model cars, planes, and rockets. "My dad is the most patient and noncritical person I've ever met," Douglas says. "He never grabbed anything and did it for me. Without that I don't think I would be here."

Despite the ability he cultivated, Douglas's desire to study at Yale University surprised his parents, who didn't think they could afford it until they discovered need-based financial aid. After studying literature at Yale, Douglas eventually opted for computer science, but then realized he didn't want a tech job. "I don't like working on things that are going to happen anyway without me," he says. "I had a gut feeling I could take programming skills into an area that was not mature and make more impact."

This thinking steered Douglas to a biophysics PhD at Harvard Medical School, working with William Shih and George Church. In mid-2005, shortly after Douglas started working with the synthetic biologists, Shih heard Caltech's Paul Rothemund talk about creating self-assembling DNA shapes and patterns. "I came back to Boston very excited, and Shawn was the first one with whom I could share the news," Shih recalls.

At first Douglas tried to enhance and expand software Shih had written to design DNA sequences that would self-assemble. Then he built his own, called caDNAno, from scratch.[1] Douglas worked on caDNAno through early 2008, limiting his work in the wet lab and leaving Shih "frustrated with his priorities." But now Douglas considers creating the program his most important accomplishment, and his former Harvard labmate Hendrik Dietz, now at Technische Universität München in Germany, agrees. "With caDNAno we could really focus on churning out sequences for all kinds of DNA objects," Dietz says.

Dietz made a breakthrough of his own on DNA self-assembly protocols in late 2007, fuelling a friendly rivalry with Douglas, with each seeking to trump the other's group meeting presentations. That relationship helped spawn two 2009 papers pushing the field from two-dimensional to three-dimensional assembly[2] and enabling twisted and curved structures.[3]

Working with Church as a postdoc, Douglas used his nascent skills in synthetic biology to develop nanoscale DNA robots. In a 2012 paper, he described DNA boxes loaded with antibody fragments that could be opened with two different molecular keys, such as cell surface antigens.[4] "This really inspired people to dream about the therapeutic potential of information-processing nanorobots made out of DNA," stresses Shih.

In 2011, Douglas established an annual competition called BIOMOD to help students enter the molecular programming community. Every year, undergraduate teams compete to build the coolest stuff using biomolecules.

Since Douglas became an assistant professor at the University of California, San Francisco, in 2013, his lab has worked towards next-generation DNA nanorobots. "The *Science* paper from 2012 was a prototype," he emphasizes. "Something that will be used in patients will probably look very different." ■

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Tools for Drools

A general guide to collecting and processing saliva

KELLY RAE CHI

Healthy adults secrete roughly 1 to 1.5 liters of saliva each day from three major pairs of glands that are in close contact with the bloodstream. Mostly water, spit also contains electrolytes and proteins, including glycoproteins that form mucus, enzymes that break down food and bacteria, and secretory antibodies.

Besides maintaining our oral health, saliva harbors clues about our ancestry and whether we might be fighting an infection, are overstressed, or have a hormonal imbalance. In the future, the watery fluid may even provide a rapid screen for a recent heart attack or distinguish between bacterial and viral infections. Indeed, characterizing the oral microbiome, the collection of all of the microorganisms in a person's mouth, and its potential links to health and disease is its own emerging field. (See "The Body's Ecosystem," *The Scientist,* August 2014)

"In the past few years, there's been a lot more optimism about what can be done [using] saliva," says Paul Slowey, CEO of Oasis Diagnostics in Vancouver, Washington. That's because technologies are now sensitive enough to detect and quantify DNA, RNA, metabolites, and proteins generally present in saliva at levels anywhere from 100 to 1,400 times lower than their concentrations in blood. Many tools are on the market, tailored for collecting samples from people of all ages, and purification techniques are also improving.

Although saliva is easier and cheaper to collect, store, and transport than blood, studies of the fluid and the development of new saliva- and oral-based diagnostics are not necessarily simple. Planning around the analyte of interest should take into consideration the myriad collection tools now commercially available, as well as suitable processing, stabilization, and freezing strategies. These factors can affect your results, especially if you're aiming for quantification.

The Scientist talked to saliva experts about their cuspidor of tricks. Here's what they told us.

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SAMPLE TYPES

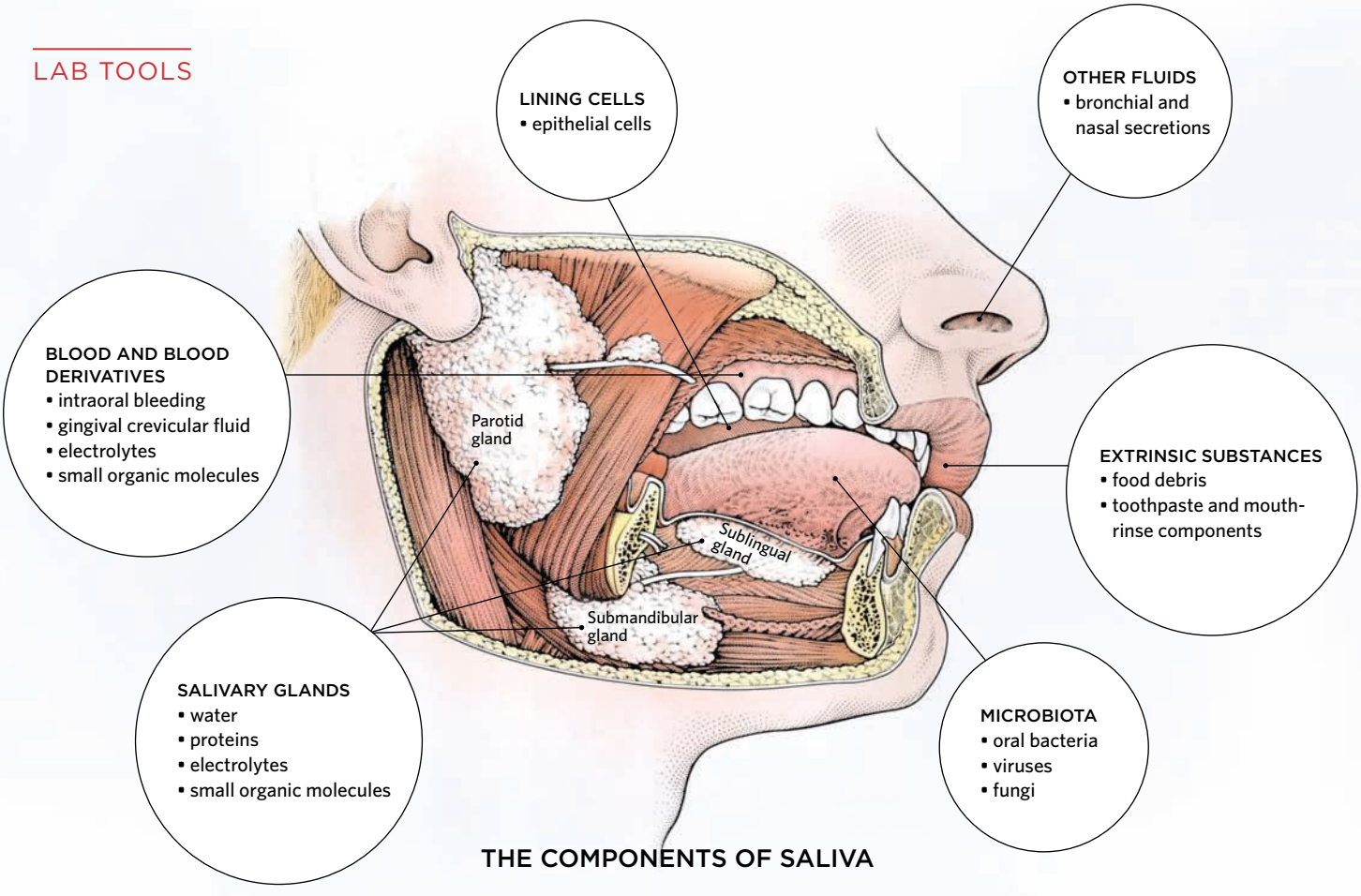
WHOLE-MOUTH SALIVA. This is salivary fluid and all the extras: cells from the mouth, nasal mucus, blood from any tiny mouth or gum sores, microbiota, and food debris. It is collected by spitting or drooling into a tube. To increase yield, you can stimulate saliva production mechanically by chewing on something that doesn't interfere with an assay, such as unflavored gum, wax, or silicon tubing. Even more saliva is produced by adding citric acid to gum, mouth rinses, or candy.

