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RAPID EVOLUTION

GENETIC CHANGE WITHIN POPULATIONS CAN HAPPEN IN MERE GENERATIONS

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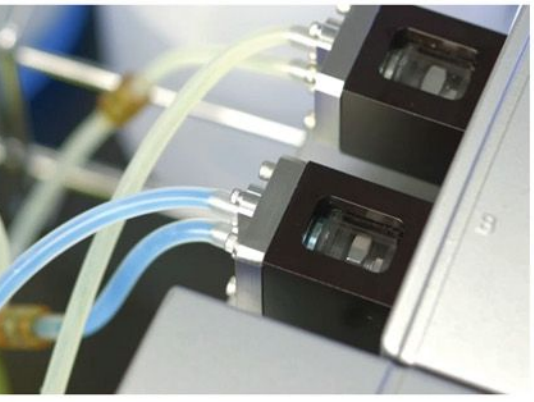

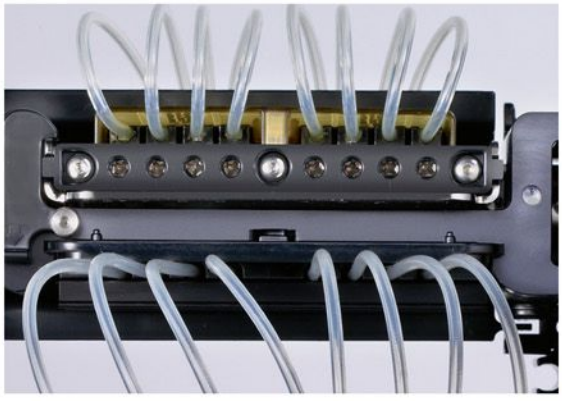

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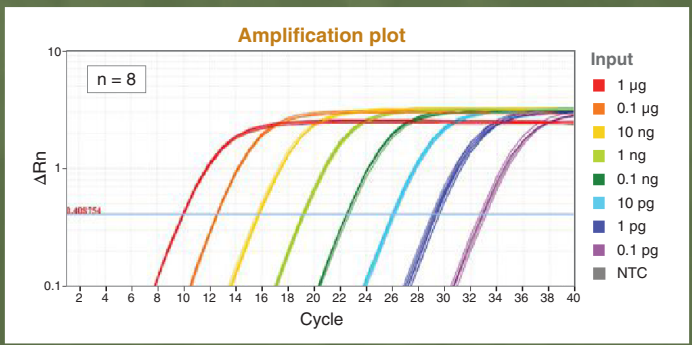

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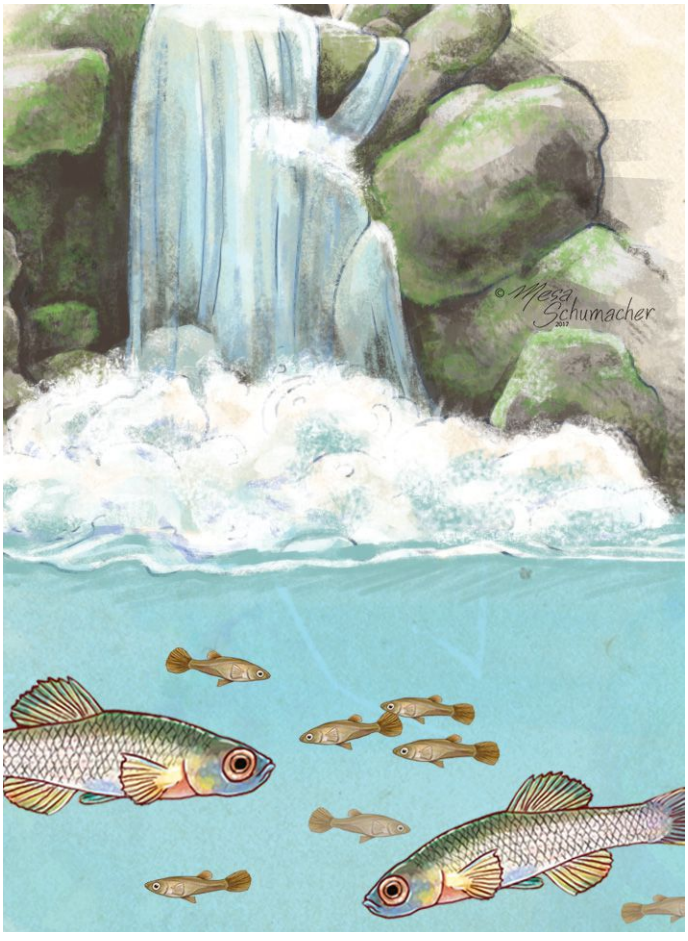

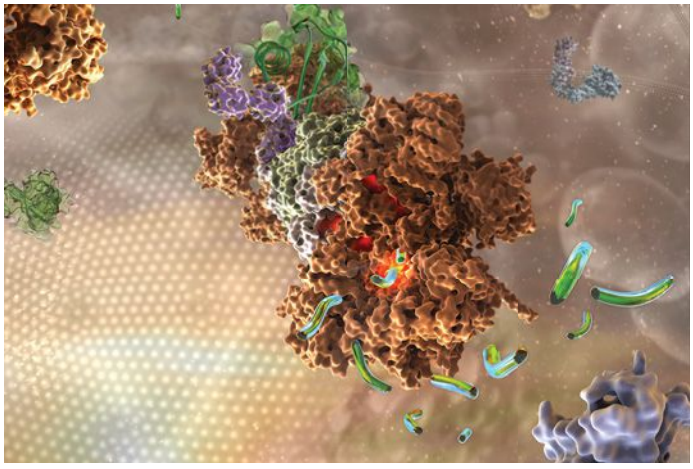

Features

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BioResearch

Lonza

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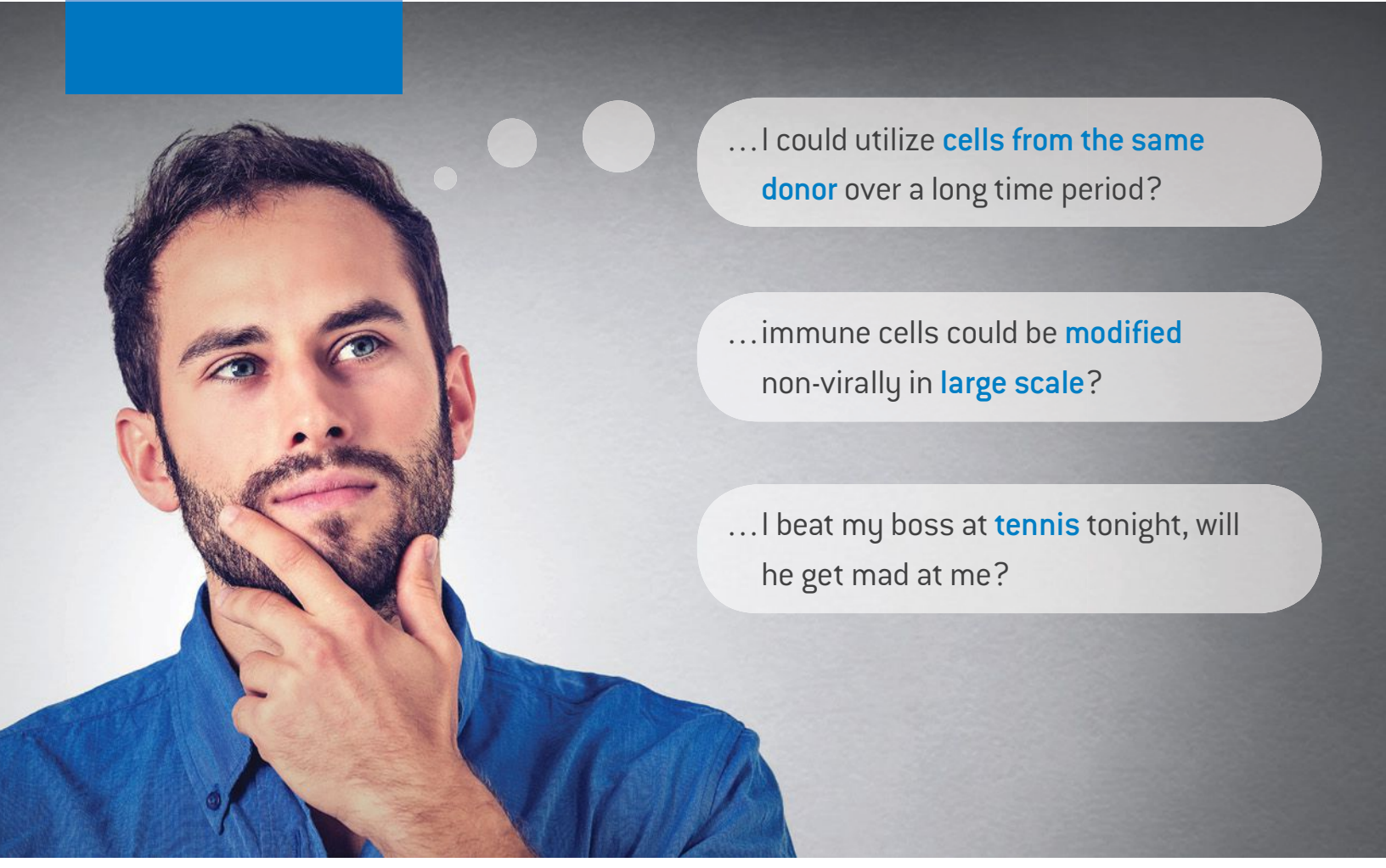

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CORRECTIONS:

In "Monumental Maize" (*The Scientist*, April 2017), the affiliations of both Edward Buckler and Sarah Hake should state that the two are researchers with the US Department of Agriculture at their respective institutions.

In "Angela Brooks: Splicing Specialist" (*The Scientist*, April 2017), the correct name of Brooks's postdoctoral advisor is Matthew Meyerson, not Michael Meyerson.

The Scientist regrets the errors.

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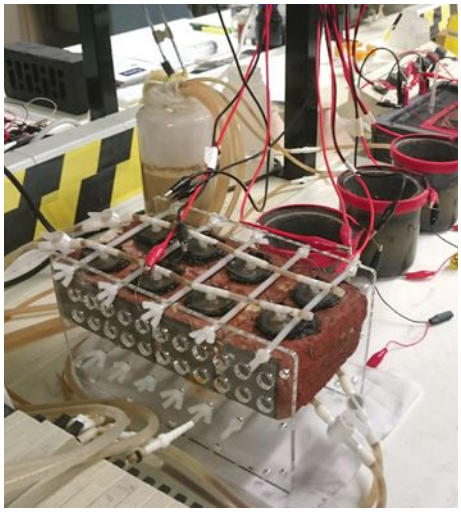

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Dragons on the Hunt
Watch Komodo dragons bring down a buffalo.

VIDEO
Myelin Basics
May profilee Ben Barres of Stanford University discusses how understanding the basic biology of myelination could help patients with multiple sclerosis and other diseases.

SLIDE SHOW
Living Bricks
Tour the Bristol Robotics Laboratory, where researchers are trying to make building materials that incorporate microbial fuel cells to clean wastewater and generate electricity.

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

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- New insights on celiac disease
- Exome-tailored diets
- The discovery of IgE
- The athlete's microbiome

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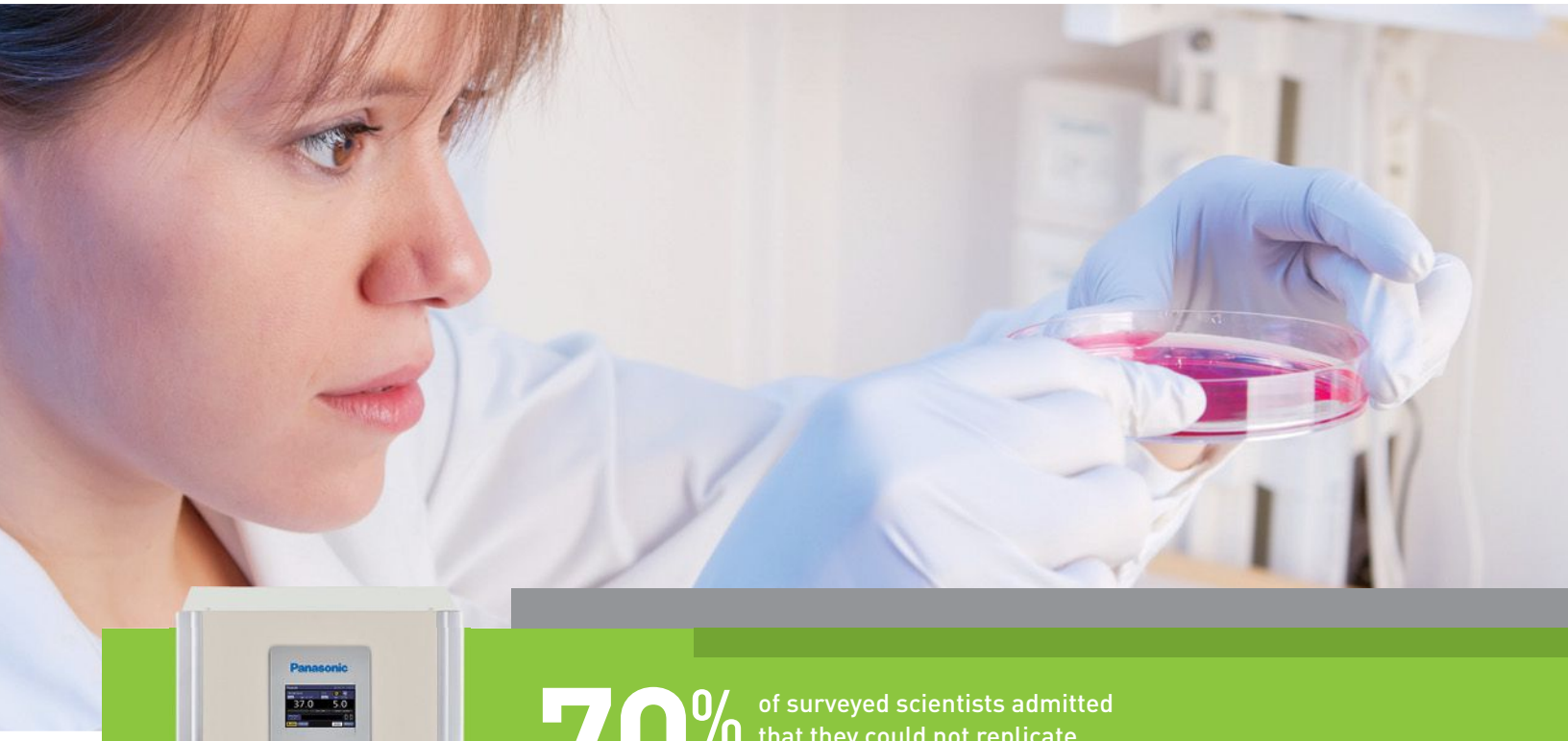

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50% admitted that they couldn't replicate their own research.[1]

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1) Baker, Monya. "1,500 scientists lift the lid on reproducibility." Nature, no. 533 (May 26, 2016): 452-54. doi:10.1038/533452a.

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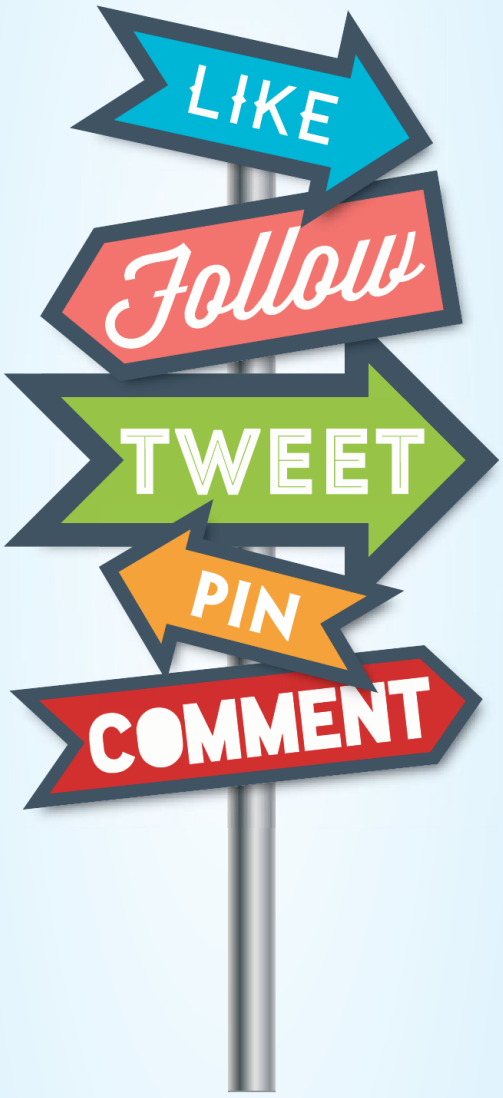

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Contributors

John Hines went to Cornell University wanting to become a veterinarian. But after spending a summer in a veterinary clinic, he decided to pursue a research career instead. As an undergrad, Hines was introduced to research in a microbiology lab that focused on metabolic pathways and bacteria. "That convinced me that I was on the right path—I looked forward to going into the lab and tinkering around," he says. That experience, along with the several months he worked in a pharmacy before finishing college, convinced Hines to focus on pharmacology for his doctoral studies, which he completed at the University of Pennsylvania. In 2001, he became a postdoctoral fellow in Craig Crews's lab at Yale University, where he is currently positioned as a research scientist studying proteasomes both as therapeutic targets and as basic research tools.

As the son of a NASA researcher, **Craig Crews** was immersed in science throughout his childhood. He majored in chemistry at the University of Virginia, then spent a year in Germany on a research fellowship in a molecular biology lab at the University of Tübingen. When he returned to the U.S., he began his doctoral research at Harvard University, probing signaling pathways in the cell. He remained at Harvard for a postdoc at the Cancer Research Institute, then joined the Yale faculty in 1995. A theme that resonates through his career, Crews says, is the "interface of chemistry and biology and how one can use chemical approaches to address biological questions." Crews also founded a company, Arvinas, to commercialize proteolysis targeting chimeras (PROTACs), a technology developed in his lab to tag and eliminate disease-causing proteins by targeting them for proteasomal destruction. "We hope to be in the clinic next year for our first two programs, in prostate and breast cancer," Crews says.

Read their feature about proteasomes on page 34.

While growing up in Wiesbaden, a city in western Germany, **Roman Liepelt** was fascinated by optical illusions. "I conducted tiny experiments by myself," he recalls. "Then, in school, I got interested in biology." This led him to study biology and psychology at Johannes Gutenberg University in Mainz, Germany, where he received his diploma. Liepelt moved to Berlin in 2006 to pursue a doctoral degree in psychology at Humboldt University, investigating multitasking and executive control functions. He then relocated again for his postdoc, this time to the Max Planck Institute for Human Cognitive and Brain Sciences in Leipzig, where he examined the behavioral and neural underpinnings of social perception and action. In 2016, Liepelt became a senior lecturer at the German Sport University in Cologne, where he is currently exploring, among other things, agency perception and body ownership—which, Liepelt says, have a lot in common with the illusions that captivated him as a child. "The principles at work there also involve multisensory integration," he says.

As an undergrad at Monash University in Melbourne, Australia, **Jack Brooks** developed an interest in proprioception. "The most interesting part for me is that, unlike a lot of other senses like vision and touch that have receptors, there's no obvious receptor that signals things like the length of your limbs," Brooks says. His curiosity in the topic grew after taking part in a study that was looking at whether muscle damage could impair the sense of position in a limb. Now, Brooks is working on his PhD at Neuroscience Research Australia in Sydney, investigating touch, proprioception, and embodiment. In the future, Brooks hopes to further understand how stable embodiment is maintained as the body changes with age, and how it manages to remain even after substantial neural damage occurs.

In "The Mind-Body Connection," page 40, Liepelt and Brooks write about how we recognize and control our bodies.

Bob Holmes studied desert grassland plants for his PhD in evolutionary biology and ecology at the University of Arizona. But by the end of his doctoral studies, Holmes knew he didn't want to be a researcher. "I used to joke that what I wanted to be when I grew up was a dilettante," he says. "It took me a few years until I finally realized that was the job description for a journalist." Holmes began the path to his new career in the science-writing program at the University of California, Santa Cruz. After graduation, he interned at *US News & World Report*. While there, Holmes connected with an editor at *New Scientist*, a magazine he's now been affiliated with for more than 20 years. In an essay (page 61) based on his first book, *Flavor*, Holmes explores the science of "our most neglected sense." "One of the really fascinating things turned out to be that every single person lives in their own unique flavor world, which means that almost certainly we perceive the world differently in terms of odor, and therefore, flavor," he says.

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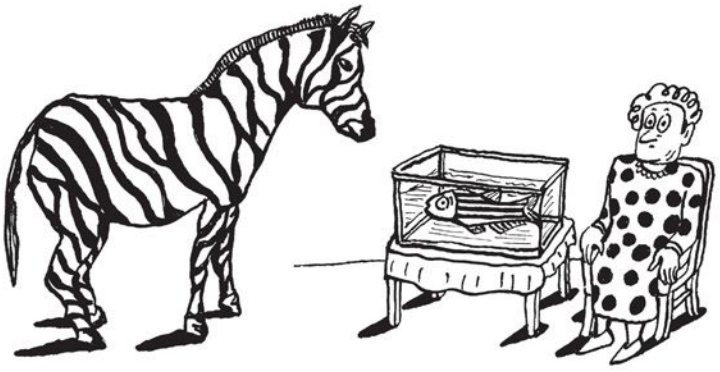
Learning Your Stripes



Science's lowest common denominator has always been patterns.

BY MARY BETH ABERLIN

As the May installment of the magazine came together, I realized that a number of this issue's articles are about patterns. Not just immediately visible ones like the stripes on a zebra, but more subtle cycles that repeat over and over again.

Noticing that the world is full of patterns has surely been central to the evolutionary success of human beings. Early cave painters depicted the spotted hides of the animals they hunted. Those hunters no doubt observed how the skins made the animals harder to pick out from the surroundings. They saw that life around them unfolded in generational cycles, and constructed creation myths to deal with what they saw and with what they feared because they could not understand it.

Before science became a formal discipline, close observation of the natural world and a keen desire to figure out what gave the patterns form and meaning sowed the seeds for its eventual blossoming. Artists depicted, philosophers cogitated, and naturalists stoked fascination with their ever-expanding collections of biological wonders.

Two of the articles in this issue deal with developmental biology. Historically, embryology undergirds the discipline, which began in antiquity with writings by Hippocrates and Aristotle, and continued to rely heavily on patterns gleaned from the study of comparative anatomy. "Failure to Recapitulate, 1874" (page 68) recounts how Ernst Haeckel promulgated the biogenetic law (almost a mantra), "ontogeny recapitulates phylogeny," using his illustrations of vertebrate embryo patterns to drive home his point that embryos of more advanced species passed through stages mirroring vertebrate evolution. (Haeckel's law was discredited because it relied on embryos' resemblance to adults of the various species.)

And then there are the more familiar patterns of an animal's stripes, the development of which is being revealed thanks to advances in microscopy and genomics that took dev bio down to the cellular level. "Macrophage Messaging" (page 48) reports on a study of stripe formation in postembryonic zebrafish, where macrophages deliver vesicles loaded with cellular signals to the melanocytes that then organize into the patterns of black stripes that give the little fish its name.

Until 30 years ago, evolution, proposed after close observations of fossil patterns in rock strata and species distributions among islands, was thought to be inexorably slow, taking place over eons. Senior Editor Jef Akst reports on the process of rapid, or contemporary, evolution (page 26): biologists can, in their own lifetimes, actually witness species change. With examples reported for dozens of animals, the concept is now well-documented, and researchers have moved on to sorting out the consequences of rapid evolution at the ecosystem level. "The complexity of both ecosystems and evolutionary theory makes these dynamics very challenging to dissect, however," Akst writes. "In addition to the logistical limitations of the research, there are also theoretical challenges." But aficionados of the field are optimistic. As one scientist puts it: "In part, it's just a matter of waiting for the data to accumulate. . . . One generation is the speed limit."

Robert Hooke and Santiago Ramón y Cajal would certainly love the Lab Tools (page 54) on machine-learning algorithms that make a cell biologist's life much more productive by number-crunching large amounts of data on a cell's structural and functional aspects after a little training from their human users. It's fun to imagine those two icons liberated from hunching over a microscope, eyes darting from the lens to the paper on which they drew the patterns they saw in plant cells and brain slices.

Zeroing in on the form and function of patterns in nature reveals broader truths. Another scientific icon, Richard Feynman, beautifully summed this up when he closed a 1964 lecture thus: "Nature uses only the longest threads to weave her patterns, so that each small piece of her fabric reveals the organization of the entire tapestry." ■

Editor-in-Chief
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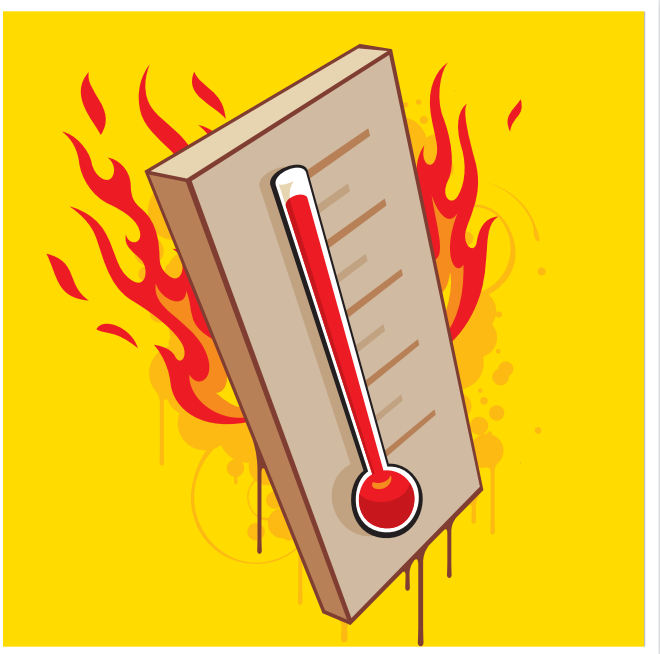
ANDRZEJ KRAUZE

Speaking of Science

[Trump's] de-funding of many of the basic scientific programs that are there to measure, to monitor what's happening with the climate, is sort of like having a child who is suffering from a very high fever, and then deciding to just stop measuring their temperature, stop taking their temperature. That's effectively what he is doing. But not just with a single human being—with our entire planet—and it's a threat to all of us.

—Pennsylvania State University climatologist **Michael E. Mann,** in an interview about his paper in *Scientific Reports* linking global warming to changes in the Northern Hemisphere jet stream (March 27)



That is not known as an objective writer or magazine.

—Chairman of the US House science committee **Lamar Smith (R-TX)**, during a hearing on climate change in which Michael E. Mann mentioned a *Science* news article criticizing Smith's politicizing of the committee (March 29)

It's not socially acceptable to say that there might be group differences in an endophenotype—in their behavior, intelligence, anything that might have any genetic component. . . . If someone's going to ask me, I'm going say, 'It could be true.'

—Science blogger **Razib Kahn**, on the controversy surrounding his writing on race, which posits a biological underpinning for what many scientists consider to be a social construct (*Undark*, March 28)

There is currently no US organization that promotes research integrity across sectors and disciplines on a continuing basis and as its core mission.

—US National Academy of Sciences (NAS) president **Marcia McNutt** and **Robert Nerem**, Georgia Tech professor emeritus, in a recent *Science* editorial announcing a new NAS report on research integrity (April 11)

What the study of complete genomes from different parts of the world has shown is that even between Africa and Europe, for example, there is not a single absolute genetic difference, meaning no single variant where all Africans have one variant and all Europeans another one, even when recent migration is disregarded. It is all a question of differences in how frequent different variants are on different continents and in different regions.

—**Svante Pääbo**, biologist and director of the Max Planck Institute for Evolutionary Anthropology in Germany, on the lack of a genetic basis underlying racial categories (February 5, 2016)

I'd much rather fight Ebola in West Africa than in West Dallas.

—Representative **Tom Cole (R-OK)**, chair of the House Labor, Health and Human Services, Education, and Related Agencies subcommittee, on proposed cuts to the Centers for Disease Control and Prevention (March 29)

Science is still the best thing that has happened to humans, even more so because it tries to understand and correct biases.

