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EXPLORING LIFE, INSPIRING INNOVATION

MODERN FORENSICS

DRUG REPURPOSING

THE IMPRINTOME

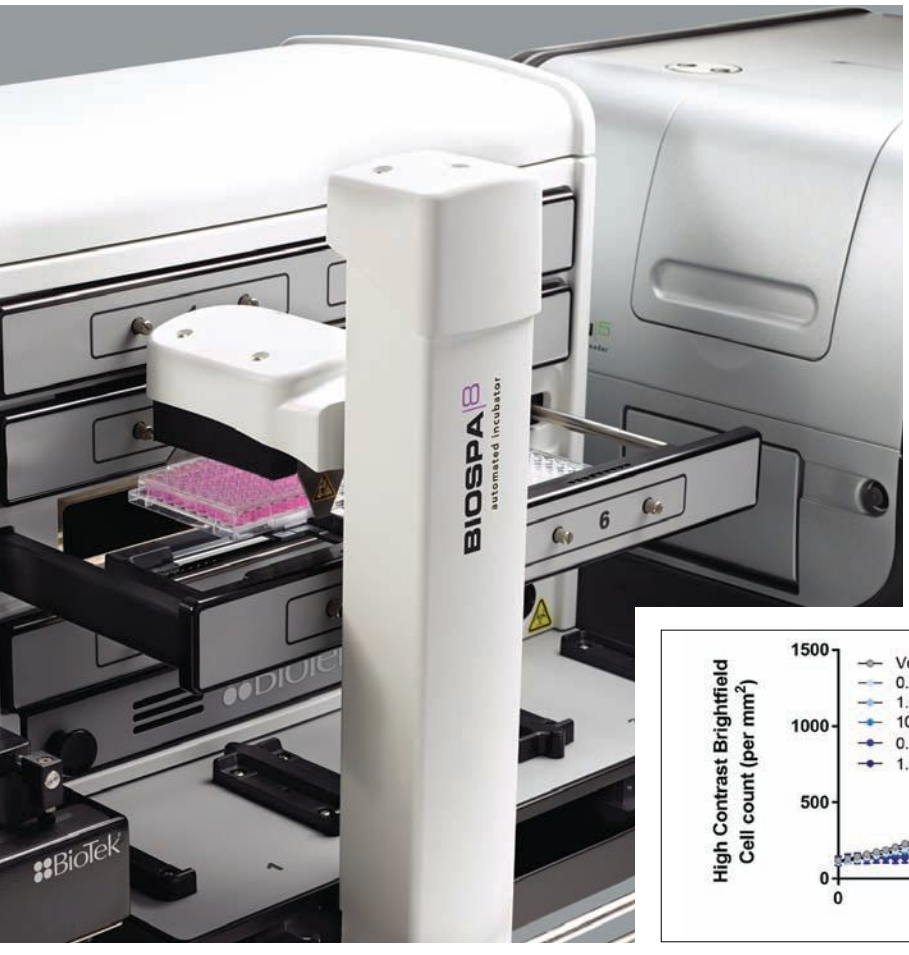
Driving Out Disease

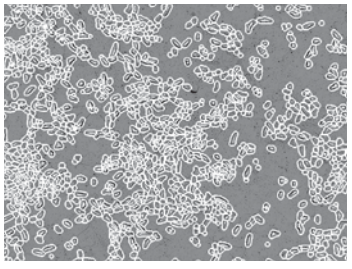
SCENARIOS FOR THE GENETIC MANIPULATION OF MOSQUITO VECTORS

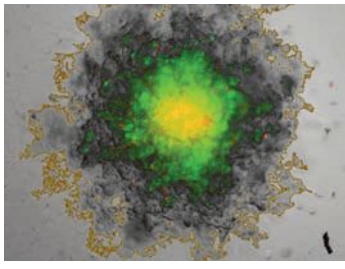
PLUS

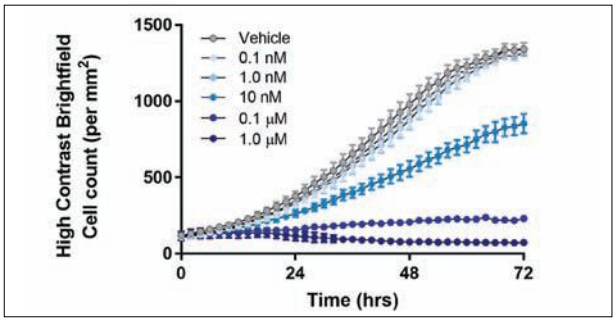
THE PhD GLUT

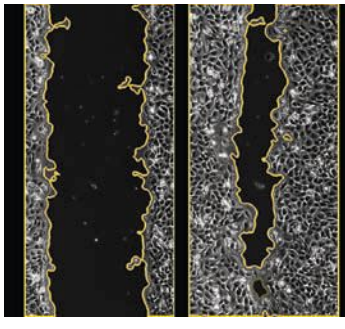
BIOSPA SYSTEM FOR AUTOMATED LIVE CELL ANALYSIS











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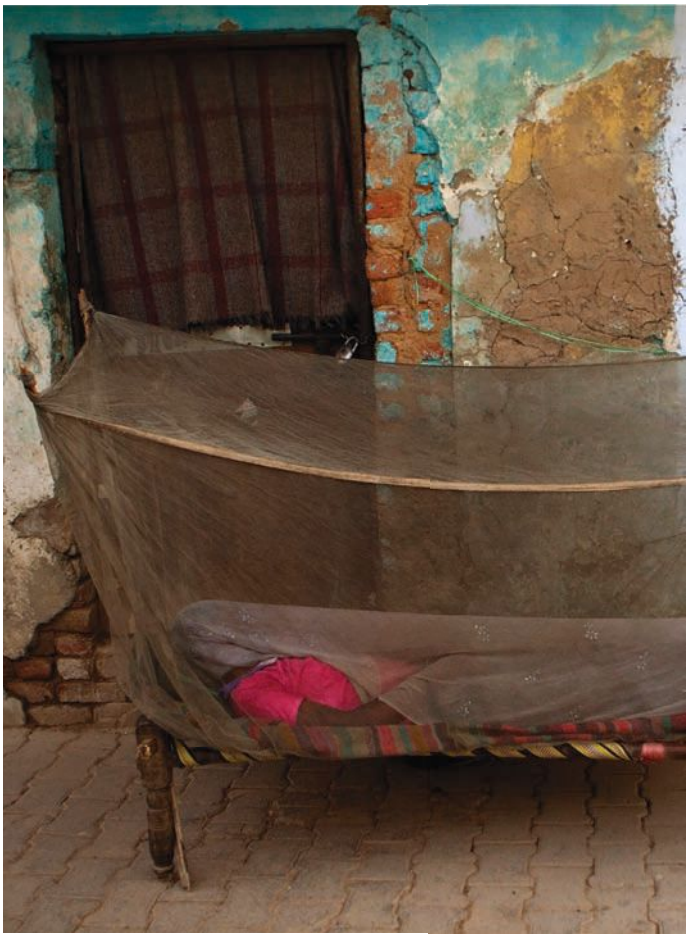

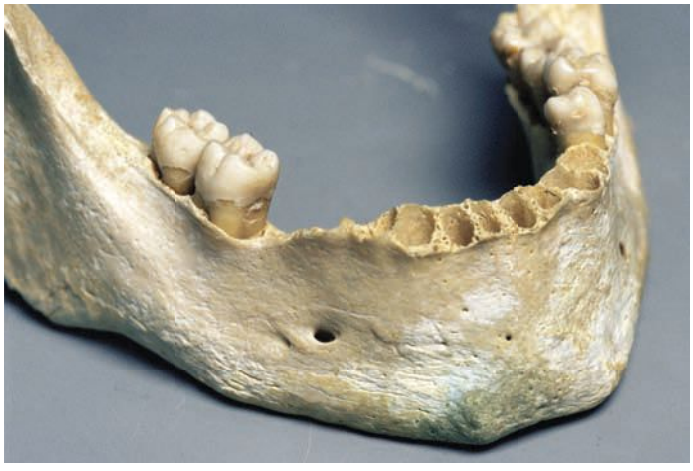

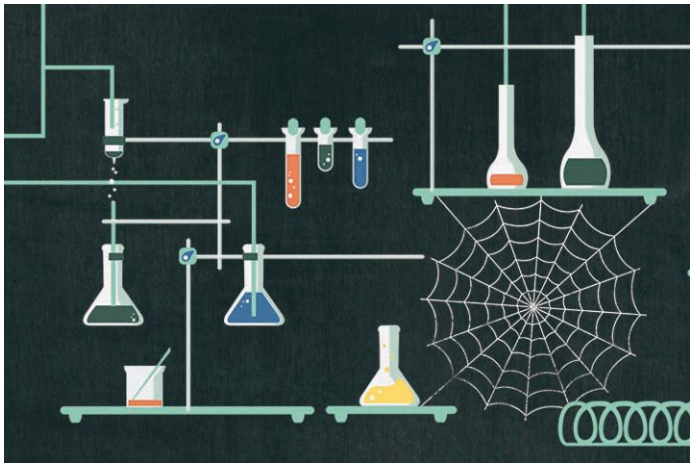

Features

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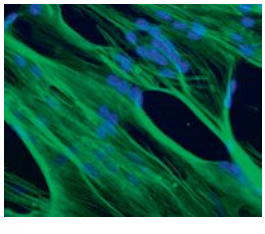

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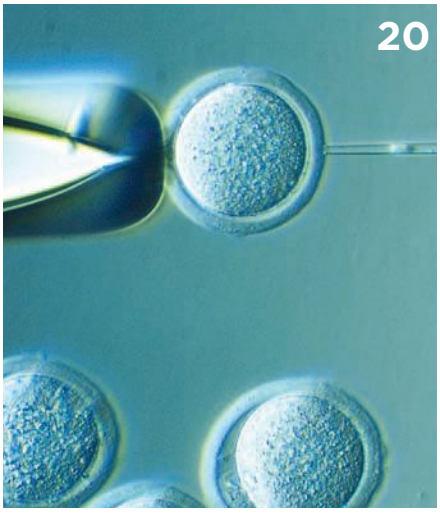

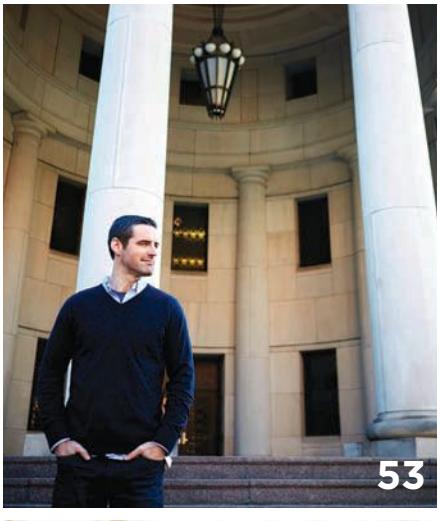

Online Contents

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Pharma Redo
Steve Braun of Cures Within Reach, a nonprofit focused on breathing new life into old medicines, describes the potential benefits of drug repurposing.

VIDEO
Island Adaptation
Anole lizards inhabiting the Caribbean islands illustrate some of the key principles of evolution.

SLIDE SHOW
Historical Hunts
See images from decades of fur trapping and hunting in the Amazon basin.

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

Coming in February

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

- RNA transfer to and from plants
- Plant epigenetics
- How cells sense and propagate physical forces
- Activating silent gene clusters for drug discovery
- Advancing precision therapies

AND MUCH MORE

HISTORICAL HUNTS: HERNONDINO CHAGAS AND CATHARINA VERGOLINO DIAS. VIRTUAL LIBRARY OF THE BRAZILIAN INSTITUTE OF GEOGRAPHY AND STATISTICS. PLANT: © ISTOCK.COM/COLOR_BRUSH

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Contributors

Andrea Crisanti is a professor of molecular parasitology at Imperial College London and editor-in-chief of *Pathogens and Global Health*. He got his start in immunology, primarily researching malaria. Crisanti graduated from Sapienza University of Rome with a medical degree in 1979, served two years as a medical officer in the Italian army airborne division, and then earned his PhD from the Italian National Research Council. At the time, the push to develop a malaria vaccine was progressing slowly, so Crisanti turned his attention to the vectors of the malaria parasite, mosquitos, a focus he believed could more quickly yield actionable results. Crisanti and his colleagues began tinkering with mosquito genomes using techniques developed in fruit flies, first producing mosquitos resistant to infection by the malaria pathogen and then exploring approaches such as gene drive. Without prior research in mosquitos to draw upon, "we had to invent everything from scratch," he says. Now, he predicts a gene drive designed to fight the spread of malaria will be ready for deployment in the field in a matter of years.

Tony Nolan is a senior research fellow at Imperial College London, where he has been developing techniques to stop the spread of malaria using gene drive in mosquitos since 2008. As an undergraduate at Imperial College, he was introduced to parasitology and drawn to the college's robust malaria research program. He undertook a PhD at Imperial with Crisanti that resulted in the first successful transformation of a mosquito germline. After that, Nolan and his colleagues went on to establish the fundamental techniques for engineering a gene drive, but the process of targeting a particular gene could take months on end. When the CRISPR gene-editing technique came onto the scene, the field accelerated by leaps and bounds and Nolan is now optimistic about its future. "In general, we have the major breakthroughs we need, we're just sorting out the details, the logistics, and getting regulatory approval," he says.

Crisanti and Nolan discuss the latest advances and applications in gene drive technology in "Driving Out Malaria" (page 24).

John Loike, a frequent contributor to *The Scientist*'s Critic at Large column, is director for special programs at Columbia University's Center for Bioethics. Loike spent the first stretch of his career as an immunologist after receiving a PhD in pharmacology from the Albert Einstein College of Medicine. In 1997, Loike ran some tests on samples from Dolly, the famously cloned sheep, and he began to notice that the press, despite making a stir over Dolly, did not always accurately portray the science involved. "It triggered my social responsibility, and I began writing about the ethical dimensions of technology," he says. After publishing articles about science ethics for several years, Loike was offered a position teaching a bioethics course at Columbia University. These days, he labors to convey to students the importance of contextualizing scientific decisions within their cultural backdrop. Every year, for example, he leads a small group of students on a two-week trip to Bangkok to observe cultural attitudes toward issues such as contraception and sex education.

In "The Mito-Mom Conundrum" (page 20), Loike and his coauthor Nancy Reame offer perspectives on mitochondrial replacement therapy.

Cordelia Fine is a professor of history and philosophy of science at the University of Melbourne in Australia. Fine studies the implications and often faulty scientific underpinnings of gender bias. She was appointed to Melbourne Business School in 2011 as an associate professor before taking her current position in January 2017. Fine studied psychology as an undergraduate at Oxford University and went on to earn a master's degree in criminology from Cambridge University and a PhD in psychology from University College London. She began investigating gender bias a decade ago, after noticing that neuroscientific research was frequently misinterpreted, misapplied, or overstated in the service of gender myths, which led to her 2010 book *Delusions of Gender*. Her research currently focuses on differences in risk-taking between genders and the consequences of gender essentialism for social attitudes.

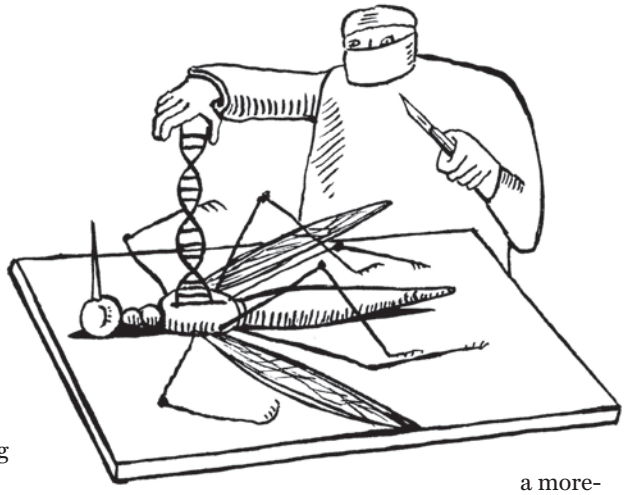
Cordelia Fine offers an essay based on her latest book, *Testosterone Rex: Myths of Sex, Science, and Society* (page 62).

COURTESY ANDREA CRISANTI; COURTESY TONY NOLAN; COURTESY JOHN LOIKE; FUTURE VINTAGE STUDIOS

Skeeter Science

From *Aedes* to *Wyeomyia*

BY MARY BETH ABERLIN



ANDRZEJ KRAUZE

At the end of October 2015, alarm bells began ringing: hundreds of pregnant women in northeastern Brazil were giving birth to babies with unusually small heads and an unusual pattern of very severe neurological damage. Epidemiologists observed an association of the condition with the mothers' infection by a mosquito-borne virus called Zika early in pregnancy, and researchers raced to demonstrate a causal relationship. The virus generated such buzz (and fear) that reports were omnipresent throughout 2016, both in the popular media and in the scientific literature. At one point, officials considered canceling the 2016 Olympic Games, which were eventually held in Rio de Janeiro in August with no cases of infection reported.

Last February, as infections continued to mount across Latin America and the Caribbean, the World Health Organization (WHO) declared Zika a "public health emergency of international concern." After visitors to countries in those regions returned home with infections, researchers confirmed that the virus could be transmitted sexually and was associated with an increased incidence of peripheral nerve damage manifested as muscle weakness and paralysis (Guillain-Barré syndrome). Then, in July, Zika-harboring mosquitoes appeared in the continental United States, with 184 locally acquired cases reported in Florida. Vigilant monitoring and heavy insecticide spraying seems to have halted active transmission there, but at the end of November, Texas became the second state to report a locally acquired case of Zika. A week earlier, on November 18, as infections had begun to wane both in the Southern Hemisphere and in the U.S., WHO changed Zika's classification from international emergency to one of "significant enduring public health challenge." Despite this status change, however, Zika—both the virus and the illness—still harbors many baffling mysteries and research continues at a furious pace.

As the epidemic evolved, life scientists reached across disciplines to immediately and openly share results that could shed light on why the virus can be so damaging and how its spread might be controlled. To search for clues on how to rein in the virus, the Zika genome was sequenced in short order, and mosquito researchers formed the *Aedes* Genome Working Group, which rushed to assemble a more-complete map of the DNA sequence of *A. aegypti,* a mosquito that transmits not only the Zika virus but also dengue and chikungunya.

While Zika hogged the headlines in 2016, a mosquito of another genus, *Anopheles,* continues to play its ages-old role as carrier of the parasite responsible for a scourge suffered by millions upon millions—malaria. Because no vaccine exists yet, researchers are investigating improved methods to manage the parasite's spread by tinkering with the mosquito itself. In this issue's cover story (page 24), Tony Nolan and Andrea Crisanti write about how knowledge of the mosquito genes responsible for key behaviors can inform the design of gene drives—the insertion of genetic elements into the insect's DNA that are selectively inherited and could result in a radical diminution of mosquito populations. But while gene drives "are species-specific, self-sustaining, and have the potential to be long-term and cost-effective," the authors write, "the decision to deploy a gene drive technology does not, and should not, lie with the scientists who design it."

Even though the value of a gene drive that wipes out the insect carrier of a disease that affects millions is obvious, implementation of such methods remains highly controversial, and a careful appraisal of the approach's ethical and ecological implications is imperative.

Other articles about controversial issues can be found in "The Mito-Mom Conundrum" (page 20), which lays out the ethical issues surrounding mitochondrial replacement therapy, and suggestions about how to deal with the glut of life science PhDs ("Reality Check," page 59).

Scientific research is no stranger to controversy. And when we dig it up, you can be sure we will share it with you. ■

Editor-in-Chief
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2016

TheScientist TOP 10 INNOVATIONS

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3. Pacific Biosciences
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4. Axion BioSystems
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5. Thermo Fisher Scientific
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6. NanoString Technologies
nCounter Vantage 3D Panels

7. 908 Devices
ZipChip

8. Horizon Discovery
Turbo GFP Tagged HAP1 Cells

9. Photometrics
Prime sCMOS Camera

10. Thermo Fisher Scientific
GeneArt Platinum Cas9 Nuclease

Congratulations to all of our 2016 winners!
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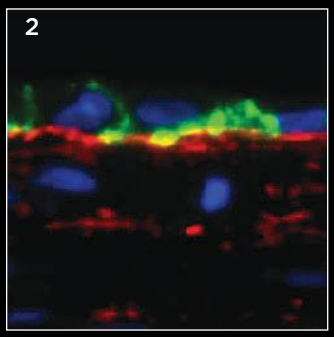

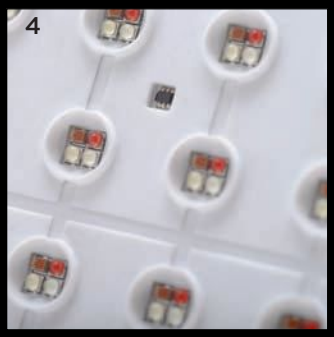

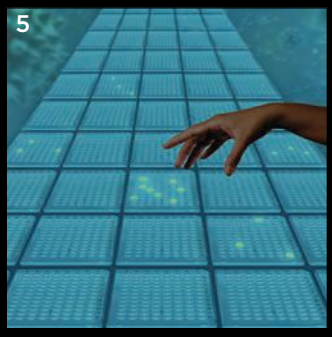

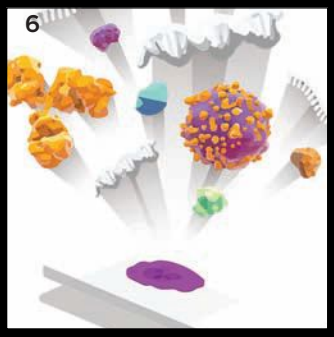

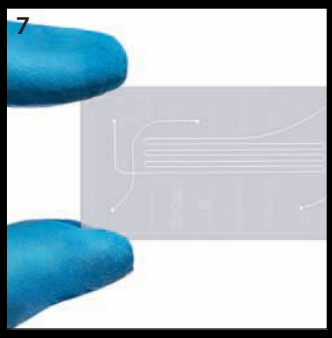

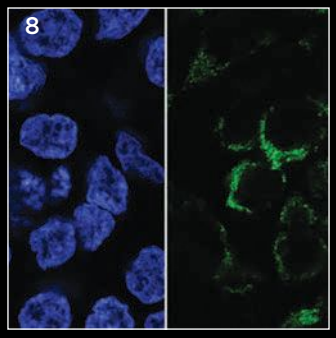

TS Webinars

COMING**SOON** | Precision Medicine: Learning Lessons from the Microbiome

The influence of gut microbiota on human health has been well documented, particularly in the case of metabolic disorders, such as type 1 and type 2 diabetes. In light of the strong association between the composition of one's microbiome and human health, researchers have begun to target these microbial populations. For a detailed look at the state of the microbiome and its role in precision medicine, *The Scientist* is bringing together a panel of experts to share their research and discuss the next steps. Attendees will have an opportunity to interact with the experts, ask questions, and seek advice on topics related to their research.

ROB KNIGHT, PhD
Professor, Departments of Pediatrics and Computer Science & Engineering
University of California, San Diego

JOSEPH F. PETROSINO, PhD
Associate Professor of Molecular Virology and Microbiology
Director, Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine
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The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- The human microbiome's influence on health
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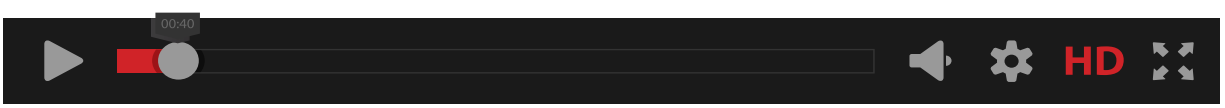
The tumor milieu is the site of complex interactions between immune cells, tumor cells, and the surrounding tissue. Innovative technologies and strategies are being employed to characterize these interactions to understand the role of the tumor microenvironment in tumorigenesis and metastasis. A panel of experts discusses advances in our understanding of the tumor microenvironment and how this knowledge is being harnessed to develop targeted therapies.



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Speaking of Science

I wouldn't say regulations are perfect but we want them to be based in science, and to come from really strong evidence. There may be a wholesale turning away from that science to this narrative of "regulations are bad."

—Biologist **Andrew Rosenberg,** director of the Center for Science and Democracy, on the prospects for evidence-based policymaking in the Trump Administration. (*The Atlantic*, December 2)

We're gearing up for a fight. We don't know exactly where that fight will be fought, because we haven't yet seen the policies that the Administration will push. But we're expecting that the First Amendment will be an area where there's some controversy. And if the speech of scientists is caught up in restrictions on speech, then we'll be there.

— ACLU attorney **Alex Abdo,** speaking to *The Scientist* about the organization's plans for defending the rights of scientists should the need arise during the Trump presidency (December 2)

To alter wild populations or bring whole species to extinction has major ethical, social, and environmental implications. Not only do we lack the knowledge and understanding to carry out such complex risk assessments, we don't even know what questions to ask. We need to pause and allow the scientific community, local communities, and society at large to debate and reflect, rather than simply allowing technology to lead us down this path. In the meantime, a moratorium is essential.

—**Ricarda Steinbrecher,** a molecular geneticist representing the Federation of German Scientists, advocating for a cessation of research on using gene drive to combat mosquito-borne diseases at the recent 2016 United Nations Convention on Biodiversity meeting in Mexico (December 5)

It is a very progressive way of promoting preprints and reasonable as a policy for a private foundation. But the hope would be that, over time, biologists grow to see the value and want to do this, just like the physicists do, rather than being forced into it. Being forced to do something is not necessarily the best way to win over someone's heart.

—**Ron Vale**, University of California, San Francisco, cell biologist and founder of the preprint advocacy group ASAPbio, on a recently announced decision by the NIH-funded research consortium the 4D Nucleome to mandate that its awardees post their manuscripts to online preprint servers prior to peer review (November 30)

Frankly, our country cannot reach its full potential if more than half of its people do not feel welcomed into the lab where their ideas, their talent, and their ambition is needed.

—Canadian Science Minister **Kirsty Duncan,** speaking in early November about the need to better represent women scientists in the Canada Research Chairs program. In December, the Canadian government announced a cohort of 203 new and renewed Canada Research Chairs that is 38 percent female, one of the largest proportions ever.

With the increased availability of legalized marijuana, there is an urgent need to understand the prevalence of its use and also its effects among older generations. The paucity of knowledge in this area constrains the care for a changing demographic of older adults with higher rates of substance use.

—**Benjamin Han,** a geriatrician at New York University's Langone Medical Center, on his recently published *Addiction* study that found a 71 percent increase in marijuana use among US adults aged 50 and older from 2006 to 2013 (December 2)

Notebook

JANUARY 2017

HIDE AND SEEK: Pelts of jaguar (*Panthera onca*), giant otter (*Pteronura brasiliensis*), neotropical otter (*Lontra longicaudis*), and ocelot (*Leopardus pardalis*) at a tannery in Manaus (Amazonas State, Brazil) during the 1950s

Hunting Through History

Wildlife ecologist Andre Antunes is the first to admit that he's not ideally suited to archival research. "My home is in the forest," he says wistfully. "It's very difficult for me to work in libraries." But in the late 2000s, while investigating the impact of commerce on Amazonian ecology over the 20th century, the Brazilian researcher had little choice but to dive into library and museum archives in search of data.