MUCOSAL TRANSUDATE AND GINGIVAL CREVICULAR FLUID. These fluids feed into spit but are generally better reflections of the blood constituents than whole-mouth saliva, says Daniel Malamud, professor of basic science and of craniofacial biology and director of the HIV/AIDS Research Program at the New York University College of Dentistry. In addition, the fluids are a good source of IgG antibodies, of which there are limited amounts in the mouth. For example, the FDA-approved OraQuick ADVANCE Rapid HIV-1/2 Antibody Test collects antibodies in the mucosal transudate—an ultrafiltrate of blood that passes through the mucosal surface of the cheek and palate—by swabbing the upper and lower gums using a flat, absorbent pad. Gingival crevicular fluid is contained in the crevice between tooth and gum. "You can collect that fluid on a paper point," Malamud says. "Although it's only a few microliters, people can do biochemistry on that."

WHAT CAN YOU ANALYZE?

DNA. Characterization of the DNA found in epithelial and white blood cells present in spit is one of the most active areas of saliva research. Investigators are probing saliva for genes associated with cystic fibrosis, autism, and other disorders. Oasis Diagnostics, for example, has validated the DNA•SAL collection device for research on a variety of genetic diseases and predispositions, such as Gaucher disease, thalassemias, and cardiovascular disease, Slowey says.

Many companies that sell collection tools also offer extraction kits that are customized for preserved saliva samples (see below). A protocol for extracting DNA from saliva for analysis with next-



THE COMPONENTS OF SALIVA

gen sequencing is available in *Journal of Visualized Experiments* (doi:10.3791/51697, 2014).

Although estimates vary, roughly 30 percent of the DNA in whole saliva comes from bacteria in the mouth. New England BioLabs offers a kit for boosting microbial DNA (NEBNext Microbiome DNA Enrichment Kit) in samples collected for oral microbiome studies.

RNA. RNA holds promise for probing more than oral disease. David Wong and his team at the University of California, Los Angeles, have recovered extracellular RNA from saliva, linking certain of those RNAs to the presence of oral and other cancers. Wong's method for profiling salivary microRNAs, a type of noncoding RNA in cell-free saliva, is available in *Methods Mol Biol,* 936:313-24, 2013. Wong has developed a collection tool (for nucleic acids and proteins) called RNAPro•SAL, which is sold by Oasis Diagnostics. It works by compressing an absorbent pad soaked in a proprietary medium to remove cells and other debris from the sample. Other researchers have developed methods for isolating RNA from the cells present in saliva (*Clin Chem,* 59:1118-22, 2013).

Much of the RNA in saliva comes from microbes in the mouth. A study by Wong's group found that an additional low-speed centrifugation step, and boosting sequencing depth, helps minimize interference from microbial RNA (*Clin Chem,* 58:1314-21, 2012).

PEPTIDES AND PROTEINS. About 30 percent of proteins found in blood are also present in saliva. The list includes cytokines, hormones, growth factors, and antibodies. Researchers are evaluating salivary proteins as potential markers of system inflammation, Alzheimer's, and diabetes. Proteins, along with RNA, are especially sensitive to degradation by enzymes and to different collection methods.

HOW DO YOU COLLECT IT?

There's no universal method for collecting saliva; it depends primarily on the age of the participant and your plans for analysis. But the way that you collect saliva, both the sampling location in the mouth and the time of day, can matter. "If there's differences in collection [and processing] it will make it challenging to compare results across different labs," Wong says. That's especially important if you are studying biomarkers that you hope will be clinically useful, he adds.

Passive drool (in which the participant allows saliva to collect on the floor of the mouth, then leans forward and dribbles into a tube) is considered the gold standard of collection methods across different analytes. However, it requires compliance from patients and might even be difficult for some adults.

Researchers whose study subjects are preterm and newborn babies collect saliva via suction, a normal procedure for a neonatologist, but one that is becoming more accessible to other clinician researchers with the development of Pedia•SAL, a device that connects with a pacifier but that doesn't require suction by the infant to work, says Jill Maron, associate professor at Tufts University School of Medicine. For older babies and toddlers, Salimetrics makes tools for collection that work even when participants are not cooperative.

Many collection tools work by absorbing saliva. It's crucial to find out whether a given tool has been validated for your molecule of interest. "We know that when you absorb a saliva sample through any kind of a foam or cotton product, you change that sample's integrity in ways that may or may not be known. Depending on what you're interested in testing, you may or may not be able to use that sample," says Douglas Granger, director of the Institute for Interdisciplinary Salivary Bioscience Research at Arizona State University and founder of Salimetrics.

In a 2013 study, for example, researchers compared protein profiles of saliva collected using passive drool, paraffin gum, or a commercially available cotton swab. The total protein concentrations the researchers obtained across the samples were similar; however, the protein profiles themselves were somewhat distinct, especially the profile of saliva collected via the swab (*Clin Chim Acta,* 419:42-46). It's best to choose the commercial collection device carefully, and to keep collection methods consistent across multicenter studies.

HOW DO YOU ENHANCE COLLECTION?

The passive drool method usually produces enough sample, but if you need to stimulate saliva flow, a recent study of meat eaters shows that the smell of microwaved bacon enhances saliva flow without interfering with hormone concentrations (*Clin Ther,* 37:515-22, 2015).

Besides a whiff of bacon, there are lozenges, mouth rinses, and chewing gums that you can use if you need to stimulate saliva production. Make sure you have established that the method you're interested in using for saliva stimulation doesn't interfere with your ability to detect your analyte of interest. Mouth rinses, for example, will dilute your sample, Malamud says.

Also, be warned: "There's a difference between nonstimulated saliva, which is just spitting it in a cup or collecting it with a tool like ours, versus stimulated saliva," Slowey says. Altering your flow rate may change the concentration of certain analytes more than others.