—Stanford University's **John Ioannidis**, in *STAT* interview about the results of his meta-analysis, published in *PNAS* (March 21), that reviewed seven types of bias across 22 scientific disciplines

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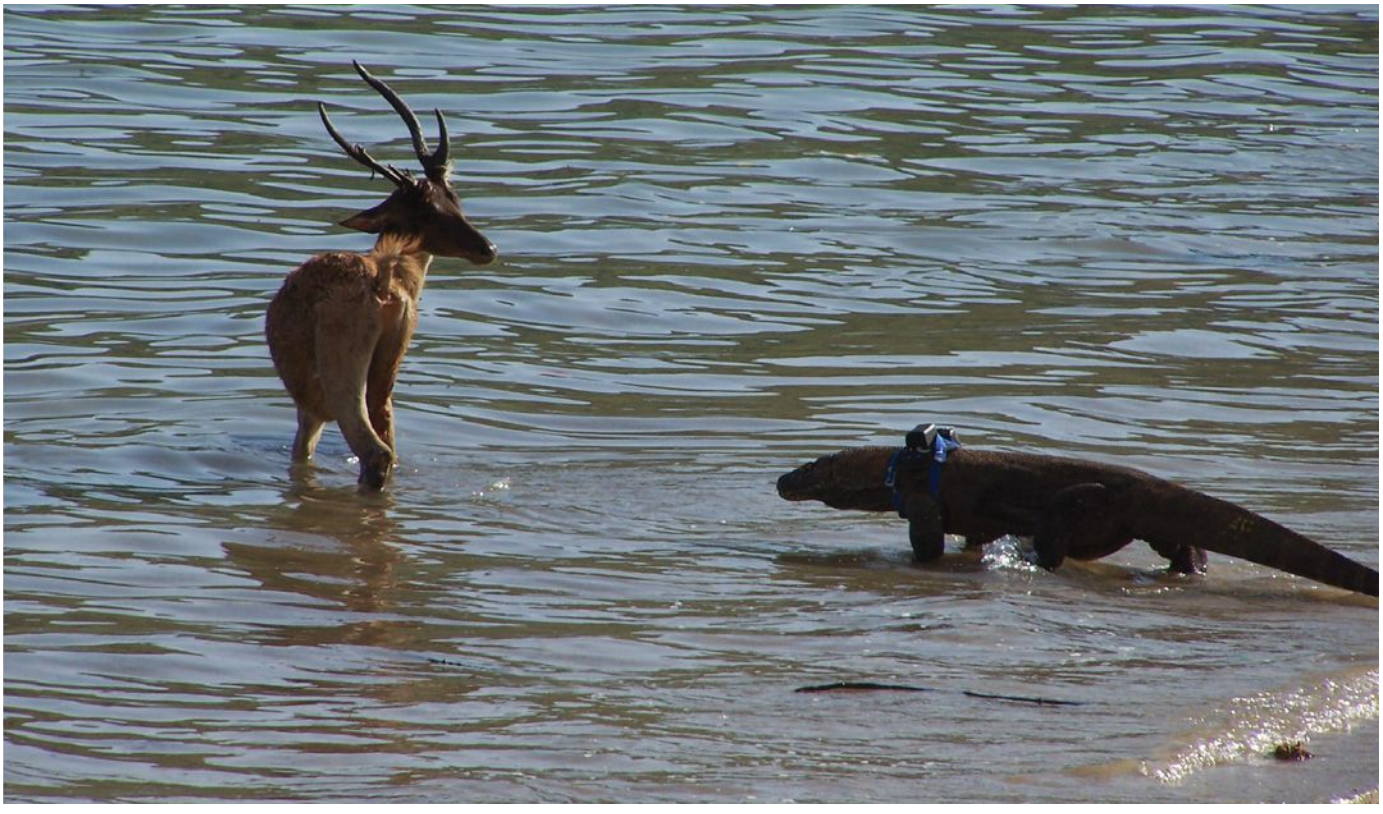


DRAGON PROBLEMS: A Komodo dragon fitted with a GPS tracking device stalks an injured Timor deer in the shallows fringing the Komodo Archipelago in Eastern Indonesia.

PLOS ONE, 5:E11097, 2010; PHOTO BY ACHMAD ARIEFIANDY

Hopeful Monsters

With the world facing an antibiotic-resistance crisis, the hunt is on for alternatives to fight bacterial infection. The quest has led peptide chemist Barney Bishop and bacteriologist Monique van Hoek, both at George Mason University in Virginia, to look for antimicrobial peptides in an unexpected place: inside the Komodo dragon.

"Natural antimicrobial peptides are found pervasively in life on this planet," Bishop says. "It's one of the most primitive immune systems; even bacteria use antimicrobial peptides against other bacteria."

Animals that live in "microbially challenging environments," could be expected to harbor numerous and varied antimicrobial peptides, says van Hoek. Alligators, for example, live in swamps teeming with microbes and regularly sustain injuries from other gators. The distantly related Komodo dragon has been thought to harbor dozens of species of potentially harmful bacteria in its saliva. The giant lizards damage their gums as they attack prey, eat carrion, or fight each other, and those oral bacteria make their way into the animals' bloodstreams. Despite regular exposure to pathogens, these reptiles rarely succumb to infection. The researchers wanted to know if antimicrobial peptides could explain why.

Bishop's team devised a way to mine antimicrobial peptides from the blood of these reptiles, using hydrogel microparticles designed to capture small, positively charged peptides. The technique allowed the researchers to pull potential antimicrobials peptides out of blood samples, then separate the hydrogel particles and determine the sequence of the peptides. A few years ago, Bishop, van Hoek, and their colleagues applied the process to blood samples from American alligators and identified 45 potential antimicrobial peptides.

"I was very surprised personally," says van Hoek, who had found only two published studies that looked at peptides in crocodile blood, both of which had identified just a few peptides that showed little antimicrobial activ-

ity. "Their protocols are quite different from ours. We have more of a firehose problem: we had so many peptides that seemed promising and potentially active that we'd have to sort through."

Using computational programs to predict antimicrobial activity—based on sequence similarity to known antimicrobials and presumed qualities of the peptides, such as hydrophobicity and charge distribution—the researchers selected eight to synthesize for testing. Five of those appeared to be potent antimicrobials (*PLOS ONE*, doi:10.1371/journal.pone.0117394, 2015).

The results motivated the researchers to keep up their pursuit. In particular, "we always wanted to do the Komodo dragon," Bishop says. There's an old hypothesis that a cocktail of nasty bacteria in Komodo dragons' mouths serves as a sort of venom. "A single bite from the Komodo dragon is often enough, over time, to take down its prey," Bishop explains. "They track prey for days until it essentially drops. This way, the Komodo can bring down animals bigger than it that could bring it harm, such as water buffalo. All they need is a quick bite, and then they wait."

While evidence to support this hypothesis remains scarce, it was enticing enough for Bishop and van Hoek to accept samples of Komodo dragon blood from the same St. Augustine, Florida, alligator farm that had provided them with gator samples. Again, the researchers found a large number of potential antimicrobial peptides—48 to be exact (*J Proteome Res*, doi:10.1021/acs.jproteome.6b00857, 2017).

But there was something different about the Komodo peptides, Bishop says. "I was really surprised that we see a very large number of histone fragments." Forty-seven of the 48 peptides were derived from histone proteins. "We see multiple different histone proteins represented," Bishop says. The researchers only tested blood samples from one Komodo dragon, but they are working to verify the results with a sample from another dragon now.

Histones themselves have been shown to have antimicrobial activity, the researchers note. Antimicrobial peptides called bufo-rins, first identified in the digestive systems of toads, for example, are derived from histones, and the fat cells of *Drosophila* produce extra histones when the flies have bacterial infections, "and that seems to be protective," van Hoek says.

When van Hoek's team tested eight synthetic replicas of the Komodo dragon peptides against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, seven of them showed strong antimicrobial activity against both (the eighth was only effective against *P. aeruginosa*). "One thing we look for in microbiology are compounds that have broad-spectrum activity," says van Hoek, which is why she tested one gram-positive (*S. aureus*) and one gram-negative (*P. aeruginosa*) organism. "We found some here, and we found some in our alligator study," she says. "We're really excited." Last month, Bishop, van Hoek, and their colleagues published evidence that a new dragon-inspired synthetic peptide, which they dubbed DRGN-1, promoted healing in mice with infected and uninfected wounds (*NPJ Biofilms Microbiomes*, doi:10.1038/s41522-017-0017-2, 2017).

> **We have more of a firehose problem: we had so many peptides that seemed promising and potentially active that we'd have to sort through.**
>
> —Monique van Hoek
> George Mason University

"It's hard to know where the next antibiotic is going to come from, so these sorts of bioprospecting approaches are pretty neat," says Marvin Whiteley, a microbiologist at the University of Texas at Austin who was not involved in Hoek and Bishop's research.

Although there is a long road to the clinic for such antimicrobial peptides, their use would not be unprecedented. A mixture of antimicrobial peptides called colistin—originally discovered in the bacterium *Paenibacillus polymyxa* in the late 1940s—has been used to treat bacterial

infections for more than half a century. Unfortunately, the treatment has caused kidney toxicity and other problems in many patients, and is now considered a drug of last resort. "People assumed that the whole class of drugs would have the same problems that colistin does," van Hoek says of why there haven't been other antimicrobial peptides that have made it to human trials yet. But, she adds, "especially in the current situation, where we're facing the problem of multidrug-resistant bacteria, we no longer have the luxury of excluding whole classes of antibiotics."

As for whether Komodo dragons harbor bacteria in their saliva as a form of venom to take down large prey—and thus need the antimicrobial peptides to protect themselves from infection—there remains little evidence to support this idea, besides the animals' sometimes impressive predatory take-downs. In 2013, Ellie Goldstein, a professor of medicine at the University of California, Los Angeles, and colleagues surveyed the oral microbiomes of 10 captive adult dragons and found no virulent species (*J Zoo Wildl Med*, 44:262-72, 2013). "It was all basically environmental flora from their food," Goldstein said. "Nothing there was pathogenic."

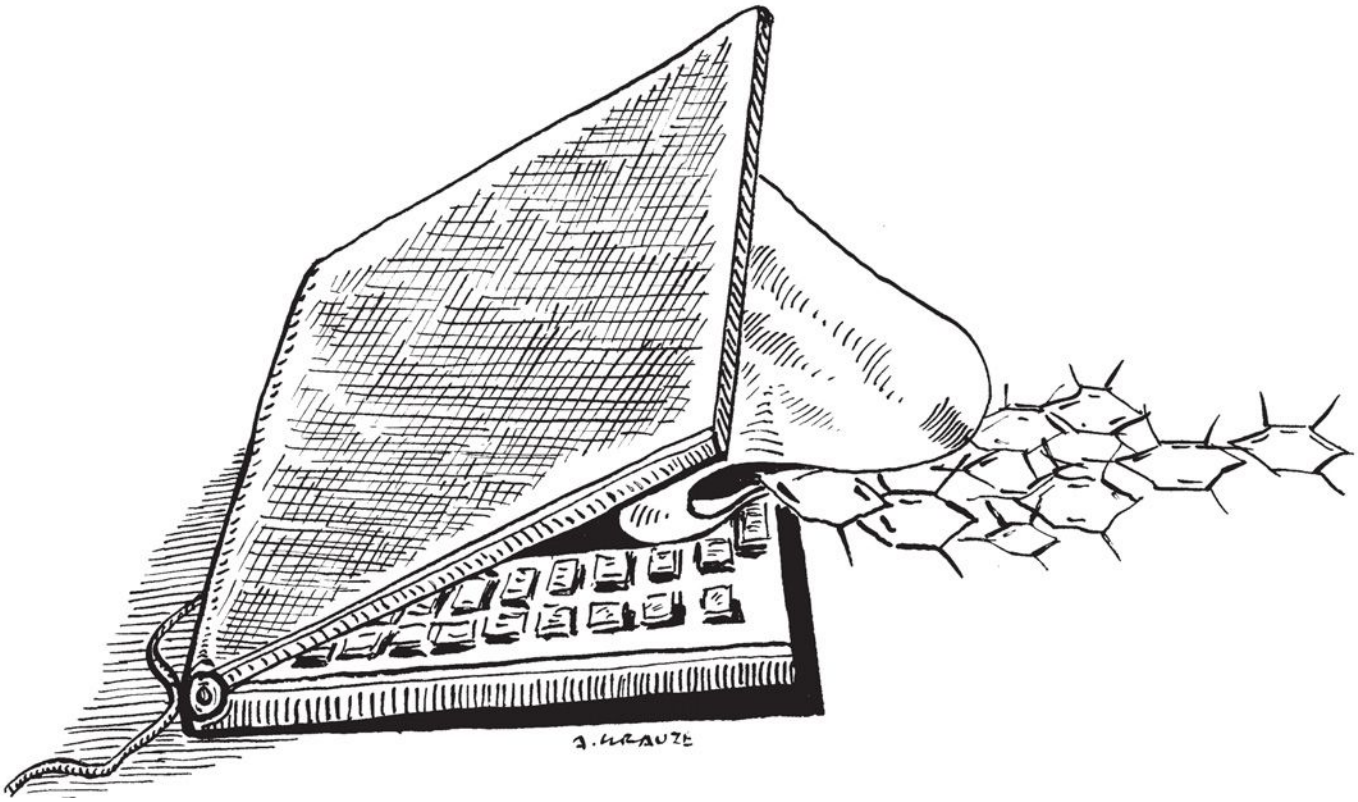
So how do Komodo dragons take down a water buffalo? One idea, put forth by the University of Melbourne venom expert Bryan Fry: the wounds inflicted by the giant lizards get infected when the water buffalo wade into stagnant watering holes. The Komodo dragons then simply take advantage of the situation. —Jef Akst

Makings Scents of Odor Data

In June of 2014, Pablo Meyer went to Rockefeller University in New York City to give a talk about open data. He leads the Translational Systems Biology and Nanobiotechnology group at IBM Research and also guides so-called DREAM challenges, or Dialogue for Reverse Engineering Assessments and Methods. These projects crowdsource the development of algorithms from open data to make predictions for all manner of medical and biological problems—for example, prostate cancer survival or how quickly ALS patients' symptoms will progress. Andreas Keller, a neuroscientist at Rockefeller, was in the audience that day, and afterward he emailed Meyer with an offer and a request. "He said, 'We have this data set, and we don't model,'" recalls Meyer. "'Do you think you could organize a competition?'"

The data set Keller had been building was far from ordinary. It was the largest collection of odor perceptions of its kind—dozens of volunteers, each having made 10 visits to the lab, described 476 different smells using 19 descriptive words (including sweet, urinous, sweaty, and warm), along with the pleasantness and intensity of the scent. Before Keller's database, the go-to catalog at researchers' disposal was a list of 10 odor compounds, described by 150 participants using 146 words, which had been developed by pioneering olfaction scientist Andrew Dravnieks more than three decades earlier.

Meyer was intrigued, so he asked Keller for the data. Before launching a



ANDRZEJ KRAUZE

DREAM challenge, Meyer has to ensure that the raw data provided to competitors do indeed reflect some biological phenomenon. In this case, he needed to be sure that algorithms could determine what a molecule might smell like when only its chemical characteristics were fed in. There were more than 4,800 molecular features for each compound, including structural properties, functional groups, chemical compositions, and the like. "We developed a simple linear model just to see if there's a signal there," Meyer says. "We were very, very surprised we got a result. We thought there was a bug."

In January 2015, the call went out to modelers to join a competition for designing the best model from data on 69 odors to predict their scent profiles. Eighteen teams submitted algorithms. They performed fairly well at estimating the presence of certain qualities in an odor—garlicky, fishy, sweet, or burnt, for example—and especially well at predicting how intense or pleasant a smell would be. "It's a very impressive effort to collect this much data, and it allowed them to model responses and descriptors better than has been done before," says Kobi Snitz, a modeling specialist in Noam Sobel's olfaction research group at the Weizmann Institute of Science in Rehovot, Israel, who did not participate in the competition.

One of the results that surprised Meyer most was the second-place performance of a linear model. That algorithm took different parts of each molecule and generated predictions of how each bit would smell—one part might evoke a bakery, for instance, and another, grass. Meyer speculates that this may reflect something fundamental about olfaction and the way odors interact with receptors. Rather than an entire molecule matching a distinct receptor, perhaps it interacts with numerous receptors, with each responding to these various molecular subunits.

Although his data set contained thousands of molecular features, Keller says very few were required to describe each molecule's smell. "If you know the features of the molecule that make something smell like garlic, you can look at those few and have a pretty good prediction," he says. "A nice step would be to see how that relates to the binding of odor molecules to odor receptors. If you only have a few features that are important, it becomes a more tractable problem."

Keller says there's no consensus in the olfaction field about how the sense works. "The basic science issue is we really have no idea what's in the odor that makes us [perceive a certain smell]," agrees Johan Lundström, who leads olfactory research groups at the Karolinska Institute in Stockholm and the Monell Chemical Senses Center in Philadelphia. Keller's database could offer some insight as researchers continue to probe it (the team has made it publicly available), but there's a limitation: it only includes pure odors, rather than mixtures. "Most odors are not monomolecular," says Lundström. "Ninety-nine-point-nine percent are complicated mixtures that consist of anywhere from two to 500 different chemicals."

Several years ago, Snitz and colleagues developed an algorithm to predict the similarity of certain odor mixtures (*PLOS Comp Biol*, 9:e1003184, 2013). "It turned out that the model works better when mixtures are represented as a single entity rather than as a collection of distinct components," Snitz says.

Keller is already working on a data set of odor mixtures, using an approach similar to Snitz's study, but asking study subjects to rate similarities between different smells, rather than to use semantic descriptors. Until this collection is ready, researchers can play around with the data set used in the DREAM challenge. And for eager modelers, Meyer and other DREAM leaders create new challenges every six months. "It's a very nice idea to have this kind of competition," says Lundström. "Scientists are naturally competitive. This way you can use that competition to do something great for the community."

—Kerry Grens

Probing Pests

Aphids are some of nature's most notorious pests. These tiny, sap-sucking insects munch on farmers' crops, which causes physical damage and transmits pathogens that often render plants unsuitable for human consumption.

While most aphids are only able to colonize one or a few plant species, the green peach aphid (*Myzus persicae*) isn't picky about its food. The polyphagous, or generalist, pest can feed on more than 100 species from around 40 different plant families. "Polyphagous insects always have a host somewhere, so they can keep going and can become massive pests," says Saskia Hogenhout, a project leader in plant-biotic interactions at the John Innes Centre in the United Kingdom.

Green peach aphids are not the only polyphagous pests—others include spider mites and locusts. However, unlike many other generalists that only reproduce sexually, *M. persicae* also spawns clones in the

NOT JUST PEACHY: A green peach aphid, surrounded by her progeny, feeds on a leaf (top). The insect feeds on dozens of different plant species.

ANDREW DAVIS, JOHN INNES CENTRE

spring. "So you could say that *Myzus persicae* is a true generalist in the sense that genetically identical individuals can go to different plant species," Hogenhout says.

Asexual reproduction in aphids is also, coincidentally, a boon for experimenters, says Thomas Mathers, a postdoctoral scientist at the Earlham Institute in the United Kingdom. If researchers keep aphids constantly under springtime conditions, they will only reproduce clonally, providing a population of genetically homogenous specimens to study.

Hogenhout, Mathers, and colleagues recently set out to see what gave *M. persicae* its remarkable ability to colonize such a huge variety of plants. To do so, they first sequenced *M. persicae*'s genome and compared it to that of the pea aphid, *Acyrthosiphon pisum*, a specialist that only occupies a few host plant species.

Much to the authors' surprise, *A. pisum* had a genome nearly double the size of *M. persicae*'s. "[This] is very surprising and counterintuitive," says Chris Bass, a University of Exeter professor who was not involved in the work. "You might expect that this aphid, with its very large host range, has a larger complement of genes that might allow it to feed on a different number of hosts—but it looks like the opposite is actually the case."

The group then investigated the transcriptional changes that occurred when *M. persicae* colonizes a new host. From this analysis, they found that gene expression changes in two gene families, *cathepsin B* and *RR-2 cuticular protein*, occurred rapidly—a mere two days after the aphids were transferred to a new plant host. When the researchers generated transgenic plants that could knock down *cathepsin B* gene expression in the insects, the aphids' ability to survive and reproduce on a new host was significantly reduced.

You could say that *Myzus persicae* is a true generalist in the sense that genetically identical individuals can go to different plant species.

—Saskia Hogenhout, John Innes Centre

The team also discovered that the genes involved in host-switching were older gene duplicates that arose during aphid evolution. "It was as if *Myzus persicae* was able to take pre-existing genetic diversity in the genome and then just fine-tune the expression of those genes to be able to colonize a new host, rather than having more duplicated genes to colonize these different plant species," Mathers says.

"[This is] an important study because we do need to understand what makes a species a generalist or a specialist, what makes them able to switch to new hosts,

and to predict whether they might become pests to other plant species," says Julia Ferrari, a biologist at the University of York who was not part of the work.

Not only is *M. persicae* able to live and dine on multiple plant hosts, but it can also rapidly develop resistance to pesticides. "Understanding the genetic basis of resistance can be very useful in terms of trying to come up with strategies to slow, overcome, or prevent resistance emerging," Bass says.

According to Hogenhout, it's possible that the ability to transfer rapidly between hosts helps *M. persicae* develop resistance to pesticides as well. If a pesticide is applied to a crop, these aphids can quickly move away to another crop after only experiencing sublethal doses of pesticides. A few years ago, a group at Imperial College London reported evidence for this type of mechanism (*Pest Manag Sci*, 70:88-96, 2014). "It's a bit like antibiotics. If you don't finish your antibiotic treatment, you may generate resistant bacteria," Hogenhout says. "If we can prevent the insects from moving away from the crop when insecticides or other control methods are being applied, then we can also maybe delay the time by which the insects can develop resistance."

The group is now looking into the mechanisms that might be behind the green peach aphid's changes in gene expression. "We found all the things that have changed, but we are right at the beginning of finding out how that really works—so what the transcription factors or the epigenetic mechanisms that underline this transition are," Mathers says. "Maybe going after things relating to that might be most useful if you want to find something that you can target for stopping [these pests] from being so successful."

—Diana Kwon

The Walls Are Alive

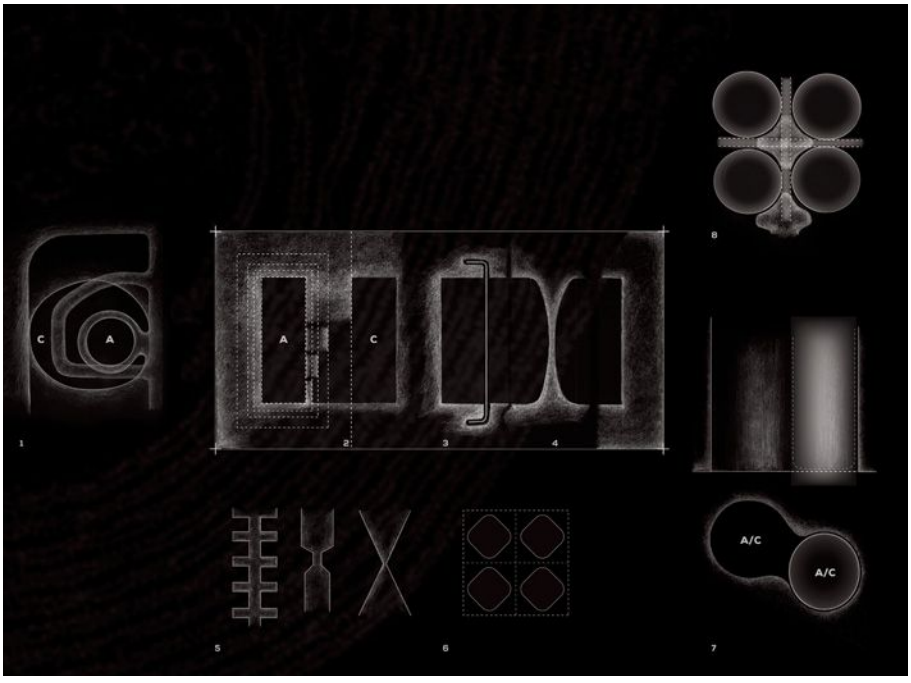
Bristol Robotics Laboratory, on the grounds of the University of the West of England (UWE Bristol), is a clean, dynamic place. Thin partitions separate researchers sitting next to their meticulously designed robots in a huge, open-plan think tank. But down the hall is a separate room, cut off from the hubbub.

Ioannis Ieropoulos and Gimi Rimbu have not shared why their lab is sealed off from the rest of the site as we open the door and take our first step inside, so it's a shock when we're met by an odor that brings tears to the eyes. The vile smell that Ieropoulos's and Rimbu's acclimated nostrils barely notice? Wastewater—delivered a few days prior from the local sewage treatment plant and siphoned into thumb-sized holes in various different standard bricks that look like they are on life-support machines.

Rimbu describes how each six-hole brick houses three microbial fuel cells (MFCs). In one hole sits an anode and, in another, the cathode. "In the hole with the organic waste, water is oxidized," he says. "And in the other, the water is recombined on a carbon substrate which is the electrode. And from this, you clean the water, produce electricity, and harvest small quantities of useful compounds." Connected with crocodile clips and wires, two of these bricks can power a digital thermometer.

The malodorous bricks in Bristol Robotics Laboratory are part of the wider Living Architecture (LIAR) project, an exploratory multinational collaboration funded by the European Union's Horizon 2020 Framework Programme to build a prototype freestanding partition composed of up to three types of bioreactor building blocks. The first is Ieropoulos and Rimbu's MFC. The second is an algae bioreactor, a sealed container of algae that grows and produces biomass or sucks up CO_2 from the surrounding air. The third involves programmable synthetic microorganisms that can act like a metabolic app to produce any product the researchers desire.

LIAR was originally conceived on Christmas Eve 2013 when Andrew Adamatzky, an expert in unconventional computing from UWE Bristol, and Rachel Armstrong, a University of Newcastle experimental architect, started talking. They envisioned "a gut-



SIMONE FERRACINA/LIVING ARCHITECTURE CONSORTIUM

SHIT BRICKS: These diagrams outline the construction of microbial fuel cell-containing building materials that could one day turn wastewater into energy.

Only the insane would want urine running inside the walls of their house.

—Andrew Adamatzky
University of the West of England, Bristol

building or kidney-building which takes away your metabolites," says Adamatzky. "But we realized gut analogies are not that sexy for the general public." Since then, the project has morphed into a mission to make buildings literally come alive brick by brick. Essentially, LIAR's living bioreactor bricks will use sunlight, wastewater, and air to generate oxygen, electricity, proteins, and biomass.

Biomimicry is fashionable in architecture and engineering, but LIAR is a step beyond simply aping natural processes or forms. "Living architecture is where things are not 'like' biological systems, they have original physiology and perform 'as' living things," Adamatzky explains.

Each bioreactor acts as an elementary processor that can communicate locally with its neighbors, meaning an array of bioreactors arranged as a wall acts as parallel processor. "Bioreactors have several innate capabilities, such as sustainability, adaptability, and parallel operation, which traditional silicon-based electronics struggle to replicate," adds UWE Bristol's Neil Phillips. LIAR aims to utilize these abilities to provide a type of unconventional computing capability, which would be difficult or impossible to achieve through established technology.

For example, using the electricity it produces, a living-wall bioreactor array could self-assess its own health and even solve tasks a conventional computer solves using silicon chips. As an analogy, the GeForce GTX 780 graphics card has 2,304 CUDA cores. Likening each brick to one computing core, the ~20,000 bricks needed to construct a typical 4–5 bedroom detached house represent the same computing power as 10 GeForce graphics cards. "That is enough computing—in principle and we are talking about the distant future—to do real-time physical modeling of the behavior of all occupants," essentially merging the fields of artificial intelligence, supercomputing, and green energy production, explains Adamatzky.

While a programmable living house may sound outlandish, Armstrong is keen to highlight that LIAR's aims are grounded. "This is not a paper project," she says. "It is not speculative; it's high-risk, but implementable." Others working in microbial electrochemical technologies see great potential in the LIAR project. "This would be an innovative approach to exploit the capabilities of MFCs for sustainable architecture," says Iowa State University mechanical engineer Nicole Hashemi, who recently demonstrated electricity production from the world's first paper-based MFC. But, she warns the MFCs "may face issues with biofouling or specifically macrofouling in regions where biofilm formation is not intended."

Korneel Rabaey from Ghent University in Belgium sees further problems. "Do not expect any notable current to come from these systems," he says. "Also, stability in the longer term may be challenging. If the system requires maintenance then logistics will need to be developed, and an additional challenge is to show that the power coming off the wall can be harvested."

Back in Bristol, Rimbu has two goals for the next few months: to build an actual 3 × 3 meter prototypical wall made of MFC bricks and to replace wastewater with another renewable resource—human urine. "The electrical output should be two or three times better, as there are some chemicals and ions in urine that the microbial fuel cells like," he notes.

