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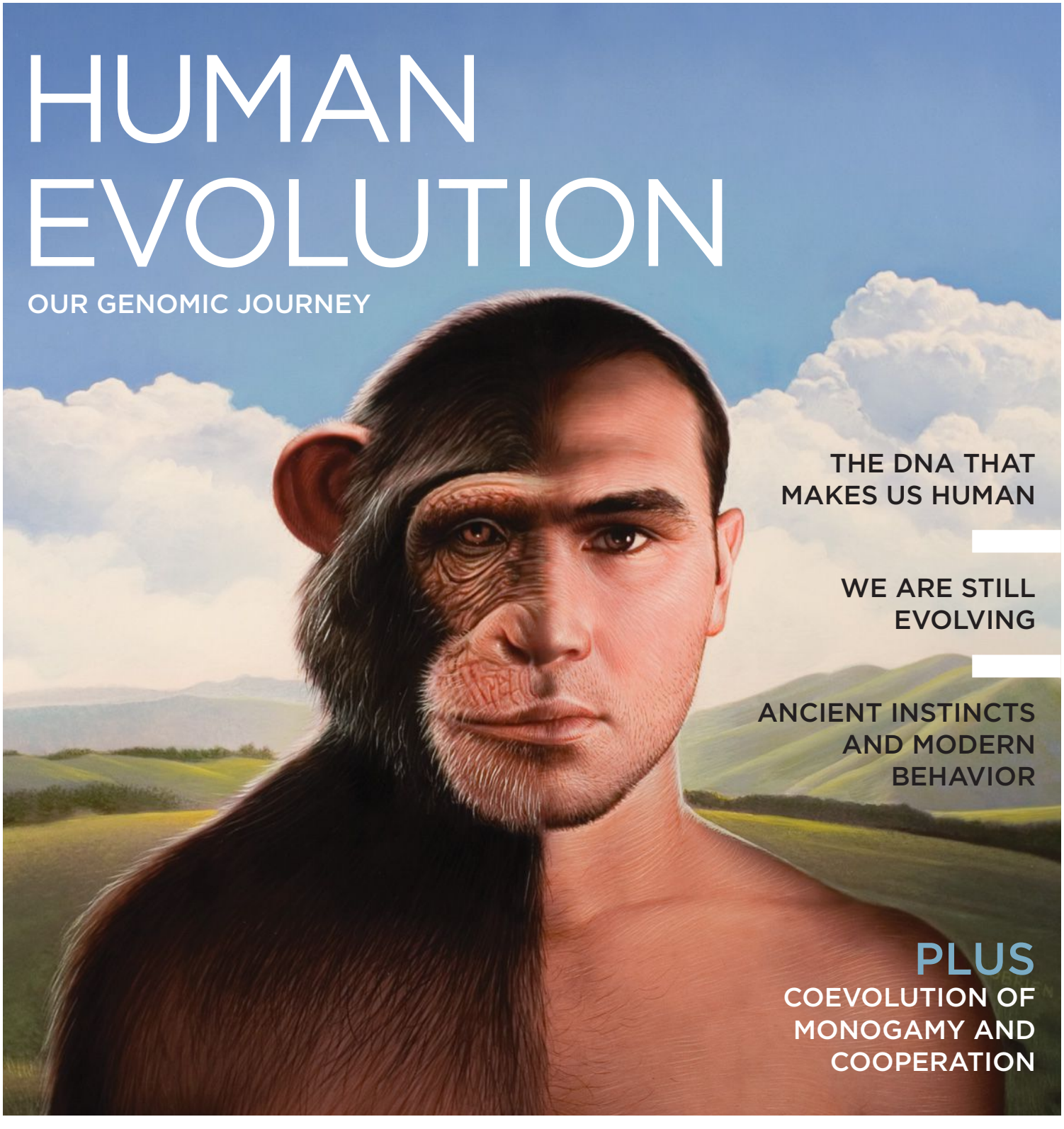
HUMAN EVOLUTION