HOW DO YOU ACCOUNT FOR DIFFERENCES IN FLOW RATES?

Besides the stimulation of saliva, many different factors (such as age, exercise, smoking habits, alcohol use, time of day, and overall oral health) affect the flow rates of saliva. Accounting for differences in individuals' saliva flow rates can be important if you're collecting quantitative data. Some researchers directly measure a person's saliva flow rate: the volume of saliva collected (measured using a pipette) divided by the time it took to produce the amount.

Another way to circumvent differences in flow rates is to use a device aimed at collecting a fixed amount of fluid. Vivek Shetty at UCLA and his collaborators developed such a device, which collects 100 µL, and are selling it through the Japanese company Nanbu Irika for $10 per device (or $280 for 30 devices).

Internal controls are also important when you're adjusting for differences in flow rates or variations in sample quality. Total protein concentration is one approach; a small panel of RNA markers is another. Check to see if anyone has measured your analyte of interest, and whether there are any validated references.

HOW DO YOU PRESERVE IT?

Saliva contains enzymes that can rapidly degrade your analyte. Some analytes, such as cortisol or melatonin, are more stable at room temperature than others. But in general, it's a good idea to chill saliva in the fridge immediately after collection, and "clean it up" before you freeze it, Slowey says.

The particulars of purification depend in part on whether your molecule resides inside or outside of the cell, but there are commercial extraction kits available for either option. For extracellular RNAs, researchers spin cells down and analyze or freeze only the supernatant. Researchers studying human DNA, on the other hand, use the pellets of cells, lysing them and employing spin columns that bind genomic DNA and allow the rest to wash off.

Stabilizing buffers (sold by Norgen, Qiagen, and others) help neutralize enzymes that can degrade DNA and RNA. If you can't get samples to a freezer right away, then these reagents (such as Qiagen's RNAprotect Saliva Reagent) can help. DNA Genotek's Oragene family of kits for the collection of human DNA and RNA in saliva contains preservatives that allow researchers to store samples at room temperature.

For those researchers interested in microbial DNA and RNA, DNA Genotek's OMNIgene Discover kits for microbial DNA and RNA (OM-505) or DNA only (OM-501) are also ideal for long-term storage, notes David Speicher, a researcher at Griffith University in Queensland, Australia, who has studied extraction and storage methods.

Researchers are probing saliva for genes, microRNAs, and protein markers associated with a wide variety of diseases.

Proteins are the most labile of all the analytes, and DNA and RNA stabilizing solutions, many of which work by inhibiting enzymes, do not help preserve proteins. This is an active area of work, Speicher says. To solve the protein problem, Wong's group has come up with a stabilizing solution that is pre-added to RNAPro•SAL collection tubes (*Anal Chim Acta*, 722:63-69, 2012).

HOW LONG CAN YOU FREEZE IT?

A –80 °C freezer can preserve saliva for a long time, but for a given analyte, the limits are untested. Recent evidence from Speicher suggests a note of caution: salivary DNA with no stabilizing solution, stored at –80° for 14 months with no freeze-thaw cycles, is partially or completely degraded (*Diagn Microbiol Infect Dis,* 82:120-27, 2015).

As a general rule, aliquot your samples to minimize the freeze-thaw cycles. "We always suggest that samples are kept cold after they're collected and that the cold chain is managed very thoughtfully until they can be frozen," Granger says.

And if, after reading the many reviews now available on saliva collection and processing, you're still lost, then pick up the phone and call someone who's been doing what you aim to do. "Most scientists are more than happy to talk," Malamud says. ■

Breaking Down Barriers

Finding and recruiting diverse populations for clinical studies

BY CARINA STORRS

Surveys of clinical research tell a bleak tale about diversity in study populations. A review of cancer treatment trials published between 2001 and 2010 reported that 80 percent of participants were white and 60 percent were male (*Cancer,* 119:2956-63, 2013). Another survey found that less than 5 percent of NIH-funded studies of respiratory diseases in the last 20 years included minority (nonwhite) participants (*Am J Respir Crit Care Med,* 191:514-21, 2015).

This lack of diversity means that many questions go unanswered about the benefits and risks of drugs in minorities, women, and the elderly—groups that are typically underrepresented. What's more, different ethnic groups have different propensities toward certain diseases—Hispanic people are more likely than whites to be diagnosed with diabetes, for example—making the study of treatments for these groups even more important.

Enrolling the necessary clinical trial participants from any demographic can be a challenge (see "Clinical Matchmaker," *The Scientist,* June 2015), but there are special barriers to recruiting underrepresented groups. Minority groups often harbor a general distrust of the medical community, dating back to the infamous Tuskegee syphilis trial with African American men in the 1930s. Practicalities also stand in the way: time commitment and transportation to the study site can be particularly challenging for women and the elderly, respectively.

Researchers are becoming increasingly aware of this problem, but "there is clearly a long way to go" to fix it, says Romana Hasnain-Wynia, program director for addressing disparities at the Patient-Centered Outcomes Research Institute (PCORI), a nongovernmental organization that supports and communicates research, including research on health disparities, to patients. On the bright side, however, "there are more tools and resources for working with disparate populations than there were even 10 years ago," she says.

Here, *The Scientist* takes stock of resources that aim to help researchers increase diversity in clinical research.

Finding study participants in the general population

A growing number of Web-based tools can help clinical researchers connect with underrepresented groups. One example is the Clinical Trial Recruitment Center (CTRC), which is hosted by the Clinical Trial Engagement Network (CTEN). CTEN is a set of tools for researchers that was launched in March 2014 by the National Minority Quality Forum (NMQF), a nonprofit organization that aims to improve health care for minority populations.

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CTRC is building a nationwide registry of patients and healthy individuals interested in participating in clinical trials. Prospective trial participants sign up through the I'm In website, which was created by the NMQF and the Pharmaceutical Research and Manufacturers of America (PhRMA) to increase awareness about the importance of diversity in clinical trials. As of April 2015, the registry included about 200 volunteers, but NMQF is working to increase numbers with a website redesigned for user-friendliness and by holding events to promote awareness. One-time access to search the registry generally costs $250; ongoing access runs from $50,000 to $250,000, depending on the study budget.

Researchers can use CTRC's "Map Datasets" function to view the location of registered volunteers across the U.S. Each volunteer is represented by a purple dot; clicking on a dot opens a window with an individual's nonidentifiable information: unique CTRC ID number, gender, race, zip code, and the medical condition(s) that she would like to help researchers study. (A feature that allows searching by specific demographics is in the works.) Investigators provide the ID numbers of volunteers that fit their study to NMQF, and NMQF provides those volunteers with information about the trial and the option to enroll. Researchers can also search the CTRC Investigator Registry

for other network users who may want to collaborate and who focus on underrepresented groups.

"We are helping the investigator understand the full landscape of where patients are and where potential investigators might be found," says Gary Puckrein, president and founder of NMQF. The map also displays hospitals that focus on minority populations, sites of other clinical trials, and the prevalence of certain medical conditions in each zip code.