As part of a conservation management project in the lower Purús River, a tributary of the Amazon that rises in Peru and empties into the Amazon upstream of Manaus, Brazil, Antunes was reviewing the history of natural resource use in the region. The work entailed countless hours hunting down old documents—port registries, export records, and steamship cargo manifests—to get a picture of how forest products were traded throughout the region. But what started as a daunting task of rifling through piles of yellowing papers—"I was scared to start!" Antunes recalls—soon turned into the basis for a whole new scientific undertaking.

"I found a cargo manifest detailing numbers of [animal] hides of specific species, with trade date and locality," he says. "I searched for similar boxes and counted maybe 200 boxes with another 3,000 documents like that. Then I really glimpsed: there's an interesting research project here."

The Amazonian trade in furs and skins has a long history, kicking off around 1912 after international rubber prices collapsed and forced local enterprises to find alternative sources of income. In areas of the Amazon that lie in Brazil, authorities made several attempts to curtail the practice—including the Faunal Protection Law of 1967 and the ratification of the Convention on International Trade in Endangered

Species of Wild Fauna and Flora in 1975, which Antunes says essentially criminalized hunting in the region. Yet, trade persisted throughout most of the 20th century and is thought to have caused large-scale defaunation. As Antunes collated the archive records, he realized he had an unprecedented opportunity to analyze this impact on Amazonian species, using an untapped source of information.

The animal hide data alone is a "goldmine," says Richard Bodmer, president of nongovernmental organization (NGO) FundAmazonia and director of the Museum of Indigenous Amazonian Cultures in Iquitos, Peru. "It's a very important discovery in terms of data and in terms of understanding the longer trends that have happened in the Amazon."

To investigate the effects of hunting on populations of animals killed for their hides, Antunes knew he would need a careful statistical approach. But at the time, "I was a bit naive" about the methods required, he says. So, he enlisted the help of University of Auckland statistician Rachel Fewster, and in 2014, he moved to New Zealand for several months to work on the data.

The main challenge, the pair quickly realized, was extracting information from the various records Antunes had collected. "The problem is that they often just lump animal hides together as one category," says Fewster. "We wanted to distinguish between different types of species." In the end, Fewster says, she and Antunes "devised a whole new modelling framework" to compare data from two peak periods of hunting—the 1930s and the 1960s—that used the more detailed records to fill in gaps in "lumped" records, and accounted for improvements in data quality as the 20th century progressed.

The results weren't uplifting: records indicated that at least 23 million individual animals, representing 20 species of mammals and reptiles, were slaughtered for their hides between 1904 and 1969. The team's analysis also showed differences in how this mass hunt affected aquatic and terrestrial species. The researchers found that populations of aquatic species appeared to collapse, with 1960 harvests

At least 23 million individual animals, representing 20 species of mammals and reptiles, were slaughtered for their hides between 1904 and 1969.

of giant otters, black caimans, and manatees falling to around 10 percent of what they'd been three decades earlier. Terrestrial species, on the whole, fared much better. Although one species, the white-lipped peccary—a herd-living, hog-like animal—showed significant decline, others, including red brocket deer, showed higher harvests in 1960 than in 1930, suggesting substantial resilience to hunting pressure (*Science*, 2:e1600936, 2016).

"I wasn't expecting that," says Antunes of the aquatic-terrestrial split. "In the initial stages of research, I expected reproductive rate would be the most important factor." However, on reflection, he notes, the results are intuitive. Aquatic habitat accounts for just 5 percent to 12 percent of the central-western Brazilian Amazon across low- and high-water seasons, meaning aquatic species occupy a much smaller area than their terrestrial relatives. Amazonian waterways are also more accessible than terrestrial habitats to hunters, who traditionally live in settlements clustered along riverbanks.

This spatial arrangement of hunters probably explains a lot of the results, suggests the Wildlife Conservation Society's (WCS) executive vice president for conservation and science, John Robinson, although other factors, such as reproductive rate, are certainly important in determining a hunted species' resilience. "At its core, this is a really important paper," he says. "It pulls together the history of commercial hunting in Amazonia in a really powerful way."

Encouraged by the apparent resilience of the terrestrial species, Antunes, now a researcher with WCS, is taking part in workshops in Manaus this year to bring together government, NGOs, and locals to discuss decriminalizing subsistence hunting—a practice still integral to the livelihood of many indigenous and forest communities in the Brazilian Amazon. It's a move both he and Bodmer argue would allow better management of resources in the region, and could bring Brazil in line with other Amazonian countries, such as Peru, that already favor community-based conservation programs.

Antunes now spends much of his time working with indigenous and forest communities—a relationship that benefits both sides, he notes. "They really know the wildlife better than any biologist," he says. "They live there, day after day, whereas we are like visitors. There is no way to conserve the Amazon if we ignore these people."

As for going back into the archives? With the work he's doing now, "I'm not sure if I'll carry on my research in libraries," Antunes laughs. "If I have to learn about any historical documents, probably I'll have a look."

—Catherine Offord

Breaking the Bottleneck

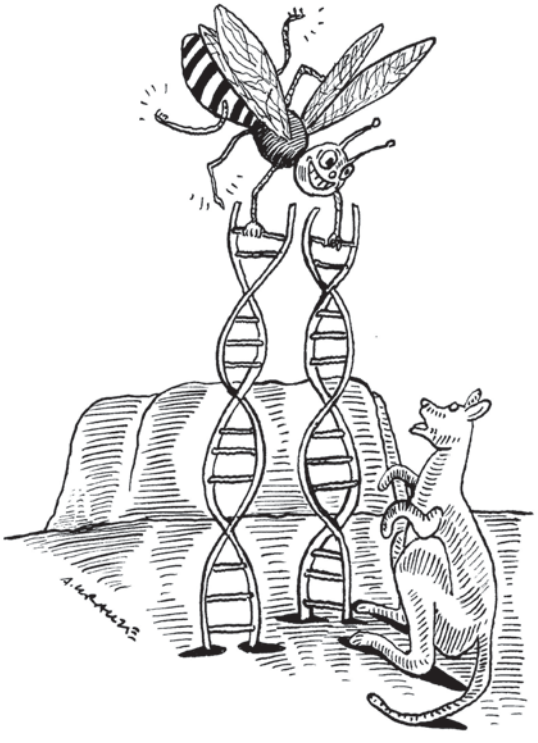
In the famous case of Darwin's finches, natural selection acts decisively, elevating a trait in the population, weeding it out, or simply ignoring it. But in actuality, natural selection can sometimes exert a more complicated influence. An unusual pattern of genetic selection turns out to be responsible for the rampant spread of the invasive Asian honeybee (*Apis cerana*) from Papua New Guinea into Australia, a pattern that Rosalyn Gloag of the University of Sydney and her colleagues have managed to decode.

The first Asian honeybees reached Australian shores in 2007, probably on the mast of a ship or stowed away in a shipping container. The bees made landfall in Cairns, a city on the continent's northeastern coast, and spread rapidly from there. Bruce White, a retired government apiculture and biosecurity specialist, says that Australian officials who responded to the invasion were ill equipped to contain the bees and did not realize how quickly colonies could disperse. Concerned beekeepers

alerted authorities to *A. cerana* hives that appeared in their yards, but the eradication effort proved futile.

Up until now, the success of *A. cerena* in Australia has posed something of a biological puzzle. Under the normal rules of evolution, the invasive bees should have been hamstrung by what's known as the founder effect—reduced genetic diversity when a population takes root from just a small number of individuals. Based on a preliminary study of neutral genetic markers by Gloag and her colleagues, *A. cerana* appears to have arrived in Cairns only once, with just one or a few colonies, meaning that no secondary arrivals infused the population with fresh genes to counteract the founder effect.

Low genetic diversity can have various ramifications, but in particular it should have tampered with sex determination in the bees. In *A. cerana*, diploid progeny develop as females while haploids develop as males. A single gene, *csd*, governs this system, with an important caveat: a larva that is diploid and homozygous at *csd* is inviable. In a large, diverse population with many *csd* alleles, the odds of getting two of the same allele are so low that *csd* homozygosity rarely causes problems. But in populations with only a few *csd* alleles, homozygotes are more common and can drag down the colony's reproductive output. Theoretically, this system should render bees highly susceptible to the founder effect, a prediction that has been borne out in other social insects with similar sex determination mechanisms.



To uncover how *A. cerana* circumvented the problem, the University of Sydney team sequenced the *csd* genes of bees every year after the invasion, using samples preserved by government eradication efforts. They also sampled *A. cerana* populations in the insect's native range in China. They found a stock of 22 different *csd* alleles among native bees, but only seven *csd* alleles among the invasive colonies. Only three of those alleles were common during the first few years after the invasion—a massive curtailing of genetic diversity that should have doomed the Asian honeybees in Australia.

In small populations, rare alleles are likely to disappear by random chance. In this case, *csd* alleles that were rare in the founding population, rather than disappearing, increased in frequency over the next several years, Gloag and her colleagues reported in *Nature Ecology and Evolution* (doi:10.1038/s41559-016-0011, 2016). The frequency of all seven alleles in the gene pool converged toward equilibrium.

The researchers were observing a phenomenon called negative frequency-dependent selection, which occurs when alleles become less and less advantageous the more common they are in a population. In the early invading population, individuals that carried rare *csd* alleles would almost always produce viable offspring, since the odds of a diploid larvae ending up with two identical rare alleles is close to zero. On the other hand, individuals with common alleles would frequently produce inviable homozygotes. As the bees proliferated in their new home, rare alleles spread through the population, pushing the gene pool toward an ideal equilibrium where every *csd* allele is equally common.

It's an unusual case in which evolution has favored diversity over any one particular gene. Most evolutionary trends are typically studied over enormous time scales, so Gloag did not expect to see negative frequency-dependent selection unfold over the course of just one decade. "What surprised us is how quickly the effects of that selection are felt," Gloag says. "Evolution can act fast—much faster than we often give it credit for."

Salvaging the seven *csd* alleles was the Asian honeybee's ticket to survival in Australia. By 2011, officials had shifted from an eradication program to a focus on containing and monitoring the invasive bees, says Gloag. "They were no longer under the illusion that they were going to get rid of them."

Asian honeybees are harmless invaders on their own, but they are known to carry parasitic mites, *Varroa destructor* and *Varroa jacobsoni*, that wreak havoc on European honeybee populations. *V. destructor* was constrained to Asian honeybees until it jumped hosts to the European variety and spread across the globe with disastrous impact—and scientists believe *V. jacobsoni* may be poised to jump hosts too.

For now, Australia is one of the few places still free of *V. destructor*. "We're pretty much the only holdout," Gloag says, "but we've seen the damage it's done to pollination services in other parts of the world, and we're very worried."

In the face of a looming ecological threat, Gloag finds a positive message in her team's discovery: If the invasive bees could recover from what seemed like terminal genetic loss, then perhaps endangered species that have sustained similar losses could demonstrate similar resilience. Australia's bee problem illustrates how species bounce back. "You can have healthy populations that have been through extreme bottlenecks," she says. "Nature finds a way."

—Ben Andrew Henry

Darwinian Lizards

It's not easy to snare a lizard. Evolutionary biologist Michele Johnson affixes a noose made of dental floss to a telescopic fishing rod to reach into the bushes and tree can-

ANDRZEJ KRAUZE

Anoles really are a good model system for lots of questions, from very small-scale molecular work all the way up to adaptive radiation.

—Jerry Husak, University of St. Thomas

opies where Caribbean anoles live. By the end of the summer field season, her students from Trinity University in San Antonio, Texas, develop a knack for it. "We almost always catch our lizards," says Johnson.

She doesn't just collect field measurements and observations; she's taken 30 different species of anoles back to her lab to analyze their physiology. Anoles have become a favorite model for evolutionary biologists because of their extraordinary diversity—there are more than 400 species in genus *Anolis*—and because of how they originally populated the Caribbean islands. The relative scarcity of mammals, snakes, or birds on the islands left many niches open for the lizards to occupy.

As anoles—which also inhabit Central and South America—reached individual islands, their populations diversified into island-specific forms that occupy certain niches. For example, each of the four largest islands in the Greater Antilles (Hispaniola, Cuba, Puerto Rico, and Jamaica) hosts one or more species that are green lizards hanging out in the lower canopies of trees, and another group of short-limbed, slow-moving reptiles that perch on twigs. These are two of the six "ecomorphs" that scientists who study Caribbean anole species have defined. To be considered an ecomorph, a set of habitat specialists must exist on more than one island, though the species in each group differ between islands. And yet, other anole species belong to no particular ecomorph class.

Caribbean anoles offer scientists a sort of "natural experiment," explains Luke Mahler, an evolutionary biologist and herpetologist at the University of Toronto. Each isle, with similar environments, acts as a replicate for how anoles underwent convergent evolution into ecomorphs. As a result, evolutionary studies of anoles have flourished in the past couple of decades—think Darwin's finches, but scalier.

"They really are a good model system for lots of questions, from very small-scale molecular work all the way up to adaptive radiation," says Jerry Husak, a physiologist at the University of St. Thomas in St. Paul, Minnesota.

The basic anole ecomorphs go way back in evolutionary history, found Jonathan Losos, an evolutionary ecologist at Harvard University. Emma Sherratt, now at Australian National University in Canberra, got a hold of 20 fossil anoles while a postdoc in Losos's lab. The fossils dated back 15 million to 20 million years, when the lizards were preserved in amber on the island of Hispaniola. Some were in museums, others in private collections. Using CT scans, the Losos team examined

NOTICE ME!: A male *Anolis stratulus* (barred anole) extending his dewlap in Puerto Rico

anatomy to confidently assign these fossils to four of today's ecomorphs; a couple other fossils might be part of a fifth (*PNAS*, 112:9961-66, 2015). "At least several of the habitat specialist types already existed," concludes Losos.

Despite the countless hours biologists have spent studying Caribbean anoles, the genus seems to have plenty of surprises still in store. In addition to her ongoing studies of physiology and behavior in diverse anole species, Johnson has recently focused on how her local Texan anole, *Anolis carolinensis*, determines dominance. *A. carolinensis*, like many other anole species, adopts a strict mating hierarchy in captivity, with males battling each other for access to prime habitat and to females. In the field, the hierarchy is more complicated—a lizard defending his own territory is more likely to win a fight, she thinks. She figured the biggest males would also be more likely to triumph, either in the lab or the field, and thus achieve larger territory and more females to court.

In order to correlate body characteristics and behaviors with dominance, Johnson's group set up a sort of lizard fight club, pitting anoles against each other in one-on-one cage matches, with a single perch to battle over. Winners tended to execute more visual displays, performing push-ups and head-bobs and expanding the showy throat skin known as a dewlap. They also chased and bit the losers, who tended to back away and to hide in a corner.

But larger anoles weren't always the winners in captivity or in the field. "Body size doesn't predict who wins these fights at all," says Johnson. Instead, behaviors made a huge difference—the most aggressive lizards won their matches. A longer head also helped, perhaps because it looked to opponents like a serious biting weapon. In the field, animals with a wider head and powerful jaws occupied larger territories with more females present (*Anim Behav*, 118:65-74, 2016).

Body size still probably matters, Johnson says. She has not yet tested in field studies whether size might help an *A. carolinensis* male establish his territory or take over a vacated area. And at least in other anole species, bigger males sire more offspring.

Mahler also got a surprise from the anoles when, in 2010, he received an email from Miguel Landestoy, a Dominican naturalist who claimed he'd seen a new species. Mahler was initially skeptical. "Everybody thinks they've got a new species," he says, yet "the Caribbean anoles are the best known anoles, by a long shot."

Then Mahler opened Landestoy's pictures. "Holy crap," he said. "That doesn't look like anything we've seen on Hispaniola." The critter was huge, by anole standards—about a foot from nose to tail tip. It had short legs, a short tail, and a mottled greenish-gray pattern that suggested it could easily blend into a mossy or lichen-covered branch. "I bought the first cheap flight I could find," recalls Mahler.

The other thing that struck Mahler about the new species—which he and his colleagues dubbed *A. landestoyi*—was that it looked similar to anoles found in Cuba. Their clade is called *chamaeleonides* for their creeping, chameleon-like movements and camouflage prowess. These particular kinds of anoles, scientists had assumed, were unique to Cuba. But here was another species, making its living in many of the same ways, on Hispaniola (*Am Nat*, 188:357-64, 2016). "This is an example of what might be a seventh ecomorph. . . . Evolution is more predictable than we have yet given it credit for," says Johnson, who was not involved in the project.

"It's amazing, in part, that anything new there could be found after all these years," adds Losos, a coauthor on the study. "The age of discovery is not yet over."

—Amber Dance

A Slippery Problem

In the early 20th century, Danish biologist Johannes Schmidt solved a puzzle that had confounded European fisherman for generations. Freshwater eels—popular for centuries on menus across northern Europe—were abundant in rivers and creeks, but only as adults, never as babies. So where were they coming from?

In 1922, after nearly two decades of research, Schmidt published the answer: the Sargasso Sea, the center of a massive, swirling gyre in the North Atlantic Ocean. Now regarded as some of the world's most impressive animal migrators, European eels (*Anguilla anguilla*) journey westward across the Atlantic to spawning sites in the Sargasso; their eggs hatch into larvae that are carried back across the ocean by the Gulf Stream, arriving two or three years later to repopulate European waterways. For decades, researchers have assumed that adults made the journey in one short and rapid migration, leaving European coastlines in autumn and arriving in the Sargasso Sea, ready to spawn, the following spring. But this assumption rests on surprisingly little evidence, says behavioral ecologist David Righton of the UK Centre for Environment, Fisheries, and Aquaculture Science.

"Since Johannes Schmidt identified this spawning area in the Sargasso Sea,

PHOTO BY MICHELE JOHNSON

people have been wondering about that great journey and trying to figure out how to follow the eels," says Righton, whose work on epic marine migrations includes appropriately titled projects such as CODYSSEY and EELIAD. "But the technology hasn't been available. . . . They just slip away into the darkness, really, in autumn, and no one knows what happens to them."

This information gap is of particular concern to conservationists. European eel recruitment (the number of babies added to a population each year) is thought to have declined more than 90 percent in the last 45 years. In 2008, the International Union for Conservation of Nature classified the fish as "critically endangered," citing a concerning lack of data on their life histories. "We have a black hole, or a 'blue hole,' out in the ocean in terms of adult migratory behavior," says Kim Aarestrup, a senior researcher at the National Institute of Aquatic Resources, Denmark, and one of Righton's collaborators. "It's really hard to do population ecology and management if you have a hole in the life cycle."

In the mid-2000s, with these concerns in mind, Righton, Aarestrup, and other European colleagues set out to fill in the blanks. Taking advantage of recent improvements in animal telemetry, they tagged more than 700 large (therefore female) European eels—no easy task, Aarestrup admits, since "they're pretty slimy." The team planned to track their slippery quarry across the Atlantic. As eels swim too deep—usually at least 200 meters down—to be tracked using GPS, tags logged environmental data to provide indirect clues about the eels' whereabouts. When a tag's battery died after several months, it detached and floated to the surface, where, depending on the type of tag, it either relayed data via satellite or drifted back to shore for collection.

As the data rolled in, the researchers realized there was more to European eel migration than previously thought. Just a fraction of tagged eels made it to the ocean: only 87 tags collected data beyond the coastline. Many of those 87 were soon separated from their eels by predation, and none made it beyond the Azores—a result that highlights the peril inherent to the transoceanic journey, Righton notes.

The partial trajectories recorded by the tagged eels that did make it to the ocean revealed further surprises. For starters, rather than take a direct route, eels apparently meandered their way to the Sargasso. "They're taking a much longer route than 'as the eel swims,'" says Righton, meaning that many models of eel migration are likely inaccurate. What's more, the team found, eels don't seem to swim with nearly enough urgency to reach the Sargasso in time for spawning early the following year.

Using eel larvae catch data from the spawning region, the researchers had estimated larval growth rates and extrapolated backwards in time to predict hatching times. From those estimates, they'd calculated that peak spawning must occur as early as February. But comparing this timeline with tag data revealed that eels leaving Europe in the autumn would have trouble reaching their destination fast enough at the pace they were going. "An eel has to go, for example, from Denmark at 55 kilometers a day to make it," says Aarestrup. Tags instead showed maximum speeds of around 47 kilometers per day for the largest—and presumably strongest swimming—fish in the population.

The findings point to a different story from the one told until now, the researchers argue—at least some of these eels weren't destined for the first spawning season at all, but the second (*Sci Adv*, 2:e1501694, 2016). "We can say that it's highly unlikely that a significant number of the eels leaving Europe will actually make it down to the Sargasso Sea ready for the coming spawning," explains Aarestrup. "Which then leads us to the conclusion that they're probably part of the next spawning, which happens 12 months later."

The claim is certainly a surprising one, says Michael Miller of Nihon University in Japan. "They propose the hypothesis that maybe there's a mixed strategy—some go quickly and some go slowly," he says. "It's a completely new idea." However, he emphasizes that without full data sets, there may be other explanations that can't yet be ruled out. As Aarestrup and his coauthors acknowledge, larval growth rates might be underestimated using catch data, due in part to the biasing size-selectivity of fishing nets, meaning that peak spawning could actually occur later in the year. Alternatively, Miller adds, "eels that leave too late might just not succeed in spawning."

Getting to the bottom of the mystery will probably require better tags that last longer and can be attached to a wider range of eels, not just the largest, notes Righton. "We're waiting for a revolution in technology," he says.

In the meantime, the study raises important questions about the ecology of these "amazing fish," Miller notes. "The hypothesis is most interesting, because it could be true, or it could be not true," he says. "Either way, they've proposed the hypothesis and it's going to stimulate a lot of interesting research."

—Catherine Offord

REV-EEL-ING SCIENCE: Researchers studying the European eel (*Anguilla anguilla*) are starting to answer age-old questions about the fish's life cycle.

The Mito-Mom Conundrum

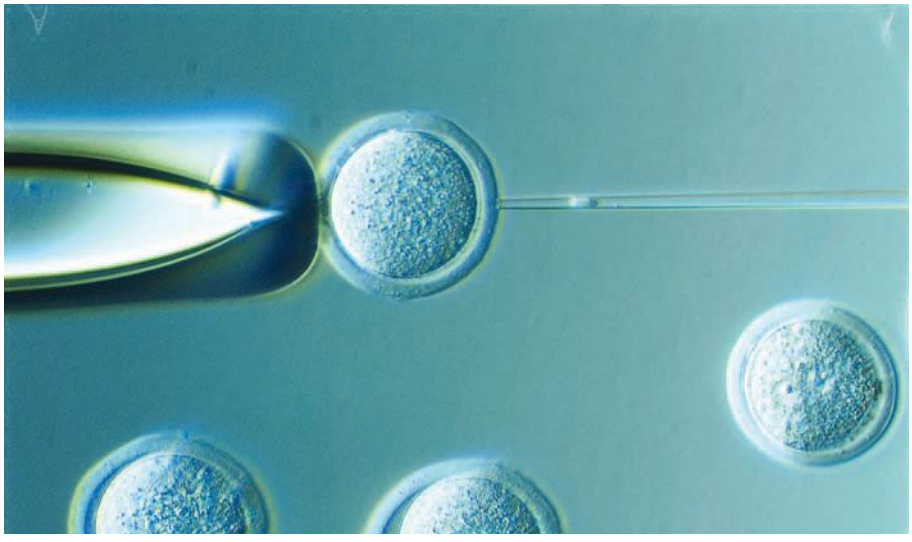
Mitochondrial replacement therapy raises important societal and ethical concerns, but should be embraced for its utility in preventing disease.

BY JOHN D. LOIKE AND NANCY REAME

In 2015, the US Congress banned the use of gene-editing techniques, such as CRISPR, for the creation of genetically modified human embryos. President Obama also signed into law a policy that precludes modification of the human germline. An unresolved issue with Congress's ban and President Obama's law is whether they encompass mitochondrial replacement therapy (MRT), a procedure that allows women with mitochondrial disease to give birth to unaffected children by inserting a nucleus from one of her eggs into an enucleated egg from a woman with healthy mitochondria, followed by in vitro fertilization.

A recent National Academies of Sciences, Engineering, and Medicine report stated that MRT is ethical if conducted exclusively in male embryos and limited to women with mitochondrial diseases. And recent studies have shown that mitochondrial donation has broader clinical applications beyond treating mitochondrial diseases, specifically in relation to infertility problems typically suffered by older females. In 2015, for example, a private research enterprise made headlines when its scientists revitalized senescent eggs through MRT before fertilizing them in vitro, helping an infertile couple in Canada give birth to a healthy baby boy. These expanded indications for MRT only strengthen the rationale for its use as an approved form of germline intervention.

MRT does raise a number of social and ethical challenges, however. One of the most debated aspects of mitochondrial donation is its potential for enabling scientists to "play God." But, humans have engaged in genetic modifications of plants and animals for thousands of years. Moreover, preimplantation genetic diagnosis (PGD), a genetic profiling technique for IVF-generated embryos, can be viewed as a form of genetic selection to alter the human population's genome pool by eliminating embryos with various genetic diseases. We argue that MRT is akin to these long-standing practices.



Another scientific and legal point of contention is how the term "genetic intervention" is interpreted. Many ethicists restrict its use to the exclusive involvement of germline modification of nuclear DNA. We and others propose that any genetic modification of germ cells—whether in the DNA of the nucleus or the mitochondria—constitutes germline intervention because the donor is providing essential genetic information to the child. Similar to sperm or egg donation, we argue that MRT should be considered a form of genetic intervention and that the congressional and presidential bans be reconsidered.

Concerns have also been raised about the unintended consequences of "three-parent" children. A key consideration here is whether mitochondrial donation should be regulated in the same way as a conventional organ donation—in which case the recipient acquires all the rights and responsibilities of the donated organ and the donor has neither parental rights nor legal responsibility for any unforeseen genetic diseases that the recipient receives from the donation—or be treated as egg donation, which carries unique ethical and legal considerations. Because the genetic contribution from the mitochondrial DNA donor is essential and critical for the development of a healthy child, and because emerging studies show that several mitochondrial genes affect mental illness, we argue that the genetic contribution from mtDNA should not be considered irrelevant to the status of parenthood. But there are legal ways to navigate this ethical challenge, similar to the avenues for giving up parental rights in the case of adoption.

As is the case with adoption as well as with other third-party reproductive

technologies, such as egg donation and gestational carriers, MRT also raises the question of whether the resulting offspring have a right to know their genetic parentage and lines of ancestry. This could compromise donor privacy. But again, there are now ways to deal with such challenges. Namely, as the price of gene sequencing decreases, every child can ultimately have his or her genetic information available without revealing the identity of the genetic contributors.

There are two other ethical concerns to MRT that are related to its high costs, which run between $25,000 and $50,000. The first is the fair distribution of medical resources to make this therapy available to all patients regardless of their financial capabilities. This concern can be circumvented, in part, by virtue of its potential eligibility as an approved treatment for infertility or for mitochondrial mutations, as many US states allow insurance providers to cover some costs of IVF for infertility. The second concern is that if the access to this technology is not fairly distributed, then economically disadvantaged women may be at risk of exploitation because of the excessive compensation paid to mitochondrial donors. This financial coercion of impoverished egg providers is already a global problem and serves as a cautionary warning for mitochondrial donation. There should be strict legal safeguards against such practices.