Whether or not home owners will buy into the idea of their dwellings digesting their own waste matter may be a moot point as "only the insane would want urine running inside the walls of their house," jokes Adamatzky.

But Ieropoulos argues that we already have toilet plumbing running through our households, and there is only odor when something goes wrong. "The same applies for living architecture," he says. "Especially since the technology is actually helping bring down the smell from human waste." —Benjamin Skuse

Birds of a Feather?

Taking into account the interaction of nuclear and mitochondrial genes in birds holds the promise of more objectively defining what constitutes a species.

BY GEOFFREY E. HILL

What defines a species? Because the boundaries between species can appear so fluid, pursuing such a question seems, at times, like academic esoterica—little different than discussing how many angels can dance on the head of a pin. But accurate species definitions lie at the heart of biological investigations and management of natural resources (e.g., the US Endangered Species Act). It is troublesome, therefore, that new information on the genetic structure of long-recognized species of birds could jeopardize their status as full species.

The problem, in a nutshell, is that the DNA of many familiar species of birds holds signatures of substantial exchange of nuclear genes with other bird species. Such gene exchange matters because, by decades-old definitions, it is the isolation of gene pools that defines species. Substantial genetic exchange raises questions about whether these populations truly constitute species.

A case-in-point concerns the blue- and golden-winged warblers, two beautiful and very distinctive little songbirds that have long been regarded as separate species. A recent study, however, showed that these two "species" share more than 99 percent of their nuclear genes—much more gene sharing that we would expect between full species. There is evidence that this shared nuclear genotype is a product of regular interbreeding between individual golden- and blue-winged warblers for millennia. In other words, there appears to be poor isolation of gene pools between these two songbird species. These data are leading some biologists to question whether blue- and golden-winged warblers really are separate species. The golden-winged warbler is currently a threatened species

Blue-winged warbler

Golden-winged warbler

receiving conservation attention, so losing status as a separate species would change not only birdwatching field guides, but also federal and state funding to protect threatened avian populations.

There are many other examples of iconic birds with uncertain species boundaries. The Baltimore oriole, namesake of a professional baseball team, was merged with other orioles to form the not-so-iconic "Northern oriole," before a committee of the American Ornithologists' Union voted the Baltimore oriole back to full-species status.

Most of the focus on the flow of genes between species concerns genes in the nucleus, which harbors the large majority of genes in all animals. But complex organisms also have a small set of genes in the mitochondrion that is distinct from nuclear genes. An interesting pattern is emerging repeatedly in genomic comparisons among closely related bird species: even as variation in nuclear genotypes indicates fuzzy boundaries between species, differences in mitochondrial genotypes form distinct and abrupt boundaries between species.

For instance, blue- and golden-winged warblers show a level of genetic differentiation in mitochondrial genotype that is typical of separate species. An abrupt transition in mitochondrial genotype exactly matches the abrupt transition in plumage color and song between these two species of warblers.

Time and again, the nuclear genetic boundaries between avian species are fuzzy while the mitochondrial genetic boundaries are clear. A possible implication is that mitochondrial genes play a special role in speciation.

In a paper published online on March 8 (*The Auk*, 134:393-409, 2017), I have proposed a new definition of species, which could explain these patterns of variation in nuclear and mitochondrial genes. I argue that what really makes each species distinct is a set of mitochondrial and nuclear genes—about 200 genes in total—

that have evolved to work well together. I call this a "coadapted set of mitonuclear genes," and present the idea that it is uniquely coadapted sets of mitochondrial and nuclear genes that define a species. Because most nuclear genes are not coadapted with particular mitochondrial genes, the large majority of nuclear genes can move across species' boundaries during hybridization events without compromising species integrity, forming the fuzzy boundaries between species in nuclear genotype. Mitochondrial genes and the small number of nuclear genes that interact with them are prevented from flowing into other species because mismatches in these coadapted genes produce low-quality individuals that do not persist.

In my paper, I applied this mitonuclear compatibility species concept only to birds, but there is no reason to believe that speciation in birds is fundamentally different than speciation in other complex animals. Birds provide an ideal test case for species concepts because we have detailed knowledge of their distributions and patterns of interbreeding, and because the traits that birds use in sexual signaling—coloration and song—are conspicuous to human sensory systems. Also, because of their very high respiration rates (the body temperature of most birds is around 105 °F), birds are the animal group that is likely to suffer the highest fitness costs when mitonuclear genes are mismatched across species boundaries, as the loss of function is targeted to cellular respiration.

Even as variation in nuclear genotypes indicate fuzzy boundaries between [bird] species, differences in mitochondrial genotypes form distinct and abrupt boundaries between species.

The good news about the mitonuclear compatibility species concept is that it makes specific predictions that can be tested with the burgeoning databases on both the nuclear and mitochondrial genotypes of birds. If the hypothesis is supported in birds, then it can be applied to other vertebrates and to other animal groups.

A species concept that can be objectively applied to define species would be a boon to taxonomists and conservation biologists alike. And it is somehow reassuring that the instincts of 19th century naturalists regarding the boundaries between species might be confirmed with 21st century genomic analysis. ■

Geoffrey E. Hill is a professor of biological sciences and curator of birds at Auburn University in Alabama. A version of this story was published at the-scientist.com on March 10, 2017.

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TheScientist TOP 10 INNOVATIONS

Phosphorylation at the Flick of a Switch

Incorporating light-controlled dimerization domains into kinases provides tight regulation of these enzymes.

BY RUTH WILLIAMS

Controlling a protein's activity with light enables spatial and temporal regulation that would be practically impossible otherwise. Such fine control is desirable for teasing out the molecular details of cellular processes and for initiating the actions of therapeutic proteins in precise locations in the body.

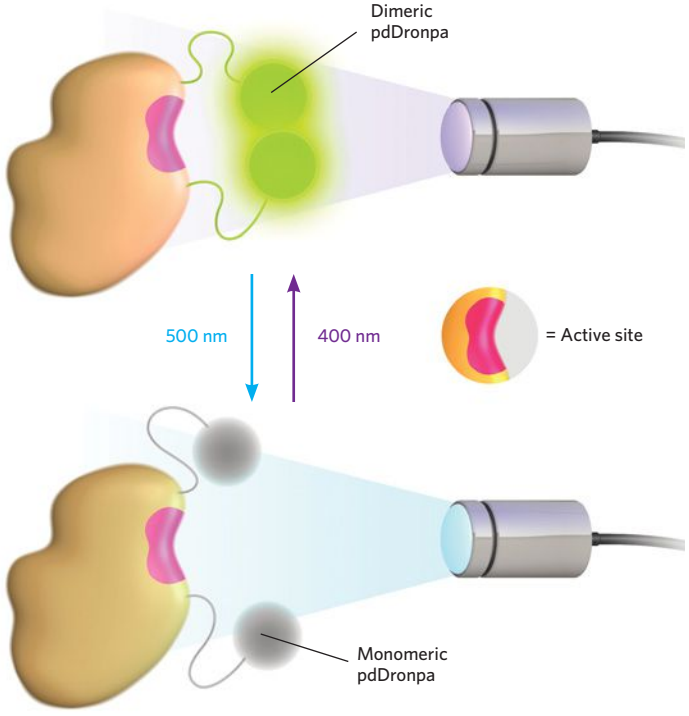
Molecular biologists, including Michael Lin of Stanford University, are hard at work developing and improving such protein technology. And Lin's latest approach is "particularly remarkable," says Harald Janovjak of the Institute of Science and Technology in Austria.

The principal component of Lin's system is an engineered protein dimer (a green fluorescence protein) that, upon exposure to blue light (500 nm), converts to two monomers. Upon violet light (400 nm) exposure, the monomers revert to the dimeric form. Without violet light, the monomers will slowly dimerize in approximately 15 minutes, says Lin.

By encoding these monomers as domains on either side of the active-site sequences of kinases, Lin's team has created enzymes that are inactive when the domains are dimerized and active when the domains separate into monomers. Janovjak likens these engineered kinases to people folding and unfolding their arms. "If I were to cross my arms in front of me, that makes interactions with other people more difficult," he says.

What's more, the fluorescent protein itself changes brightness from high to low as it switches from dimer to monomer, providing a visual indication of kinase activation, Lin explains.

Using this dimerization technique, Lin's team has made four photoswitchable kinases, which the researchers have shown work as well as the endogenous enzymes in both cells and animals. But, the concept could be readily applied to other types of proteins, says Lin. (*Science*, 355:836-42, 2017) ■



FLIPPING OUT: Researchers have designed kinases that can be inactivated and activated by light. In violet light, engineered green fluorescent domains (called pdDronpa) dimerize, glow, and block the enzyme's active site (top). In blue light, the domains break into monomers, lose their fluorescence, and uncage the kinase's active site (bottom).

AT A GLANCE

KINASE PHOTOACTIVATION TECHNIQUE	HOW IT WORKS	ENGINEERING REQUIRED	REVERSIBLE ACTIVATION	SUITABLE TARGETS
Light-induced kinase activator localization	The system uses the light-induced binding of a phytochrome (Phy) to phytochrome interaction factors (PIF), all found naturally in photosynthetic organisms. A kinase activator is engineered to contain a PIF domain, and a Phy domain is tethered to a location of interest—say, the plasma membrane. Light prompts recruitment of the activator to the desired site (by Phy-PIF interaction) and consequent kinase activation.	Two recombinant proteins	Yes	Kinases regulated by localization activators
Single-chain photoswitchable kinases	A kinase is engineered to contain two monomer domains that interact to form a dimer. This dimerization folds and inactivates the kinase. Blue light separates the monomers, thus unfolding and activating the enzyme.	One recombinant protein	Yes	In theory, all kinases

THE TEMPO OF EVOLUTION

Genetic change can occur on timescales that influence ecological dynamics.

BY JEF AKST

Starting in the late 1970s, aspiring evolutionary biologist David Reznick became intent on documenting evolution in action. Although he had learned in school that observable change took place over millennia, the young biologist questioned that notion, and set out to observe genetic adaptation in real time. "Prominent evolutionary biologists were skeptical you could see it happening," Reznick recalls. "I guess it was a gamble, but it seemed like one that was worth taking."

He chose Trinidadian guppies as his study system, and as a graduate student at the University of Pennsylvania Reznick flew to the Caribbean island off the north coast of Venezuela in 1978 to observe and collect guppies, and again in 1981 to shuffle the fish between streams. He moved guppies that were living among cichlids and other larger fish to low-predation sites that also lacked other guppies. (See illustration on pages 30-31.)

Within four years—just eight generations—Reznick saw the populations change. Guppies transferred to low-predation environments matured and reproduced later, and grew to a larger size. Guppies from the original high-predation environment did the exact opposite.[1] Females in low-predation environments also began having fewer, but larger young, and the interval between their litters (guppies give live birth) got longer. Back in the lab, Reznick bred guppies from the two different community types for two generations and found that the grandchildren of the wild-caught fish maintained their life history differences when raised in a common environment.[2] This demonstrated that the phenotypic changes were genetically encoded, not simply due to phenotypic plasticity, those morphological, physiological, and behavioral adjustments organisms can make in their lifetimes. Here was evolution—genetic change at the population level—happening right before Reznick's eyes.

"People thought, if we want to understand the process of evolution, we look at the fossil record," says Reznick, now a biology professor at the University of California, Riverside. But by averaging phenotypic change across tens of thousands or millions of years, the fossil record underestimates rates of change on shorter timescales, so "for a very long time, people were looking at a biased image of how evolution happens."

PAUL BENTZEN

GUPPY GROUPIES: In the late 1980s, the University of California, Riverside's David Reznick peers into a microscope, inspecting a guppy from a Trinidadian stream for colored marks he and his colleagues had injected just under the skin of hundreds of fish for a mark-recapture study. (In the foreground, Helen Rodd, then a student working with Reznick and now a professor at the University of Toronto, does the same.)

Around the same time, researchers were observing similarly high rates of change in other animal populations. As Reznick was puddle-jumping across Trinidad, famed husband-and-wife evolutionary biologists Peter and Rosemary Grant in the Galápagos Islands were documenting changes in the size and shape of finch beaks following environmental fluctuations.[3] These natural experiments revealed bursts of change in the birds' beaks after an exceptionally strong El Niño event in the early 1980s, for example,[4] and in response to heightened competition for food between the medium ground finch (*Geospiza fortis*) and the large ground finch (*G. magnirostris*), after the latter's migration to Daphne Major, the Grants' research site, in 1982.[5] Statistical analyses involving pedigrees that demonstrated the high heritability of beak morphology determined that these changes were primarily the result of genetic evolution, not phenotypic plasticity.

These studies weren't the earliest to document quick shifts in a population's traits, but "the work came out at just the right time," says McGill University evolutionary ecologist Andrew Hendry. "People were ready for the idea that evolution occurred rapidly. . . . The finch and the guppy work [served as] the empirical exemplars from which one could say that this is a general phenomenon."

Adding to those examples, evidence of evolution occurring faster than previously appreciated has continued to accumulate over the past few decades. It is now clear that, while observed rates of change may well be rapid relative to the evolutionary timescales that Darwin theorized about, they're not at all exceptional. "Within evolutionary biology there really has been an unheralded paradigm shift between 1980 and now," says Reznick. "Most evolutionary biologists consider it routine to think of evolution as a contemporary process."

The concept, appropriately termed contemporary evolution, is now well accepted, agrees Stephen Ellner, an ecologist and evolutionary biologist at Cornell University. "At this point, there's a general understanding that this is happening, and it's happening all over." The research has now shifted from documenting this phenomenon to studying its consequences.

How fast is fast?

When discussing evolution, defined as changes in the genetic makeup of a population, the relevant unit of time is the generation: alleles that aid in the organisms' survival and reproduction are likely to increase in frequency from one generation to the next, while those that reduce fitness will likely become less common. For this reason, it is easier to study evolutionary change in organisms with shorter generation times.

The other key factor dictating the rate of evolutionary change is the strength of selection. The rapid appearance of resistance mechanisms in bacteria exposed to antibiotics is the epitome of what can happen when extreme selection is imposed on a species with short generations. While researchers have long recognized that antibiotic resistance can develop quickly in infection-causing pathogens, the idea that contemporary evolution could be detected in macrofauna species remained on the scientific fringe for years. But, Ellner posits, if it's happening in bacteria under intense selection, why not in other organisms under other conditions? "A priori there's no reason [to think] the same sort of thing wouldn't be happening when generation times are longer and selection isn't that strong."

MOST EVOLUTIONARY BIOLOGISTS CONSIDER IT ROUTINE TO THINK OF EVOLUTION AS A CONTEMPORARY PROCESS.

—David Reznick, University of California, Riverside

The literature on "rapid evolution" is now 30 years deep. Using what are known as common garden experiments—raising animals from different populations in the same controlled environment (as Reznick did with the guppies)—researchers have observed some astonishing rates. In 1997, Reznick and his colleagues calculated rates of change in his guppy experiments of "up to seven orders of magnitude greater than rates inferred from the paleontological record," the authors wrote in *Science*.[6] That same year, Harvard University's Jonathan Losos, then at Washington University in St. Louis, and collaborators published a *Nature* paper documenting the differentiation of anole populations over a decade and a half following the release of the lizards onto 14 small islands in the Bahamas in 1977 and 1981.[7]

COURTESY OF DAVID REZNICK

The studies caught the attention of Hendry and Michael Kinnison, then fellow graduate students in Thomas Quinn's lab at the University of Washington. Both young researchers were studying rapid evolution in salmon, but despite the focus of their studies, neither had given much thought to quantifying the rates of change in a way that would be comparable across species or defining what should count as rapid. "A lot of [researchers] were working under the assumption that, if you could see it, it was rapid. If you could see it within a human life span, then it must be exceptional," says Kinnison, now a professor at the University of Maine. "What is rapid or not rapid should have some measurable frame of reference."

After the *Nature* and *Science* studies came out, Hendry and Kinnison got together to develop a framework for quantifying rates of evolutionary change. They promoted the use of the haldane (a change of one standard deviation in a phenotypic trait per generation; named after evolutionary thinker J.B.S. Haldane) over the darwin (the proportional change in a phenotype per million years; named after you-know-who), and encouraged researchers to provide confidence intervals and measures of statistical significance. If done correctly, "evolutionary rates provide a convenient way to compare the tempo of evolution across studies, traits, taxa, and time scales," Hendry and Kinnison wrote in 1999.[8]

FINCH FLUCTUATIONS: Evolutionary biologists Peter and Rosemary Grant documented changes to the beak size and shape of the medium ground finch (*Geospiza fortis*, above) following the 1982 arrival of the large ground finch (*G. magnirostris*) to Daphne Major.

In November 2001, the researchers published a meta-analysis of patterns in reported rates of contemporary evolution.[9] Mining the literature for usable data sets, Kinnison and Hendry found no shortage of studies documenting change over the course of a study. "There was a lot more out there than we even suspected ourselves," says Kinnison. "The more and more we dug, the more and more cases that we found . . . all sorts of species spanning all taxonomic breadth."

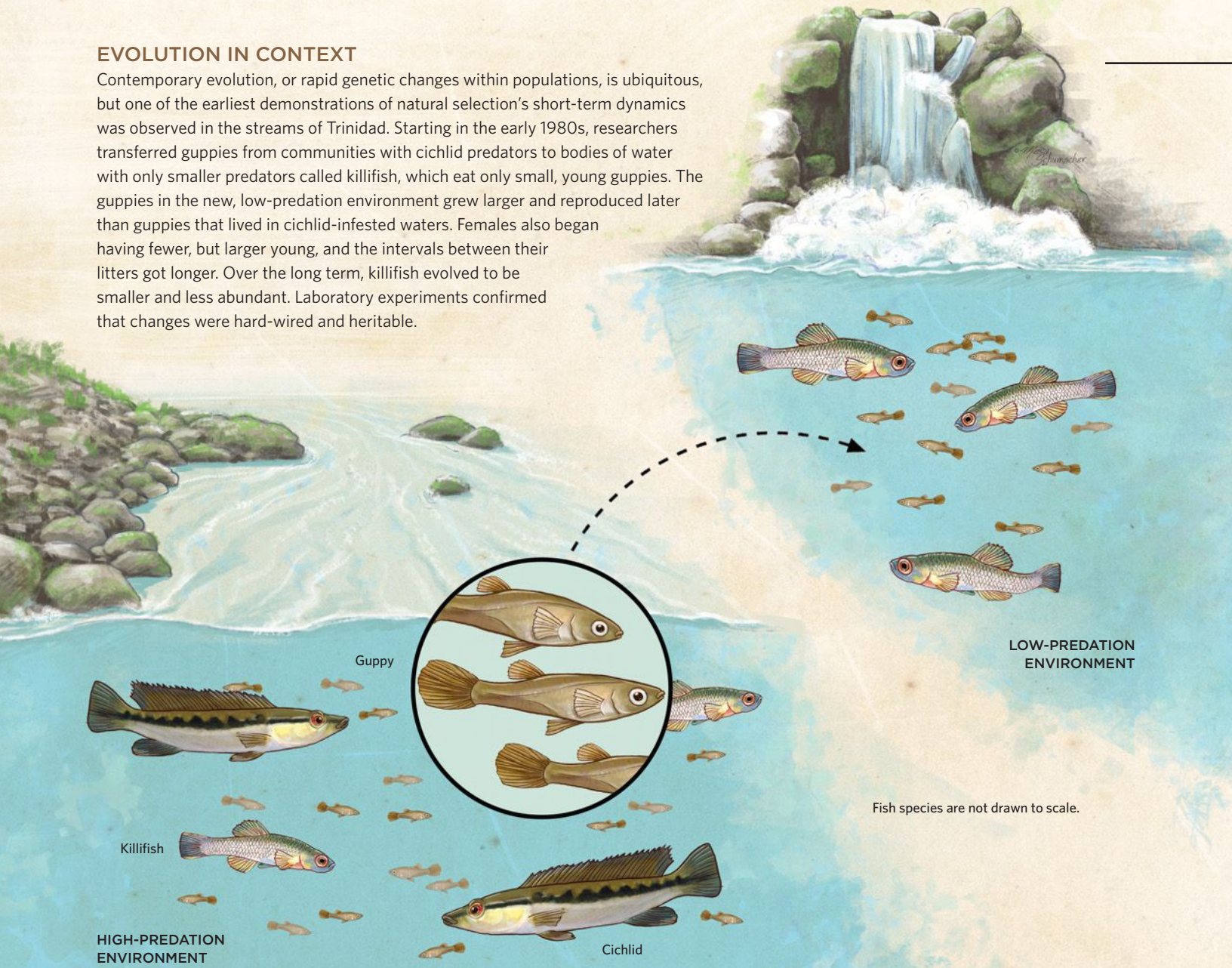
In total, the researchers gathered data on 30 different animal species, for a total of 2,151 evolutionary rates calculated in haldanes, and another 2,649 in darwins. The sources included both "genetic" studies—those that performed common garden experiments or used quantitative genetics to infer genetic change in wild populations—and "phenotypic" studies, which just measured change in a trait over time, and thus represented the combined effects of genetic adaptations and phenotypic plasticity. Analyzing all the data together or the genetic data separately yielded similar results. As it turned out, part of the reason so many scientists had been able to document dramatic change over short time frames was precisely because they were limiting the duration of observation; in the short term is when evolution's at its fastest.

WIKIMEDIA COMMONS/HANS HILLEWAERT; © ISTOCK.COM/PATRICK_GIJSBERS

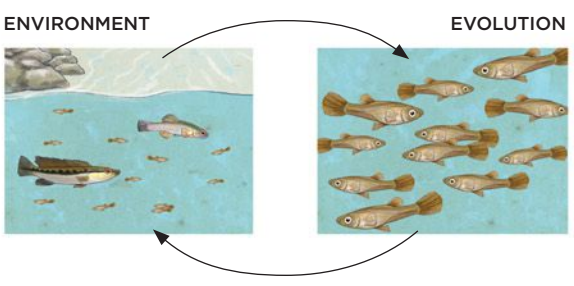
LIZARD LINEAGES: Harvard University's Jonathan Losos and colleagues have found that the diversification of anole populations released onto 14 small islands in the Bahamas in 1977 and 1981 was driven largely by the environment.

EVOLUTION IN CONTEXT

Contemporary evolution, or rapid genetic changes within populations, is ubiquitous, but one of the earliest demonstrations of natural selection's short-term dynamics was observed in the streams of Trinidad. Starting in the early 1980s, researchers transferred guppies from communities with cichlid predators to bodies of water with only smaller predators called killifish, which eat only small, young guppies. The guppies in the new, low-predation environment grew larger and reproduced later than guppies that lived in cichlid-infested waters. Females also began having fewer, but larger young, and the intervals between their litters got longer. Over the long term, killifish evolved to be smaller and less abundant. Laboratory experiments confirmed that changes were hard-wired and heritable.

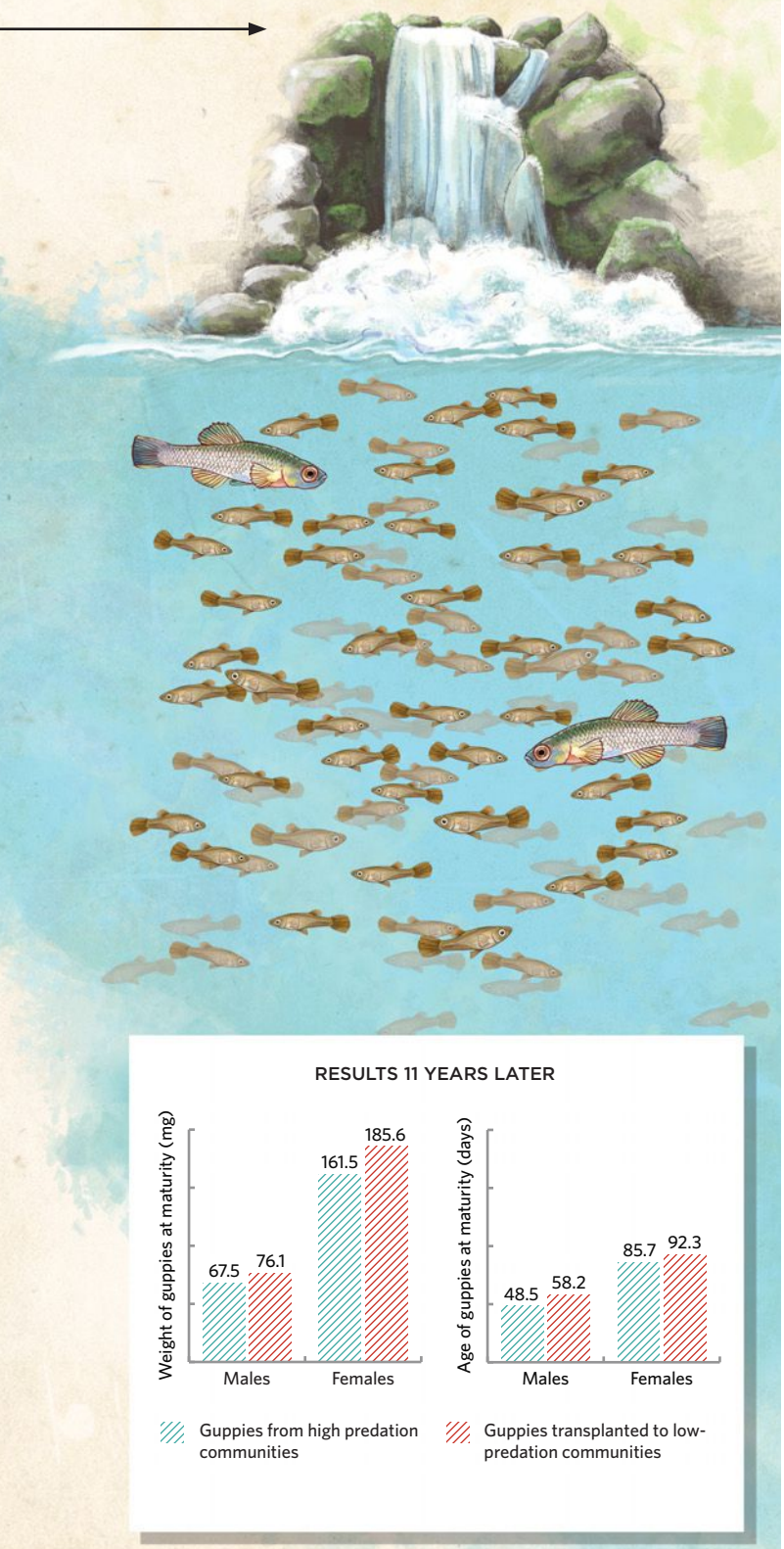


Fish species are not drawn to scale.



ECO-EVO FEEDBACK

As the guppies became more abundant in the low-predation habitat, they faced more competition. It turns out that high guppy densities—and the subsequent depletion of resources—is the main driver of guppy evolution, not the higher probability of survival in the low-predation communities (Reznick et al., in review). Moreover, as the guppies evolved, they further influenced the environment, affecting not only individuals in their own population, but all the other organisms in the community, which were also evolving.



It makes sense, says Kinnison. "If populations are really tracking dynamic environments—year-to-year variation in climate, other species that they depend upon or compete with, or the like—then you would expect their traits to be bumping around pretty rapidly. But over the long term, a lot of these processes average out, [which] flattens those rates down."

The study was published in a special issue of *Genetica*, devoted to the topic of contemporary evolution and edited by Hendry and Kinnison. "I guess what Mike and I did was, by bringing these 30 papers about rapid evolution together, we made it clear that these weren't isolated phenomena that were just weird exceptions," Hendry says. "They were actually kind of common, and you could use that information to ask questions that weren't just about guppies or finches."

Ecological theory evolves

At the 2005 annual meeting of the Ecological Society of America (ESA) in Montreal, Hendry organized a symposium dedicated to contemporary evolution. "All the main players were there," Hendry says. At that meeting, Tom Whitham of Northern Arizona University gave a talk about what he called "community genetics," describing how genetic variation within populations of cottonwood trees had ecological consequences, such as effects on the insect and microbial communities. "One genotype of cottonwood tree will create a very different environment than another genotype," Hendry recounts. "That was, for me, the realization that rapid evolution wouldn't just have consequences for the organisms themselves, but for the rest of the community also and for the ecosystem."

Traditionally, ecologists modeling communities and biomes have treated species as static organisms, assuming that evolution takes place on timescales that are far too slow to influence ecosystem dynamics, which operate on the order of weeks, months, and years. But as many studies have now shown, organisms are not constant at all, and "the rate at which they change is comparable to the rate at which ecological interactions are happening," says Reznick. "It can have a profound effect."