ResearchMatch is another tool that lets investigators working at member research institutions, of which there are currently 105, look through its registry of de-identified patients and healthy individuals (*Acad Med,* 87:66-73, 2012). Researchers working at one of these institutions can sign up using their institution name and institutional e-mail address. This allows them to search the database for free by criteria such as age, race, and body mass index (BMI) range. ResearchMatch, which was developed at Vanderbilt University School of Medicine and launched in 2009, has been used by more than 2,600 researchers and has signed up more than 77,000 volunteers interested in participating in clinical studies or taking clinical surveys. Research institutions must be not-for-profit to join the ResearchMatch network.

Beyond such networks, some academic medical centers allow outside researchers to query their de-identified electronic medical records for patients who fit a certain profile, including age, race, and disease. Tips on how to query these databases are often available through clinical translational science offices. Many institutions have such offices, including all 62 that are funded through NIH Clinical and Translational Science Awards (CTSA).

Connecting with specific disease groups

Some disease groups work with researchers to help them connect with underrepresented patients. For example, the North American Research Committee on Multiple Sclerosis (NARCOMS), which was established in 1993, maintains the world's largest registry of adults diagnosed with MS who provide voluntary information about their age, sex, race, and details about their disease. Researchers can apply for recruitment assistance to have NARCOMS notify participants who meet demographic or other requirements for a study and solicit their participation. The Cystic Fibrosis Foundation (CFF) also collects age, sex, race, and other demographic and health data for people who have the disease. Investigators can apply for access to de-identified information about patients with particular demographics for observational studies, and can also connect with one of the more than 110 CFF-accredited care centers to find patients who meet their study criteria.

In the last two years, PCORI has been supporting the creation of disease-specific patient groups, called Patient-Powered Research Networks (PPRNs), which help advance health research by connecting members with clinical trials and communicating health information. There are currently 18 PPRNs across the U.S. focusing on conditions ranging from arthritis to Crohn's disease to sleep apnea. Joe Selby, the executive director of PCORI, encourages researchers to contact a network's principal investigator, listed on each network's page, to inquire about working with its patients. Some networks, such as the one for muscular dystrophy, are already connecting researchers and patients, but all should be ready to offer this assistance by the end of 2015.

Broadening recruitment strategies

Patient registries can hook up investigators with people who already want to participate in clinical research. But these registries do not always have enough participants in the right geographical areas, so researchers end up having to do their own outreach to inspire interest in their study—not to mention in the notion of clinical research itself.

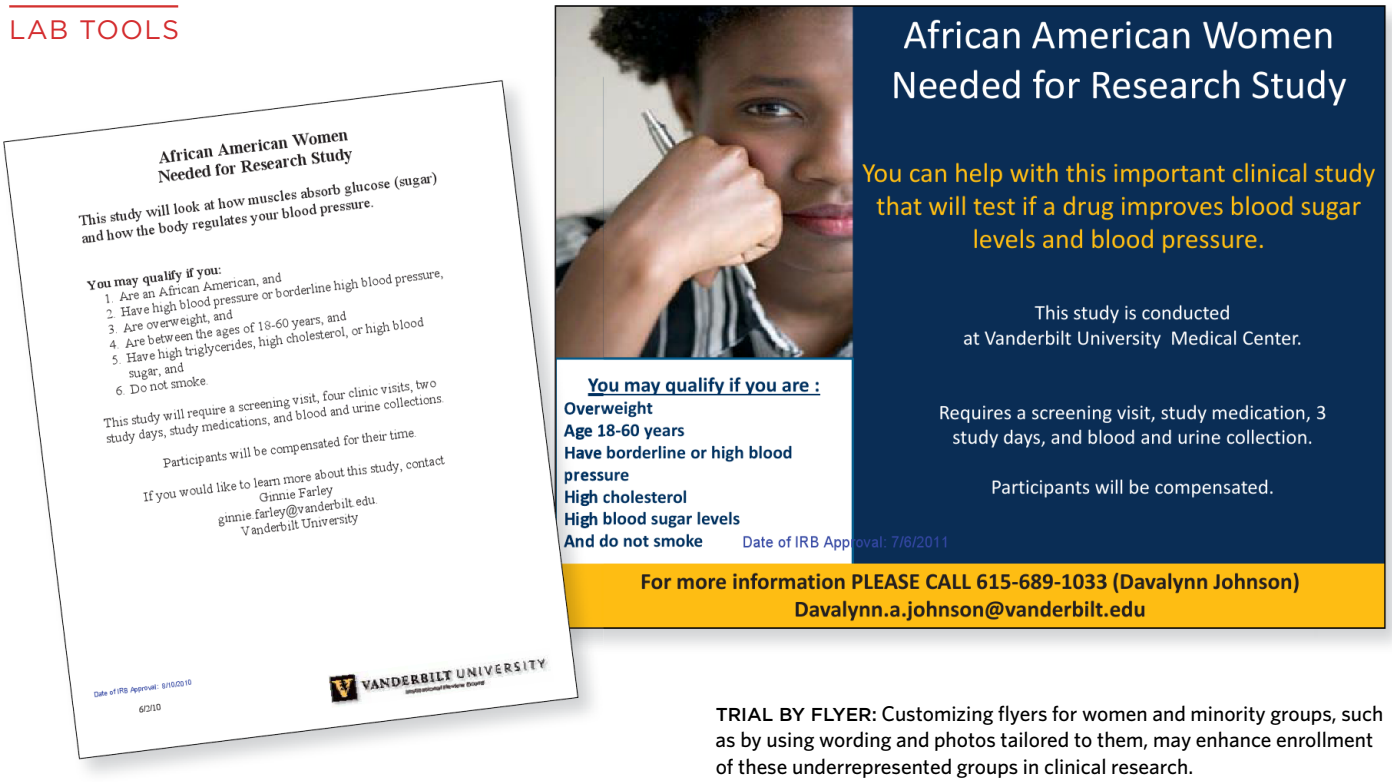
A comprehensive model where researchers are actively involved in improving community health can rebuild trust in black and Hispanic communities.

Stephen Thomas,
University of Maryland Center for Health Equity

Often, this involves creating recruitment materials such as flyers or other types of ads that are posted in hospitals, clinics, or elsewhere in the community. Thinking carefully about how those materials are designed is key to encouraging enrollment of underrepresented groups and to overcoming barriers such as distrust and concern about being treated fairly, says Consuelo Wilkins, executive director of the Meharry-Vanderbilt Alliance, which supports translational research at Vanderbilt University and Meharry Medical College.

In one case, Wilkins and her colleagues were able to boost enrollment of African American women in a study by redesigning the recruitment flyer to include a photo of an African American woman and colorful text blocks instead of a simple black-and-white layout (*Clin Transl Sci*, doi:10.1111/cts.12264, 2015). The language in the flyer or ad is also important; words such as "obese" and "qualify (for a study)" can have especially negative connotations among minority groups. For example, Wilkins says that African American men did not respond to one particular study about prostate cancer because the flyer prominently featured the word "profiling" (referring to genetic profiling), which some associated with racial profiling. Wilkins recommends that researchers ask members of their target community to look over recruitment materials to ensure the language is not insensitive.