Mitochondrial replacement therapy will probably emerge as an effective method to enable women with mitochondrial disease to have healthy children, among other possible medical benefits, and should not be banned because of presumed social or ethical complexities.

MRT will probably emerge as an effective method to enable women with mitochondrial disease to have healthy children, among other possible medical benefits, and should not be banned because of presumed social or ethical complexities. Given its potential for permanent alterations of DNA, this technology should not be viewed as equivalent to classical organ donation but rather treated with precautions in line with other germline interventions, such as egg or sperm donation, for which regulatory practices are already in place. Using these as a framework, governments and the scientific community should invest time and money into making MRT widely available to patients. ■

John D. Loike and Nancy Reame are faculty members at Columbia University College of Physicians and Surgeons.

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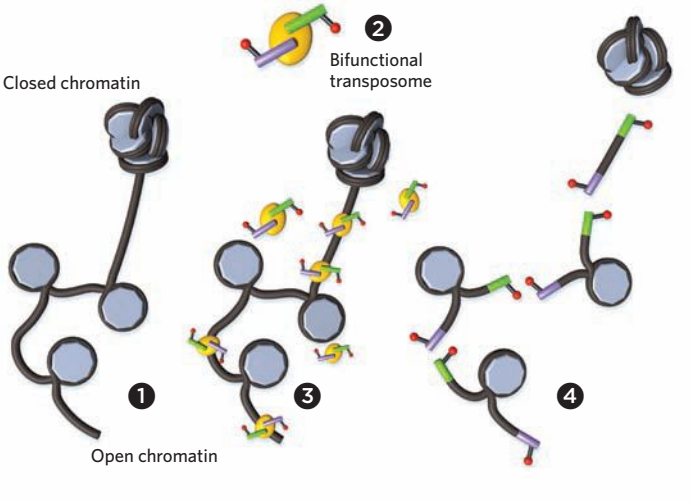
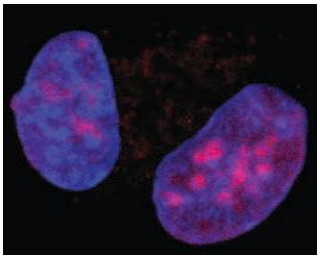
Hailing ATAC-see

A test tube-based genome-labeling technique has been brought under the microscope.

BY RUTH WILLIAMS





5 → Amplify and sequence

ATAC DO: The next generation of a technique called ATAC-seq, which captures and sequences active regions of DNA 1, allows for the visualization of these regions as well. In ATAC-see, a so-called transposome 2 uses a transposase enzyme (yellow) to insert a pair of fluorescent DNA tags into open regions of chromatin 3. Upon insertion, the DNA is cleaved 4 and the tags are visible under a microscope (5 human cells' open chromatin labeled red). These tagged sections of DNA are then sequenced.

In the limited space of the nucleus, most of the genome is tightly folded, leaving accessible only the parts that need to be transcribed. There is "huge interest" in determining which elements of the genome are active in a given cell type, says Howard Chang of Stanford University. This was Chang's motivation for developing a technique called ATAC-seq (assay for transposase-accessible chromatin)—in which DNA tags (acting as transposons) are enzymatically integrated into open regions of the genome and then used to identify those regions through sequencing. (See "Reveling in the Revealed," *The Scientist*, January 2016.)

Chang described ATAC-seq in 2013, but says, "we were breaking the cell open to get this information, so we didn't have any sense of the 3-D organization of the [accessible] elements."

Fast-forward three years, and Chang has now developed ATAC-see. This technique uses the same enzymatic approach as ATAC-seq to integrate DNA tags, but fluorophores conjugated to the tags allow for their visualization in 3-D, fixed nuclei. And, once the cells have been observed, sequencing can identify the tagged regions.

Using ATAC-see, Chang's group has discovered that in neutrophils, unlike in most cells, accessible chromatin tends to be located at the nuclear periphery. This arrangement seems to facilitate formation of neutrophil extracellular traps—strings of discharged genomic DNA that are used to catch and kill pathogens, but that also enable cancer metastasis.

The team has also used ATAC-see to examine how accessibility changes across the genome during the cell cycle.

"One of the big challenges in the field of chromatin study is to integrate data obtained by imaging with data obtained by genomic methods," says Job Dekker of the University of Massachusetts Medical School who was not involved in the study. "So what I like about this assay is that it combines these two things." (*Nature Methods*, doi:10.1038/nmeth.4031, 2016) ■

AT A GLANCE

ACCESSIBLE CHROMATIN ASSAY	HOW IT WORKS	TECHNICAL EASE	VISUALIZATION	NUMBER OF CELLS REQUIRED FOR SEQUENCING
DNase-seq	DNase I preferentially digests DNA that is free of nucleosomes (accessible). Nucleotide linkers are ligated to the digested strands and used for isolating and sequencing the digested regions.	Difficult, especially optimizing digestion conditions	No	Millions
ATAC-see	A transposase enzyme inserts fluorescently labeled DNA sequencing adaptors (tags) into accessible chromatin, and the tags are used to visualize, amplify, and sequence the DNA.	Relatively easy for those familiar with standard molecular techniques	Yes	50,000. ATAC-seq (the protocol lacking fluorescent visualization) has been performed on single cells, so this might be possible.

DRIVING OUT MALARIA

Introducing genetic changes into mosquito populations could be key to effective malaria control.

BY TONY NOLAN AND ANDREA CRISANTI

In recent years, researchers have sequenced the genomes of several *Anopheles* mosquito species, including those responsible for nearly all of the malaria transmission in Africa. With this information, they have begun to identify the genes underlying the insects' ability to colonize human habitats, their reproductive biology, and their susceptibility to infection by the malaria parasite (*Plasmodium* spp.). If we know the genes, or variants of genes, that are responsible for key mosquito traits, such as parasite clearance or egg laying, we can theoretically introduce a genetic modification into the insects that reduces malaria transmission.

The idea has been percolating in the minds of scientists for nearly 20 years, ever since researchers developed a way to introduce genes into mosquitoes. But one challenge has hindered all such efforts to date: how to encourage the spread of a gene modification from a few lab-reared mosquitoes to an entire wild population over a relatively short time frame. In nearly all cases, the genetic modification encoding the trait of interest is very unlikely to confer any positive fitness effect to the mosquito, and therefore its frequency is not likely to increase over time as a result of natural selection. Moreover, even a very large laboratory population released into the wild will represent only a tiny fraction of *Anopheles* in Africa. Now, however, researchers may finally have a plausible solution: gene drive, a technique that introduces a genetic element (also referred to as a gene drive) that cheats the normal rules of Mendelian inheritance.

Gene drives use a variety of tricks to ensure that they, or the chromosomes that contain them, are selectively inherited.

In sexually reproducing organisms that have two copies of every chromosome, any single copy of a gene normally has a 50 percent chance of being passed on to an individual's offspring. Gene drives, on the other hand, use a variety of tricks to ensure that the genetic elements, or the chromosomes that contain them, are selectively inherited. The most efficient gene drives might ensure an inheritance bias of nearly 100 percent, such that the genetic sequence doubles in frequency, relative to a similar genetic element not displaying drive, in each generation. All things being equal, after 10 generations the gene drive would have increased its frequency in the population by a relative 1,024-fold (2^{10}).

There have been several documented cases of gene drives existing in nature, with selfish genes or selfish chromosomes invading populations of several different insect species. By understanding how these selfish elements work, researchers

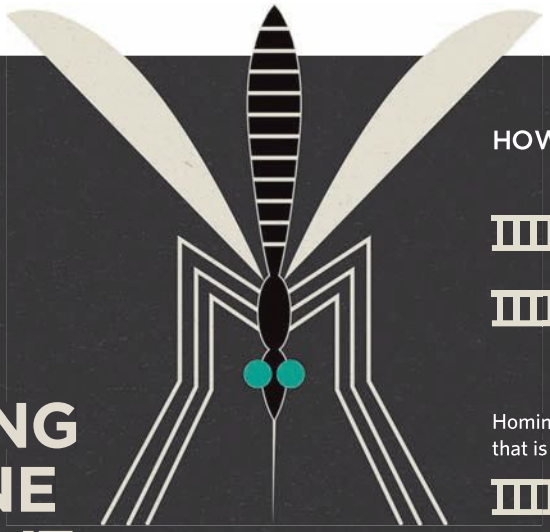
hope to use gene drive to spread modifications into a mosquito population even if those modifications are detrimental to individual fitness. If successful, the strategy could be the beginning of the end of one of the world's most devastating and persistent infectious diseases.

A formidable foe

In the early 20th century, Italian physician and zoologist Giovanni Battista Grassi and British army surgeon Ronald Ross determined that malaria is caused by a parasite transmitted from human to human exclusively by the bite of female mosquitoes belonging to a limited number of species from the genus *Anopheles*. Their discovery has motivated more than a century of research into mosquito biology, diversity, and ecology. And this knowledge of the malaria vector has directly led to successful examples of malaria control, and even elimination in some regions.

Local malaria eradication has only been achieved in areas such as southern Europe and several southern US states, where a combination of factors allowed for transmission control—in particular, low numbers of infected people and a low density of efficient mosquito vectors in close proximity to humans. In some regions of sub-Saharan Africa, where 200 million people are infected with malaria every year, infected mosquitoes may bite a person almost every single day during the area's transmission season. For these reasons, among others, Africa suffers the brunt of the malaria burden, accounting for more than 90 percent of the 438,000 malaria-caused deaths in 2015.

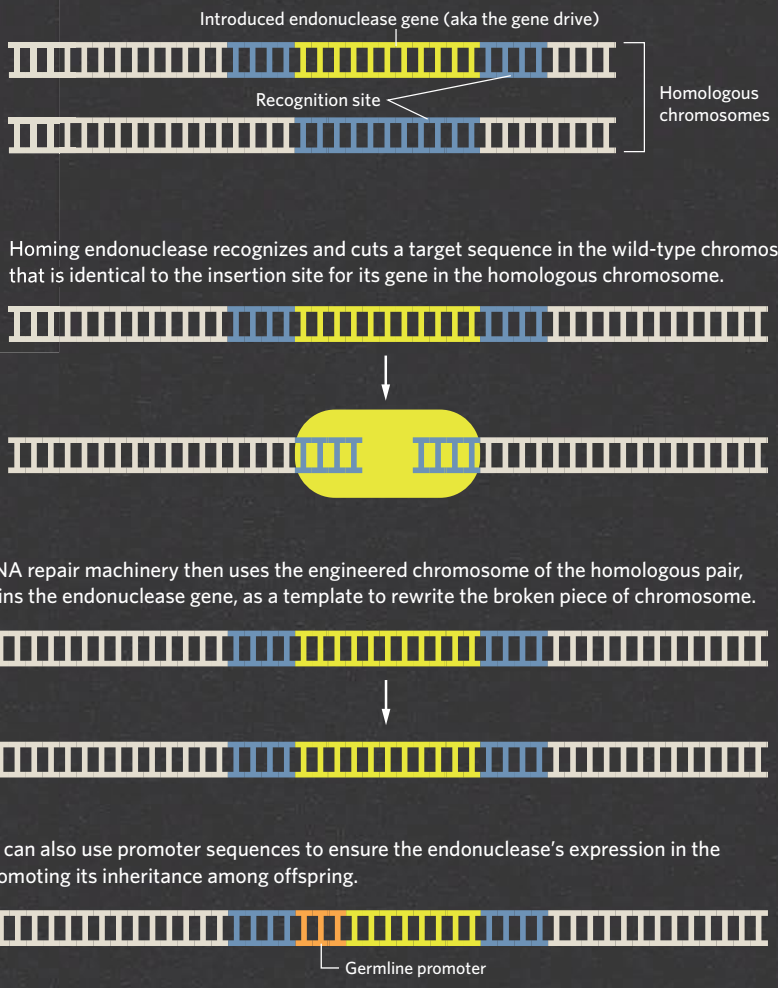
With current methods of malaria control—including large scale removal of mosquito breeding areas, the use of insecticide-treated bed nets, and through regional insecticide spraying—and other conventional tools such as vaccines and drugs currently proving insufficient to eliminate the disease, some researchers have turned to methods for genetically controlling the mosquito population. These approaches have the potential to be species-specific and self-sustaining with-



USING GENE DRIVE TO CONTROL MALARIA

For years, researchers have looked to genetically modify mosquitoes to prevent the spread of malaria. But while introducing genetic changes has long been supported by advancing technologies, getting those modifications to spread through wild mosquito populations has remained a challenge. Now, the newly understood concept of gene drive, in which genetic elements can spread more rapidly than those following traditional Mendelian inheritance principles, may finally solve the problem.

HOW GENE DRIVE WORKS



out requiring repeated releases or high levels of public infrastructure.

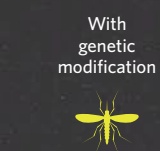
Several examples of naturally occurring gene drive elements have been documented in insect populations in the past. For example, in *Drosophila melanogaster*, the transposable element P is able to copy and paste itself semi-randomly at several sites in the genome so efficiently that last century it swept through all wild populations in less than 60 years. The intracytoplasmic bacterium *Wolbachia* can also be considered a selfish genetic element. Found in around half of all insect species, *Wolbachia* is transmitted through the germline and can provide a relative fertility advantage to infected females. Theoretically, either of these genetic elements could be linked to a modification that affected mosquito fitness or the insects' ability to fight the malaria parasite. However, the indiscriminate nature of transposable element movement in the genome and the difficulty associated with establishing *Wolbachia* infections in the large majority of malaria-carrying mosquito species has so far hindered the development of these approaches for malaria control. (See "Evolution Resisted," *The Scientist*, October 2009.)

If we know the genes that are responsible for key mosquito traits, we can theoretically introduce a genetic modification into the insects that reduces malaria transmission.

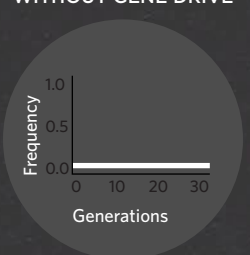
Another type of gene drive proposed to alter mosquito populations was inspired by a class of natural selfish genetic elements called homing endonucleases that exist in single-celled eukaryotes such as yeast and algae.[1] Homing endonucleases are DNA-cutting enzymes that are extremely precise in their action because they recognize a very long (and therefore very rare) target sequence on a chromosome and insert the endonuclease-encoding gene within this sequence, making it resistant to further cleavage. When the endonuclease comes into contact with its chromosome's homolog that does not contain the endonucle-

HOW GENE DRIVES SPREAD

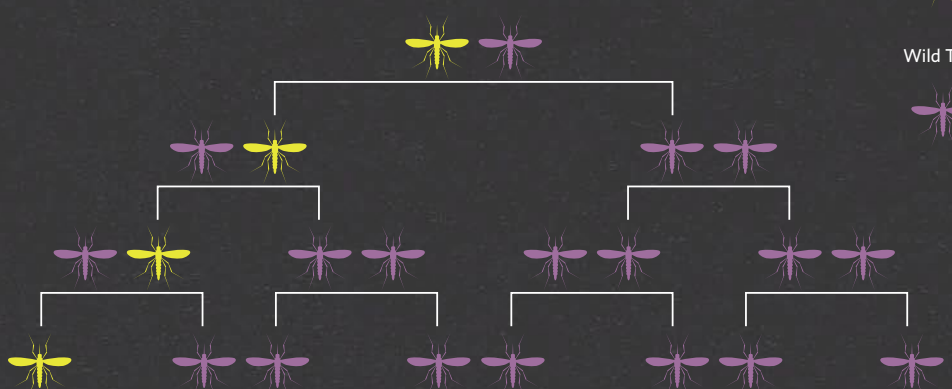
Without gene drive, an allele will be passed from generation to generation via traditional Mendelian genetics. That is, when a heterozygous individual carrying only one copy of the allele mates with an individual lacking it altogether, only half of their offspring will inherit the genetic segment. But in a gene drive that spreads the allele to the homologous chromosome in the germline, all progeny will receive a copy. With gene drive, it's even possible for a deleterious allele to spread though the population, despite imposing a severe fitness cost.



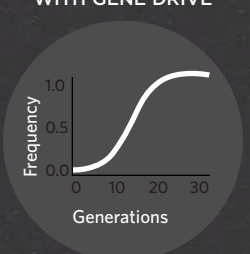
WITHOUT GENE DRIVE



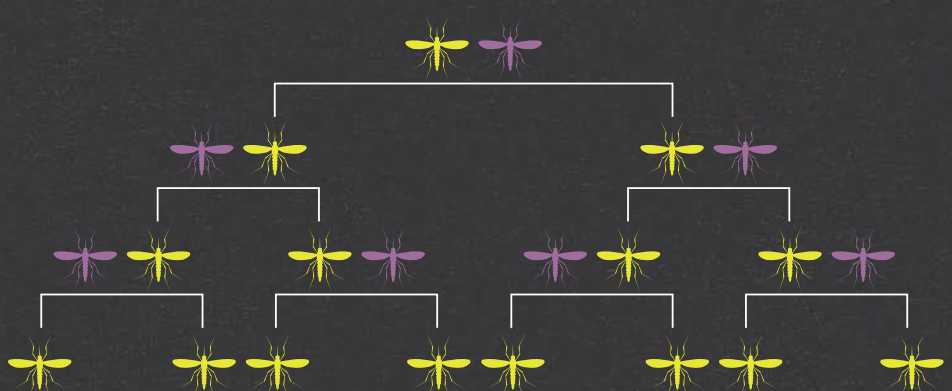
If the genetic modification has no negative fitness consequences, its frequency in the population will remain at whatever the initial introduced frequency was.



WITH GENE DRIVE



With gene drive, the genetic modification will quickly increase in frequency until it is carried by all mosquitoes in the population.



ase gene, it cuts the intact target sequence, prompting the cell's DNA repair machinery to use the intact chromosome, which contains the endonuclease gene, as a template to mend the break. (See illustration on page 26.)

Several years ago, our group decided to investigate whether these homing endonuclease genes could be adapted to work in the mosquito. First, we artificially inserted the natural target site of a homing endonuclease into the mosquito genome and then tested the ability of an inserted endonuclease to cut its target site and spread to homologous chromosomes. By using promoter sequences to ensure the endonuclease was specifically expressed in the germline of the mosquito, we showed that it could act as a gene drive by biasing its inheritance among the sperm or eggs that result in offspring.[2]

This type of biased inheritance is dependent on the pathway the cell uses to repair the broken chromosome rather than on the nuclease itself. Indeed, we and others showed that this type of gene drive could be constructed with any type of sequence-specific DNA cutting enzyme,[3] including CRISPR,[4,5] by inserting these nucleases within their target site on the chromosome. CRISPR gene editing requires only a single protein (Cas9) and a small guide RNA that determines specificity by simple complementarity to the DNA target, making it relatively easy to engineer a nuclease protein to recognize sites of interest in the mosquito genome. In combination with years of research on which type of genetic modifications are most likely to reduce malaria transmission, researchers now have the tools they need to develop effective malaria control using a gene drive approach.

Implementing a gene drive against malaria

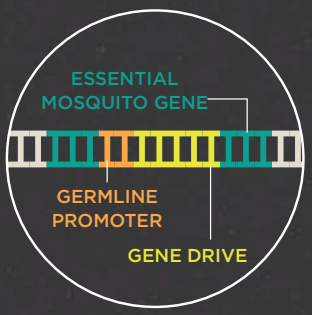
When it comes to genetically modifying mosquitoes to control the spread of disease, there are three broad strategies: spread a deleterious modification through the mosquito population to reduce overall mosquito numbers; bias the inheritance of the male sex chromosome, distorting the

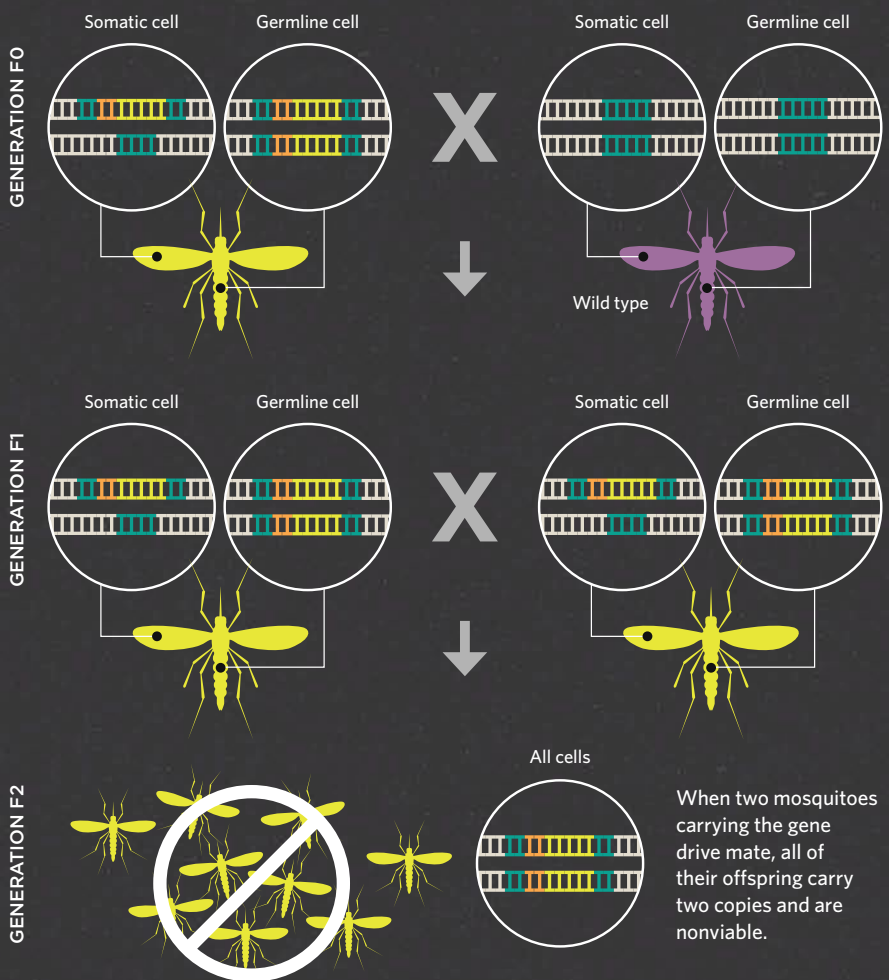
HOW GENE DRIVE COULD BE USED TO CONTROL MALARIA

There are three general approaches to implementing gene drives in mosquito populations for the control of malaria: (1) spread a deleterious mutation to reduce mosquito numbers, (2) distort the sex ratio of the population, or (3) deliver cargo to render mosquitoes resistant to the malaria parasite.

(1) POPULATION-WIDE GENE KNOCKOUT

A gene drive can be designed to disrupt a mosquito gene essential for survival or reproduction. In this case, researchers would restrict its expression to the germline, such that individuals that have one copy of the inserted genetic element (aka gene drive) still have a functioning copy of the mosquito gene in the rest of their body where it is needed and therefore act as carriers of the gene drive. In the germline, however, the gene drive spreads, increasing the proportion of gametes that carry it. When released into the wild, these animals would mate and pass on the gene drive to all offspring. When two mosquitoes carrying the gene drive mate, none of their offspring would inherit a functional copy of the essential gene, decimating the population.

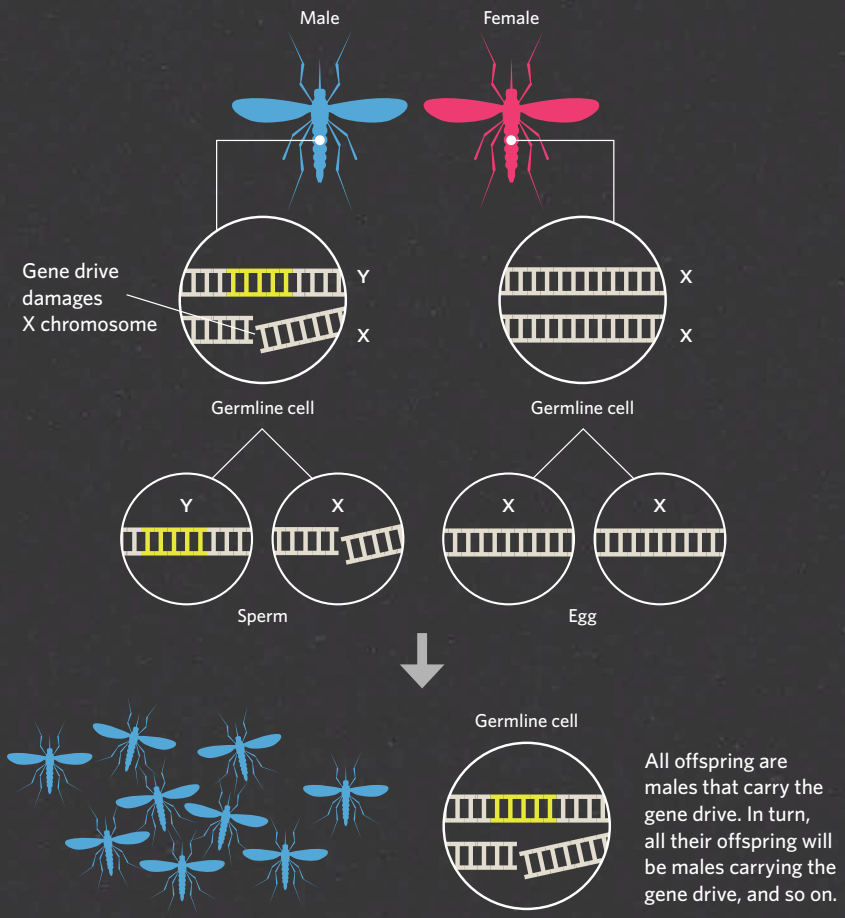




When two mosquitoes carrying the gene drive mate, all of their offspring carry two copies and are nonviable.

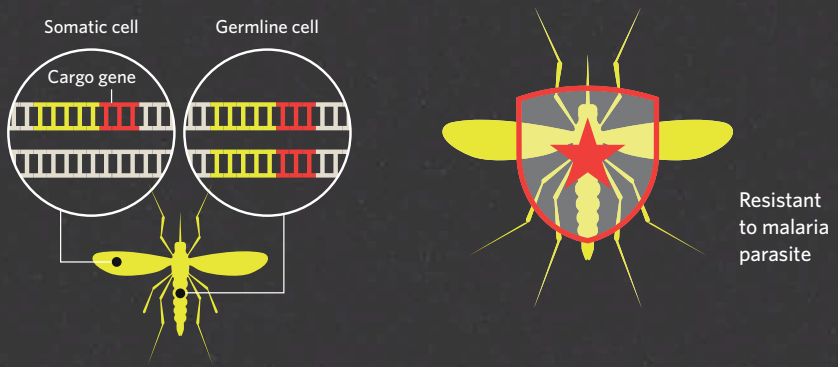
❷ SKEWED SEX RATIO

A gene drive designed to selectively destroy the X chromosome in *Anopheles gambiae* sperm will result in the nonviability of those sperm, leaving only sperm carrying Y chromosomes. This will lead to a sex ratio greatly skewed toward males, which will eventually cause the population to crash.