Two years before the ESA meeting, working in collaboration with the lab of Cornell colleague Nelson Hairston, Ellner and colleagues published the first experimental demonstration of this effect in the lab, showing that the rapid evolution of algae (prey) in response to oscillating densities of rotifers (predators) substantially altered the overall predator-prey dynamics.[10] "Often when you look at natural populations of predators and prey, you get cycles of abundance," says Reznick. "What they showed was that those oscillations change in a very dramatic way if the prey are evolving as part of the cycle; the whole structure of the cycle is different."

Studying such eco-evolutionary dynamics in natural systems "is vastly harder to do," Hendry says. But a handful of studies make a strong case that the dynamics researchers observe in the lab are also operating in nature. In 2011, for example, Reznick worked in collaboration with Martin Turcotte's group at the University of Pittsburgh to demonstrate that evolving field populations of green peach aphids

THE GUPPY EFFECT: In experiments interrogating the impacts guppies have on their environment, the University of California, Riverside's David Reznick and his colleagues used electric "exclosures" to deliver a weak pulse of electricity every few seconds, deterring guppies (but not invertebrates and other smaller species) from feeding on the algae. When the grid is electrified (top: right; bottom: left), diatoms quickly accumulate.

grew significantly faster and reached higher densities than control populations that could not evolve because they harbored no genetic variation.[11,12] In 2014, Tim Farkas, a postdoc at the University of Connecticut, reported similar dynamics in experimental populations of stick insects, where the relative fitness of two morphs—equally represented at the outset—varied according to the density of the founding populations.[13] And in the streams of Trinidad, Reznick's team has observed that guppy evolution triggers a ripple of change through the ecosystem. Guppies play an important role in limiting algal growth in the streams, for example, and the fish prey on young killifish, creating a complex predator-prey dynamic. "Guppies have always been the victim—killifish eat baby guppies—but guppies eat baby killifish," Reznick says, and the two species compete. As the guppies evolve, changes in them can affect all of these interactions, which can further affect the environment and all the organisms evolving within it.

"We've got all of these interesting examples of what you would call eco-evolutionary effects," says Kinnison—"places where evolution is feeding [back on] some ecological aspect."

From field studies like these that validate laboratory findings and theoretical work, researchers know that individual genotypes—and diversity itself—have ecological consequences, says Hendry. And the general rule is that more individual genotypes equates to better ecological function. In fact, he says, "evolution within a community will tend to maintain diversity in that community, on average . . . generally enhancing ecosystem function, productivity, nutrient cycling, things like that."

The complexity of both ecosystems and evolutionary theory make these dynamics very challenging to dissect, however. In addition to the logistical limitations of the research, there are theoretical challenges. For example, most studies of contemporary evolution follow a focal species, when in reality, every species in a community is evolving at the same time, Hendry says. And it's very possible that eco-evolutionary dynamics are yielding "cryptic" effects. "The classic measure of eco-evolutionary dynamics is a [phenotypic] change," says Hendry. "But what evolution often does is it generates stability, so you see no change. So you have this paradox where the lack of apparent dynamics actually probably reflects a huge underlying component of eco-evolutionary dynamics, and that's just super hard to study."

In 2005, Ellner, Hairston, and colleagues outlined ways of assaying how important evolutionary change is for ecological dynamics.[14] And in 2011, the researchers published an updated protocol, emphasizing the data and statistical analyses needed to determine whether observed changes are the result of evolution or of differing ecological conditions.[15] "By hook or by crook, do the full factorial of the population before and after the evolutionary change in the environmental conditions. Then you can parse out the contributions," says Ellner.

The field is young, and more than anything, it needs more time, he adds. "In part, it's just a matter of waiting for the data to accumulate. . . . One generation is the speed limit."

The human factor

Although researchers aren't entirely sure how evolutionary and ecological forces interact, these newly appreciated dynamics demand consideration in a number of different contexts, perhaps most obviously, conservation. Organisms are likely evolving in response to the degradation of their habitats, for example, not just dying off randomly such that the population's genetic mix remains the same even as it dwindles toward extinction.[16]

For example, the occurrence of rapid evolution could have implications for the world's fisheries. "When we are not targeting the small juveniles and babies that natural predators would, but we are actually taking out the big adults that are in their reproductive prime, it makes sense to every evolutionary ecologist that we are causing evolutionary changes, genetic changes," says Silva Uusi-Heikkilä, an evolutionary biologist at the University of Turku in Finland.

Indeed, Kinnison and his colleagues have calculated that populations harvested by fishermen, hunters, or plant cultivators evolve some three times faster than organisms subjected to other types of selective forces.[17] In addition to the severity of harvest—with people effectively clearing out all individuals of a particular size range, for example—the practice also exerts a very consistent selective pressure, in contrast to the instability of many environmental factors, Kinnison explains. "Harvest is the champ," he says. "Harvest drives evolution faster than everything else."

In the laboratory, Uusi-Heikkilä and her colleagues have found that size-selective harvesting of fish can indeed drive their evolution. Starting as a graduate student at the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Berlin, she raised zebrafish in large tanks, periodically scooping them out to measure them all and removing 75 percent of the largest fish, to mimic the situation in recreational and commercial fishing. "We were able to show that only five generations of size-selective harvesting caused genetic changes in these populations," Uusi-Heikkilä says. Those populations that were "fished" were smaller as adults and had lower reproductive output. Females produced fewer eggs

RON BASSAR

and spawned less frequently. The zebrafish were also less active, less explorative, and less bold.

But the importance of evolution has remained controversial in the fisheries industry, says Uusi-Heikkilä, largely because it's difficult to demonstrate that evolutionary changes in wild populations are the result of size-selective harvest and not some other factor. And fisheries biology is a contentious field. "It can be difficult; there's a lot at stake," Uusi-Heikkilä says. In addition to being a scientific endeavor, "this is an economic [and] political issue."

IT MAKES SENSE TO EVERY EVOLUTIONARY ECOLOGIST THAT BY FISHING WE ARE CAUSING EVOLUTIONARY CHANGES.

—Silva Uusi-Heikkilä, University of Turku

Contemporary evolution also throws a wrench into the use of conservation hatcheries, captive-breeding programs initiated to help repopulate wild fish stocks. In 2008, Mike Blouin's group at Oregon State University, along with NOAA researchers at the Northwest Fisheries Science Center in Washington State, published a study that provided evidence that fish were adapting to captivity in ways that made them less fit when released into the natural environment. The team collected steelhead trout from the Hood River, a tributary of the Columbia River a few hours' drive north of the university, and bred them in captivity for two generations. The researchers then bred the captive fish with wild-caught fish and raised those offspring in the hatchery until they matured, alongside the offspring of two wild fish to control for the effects of the rearing environment. They then released this fish to breed in the wild, and for three years sampled wild trout for genetic analyses to determine if they were descendants of the fish raised in captivity. They used this information to calculate the reproductive success of the captive-reared trout, and compared fish whose genomes came entirely from wild trout with those that had one captive-raised parent.[18]

"[It was] a really powerful experiment," Kinnison says. "They estimated that the loss of fitness in the wild from just one generation of having parents bred in captivity was pushing upwards of a 40-percent reduction in performance. It was shocking."

How eco-evolutionary dynamics could inform the development of sustainable harvesting practices remains an open question. But the importance of considering these forces when developing fisheries-management strategies is slowly being recognized by the fishing industry. Five years ago, an international group of researchers suggested the implementation of evolutionary impact assessments "as a structured approach for assessing the evolutionary consequences of fishing."[19] And in November 2015, researchers published the first peer-reviewed evolutionary impact assessment, that of the North Sea plaice fishery.[20] "I think it's really now starting to catch," says Kinnison.

Uusi-Heikkilä knows without more solid fieldwork there's still a steep hill to climb to convince skeptics; laboratory studies alone just won't cut it. "I have to be very careful when I mention zebrafish and fisheries in the same sentence," she admits. "But what we are able to do with this model system and experimental studies is disentangle the plastic and evolutionary effects to show that it is possible. Because I think previously people didn't even believe this is possible. I mean, evolution can't happen in a few generations, it's something that takes millions of years." ■

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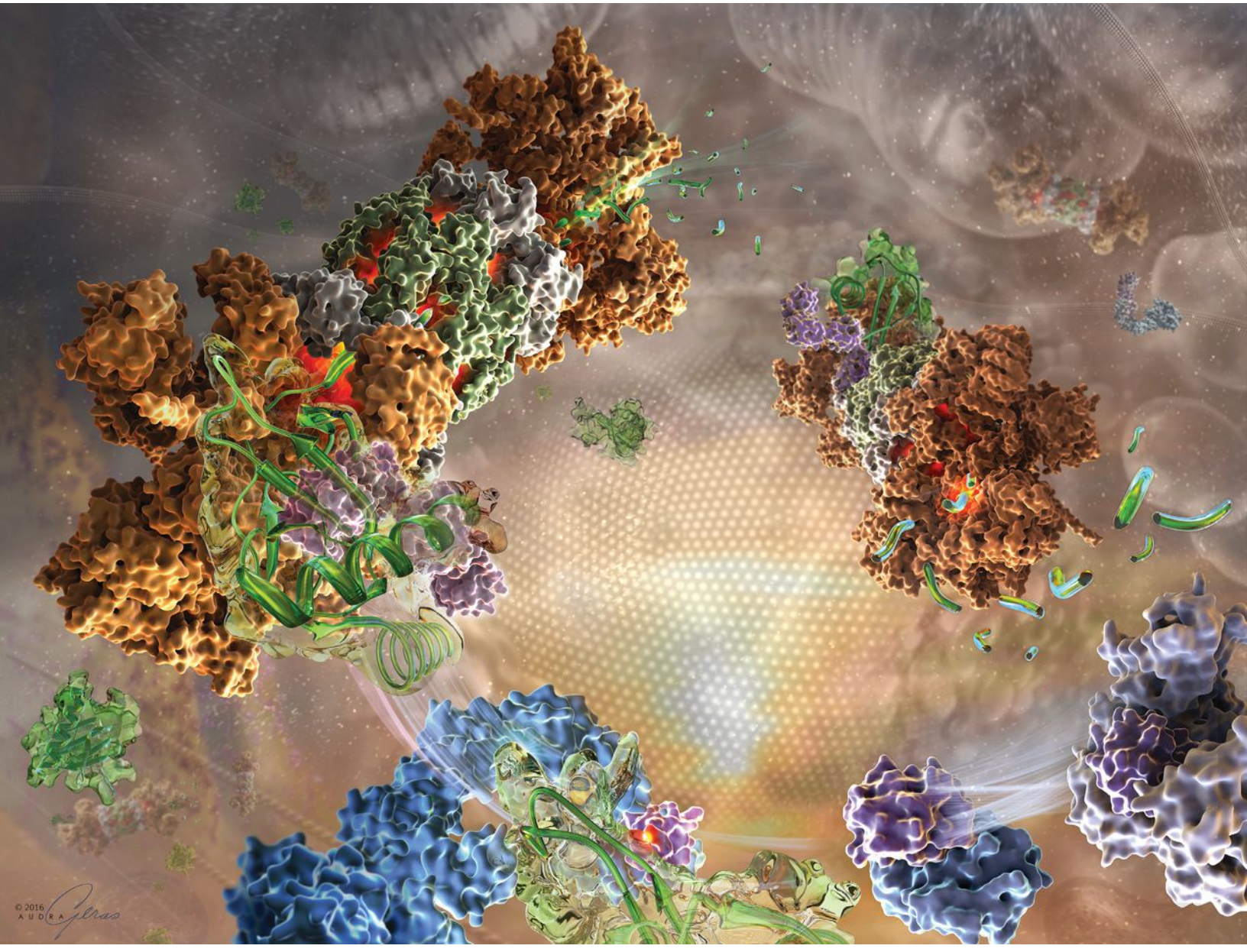


The Protein Recycler

Proteasomes serve as powerful tools in research and medicine.

BY JOHN HINES AND CRAIG M. CREWS

Although they are not alive themselves, proteins nonetheless progress through a life cycle of sorts: they are created by the cell, serve a specific purpose in the organism, and ultimately expire either by passive accumulation of structural defects or through active metabolic processes. As in ecological circles of life, the dead are degraded for their core components. But rather than the scavengers and microbial decomposers at work in macroscale habitats, much of the protein recycling work within the cell falls to a barrel-shaped protein complex known as the proteasome.

Found in most known organisms, the proteasome is the crucial component of ubiquitin-mediated protein degradation. It complements the numerous proteases that degrade proteins in the cell. Protease targets can be very broad, even random, yet at the same time, the proteases themselves can be quite limited in the extent to which they break down those molecules. On the other hand, the substrate selection for the proteasome is a tightly controlled process in which chains of ubiquitins attach to proteins destined for extensive degradation by the proteasome. Thanks to its prominent recycling role, the proteasome has offered researchers a tool to manipulate levels of a specific protein and test its function, or to treat cancer, inflammation, and other diseases.

High-resolution structural microscopy techniques and innovative chemical biological approaches have allowed researchers to unveil the different roles proteasomes play in cellular maintenance and adaptation to changing biological environments. Scientists now know there are different kinds of proteasomes, some of which are highly specialized. The constitutive proteasome is present in all cells, while the others—the immunoproteasome, the thymoproteasome, and the spermatoproteasome—are found in immune cells, the thymus, and the testes, respectively.

The more researchers learn about proteasome structure and function, the better equipped they are to harness its power for their own purposes, turning the cell's protein decomposer into an invaluable biological apparatus.

Proteasome structure and function

The proteasome weighs in at an impressive 2,500 kilodaltons, making it comparable in size to the ribosome. This beefy complex is composed of more than 60 protein subunits that act together to hydrolyze targeted proteins into short peptides of just 3 to 15 amino acids. These peptides are then broken down further into their constituent amino acids by cellular proteases.

The proteasome has offered researchers a tool to manipulate levels of a specific protein and test its function, or to treat cancer, inflammation, and other diseases.

The proteasome can be divided into two main components, the core particle and the regulatory particles. The core particle is formed from four rings, each composed of seven subunits, that are stacked to form the proteasome's barrel structure, which measures approximately 5 nanometers in diameter—almost as thick as the cell membrane. The two outer rings are constructed of alpha subunits that constrict the ends of the barrel to control access to the lumen. The two inner rings contain proteolytic beta subunits that degrade protein chains as they pass through. The regulatory particles form "lids" on either end of the barrel, unfolding polyubiquitinated target proteins and threading them through the narrowed opening of the core particle into the lumen. The proteasome is a two-way street; proteins can enter and exit either side.

The proteasome is responsible for three types of ATP-dependent proteolytic activity: chymotrypsin-like, which cleaves on the carboxyl side of a target protein's hydrophobic amino acids; trypsin-like, which chops up the carboxyl side of basic amino acids; and caspase-like, which cuts the carboxyl side of acidic amino acids. Each of these protease activities is encoded in separate beta subunits in the core particle—β5, β2, and β1 subunits, respectively. Despite targeting different parts of a protein for cleavage, all of these subunits act via a similar mechanism: a threonine residue in each β subunit attacks the peptide bond of its target amino acid. Last year, Michael Groll of Technische Universität München and colleagues found that, in order to keep the proteasome from degrading itself during its initial assembly, these reactive threonine residues are masked by protective amino acids that are snipped off in the final assembly step.[1]

Specialized proteasomes are structurally similar to the constitutive proteasome, but do have some unique alpha and beta subunits that impart functional differences. For example, due to its beta subunits β1i, β2i, and β5i, immunoproteasomes have dramatically reduced caspase-like proteolytic activity and greatly increased chymotrypsin-like activity, which is believed to assist in creating peptide fragments that are better suited for binding to the major histocompatibility complex during antigen presentation.

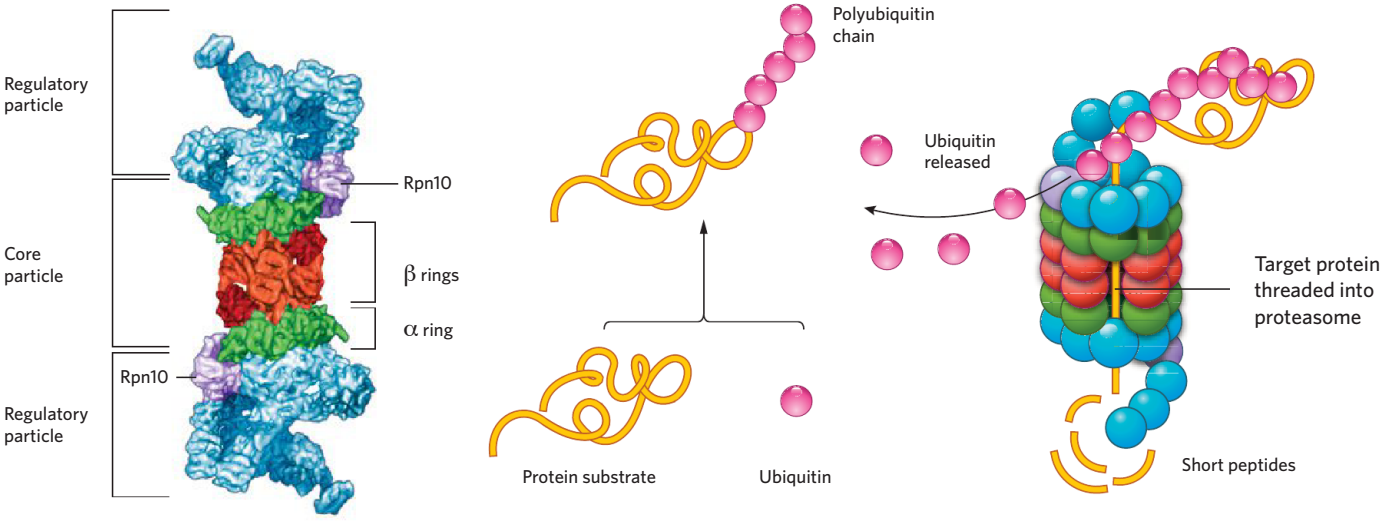
Conversely, the thymoproteasome-specific β5 subunit (β5t) gives that complex much lower chymotrypsin-like activity than constitutive proteasomes, a difference that appears to be instrumental for the thymus to select $CD8^+$ T cells for survival and maturation. Knockout mice that lack β5t lose more than 80 percent of their cytotoxic $CD8^+$ T cells and succumb to infections that wild-type mice overcome.[2] Finally, the altered alpha subunit composition of the spermatoproteasome may contribute to the degradation of acetylated core histones, an important step in chromosome condensation during spermatid differentiation and spermatogenesis.

Proteasome-based medicine

The rapid proliferation of cancer cells demands fast turnover of expired proteins, as well as the efficient disposal of proapoptotic and tumor suppressor proteins. As a result, malignant cells are more susceptible to the cytotoxic effects of inhibiting protein degradation than normal cells.

PROTEASOME BASICS

The structure and function of the cell's protein-degrading machine



The proteasome can be divided into two main components: the core particle, four stacked rings that form the proteasome's barrel structure, and the regulatory particles that form "lids" on either end of the barrel. At the junction of the lids and bases of the regulatory particles is Rpn10, which binds the polyubiquitins on targeted proteins. Other subunits in the regulatory particle release the ubiquitin and help unfold the proteins. Inside the core particle, the proteolytic β rings, each of which have seven subunits, degrade the protein chain into short peptides of just 3 to 15 amino acids.

Over the past couple of decades, scientists have discovered a number of small-molecule inhibitors of the proteasome. Inhibitors belonging to the peptide aldehyde group inhibit the proteasome reversibly and are used extensively in research. However, they can also block the action of cellular proteases, limiting their utility as a research tool and as a potential drug. Beta-lactone proteasome inhibitors bind irreversibly to the active-site threonine of the β subunits in the core particle, but also are not completely proteasome-specific. Members of the boronic acid and epoxyketone classes of inhibitors are more specific for the proteasome and as such have successfully made the leap from research to therapeutic applications.

In 2003, the boronic acid class inhibitor bortezomib (Velcade) became the first therapeutic proteasome inhibitor approved by the US Food and Drug Administration (FDA), which green-lighted the compound as a multiple myeloma treatment. Enormously successful, bortezomib has been shown to have a response rate of 50 percent to 90 percent in multiple myeloma patients who have relapsed following established front-line therapies.

Bortezomib treatments can cause peripheral neuropathy and a decrease in blood platelets, however, thereby limiting the dose that patients can be given. In 2015, the FDA approved an epoxyketone class inhibitor that we helped develop, called carfilzomib (Kyprolis), for the treatment of refractory myeloma. Clinical trials have shown that carfilzomib also has a high patient-response rate, but does not cause the peripheral neuropathy seen with bortezomib.[3] Another boronic-acid-class inhibitor, ixazomib (Ninlaro), was approved in 2015 by the FDA for the treatment of myeloma. Unlike carfilzomib and bortezomib, which need to be administered intravenously, ixazomib is formulated to withstand the acidic environment of the stomach and is taken orally.

However, the use of these drugs can lead to the development of resistance. (See "Resist or Desist," *The Scientist*, April 2017.) Studies aimed at determining the molecular basis for acquired resistance have found that mutations that confer resistance to bortezomib also reduce carfilzomib effectiveness.[4] Thus, the effort to develop the next generation of cancer-fighting proteasome inhibitors continues.

In the meantime, researchers are also exploring the potential of proteasome inhibitors to treat a variety of other diseases. While the inhibitors presented so far block all proteasomes, the specialized biology associated with the nonconstitutive varieties has inspired a hunt for drugs that specifically target a particular type. Given the immunoproteasome's continuous expression in cells of the immune system, and the stimulation of its production in other tissues during heightened immune activity, researchers reasoned that selective inhibition of the protein-degrading machine might allevi-

ate chronic inflammatory diseases without incurring side effects (neuropathy, platelet suppression) from blocking the other proteasomes. In a mouse model of lupus, inhibition of the immunoproteasome by the compound ONX-0914 halted the progression of the disease state.[5] Much research has been conducted to develop inhibitors with even greater selectivity for the immunoproteasome,[6] and scientists, clinicians, and patients alike hope that immunoproteasome inhibitors will soon begin clinical testing against autoimmune disorders.

Proteasome inhibition may also be of value in the treatment of neurodegenerative disorders. The process of neurodegeneration is driven in part by inflammation, and inflammation depends on active proteasomes. Indeed, regions of Huntington's disease–afflicted brains containing the trademark pathological aggregates also possess neurons with high levels of immunoproteasomes that exacerbate the inflamed state of the tissue. Inhibitors would help dampen that neuronal inflammation. In addition, because proteasome inhibitors also increase production of nerve growth factor, which can heal mildly damaged nerve cells, they might actually have a dual-action neuroprotective effect.

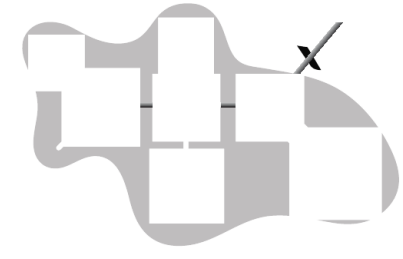
Cardiac tissue following oxygen deprivation (ischemia) may also benefit from proteasome inhibition. But, it is critical to limit the inhibitors' effect to only the chymotrypsin-like activity to protect the heart from damage. Selective inhibition of increased chymotrypsin-like activity during ischemia preserves proteins critical for normal cardiac functioning; but general inhibition of all three proteasome activities can enhance cardiac cell death.

Finally, proteasome inhibitors may prove useful in fighting certain infections. Researchers have begun to develop inhibitors that selectively block the proteasomes in infectious organisms while sparing human proteasomes. An early example is *Mycobacterium tuberculosis,* an unusual bacterium because it has proteasomes (most bacteria do not).[7] Scientists have developed inhibitors that are over 1,000-fold more selective for the *M. tuberculosis* proteasome over the human counterpart in vitro; the next step is to test their safety and efficacy in people. The emergence of multidrug-resistant strains of *M. tuberculosis* and the estimated 2 million deaths annually from the disease has led the World Health Organization to declare tuberculosis a global health emergency. Given the urgency, the realization of a clinically useful proteasome inhibitor to combat tuberculosis would be invaluable.

Inhibitors with selectivity for the proteasome found in disease-causing parasites such as *Plasmodium falciparum* (malaria), *Leishmania* spp. (leishmaniasis), and *Trypanosoma* sp. (Chagas disease) are also in varying stages of development. Although not yet sufficiently advanced for clinical testing, these proteasome inhibitors offer a hope to fill the clear and compelling need for new pathogen-fighting agents.

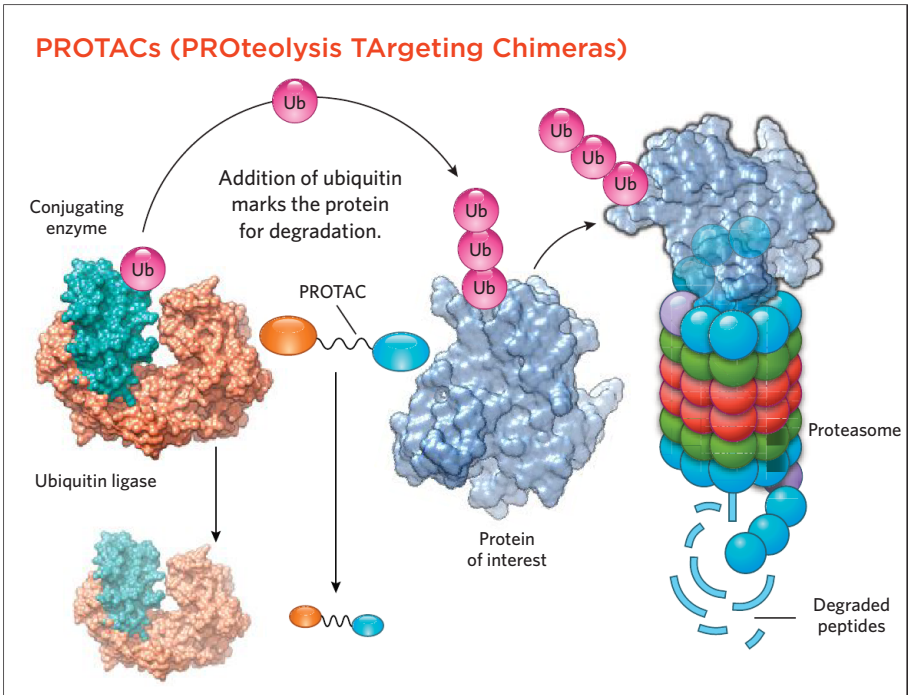
PROTEASOME ACTIVITY

In contrast to cellular proteases, which cleave proteins at specific sequences, the proteasome hydrolyzes targeted proteins at certain types of individual amino acids. Threonine residues in three separate β subunits of the core particle attack the peptide bond of its target amino acid (colored arrows).



- Chymotrypsin-like (blue arrows): Cleaves on the carboxyl side of the target protein's bulky hydrophobic amino acids
- Trypsin-like (purple arrows): Cleaves on the carboxyl side of basic amino acids
- Caspase-like (red arrows): Cleaves on the carboxyl side of acidic amino acids

Shown here is the amino acid sequence of DNA-dependent protein kinase (DNA-PK). The cellular protease granzyme B cleaves DNA-PK only following its consensus sequence (italics; black arrow), which exists only twice in the entire protein, while the proteasome cleaves DNA-PK hundreds of times over its length (colored arrows).



UBIQUITIN BOOST: PROTACs help catalyze the ubiquitination of target proteins. PROTACs require only brief contact to trigger ubiquitination, meaning that one PROTAC molecule can flag multiple copies of its target for disposal.