Vanderbilt University's Institute for Clinical and Translational Research developed an approach for getting this type of feedback, called the Community Engagement Studio (*Acad*



TRIAL BY FLYER: Customizing flyers for women and minority groups, such as by using wording and photos tailored to them, may enhance enrollment of these underrepresented groups in clinical research.

Med, in press). Researchers spearheading a particular study hold a workshop for community stakeholders such as health-advocacy groups, members of neighborhood centers and churches, and patients to explain various aspects of the study including recruitment strategies. This type of effort makes sense for investigators or centers that are creating an ongoing research program around a population, Wilkins says. She and her colleagues have developed an online toolkit to help researchers implement this approach for their own studies.

Many of the clinical research offices at CTSA-funded institutions also help researchers design their flyers and ads to ensure the language is appropriate and easy to understand. Some offices, such as the University of North Carolina's TraCS Institute, have templates that researchers can start with. Recruitment specialists in these offices also advise about the best locations in the community for posting flyers or ads and help researchers connect with outlets for posting.

However, according to Stephen Thomas, director of the University of Maryland Center for Health Equity and a professor in the university's School of Public Health, this kind of marketing can only take researchers so far. "A comprehensive model where researchers are actively involved in improving community health can rebuild trust in black and Hispanic communities," Thomas says. His center partners with health groups that regularly go into the community—for example, to the center's network of 11 barbershops in Maryland and DC—where they offer basic health services such as blood pressure screening, smoking cessation assistance, and health insurance registration help. Investigators who collaborate with the center work with this same network, known as the Health Advocates In-Reach and Research (HAIR), to offer people the opportunity to participate in clinical studies.

Indeed, several studies have demonstrated that barbershops and beauty salons can help recruit community members into studies, such as prostate cancer screening (*Am J Prev Med,* 47:77-85, 2014). There is a "realization that barbershops and beauty salons have been underutilized as trusted venues to reach people," Thomas says.

The center's Maryland Community Research Advisory Board (MD-CRAB) is made up of community members who give feedback to researchers about recruitment strategies, study design, and other study aspects. Several other places have similar groups, such as the University of Pittsburgh and the Mayo Clinic in Rochester, Minnesota. The Maryland Center for Health Equity provides technical assistance to anyone interested in setting up a CRAB, Thomas says, adding that it is not difficult.

Funding research with a focus on diversity

PCORI can be a good source of funding for clinical studies that make an effort to include underrepresented groups. Although PCORI's main focus is on research that compares outcomes between two clinically acceptable treatments, it also prioritizes research that could reduce health disparities. "You get a lot of points for focusing your questions on minority or other underserved, understudied, or disparate communities," says Selby, executive director of PCORI. Researchers can apply for funding for individual studies through one of PCORI's targeted announcements in specific study areas or through a broad announcement in an area the researchers propose. Funding opportunities are available through at least 2018, Selby says. ■

COURTESY OF DR. CYNDYA SHIBAO

Staying Active in the Lab

Retiring as a professor, and even shutting down your own lab, doesn't necessarily mean quitting research.

BY JENNY ROOD

John Dowling never did a postdoc. In what was a fairly typical career transition in 1961, he went directly from Harvard graduate student to Harvard assistant professor, and, after a few institutional moves, eventually settled at the university as a full professor in 1971. (See "An Eye for Detail," *The Scientist*, October 2014.) Now retired at the age of 79, the vision researcher plans to make up for skipping what has become a critical part of every new scientist's career by joining the labs of his younger colleagues, neuroscientists Jeffrey Lichtman and Joshua Sanes, as a postdoctoral fellow. Dowling believes that older researchers can still be "quite creative and contribute substantially to the effort. . . . I always loved doing experiments and wanted to stay active the field."

Retirement has not been mandatory for professors in the U.S. since 1994, but many still choose to give up their teaching and administrative duties—and their salaries—while continuing to spend time in the lab. Some, like Dowling, choose to take a step down the academic ladder by pursuing post-retirement postdocs; others find themselves able to start or continue leading small labs. While doing research in retirement is unconventional, options and support systems for many professors contemplating their research future are out there and growing more plentiful. Some universities have even set up special associations for retired faculty members. Here, *The Scientist* speaks with a handful of researchers entering retirement but not quite ready to walk away from science altogether.

Preparing for retirement

For researchers who plan to retire, the first step is crafting an exit strategy. Dowling aimed to cut his lab in half by the time he was 70, and at 75, to shut it down entirely—a goal he finally reached in the summer of 2014, as he turned 79. Similarly, organic chemist Joseph Lambert, who retired five years ago after 45 years at Northwestern University in Illinois, stopped taking new graduate students five years before his planned retirement age of 70.

TRINITY UNIVERSITY, JENNIFER GOODRICH BALREIRA

CONTINUING TO MENTOR: Joseph Lambert, a retired researcher formerly of Northwestern University, discusses research results with Allison Levy, an undergraduate student at Trinity University, where Lambert continues research on a volunteer basis.

A second consideration is location. Many researchers, like Dowling, choose to stay at their own institution, and simply move down the hall to the lab of a colleague. Plant biochemist and photosynthesis expert Andre Jagendorf, who retired from Cornell University in 1996, didn't even have to go that far: he remained in his own longtime laboratory space as a member of Robert Turgeon's group, which moved in upon Jagendorf's retirement. "The standard model of retire, no longer teach, no longer draw a salary is quite viable at your home institution," Lambert says. "But moving to another place is a bit more unusual."

Knowing he wanted to move back to his hometown of San Antonio, Texas, where he had recently purchased his childhood home, Lambert spent his final years at Northwestern tailoring his research group to match a hoped-for position at local Trinity University. He shifted his lab to one rich in undergraduates and postdoctoral fellows to prepare for a similar composition at Trinity, which does not have graduate programs in chemistry. He also changed his research focus from wet-lab chemistry to a less resource-intensive side project he'd been pursuing on and off since the 1970s: investigating the chemical properties of amber. Meanwhile, he and then Trinity chemistry department

chair Steven Bachrach negotiated with the administration to set up a unique position for Lambert: research professor of chemistry. The nonsalaried role doesn't take up lab space or take away a hiring position from a younger colleague, but it still allows Lambert to mentor students and conduct research. He continues to publish three or four articles a year on the nuclear magnetic resonance spectroscopy of amber, for example, and has already written two books. "I'm a member of the Trinity staff, and I feel like I'm contributing to their objectives," he says.

Nancy Mills, who retired this year from the chemistry department at Trinity University, also knew she wanted to relocate after retirement, but she didn't know where exactly. So she used her last sabbatical, in 2008, to work in Mike Haley's group at the University of Oregon to test out the town of Eugene. She found it to be a good fit, so Mills and her husband began planning their move, and Mills began strategizing how she'd continue her research once outside the comfort of her Trinity lab. While her work had long relied on both computational and wet-lab experiments, she realized that the latter would be hard to conduct after her retirement, due to a lack of access to the necessary space and equipment. So she started focusing her last years at Trinity on experiments in the lab. "I began to take all the computational projects I was envisioning and just put those on the back burner," she says, saving them for her retirement.