❸ POPULATION-WIDE GENE KNOCK-IN

A gene drive can also be designed to deliver a cargo, such as an antimicrobial peptide, that makes mosquitoes resistant to the malaria parasite. In this case, the antimalarial cargo would be expressed wherever the parasite is most vulnerable, whereas germline expression of the gene drive would guarantee its spread through the population.



sex ratio toward males, which do not bite humans; or equip mosquitoes with antiparasite cargo such as an antiparasitic peptide. Each of these approaches has shown promise in early-stage lab work, and each has its own merits and considerations.

Disrupt an endogenous mosquito gene

The easiest way to imagine reducing a mosquito population would be to target a gene essential for female fertility. Individuals with only one copy of the defective gene are fertile, but the gene finds its way into all of their gametes, such that they transmit the gene to all of their offspring. As the gene drive element increases in frequency in the population, so does the probability of offspring receiving a copy of the gene drive from both parents and thus no functional copy of the female fertility gene. All such females in this class will be sterile, while males will continue to transmit the gene drive to all offspring.

Our group has recently identified three mosquito genes with roles in female fertility that may be suitable targets for such an approach, and we have shown in small laboratory populations that a gene drive specifically designed to disrupt one of these genes can spread through the population.[4] An alternative but similar approach would be to target a mosquito gene essential for parasite transmission, such as a specific receptor to which the parasite binds in order to enter the cell. No such specific receptors have yet been identified for the human malaria parasite.

Distort the sex ratio

Because only female mosquitoes transmit malaria, any intervention that skews the sex ratio of a mosquito population toward males should lower disease transmission. Our group has also shown that the X chromosome in *Anopheles gambiae* can be selectively destroyed during sperm maturation in males by expressing nucleases that specifically target a repetitive sequence only found—in several hundred copies—on the X chromosome.[6,7] The nucleases shred the X chromosome, causing nonviability in sperm that inherit it.

As a result, 95 percent of all viable sperm contain a Y chromosome and produce male offspring containing the nuclease. We are currently working to link the endonuclease gene to the Y chromosome so that the nuclease can act as a gene drive to spread through the population.[1] Eventually, the population would be expected to crash due to a shortage of females needed for mating.

Add a cargo

A third option is to equip mosquitoes with some sort of cargo that directly fights the malaria parasite. When the gene drive is designed to bring along such a payload, the desired effect is dependent on the nature of the cargo rather than the genomic location of the gene drive. Researchers have proposed a range of antimicrobial peptides, both natural and synthetic, for this purpose, and in 2015 researchers successfully constructed a gene drive in *Anopheles stephensi* that included genes encoding single-chain antibodies designed to bind and inhibit surface proteins on the malaria parasite.[5] This study showed biased inheritance of the gene drive in a single generation; however, the effect on the parasite has not been demonstrated, and this approach has yet to be tested in a caged mosquito population.

Gene drive considerations

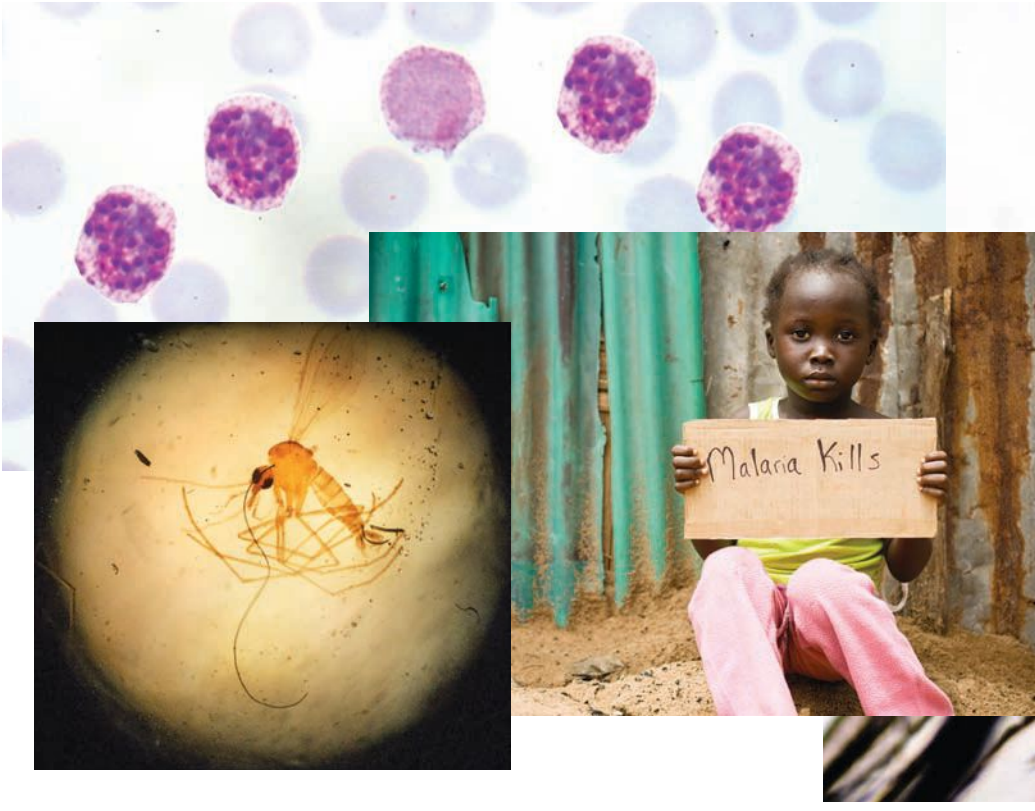
Gene drives have enormous potential for the control of populations of insect vectors and pests. They are species-specific, self-sustaining, and have the potential to be long-term and cost-effective. Gene drives can complement existing approaches, such as vaccine development, antimalarial drugs, and conventional vector control approaches, including insecticides, bed nets, and other barriers.

The building of functional gene drives for malaria control is still in its infancy, however, and each of the various approaches must overcome considerable technical hurdles. Although gene drive is deliberately designed to spread despite causing a negative fitness effect on the mosquito, it will nonetheless generate a selection pressure for the evolution of resistance in the mosquito population. This selection pressure is likely to be higher for strategies that aim to reduce the reproductive output of the population than for those that alter the insect's ability to transmit the parasite.

The most obvious way resistance might manifest is sequence variation at the target site of the endonuclease. Such variation may already exist in the population or it can accrue at a low rate if the broken target site is repaired by a DNA repair mechanism called nonhomologous end joining, rather than by copying the homologous chromosome (homology-directed repair). This risk can be mitigated in several ways, such as choosing target sites where there is very strong sequence conservation and/or multiplexing of gene drive elements. For example, a CRISPR-based gene drive might be designed to include multiple different guide RNAs so that likelihood of resistance to cleavage by all guide RNAs is reduced, akin to combination therapy with antibiotics. Engineering tolerance into the nucleases so that the enzymes cleave target sequences despite single-base-pair variants could also help combat mosquitoes evolving resistance to the approach.

On the other hand, with a gene drive carrying antiparasitic cargo, the malaria parasite will be under selection for resistance, and the robustness of the antiparasitic effect might vary with the genetic backgrounds of the mosquito and *Plasmodium* strains, as well as with environmental factors such as temperature. Additionally, gene-drive elements carrying a cargo might lose it, either through mutation or recombination, but still show drive and might outcompete those with the cargo. This is not a concern for gene drives targeting an endogenous gene as there is no additional cargo to lose. Ultimately, the choice of which approach to take will depend on the specific disease-transmission scenario and the ecology of the vector species.

Any gene drive approach that shows efficacy in the laboratory must be subject to a lot of testing in strictly confined large



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cages to see how the intervention scales up, how stable the gene drive elements are, and whether any signs of resistance or off-target effects crop up. In the field, background studies to look at the distribution, genetic heterogeneity, and gene flow of mosquito species in a given area are essential.

In addition, as with any new technology, a careful appraisal of the approach's ethical and ecological implications is imperative. In this light, we have welcomed recent efforts by the research community to agree on a set of principles for the safe development, implementation, and containment of gene drives.[8] A recent report by the National Academy of Sciences encouraged a cautious, step-wise approach to building, testing, and implementing gene drives.[9] But while overarching guidelines are helpful, deploying gene drives for control of any population should be assessed on a case-by-case basis. When it comes to targeting populations of *Anopheles gambiae* mosquitoes, regulators should weigh risks against the potential benefits of controlling a disease that is still causing a huge health and economic burden in the face of current malaria-control strategies.

Lastly, and most importantly, the decision to deploy a gene drive technology does not, and should not, lie with the scientists who design it. This will be a decision to be made by those countries and communities that are affected by the disease and will require an open dialogue to engage stakeholders at all levels and to ensure that consensus is reached before the technology is implemented. ■

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Tony Nolan is a molecular biologist and senior research fellow at Imperial College London, where Andrea Crisanti is a professor of molecular parasitology.

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CRIME SCENE DO

CUTTING-EDGE FORENSICS

Modern technologies are helping to solve the case of skeleton-filled coffins found in a Missouri man's home, and other unsolved mysteries.

BY BOB GRANT

Forensic anthropologist Lindsay Trammell had only just received the human remains and she already knew that she'd need help with this case. It was the summer of 2014, and 15 skeletons had arrived at the St. Louis Medical Examiner's Office as a jumble of bones inside four wooden coffins. Some of the bones looked ancient; they were "falling apart," Trammell recalls. But others were in relatively good shape. "There were different levels of preservation throughout the remains."

She photographed, inventoried, and measured the skeletal elements employing the standard biological techniques typically used by forensic anthropologists, who are still by and large not regular fixtures in crime labs. Those analyses indicated that some of the skulls bore characteristics of people with African ancestry while others did not. "Just by looking at them, my inclination was that they were from different ancestral groups," Trammell says.

Something wasn't adding up. Earlier that summer, Tony Wheatley, then a detective at the Morgan County Sheriff's Office, went to a home just outside of Versailles, Missouri, to investigate a reported suicide attempt. Police knocked on the door of retired archaeologist Gary Rex Walters, but no one answered. So they entered to ensure the safety of any occupants. Wheatley says that he and his fellow officers did not find any people inside, but they did spy marijuana pipes in plain view. The officers left the premises and applied for a search warrant. When they later reentered the home, they found four open wooden coffins full of human remains—bones, teeth, and skulls.

Wheatley called the Morgan Country Coroner, who indicated that the remains shouldn't have been stored in Walters's home. The detective confiscated the coffins and their grisly contents. "We went ahead and secured them for safe keeping until we could figure out what was going on," Wheatley says. "I'm not trained in anthropology or anything like that, so I didn't know how old they were or what they were."

Walters argued that he had permission to own the skeletons contained in the coffins, saying that he had excavated the remains near Iztapa, Guatemala, sometime in the 1970s. He even produced decades-old documentation from the Guatemalan government to prove the legality of his cache.

But Wheatley says he "still wasn't convinced that those documents covered those remains that we had seized. . . . We wanted to make sure that they weren't newer bones that [Walters] had come across locally." He added that there are several unmarked Native American burial sites in central Missouri and that there was a black market for items recovered from those sites. "We didn't want him trying to sell Native American bones," Wheatley says.

Wheatley's concerns only grew as he looked into the archaeologist's background. In the 1990s, Walters had been accused of stealing human remains that he was supposed to help relocate from a historic African American cemetery in St. Louis to make room for transportation infrastructure in the city. That incident, which revolved around his insistence that he was owed money for his work on the cemetery relocation project, was apparently resolved when he returned 28 bodies and received $90,000 for his work.

> As an anthropologist, our job mostly in the past was focused on creating what we call a biological profile from the skeleton to help identify them. As technology is changing, our rules are honestly shifting.
>
> —Lindsay Trammell, St. Louis Medical Examiner's Office

The discovery of the human skeletal remains in Walters's home reanimated old suspicions. "[The investigators] were just trying to verify whether he was telling the truth or whether these were skeletal remains of modern individuals that had gone missing or been killed," Trammell says. "So that was one of the reasons they called me—to see if we could discern, based on looking at the remains, were they from Guatemala, were they from this cemetery, or were they from somewhere different altogether?"

But Trammell's preliminary work left her puzzling over what seemed like a mixed bag of remains. So she called for backup. Trammell sent samples to Cris Hughes of the University of Illinois at Urbana-Champaign and Chelsey Juarez of North Carolina State University (NCSU), who performed genome sequencing and isotope analysis, respectively, to provide more detailed information on the skeletons that would either corroborate or contradict the archaeologist's story.

"Really, as an anthropologist, our job mostly in the past was focused on creating what we call a biological profile from the skeleton to help identify them," Trammell says. "As technology is changing, our rules are honestly shifting, because now you do have DNA and now you do have isotopes, where these types of tests can tell you quite a bit from the skeleton."

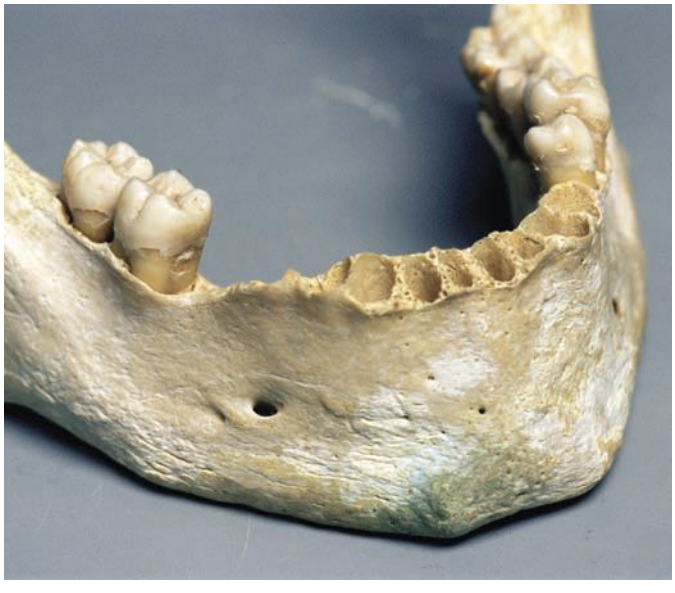
The archaeologist's bizarre case is one of only a handful of examples where such techniques have been applied to criminal investigations. While searching for DNA matches to established databases or suspect samples is common practice in many crime labs, genome sequencing to establish a body's likely ancestry and isotopic analyses to suggest its possible geographic origins remain exceptions to the rule of standard forensic workups. But as technologies mature—from single-cell sequencing to epigenetic analyses—investigators are beginning to rely more and more on advanced forensic methods, yielding unprecedented insights into victims, perpetrators, and their crimes.

"It's taken us a while to get to this point, truthfully," says Seth Faith, a researcher at NCSU's Forensic Science Institute. "But now we have this wonderful technology in front of us and people who are trained in how to use it and interpret that data."

The devil's in the DNA

Because they had collaborated on cases before, Trammell knew that Hughes, who also serves as a deputy forensic anthropologist at the Champaign County Coroner's Office, was clued in on the latest in genome sequencing as applied to forensics. Trammell was also aware that Hughes trained under University of Illinois ancient DNA researcher Ripan Malhi, who has used advanced sequencing techniques to recover and decode DNA from 6,000-year-old human remains. "[Hughes is] definitely the expert when it comes to DNA," Trammell says. "I knew that she could test for mitochondrial haplogroups"—genetic signatures that are associated with geographic regions—"and [determine] whether or not they were consistent with an individual that was African or Native American."

Trammell sent small bone and teeth samples from 10 of the crania found in the archaeologist's wooden coffins. Of those, Hughes took subsamples from six and sequenced hypervariable region 1 (HVR1) of the mitochondrial DNA, which can help determine an individual's haplogroup. "To jibe with [the archaeologist's] story, I would have to see something that was a Native American haplogroup," Hughes says. Of the four samples that yielded sequenceable DNA, three did have HVR1s consistent with Native American ancestry. However, one sample's HVR1



placed the mitochondrial genome within West African haplogroups, suggesting that not all of the remains in the wooden coffins were from Guatemala after all. "Some of those materials in there were basically raising a red flag and were not consistent with his story," Hughes says.

Hughes knew that Iztapa was abandoned by natives in 1350 CE, before Europeans arrived in the area, meaning that "if we're looking at the DNA, and we're looking at maternal ancestry, you should expect to only see Native American links," she says. "We shouldn't see anything that's European or African, because there was no contact. They hadn't come over yet."

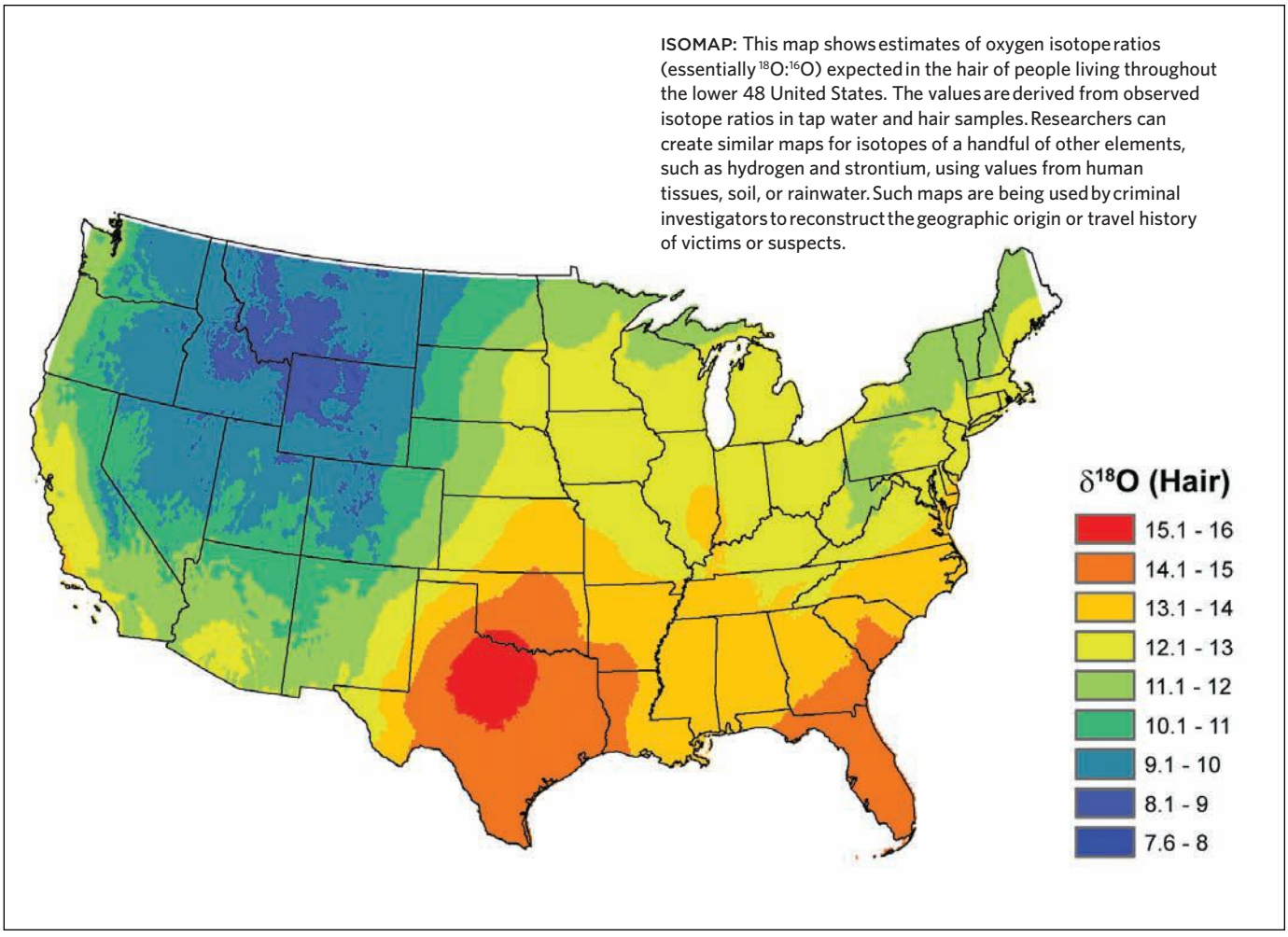
But Hughes knew of another analysis that could provide further evidence of the skeletons' origins, so she suggested Trammell contact Juarez to analyze the relative abundances and ratios of isotopic forms of common elements, which can yield clues about where the deceased individual lived from birth to death.

Isotopes tell most

Juarez also received bone and tooth samples from the same 10 crania that Trammell had sampled for Hughes. Using mass spectrometry and focusing on the crown of the first permanent molar—which starts forming in utero and finishes developing by age 3—she performed analyses that quantified the amounts of isotopic strontium in the skeletal materials. She then compared those levels with maps of known isotope distributions in both coastal Guatemala and the St. Louis region. "We did not find overlap in the Latin American region where [the archaeologist] was claiming that the bones were from," Juarez says. "We did find overlap in the St. Louis area."

Juarez's results provided further support that the bones in the archaeologist's skeletal stockpile were not all from ancient Guatemala, as he had contended. "Based on the results that we were getting, my first thought was that these remains were coming from multiple different archaeological sites from various regions," says Trammell. "To me it seemed like this was something that he was possibly collecting throughout his career. But without testing every single element, there's no way to prove that."

Hughes and Juarez submitted their data and reports on their analyses to Trammell, who passed those findings onto investigators in Morgan County.



ISOMAP: This map shows estimates of oxygen isotope ratios (essentially ^{18}O:^{16}O) expected in the hair of people living throughout the lower 48 United States. The values are derived from observed isotope ratios in tap water and hair samples. Researchers can create similar maps for isotopes of a handful of other elements, such as hydrogen and strontium, using values from human tissues, soil, or rainwater. Such maps are being used by criminal investigators to reconstruct the geographic origin or travel history of victims or suspects.

ANNU REV EARTH PLANET SCI, 38:161-87, 2010

The reach of forensics

In a handful of other cases, investigators are also turning to advanced genetic and isotope analyses to shed light on their investigations. And as the technologies continue to mature, the practice is likely to become much more common. Hughes sequenced the mitochondrial genomes from Trammell's samples using older Sanger sequencing, for example, but other researchers are employing more-advanced next-generation sequencing to generate more-complete genomics profiles with greater efficiency and speed.

One of the best and broadest examples of applying next-gen tools and methodologies to forensic sequencing can be seen in a project at the Armed Forces DNA Identification Laboratory (AFDIL). Researchers there are using next-gen sequencing to sift through the genomes of fallen service members whose remains were recovered on foreign battlefields from World War II, the Korean War, and Vietnam.

> We are using the very sensitive methods of next-gen sequencing to recover 55- to 75-base-pair fragments, which are not amenable to Sanger sequencing strategies that require 20-base-pair primers on either end.
>
> —Charla Marshall, Armed Forces DNA Identification Laboratory

Charla Marshall, chief of the emerging technologies section at AFDIL, headed up the validation of a sequencing protocol that investigators are now using to repatriate the remains of more than 800 Korean War veterans. The skeletal remains were buried in the National Memorial Cemetery of the Pacific in Hawaii (colloquially referred to as the "Punchbowl") in 1953 after being disinterred from their original burial grounds near Korean battlefields, shipped to Japan, preserved in formalin, and shipped to Hawaii. In the late 1990s, the AFDIL retrieved the bodies from the Punchbowl to begin identifying the soldiers. But the bodies had been fixed in formalin, which induces DNA crosslinks that disrupt sequencing reads; so the researchers were unable to extract DNA fragments long enough to sequence with the Sanger method.

"Right now we are using the very sensitive methods of [next-gen sequencing] to recover 55- to 75-base-pair fragments, which are not amenable to Sanger sequencing strategies that require 20-base-pair primers on either end," Marshall says.

In July of this year, the Defense POW/MIA Accounting Agency (DPAA) announced that researchers at AFDIL had identified US Army Corporal Charles White, who died fighting in North Korea in 1951 at the age of 20. Using Marshall's protocol to sequence Cpl. White's mitochondrial genome, the team matched it to mtDNA provided by a niece, a nephew, and a sister. Last summer, his remains were returned to his family in Lexington, Ohio, and he was buried with full military honors. And Cpl. White is just one of many. "Between March of 2016 and September 30 of 2016, we have processed 80 samples through the [sequencing] procedure," says Tim McMahon, chief of AFDIL. "And we're running at about a 45 percent success rate," in terms of pulling useable sequences from the chemically degraded DNA.

Isotope analyses, which can narrow a person's travel and life history down to a set of geographic locations, are also becoming more common in forensics labs. In 2015, scientists working on the murder of 2-year-old Bella Bond, whose virtually unidentifiable body was found in a trash bag near Boston Harbor, used isotopes to help determine where she may have lived during her tragically short life. By comparing oxygen isotopes in her hair and teeth to known values of oxygen isotopes in drinking water throughout the country, investigators suspected that she had spent most of her life in the New England area, information that helped police narrow the search for her murderers and arrest the girl's mother and her boyfriend for the crime in September 2015.

Lesley Chesson, president of Salt Lake City–based IsoForensics, says that her company gets requests from investigators all over the country for isotope analyses like the one used in the Bella Bond case. "This application for modern casework has really been taking off in maybe the last 10 or 15 years," she says. "We work with folks from all over the United States—police departments, state bureaus of investigation, and sheriff's departments."

IsoForensics was spun out of the University of Utah, where geochemist Gabe Bowen conducts research on the cutting edge of isotope analyses with an approach called isomapping or isoscapes. Isomaps are predictive maps that display the distribution of isotopes of a particular element that can be compared with isotope ratios in bones, teeth, hair, or other tissues to estimate the geographic origins, travel histories, diets, or date of death of a person.

"Isoscapes are trying to take our first-principles—understanding of the physical, biological, and chemical systems that control isotope variation—and turn those into predictive maps that provide a fingerprint for interpreting forensic data," Bowen says. "It would be like if you wanted to know where a letter came from in the mail. It's got a ZIP code on it. You need a map of the ZIP codes across the U.S. to interpret that to figure out where it came from."

But building isomaps is a slow, ongoing process. "These days, we've got massive [DNA] databases against which you can compare a sample," Bowen says. "We're not at that point yet [with isomaps]. And, unfortunately, there hasn't really been a concerted, community-wide effort to build the necessary databases."

A lack of funding, Bowen adds, makes this a difficult proposition. "So we're left in a situation where most applications, when they come up, involve making some measurements and then scraping to fund samples for comparison or pull together data from published sources that can provide comparators," he says. "So that means there's not a plug-and-play application in most cases. Each application still, at some level, involves a lit-

ADVANCING FORENSICS SCIENCE

TRADITIONAL TECHNIQUES

Currently approved and accepted forensic anthropology methods include creating a so-called biological profile of a crime victim or set of remains. This involves taking several measurements, especially of skeletal and cranial features, that can indicate age, gender, stature, and even ancestry.

DELVING INTO DNA

Genetic analyses have been used in crime solving since the 1980s. Investigators sequence the DNA of victims and/or suspects to establish presence or absence at a scene or familial relationships, among other applications. Traditionally, this sequencing involves older technologies, such as Sanger sequencing, and targets only small portions of the genome. More recently, some forensic scientists are advocating for the use of next-generation sequencing, which can capture whole genomes and fragments of degraded DNA too small for Sanger sequencing to capture, to provide more information about the individual of interest, including clues about ancestry and phenotypic traits.