Harnessing the proteasome in basic research

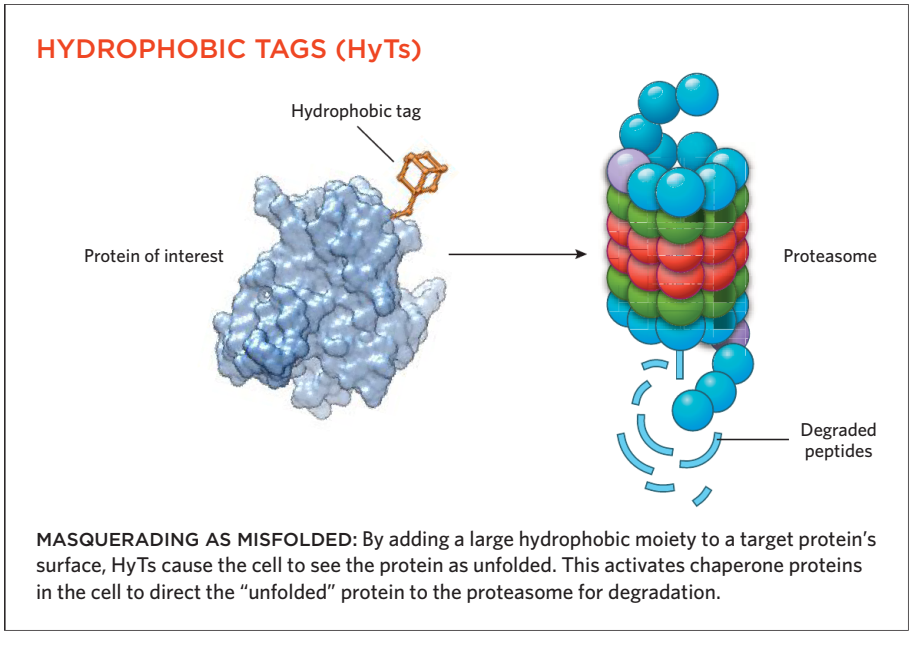
Outside of the clinic, researchers often control the proteasome to manipulate biological systems. Proteasomes are known to play a role in diverse cellular processes, including inflammation, apoptosis, circadian regulation, and more. From the study of any of these phenomena to examining the life cycle of individual proteins, proteasome inhibitors continue to be used in the lab extensively.

Inhibition, of course, isn't the only way to manipulate the proteasome. Quite the opposite, in fact. Researchers have devised strategies for directing ordinarily stable proteins to the proteasome for their degradation. While inhibiting the active sites of proteins with a small molecule permits exploration of a single biological activity, degradation of that protein eliminates all of its biological functions and allows for a more thorough consideration of its importance.

An example of such a protein "knockdown" strategy comes from the research group of Tom Wandless at Stanford University. Wandless and his colleagues identified destabilizing peptide sequences that could be spliced into a protein of interest to target it for proteasomal degradation. Wandless also developed a small molecule called Shield-1, capable of binding to and stabilizing the destabilized peptide sequence. Treatment with Shield-1 ultimately prevents recognition of the recombinant protein for ubiquitination and thereby preserves its expression.[8] While highly specific as well as adjustable, this application was nevertheless limited since it required the introduction of the recombinant/destabilized protein into the system by genetic modification. This is a relatively simple task in a dish of cultured cells, but at the level of a whole organism, genetic modification is laborious and difficult in mice, let alone humans.

For many years, our lab has been developing strategies to target proteins for post-translational knockdown without the need for any genetic modification. One class of molecules that promotes protein degradation in such a way is the hydrophobic tags (HyTs), which function by appending a large hydrophobic moiety to the surface of a target protein. The presence of the hydrophobic group mimics a partially unfolded state, thereby engaging the cell's heat shock proteins and/or other chaperones that maintain protein quality.[9] Unable to correct the faux unfolded state of the target protein, the chaperones ferry it to the proteasome for disposal.

This strategy works with many different classes of proteins, including those that lack a tractable active site and therefore cannot be inactivated by a traditional small mol-



MASQUERADING AS MISFOLDED: By adding a large hydrophobic moiety to a target protein's surface, HyTs cause the cell to see the protein as unfolded. This activates chaperone proteins in the cell to direct the "unfolded" protein to the proteasome for degradation.

While active site inhibition of proteins with a small molecule permits exploration of a single biological activity, degradation of that protein eliminates all of its biological functions and allows for a more thorough consideration of its importance.

ecule inhibitor. One compelling target for degradation is the pseudokinase Her3,[10] which plays a prominent role in ovarian and breast cancer. Moreover, the use of HyTs to degrade oncogenic proteins could overcome some forms of developed resistance to traditional inhibitor therapeutics. For example, laboratory tests have shown that androgen-based HyTs help fight the growth of castration-resistant prostate cancer cells that can no longer be controlled by androgen receptor antagonists.[9]

Another class of molecules that causes protein knockdown is known as PROTACs (PRoteolysis TArgeting Chimeras). These two-headed molecules facilitate polyubiquitination of target proteins, thereby tagging them for destruction by the proteasome. As we recently showed, PROTACs catalyze the eradication of specific proteins. One PROTAC molecule causes the degradation of multiple copies of its target.[11] PROTACs require only transient contact with their targets to cause their ubiquitination and degradation, as opposed to reversible inhibitors, which need constant occupancy to block target functioning; the latter scenario requires near-saturating concentrations of inhibitor to produce a biological result.

Since the early 2000s, our lab has created PROTACs that effectively cause the degradation of numerous protein targets, including transcription factors, enzymes, scaffolding proteins, and a variety of kinases. Only in the past few years, however, have PROTACs been successfully used in vivo. The earliest PROTACs we constructed included peptide components, which caused them to be quickly degraded in the bloodstream. In 2008, we reported our synthesis of the first PROTAC composed entirely of small molecules,[12] and we have since developed a number of others that, owing to their lack of a peptide component, are more metabolically stable and better able to enter cells. For example, our group and others have developed PROTACs that degrade the protein BRD4, which itself drives up levels of the known oncogene, Myc. We have shown that these PROTACs suppress Myc-driven tumors effectively in mouse xenograft models.[13,14] With these advances, PROTACs may soon join other proteasome-targeting therapeutics on the path to the clinic.

Most recently, we have designed PROTACs with a greater level of mechanistic sophistication. HaloPROTACs, by virtue of an incorporated chloroalkane group, irreversibly bind their target proteins such that degradation proceeds with greater expedience and with a higher degree of targeting efficiency.[15] PhosphoPROTACs make target degradation conditional on the activation of specific signaling pathways, permitting researchers to better map out the connections between downstream effectors and upstream receptors.[16]

With these and other new techniques, scientists will chip away at several remaining questions about proteasome function. Can we develop effective inhibitors for the prokaryotic proteasome, which is simpler than the proteasome of higher organisms, into an effective target to combat bacterial infections? Are there other tissue-specific proteasomes, like the thymoproteasome and spermatoproteasome, that have yet to be discovered and what are their biological roles? Can small molecules like PROTACs and HyTs be used to target proteins lacking traditional active sites that are currently considered undruggable under the modern pharmaceutical paradigm?

The answers to these questions will serve as the foundation for new and exciting developments that may transform both chemical biological investigations and modern medicine. ■

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Picasso
XXXII

The Mind-Body Connection

Understanding how people recognize and control their own bodies could help researchers develop therapies for those who've lost their sense of self.

BY ROMAN LIEPELT AND JACK BROOKS

An amputee struggles to use his new prosthetic limb. A patient with a frontal-lobe brain lesion insists that her left hand has a mind of its own. The alleged criminal claims in court that he did not fire the gun, even though several eyewitnesses watched him do it. Each of these individuals is grappling with two elements of the mind-body connection: ownership, or an ability to separate ourselves from the physical and social environments, and agency, a conviction that we have control over our limbs.

The human brain typically handles these phenomena by comparing neural signals encoding the intended action with those signals carrying sensory feedback. When we are born, we make erratic reaching and kicking movements to map our body and to calibrate our sensorimotor system. During infancy, these movements solidify our self-awareness, and around the time we first walk, we are quick to investigate a sticker placed on our forehead when looking in a mirror, recognizing the foreign object as abnormal. By the age of four, our brains are proficient at distinguishing self and other. In the amputee, the brain lesion patient, and the defendant on trial, the sense of self is disrupted due to discordance between sensory feedback from the limb and the brain's expectations of how a movement should feel.

Instead of investigating ownership and agency as two distinct concepts, recent research has sought to understand how body ownership might have developed through the sum of agency experiences that we accrue throughout our life. What we perceive as our body is not only what looks like our body, but what we typically have conscious control over. This control is asserted by learned associations between our muscular movements and the sensory feedback we perceive when performing an action—the so-called "action effects." What remains unclear, however, is just how multiple selves—including our bodily, social, and autobiographical selves—are integrated, and what kind of agency experiences drive the perception of having a single, stable self.

We are quick to investigate a sticker placed on our forehead when looking in a mirror, recognizing the foreign object as abnormal.

Bodily illusions

In 1937, French scientist J. Tastevin was testing perception of touch and finger position when he noticed that people often mistook a plastic finger protruding from underneath a cloth near their hand as their real finger. In the 1960s, French philosopher Maurice Merleau-Ponty described the way the body

feels as "my hereness" and noted that perceiving oneself in a mirror extends this to a visually perceived self that is part of the external world, which Merleau-Ponty called "my thereness." In doing so, he anticipated that self-recognition may be more than the immediate experience of the feeling of our bodies, that it may also involve the visual perception of our bodily self, which is quite similar to the way we perceive others. Over the next 40 years, research focused on the senses of touch and limb position, but little, if any, focus was given to mental representations of the body other than case studies of neurological disorders.

Fast-forward to 1998, when Princeton University cognitive scientist Matthew Botvinick headed a study on an illusion similar to the one Tastevin observed to evaluate body ownership.[1] Participants sat with one arm under a table. Researchers placed a rubber arm on top of the table in alignment with the real arm below. The experimenters stroked the participant's arm and the rubber hand either synchronously or asynchronously with a small paintbrush and asked the subjects to respond to a series of questions on body ownership. Subjects reported feeling as if the rubber hand were their own after synchronous but not asynchronous stroking. When asked to point to where they perceived their hand, participants tended to point toward the rubber hand, suggesting they had "embodied" the object as part of their own body. This rubber-hand illusion (RHI) suggests that the sense of self is highly malleable; the perceived location of the arm as estimated from the senses of touch and of limb position (known as proprioception) is superseded by visual input, as this sense is often more reliable.

Over the last 20 years, researchers have used the RHI setup to probe how we perceive our bodies. Some have measured physiological responses following threats to the rubber arm to objectively test if that limb is perceived as a part of the body. In 2003, Vilayanur Ramachandran of the University of California, San Diego, and Carrie Armel of Stanford University observed an increase in sweat production, known as skin conductive response (SCR), when they bent a finger on the rubber hand into a position that would normally be excruciatingly painful.[2] A few years later, the Karolinska Institute's Henrik Ehrsson, then at the Wellcome Trust Centre for Neuroimaging, and colleagues found that threatening the artificial limb with a needle increased activation of brain areas involved in bodily awareness and the anticipation of pain; the stronger the illusion, the stronger the activation of these regions.[3]

Knowing what we now do about body ownership, can we help amputees fully embrace their prosthetic limbs?

What happens to the "neglected" limb—the one under the table—reinforces the idea that we can learn to embody an artificial limb while disregarding the real one. In 2008, the University of South Australia's Lorimer Moseley, then at the University of Oxford, and colleagues measured a decrease in the skin temperature of the concealed limb, suggesting reduced blood flow.[4] The authors interpreted this physiological disembodiment of the real limb to be a consequence of taking ownership of an artificial body part. A few years later, Moseley and colleagues found that the real limb under the table exhibited increased histamine reactivity, a measure of the innate immune response, suggesting that the body had begun to reject the subject's actual hand as it accepted the artificial one in its place.[5]

Illusions of embodiment can be induced in ways other than discordant visual-tactile stimuli. Receptors in skeletal muscle known as muscle spindles make a large contribution to our sense of proprioception. (See "Proprioception: The Sense Within," *The Scientist,* September 2016.) Moseley and colleagues wondered if muscle spindles might also contribute to the sense of ownership. The researchers applied a nerve block to the index finger on one hand and blocked the participant's view of it, effectively removing both visual and tactile inputs, while the subject's other hand visibly grasped a rubber index finger. The experimenters found that when they synchronously moved the real index finger and the rubber finger, the participants reported feeling that the fake finger was embodied, suggesting that input from muscle spindles in conjunction with vision is sufficient to generate ownership.[6]

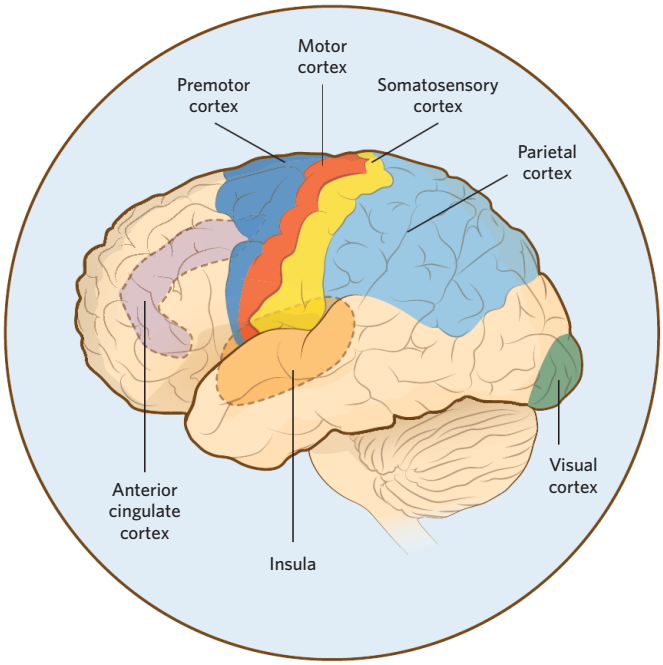
While the above experiments probing the sense of body ownership are admittedly contrived scenarios, the fact that people can learn to embody a limb that they weren't born with has major implications for amputees. Knowing what we now do about body ownership, can we help amputees fully embrace their prosthetic limbs?

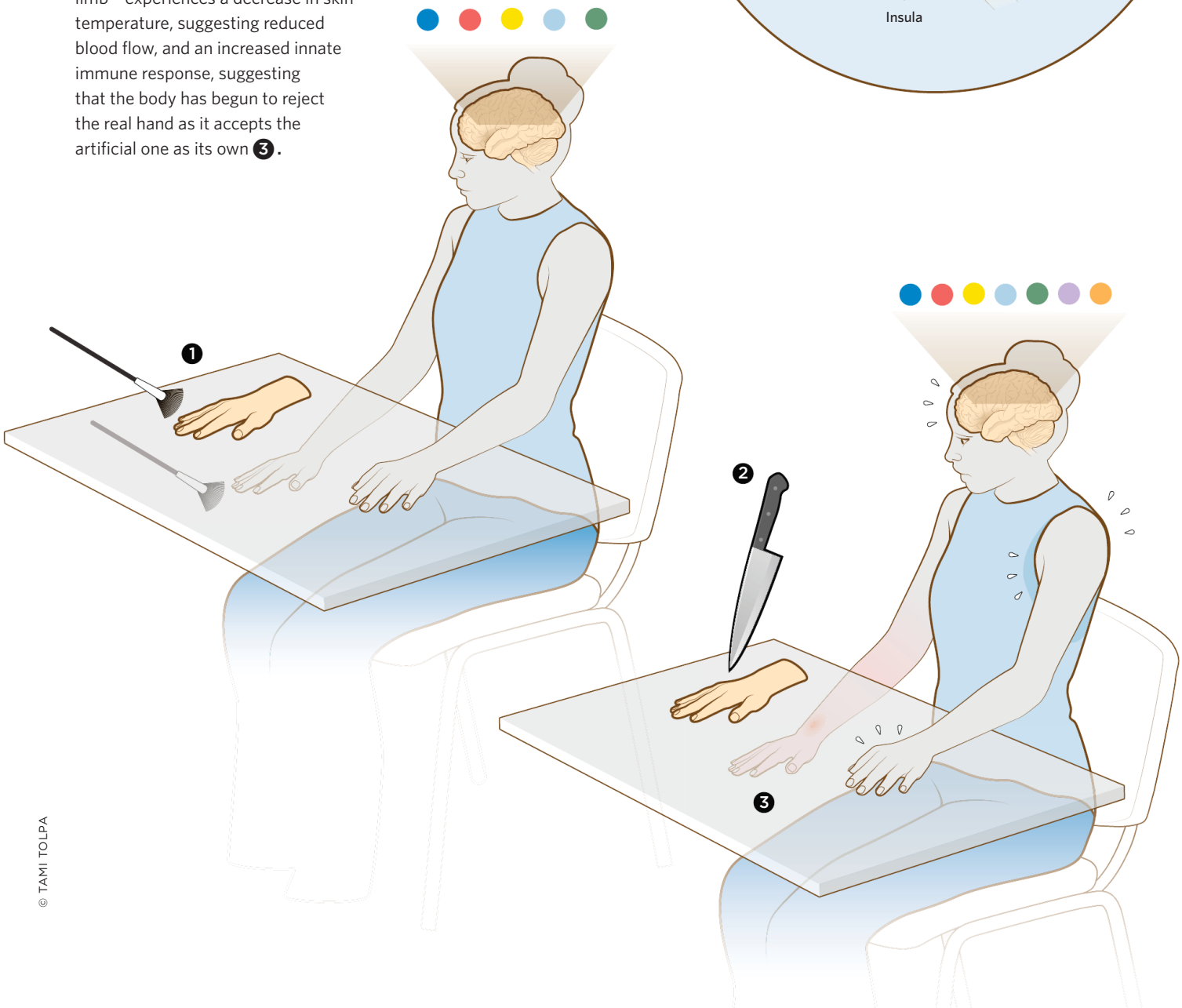
There's some evidence that we can. In the mid-1990s, researchers at the Toho University School of Medicine in Tokyo, Japan, trained macaques to use a rake to retrieve objects and found that neurons responsive to touch stimuli from the hand and to visual input now also respond to the rake when it was in use.[7] The RHI is one way to induce a person to accept an artificial limb as her own, but amputees obviously lack skin, muscles, and neurons in their missing limb to stimulate. As a workaround, researchers have begun to use electrical stimulation of the brain regions thought to be involved in representing the body to mimic the effects of stroking a real limb. Last year, working with two patients undergoing brain surgery for epilepsy, Kelly Collins of the University of Washington and colleagues stimulated the region of the somatosensory cortex corresponding to

THE RUBBER-HAND ILLUSION

A classic experiment to test the idea of body ownership is to have volunteers place a hand out of view under a table and set a rubber hand on the top the table. When researchers touch the real hand and the rubber hand synchronously, participants will feel as if the rubber hand were their own. Researchers have observed that brain areas including the premotor, somatosensory, and parietal cortices, candidate regions for identifying and representing self, are activated in response to the now-embodied fake hand ❶.

If the rubber hand is physically threatened, volunteers will often begin to sweat, indicating they feel as if they are at risk of injury. At the same time, activity increases in the insula and anterior cingulate cortex, deep brain regions responsible for bodily awareness and pain anticipation (*PNAS*, 104:9828-33, 2007) ❷. Meanwhile, the real hand that is under the table—known as the "neglected" limb—experiences a decrease in skin temperature, suggesting reduced blood flow, and an increased innate immune response, suggesting that the body has begun to reject the real hand as it accepts the artificial one as its own ❸.





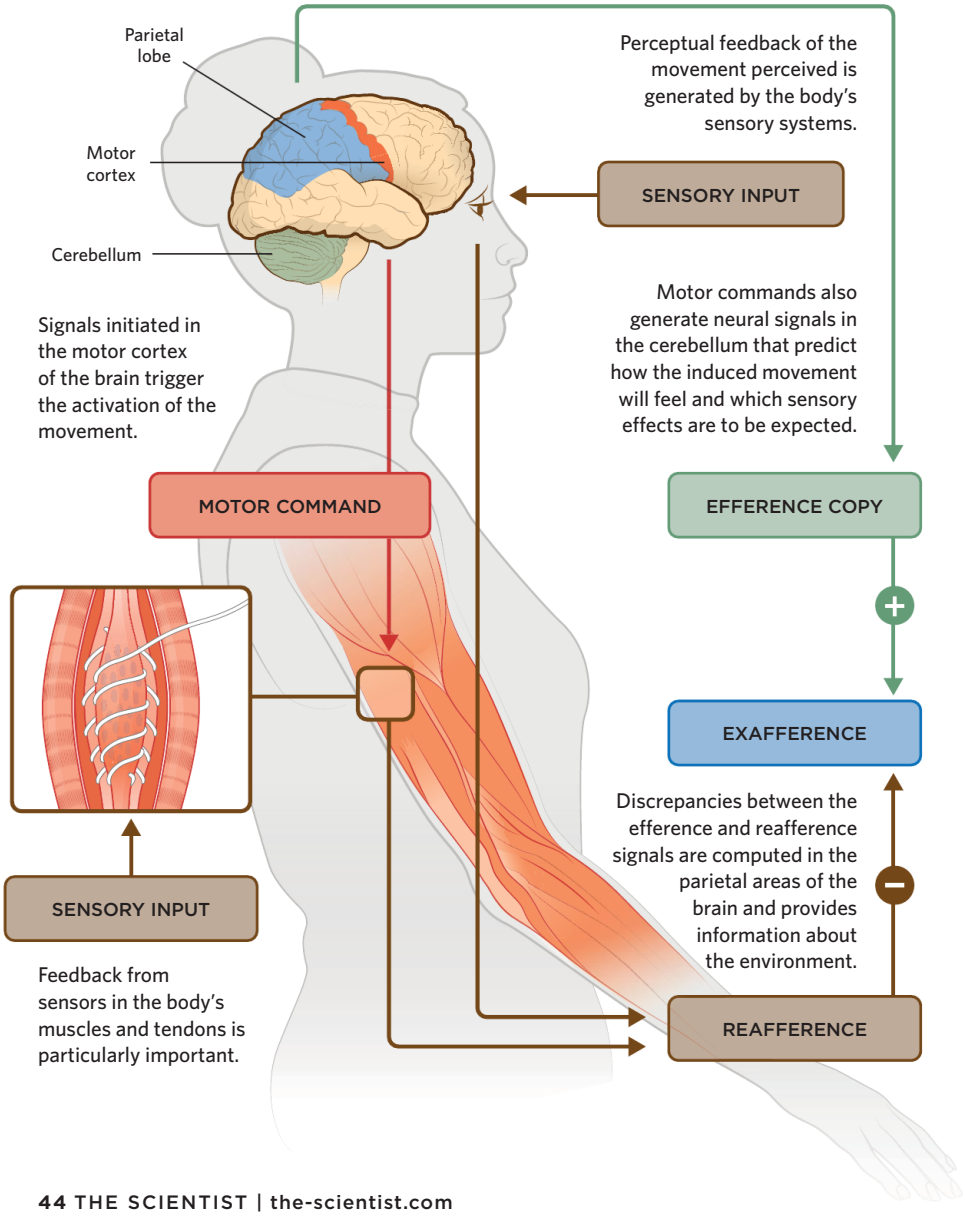
one hand while touching a rubber hand visible to the participants.[8] Both patients had a strong sense of ownership over the artificial limb, which attenuated when electrical stimulation was moved to other regions of the cortex. This study provides hope that a similar procedure could help train amputees to embody their prosthetic limbs.

Increased embodiment should not only improve amputees' control over their prosthetics, but may help reduce the phantom pain that many amputees suffer. To date, effects of the RHI on pain reduction are equivocal, but a couple of studies suggest that variations of the illusion could have potential, as they generate stronger, more holistic feelings of ownership. In 2011, Ehrsson and colleagues completed an experiment in which participants, who were upper limb amputees, viewed a mannequin through goggles that relayed a video feed with a first-person view of the artificial body. The researchers stroked either the intact hand of a complete mannequin or the stump and the area below the stump of an amputated mannequin, while at the same time stroking the stump of the participants. Although the illusion only worked under certain conditions, two of four participants did report remarkably reduced pain after synchronous stroking of their stump and the mannequin's stump or the area below it.[9] And earlier this year, James Pamment and Jane Aspell at Anglia Ruskin University in the U.K. induced a similar full-body illusion in 18 people suffering from various pain conditions. They found that the illusion reduced pain ratings by 37 percent in this cohort.[10] Together, these studies point to the possibility that whole-body illusions could be used to overcome phantom limb pain in amputees as well as other pathologies marked by pain.

A better understanding of how body ownership is encoded in the brain could also one day help treat patients with more-extreme body illusions, such as the brain lesion patient who has lost control of her left hand or the defendant who insists he did not fire the gun. Damage in multisensory areas of the brain—particularly the transition between the parietal and the temporal cortex, the so-called temporo-parietal junction, and parts of the medial frontal cortex[11]—may result in an object being incorrectly embodied or in the disembodiment of a limb or even the whole body, as in the case of patients having out-of-body experiences.[12] The neural correlates and brain mechanisms leading to self/other discrimination and out-of-body illusions could one day be targeted to help patients suffering from disorders that result in abnormal bodily consciousness.

MEETING EXPECTATIONS

The reafference principle posits that we are constantly comparing feedback from sensors in our limbs (termed reafference) and other sensory systems (e.g., visual, auditory, etc.) with our expectations based on our intended movements (termed efference copy). Disagreement between these signals (termed exafference) can lead to reduced feelings of embodiment and agency.



Being in control

Beyond ownership, the sense of agency is a conviction that we have control over the events we initiate. We have control when

we reach for a glass of water, when we kick a football, and when we put pen to paper.

Based on theoretical ideas of 19th century physician and physicist Hermann von Helmholtz, German scientists Erich von Holst and Horst Mittelstaedt demonstrated the reafference principle in 1950 to distinguish between self-generated movements and external perturbations. Any time we move, we generate a motor command (efference) to control the muscles. At the same time, we also generate a prediction—based on prior experience of the sensation resulting from the movement—termed the efference copy. The actual movement-related sensory input, which comes from receptors in the muscle and skin, is referred to as reafference. Any difference between the two signals (reafference and efference copy) is the result of environmental input, which is termed exafference. Understanding errors that may occur within this system is probably central to understanding problems in agency and ownership perception.

In a letter to Oliver Sacks, which the late neurologist and author published in his 1984 book *A Leg to Stand On,* Russian neuropsychologist Alexander Luria stated, "If a part of the body is split off from action, it becomes 'alien' and not felt as part of the body." This occurs in patients with alien-hand syndrome, for example, a debilitating condition that leaves sufferers with no control over an arm. Some patients have to strap their arm to their chest before sleep so that they don't punch themselves in the middle of the night. Luria's view suggests that, in this situation, the sensation returning from the limb is considered pure exafferent input, as if there were no conscious prediction that the limb should move, and as such it is disembodied. But are agency and ownership really this dependent on each other?

In 2005, Manos Tsakiris of Royal Holloway, University of London, and colleagues found that indeed they are. In the group's study, a lever that lifted a participant's passive right index finger was operated either by the participant's own left hand or by an experimenter, so that movement of the right hand was effected either voluntarily by the subject or externally by the researcher. Participants could not see their hands, but on a screen they saw a video stream of a gloved right hand—either their own or someone else's—with its index finger being lifted by a lever, and they were asked to say whether or not it was their own. The subjects were substantially more accurate at identifying their own hand when the movement was voluntary—and thus the motor command and sensory feedback they received were in agreement—suggesting that agency is critical to self-recognition, a key component of ownership.[13]

The rise of brain-machine interfaces and neuroprosthetics will further blur the line between "me" and "mine."

Another line of evidence in support of the interdependent relationship between agency and ownership comes from the work of Hiroshi Ishiguro of Osaka University in Japan. As humans, we can harness the power of imagination to test things out before we enact them; imagining moving a limb produces substantial activation of the limb-specific movement-planning areas of the brain, and people controlling prosthetic limbs to perform basic tasks have activity in these same areas. Ishiguro and colleagues fitted participants with a head-mounted display through which they viewed robotic hands. Participants imagined moving the hands, and the resulting neural activity was recorded via EEG and used to command the robotic hands. When participants could not move the hands, their feelings of ownership, as measured by response to limb threat, were halved, suggesting that embodying a new limb is optimized when one is able to move it and receive visual feedback.[14]

Past control over an object—the experience of agency over that object—might also contribute to embodiment. In a study performed by one of us (R.L.) and colleagues, researchers found that a rubber hand, a smartphone, and a wooden block were in principle all perceived as embodied in an adapted version of the RHI. But when participants were asked to verbally estimate the position of a limb, covered by a box placed next to the rubber hand and object, they perceived their hand as being closer to the smartphone and the rubber hand, but not toward the wooden block, which people had no previous agency experience with.[15] This finding suggests that there is a direct impact of past agency experience on ownership.

Other research has suggested that agency is partly separable from ownership, however. In 2012, Ehrsson, along with his then graduate student Andreas Kalckert, designed a rubber-gloved wooden model hand to make finger movements that were either linked by a wooden rod to (and thus synchronous with) movements of the participant's own hidden hand, or detached and controlled independently by the experimenter.[16] Initiation of synchronous movements by the participant elicited a strong sense of ownership and agency over the model hand; linked, synchronous movements initiated by the experimenter (passive movements) abolished the sense of agency, while the sense of ownership remained intact. Conversely, when the experimenters rotated the robotic hand by 180 degrees—putting it in an anatomically implausible position, with the fingers facing toward the body—participants maintained a sense of agency, but not of ownership.