Then, as luck would have it, Haley got in touch to tell Mills that the computational scientists in his group had graduated and to ask if she would be willing to fill that role. Mills jumped on the opportunity. Not only had she found a location for retirement, but her time in Haley's lab ended up landing her a job. "The connections I made on sabbatical meshed nicely with my goals for retirement," she says.

Finding funding

One of the advantages of integrating into a younger faculty member's lab—the retirement research approach taken by Jagendorf, Mills, and Dowling—is an ability to focus on the science. "He works more or less like a postdoc would, except he has his own office," Turgeon says of Jagendorf, who comes in five days a week to conduct his own research as well as help out with washing dishes or running the high-performance liquid chromatography machine. "You can work in the lab and do things you enjoy, but you're not faced with a lot of bureaucracy or teaching."

> **One can do wonders when you say, "Oh, by the way, I don't want any money, all I need is an office."**
>
> —Joseph Lambert, Trinity University

It can also simplify the funding situation. Most late-career postdocs work on a volunteer basis and don't draw a salary, and the same is true of Lambert, even though he is running his own group at Trinity. "One can do wonders when you say, 'Oh, by the way, I don't want any money,'" Lambert jokes. "All I need is an office."

"It's a lot easier," agrees Jagendorf, who is also not paid. "I don't have to write grant applications; I don't have to worry about salaries; just work."

For those in charge of their own projects, however, funding can be an issue. "The main problem is that the granting agencies don't look kindly on old people," says Ludwig Brand, 83, who retired from Johns Hopkins University in 2012 after 50 years as a professor of biochemistry there. Moreover, even those supported by their hosts' funding can encounter problems obtaining research materials, as employees not specifically listed on grants sometimes cannot order their own supplies.

For this reason, Lambert negotiated his position at Trinity to be on the tenure track, allowing him to apply for grants, such as one he has received from the Dreyfus Foundation. (In two years, at age 77, he will be up for tenure for the second time.) At Johns Hopkins, Brand has another option: the Johns Hopkins Academy. Established the year Brand retired, the academy provides retired Johns Hopkins faculty with access to facilities, including offices if their home departments don't have space, and a few thousand dollars a year to enable them to continue their research, travel to conferences, and organize symposia.

"The academy is a win-win situation . . . both for the university and the faculty," says Brand, who in 2013 published a paper on protein dynamics and

DON HAMERMAN/TRINITY UNIVERSITY

MILLS'S MILIEU: Nancy Mills brainstorms with members of her Trinity University lab before retiring to Eugene, Oregon, where she remains active in research as a part of Mike Haley's group.

COURTESY OF LUDWIG BRAND

MASTER NAVIGATOR: Circa 1992, Johns Hopkins University's Ludwig Brand, now retired but still active in research, gives a sailing lesson to graduate student Martha Brown on Middle River, Maryland.

has a second in press with the academy's support. He also continues to serve on graduate board exams, teach freshman seminars, and work with students and young faculty. "One advantage of not writing grants is you have a little more time to mentor," Brand says.

A handful of other institutions, such as Emory University and Arizona State University, have organizations known as "emeritus colleges" that provide intellectual programs for retired faculty. While some institutions may provide offices or small research grants, most are primarily focused on providing intellectual engagement for retired professors on a shoestring budget. Arizona State's emeritus college, for example, even runs an academic journal for its members. As of October 2013, there were eight emeritus colleges or equivalent institutions at American universities, and their ranks continue to grow.

Finding the right fit

Although Mills is nervous about retirement because it's a "one-way path" and you can't "unretire," she is excited that her future collaboration will continue to engage her mind. "It's potentially a godsend to be able to work with Mike Haley's group," Mills says. "Intellectual stimulation is harder when you work all by yourself."

Retired researchers can also be a boon to the younger faculty hosts. In addition to the fact that most retired professors work on a volunteer basis, contributing to the output of a lab while not requiring a salary, the wisdom of the older lab member can be a real benefit to the group, says Turgeon. "[Jagendorf] just has a lot of insight," he says. "He often has some pretty knowledgeable and inspirational things to say."

David Weiner, a pathologist at the University of Pennsylvania who hosted retired clinical dermatologist Henry Maguire as a full-time postdoc in his lab from 1997 to 2011, couldn't agree more. Earlier in his career, Maguire had worked with Martha Chase, one of the researchers who conducted the storied 1952 Hershey-Chase experiment that helped confirm DNA as the hereditary material of modern organisms. In retirement, Maguire's expertise was an invaluable asset to the group, Weiner says. "He always challenged us: What is the importance of this work? What is the point of doing this? . . . He made things kind of slow down, took you out of the rat race, and gave you time to think about things in an elegant and important way."

Maguire's experiences were also an inspiration to the next generation of scientists, Weiner adds. He recalls a group of students gathered around the 80-year-old Maguire as he slowly and methodically explained testing for delayed-type hypersensitivity, a procedure he had first championed half a century earlier to study inflammatory responses and compare in vivo immune reactions in the skin to a visual readout. "They will always remember how they learned how to do those experiments," Weiner says.

When looking for a lab to retire to, Lambert says it's all about personalities. "The most important thing is finding a friendly environment."

"Labs are like families," agrees Weiner. "Henry really contributed to that rich fabric and made it so much more wonderful." ■

The War Rages On

Conflict between science and religion continues, with effects on health, politics, and the environment.

BY JERRY A. COYNE

The battle between science and religion is regularly declared over, ended with an amicable truce. Accommodationists on both sides assure us that the disparate pursuits occupy nonoverlapping spheres of inquiry (science deals with the natural world; religion with meaning, morals, and values). After all, there are many religious scientists (two notables are evangelical Christian Francis Collins, director of the National Institutes of Health, and Brown University biologist Kenneth Miller, an observant Catholic), so how can there be possibly be a conflict?

But despite these claims, the dust hasn't settled. Why do 55 percent of Americans aver that "science and religion are often in conflict"? Why are less than 10 percent of all Americans agnostics or atheists, yet that proportion rises to 62 percent of all scientists at "elite" universities, and to 93 percent among members of the National Academy of Sciences? I consider these questions and more in my latest book, *Faith vs. Fact: Why Science and Religion Are Incompatible*.

My conclusion: the conflict between science and religion is deep, endemic, and unlikely to be resolved. For this conflict is one between faith and fact—a battle in the long-fought war between rationality and superstition.

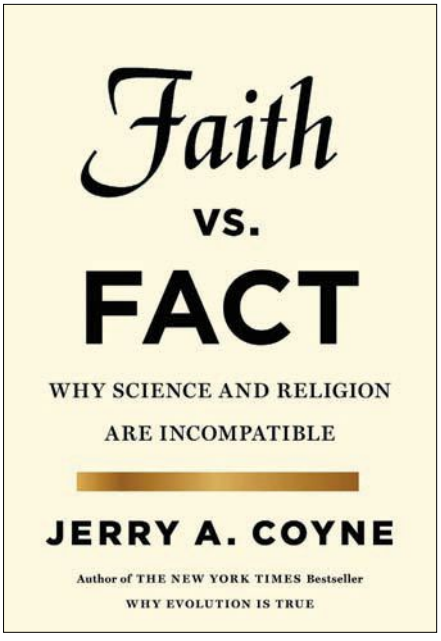
The friction exists because science and religion are both in the business of determining what is true in the universe—although religion has other concerns as well. Science's ambit is well known, but it's important to realize that religion also depends heavily on claims about what is true: claims about the existence, number, and nature of gods, what behavior one's god commands, the occurrence of miracles, and whether there are eternal souls, untrammeled free will, and afterlives.