ISOTOPE ANALYSES

Over the last decade, forensic scientists have begun to adapt the mass spectrometry used by ecologists, archaeologists, and paleoclimatologists to uncover hidden dynamics or origins using isotopic ratios. Comparing the relative levels of different isotopes of certain elements—for example, strontium, carbon, oxygen, hydrogen, and nitrogen—in hair, teeth, or bones with abundances of these isotopes in soils or drinking water can suggest a geographic origin, diet, time of death, or travel history for an individual. For example, levels of ^{18}O—a heavier stable isotope of oxygen than normal ^{16}O—in hair can indicate how closely someone lived to a coastline, because drinking water in those regions is typically more ^{18}O rich than inland areas.

INFOGRAPHIC BY JUDE BUFFUM

tle bit of research. And when you start getting into operational forensic work, that's not very attractive."

Outside the chalk outline

In addition to their use in missing-person cases, next-gen sequencing approaches may extend beyond the identification of complete or partial human genomes. In 2015, Faith's colleagues at NCSU published a paper in *PLOS ONE* that reported the sequencing of fungal DNA from nearly 1,000 dust samples collected from indoor environments across the U.S. (10:e0122605). The researchers identified as many as 40,000 fungal taxa in these samples, and developed an algorithm that can place a given sample of dust within a couple hundred kilometers of its geographic origin based on the signature of fungal species it contains.

Isotope analyses are also being developed for use in food safety, wildlife forensics, poaching investigations, and in African ivory smuggling cases.

"Being able to predict within 200 kilometers where something's come from in the United States is pretty impressive," Faith says. "There's no other technology to date that can get that type of precision." High global diversity and their resistance to desiccation make fungal taxa capable geographic identifiers. Faith adds that his group has launched a larger project in concert with the US Department of Defense to create a world map of dust fungal taxa. This, Faith says, may help investigators predict the origin and travel history of samples involved in smuggling cases, port investigations, or hazardous material trafficking, among other scenarios.

Faith has also illustrated the utility of next-gen sequencing in the context of forensic epigenetics to identify individuals (*Electrophoresis*, 35:3096-101, 2014). "Here in the lab, we've done some work to look at tissue sores to establish where the DNAs come from based on the epigenetic attributes, like methylation patterns," he says. "We're also trying to develop an approach to differentiate identical twins based on epigenetic patterns."

And the same mass spectrometry technology that fuels isomapping and other isotope analyses is also applied to characterizing the composition of other molecules hidden away in crime scene samples. "We're seeing folks using mass spec to identify the proteins in hairs, for example, and trying to make those attributable to individuals," Faith explains.

Bowen says that isotope analyses are also being developed for use in food safety, wildlife forensics, poaching investigations, and in African ivory smuggling cases. "There's a lot of different directions that people are going with it."

The mystery lingers

Back at the St. Louis Medical Examiner's Office, Lindsay Trammell is still housing piles of bones recovered from the archeologist's home in central Missouri. No charges were filed in the case, and the wooden coffins,

along with other, nonhuman artifacts contained in them, were returned to the man. According to an official with the Morgan County Sheriff's Office, however, "there are still some people in St. Louis that are trying to trace [the skeletal remains] back to some victims." For that reason, the official says, "we're not at liberty to discuss it yet."

While Trammell's traditional biological workup and the extra analyses performed by Hughes and Juarez made a compelling case that the archaeologist wasn't completely truthful about the bones found in his house, the data generated by these forensic techniques is not yet admissible as evidence in court. (See "Challenges" below.)

"It's a really interesting case, with three anthropologists in the youngest field of anthropology with different specialties," Hughes says. "Then again, it's kind of sad. No charges were filed. This is the hard part about the work. When there's no closure or there's no proper resolution for those individuals whose family members are kept in this guy's house." ■

CHALLENGES

Forensic anthropologists now have a toolbox containing advanced technologies and methodologies, so why haven't next-gen sequencing and isotope analysis become the standard protocol at crime labs across the world?

Forensics in the courtroom

Any method used for gathering information must be heavily vetted and validated before the results are admissible as evidence. This legal bar for newer technologies to generate admissible evidence is so high, says North Carolina State University forensic anthropologist Chelsey Juarez, that her isotope analyses are typically used at the front end, rather than the back end, of criminal cases. "Most of the time, the question that I deal with in terms of isotopes and forensic science . . . it's not necessarily something that would go to court," she says. "It's something that would help law enforcement try to get a lead on a case."

Researchers are now working to codify advanced forensic techniques and make the case for the methods to be established as standard practices for generating actionable evidence. Seth Faith of North Carolina State University, for example, says that he is involved in a working group led by the FBI with the aim of bringing next-gen sequencing into broader forensic use at labs associated with the FBI's Combined DNA Index System (CODIS). "We hear this time and time again from the laboratories: they're not going to move forward until they see those formal guidelines and standards issued by the FBI," Faith says. "So we're in the process of getting those out."

Lack of money

Getting the data generated by these forensic techniques admitted in a court of law isn't the only challenge. Funding on the state and local levels, where many crime investigations take place, is such that buying expensive sequencers or mass spectrometers is simply out of reach. This reality makes it even more remarkable that Lindsay Trammell of the St. Louis Medical Examiner's Office was able to enlist the help of forensic anthropologists who donated their time and analyses to the case of the archaeologist's skeletal cache in central Missouri.

Lack of experts

Another impediment to the more widespread adoption of modern forensic techniques is the fact that practitioners of such methods are few and far between, at least those who specialize in analyzing human remains. "There are extremely few forensic anthropologists who do this even now," says Juarez.

And until methods such as next-generation sequencing and isomapping make their way into more crime labs, it doesn't make sense to train and fledge students in these techniques. "I guess I'm a little torn, because I applaud and I'm excited about the new technologies that we're encountering, that we're using on casework," says Juarez. "But I also have great trepidation because I don't want to encourage an explosion of forensic anthropologists doing isotopes that they can never find a job for."

Lack of awareness

Another obstacle that may be slowing the introduction of high-tech life science tools into the criminal justice system is a level of discomfort among working forensic investigators. If researchers were better communicators, perhaps criminal investigators might be more receptive to using those tools, says Lesley Chesson of IsoForensics. "They don't know that they understand the technique 100 percent, and so they don't know whether it would help their case or not," she says. "We, as forensic scientists in the academic world, need to do a better job about explaining to law enforcement and the actual users of this who would benefit from this, here's how this can potentially help."

Pipeline Reruns

An entire industry has sprung up around resurrecting failed drugs and recycling existing compounds for novel indications.

BY ANNA AZVOLINSKY

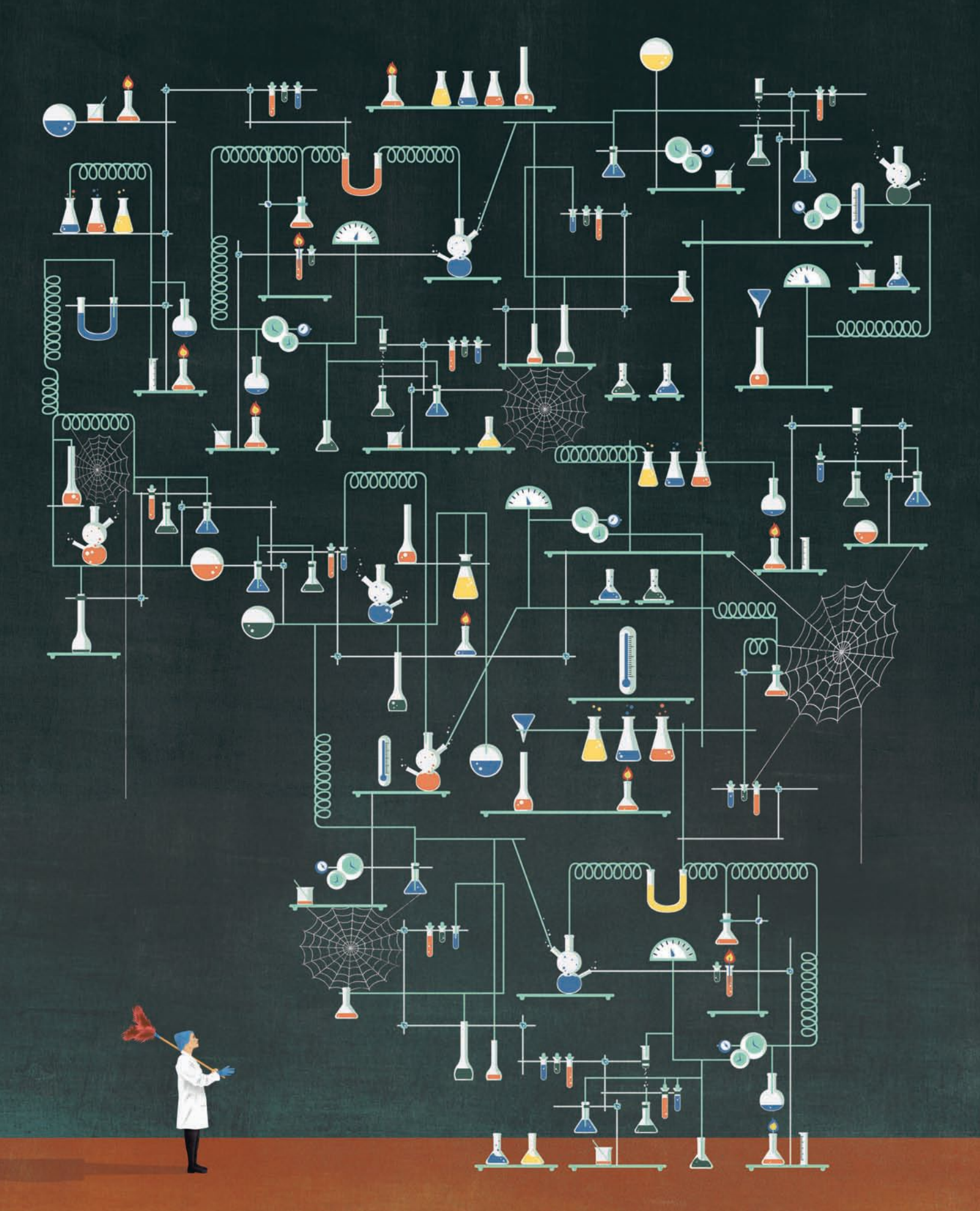
In 2010, Bruce Bloom, CEO of Illinois-based Cures Within Reach, reviewed the organization's decade-long track record of bringing new treatments to patients. He found that the nonprofit had funded 190 novel drug projects, but "couldn't find any instance where it was directly helping patients," says Bloom. Cures Within Reach had also funded 10 different drug repurposing projects, seeking to test existing drugs for novel indications. Of the 10 projects, four generated enough evidence to give physicians confidence to treat patients off-label, which doctors can do at their discretion, particularly when there is no approved therapy for a condition or when a patient has exhausted all available treatment options.

"We then polled 200 researchers and clinicians, and 66 percent of researchers told us they had a [repurposing] project ready for investigation, and 25 percent of clinicians had clinical observations they wanted to test in a trial," says Bloom. "This convinced us that there is a ton [of opportunities] out there for repurposing."

Thalidomide, originally approved in Europe in the 1950s as a sedative and in the U.S. in 1998 to treat leprosy, was one of the initial compounds researchers suggested to Bloom's organization for repurposing—in this case, to treat multiple myeloma. In 2000, Cures Within Reach—which itself receives funding exclusively from nongovernment sources including private foundations—helped support a thalidomide Phase 2 trial at the Mayo Clinic. Because the drug had already been tested as a leprosy treatment, the researchers were able to bypass Phase 1 safety and dosing trials, which can take years to complete. Based on those results, in combination with a handful of other trials of the drug, the US Food and Drug Administration (FDA) approved thalidomide for multiple myeloma in 2012. Bloom estimates it cost only $40–$80 million in total to secure this FDA approval, compared to the average of $1–$2 billion it takes to develop a drug from scratch.[1]

Other researchers are taking similar approaches to find promising therapies already developed for one disease that could help treat another. Many academics have found promise in drugs that have long been on the market—inexpensive generics whose patents have expired. And a handful of nonprofit companies have cropped up to help usher these discoveries, which lack monetary incentive, to the clinic.

Some companies hoping to recoup returns on their investments are also looking to repurpose existing drugs still under patent, such as those that were shelved after unsuccessful trials. Because resources have already been devoted to these unapproved therapies, companies see value in attempting to revamp them for a new indication. "A lot of the cost and risks of drug development has already been surpassed, which is a huge cost benefit," says Craig

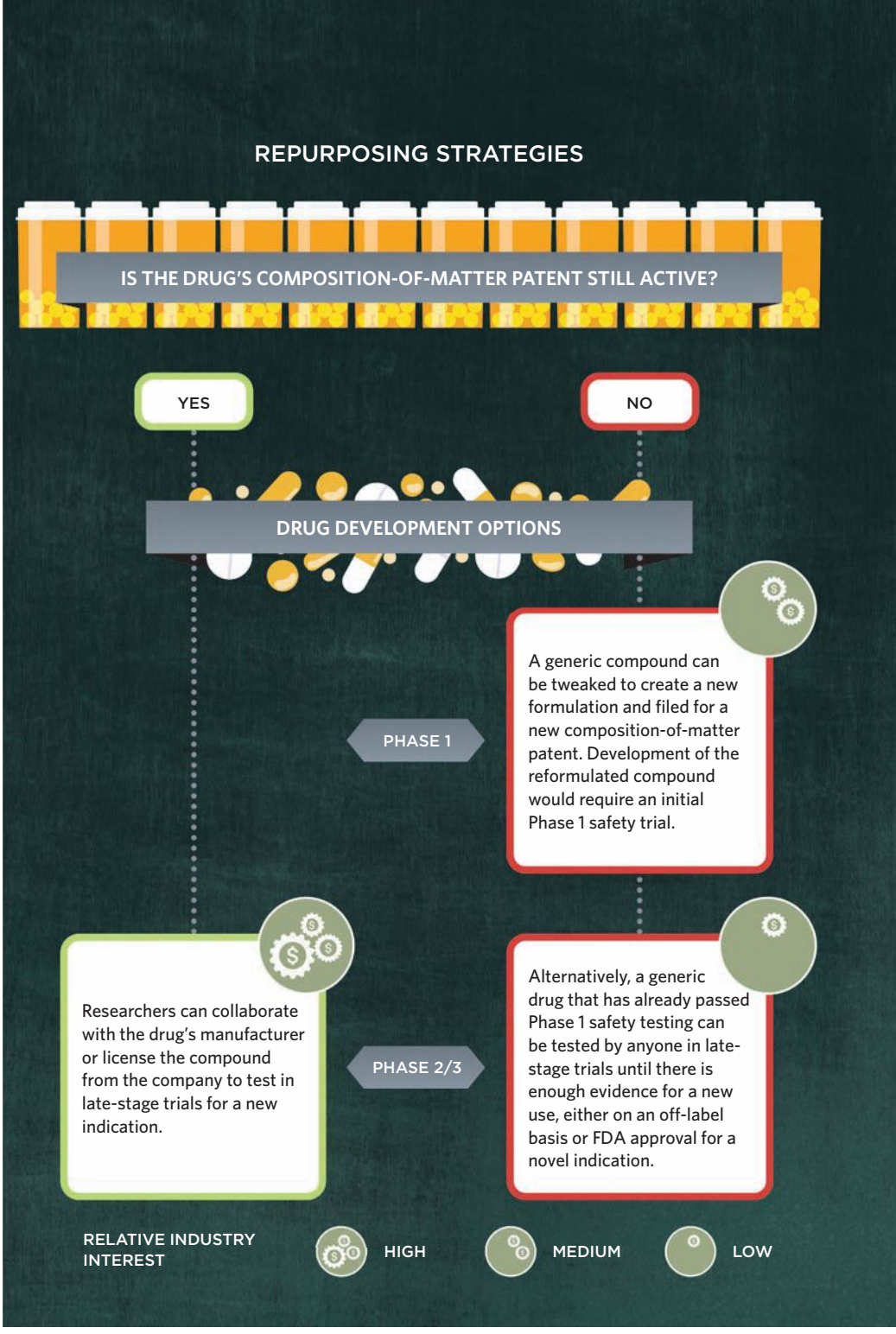


Wegner, head of AstraZeneca's Emerging Innovations Unit in Boston.

Meanwhile, the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) aims to bridge the industry-academia divide by opening pharma's storehouse of compounds to university researchers for study of their mechanisms and potential uses. The center, established in December 2011, funded nine drug projects in 2013 and another four in 2015. Ongoing phase 2 clinical trials grew out of these projects and the center announced funding of several new projects in 2017. "There has been an incredible amount of energy around repurposing in the last five years that was not there previously," says Bloom.

Most successful cases of drug repurposing have been largely serendipitous discoveries. Sildenafil, sold as Viagra since 2005, was tested as a treatment for erectile dysfunction only after erections emerged as a side effect in Phase 1 trials for cardiovascular disease. The antihypertensive minoxidil was reformulated into the topical cream Rogaine after patients using it experienced hair regrowth. But driven by such repurposing success stories, researchers are now taking more-tactical approaches to pinpoint new uses for existing and failed drugs, relying on new high-throughput techniques such as large-scale screens and bioinformatics strategies to mine data for drug-disease connections.

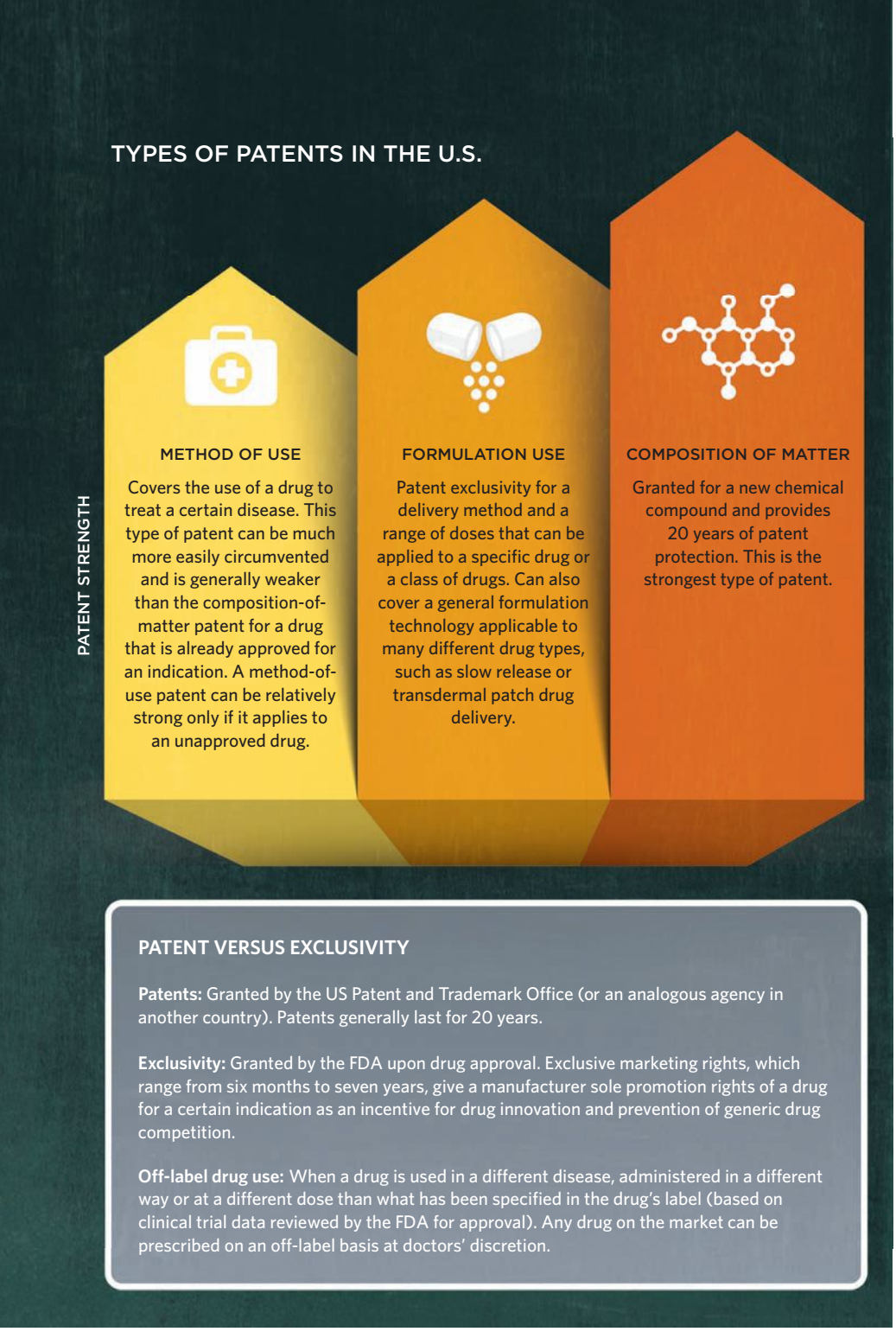
"More and more," says Bloom, "people are thinking of repurposing as a faster, cheaper, safer way to drive therapies to patients and as a method of creating a smarter way of new drug development."



Academia takes the lead

Several years ago, Heath Schmidt of the University of Pennsylvania's Perelman School of Medicine teamed up with Penn clinical researcher Rebecca Ashare to test galantamine's ability to help smokers kick their habit. Galantamine, an acetylcholinesterase inhibitor approved in 2001 for the treatment of Alzheimer's disease, blocks an enzyme that degrades acetylcholine, a neurotransmitter in the brain that's been linked to cognition that also binds to some of the same neuronal receptors that mediate nicotine's rewarding effects. "The idea is that if you can increase acetylcholine signaling in the brain, you could decrease nicotine-related behaviors such as tobacco smoking," says Schmidt.

In 2012, the team launched a Phase 2 short-term efficacy trial, and in a study published last year, found that smokers who took the acetylcholinesterase inhibitor for two weeks had decreased satisfaction from smoking and smoked an average of 12 percent fewer cigarettes compared with smokers who took a placebo.[2] "The known safety and side effects can make a trial much more efficient," says Ashare. The researchers have already launched a second Phase 2 trial to study the drug's effects on longer-term smoking cessation.



Schmidt and Ashare are not alone. Many academic researchers are turning their attention to existing drugs as a potential goldmine of therapies that are cheaper and faster to move into the clinic, and they're getting more methodical in their approach. Stephen Wong, a biomedical engineer at the Houston Methodist Research Institute in Texas, switched his focus from novel drug discovery to repurposing nine years ago when he realized the breadth and depth of clinical trials and basic science information available online. That has "really changed everything for drug development," he says. Wong's lab culls and archives publicly available omics research databases, journal articles and conference abstracts, human clinical trial data, patents, and Houston Methodist's database of longitudinal patient records, as well as privately generated omics data from preclinical disease models. The researchers then mine the information to identify molecules and combinations of molecules that match disease targets and pathways using artificial intelligence algorithms. "We call our technology the DrugX engine," says Wong. "It's like Google but for drug discovery."

The search engine spits out dozens of potential matches for laboratory and animal testing. Wong's team then turns to disease-specific clinicians and researchers who can help narrow down the list. "If we get 1,000 possibilities from our search engine, an expert can likely tell me which few we should actually validate," Wong says. The lab's efforts have led to several Phase 2 clinical trials (skipping Phase 1 safety trials in all cases), including an ongoing one testing the malaria drug chloroquine, administered in combination with chemotherapy, for metastatic breast cancer.[3]

Hua Xu's lab at the University of Texas Health Science Center in Houston also hopes to repurpose drugs, relying exclusively on clinical data. "[Doctors] monitor for bad side effects of drugs, but then we started to think, 'Why couldn't we use electronic health records to find potentially good effects of drugs?'" he says. Xu's group found, for example, that patients with breast, colorectal, or lung cancer who took metformin for type 2 diabetes had better overall survival compared with diabetic cancer patients who took other diabetes medications.[4]

In 2014, the growing popularity of drug repurposing led Hermann Mucke—a biochemist who has run his own consulting firm to advise pharma companies and academic institutions on potential repurposing opportunities for nearly 17 years—to help launch a dedicated journal, *Drug Repurposing, Rescue, and Repositioning,* currently published twice a year as special issues of *ASSAY and Drug Development Technologies*. "Our mid-term goal is to publish regularly as a stand-alone journal," says Mucke, who serves as the editor. "There is more than enough research going on in the field to warrant this."

HOW MUCH CHEAPER IS IT TO REPURPOSE A DRUG THAN TO DEVELOP A NEW ONE?

It takes about 12 years and approximately $2.6 billion to discover a candidate compound and take it through all the preclinical and clinical tests needed for US Food and Drug Administration approval, according to a recent estimate from analysts at London- and New York–based investment firm AB (formerly AllianceBernstein; *Nat Rev Drug Discov,* 11:191-200, 2012). To save money, companies, academic institutions, and nonprofits are getting into drug repurposing, where a pharmaceutical company has already done the heavy lifting of preclinical development as well as Phase 1 and sometimes Phase 2 and 3 human trials. Even with clinical data in hand, however, it is no small feat to conduct successful Phase 2 and Phase 3 trials required by the FDA for approval of a new indication. In fact, these late-stage trials are arguably the most costly phases of clinical testing. A Phase 3 trial program can cost anywhere between $40 million to $300 million, according to AstraZeneca's Craig Wegner.

"That developing a known drug for a repurposed indication would be hugely cheaper than for a single new chemical entity is a common misunderstanding," says pharmaceutical company consultant Hermann Mucke. "The most expensive parts of a drug development program, the late-stage trials, apply to a repurposed development, [including of failed drugs] to the same extent." That said, he adds, "repurposing takes some of the risk out, making drug development more predictable, particularly for rare diseases."

When the AB analysts compared traditional drug development and repurposing costs, bringing a new molecular entity to market still seemed much more expensive than drug repurposing—on the order of $1 billion to $2 billion, compared with about $300 million to repurpose—"but these figures are heavily slanted to favor repurposing," says Wegner. The $1 billion figure includes the totality of all of the compounds a company generates and tests prior to clinical trials, including those that never make it to human trials, according to Wegner. Estimated repurposing costs are "not attrition-adjusted, even though most repurposing efforts also fail," he says. "Repurposing can be less expensive, but it isn't the magnitudes people are talking about."

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While academic labs continue to churn out new leads, they often encounter difficulties garnering industry interest to support trials for a new use of a generic drug. After Eric Verdin, president and CEO of the Buck Institute for Research on Aging in Novato, California, and his colleagues identified two possible clinical uses for an aspirin derivative in mice,[5,6] for example, the team was unable to find a partner to move the compounds into clinical trials. "I'm becoming disenchanted with drug repurposing," Verdin says. "It's impossible to get funding from venture capitalists or even from our institution's intellectual property office." Verdin said he was advised to modify the molecules to make them unique, such that they would be patentable and generate revenue. "But [if the generic version works], this is completely the opposite of what one should do with this type of discovery," he says.

"Repurposed generic drugs do not appear to be good business cases," agrees researcher Michael Pollak, a cancer researcher at McGill University in Montreal. "That's the reality of repurposing." Metformin—a widely used generic and typically the first line of treatment for type 2 diabetes—is a good example. Although the drug may slow the growth of some types of tumors and may even prevent certain cancer types,[7] trial funding has come predominantly from academia. Despite hundreds of small clinical trials, a lack of coordination between academic institutions and industry has resulted in slow development and no clear answer on the drug's efficacy in thwarting cancer growth. "No company expects to make a profit from metformin's use in cancer," Pollak says.