This double dissociation suggests these two components of self are partly processed separately when deprived of the usual multisensory inputs. But in the real world, the evidence all seems

to point toward the interdependence of agency and ownership. Perhaps the best example of this is the bizarre case of Ian Waterman, one of very few people without sensation of touch or limb position below the neck, lost in an autoimmune episode when he was 19. Neurophysiologist Jonathan Cole of Bournemouth University, who has studied Waterman for many years, explains that "Ian felt 'disembodied' only at the beginning, when he had no agency" and when he was not looking directly at his body; Waterman only required vision with crude movement control to regain ownership of his body. Because Waterman receives no peripheral feedback, he has to consciously think about his movements, Cole adds, and as a result, "he feels more cognitively embodied than we might." During a recent visit to NASA, Ian was able to control a full-body robot.[17] When a trolley careened toward the robot, he immediately tried to protect his "new self" by avoiding it.

Given our seemingly boundless potential to attribute agency and ownership to inanimate extensions of ourselves, it is hard to predict how we might interact with our surroundings in the future. It is possible that we might one day control robots with our bodies and our minds. Nearer-term, the rise of brain-machine interfaces and neuroprosthetics will further blur the line between "me" and "mine," and will inform the design of prosthetics that move more naturally so that they can be more easily "embodied." A better understanding of the link between the sense of agency and actions themselves will also have implications for treating rare disorders of self, and raise ethical questions about the legal treatment of those who claim at some point to have lost control of their bodies. ■

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The Literature

EDITOR'S CHOICE IN DEVELOPMENTAL BIOLOGY

Macrophage Messaging

THE PAPER

D.S. Eom, D.M. Parichy, "A macrophage relay for long-distance signaling during postembryonic tissue remodeling," *Science*, doi:10.1126/science.aal2745, 2017.

Macrophages are increasingly appreciated as important mediators of many physiological processes, from homeostasis to tissue remodeling. But the recent discovery of a new role for the immune cells comes from an unexpected source: the stripes that give zebrafish their name.

Widely used as a model organism for developmental biology because the young are transparent, *Danio rerio* as adults have a characteristic black-and-yellow striping that runs the lengths of their bodies. "Nobody really pays much attention to the later stages" of the fish's development, says University of Virginia biologist David Parichy. "But for years, [our lab] has worked on pigmentation and pattern formation."

Zebrafish pigmentation is directed by precursors to the skin's yellow-pigment cells called xanthoblasts. During development, these cells produce long, thin filaments tipped with vesicles containing signaling molecules that land on black-pigment cells called melanophores; once docked, these vesicles help arrange melanophores into orderly black stripes.

Last year, while using time-lapse imaging to watch labeled vesicles, Parichy and Dae Seok Eom, his colleague at the University of Washington, were struck by the peculiar way they moved. "These things were so weird," says Parichy. "They cruise around like they have a mind of their own. Looking at them, we started to think, well, maybe there's something tractoring them around."

The vesicles' wanderings were reminiscent of another cell type: the macrophage. Indeed, when the pair depleted macrophages in baby zebrafish, they found that abnormal dark blotches appeared between the black stripes, indicating communication failure between xanthoblasts and melanophores.

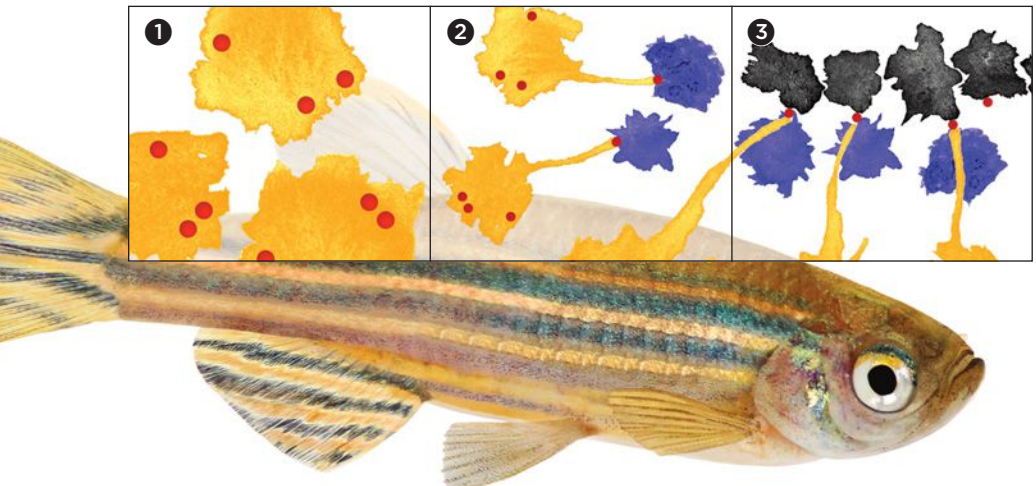
Further time-lapse imaging in normal zebrafish—this time with macrophages also labeled—revealed what was going on: the immune cells were engulfing xanthoblast vesicles and dragging them around intact. Then, on encountering a melanophore, each macrophage deposited its cargo and wandered off elsewhere.

The study provides the first evidence of macrophages physically transferring a signal in this way, notes Richard Lang of Cincinnati Children's Hospital. "From a technical perspective, it's quite gorgeous," he says. "The images give you a really important insight into the way this works."

The researchers proposed how macrophages might identify the vesicles, too. A phospholipid called phosphatidylserine—a well-known "eat me" signal recognized by macrophages—is concentrated on xanthoblasts' vesicle-forming surfaces, and fewer filaments extend from xanthoblasts in its absence. Perhaps phosphatidylserine was coopted by these vesicles to hitch a ride with macrophages, says Parichy, noting a colleague's suggestion that as vesicles are not degraded during the process, a more appropriate descriptor for this signal could be, "bite me."

For now, many parts of the mechanism remain unclear. Nevertheless, the research prompts reflection on whether other systems might use this sort of signaling. "It could be a fish-specific thing," says Parichy, "but I doubt it." Lang, who studies vascular system organization, says one could imagine "anything that requires a regular pattern" involving something similar. "It's a fascinating beginning to an unusual story." —Catherine Offord

CELL ESCORT: As they mature, zebrafish develop a pattern of black stripes made up of dark pigmented cells called melanophores. Researchers have now shown that organization of the pattern is achieved by the ferrying activity of immune cells called macrophages. First, xanthoblasts (orange)—precursors to yellow pigment cells residing in zebrafish skin—form vesicles (red) filled with signaling molecules at their surface ❶. Then, macrophages (blue) pick up these vesicles, which remain attached to xanthoblasts by thin filaments ❷. On encountering a melanophore (black), the macrophage deposits its cargo on the surface of the pigment cell ❸. This long-distance communication represents an entirely new function for macrophages.



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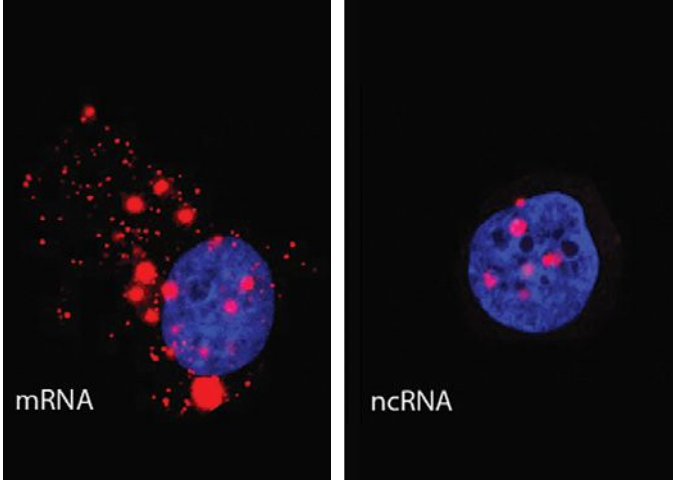
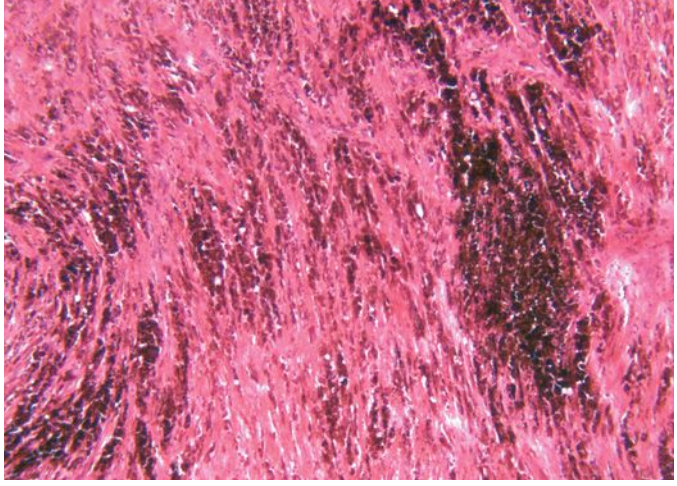


TWO-FACED: mRNA (left, red) from the *ASCC3* gene is mostly in the cytoplasm, while *ASCC3*'s noncoding RNA (right, red) is in the cell nucleus (blue).



T-CELL TARGET: Metastatic melanoma, pictured here, has been in the sights of immunotherapy developers.

GENETICS & GENOMICS

The Long and Short of It

THE PAPER

L. Williamson et al., "UV irradiation induces a non-coding RNA that functionally opposes the protein encoded by the same gene," *Cell*, doi:10.1016/j.cell.2017.01.019, 2017.

DAMAGED DNA

When its DNA is damaged, a cell activates genes to repair the lesion and slows down the transcription of many others. According to Jesper Svejstrup of the Francis Crick Institute, researchers have known about this response for a few decades. However, "that was the extent of what we knew," he says.

TWO FOR ONE

Last year, Svejstrup and colleagues identified factors associated with transcription-related changes after UV-induced DNA damage, including the transcription of *ASCC3*, which encodes a protein involved in regulating gene expression (*Cell Rep*, 15:1597-1610, 2016). In their latest study using sequencing analysis, they discovered that normally long *ASCC3* transcripts became much shorter after damage.

FUNCTIONAL SEE-SAWS

Knocking down the short *ASCC3* transcript produced after UV exposure prevented the cell from recovering normal levels of transcription. "Without the short isoform of *ASCC3*, you can no longer respond correctly to DNA damage, and cells die," Svejstrup explains. Blocking the long version, on the other hand, increased transcription levels after UV irradiation. "It's interesting because the same gene, *ASCC3*, is producing two opposed [functions]," says Alberto Kornblihtt, a molecular biologist at the University of Buenos Aires who was not involved in the work. "If the protein is made from the long pre-mRNA, then global transcription is repressed. But if the short RNA is made, it helps recover transcription hours after damage."

UNCOVERING MECHANISMS

How the short isoform aids repair remains unknown. "The most logical, simple explanation is that the [noncoding RNA] counteracts the protein encoding form," Svejstrup says. "Perhaps [it] binds to ASCC3 protein—but we haven't been able to get clear evidence for that [yet]." **—Diana Kwon**

IMMUNOLOGY

Rare but Special

THE PAPER

A.G. Chapuis et al., "Tracking the fate and origin of clinically relevant adoptively transferred $CD8^+$ T cells in vivo," *Sci Immunol*, 2:eaal2568, 2017.

T-CELL THERAPY

One approach used in cancer immunotherapy is to extract T cells from a patient's blood, select a single clonotype that binds to a tumor antigen, expand it in culture, and reintroduce the cells to the body. Reaching therapeutic levels, however, might take several months, sometimes too late to save the patient.

THE SOLUTION

Rather than generating a therapeutic population of lymphocytes from a single T cell, Fred Hutchinson Cancer Research Center immuno-oncologist Aude Chapuis and her colleagues decided to infuse patients with a polyclonal group of cells stimulated by a particular tumor antigen. "Instead of picking one cell and growing it out, we're taking a lot of cells and growing them a lot less," on the order of just four to six weeks, Chapuis says.

THE RESULT

The researchers tested the approach in 10 metastatic melanoma patients, tracking T cells in the blood via high-throughput sequencing. They found that, not only did the cells persist, the immunodominant clones that emerged existed only in very low frequencies in the body prior to the therapy. "The results demonstrate that long-lasting T cells are derived from a very rare, extant pool of largely inexperienced and probably naive T cells," says coauthor Cassian Yee of the MD Anderson Cancer Center.

THE LONG VIEW

In the two patients who had complete remissions, a single dominant clone took over. "Sometimes there's just this perfect fit and [one T cell type] just goes to town," says Nicholas Restifo, who studies T cell–based immunotherapies at the National Cancer Institute and was not involved the study. **—Jef Akst**

PROFILE

Glia Guru

Ben Barres recast glial cells from supporting actors to star performers, crucial for synaptic plasticity in the brain and for preventing neurodegenerative disorders.

BY ANNA AZVOLINSKY

In his first two years of graduate school, Ben Barres (then Barbara Barres) was a neuroscience graduate student by day and a neurologist on nights and weekends. He began his PhD at Harvard Medical School in 1983 after completing full medical training: an MD from Dartmouth Medical School followed by four years of a neurology-focused residency at Cornell University hospitals in New York City.

"When I started graduate school, all of my medical school loans came due. I had to start paying them back, but my graduate-student stipend was $6,000—barely enough to live on. So I started to moonlight as a neurologist. I was covering a neurology practice at a local hospital, seeing patients and covering emergencies Friday night to Monday morning. On Monday mornings I would drag myself to the lab. It was very hard to take courses, be in the lab, and to work as a neurologist. After about two years, I came in Monday morning and my advisor saw how bad I looked and asked why I was doing this to myself. I told him that I had loans to pay and couldn't on my stipend alone. And he said, 'Oh, is that all? That's the problem? That's why you're working?' And I said, 'Yes.'" Barres's advisor was David Corey, then a young investigator who had Howard Hughes Medical Institute funding. "He had slots to pay postdocs and I was still the only one in his lab, so he said to me 'Since you have an MD, even though you are a graduate student, you can also be a postdoc and I can pay you a postdoc salary,' which was $17,000 at the time. 'Would that be enough for you to quit the neurologist job? Will that work for you?' And I said 'Yes!' I immediately started sleeping at night and doing well in the lab and enjoying life because I was not exhausted all the time. I realized that I am not a good multitasker. That was an early insight: it was not going to work for me to simultaneously be a clinician and a researcher. But I was glad I did the neurology training. My work on glia came directly from my medical training, but I have never tried to practice medicine again. And that was David Corey for you, the world's best graduate advisor. He is wonderful and I am very lucky to have ended up in his lab."

> "Once I get in the lab, I can't leave, I just get so excited. It's an addiction. I will always choose doing experiments over sleeping."

Over the last 25 years, Barres's research on glia has fundamentally changed the image of these cells—highly abundant non-neuronal cells in the brain that were previously relegated to a supportive and structural role (glia means 'glue' in Greek)—delineating the important roles they play in how the brain functions in sickness and in health.

Here, Barres describes how his career path in science began when he was a six-year-old, talks about what ignited his interest in neurobiology, and tells how he changed his life at age 42.

BARRES BEGINS

Budding scientist. Barres was born in 1954 and raised in West Orange, New Jersey. "When I was about six years old, I remember I decided to be a scientist and my fraternal twin sister decided to be a nurse, and I went on to become a scientist and she became a nurse. There were no nurses or scientists in our family, so who knows where we got this idea," he says. "I was a young geek; I was interested in science, period." Computers and computer programming were just entering the mainstream when Barres started high school, and he tried to get as much experience as possible using these early computers and writing code for them. During high school and college, he spent his summers working at Bell Labs in New Jersey and took part in a science honors program at Columbia University, in which the university's professors taught weekend science and computer classes to high school students.

Sexism encounters. At age 13, Barres decided he would attend MIT. "Don't ask me why. I think I met someone that I admired who went to MIT," he says. As a high school senior, he applied for early decision and was accepted. He entered MIT in 1972 thinking he would major in computer science. "I had a take-home, five-question final exam due at midnight that took me all day. I solved the last question late at night; I just suddenly saw the answer. The next day, the professor passed back the exams and said that no one had solved the last question. I went to him after class and told him that I had solved the problem and showed him my paper. He looked at me with disdain and said, 'Your boyfriend probably solved it for you.' He just couldn't imagine, in 1973, that a woman could solve a problem that hundreds of men couldn't solve. I was kind of indignant that he accused me of cheating, but it really didn't occur to me until years later that it was sexism. I didn't really think about those things then. I saw myself as a guy and felt that I was a guy inside, even though I was a woman. So I was a bit oblivious to stuff like that." Barres also had a hard time getting into a lab to do an undergraduate thesis project, even though his male counterparts had no trouble finding a professor mentor. He did eventually join the biochemistry laboratory of Maria Linder, one of the few female

STANFORD PRESS OFFICE

BEN BARRES

Professor of Neurobiology
Stanford University School of Medicine
Member of Stanford Neurosciences Institute

Greatest Hits

- Devised a purification technique called panning to isolate rat ganglion cells and then different types of rodent glial cells
- Demonstrated that glial cells are necessary for neuronal formation of functional synapses and to maintain synaptic function in culture
- Identified the important synapse-inducing molecules secreted by astrocytes, including thrombospondins
- Found that microglia and astrocytes phagocytose synapses both during development and in the mature brain, providing evidence that glia are involved in synaptic plasticity throughout life
- Found that a type of highly neurotoxic "reactive" astrocyte is generated after acute brain injury and in neurodegenerative diseases

members of MIT's science faculty at the time. "Still, I loved it at MIT. I had a great time. The most famous faculty teach undergrad courses there. I had Salvador Luria, who had a Nobel Prize, as my first biology professor. They all radiated such passion and talked about their latest research in class. I loved science when I came in and still loved it when I came out, and that's all that mattered."

Neuroscience spark. As a sophomore, Barres took a course called Psychology and the Brain, taught by neuropsychologist Hans-Lukas Teuber. It was 1973 and the term "neurobiology" had not yet been coined. "He talked about figuring out what parts of the brain did what and just hooked me. That was when I got the idea to become both a neurologist and a neuroscientist," he says. Barres switched his major from computer science to premed. His goal was to get his medical degree first and follow it with a doctorate in neurology. After graduating in 1976, Barres entered Dartmouth Medical School. "I started with medical school first, which turned out to be fortuitous for me. When I started the PhD program at Harvard, I was more mature about the way I studied and more focused. I had decided to do a PhD rather than a specialized postdoc after residency because I didn't feel I had broad enough training in neurobiology, which was just starting to explode," he says. But before Harvard, Barres completed four years of a residency program in neurology and became a certified neurologist. "Although my plan was to simultaneously practice and do research, I was much more aware after my residency that there was very little that neurologists could do to help their patients. There were few actual treatments for the conditions I was seeing, so it was becoming less appealing to me to actually practice neurology."

First glimpses. In Corey's lab at Harvard, Barres learned how to patch clamp and made recordings from glial cells, which happened because Corey was just setting up his own lab there and Barres had just learned how to culture rat-derived glial cells in his prior rotation. "The patch clamp was the first time you could do good recordings, because glia are such tiny cells." At the time, glial cells were thought to be passive neuron-supporting cells. But Barres began to notice that different types of glial cells had different types of ion channels. He had become captivated with these highly abundant brain cells back in medical school because their role in the normal brain was a mystery. While in Corey's lab, Barres cranked out six publications, including five in *Neuron*. In 1988, he and Corey were among the first to show that glial cells do indeed have ion channels. Barres also developed an antibody-based technique, called panning, to purify glial cells, which demonstrated a range of glial cell types in

the rat brain. "I had these beautiful glial cells to record from, but we couldn't keep them alive in culture for more than a few hours because the necessary growth factors had not been identified yet."

BARRES BUBBLES UP

Moving on. Barres first encountered his postdoc advisor while poring over the literature on glial cells. "It was all dreck, really descriptive and not clear, except for Martin Raff's work. He was defining glial-cell markers." Barres met Raff during a visit Raff made to neighboring MIT, and after the British researcher served as Barres's unofficial second graduate advisor, Barres joined Raff's University College London lab in 1990. There, he adapted his panning technique to purify each of the major classes of glial cells, including astrocytes and oligodendrocytes. His motivation was to combine glia and neurons in culture to begin to tease apart their interactions. Barres went on to show that about half of oligodendrocytes in the rat optic nerve die during development and to identify the growth factors necessary for their survival in culture. He also demonstrated that the point of glial apoptosis was to form a one-to-one match with a myelinated axon.

More than glue. Barres moved to Stanford to set up his own lab in 1993. As a graduate student and postdoc, he had devised tools to purify and culture glia. In his own lab, he began to study how glial cells communicate with neurons, and to what end. Barres and then postdoc Frank Pfrieger showed that, in vitro, glial cells are necessary for the formation of functional synapses between neurons. Then in 2001, Barres's lab found that neurons need glial cells to make and stabilize mature synapses in vivo as well. "That was a complete surprise. The dogma was that neurons intrinsically have all of the machinery to form synapses," he says.

Communication enablers. Barres was on his way to studying the function of glia cells in vivo, but first, he needed to get a handle on some of the molecules glial cells used to communicate with neurons. "If we didn't know the glia-secreted molecules, we couldn't do a knockout mouse and ask what glial cells are doing in vivo. The purified cell experiments were tools to generate hypotheses that we could test in vivo," says Barres. In 2005, the lab showed that two thrombospondins, glycoproteins secreted by immature astrocytes in the developing brain, promote synapse formation both in culture and in vivo, and that thrombospondin was sufficient to induce in vitro formation of neuronal excitatory synapses—structurally complete and presynaptically active, but postsynaptically silent—even in the absence of astrocytes. Then, in 2012, Nicola Allen, a postdoc in the lab, found that two other astrocyte-secreted molecules, glypican 4 and 6, are necessary for neurons to form fully functional glutamate receptor–dependent synapses. The lab and Barres's former students are still working to understand the other molecules that work together to sculpt neuronal synapses.

Smart glia. Another function of astrocytes, discovered by then postdoc Won-Suk Chung, was that astrocytes eat synapses by phagocytosis not only during developmental pruning, but also in the adult brain. "Astrocytes are sensing neuronal activity and making decisions about which synapses to eat or not eat, and we think this implies a critical role for astrocytes in synaptic plasticity that underlies experience. It's another demonstration of how smart glia are and the remodeling and restructuring that goes on in the brain," says Barres.

Glia gone bad. Most recently, Barres's lab showed that astrocytes can go rogue. Aberrant astrocytes in the mouse brain, rather than promoting neuronal connections, induce death of other types of glial cells and of neurons. The team detected this type of astrocyte activity in brain samples from patients with multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases, and in individuals with brain injury. Barres is now trying to address how these rogue cells arise, what neurotoxins they secrete, and how they may be involved in neurodegenerative diseases. "We now have some evidence that it is neuronal sickness or injury that induces these astrocytes, and the implication is that this glial reaction may be partly causing the degeneration in the brain. We haven't proven that but that's the next exciting paper!"

BARRES BELIEVES

Being different. "I was at Stanford with my own lab for two years before I changed sex. But I had been confused about my gender from when I was a little kid, maybe even 3 years old. I knew there was something different about me and I was confused and ashamed about what it was. I never discussed it with anyone until I decided to change sex. But my parents must have been aware of it, because every Halloween I was dressing as a football player or an army man, and whenever I was allowed to choose my dress, I was dressing as a guy. And I am sure my parents thought 'What is going on with this kid?' But we never discussed it."

An eye on human disease. Stemming from his medical and graduate school experiences, Barres created and is the director of the Masters of Science in Medicine program at Stanford, with the goal of exposing PhD students in basic science to clinical medicine. "Twenty, twenty-five years ago, basic scientists were not expected to work on disease. Studying disease was considered a second-class scientific activity. The focus in graduate programs is on the model systems, and we never gave them the tools to study human disease. We want to enable researchers to study human diseases. [The program] teaches students the language, anatomy, and pathology, and the major questions in the field we have, which leads to curiosity," says Barres.

Lab addict. "Once I get in the lab, I can't leave, I just get so excited. It's an addiction. I will always choose doing experiments over sleeping. I would still probably be in Corey's lab if he hadn't stopped paying me." ■

Valerie Horsley: Skin Deep

Associate Professor, Departments of Molecular, Cellular, and Developmental Biology and Dermatology, Yale University. Age: 40

BY KERRY GRENS

Valerie Horsley was surrounded by academics from a young age. Raised by a single mother who began her doctoral work in industrial engineering when Horsley was seven, she spent a lot of time with graduate students who served as her babysitters. When it came time for her to graduate from Furman University in South Carolina with a biology degree, Horsley knew she needed her own PhD to become a professor and stay in academia.

She opted for Emory University, where her lack of in-depth molecular biology background became apparent. Horsley's undergraduate career had been wide-ranging but not marked by copious research experience. "I felt completely overwhelmed," she says. "But I think it actually helped me, because I learned to think broadly before I started thinking about specifics."

Horsley joined the lab of Grace Pavlath, where she explored the role of a transcription factor called NFATc2. Pavlath's team had previously found that mice lacking NFATc2 had smaller muscles, but they didn't know why. Horsley found that the protein was critical for cells called myoblasts to fuse and form mature, multinucleated muscle fiber cells.[1] "It revealed a novel step in the myogenesis pathway," says Horsley, who also showed that NFATc2 regulated the transcription of a cytokine, IL-4. "Nobody knew muscle made IL-4," says Pavlath. "It's considered a landmark paper."

For her postdoc, Horsley wanted to move out of muscle and into a different tissue, so she joined the Rockefeller University lab of Elaine Fuchs and dove into skin. One of her projects started with another transcription factor, Blimp1. When she knocked out the gene that encoded Blimp1, mice got oily skin. It turned out that Blimp1 was regulating the size of the sebaceous gland.[2]

In 2009, Horsley set up her own lab at Yale University. Among several lines of inquiry, including characterizing the stages of keratinocyte specification and understanding adhesions between these epidermal cells, she's been collaborating with her husband, Yale's Matthew Rodeheffer, on the role of adipocytes in hair growth. They found that in the skin, adipogenesis occurs in parallel with the growth of hair, and regresses when hair follicles die.[3] Although she wasn't sure at first whether she wanted to collaborate with her husband, the partnership yielded an entirely new appreciation for fat cells in hair growth. "No one had looked at it before," says Horsley. "It's pretty much what my lab has been working on since."

Horsley earned tenure last year. Away from the bench, she has been a leader in her department, and beyond. Yale's Thomas Pollard, who helped hire Horsley, says that shortly after Horsley joined the university she began expanding the regular junior faculty lunches to other biology departments. Then she reached out to chemistry and physics PIs; then to all of arts and sciences, and eventually to the medical school as well. "No one asked her to do this. She just recognized that it was a good idea," says Pollard. "This is not an exceptional thing for her to do. She does this all the time." ■

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Picking Out Patterns

Machine-learning algorithms can automate the analysis of cell images and data.

BY KELLY RAE CHI

It takes a trained eye to determine whether you've succeeded in turning a skin cell into a stem cell, or to distinguish between two related cell populations based on a handful of their surface markers. And even when such distinctions become obvious, looking for them in thousands of samples gets tedious. The appeal of machine learning is that a computer program can take over this heavy lifting for you—and do it even better, by seeing what you can't.

Machine learning aims to make accurate predictions from large sets of data based on prior training using a smaller set of examples. In cell biology, this could mean, for example, being able to predict a cell's phase or its identity based on its shape, size, or staining pattern.

Cell biology will increasingly rely on machine learning and other computational approaches as automated fluorescence microscopy (high-content screening) continues to capture massive sets of images that can be mined in multiple ways. Imaging applications of machine learning work by breaking an image down into numerical or other descriptors, called "features." The algorithm then selects and classifies those features. In a branch of machine-learning methods called supervised learning, those classifications are tested for accuracy by measuring against the test set of data. Once the machine-learning algorithm or program is "trained," it can be applied to a larger set of data. In contrast, unsupervised machine-learning methods mine the data and infer its structure without any training.