But while science and religion both claim to discern what's true, only science has a system for weeding out what's false. In the end, that is the irreconcilable conflict between them. Science is not just a career or a body of facts, but, more important, a set of cognitive and practical tools designed to understand brute reality while overcoming the human desire to believe what we like or find emotionally satisfying. The tools include observing nature, peer review, independent replication of results, and above all, the hegemony of doubt and criticality. The best characterization of science I know came from physicist Richard Feynman: "The first principle is that you must not fool yourself—and you are the easiest person to fool. So you have to be very careful about that."

In contrast, religion has no way to adjudicate its truth claims, which rest on ancient scripture, revelation, dogma, and above all, faith: belief without strong evidence. The problem, of course, is that faith is no way to decide what's true. It is, à la Feynman, an institutionalized way of fooling yourself. The toolkit of science is—and will remain—the only way to discover what's real. Religion can offer communality and can buttress morality, but has no purchase on truth.

But even if science and religion are incompatible, what's the harm? Most of the damage comes from something inherent in many faiths: proselytizing. If you have a faith-based code of conduct attached to beliefs in absolute truths and eternal rewards and punishments, you're tempted to impose those truths on others. The most obvious subjects are children, who are usually indoctrinated with their parents' brand of faith. That can cause real physical harm: 43 of 50 US states, for instance, have codified legal protections for parents who harm their sick children by rejecting science-based medicine in favor of faith healing. Forty-eight of our 50 states allow religious exemptions from vaccination. The results are predictable: children needlessly become sick, and some die. And we in America are familiar with religious incursion into the public sphere, such as the persistence of creationism in schools.

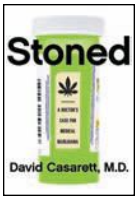
In the end, in both science and everyday life, it's always good policy to hold your beliefs with a tenacity proportional to the evidence supporting them. That is the foundation of science and the opposite of religion. As the philosopher Walter Kauffman noted, "Belief without evidence is not a virtue, but opens the floodgates to every form of superstition, prejudice, and madness." ■



Viking, May 2015

Jerry A. Coyne is a professor of ecology and evolution at the University of Chicago. His 2009 best-seller, Why Evolution Is True, *was one of* Newsweek*'s "50 Books for Our Time." Read an excerpt of* Faith vs. Fact: Why Science and Religion are Incompatible *at the-scientist.com.*

Stoned:
A Doctor's Case for Medical Marijuana
David Casarett
Current, July 2015



The science (or lack thereof) behind the use of medical marijuana is a hotly debated topic in scientific circles these days. As more states legalize marijuana for therapeutic or recreational use, it's never been more urgent to fully understand the health effects of the drug, which the federal government still considers a compound that is devoid of any medicinal virtue. Such is the quest that propels University of Pennsylvania researcher/physician David Casarett in *Stoned*.

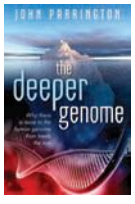
With a flair for narration, Casarett seeks clarity on the issue of medical marijuana, traveling around the world to labs, clinics, and patients' homes. The author even experiments with different marijuana-laced products using himself as subject. Although Casarett clearly favors the use of medical marijuana, *Stoned* gives a reasonably balanced account of the benefits and risks of marijuana as far as research has been able to determine. Uplifting and heartrending in turns, the book stays in a narrative groove that makes reading it as pleasurable as it is informative and thought-provoking.

Anxious: Using the Brain to Understand and Treat Fear and Anxiety
Joseph LeDoux
Viking, July 2015

Anxiety is on the rise. Nearly 20 percent of Americans experience some form of anxiety disorder. And the key to understanding and stemming the modern tide of agita may lie deep in the very brains from which it emanates, according to New York University neuroscientist Joseph LeDoux in his new book, *Anxious*.

By considering anxiety in tandem with its evolutionary partner, fear, researchers and physicians can develop a better understanding of the conditions under which anxiety stops being adaptive and starts being disruptive. By contextualizing anxiety disorders in the study of conscious vs. unconscious emotions in the human brain, LeDoux argues, science will glean crucial information about how to better treat the increasingly prevalent malady. Meditation, deep-brain stimulation, and gene therapy are all on the table, according to the author. "For therapy to have maximally beneficial and persistent effects, it may well be necessary to change both conscious and nonconscious memories that contribute to distress," LeDoux writes.

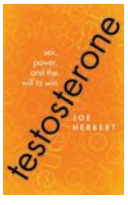
The Deeper Genome:
Why There is More to the Human Genome Than Meets the Eye
John Parrington
Oxford University Press, July 2015



By now everyone knows the tale of how Watson and Crick, Franklin and Wilkins worked together (or not) to discover the structure of DNA. The more contemporary story of the race to sequence the human genome is equally familiar. But these days, a new story about the genome is emerging: a more complicated one about how the genetic code is controlled. In *The Deeper Genome* Oxford University researcher John Parrington gives a nice overview of the regulatory elements, RNA varieties, and epigenetic changes that play crucial roles in translating genetic information into palpable, biological material and behaviors.

Because these insights represent a steadily building body of knowledge that continues to uncover complexities buried deep within a deceptively simple string of just four letters, they don't make for a sexy history-of-science tale. Nonetheless, understanding those processes is crucial to putting the genetic code to work in science, medicine, industry, and agriculture. Parrington's book serves as a good primer on the subtlety and complexity of the genome, especially the human genome, new facets of which emerge on a regular basis from labs around the world.

Testosterone:
Sex, Power, and the Will to Win
Joe Herbert
Oxford University Press, July 2015



It is the best of hormones, it is the worst of hormones. In *Testosterone*, Cambridge University neuroscientist Joe Herbert leads a guided tour through human evolution using the multifaceted hormone as his lens and vehicle. "Testosterone," he writes, "has only one function—to enable a male to reproduce." But in humans (and other animals), the potent chemical exerts its effect all over male bodies, including brains. The fuel for antler growth, aggression, and inventiveness, testosterone plays a crucial role in mammalian reproduction, was central to early human evolution, and continues to shape the social landscape of today's world.

Lest his perspective on testosterone be deemed too male-centric, Herbert explains that the chemical's effects reach far outside the male body. "The tendrils of its powerful actions creep into much of what we do," he writes. "By 'we' I do not simply mean men: the dramatic, but often unrecognized, influence it has on men is reflected in . . . the lives of women. Testosterone has important roles in women, too, . . . though these are less often recognized than those in men, and thus on another dimension of the formation and structure of the world we all live in."