Due to this lack of monetary incentive, "generic drugs found to work for a new disease are in a state of purgatory," says Wegner. Indeed, no generic drug has ever been approved for a new indication by a manufacturer without modification of the drug's delivery or its dose, which would provide renewed patent protection. Someone needs to step up to help move preliminary findings about these cheap and available drugs into the clinic where they

can help patients, Wegner adds. "This is where foundations, advocacy groups, and the NIH can play a huge role."

Nonprofits tackle generics

This unused surplus of widely available, cheap, and potentially beneficial therapies is exactly what the Massachusetts-based nonprofit GlobalCures wants to tap into. "Our goal is to repurpose 'financial orphans'—drugs for which there is evidence of efficacy but that have not gone through rigorous Phase 3 trials because there has been no financial incentive," says cofounder Vikas Sukhatme. GlobalCures catalogs case reports and anecdotal remissions submitted by patients, as well as published preclinical and retrospective human data on noncancer drugs that show promise as anticancer therapies.

"We have trial protocols written and principal investigators all ready to go," says Sukhatme, who studies tumor metabolism and immunotherapy at Harvard Medical School. "It costs $5 million to $10 million for a [small] trial, and we have ideas for 10 to 20 such studies that could be started immediately." Which of those trials will move forward depends on funding, which GlobalCures hopes to receive from NIH grants, private foundations, and donors. "Priority is given to studies that might have the greatest impact in the shortest time frame and use inexpensive medications," says Sukhatme.

The Belgium-based nonprofit Anticancer Fund also supports trials testing agents that have "low commercial interest for industry but that have potential to help patients," says Gauthier Bouche, the organization's medical director. Leveraging its network of collaborators, the Anticancer Fund sifts through published human data, anecdotes regarding off-label drug use, and high-throughput screening results in cultured human cells to decide which approved compounds are worthy of clinical trials for new indications. The organization has teamed up with GlobalCures to write manuscripts and editorials summarizing the outcomes of studies investigating noncancer drugs for different tumor types, and the researchers are working to better understand the regulatory hurdles when seeking to test a drug for a new indication.

A repurposed drug does not necessarily need approval to be considered a success, however. Bloom says that about 80 percent of repurposing efforts at Cures Within Reach aim to demonstrate efficacy of generic drugs for a new indication, providing doctors with enough information to make an informed decision on off-label use. "Our goal is to complete a robust proof of concept trial that gives physicians enough information for off-label use in a patient population that has no other reasonable treatment," he says. To earn FDA approval, the nonprofit would have to secure millions of dollars to run large clinical trials. "The cost of securing marketing approval far outweighs the possible financial return," says Bloom.

Generic drugs found to work for a new disease are in a state of purgatory.
—Craig Wegner, AstraZeneca

This was the approach the organization took when it started investigating the use of the generic mTOR inhibitor sirolimus for pediatric autoimmune lymphoproliferative syndrome (ALPS), a chronic disorder in which blood cells accumulate in the body, causing damage to many organs and sometimes leading to lymphoma. In 2008, in a small trial funded by Cures Within Reach, five of six patients treated had complete remissions.[8] After publishing the results the following year, the news began to spread among clinicians and patients. The inexpensive drug, originally approved in 1999 as a prophylactic treatment to prevent rejection of renal transplants, is now prescribed off-label for ALPS (and, more recently, for other similar autoimmune disorders in children).[9]

"Prior to the work in ALPS, the kids that were refractory to steroids or other drugs had no therapy; they suffered and died," says Bloom. "Now they have a therapy, and physicians know it is available, and it works. The patients are getting the care, and that is a success."

In bed with industry

There are also players in the repurposing field looking to turn a profit. Like academics and nonprofits in the field, biotechnology companies focused on drug repurposing are also finding innovative ways to mine publicly available information on existing compounds to uncover new drug-disease connections. (See "Teaching an Old Drug New Tricks," *The Scientist,* April 2011).

In 2008, University of California, San Francisco, pediatric endocrinologist and bioinformatician Atul Butte launched NuMedii to capitalize on his new data-mining technology that identifies potential links between drug profiles and the molecular pathways of disease. "All of the information we put into our system"—including available data on marketed drugs, generic compounds, and unapproved drugs abandoned by pharmaceutical companies during development —"is carefully curated to enable us to come up with potential clinically and commercially viable data," says Gini Deshpande, NuMedii's cofounder and CEO. "We use a lot of omics data and take an unbiased perspective to find where there may be yet undiscovered biology that we can leverage." The company then tests the most promising candidates in animal models. NuMedii has yet to take a candidate drug into the clinic, but has several "clinic-ready" compounds, according to Deshpande.

If one of the drug candidates NuMedii revives is not a generic, but a shelved, patented drug owned by a pharmaceutical company, the biotech can partner with the pharma firm for further development or obtain rights to the compound and carry on solo. But other routes exist. Some in the field are pulling for collaboration—not just among companies, but with academics of diverse expertise as well. "Drug development using bioin-

formatics is incredibly complex," says Bloom. "Right now, different companies and labs have each started to figure out a piece of the puzzle." Mucke adds: "The real interesting things will come if you use each to its full advantage and cross-link them together."

Fostering such collaborations is one of the main goals of the NIH's NCATS program, which aims to uncover new uses for the compounds pharmaceutical companies still own but whose development has been halted. NCATS asks that companies make some of these shelved compounds—and the accompanying preclinical data—available to academic researchers at no cost. The program then provides this information as well as a supply of the drug to research labs that can study new, clinically relevant activity of the drug. The company retains full control of the rights to the drug and the ability to file for the new indication. For U.K.-based AstraZeneca, the program allows the company to tap the knowledge and experience of outside experts for a disorder that may not be on the company's radar, says Wegner. "The program can benefit patients, the investigators, possibly AstraZeneca, and at the minimum, advance science."

"There have even been instances where our program elected not to fund a project, and the pharmaceutical company stepped in and provided funding, working with researchers on their own," says Christine Colvis, who heads the NCATS program. NCATS currently has 10 ongoing projects, including eight Phase 2 clinical trials. One of NCATS's most advanced projects involves AstraZeneca's shelved cancer drug saracatinib, which in 2012 was found to target amyloid-β signaling in the brain and to rescue synapse loss in mice.[10] A Phase 2 trial of saracatinib for Alzheimer's patients completed enrollment at the end of 2016.

"What I like about drug repurposing is that it can solve two issues: improved health-care impact and reduced health-care cost," says Bloom. "That's a big driver for us." ■

More and more, people are thinking of repurposing as a faster, cheaper, safer way to drive therapies to patients and as a method of creating a smarter way of new drug development.

—Bruce Bloom, Cures Within Reach

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The Literature

EDITOR'S CHOICE IN GENETICS & GENOMICS

Unfolding a Mystery

THE PAPER

J.U. Guo, D.P. Bartel, "RNA G-quadruplexes are globally unfolded in eukaryotic cells and depleted in bacteria," *Science*, doi:10.1126/science.aaf5371, 2016.

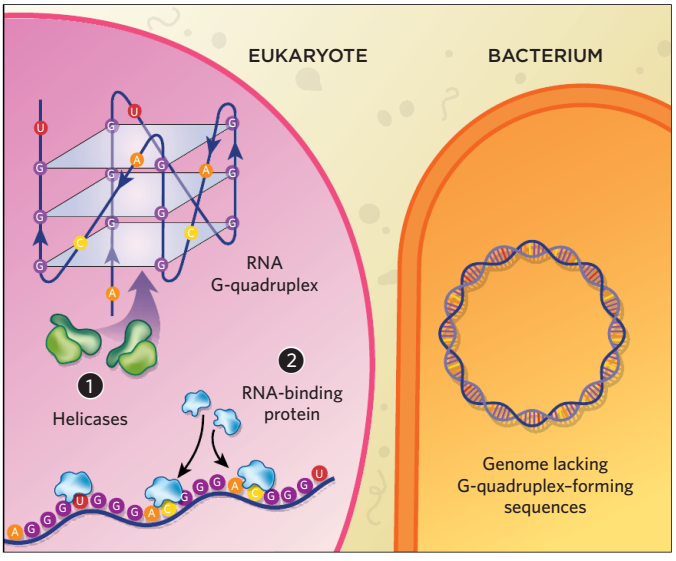
RNA doesn't lie flat. Interactions between nucleotides can turn sections of transcripts into loops, bends, and knots, some of which have regulatory functions in the cell. It was with these tangles in mind that Junjie Guo, a postdoc in David Bartel's lab at MIT, developed an in vitro chemical probe to detect folded regions of RNA. "We were trying to identify all the structures that can stably form in vitro," he says.

Testing the technique on transcripts extracted from mouse embryonic stem cells, Guo found that one particular conformation was unexpectedly abundant: RNA G-quadruplexes—stable, guanine-rich regions folded into four-stranded structures. "Only dozens of [these] regions have been studied previously," Guo notes. "But we identified thousands of endogenous RNA regions that can form these structures in vitro."

RNA G-quadruplexes, first identified more than five decades ago, were for a long time mysterious in terms of function, Guo says. There's evidence now that they influence translation, and they may be involved in diseases including cancer and neurodegeneration. But although quadruplexes are thought to form readily in the chemical environment of the cytoplasm, they have not been studied in action because they are difficult to identify inside living cells. So Guo and Bartel used their new probe to do just that.

They didn't find quite what they were looking for. It turned out that the quadruplex-forming regions detected in vitro weren't actually folding up inside mouse cells, Guo explains. "We found that most of these structures are in an open state." And it wasn't just mammalian cells. Using the technique in yeast yielded similar results: the transcriptome contained regions that formed quadruplexes in vitro, but these regions were not folded in vivo. The results suggest eukaryotes possess machinery to prevent quadruplex formation.

Looking for further insights, the researchers turned to bacteria. Unlike eukaryotic RNA, *Escherichia coli*'s transcriptome exhibited no obvious quadruplex-forming regions. However, when Guo introduced specific guanine-rich regions to cells via a plasmid, he found the mRNA easily folded into quadruplexes, triggering abnormal protein translation and reduced growth rates. The results "could partly speak to why eukaryotes have come up with machinery that suppresses formation of these structures," Guo says. The findings also imply that, instead of developing similar machinery, bacteria have eliminated quadruplex-forming sequences over the course of evolution.

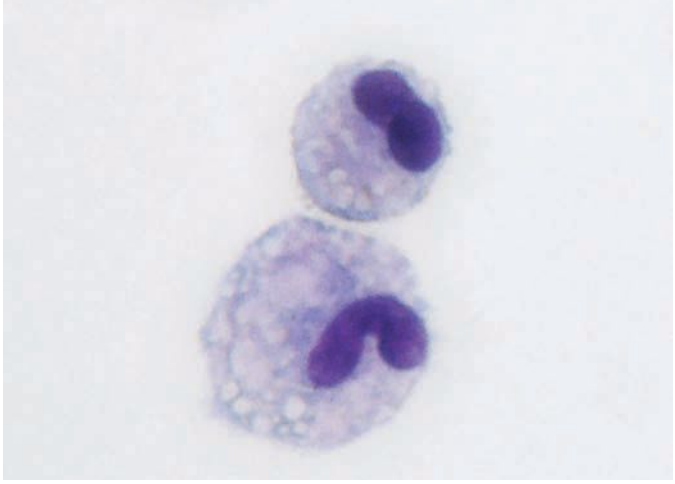


IN A TWIST: Certain guanine-rich regions of the transcriptome can fold up into four-stranded structures called RNA G-quadruplexes that are thought to have harmful effects in living cells. Both eukaryotes and bacteria have evolved mechanisms to keep these structures unfolded, according to a new study. Eukaryotes likely have protein machinery—possibly helicases to unravel the structure 1 and proteins that bind to the transcripts to keep them from reforming 2. Bacteria appear to have depleted quadruplex-forming sequences from their genomes during their evolution.

Stephen Neidle, an emeritus professor of chemical biology at University College London, says Guo and Bartel's eukaryotic results have particular relevance for the study of disease. "[They're] saying that the prevalence of sequences does not directly map onto the prevalence of stable structures," he notes. "There's significant literature discussing isolated RNA quadruplexes as therapeutic targets—this paper is putting much of that work on its head and saying, actually, those targets [may be] illusory."

Guo says he and Bartel are now looking for components of the eukaryotic unfolding machinery by identifying proteins that bind quadruplex-forming regions of RNA. "We're getting some very interesting candidates," he says, and adds that while "some have previously been shown to bind G-rich RNA, some have never been characterized."

—Catherine Offord



LAST ONES STANDING: Unlike other immune cells, these vaccine-induced macrophages from a mouse's lung manage to withstand chemotherapy treatment.

IMMUNOLOGY

Hidden Immunity

THE PAPER

A. Kamei et al., "Exogenous remodeling of lung resident macrophages protects against infectious consequences of bone marrow-suppressive chemotherapy," *PNAS*, doi:10.1073/pnas.1607787113, 2016.

BLOODLETTING

Chemotherapy wipes out cancerous cells and dividing normal cells alike, often particularly damaging those in bone marrow that produce white blood cells. As a patient's immune system is weakened, even minor infections can become life-threatening. Researchers are exploring ways to circumvent this problem by "remodeling" the immune system prior to chemotherapy.

BELOW THE RADAR

Akinobu Kamei of St. Jude Children's Research Hospital and his colleagues identified a class of white blood cell that only becomes active in the lungs of mice following vaccination for a common bacterial strain that causes pneumonia. Like some other immune cells in the lungs, these so-called vaccine-induced macrophages, or ViMs, do not originate in bone marrow, but reside solely in the lungs, likely having derived from progenitor cells in the lungs during embryogenesis.

SURVIVORS

The St. Jude team found that ViMs are not decimated by chemotherapy like other immune cells—in fact, their numbers don't dip at all. It's not clear how ViMs manage this feat, says Kamei, but mice that were vaccinated before chemotherapy, triggering ViMs, survived bacterial infections at much higher rates than unvaccinated mice.

OUTLOOK

"The future plan," says Kamei, "is to induce lung tissue [immune] remodeling to compensate for bone marrow suppression after chemotherapy." Immunology researcher Sandro Vento of Nazarbayev University in Kazakhstan pointed out in an email to *The Scientist* that the animal-model work is only preliminary. "This is an initial study which opens a new area of research, and it will be important to understand the mechanisms which allow vaccine-induced macrophages to survive chemotherapy." —Ben Andrew Henry

PARENTAL MEMORIES: Spotted lady's thumb plants whose parents survived drought conditions are more hardy, perhaps due to DNA methylation patterns.

GENETICS & GENOMICS

Hand-Me-Downs

THE PAPER

J.J. Herman, S.E. Sultan, "DNA methylation mediates genetic variation for adaptive transgenerational plasticity," *Proc R Soc B*, doi:10.1098/rspb.2016.0988, 2016.

CONVENTIONAL WISDOM

The notion that organisms pass down adaptations acquired during their lifetimes to their offspring was overturned long ago by Darwinian evolution. But the concept is getting a second chance, with more nuance. Growing evidence shows that a parent's environment sometimes does influence offspring, though the underlying process is something of a black box.

GROWING UP HARD

Sonia Sultan and Jacob Herman of Wesleyan University in Middletown, Connecticut, peeked into that box with experiments on a small flowering annual, *Polygonum persicaria*. They grew some plants in dry soil and other plants in normal soil, then raised offspring from all plants in dry soil. In some members of the progeny, they disrupted 15 percent to 20 percent of DNA methylation—a means of epigenetic regulation—across the genome.

LESSONS LEARNED

Plants whose parents endured drought were better prepared to face the same hardship, growing larger as seedlings, setting down deeper roots, and making broader leaves. But in demethylated plants, that effect went away, lending evidence to the idea that epigenetics had a role in this form of adaptation.

NEW SCHOOL

Adjusting offspring traits to better suit the parental environment is itself an evolved trait, notes Julie Etterson, an evolutionary ecologist at the University of Minnesota Duluth who was not involved in the research. The authors used plants from 12 genetically distinct populations and not all of them showed the transgenerational effect, raising questions about when and why this trait is advantageous. Sultan says these environmental hand-me-downs are a phenomenon "not accounted for by standard models of evolution." —Ben Andrew Henry

ST. JUDE CHILDREN'S RESEARCH HOSPITAL; WIKIMEDIA COMMONS/JAVIER MARTIN

PROFILE

Methylation Maestro

After initially discovering that DNA methylation represses transcription, Howard Cedar continues to explore how the epigenetic mark regulates gene expression.

BY ANNA AZVOLINSKY

In 1963, when Howard Cedar was a junior-year physics major at MIT, a specific event changed the course of his life, he says. He had just gotten back a graded exam in an atomic physics course. "I got a 95 percent, but when I went over the exam, I noticed that for one of the questions, I had done the calculations correctly yet had written an answer that was about 30 orders of magnitude from the correct answer. The professor had seen that I had done the calculations and had just made a mistake in the final answer and I got almost full credit. But when I saw that, I said to myself, 'How can you call yourself a physicist if you don't know the difference between one and 10 to the power of 30? Something's wrong here! I realized I was only getting by in physics because I was good at math," says Cedar, now a professor of molecular biology at Hebrew University in Jerusalem.

"Today, when we don't know what causes a disease, we say it's likely epigenetic. In a sense, I think this epigenetic viewpoint is right, but we need much more evidence to get a defined picture."

Cedar then changed his plan to become a physicist and applied to medical school. "It was a practical decision. I thought I could be a good enough doctor." Cedar entered New York University (NYU) in 1964. When he arrived, he learned about a brand-new six-year program initiated by the US government to support the training of MD/PhDs. "This was the first year of the program. I only heard about it when I arrived at NYU and leapt at the chance because the program paid for students' tuitions. My parents were not wealthy and had difficulty paying for my tuition at MIT. I wanted to be independent and relieve some of the pressure on them." Cedar was one of five students accepted to the NYU arm, and he became the first person in the U.S. to complete the newly minted national program. He then pursued an academic career, becoming a pioneer in the study of eukaryotic gene expression, figuring out the rules for how DNA methylation affects gene activity.

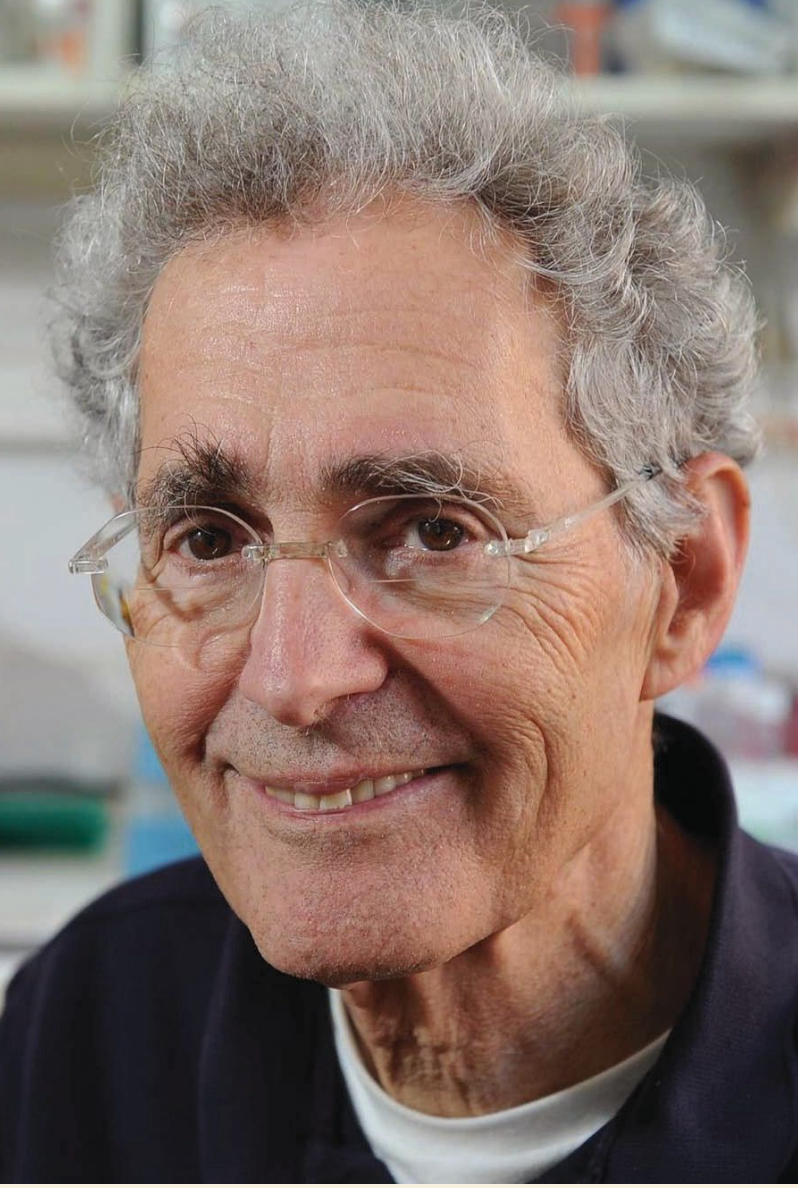
Here, Cedar recalls how his choice of a postdoc set him on a course to study DNA methylation and gene expression, why he chose to launch his laboratory in Israel despite advice to remain in the U.S., and what it's like to preside over a family of six children and 22 grandchildren.

CEDAR'S CALLING

Logical beginnings. Cedar was born in Brooklyn in 1943, during World War II. "After the war, there were many new children and a lot of excitement. I grew up in a very encouraging and warm environment," he says. Cedar's father, who was born to immigrants from Poland, was a manager at a factory, and his mother, an immigrant from Ukraine, stayed home with him and his sister. As a child, Cedar loved math puzzles and problem solving. "My parents used to tell me that I would figure out the shortest route to get somewhere or the optimum time for an activity to be most efficient. This has remained part of my being throughout my life," says Cedar. Attending high school in New Jersey after his father's factory was relocated to Morristown, Cedar excelled in math and physics and in 1960 set off for MIT. "MIT was an amazing place for me," says Cedar. "MIT had a philosophy that every student, no matter the major, needs to come out with a solid foundation in the basic sciences. I find myself still using that knowledge."

Lab rat. Cedar says he was not the best student in NYU's MD/PhD program, but he did enjoy learning about the life sciences. "I was infatuated with general biology principles. It was the beginning of the molecular biology revolution," says Cedar. After three years of medical school and clinical rotations, Cedar chose to work with James Schwartz, who started at NYU as a microbiologist and then became a neurobiologist, using the sea slug *Aplysia* to study how memory works. Part of Schwartz's research included examining how learning affects chromatin structure. Cedar was Schwartz's first graduate student. "It's very good to be the first graduate student; you get a lot of attention," says Cedar. He studied *E. coli*'s synthesis of L-asparaginase, which catalyzes the hydrolysis of the amino acid asparagine to aspartic acid, and purified asparagine synthetase from the bacterium to study how that enzyme catalyzes the reverse reaction, using aspartate to produce the amino acid in vitro. Cedar enjoyed working in the lab and the make-your-own-hours lifestyle so much that, by end of his PhD, he decided to go the research route rather than practice medicine.

A blank slate. Cedar did a one-year internship in the neuroscience lab of future Nobel Laureate Eric Kandel, who was then at NYU, working on the role of cAMP metabolism in memory formation in *Aplysia*, and then obtained a research fellowship at the National Institutes of Health (NIH) in Bethesda, Maryland. At the NIH, Cedar chose to work with Gary Felsenfeld, whose lab



HOWARD (CHAIM) CEDAR

Professor of Molecular Biology, Faculty of Medicine,
Hebrew University in Jerusalem, Israel

Greatest Hits

- Demonstrated, with Richard Axel and Gary Felsenfeld, that chromatin can restrict transcription by globally blocking most of the genome while allowing access to specific genes
- Along with Aharon Razin, showed that methylated DNA is stably propagated to daughter cells following mitotic cell division, and outlined the mechanism by which methylation sites are maintained through cell division
- Was first to demonstrate that DNA methylation inhibits transcription and that undermethylated DNA is associated with actively transcribed genes
- Provided some of the first evidence for epigenetic reprogramming by proving that methylation patterns are erased in the early embryo
- Discovered the molecular rules involved in establishing DNA methylation patterns during development

was beginning to study the relationship between chromatin structure and gene expression. "It was a marriage made in heaven," says Cedar, who initially didn't know anything about chromatin or nucleic acids, he says. "Gary was a real pioneer in nucleic acid biochemistry and an exceptionally good chemist who had studied with Linus Pauling. He tried to apply this chemistry to biology. My entire understanding of the chemistry of nucleic acids, which not too many people learn anymore, I got from him."

In 1971, when Cedar arrived at the NIH, scientists had not yet figured out chromatin's structure because they did not know how to isolate it. "We knew there were histones but we didn't know anything about nucleosomes, and we didn't know that chromatin had a well-defined structure. Gary pioneered working with chromatin, but from a chemist's perspective. We used sophisticated chemistry tools in his lab, but primitive ones for understanding biology." When Cedar arrived, he began to perform some of the first experiments addressing the biology of chromatin.

Cedar figured out how to solubilize chromatin and used purified *E. coli* RNA polymerase to understand how the enzyme interacts with chromatin. In 1973, he published a paper describing an in vitro transcription system using either chromatin or protein-stripped DNA from the calf thymus. Cedar observed that initiation sites on chromatin are much less frequent compared to those on DNA, and that transcript elongation occurs three times more slowly on chromatin. "We learned a lot about the ways that chromatin restricts accessibility to RNA polymerase, which in a sense is a key element of chromatin—it restricts access. These were the first experiments to really show that," says Cedar.

CEDAR CHURNS

A window into accessibility. After Richard Axel—a 2004 Nobel laureate—arrived in Felsenfeld's lab to do a postdoc, he and Cedar set out to show that RNA polymerase has selective access to certain genes. They used a single-stranded piece of DNA that was complementary to transcripts of the *globin* gene to detect whether the transcript was forming in vitro. When they combined purified RNA polymerase with duck reticulocyte–derived chromatin in a test tube, the polymerase was able to transcribe the globin gene. In chromatin derived from other tissues, however, RNA polymerase did not produce a globin transcript. "The experiment was the first, really, to show that gene regulation is controlled by accessibility. What we had known about gene regulation had all come from bacteria, and this was a whole new concept that is now the basis of all gene regulation in eukaryotes," says Cedar.

A major move. Cedar and his wife wanted to build a life for themselves and their two children in Israel, so after two years in the Felsenfeld lab, he applied for faculty positions there. "We went to Israel for idealistic reasons. We both wanted to be part of the new state that was established in 1948." Cedar was offered and accepted a faculty position at the Hebrew University Medical School in Jerusalem in 1973. In his new lab, he continued to study how chromatin interacts with proteins in the cell and to investigate chromatin's structure. Two years later, Roger Kornberg's lab digested chromatin with micrococcal nuclease and ran the digest on a gel, showing repeated 200-base-pair nucleosome units. "Kornberg's lab immediately saw the DNA ladder and that gave away the basic structure of chromatin. We didn't have gels in Gary's lab, we only used ultracentrifugation, which only gave us vague properties of chromatin."

Epigenetics 101. At Hebrew University, Cedar began to focus on the role of DNA methylation in modulating gene expression. Since the 1940s, researchers had known that 5-methylcytosine is found in the genomes of prokaryotes and eukaryotes. In prokaryotes, DNA methylation serves as a way to protect the genome from the integration of foreign DNA. "That's basically what was known about methylation. There was an idea going around that, maybe in animal cells, DNA methylation is involved in regulating genes, but it wasn't based on much evidence. So we just basically started playing around with it," says Cedar.