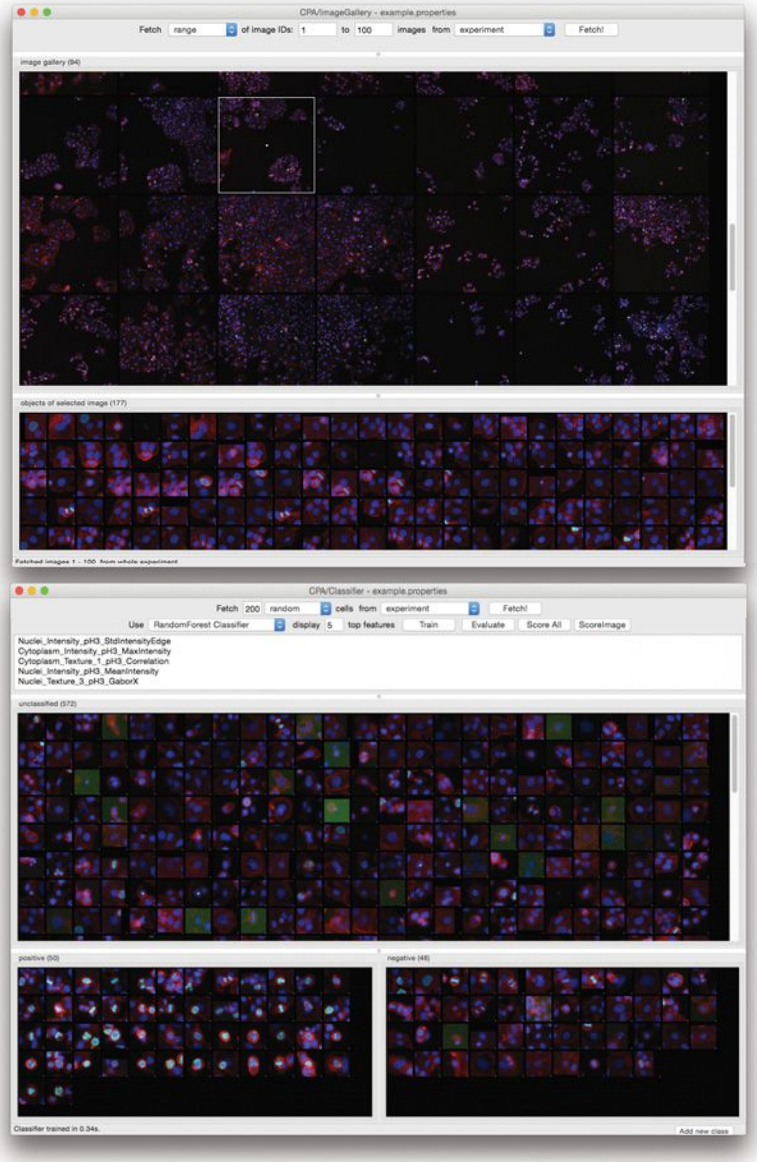
Of course, there's a level of trust involved in allowing machine learning to take the reins. *The Scientist* spoke with developers of machine-learning approaches in cell biology to help demystify these tools. Here's what we learned.

CELLPROFILER ANALYST

cellprofiler.org/cp-analyst/

INTRO: Soon after the launch of CellProfiler—a popular imaging software platform that allows biologists to recognize different cell types, phases, and conditions—its users were faced with a new problem: How do you process the thousands of measurements for each of hundreds of cells in a single image? "In many cases the data don't even fit into Excel, and certainly the tools there are limiting," says developer Anne Carpenter of the Broad Institute of MIT and Harvard University.

To address the data problem, Carpenter and her colleagues developed CellProfiler Analyst, an open-source platform that allows researchers to explore and visualize their data. The latest version of the software, 2.0, is rewritten in Python and is equipped with several machine-learning algorithms that classify multiple biological phenotypes. The original version of Analyst, coded in Java, classified only single phenotypes. Also, a new visualization tool allows researchers to see their results overlaid on their multiwell plate experiments (*Bioinformatics,* 32:3210-12, 2016).



CLASS PICTURES: In 2016, CellProfiler Analyst got an upgrade. The 2.0 version comes with a new Image Gallery function (top image) to explore and visualize images. Images can be dropped into the platform's classifier window (bottom image), which is equipped with several different popular machine-learning algorithms.

BIOINFORMATICS, 32:3210-12, 2016

APPLICATION EXAMPLE: Aiming to create human replacement livers, Sangeeta Bhatia's MIT lab cocultured two cell types, fibroblasts and hepatocytes. Hepatocytes don't proliferate in culture, so the group created a screen for compounds that would cause the cells to self-renew. CellProfiler Analyst enabled the scientists to classify cells within the screened coculture as being either hepatocytes or fibroblasts (*Nature Chem Biol*, 9:514-20, 2013).

GETTING STARTED: Users can download CellProfiler Analyst 2.0, which is Mac- and Windows-compatible, via its website (www.cellprofiler.org). General and application-specific tutorials are also available on CellProfiler's site (cellprofiler.org/tutorials/). Training the program takes from half an hour to an hour to recognize the majority of phenotypes accurately, Carpenter says.

CONSIDERATIONS: CellProfiler Analyst's versatility extends beyond traditional microscopy data; it was recently used to analyze data from imaging flow cytometry, an emerging method that captures several shots of each of thousands of single cells as they pass through a conventional flow cytometry system (*Methods,* 112:201-10, 2017)

Besides CellProfiler Analyst, another user-friendly machine-learning program that complements CellProfiler is called ilastik (*Methods,* 96:6-11, 2016). Ilastik's pixel-based classifier can process images that can then be exported into a CellProfiler pipeline. You can download ilastik for free via its site (ilastik.org/download.html), and it is Windows-, Mac-, and Linux-compatible.

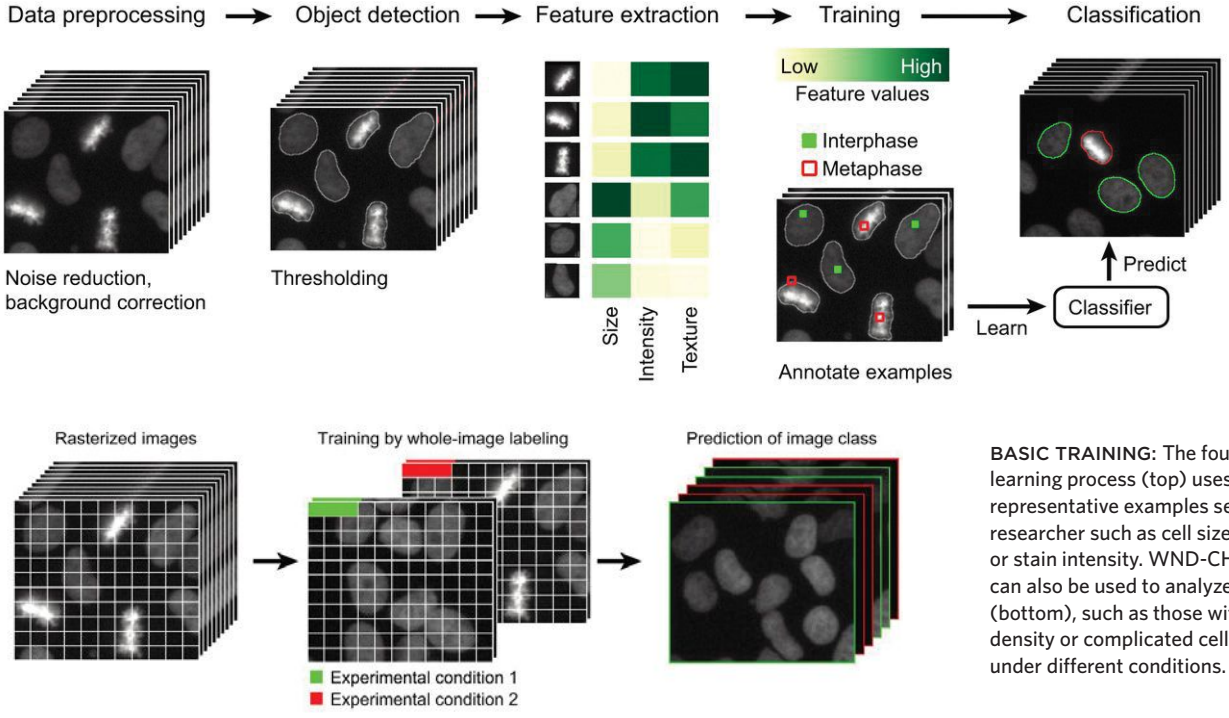
FUTURE: If the classical machine-learning algorithms in CellProfiler Analyst are not effective for identifying the phenotype you want to study, then you might need to move on to deep learning, Carpenter says. Deep learning is a type of machine learning that uses more layers of features that form a hierarchy, and often shows far superior performance than classical algorithms. For example, "identifying the stages of malaria infection in red blood cells is impossible using classical machine learning methods but our recent work has shown a deep-learning model can match the accuracy of experts," she adds. There are currently no user-friendly tools allowing biologists to readily apply deep learning to their imaging problems, but Carpenter says her lab is working on this.

WND-CHARM

github.com/wnd-charm/wnd-charm

INTRO: Developed by researchers at the National Institutes of Health, WND-CHARM (Weighted Neighbor Distances using a Compound Hierarchy of Algorithms Representing Morphology) comprises a four-step algorithm for pattern recognition: extract features, reduce their dimensions, classify them, and validate them. It is available as an open-source command-line program via GitHub (*Pattern Recognit Lett,* 29:1684-93, 2008).

A key distinction of WND-CHARM is that it extracts 10 to 100 times more features compared with other approaches. "We want to describe an image numerically every which way we can," says developer Ilya Goldberg, formerly of the National Institute on Aging and now chief technical officer of the Seattle-based diagnostics company Mindshare Medical. "We have around 4,000 features we compute." Another algorithm within the program narrows the number of features to help reduce the dimensionality of the data into a more manageable set, he says. Users can also hand-select image features for their particular problem. The classifier figures out how to combine these features to generate predictions.



BASIC TRAINING: The four-step machine-learning process (top) uses a set of representative examples selected by the researcher such as cell size, morphology, or stain intensity. WND-CHARM software can also be used to analyze entire images (bottom), such as those with high cell density or complicated cell structures, under different conditions.

J CELL SCI, 126:5529-39, 2013

APPLICATION EXAMPLE: WND-CHARM's developers have deployed the tool in more than a dozen different imaging applications and across imaging modalities ranging from fluorescent microscopy to computed tomography scans. One example is the use of WND-CHARM to determine the age of individual *Caenorhabditis elegans* worms, using images of body wall muscles and a body part involved in feeding. Even individual worms within a synchronized population do not die on the same day, even though their genes and environment are the same.

GETTING STARTED: You can download WND-CHARM on GitHub (github.com/wnd-charm/wnd-charm). Users should be comfortable with a command-line interface and have access to a Linux terminal. On the other hand, the set-up is relatively straightforward in that users simply put images into folders and then tell the program to operate on those folders, Goldberg says. WND-CHARM generates an html or plain-text report containing classifier statistics.

CONSIDERATIONS: You can now use WND-CHARM, or something like it, within CellProfiler. Last year, Carpenter and others at the Broad Institute described a new algorithm based on WND-CHARM, called CP-CHARM, which aims to preserve the functionality of the former but make it more user-friendly, namely by incorporating the feature-extraction step into CellProfiler (*BMC Bioinformatics*, 17:51, 2016).

MACHINE LEARNING IN FLOWJO

www.flowjo.com/
exchange.flowjo.com/

INTRO: Commercialized in 1997, FlowJo is a flow-cytometry-analysis pipeline that allows scientists to analyze their single-cell phenotyping data. "The first data problem that FlowJo really addressed was: How do we analyze many thousands of cells for several markers that are on each individual cell?" says Michael Stadnisky, chief executive officer of the Oregon-based company.

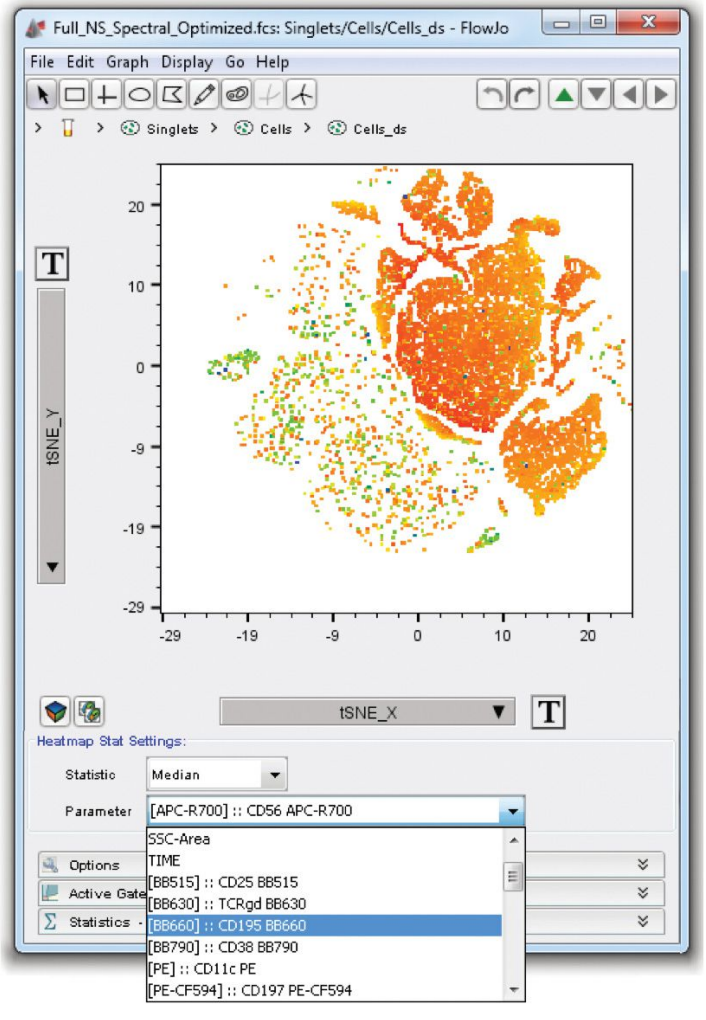
The traditional approach in flow cytometry of manually parsing, or gating, cell types has become even more labor-intensive because flow cytometers can now capture 40+ features of an individual cell, and the throughput of instruments has risen considerably. Gating is also difficult to reproduce. "If you're thinking about those same approaches we've always done, it gets difficult," Stadnisky says.

The company offers a handful of machine-learning plug-ins, both within FlowJo and in an open-source portal where users can also deposit their own plug-ins. These tools can be deployed in various steps throughout the flow cytometry workflow. FlowMeans, for example, clusters cell types automatically through an algorithm called k-means clustering, which has been optimized for flow cytometry data. Another, tSNE (for T-distributed stochastic neighbor embedding), reduces many dimensions of data down to two newly derived parameters. Both plug-ins can help expedite or complement gating.

GETTING STARTED: Users can find the price list for individual or group licenses on FlowJo's website. The tSNE and FlowMeans plug-ins are available on FlowJo's open-source exchange site (exchange.flowjo.com), where developers can also share their own custom plug-ins. Github has sample code that researchers can use as a starting point for developing their own, Stadnisky says. "We know we can't write every machine-learning algorithm for every situation," he adds. "So what folks who are writing algorithms can do now is wrap their algorithm in a plug-in or app from FlowJo."

APPLICATION EXAMPLE: Using tSNE on a publicly available immunology data set, FlowJo scientists and their collaborators were able to identify a new subtype of $CD8^+$ T cells. Moreover, the company's analysis shows that tSNE outperforms both manual dimensionality reduction and a more traditional method for mining high-dimension data called principal component analysis (PCA). ■

GOING WITH THE FLOW: FlowJo's tSNE plug-in can be used to visualize and explore high-dimensional flow cytometry data, to help discover new types of cells that have been missed during gating.



COURTESY MICHAEL STADNISKY

Passing the Buck

The scientific community struggles to define the responsibility of collaborators when research goes bad.

BY CATHERINE OFFORD

When cancer researcher Ben Bonavida accepted a visiting graduate student from Japan into his lab at the University of California, Los Angeles (UCLA) just over a decade ago, he treated Eriko Suzuki like every other student he had supervised for the past 30 years. "I met with her regularly," Bonavida recalls. "We went over her data, she showed me all the Westerns, all the experiments." After months spent working on the cancer therapeutic rituximab's mechanism of action, "she presented her findings to me and the other collaborators in the lab, and based on that we published a paper in *Oncogene*."

Appearing in 2007, the paper accrued nearly 40 citations over the next seven years. But in April 2014, the study gained a less favorable mention on PubPeer, a website where users anonymously discuss research articles, often raising possible causes for concern. One user noted that some of the Western blots used to support the paper's conclusions looked suspicious. In particular, one figure appeared to contain a duplicated and slightly modified part of another image.

PubPeer's readers didn't have to wait long to find out if their suspicions were grounded. Within the week, Bonavida's visiting student—by then an assistant professor at Tokyo University of Agriculture and Technology—had confessed to image manipulation, and the paper was eventually retracted in 2016, with a brief statement citing "data irregularities." In UCLA's ensuing investigation, Bonavida was cleared of wrongdoing; nevertheless, he says, he was left in shock. "It affected me very deeply," he says. "I have trained over a hundred students through my career. Nobody has done something like that with my work before."

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These days, Bonavida's experience is becoming all too familiar. Scientific retractions are on the rise—more than 650 papers were pulled last year alone—and, more often than not, they're the result of misconduct, whether image duplication, plagiarism, or plain old fraud. The pressure is now on the scientific community to address the issue of research integrity—and the role of coauthors like Bonavida in maintaining the veracity of research to which they contribute and ultimately support for publication. Even when coauthors have no involvement in the misconduct itself, is there something they should have done differently to avoid publication of the research in the first place?

The answer depends on who you ask, says Hanne Andersen, a philosopher of science at Aarhus University in the Netherlands. While some papers containing misconduct are the work of serial fraudsters who have deliberately duped their coauthors, many cases are not so clear-cut, and there's a whole spectrum of opinions as to the level of the collaborators' responsibility to verify the authenticity of all elements of the research project, not just their own contributions. In short, Andersen says, "the scientific community doesn't have a uniform view."

Risky business

Over the past century, the average number of coauthors on a paper has climbed

from essentially zero to between two and seven—with one of the most rapid increases seen in the biomedical sciences (*PLOS ONE*, doi:10.1371/journal.pone.0149504, 2016). "Multiauthored papers, often with more than 10 authors, are becoming commonplace," wrote David Goltzman, a professor of medicine and physiology at McGill University, in an email to *The Scientist*. "In many cases, it is a major advantage to bring the expertise of scientists who have different research focuses together. [It] facilitates tackling scientific problems which could otherwise not be addressed."

But this rise in coauthorship also exposes a vulnerability inherent to scientific research—that collaborations are fundamentally based on trust. "Trust is needed in science," says Andersen. "If we didn't trust each other, we would need to check everything everyone else did. And if we needed to check everything everyone else did, why collaborate in the first place?"

Carlos Moraes, a neuroscientist and cell biologist at the University of Miami who found himself in a similar position to Bonavida when a colleague's misconduct led to the retraction of multiple coauthored papers, agrees. "If you are the main author of a 'several pieces' type of work, you can do your best to understand the raw data and the analyses," he wrote in an email to *The Scientist*. "Still, trust is a must when the technique or analysis is beyond your expertise."

But trust between collaborators can be violated, and when papers turn out to contain errors or falsified data, the damage is not limited to the guilty party. While scientists who issue corrections quickly and transparently may be unscathed or even rewarded for doing the right thing (see "Self Correction," *The Scientist*, December 2015), recent research suggests that a coauthor's career can take a hit after retractions—particularly if misconduct is involved—even if they are cleared of wrongdoing (*J Assoc Inf Sci Technol*, doi:10.1002/asi.23421, 2015). In cases where one or a few researchers commit fraud, "other authors are in effect 'victims' of the scientific misconduct," says Goltzman, who has had his own experience of retraction fallout after a colleague was found to have falsified large amounts of data.

If we didn't trust each other, we would need to check everything everyone else did. And if we needed to check everything everyone else did, why collaborate in the first place?

—Hanne Andersen, Aarhus University

Some see the issue as more nuanced, however. "It's quite odd that you would consider authors of a fraudulent paper to have no responsibility," says Daniele Fanelli, a Stanford University researcher who studies scientific misconduct. "But that's because we're in a system that those authors would be getting undue credit for that paper if the problems hadn't been discovered." In Fanelli's view, the issue boils down to ambiguity about what coauthorship entails, particularly when ensuring the manuscript is accurate and complete. It's a subject that has "almost willfully been ignored," he says.

Defining responsibility

Indeed, despite the growing abundance of collaborations in the global scientific community, the duties of individual researchers and their role in upholding a study's integrity are rarely defined. During the UCLA investigation, for example, Bonavida says he and his colleagues realized that, even though Bonavida was not only a coauthor but the lab head, the university had no protocol outlining his responsibility for verifying the paper's results. "They didn't have any rules for the faculty that you need to keep documents and original data for so many years, and so forth," he says. "They never made any such guidelines."

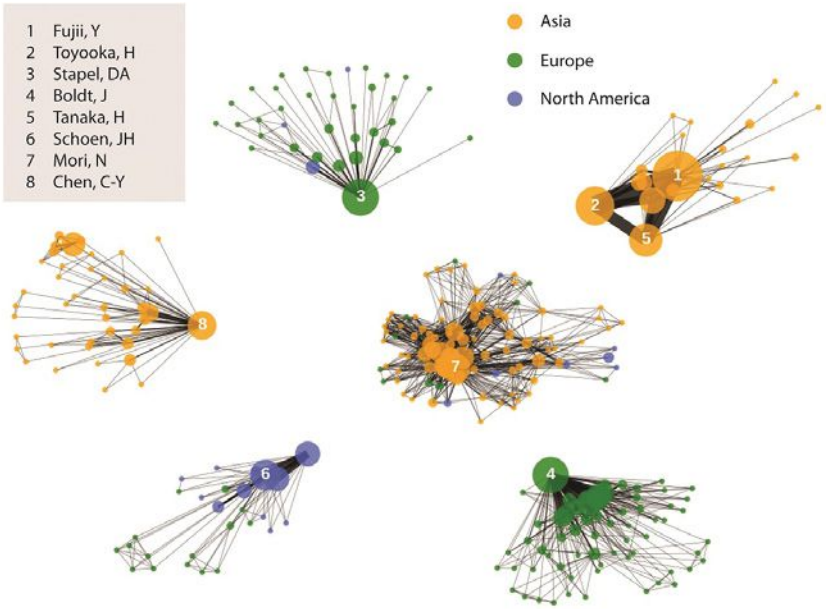
A similar lack of procedure is also true of the journals that publish the research. Although some journals now require authors to itemize their contributions, there are no hard-and-fast standards about what coauthorship entails. "It's dicey," says Geri Pearson, co-vice chair of the Committee on Publication Ethics (COPE), a nonprofit organization that provides guidelines to journal editors on how to handle disputes in scientific publishing. "There's a lot of fuzziness about authorship."

Some journals have maintained that authors should accept equal responsibility for a paper—meaning both credit for its success or blame for its flaws. In 2007, an editorial in *Nature* suggested an alternative—journals should require at least one author to sign a statement vouching for the paper and claiming responsibility for any consequences should the study be found to contain "major problems."

But such "solutions" are generally criticized as unrealistic. *Nature*'s proposal attracted dozens of responses on its site, almost all of them negative. "What does it even mean?" Ferric Fang, a microbiologist at the University of Washington who also studies scientific misconduct, tells *The Scientist*. "That there should be an individual who flies around to each person's lab and does an inspection? Even then, how could you be sure that someone wasn't doing something unethical? . . . To act as if we can declare that [one person is] fully responsible and that makes it so, I think it's kind of ridiculous."

Rather than making a single, broad definition of coauthor responsibility, then, some researchers instead argue for complete transparency when a paper is found to contain flaws. Retracted papers are notoriously persistent in the literature, continuing to accumulate citations long after their findings have been debunked. (See "The Zombie Literature," *The Scientist*, May 2016.) The UCLA group's *Oncogene* paper, for example, was cited at least 15 times between being flagged on PubPeer in 2014 and being retracted two years later. Moreover, retraction notices themselves are often opaque, making it unclear what exactly led to a paper's retraction, or how authors behaved during the process.

To address this problem, some researchers have proposed standardized retraction forms (see "Explaining Retractions," *The Scientist*, December 2015), and in 2015 the Center for Open Science and the Center for Scientific Integrity, the parent organization of *Retraction Watch*,



WEB OF RETRACTIONS: One author's misconduct can have profound effects on the research community. The eight researchers with the highest individual retraction counts in the scientific literature—many of them for misconduct—have together coauthored problematic papers with more than 320 other researchers (circles, sized by retraction count and colored by continent of primary affiliation). The number of retraction-producing collaborations (black lines) between any two researchers varies, but in several cases, researchers produce multiple problematic papers with the same individuals or groups, leading to highly interconnected clusters of scientists linked by their retraction history.

announced their joint effort to create a retractions database, searchable by various classifiers, including all coauthors, journal of publication, and the reason for the retraction. The tool, a preliminary version of which went live at retractiondatabase.org in December 2016, could aid the monitoring of published research itself, as well as help identify labs or individuals who are continually linked to misconduct, notes Andersen. "If you're associated with it once, it would be a pity if you are punished for what someone else did," she says. "But if you're repeatedly associated with it, maybe that's not a great lab for training young scholars."

Addressing the cause

Even without a solid definition of coauthor responsibility, most researchers agree that scientists themselves can help combat misconduct with a more prudent attitude towards collaboration. "You see reports afterwards where people say, 'Well, this looked almost too good to be true,'" says Andersen. "But nobody intervened." Individual researchers could be more vigilant, she adds, particularly in the supervision of junior researchers. Bonavida says that he now takes more effort to explain to graduate students how to correctly present their data. And Moraes says he has become "a lot more careful when scrutinizing the raw data." His advice: get all the data, "including the so-called 'unimportant controls,' and not only the final bar graph."

In cases where one or a few researchers commit fraud, other authors are in effect "victims" of the scientific misconduct.

—David Goltzman, McGill University

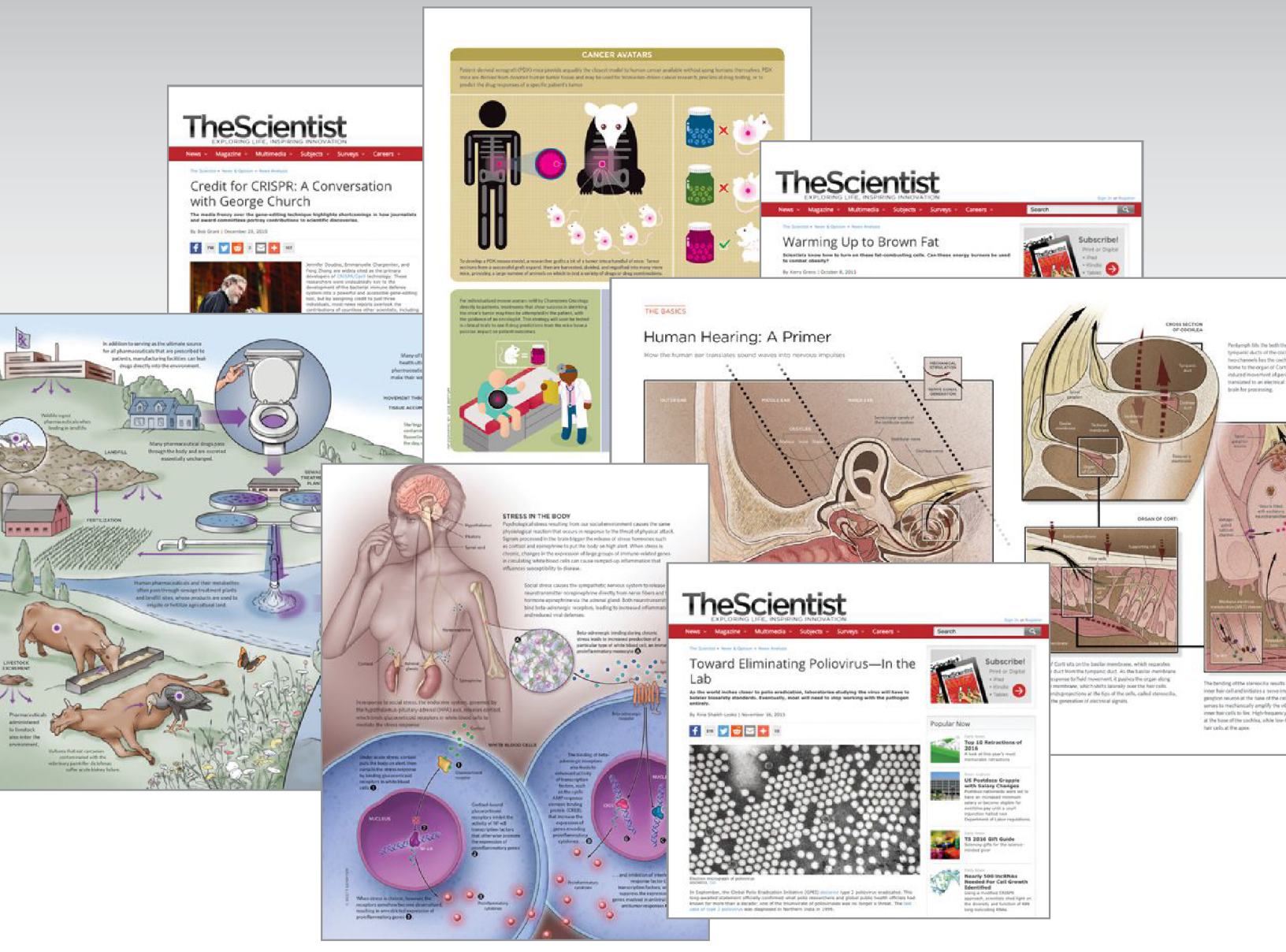
Researchers can also help combat misconduct by making adjustments to the way they organize their collaborations. Goltzman wrote that his group, part of a multicenter study on osteoporosis that uses considerable volumes of medical data, has now adopted procedures that encourage greater transparency. For example, "we previously allowed each investigator to mine all the data they deemed necessary for their study by access to a central database," he explained. "We are now asking each investigator to request the data they need from a statistician . . . so that we know exactly what data is required and provided."