—Bob Grant

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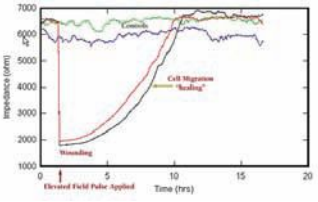

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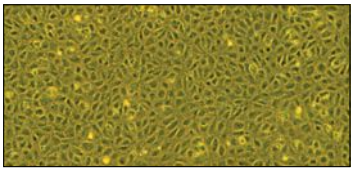

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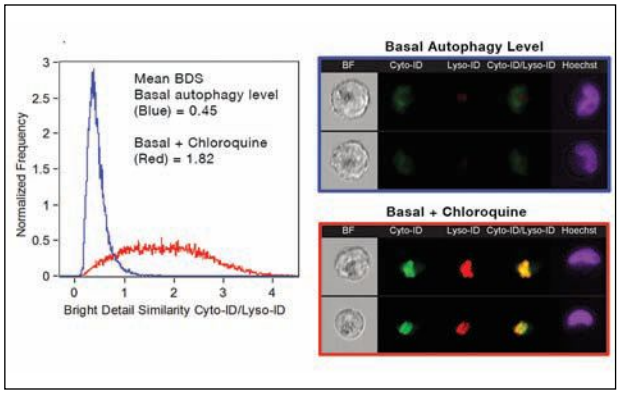

Autophagy is a process in which normal cellular components that accumulate during growth and differentiation are subjected to lysosomal degradation. Here we demonstrate an evaluation of autophagic flux by measuring co-localization of Cyto-ID® (autophagic vacuole marker) and Lyso-ID® (lysosomal marker) from Enzo Life Sciences using the Amnis® ImageStream®X Mark II (EMD Millipore) imaging flow cytometry platform.

Cells treated with chloroquine have decreased lysosomal autophagosome degradation. To demonstrate this, Jurkat cells were treated for 4 hours with chloroquine or left untreated. The cells were then labeled with Lyso-ID, Cyto-ID and Hoechst and imagery acquired on the ImageStreamX Mark II cytometer.

The Amnis IDEAS software was used to evaluate the autophagic flux by measuring co-localization using the Bright Detail Similarity (BDS) metric, as previously reported by Phadwal, *et al; Autophagy 8, 677* (2012). BDS compares the localization of the bright detail of two images, in this case the Cyto-ID and Lyso-ID markers. A high BDS score indicates that the two markers are co-localized in the cell; whereas a low BDS score indicates that they are not co-localized. The results show quantitatively that chloroquine inhibits autophagosome degradation in the lysosome.

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Half Mile Down, 1934

BY JENNY ROOD

On August 15, 1934, two tall, lanky men squeezed through the tiny hatch of a 57-inch-wide steel ball that was then dropped into the deep sea off the coast of Bermuda. Naturalist William Beebe and the orb's inventor, Otis Barton, were already familiar with the damp, uncomfortable quarters inside the sphere. Despite the danger of being suspended by a single cable, Beebe and Barton had made numerous dives during the previous four years observing marine life and mapping the contours of the underwater volcano beneath the islands from inside the capsule. On that summer day, however, the two would descend 3,028 feet, deeper than any human had been before or would be again for nearly 30 years.

In a 1926 article in *The New York Times*, Beebe, a noted American ornithologist-turned-marine biologist had outlined his wish to go deeper than the 60 feet his diving suit would allow. In response, Barton, an engineer studying natural history in graduate school at Columbia University, designed the bathysphere to protect against the high pressure of the ocean depths. By the 1930 summer research season, the 5,400-pound sphere made of inch-and-a-half-thick steel was ready for its inaugural dive. The first manned descent lasted 15 minutes and only reached 45 feet below the surface, but after testing unmanned descents to greater depths and working out some kinks, Beebe and Barton progressed to depths of a quarter of a mile.

On September 22, 1932, Beebe conducted the first-ever international live broadcast from the U.S., speaking to radio listeners from the bathysphere 2,200 feet down. Peering out of a single three-inch-thick window of fused quartz, Beebe described the strange, unknown life-forms he saw. In *Half Mile Down*, his book describing the bathysphere's journeys, he wrote of siphonophores "lovely as the finest lace" and "pteropods, or flying snails, each of which lived within a delicate, tissue shell, and flew through life with a pair of flapping, fleshy wings." Beebe's vivid descriptions inspired underwater explorers like Jacques Cousteau for decades to come, says Beebe biographer Carol Grant Gould.

BEEBE IN THE BATHYSPHERE: Naturalist William Beebe peers out of the access hatch of the bathysphere, a metal capsule he used to probe the contours of the ancient volcano under Bermuda and to study how light and marine life changed at ocean depths previously inaccessible to human explorers. "There's an amazing sense of wonder and awe when he's looking through the bathysphere window," says Carol Grant Gould, author of a biography of William Beebe. "He never, ever lost that."

COURTESY WILDLIFE CONSERVATION SOCIETY ARCHIVES. IMAGE © WCS.

These days, visitors can see the brilliant blue bathysphere, which belongs to the Wildlife Conservation Society, the successor of the New York Zoological Society that funded Beebe's first expeditions, at the entrance to the New York Aquarium, where it was placed last year in honor of the 80th anniversary of Beebe and Barton's record-setting dive.

"His meticulous and careful descriptions and the illustrations that came out of those descriptions were really valuable in terms of . . . deepening our realization of how diverse and odd a lot of that life was," says the Aquarium's vice president and director Jon Dohlin. "The idea of taking the bathysphere down a half mile . . . was so far ahead of its time." ■

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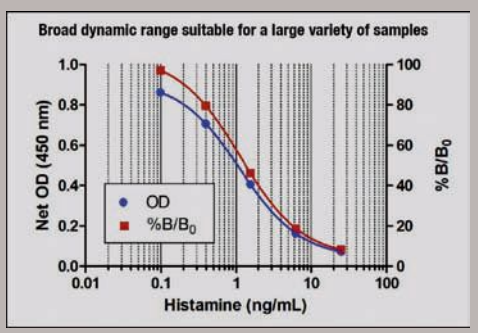

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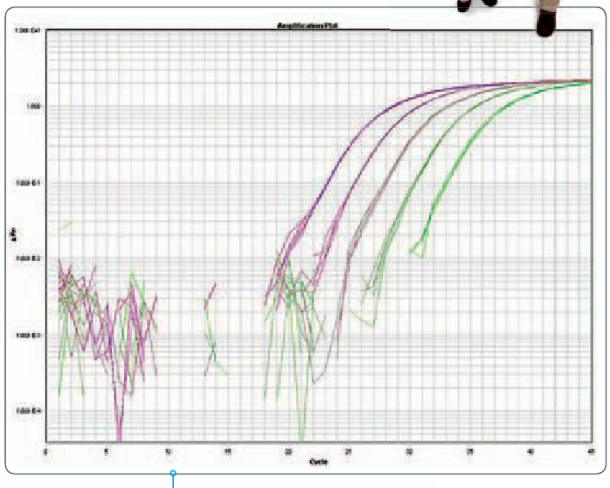

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