He teamed up with another faculty member, Aharon Razin, who was working on DNA methylation in bacteria, and in 1977 the two initially showed that 5-methylcytosine is concentrated in the parts of chromatin that are protected from nuclease digestion, suggesting a connection between methylation and a closed chromatin structure. Cedar and Razin next built a system to test whether DNA methylation can affect gene expression, using bacteria-derived restriction enzymes that do not cut DNA at methylated sites. Working together, the two labs introduced foreign methylated or unmethyated DNA into mammalian cells in culture and demonstrated, using the restriction enzymes, that methylated sites remained methylated after DNA replication in the daughter cells.

"We knew that about 80 percent of CG pairs in eukaryotic genomes were methylated, but we didn't know how methylation was distributed, and we knew nothing about its metabolism," says Cedar. "Our experiment showed that DNA methylation was a stable part of the genome and that it was not a random distribution of methylation but that it was specific."

The how of methylation. Cedar and Razin next tackled the substrate for DNA methylation. Using purified eukaryotic DNA methylase, they showed that, in vitro, the enzyme prefers to methylate only the DNA strand already bearing the groups, suggesting that cells maintain strand symmetry of DNA methylation. Then, using gene transfer, the two labs showed that site-specific DNA methylation could inhibit transcription in mouse cells. Cedar's lab also used DNA-targeting enzymes to show, genome-wide, that actively transcribed genes are generally undermethylated.

CEDAR CRESTS

Critical mark. In the 1990s, Cedar's lab turned to understanding the dynamics of DNA methylation during early development. An earlier study had showed that levels of DNA methylation are lower in the preimplantation embryo than would have been predicted from the amount of DNA methylation in germ cells. In 1992, Cedar and Razin's labs together provided more-concrete evidence that methylation marks are erased in the early embryo. In tissues, as recently as a year ago, it was not clear whether demethylation of a gene is required to turn on transcription. "This has been a big bone of contention, and a lot of critics were saying that demethylation is just part of the deactivation process, that you don't really need it as long as the transcription factors are there," says Cedar. In May 2016, along with Yehudit Bergman, also at Hebrew University, Cedar's lab demonstrated in B cells that demethylation is indeed necessary for proper tissue-specific transcription, independent of transcription factors.

Driver of change. Cedar's lab is now trying to address how environmental changes can modify patterns of DNA methylation using male and female mice. "The question is whether the environment can actually change the genome template in a stable manner. And I don't think we really know the answer to that question, even though I would guess the answer is 'yes.' When I was a medical student, every time my professors didn't know what caused a disease, they would say it was caused by a virus. Today, when we don't know what causes a disease, we say it's likely epigenetic. With this point of view, we get things wrong sometimes, which leads to some misconceptions. In a sense, I think this epigenetic view point is right, but we need much more evidence to get a defined picture [of how far epigenetic functions extend]."

Research personalities. "I came to Israel at point zero in my career. Many people told me that I was making a sacrifice to leave the U.S. because it was the real center of scientific activity, and that Israel was a new country that didn't have a developed research infrastructure. Despite that, I went, and I was not disappointed. Israel turned out to be a powerhouse of science, but with a different scientific personality than that of the U.S. In the U.S., there is an emphasis on big science. Some of the best labs are big labs with a lot of postdocs and students and they tackle big problems, which you can only do with a lot of funding. In Israel, the labs are smaller and there is an emphasis on basic science, which changes the nature of the lab. It encourages younger students to be more involved, and they are allowed much more creative freedom."

A tree grows. Cedar and his wife, a teacher and psychodramatist who treats children through group interventions, have six children, all living in Israel, and 22 grandchildren. Their oldest son is a screenwriter and director; another son is an epidemiologist. "The holidays, when we all get together, are very interesting. We all have a lot of fun together." ■

SCIENTIST TO WATCH

Jeremy Day: Reward Researcher

Assistant Professor, University of Alabama at Birmingham. Age: 35

BY CATHERINE OFFORD

As an undergraduate at Auburn University in the early 2000s, Jeremy Day was thinking of becoming an architect. But an opportunity to work on a research project investigating reward learning in rodents changed the course of his career. "It really hooked me," he says. "It made me immediately wonder what mechanisms were underlying that behavior in the animal's brain."

It's a question Day has pursued ever since. In 2004, he enrolled in a PhD program at the University of North Carolina at Chapel Hill and began studying neural reward signaling under the mentorship of neuroscientist Regina Carelli. "He was a stellar student by all accounts," Carelli recalls. "He was very clear on the type of work he wanted to do, even that early on in his career." Focusing on the nucleus accumbens, a brain region involved in associative learning, Day measured dopamine levels in rats undergoing stimulus-reward experiments. Although a rat's brain released dopamine on receipt of a reward early in training, Day found that, as the rodent became accustomed to specific cues predicting those rewards, this dopamine spike shifted to accompany the cues instead, indicating a changing role for the chemical during learning.[1]

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Day completed his PhD in 2009, but realized that to better understand dopamine signaling and errors in the brain's reward system that lead to addiction, he would need a broader skill set. "I had a strong background in systems neuroscience, but my training in molecular neuroscience was not as strong," he explains. So he settled on "a field that I knew almost nothing about"—epigenetics—and joined David Sweatt's lab at the University of Alabama at Birmingham (UAB) as a postdoc. For someone used to a field where "data come in as it's happening," Day says, "transitioning to a molecular lab where you might do an assay and you don't get an answer for a week or two was a culture shock."

Initially, Day investigated epigenetic modification in the nucleus accumbens. "The idea was that we'd block DNA methylation and see if we could also block learning," he explains. But things didn't go according to plan. "We worked on that for a couple of years and, basically, all the results from that experiment were negative."

Instead of giving up, Day refocused. "He demonstrated a lot of perseverance," recalls Sweatt. "It really took commitment and determination to stick with the project." Leaving the nucleus accumbens, Day tried similar experiments in another site involved in dopaminergic pathways. "We found that if we blocked DNA methylation in that region, we could completely block an animal's ability to learn about rewards," Day says.[2]

In 2013, Day received a grant from the National Institute on Drug Abuse (NIDA) that helped him set up as an assistant professor at UAB the following year. He has continued collaborating with Sweatt—now at Vanderbilt University School of Medicine—who calls his former postdoc "a rising star in the discipline." In 2016, they published evidence that extra-coding RNAs—noncoding RNAs whose sequences overlap with protein-coding regions—help regulate neuronal DNA methylation in an activity-dependent manner.[3]

Now, Day is most excited about CRISPR-Cas9's potential to explore epigenetics in the brain. "For the first time, we have the ability to look at the causal role for these modifications in gene regulation, neural function, and behavior," he says. "It's a really fun time to be in the field." ■

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Parent Trap

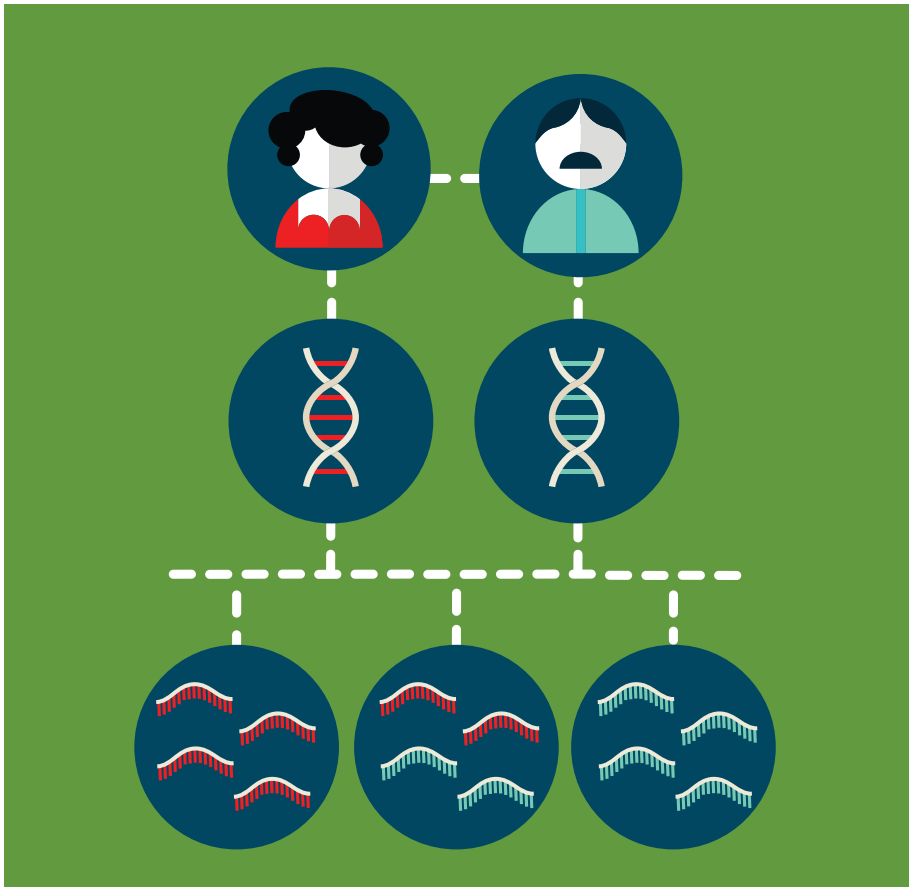
Techniques for identifying imprinted genes

BY AMBER DANCE

Throughout the genomes of mammals and plants, certain genes carry marks that indicate whether they came from mom or dad. Typically, these marks are methyl groups that regulate gene expression so that one parent's allele is selectively expressed. Together, these imprinted genes make up the imprintome.

Scientists used to search for imprinted genes one by one, but thanks to modern sequencing techniques, they can now scan entire genomes. The precise size of the imprintome is uncertain, particularly since imprinting patterns vary among tissues and at different times in development. Estimates suggest there are approximately 100 to 150 or so imprinted genes in humans and in mice, and 90 or more in the plant model *Arabidopsis thaliana*. Many imprinted regions of the genome can contain sequence variants linked to human diseases, such as diabetes. Because only one copy of an imprinted gene is expressed, loss-of-function mutations are more likely to cause problems in an imprinted situation.

Identifying a full list of imprinted genes for humans and model organisms will give scientists a springboard to characterize the mechanisms and functions of imprinting, says Ian Morison of the University of Otago in Dunedin, New Zealand. That's an ongoing effort, and there have been plenty of hurdles along the way. Piroska Szabó of the Van Andel Research Institute in Grand Rapids, Michigan, was once excited to think she'd discovered a new gene that expressed only the maternal allele—until she realized that the RNA sequences she was looking at were from a gene that had been misannotated as a nuclear gene, explaining the maternal-only inheritance.



There are other ways, too, of being misled. Some papers have identified upwards of 1,000 potentially imprinted genes, only to have most shot down later as false positives. These false hits can arise when cells select one allele for other reasons—it could be random or specific to the allele's sequence, rather than its parent of origin.

The need to accurately evaluate sequences makes a bioinformatician a key member of any imprintome team, advises David Monk of the Bellvitge Institute for Biomedical Research in Barcelona, Spain.

The Scientist asked imprinting experts to share their techniques for teasing out the imprintomes of mouse, *Arabidopsis*, and human, and to propose some ideas for new technologies and investigations that would move the field forward.

TEST CASE

RESEARCHER Piroska Szabó, Associate Professor, Center for Epigenetics, Van Andel Research Institute
ORGANISM Mouse
METHOD RNA-seq

In 2014, Szabó and colleagues reported how they'd used mouse embryonic fibroblasts to test whether the relatively new method of RNA sequencing could reveal known and novel imprinted genes.

The team started with two different strains of mice, which had known genomic differences. They crossed these strains and sequenced the RNA of the resulting offspring.

For any gene in which the maternal and paternal genomes differed in sequence, the researchers could look at the RNAs for that gene, and ask which alleles were transcribed. For most genes, they'd see a 50:50 split between maternal and paternal codes. But for imprinted genes, they'd expect to see mostly maternal, or mostly paternal, codes represented in the RNA.

FINDINGS The researchers identified 32 known imprinted genes, but no new ones, implying that the list of imprinted genes in the mouse—at least in embryonic fibroblasts—is nearly complete, says Morison, who was not involved in the study (*Nucleic Acids Res*, 42:1772-83, 2014).

PROS

- Performing both versions of the cross, with each strain standing as the mother or father, helps confirm the imprinting.
- The team used paired-end sequencing, which starts reads from both ends of a cDNA, and can help identify genes in which only certain splice variants are imprinted.

CONS

- Genes expressed at low levels are susceptible to being falsely identified as imprinted, because there are only a few transcripts that might randomly lean toward one or the other parental allele. Szabó and colleagues required a minimum of 10 sequence reads to call a gene as imprinted.
- Even dubbing highly expressed genes as imprinted is fraught with uncertainty, as some imprinted genes aren't expressed in an all-or-nothing manner. The researchers set a cutoff, 80 percent expression of one allele or the other, for calling a gene as imprinted. Different groups choose different cutoffs, and a too-lenient cutoff could yield false positives.

WISH Szabó would like to see techniques for single-cell imprintome analysis, as well as more studies of different tissues at different times during development, which might still yield more imprinted genes.

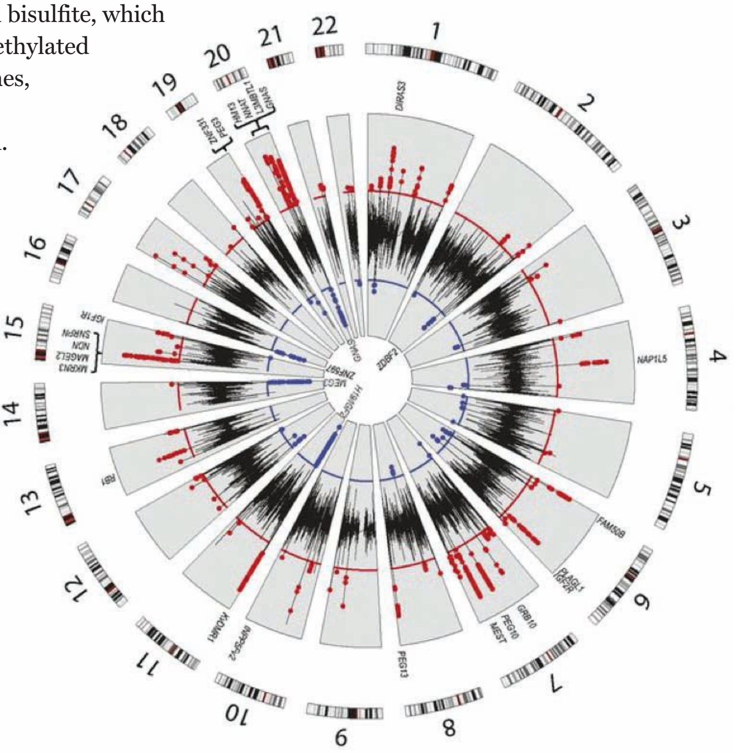
ONE-PARENT SAMPLE SET

RESEARCHERS Kazuhiko Nakabayashi, Division Chief, Department of Maternal-Fetal Biology, National Research Institute for Child Health and Development, Tokyo, Japan; David Monk, Principal Investigator, Epigenetics and Cancer Biology Program, Bellvitge Institute for Biomedical Research
ORGANISM Human
METHODS Bisulfite-seq; bisulfite-chip

Methylation is typically associated with the silencing of the nonexpressed allele, making it a convenient marker for imprinted genes, though it's possible for patterns of differential methylation to exist in tissues where both alleles are expressed. Nakabayashi, Monk, and collaborators studied methylation patterns in adult and umbilical blood and placenta cells from healthy volunteers; brain tissue from a brain bank; and a cultured liver cell line. The team treated genomic DNA with bisulfite, which converts only unmethylated cytosines to thymines, leaving methylated cytosines unaltered. By sequencing, they could determine gene methylation patterns. The researchers figured that genes that were consistently half-methylated across a variety of tissues were possibly imprinted.

To confirm imprinting and identify the parent of origin, the authors then compared those methylation patterns to methylation in tissues affected by a phenomenon called uniparental disomy, in which both copies of a genome (or a chromosome or partial chromosome) come from one parent. One such sample was from growths called hydatidiform moles that develop in unviable pregnancies, when an egg lacking a nucleus is fertilized by two sperm, or by one sperm that has its genome duplicated. The other samples were from people who carried blood cells with duplicated chromosomes from either their mother or their father.

The researchers used Illumina microarrays to identify methylated spots in the disomy tissue samples, and compared them to methylation patterns from blood cells with typical chromosome sets. In most cases, these should match up, but the methylation patterns would differ in imprinted genes, where one copy would be methylated in the normal blood



PARENTAL GUIDANCE
RULES: Maternally methylated (red dots) and paternally methylated (blue dots) regions across the human autosomal chromosomes, based on studies of uniparental disomy samples

GENOME RES, 24:554-69, 2014

cells, but neither or both copies would be methylated in the disomy tissues. For example, in a hydatidiform mole, all genes come from the father, so genes that are normally methylated only on dad's copy would be methylated on both alleles within these tissues.

FINDINGS The authors picked up 21 novel sites of differential methylation, 15 of which occur only in the placenta—and none of which were imprinted in mouse crosses they conducted (*Genome Res,* 24:554-69, 2014). "Imprinted loci are newly gained, and probably lost, during evolution," says Nakabayashi.

PROS

- Monk prefers the bisulfite-sequencing approach because most imprinted genes are differentially methylated, even if those genes aren't expressed in the tissue under analysis. "We use methylation as a sort of flag for where to look in the genome, rather than going directly to gene expression, which can be very complicated."
- Uniparental disomy tissues help to confirm the identity of imprinted genes.

CONS

- Sequencing is "hugely expensive," says Monk, estimating the cost at $6,000 per sample.
- The Illumina Infinium HumanMethylation450 BeadChip arrays only contain probes for 450,000 possible methylated regions, so some imprinted genes could be missed. The newer MethylationEPIC kit contains probes for 850,000 sites.
- Given that alleles may be differentially methylated but not differentially expressed in some tissues, methylation sequencing doesn't confirm imprinting at the RNA level.

WISH With human tissues hard to come by and the mouse imprintome not matching the human one, Nakabayashi would like to see more research on primate imprinting.

"Scalability is the next question," adds Monk. He'd like a way to perform single-cell analysis using microarrays, instead of full sequencing, but chip-based bisulfite methods require more nucleic acid—about a microgram—than one cell can provide.

PARENTAL CONFLICTS

RESEARCHER Mary Gehring, Member, Whitehead Institute and Associate Professor of Biology, MIT, Cambridge, Massachusetts
ORGANISM *Arabidopsis thaliana* and *A. lyrata*
METHODS RNA-seq and bisulfite-seq

In plants, imprinting only occurs in the endosperm, the triploid seed component that nourishes an embryonic plant. Many scientists suspect that imprinting, in both animals and plants, happens because the paternal genome promotes growth of the biggest possible offspring, while the maternal genome promotes conservation of limited resources. Gehring and colleagues were curious whether less imprinting would occur in *A. thaliana*, a self-fertilizing plant in which the parental interests ought to be aligned, than in the outcrossing *A. lyrata*. They crossed two *A. lyrata* strains, dissected the seeds by hand, and performed paired-end RNA-seq and bisulfite-seq on the resulting endosperms to identify the species' imprintome. They compared this to the imprintome they had previously determined for *A. thaliana* (*Nat Plants*, 2:16145, 2016).

FINDINGS In fact, the list of imprinted genes was mostly conserved between the two species. But the authors did observe a difference in the placement of the silencing methyl groups between the two species, suggesting their imprinting mechanisms differ.

RESOURCES FOR IMPRINTING

Genomic Imprinting website geneimprint.com	Geneimprint includes a list of imprinted genes, by species, as well as articles, reviews, and lectures on the topic.
Catalogue of Parent of Origin Effects igc.otago.ac.nz/home.html	Users can search for genes impacted by parent of origin, including imprinting and other effects such as differing mutation rates in each parent, in a variety of species. (*Nucleic Acids Res,* 29:275-76, 2001)
WAMIDEX atlas.genetics.kcl.ac.uk	This list of genes imprinted in the mouse is based on literature search or microarray expression data. (*Epigenetics*, 3:89-96, 2008)
MouseBook Imprinting Resource mousebook.org/mousebook-catalogs/imprinting-resource	MouseBook, which lays out the stock strains at MRC Harwell, includes lists and maps of imprinted genes. (*Nucleic Acids Res,* 38:D593-99, 2010)
GTEx Portal genome.gov/gtex/	A consortium provides genome and RNA sequences from human postmortem tissues. (*Nat Genet,* 45:580-85, 2013)

PRO
- As with Szabó's study, the researchers know the parental genotypes, so they can identify those alleles in the seeds.

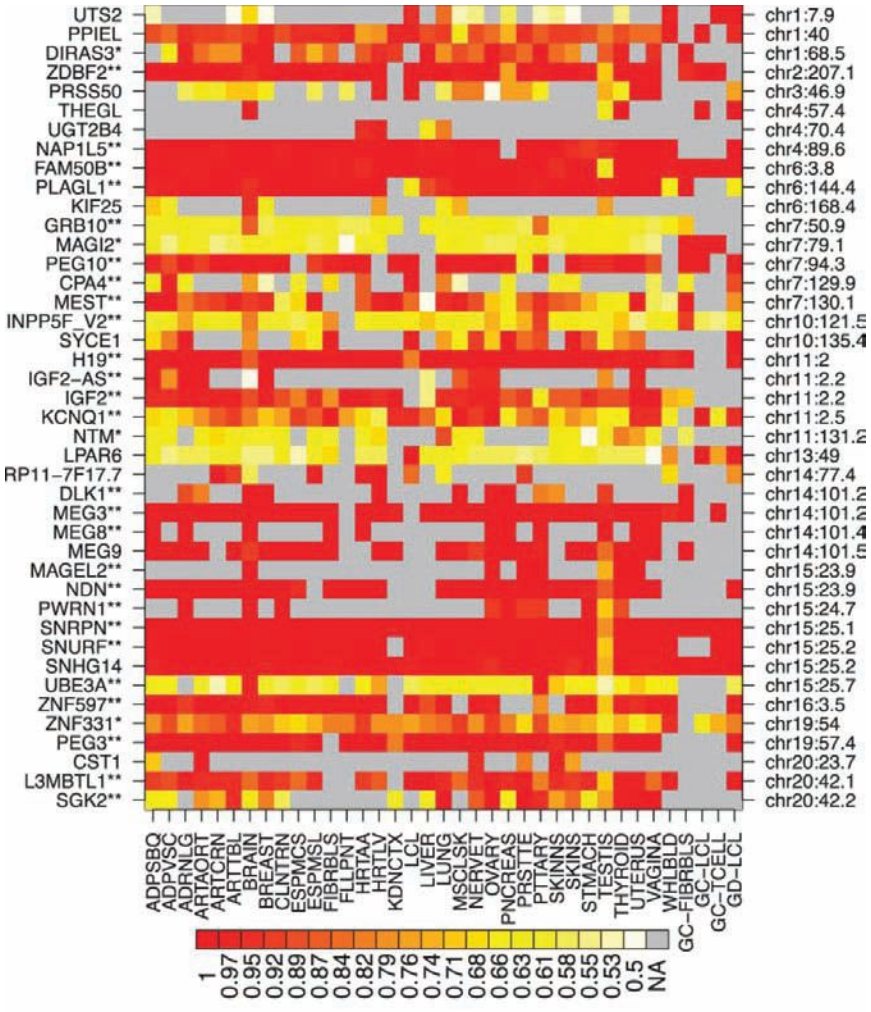
CONS
- If there's no genetic difference between the two parental strains at a given gene, any imprinting will be invisible.
- "A main challenge is understanding what's significantly different," says Gehring. Is 80 percent or 90 percent maternal expression evidence for imprinting? Different research groups set different cutoffs.

WISH Gehring would like to isolate endosperm without dissecting tiny seeds by hand; she hopes that the triploid nature of endosperm tissue (with two maternal and one paternal genomes) will help her to isolate it from other, diploid cells by flow cytometry.

DATA MINING

RESEARCHER Tuuli Lappalainen, Junior Investigator and Core Member, New York Genome Center and Assistant Professor, Department of Systems Biology, Columbia University
ORGANISM Human
METHOD Mining sequencing data

Thanks to widely available transcriptome databases, some scientists don't even have to collect new tissue to search for imprinted genes. Lappalainen is a collaborator on the Genotype-Tissue Expression (GTEx) database (see table on opposite page), which includes genotyping and RNA sequencing from postmortem samples of multiple tissue types. In a recent study, she and colleagues used data from that collection to hunt for imprinted genes. In any case where an individual was heterozygous at a given gene, the researchers could look for allele-specific expression. They did their best to filter out genes known to have random monoallelic expression, single-allele expression due to sequence variants, or RNA patterns that might look like monoallelic expression due to technical issues with the sequencing.



IMPRINTOME STOP SIGNS: Gene expression patterns for 42 imprinted genes (y axis) vary across diverse tissues (x axis), with redder boxes denoting stronger skewing towards just one allele.

FINDINGS The researchers identified imprinting in 42 genes, 12 of which were novel (*Genome Res,* 25:927-36, 2015).

PROS
- GTEx includes samples from a variety of tissues, covering systems including circulatory, nervous, and gastrointestinal.
- Having a large sample—1,582 tissues from 178 people—made it easier to confirm that imprinting occurs across the population.

CONS
- From just the GTEx data, the scientists don't know which parent a given allele came from. They had to use additional data, such as family samples, to determine which genes were paternally or maternally imprinted.
- This large-scale approach works when imprinting patterns are the same across many individuals, but it's possible the strength of imprinting varies across the population, Lappalainen cautions.

WISH The ideal way to do this kind of analysis would be with large family data sets with diverse tissue types, so that the researchers know the parental genotypes, but Lappalainen says only data sets from blood samples are currently available. ■

GENOME RES. 25:927-36, 2015

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Reality Check

Researchers and institutions seek solutions to biomedical science's growing PhD problem.

BY CATHERINE OFFORD

PhD degrees aren't what they used to be. In 1973, more than half of doctoral degree graduates in biological sciences landed a tenure-track position within six years. Three decades later, that fraction has dropped to 15 percent. Demand has not kept pace with supply, says Bruce Alberts, a professor of biochemistry at the University of California, San Francisco (UCSF), and cofounder of the nonprofit organization Rescuing Biomedical Research (RBR). "The real world for [biomedical PhD students] is that maybe a fifth will ever get academic jobs," he says. And it's not just academia that's overpopulated, he adds. "There aren't even enough jobs currently in the private sector to make it possible for all of them to get research jobs."

As a result, trainees spend more and more time in postdoctoral positions, and even then, their chances of landing a tenure-track position are in decline. Several years of survey data collected by the American Association for the Advancement of Science (AAAS) show that, although the percentage of postdocs expecting to land a tenure-track faculty position stayed above 50 percent from 2010 to 2012, the percentage who actually do so fell from 37 percent to 21 percent. Unemployment following a postdoc position, meanwhile, rose from 2 percent to 10 percent over the same time period.