Of course, while these measures may make getting away with misconduct more difficult, there's only so much collaborators can do. Preventing misconduct altogether is a challenge that many argue requires a long hard look at the scientific community in general, including the pressures it places on researchers. Misconduct and retractions "are just symptoms of a process that's not working at optimal efficiency," says Fang. "What's really needed is a more wholesale rethinking about how scientists are supported." Solutions that don't address the related problems of too little funding for too many researchers and the publish-or-perish mentality that still pervades the academic community are mere tweaks to a flawed system, he adds.

In the meantime, though, there's a growing appreciation that research integrity is not black or white. "It's not 'Everything is well and good,' or, 'We're moving into misconduct,'" says Andersen. In recognition of the gray areas of research conduct, there are now initiatives aimed at getting wayward scientists back on track. A National Institutes of Health-funded researcher rehab, for example, is currently working with scientists whose misconduct, or oversight of misconduct, has led to the publication of problematic papers. The organizers of the program, which includes a three-day workshop and follow-up contact over three months, claim that participants show tangible improvements in the way they manage their labs and conduct research.

Such efforts mark a move in the right direction, notes Andersen. "If we can catch [questionable conduct] early on, and train people, and make sure they realize that this is questionable, we can make them better scientists," she says. "That would be far better than catching it late—so late that we end their careers." ■

ROMANO FOTI



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TheScientist

Taste Makers

A person's flavor experience is shaped not only by their genome but also by unique associations linked to their culinary history.

BY BOB HOLMES

As soon as I decided to write a book on the science of flavor, I knew I wanted to have myself genotyped. Every one of us, I learned through my preliminary research for *Flavor: The Science of Our Most Neglected Sense*, probably has a unique set of genes for taste and odor receptors. So each person lives in their own flavor world. I wanted to know what my genes said about my own world. Sure enough, there was a lesson there—but not the one I expected.

Our senses of smell and taste detect chemicals in the environment as they bind to receptors on the olfactory epithelium in the nose or on taste buds studding the mouth. From these two inputs, plus a few others, the brain assembles the compound perception we call flavor. Taste is pretty simple: basically, one receptor type each for sweet, sour, salty, and the savory taste called umami, and a family of maybe 20 or more bitter receptors, each of which is sensitive to different chemicals. Smell, on the other hand, relies on more than 400 different odor receptor types, the largest gene family in the human genome. Variation in any of these genes—and, probably, many other genes that affect the pathways involved in taste or smell—should affect how we perceive the flavors of what we eat and drink.

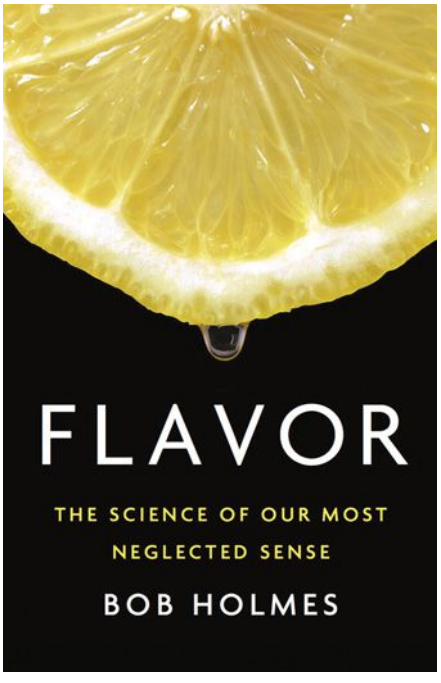
Hence the genotyping. One April morning a few years ago, I drooled into a vial and sent that DNA sample off to the Monell Chemical Senses Center in Philadelphia, home to what is likely the world's biggest research group dedicated to the basic science of flavor. A few months later, I visited Monell to take a panel of perceptual tests and compare the results to my genetic profile.

I had already learned that I'm a so-called supertaster from another researcher, who had offered that conclusion after counting the taste-bud-bearing fungiform papillae on my tongue. "Supertaster" is an often-misused term for someone who is acutely sensitive to bitter tastes, and probably other ones as well. Despite the "super," it's generally not something to be proud of—because their taste sensations are so intense, supertasters often confine themselves to narrow, bland diets. But not me. I love all sorts of intense, bitter foods: black coffee, highly hopped beers, bitter green vegetables like broccoli rabe and collard greens.

My genotype yielded equally puzzling results. My version of the sweet-receptor gene is less responsive than normal, which should make me prefer sweeter food and drinks to get the same flavor bang. And sure enough, on the perceptual tests I'd rated a 12 percent sugar solution as highly pleasant, while many people find it far too sweet. But that doesn't match my eating habits: I'm generally indifferent to dessert, and I detest sweetened coffee or tea.

The picture wasn't any clearer for the few odor-receptor genes I was tested for. Most of the time, my ratings of the pleasantness and intensity of odors like earthy 2-ethylfenchol or sweaty isovaleric acid aligned poorly with the predictions based on my genes for the relevant odor receptors.

So why don't my genes match my culinary preferences? The reason, probably, is that nurture matters at least as much as nature in molding anyone's flavor destiny. Like many people, I've learned to like my coffee unsweetened, and that main courses are more interesting than desserts. We're also good at learning to link flavors to consequences. We associate coffee's bitterness with the wake-up jolt of caffeine it delivers, so what was once a warning—bitterness—becomes a beacon. And we pair the taste of beer or gin and tonic with the pleasure we get from an evening out with friends. With associations like that, it's no wonder so many of us love what are, objectively speaking, such nasty, bitter flavors. ■



W.W. *Norton, April 2017*

Bob Holmes is a correspondent for New Scientist. *Read an excerpt of* Flavor: *The Science of Our Most Neglected Sense at the-scientist.com.*

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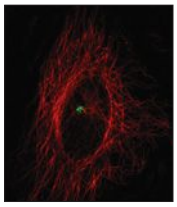

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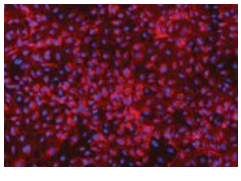

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To target diseases such as cancer and HIV, scientists are now successfully programming the immune system to seek out and destroy the agents of disease, even when those agents are the body's own cells. Immunotherapy has demonstrated remarkable efficacy in treatment of previously undruggable diseases, leading to calls for standardization and additional research. *The Scientist* is bringing together a panel of experts to review the current knowledge base, share their recent work, and discuss the future of immunotherapy. Attendees will have the opportunity to interact with the experts, ask questions, and seek advice on topics that are related to their research.

LEENA GANDHI, MD, PhD
Director, Thoracic Medical Oncology,
NYU Langone Medical Center
Associate Professor, Department of Medicine
NYU Perlmutter Cancer Center

LAURENCE COOPER, MD, PhD
Chief Executive Officer
Ziopharm Oncology, Inc.

THURSDAY, MAY 18, 2017
2:30–4:00 PM EASTERN TIME

REGISTER NOW!
www.the-scientist.com/immunoadvances
The webinar video will also be available at this link.

TOPICS TO BE COVERED:

- Progress in immunotherapies, from cancer to HIV
- Immunotherapy research and development

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ONDEMAND | 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* brings together a panel of experts to share their research and discuss the next steps.

WATCH NOW! www.the-scientist.com/microbiomemedicine

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

ROBERT A. BRITTON, PhD
Professor, Department of Molecular Virology and Microbiology
Member, Alkek Center for Metagenomics and Microbiome Research
Baylor College of Medicine

TOPICS COVERED:

- The human microbiome's influence on health
- Studying the microbiota of disease in order to better target treatments

WEBINAR SPONSORED BY:

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ONDEMAND | Single-Cell Phenotypic Analysis: Breaking the Law of Averages

Population-scale cellular analysis has provided many valuable insights into general trends of cell behavior and expression, but rare and low-level events can get lost in the lysate. Averaging the signals from an entire culture can drown out the least prevalent but most important phenotypes. That's why single-cell analysis has made such an impact, enabling higher throughputs, rare-event detection, and more-precise and meaningful analyses. To explore this exciting new frontier, *The Scientist* introduces an expert who shares his experience with single-cell phenotypic analysis and reflects on the clarity it provides.

WATCH NOW! www.the-scientist.com/singlecellphenotype

RYAN BRINKMAN, PhD
Professor, Medical Genetics
University of British Columbia
Distinguished Scientist, British Columbia Cancer Agency

TOPICS COVERED:

- Quantitative analysis of cell phenotype at the single-cell level
- Interpreting phenotypic data for optimal value

WEBINAR SPONSORED BY:

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32nd Congress of the International Society for Advancement of Cytometry

BOSTON
CYTO 2017

June 10 - 14, 2017 | Hynes Convention Center | Boston, Massachusetts, USA

The Science of Cytometry

CYTO is the premier meeting for showcasing the art and science of cytometry. This year, we will be in Boston, Massachusetts, USA from June 10-14, 2017, at the Hynes Convention Center, and we want you to join us.

The Congress will include elements for scientists working in all areas of cytometry and at all career stages. Top faculty and leaders in the industry will contribute their time and talent to CYTO Innovation, State of the Art Lectures, Scientific Tutorials, Workshops, Networking Opportunities, and Plenary Sessions featuring cutting edge cytometry technology and applications on the following topics:

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- Single Cell & Systems Biology
- Intravital Imaging
- Microbiome
- Immunotherapy
- Novel Fluorochromes
- Mass Cytometry
- Immune Monitoring
- And many more!

Key Note Speakers

Hooke Lecture

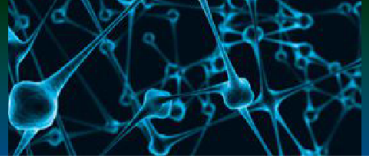
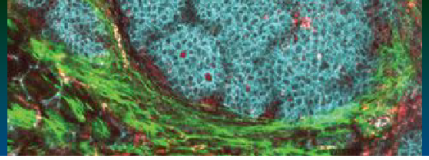
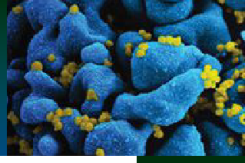
Illuminating Biology at the Nanoscale with Single-Molecule and Super-Resolution Imaging
Xiaowei Zhuang, *STORM and MerFISH*

Tsien Lecture

Optogenetics and Expansion Microscopy
Ed Boyden, *MIT Media Lab, Optogenetics & Expansion Microscopy*

Plus, don't miss CYTO Innovation, which aims to serve attendees seeking to translate their research into high-impact products and services. It is open to Full Congress Registrants; no additional fees apply! View the full program at www.cytoconference.org.

Register today at www.cytoconference.org!

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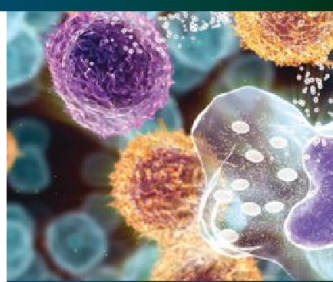
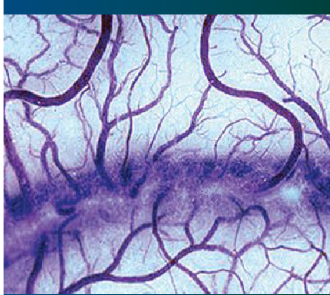
Maternal-Fetal Cross Talk: Harmony vs. Conflict (T2)
Regenerative Biology and Applications: Cell Differentiation, Tissue Organization and Biomedical Engineering (T3)
Antimicrobials and Resistance: Opportunities and Challenges (T4)
Frontiers of Serotonin Beyond the Brain (T5)
Heart Failure: Crossing the Translational Divide (A1)
State of the Brain: Genetic Dissection of Brain Circuits and Behavior in Health and Disease (A2)
T Cell Dysfunction, Cancer and Infection (A3)
Plant Signaling: Molecular Pathways and Network Integration (A4)
Natural Products and Synthetic Biology: Parts and Pathways (J1)
Tumor Metabolism (A5)
Cell Death, Inflammation and Adaptation to Tissue Stress (A6)
Organ Crosstalk in Obesity and NAFLD (J3) *joint with* **Bioenergetics and Metabolic Disease (J4)**
DNA and RNA Methylation (A7) *joint with* **Ubiquitin Signaling (A8)**
Translational Systems Immunology (A9)
Precision Genome Editing with Programmable Nucleases (B1)
Emerging Technologies in Vaccine Discovery and Development (J5) *joint with* **Progress and Pathways Toward an Effective HIV Vaccine (J6)**
Atherosclerosis: Lessons Learned and Concepts Challenged (B2)
Frontiers in Islet Biology and Diabetes (B3)
Cryo-EM from Cells to Molecules: Multi-Scale Visualization of Biological Systems (F1)
Cancer Epigenetics: New Mechanisms, New Therapies (B4)
Phosphoinositide Biology: New Therapeutic Targets Beyond Class I PI3K (B5)
Emerging Cellular Therapies: T Cells and Beyond (B6) *joint with* **Lymphocytes and their Roles in Cancer (R1)**
Mobile Genetic Elements and Genome Plasticity (B7)
GPCR Structure and Function: Taking GPCR Drug Development and Discovery to the Next Level (B8)
Regulation and Dysregulation of Innate Immunity in Disease (B9)
Antibodies as Drugs: Translating Molecules into Treatments (C1)
Noncoding RNAs: Form, Function, Physiology (C2)
Endoderm Development and Disease: Cross-Organ Comparison and Interplay (C3)
Uncomplicating Diabetes: Reducing the Burden of Diabetes-Related End-Organ Injury (J7) *joint with* **Vascular Biology and Human Diseases: From Molecular Pathways to Novel Therapeutics (J8)**
Immunological Memory: Innate, Adaptive and Beyond (X1) *joint with* **Aging, Inflammation and Immunity (X2)**
Manipulation of the Gut Microbiota for Metabolic Health (X3) *joint with* **Microbiome, Host Resistance and Diseases (X4)**
Cells vs. Pathogens: Intrinsic Defenses and Counterdefenses (C4)
Cancer Immunotherapy: Combinations (C5)
Chromatin Architecture and Chromosome Organization (X5) *joint with* **Gene Control in Development and Disease (X6)**
The Resolution of Inflammation in Health and Disease (C6)
iPSCs: A Decade of Progress and Beyond (C7)
Organs- and Tissues-on-Chips (D1)
Myeloid Cells (D2)
Therapeutic Targeting of Hypoxia-Sensitive Pathways (V1)
Pushing the Limits to Healthspan and Longevity (D3)
Tuberculosis: Translating Scientific Findings for Clinical and Public Health Impact (X7) *joint with* **HIV and Co-Infections: Pathogenesis, Inflammation and Persistence (X8)**
Mitochondrial Biology (Z1) *joint with* **Selective Autophagy (Z2)**
Precision Medicine in Cancer (E1)
Exosomes/Microvesicles: Heterogeneity, Biogenesis, Function and Therapeutic Developments (E2)
One Million Genomes: From Discovery to Health (G1)
Novel Aspects of Bone Biology (E3)
B Cells: Mechanisms in Immunity and Autoimmunity (E4)

60 CONFERENCES



7 NEW JOINT PAIRINGS



25 VENUES GLOBALLY

Conferences listed in chronological order. View program details for each at **keystonesymposia.org** followed by a **/18** and the alpha-numeric **program code** (e.g., **www.keystonesymposia/18A1**).

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Failure to Recapitulate, 1874

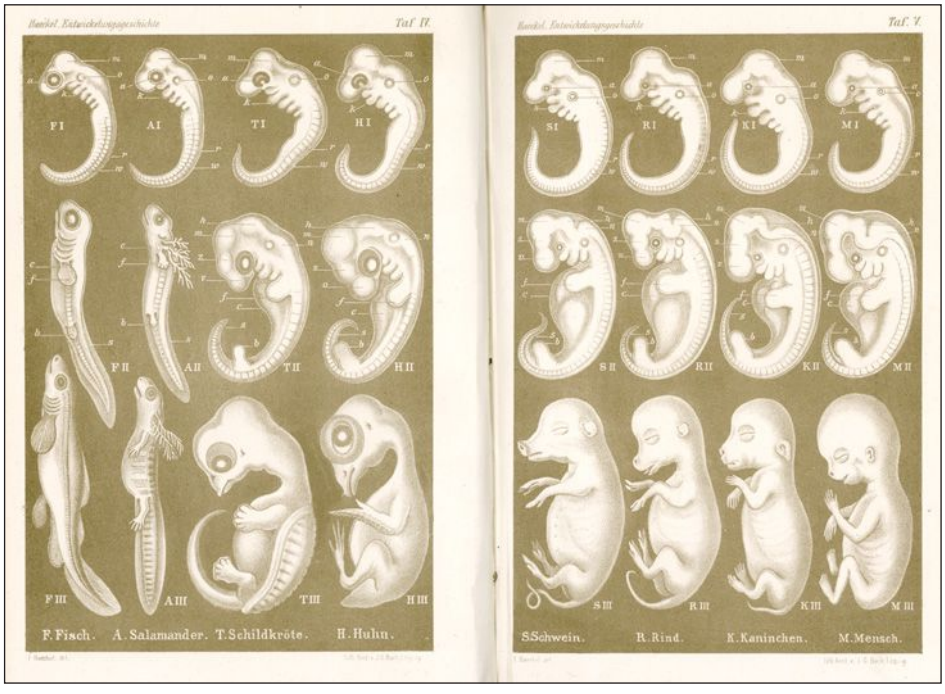
BY DIANA KWON

Ernst Haeckel, a biologist, artist, and philosopher born in Prussia in the 1830s, played a key role in spreading Darwinism in Germany. He was also deeply fascinated by embryology and illustrated some of the most remarkable comparisons of vertebrate embryos in his day. These images were widely printed and copied, both to argue for Haeckel's controversial evolutionary theories and to debunk them.

Haeckel's most influential idea was his now-infamous biogenetic law, summarized by the phrase "ontogeny recapitulates phylogeny"—in other words, an organism's embryo progresses through stages of development that mirror its evolutionary history. According to this theory, embryos of more advanced species—humans, for example—would pass through stages in which they displayed the adult characteristics of their more primitive ancestors (such as fish gills or monkey tails).

The biogenetic law was popular among scientists at the time, including Darwin, and Haeckel used his drawings of embryos to support his own theory. His textbook on comparative embryology, *Anthropogenie*—in which he published some of his most famous illustrations of embryos—was devoted to this idea, says Nick Hopwood, a historian of science and medicine at the University of Cambridge and author of *Haeckel's Embryos: Images, Evolution and Fraud*, published in 2015.

A number of Haeckel's contemporaries, such as Wilhelm His Sr., a Swiss anatomist, challenged the biogenetic law and alleged that Haeckel's drawings contained inaccuracies and misleading representations. One such accusation was that Haeckel had reprinted a single woodcut to create illustrations of a mammal, a bird, and a reptile in his first book, *Natürliche Schöpfungsgeschichte*. Haeckel admitted to this malpractice and apologized for it in a later edition of the book.



EMBRYONIC EVOLUTION: This comparative illustration of eight species' embryos from Haeckel's *Anthropogenie* (1874 edition) is among the most well-known of the German scientist's images. The rows represent three developmental stages and the columns correspond to different species (fish, salamander, turtle, chicken, pig, cow, dog, and human).

Haeckel's embryo drawings were widely circulated. They appeared in some mid-20th century high school and college biology textbooks in the United States, often bearing the name of a Canadian-British evolutionary biologist and physiologist, George John Romanes, who had copied Haeckel's work. Authors and publishers used Romanes's facsimile to dispute Haeckel's own theories, unaware that Haeckel himself had drawn the original content, Hopwood says.

In later years, Haeckel's original images reappeared, this time in the developmental biology literature, often to argue for similarities across species during embryonic growth. Although biologists still criticized the drawings for containing inaccuracies, the idea that early commonalities exist more closely aligns with what scientists believe today.

"We now think that embryos resemble not the adults of ancestral species, but the embryos of ancestral species," Michael Richardson, a professor of evolutionary developmental zoology at Leiden University in the Netherlands, wrote in an email. He adds that there is still the belief among biologists that a "phylotypic period," when embryos share strong similarities across species, exists, as Haeckel often demonstrated in the top row of his drawings. However, according to Richardson, more recent evidence points to commonalities at the molecular level.

"I think it's fascinating that [Haeckel's drawings] are some of the most controversial pictures in the history of science and yet became some of the most routinely used," Hopwood says. ■

NICK HOPWOOD

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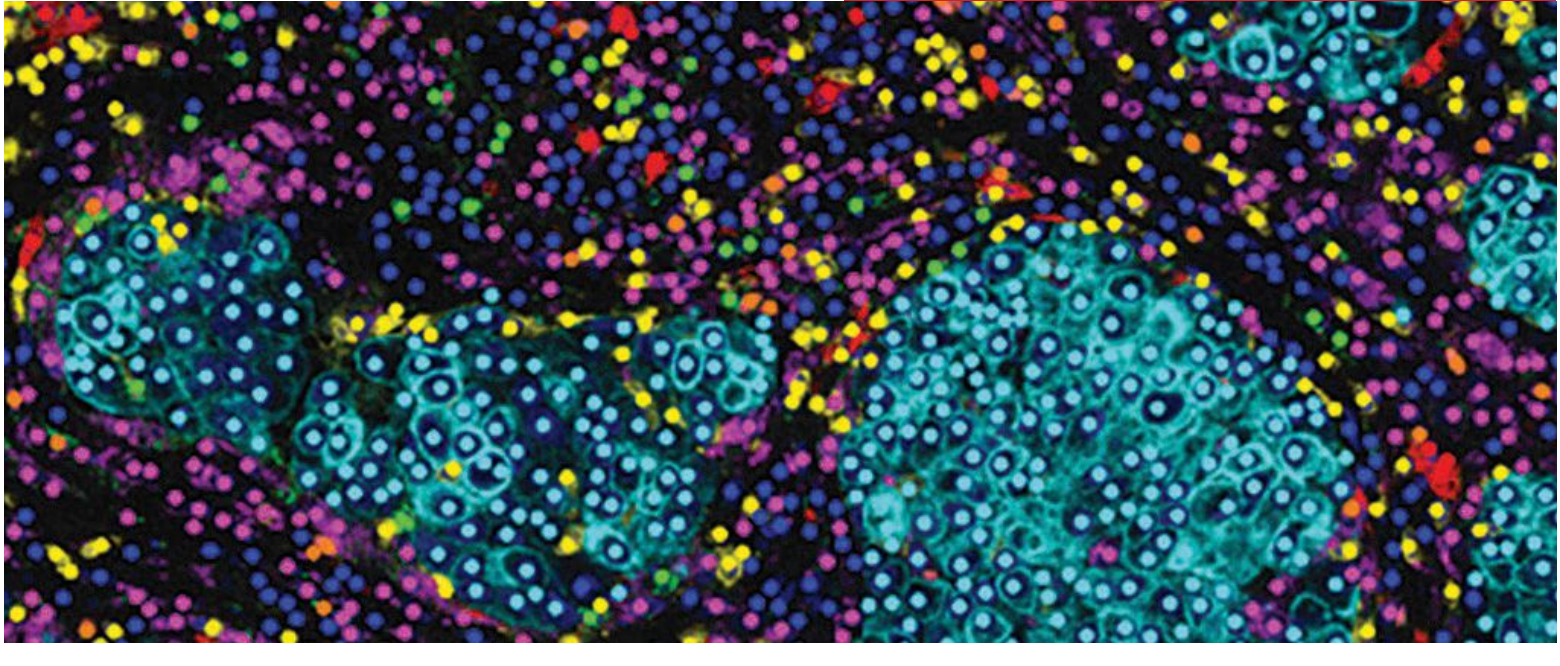
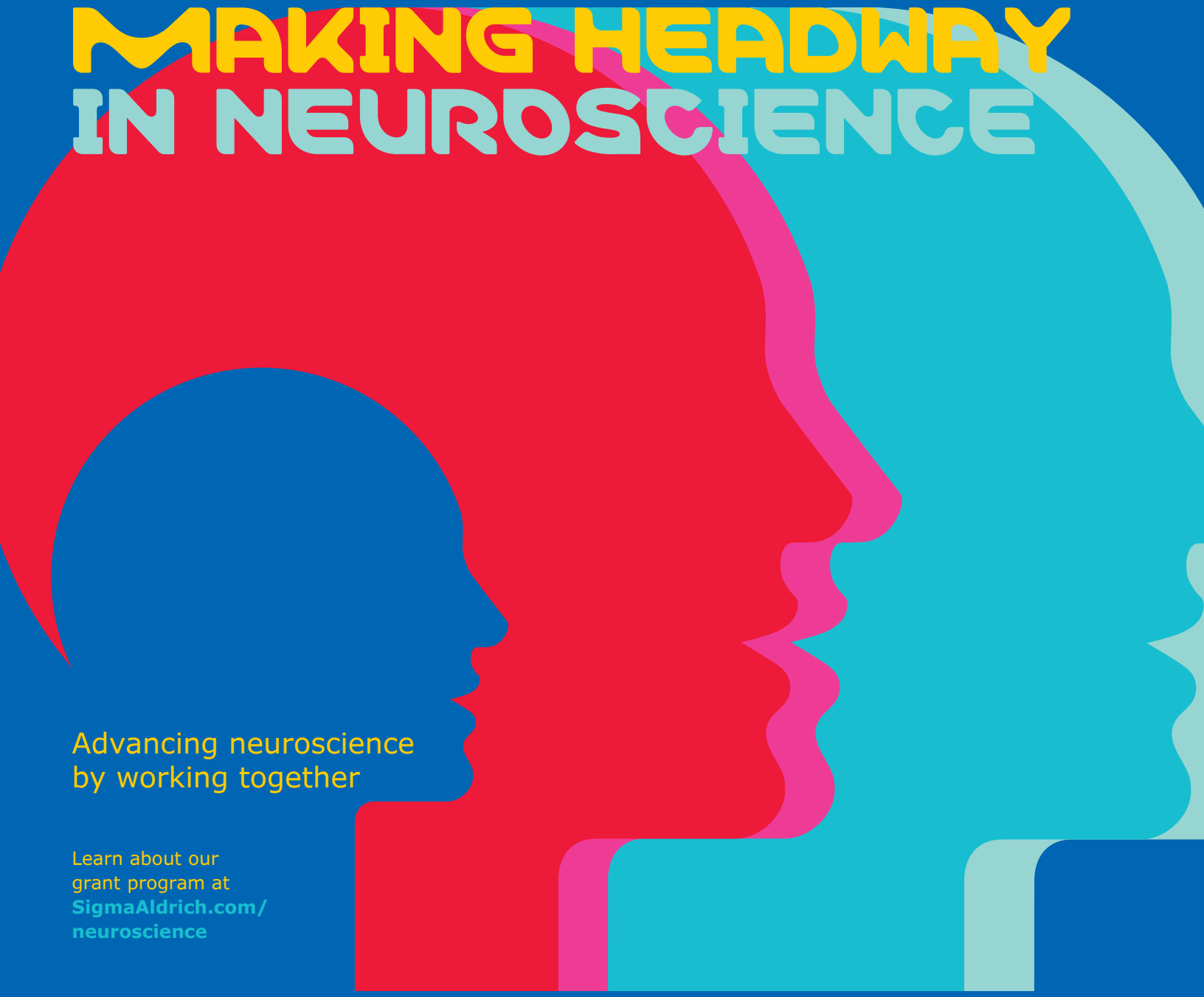


MAKING HEADWAY IN NEUROSCIENCE



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