Yet despite these sobering statistics, PhD programs continue to grow—in the U.S., the life sciences saw an increase from around 8,000 doctoral recipients in 2004 to more than 12,500 a decade later—and show no signs of leveling off. "Graduate students are fundamental to the vitality of the research enterprise," notes Alberts, who served for more than a decade as president of the National Academy of Sciences, adding that many university departments see little reason to stem the inflow of this young (and cheap) workforce.

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The resulting situation for today's PhDs—a hypercompetitive climate during graduate school and limited research-focused career prospects afterward—represents what many see as a systemic flaw in biomedical education that is impractical and unethical to maintain. It's a problem that a growing number of students, researchers, universities, and companies are now attempting to address, some through initiatives to inform and better prepare current students for nonacademic and potentially nonresearch career paths, others through longer-term efforts to modernize PhD admissions and education. Given the current job climate, Alberts says, "we have an obligation to tailor our graduate programs differently."

Bridging worlds

Because leaving academia is inevitable for the majority of today's biomedical PhDs, several institutions have established initiatives to help smooth the transition. The National Institutes of Health's Broadening Experiences for Scientific Training (BEST) program, for example, provides funding to 17 US universities for workshops, internships, and other training to expose students and postdocs to a variety of science-related careers. Meanwhile, AAAS holds

regular workshops on science policy and communication, and Johns Hopkins University School of Medicine offers a PhD program to train industry scientists. Other universities, such as the University of Wisconsin–Madison, also offer courses in nonacademic work experience for biomedical students. (See "Making the Most Out of School," *The Scientist*, May 2016.)

Nevertheless, these opportunities are few and far between, and career development classes remain optional for most PhD candidates. A 2015 survey by *Nature* of more than 3,400 graduate students worldwide—most engaged in biological, chemical, or medical research—found that less than 20 percent (30 percent in the U.S.) reported obtaining useful career advice from their institutions, and just one-third had attended career workshops or used other institution-provided resources. Although some respondents noted that these services were not available, many had not taken advantage or were simply not aware of what was on offer.

This lack of engagement is partly due to a lack of forethought: an earlier *Nature* survey of 5,000 people found that less than half of incoming graduate students had considered their job preferences before enrolling. There is also a latent disdain for work outside academia, says Mike D'Ecclessis, a graduate student in cell and developmental biology at Rutgers University. "Most PhD programs are not oriented to looking outside of the lab," he says. "It's still treated like . . . if you do something else, you're veering off course." Indeed, a 2012 study found that half of PhD students in the life sciences felt strongly encouraged by their departments or advisors to pursue a faculty research position, despite the dearth of opportunities in academia.

To help tackle these attitudes, several websites—increasingly run by students themselves—have sprung up over the last decade to provide information about the options available to newly minted PhDs. (See "Where Do They Go?" on opposite page.) U.S.-based Versatile PhD, for example, maintains a database of research and nonresearch career options, job opportunities, and testimonials from PhDs who have found employment outside academia. D'Ecclessis, meanwhile, assembled information he's gathered during his time at Rutgers to set up the PhD Career Guide, which hosts information on employers, salaries, and working hours for various careers.

The real world for biomedical PhD students is that maybe a fifth will ever get academic jobs.

—Bruce Alberts
University of California, San Francisco

A handful of private companies also offer career support for trained researchers. The key is to get people thinking about what they can offer in addition to their science, says Larry Petcovic, cofounder and vice president for communications at SciPhD, a company that provides services from basic career planning to interview preparation. "Most PhDs are self-limiting in terms of how they see their talent," he says. "It's like they've been conditioned to think the only thing they can do is their science in a research environment. But there's a lot more there."

These services can help PhDs develop the soft skills necessary to land a professional job, including communication and project management. If students start thinking about their careers early enough, they may also have the opportunity to take courses in finance, policy, communication, or other fields outside their department. "Anything else you can do in addition to your science makes you a hot commodity," Petcovic notes.

Most of these efforts are relatively recent, but there's at least anecdotal evidence that they're already having an impact. Jasmine Hughes, a bioengineering PhD student and codirector of University of California, Berkeley-based career organization Beyond Academia, says that she's starting to see graduate students think about careers sooner. "We still get people coming to us who have no idea what they're going to do," she says. "But the later-stage panic is not as common as it used to be, because people have had the opportunity to explore it earlier—which is great. They can be more concentrated on narrowing down what their focus should be."

A permanent fix

While exploring nonacademic careers may improve a PhD's employability, some argue a longer-term solution is needed to address the gap between the number of graduating PhDs and the number of jobs requiring the qualification. Although the unemployment rate for all bioscience PhDs was around just 2 percent in 2013, "you cannot look at the fact that these people are not unemployed and infer that there's no problem," says Paula Stephan, an economist at Georgia State University. "Yes, unemployment is low for people with PhDs, but that doesn't tell you anything about whether they're *underemployed* or whether they're using the kinds of skills they were trained with."

Data on PhD-holders' career outcomes are thin, but there are hints that underemployment may be an issue. A 2014 report from the American Institutes for Research found that a third of PhD holders in the U.S. reported themselves underemployed, and a study published last year as part of UMETRICS—a University of Michigan project that collects data on scientists' employment—found that the average salary for biology PhDs was just $36,000 in their first year of work, a result the authors speculated could be partly explained by low postdoctoral salaries. Although PhDs may ultimately earn a higher salary than less qualified workers, the years lost at the beginning of their careers could cost them in the long run.

It's not just PhD students who suffer from the imbalance between degree programs and jobs, either; Stephan notes that there are significant societal costs of training thousands of underused PhDs a year. "People will say, 'Well, no matter what, a PhD is a great degree; it trains you to do all kinds of things,'" she says. "But if a number of the jobs that PhDs end up getting could have been done by

people with less training, it's not a very efficient use of resources."

One possible solution to the problem is simply to cut graduate school admissions, creating a better match between the number of researchers trained and the number of research jobs available. The idea is not new: Stephan has advocated for such academic "birth control" for four decades, and Alberts and his colleagues at RBR made a case for a broad approach to reducing entrant numbers in a 2014 *PNAS* article (111:5773-77). But such proposals have so far proved unpopular. At a workshop following the publication of RBR's paper, for example, "the recommendation to cut PhD programs got the strongest pushback from the audience," says Alberts. "People argued that we don't know who will be a great scientist, and the people who will be great scientists can't possibly know either, so we need to cast as wide a possible net for talent." What's more, not all graduate programs are equal, he adds—some have higher placement rates in academia or industry than others, and it's not clear how cutting PhD programs should take this into account.

Other suggestions include requiring students to complete a master's program before embarking on a PhD in an attempt to select for dedicated researchers earlier; increasing the proportion of students funded by individual fellowships and training grants, rather than faculty research grants, to dissociate university admissions from the funding of specific labs or professors; and hiring more staff scientists to carry out day-to-day lab work to circumvent the need for a workforce of PhD students. But, such adjustments will take time to implement. "There's a huge amount of inertia," says Alberts. "To accomplish any of these changes you need to create a movement, you have to get institutions on board, scientific societies, the NIH. . . . We're going to have to work with our colleagues to make these things happen. They won't happen just because we say them."

In the meantime, Alberts is working toward a more immediate solution to achieve the same goal: show prospective students the data. RBR advises universities to detail on their websites "the actual outcomes, as far as they can tell, for their PhD graduates," Alberts explains, adding that five universities—Johns Hopkins, Duke, Harvard, Princeton, and UCSF—have already committed to adopting this practice. D'Ecclessis is in favor of the approach. "It would be incredibly helpful for people who are considering a program to see what it actually entails—what the pitfalls are, what the opportunities will be," he says. "There are still a lot of people who are interested in academia who don't understand the recent trends."

Alberts and others hope to make a dent in the biomedical PhD's sustainability problem by warning prospective students about the long-term risks of a small job market and by helping current students prepare for the options available to them after graduation. But just as important, notes Alberts, will be an evolution in the attitude of the academics who train these students.

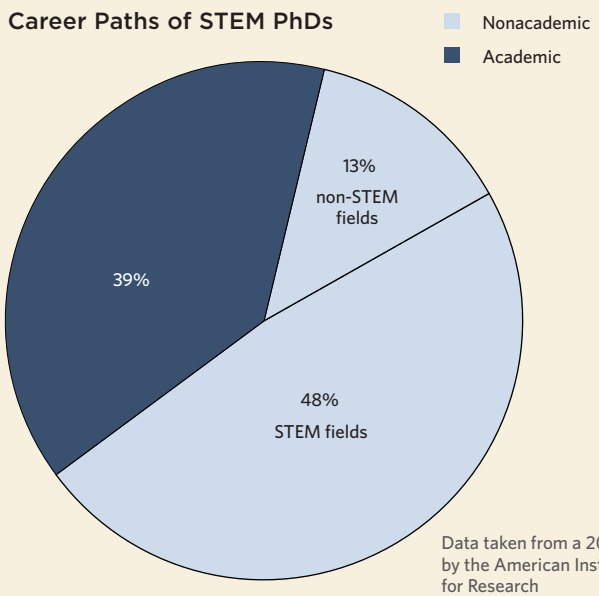
In the past, professors could get away with thinking that "anyone who's any good will get a job they want in academia or biotech," he says. "But now we know that's not true. Those of us that are older, who lived in an era where that was true, have to wake up and look at the data." ■

WHERE DO THEY GO?

Data on PhDs' career paths are hard to come by, but a handful of recent studies provide a glimpse of where graduates end up after completing their programs. A 2014 report by the American Institutes for Research, for example, found that 61 percent of science, technology, engineering, and math (STEM) PhDs (and 52 percent of biological science PhDs) were working in nonacademic careers in 2010, primarily in private, for-profit companies. Around half of these nonacademic positions were in development or applied research, although 13 percent of STEM PhDs had left these disciplines altogether.

Some universities, too, are beginning to collect their own, more detailed data. A recent project by Stanford University found that of 308 students completing bioscience PhDs at the university either between 2002 and 2004 or between 2007 and 2009, just under half continued in academia, with a large number staying at Stanford. Another 18 percent found employment in industry, 4 percent went into government, and 9 percent joined nonprofit organizations. (The remaining 23 percent had not made their information public.)

"They're really not 'alternative' careers anymore," says Larry Petcovic of careers services company SciPhD. Rather, for today's bioscience PhD workforce, "it's academia that's the alternative now."



Data taken from a 2014 report by the American Institutes for Research

Fossilized Thinking

Science is burying the myth that there are simple biological differences between males and females.

BY CORDELIA FINE

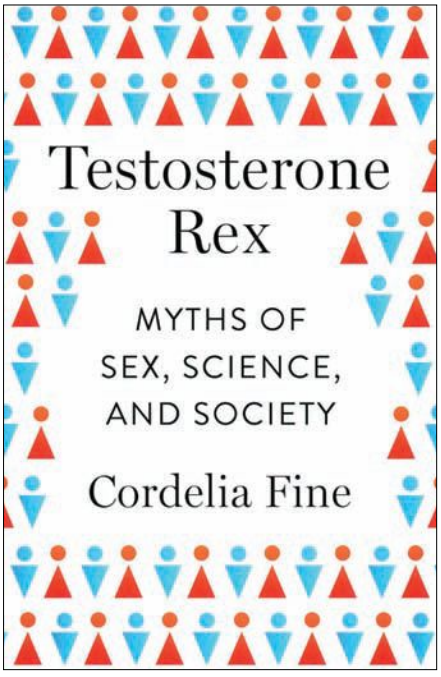
There is a myth that male and female natures are distinct, shaped by ancient evolutionary pressures and transmitted faithfully and timelessly via sex chromosomes and hormones, against which equal opportunity laws and optimistic feminists are no match. This myth is so familiar that every reader can fill in the chain of argument required to get from "cheap sperm" to an explanation of why there are many more male than female Nobel Prize winners. What's more, the issues I dissect in my latest book, *Testosterone Rex: Myths of Sex, Science, and Society*, are part of every scientist's life. Of course only a subset of scientific research examines the often-contentious zone of how systems of sex, gender, or both impact the brain and behavior. But just about every researcher is, at some point, part of conversations and disagreements as to why men predominate in a particular scientific field, or why solid female representation at junior levels falls away with seniority.

The debates are ubiquitous, whether they concern gender gaps in science, senior leadership roles, or entrepreneurship. The "nature" side claims a small triumph or two: some hormonal effect, anatomical brain difference, or evolutionary principle that seems to prove the naturalness of a particular gender gap. But then the "nurture" side enjoys a few gains—a demonstration of workplace gender bias, an unfavorable comparison with a more egalitarian country, or a study showing that in some situations the sexes aren't so different after all—that seem to finger stereotypes and sexism as culprits. And, of course, each side regularly challenges claims made by the other, dismissing the reliability, validity, or applicability of this or that finding.

Testosterone Rex seeks to transform the debate by taking a closer look at phenomena we usually think of as falling into the "nature" basket: the development of sex-specific adaptive behaviors, the role of sex in brain development, and the effects of higher testosterone levels. For example, evolutionary biologists have documented evidence, both across and within species, showing that biological sex doesn't have straightforward consequences for courtship patterns or mating behaviors. Sperm provisioning isn't as biologically cheap as was traditionally assumed, nor are competition and social dominance as irrelevant to females' reproductive success.

In neuroscience, the traditional model in which sex has a powerful, monolithic effect on the brain is being replaced by one in which the genetic and hormonal components of sex are among many factors that interact in complex ways. There is also growing appreciation that sex can also act indirectly on the brain and behavior through tangible effects on size, strength, smell, appearance, and so on. In other words, gender socialization isn't something that tinkers around the edges of the real developmental work of sex, but is an integral part of it. And in behavioral endocrinology, scientists are building on animal research that shows the importance of steroid hormones such as testosterone for behavioral plasticity to help unravel the biological links between gender constructions and hormonal state.

The Testosterone Rex myth persists in popular understanding of the gender dynamics of society. It also lurks in outdated models and assumptions in some areas of research, pinning the gender gap in retirement savings or even the global financial crisis on men's higher testosterone levels. But science has evolved, and the Testosterone Rex myth is as extinct as the *T. rex*. It's time to say good-bye, and move on. ■



W.W. Norton, January 2017

Cordelia Fine is a professor in the History and Philosophy of Science Programme in the School of Historical and Philosophical Studies at the University of Melbourne in Australia. Read an excerpt of Testosterone Rex *at www.the-scientist.com.*

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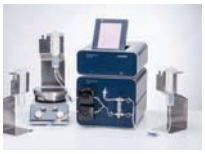

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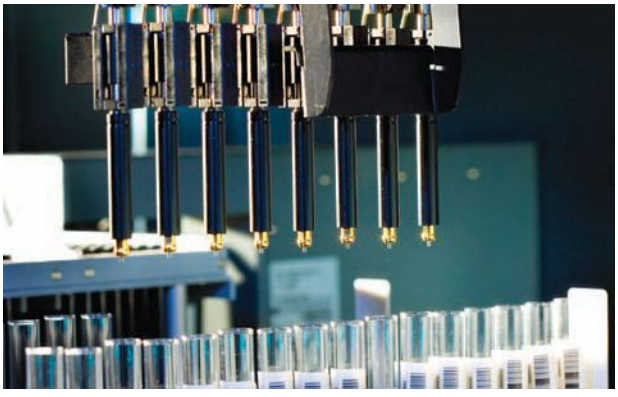
Explore Lonza's Innovative Research Solutions at SLAS2017



PyroTec™ Liquid Handling System

Lonza will be exhibiting innovative research solutions at SLAS2017 (4-8 February, Washington). Greg Alberts, Global Subject Matter Expert at Lonza, will present a poster titled "Streamlining your QC Testing: The Efficiency and Accuracy of Automated Endotoxin Testing and Process Optimization". Data in the poster will show the ability of robotic systems to reproducibly and reliably perform tasks necessary to endotoxin testing, and demonstrate the efficiency and accuracy of an automated solution when implemented in a large-scale fashion.

On booth 547, the Lonza team will showcase the 4D-Nucleofector™ LV Unit and the HT Nucleofector™ Device, both of which use Nucleofector™ Technology to enable efficient transfection of a multitude of different cell types using minimal amounts of substrate. Combining Nucleofector™ Technology with the 4D-Nucleofector™ LV Unit, provides a closed system for sterile transfection of up to $1x10^9$ cells, and enables easy scaling up of transfections without the need for re-optimization. Furthermore, Lonza's HT Nucleofector™ Device enables rapid, high-throughput transfection of cells with substrates ranging from small molecules to CRISPR components, in 384-well format.

Lonza's WinKQCL™ Endotoxin Detection and Analysis Software is a highly integrated software solution that increases throughput and accuracy of testing, and enhances data integrity. At SLAS2017, delegates can explore how the WinKQCL™ Endotoxin Detection and Analysis Software can be integrated into simple robotic platforms.

Lonza's recent acquisition of Triangle Research Laboratories (TRL), has enabled Lonza to expand its portfolio to now include a diverse range of ADME-Tox products. These cells and supporting materials are invaluable *in vitro* drug screening tools, enabling drug candidates to be assessed for a range of characteristics.

Discover more about all these innovative research solutions on booth 547 at SLAS2017.

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Genomics Topics

- Single Cell Sequencing
- CRISPR Applications
- Approaches to Clinical Sequencing
- Genomic Data Analysis
- International Genomics Forum
- **Plenary: Barbara Wold**

Trending Topics

- Flow Cytometry
- 3D Printing
- Antibody Technology
- Bioinformatics
- Information Technology (BioIT)
- International Core Associations Roundtable

Proteomics Topics

- Practical Quantitative Proteomics
- Realistic Workflow Reproducibility
- Reproducible Bioinformatics & Data Analysis
- Mass Spectrometry for Metabolomics & Antibodies
- **Plenary: Bernhard Kuster**

Bridging Topics

- Integrated Core Model
- Electron Cryo-Microscopy
- Imaging Mass Spectrometry
- Management Skills for Scientists

Imaging Topics

- Live-Cell Imaging
- 3D Super-Resolution on Commercial Systems
- Administration, S10 & MRI Grants
- Deep Imaging
- Light Sheet & 3D Reconstruction
- **Plenary: Hari Shroff**

Core Administration Topics

- Measuring Return on Investment
- Implementing Standards for Rigor & Reproducibility
- Developing Core-Core & Core-Commercial Partnerships
- Core & Graduate Education Collaboration
- 'Admin is In' Booth
- NIH & Federal Policy Updates

The 2017 ABRF Award for Outstanding Contributions to Biomolecular Technologies:
Shankar Balasubramanian & David Klenerman
for Invention of "Sequencing by Synthesis," a revolutionary DNA sequencing method

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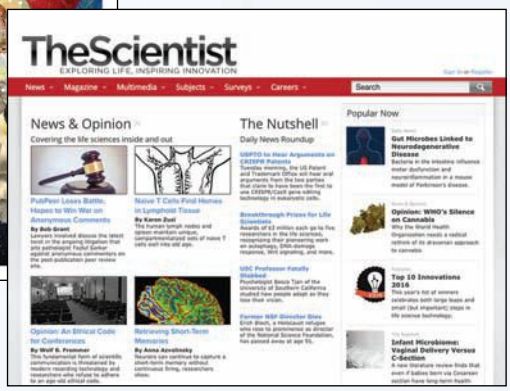

We are pleased to announce that *The Scientist* won a 2016 FOLIO Eddie award for our March 2016 issue and garnered an honorable mention in the Eddies Digital category for our online news coverage. FOLIO awards are presented annually and the Eddies recognize editorial excellence.

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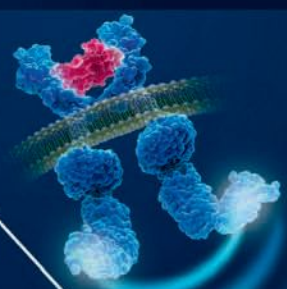

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Newspaper Dogs, 1925

BY BEN ANDREW HENRY

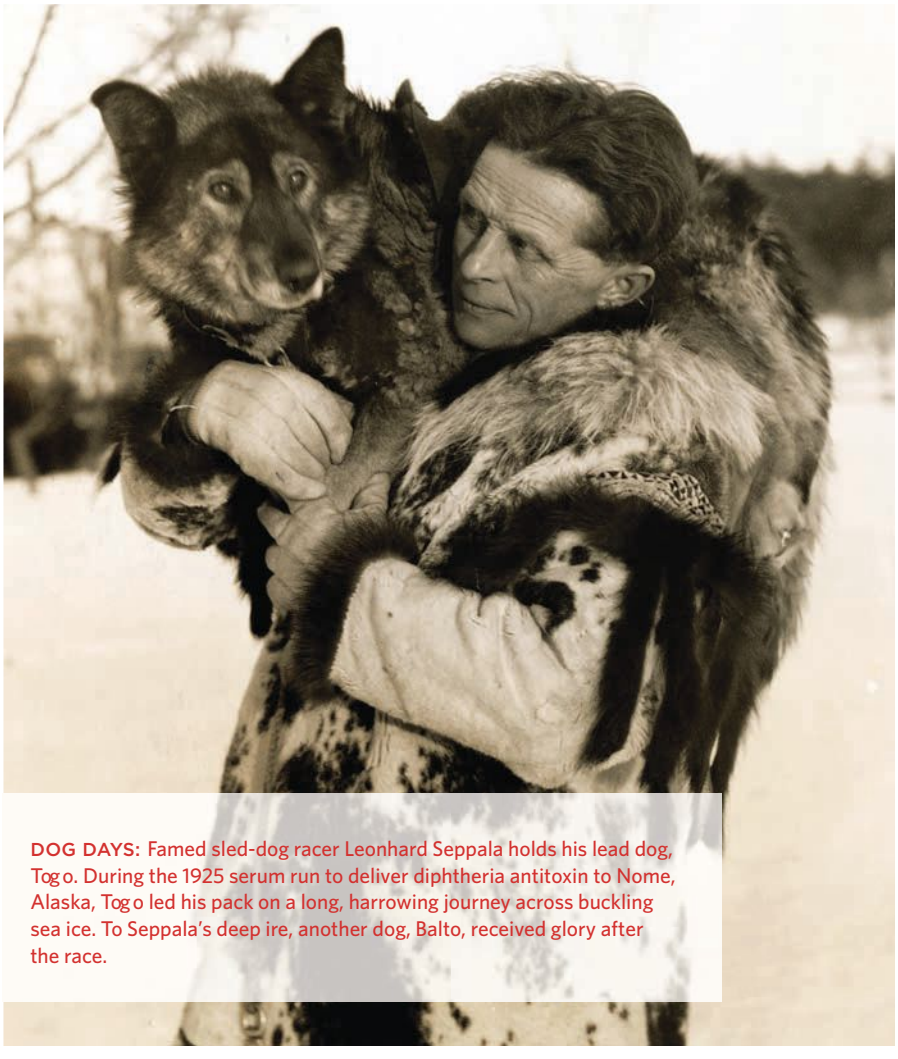
On a dawnless morning in the winter of 1925, a sled dog named Balto became an American hero. Nome, an Alaskan mining town past its heyday, teetered on the brink of a diphtheria outbreak. Children had begun to succumb to the disease, a bacterial infection that coats the esophagus in a suffocating layer of necrotic tissue. The city's meager supply of antitoxin serum had passed its expiration date. Desperate, the only doctor in town placed sick children into quarantine and radioed for help.

Boats, impeded by sea ice, did not visit Nome in winter, and bush planes were not safe in the cold due to their open cockpits and water-cooled engines. Public health officials agreed that dog sleds were their only hope to save Nome's children. On January 27, a relay of 20 dog teams and drivers set out to haul serum from Nenana, 674 miles away, to Nome, through foul storms over the course of a week.

Exhausted dogs died on the snow while their drivers lost fingers and toes to temperatures that sank below –60 °F. Telegrams from rendezvous points along the way kept the American public riveted to this days-long ordeal. Leonhard Seppala, considered one of the greatest mushers and dog breeders in Alaska, drove the hardest and longest stretch, crossing treacherous sea ice in the black of night with his lead dog, Togo, at the front of the pack.

A photographer awaited the last team in Nome, where Gunnar Kaasen stumbled off of his sled and, legend has it, uttered "damn fine dog" in reference to his lead dog Balto, before collapsing from fatigue.

Balto was Seppala's dog, on loan to Kaasen, and when Seppala toured the country following what became known as The Great Race of Mercy, Balto was lavished with praise. He became a centerpiece in the narrative of American bravery that recounted the events of the serum run. Ten months afterward, a statue of Balto was erected in Central Park.



DOG DAYS: Famed sled-dog racer Leonhard Seppala holds his lead dog, Togo. During the 1925 serum run to deliver diphtheria antitoxin to Nome, Alaska, Togo led his pack on a long, harrowing journey across buckling sea ice. To Seppala's deep ire, another dog, Balto, received glory after the race.

PHOTO BY GEORGE RINHART/CORBIS VIA GETTY IMAGES

The press attention galled Seppala. In his view, the papers had the wrong champion. The dog he thought deserved praise, instead of Balto, was Togo. Seppala had been winning races with Togo for years and considered Togo his finest dog, says Laney Salisbury, coauthor of *The Cruelest Miles*, a history of the serum run. "The dog that always got him through all his trouble," says Salisbury, "was always Togo."

Seppala "always gave all the credit to his dogs," says Helen Hegener, author of a number of books on Alaskan history and dogsledding. He raised Togo from a puppy and devoted exceptional time and attention to the dog, Hegener says. "He took [Togo] everywhere with him." When the papers covered Balto instead of Togo, "it was like the media was not giving his best friend his due," Salisbury says.

According to Salisbury, Seppala later commented to his biographer that "in Alaska, our dogs mean considerably more to us than those outside can appreciate. . . It was almost more than I could bear when the 'newspaper dog' Balto received a statue for his 'glorious achievement.'" ■

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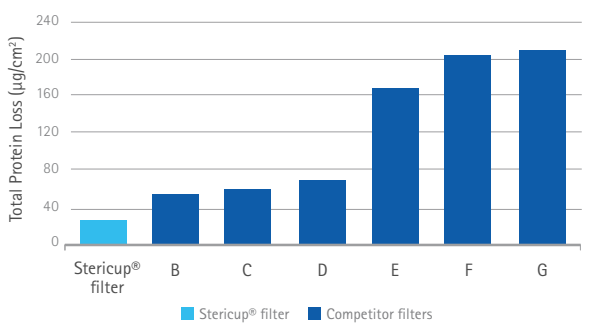

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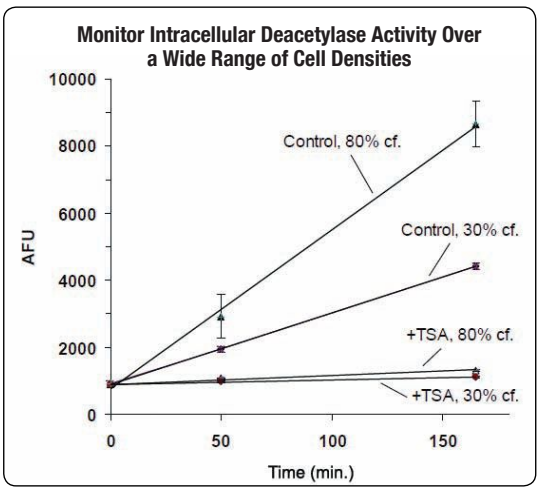

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