OUR GENOMIC JOURNEY



THE DNA THAT MAKES US HUMAN

WE ARE STILL EVOLVING

ANCIENT INSTINCTS AND MODERN BEHAVIOR

PLUS

COEVOLUTION OF MONOGAMY AND COOPERATION

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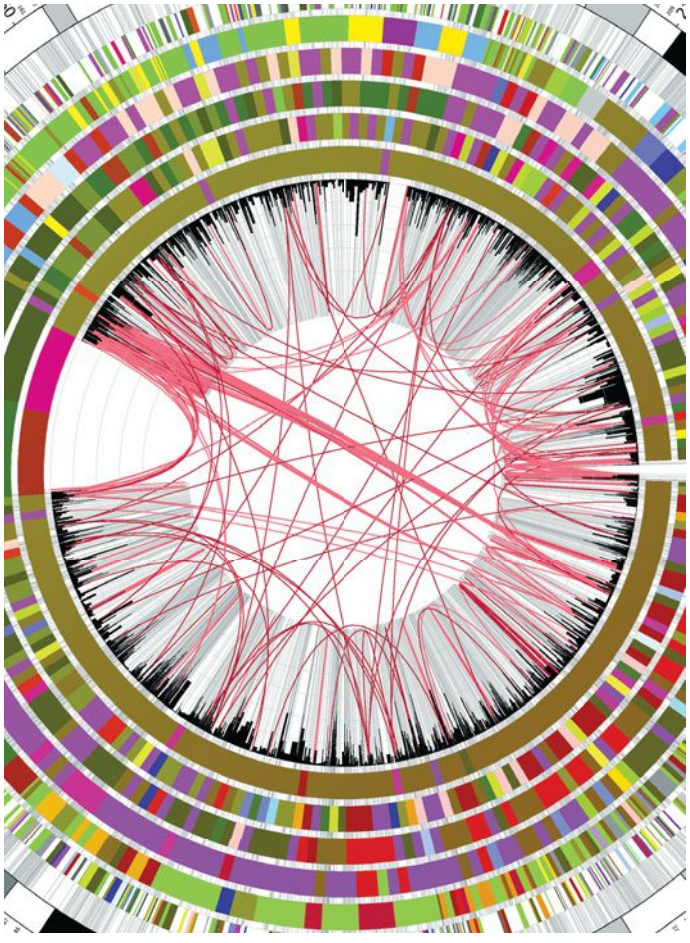

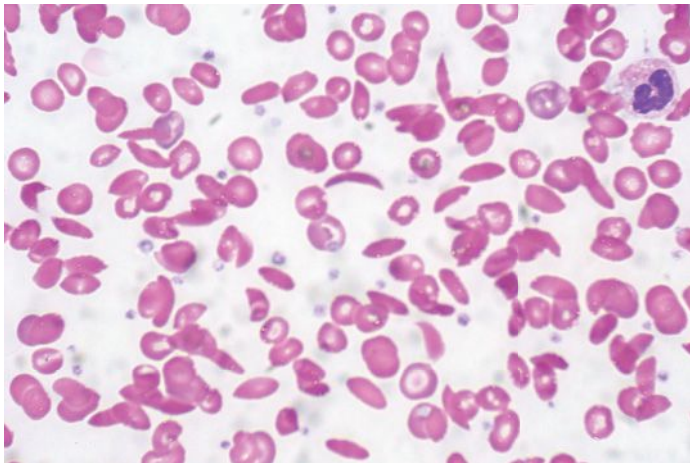

Features

ON THE COVER: ILLUSTRATION BY TIM O'BRIEN

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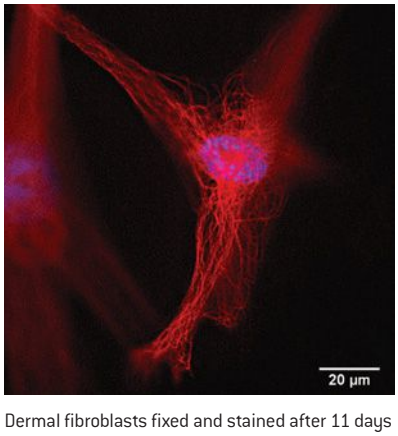

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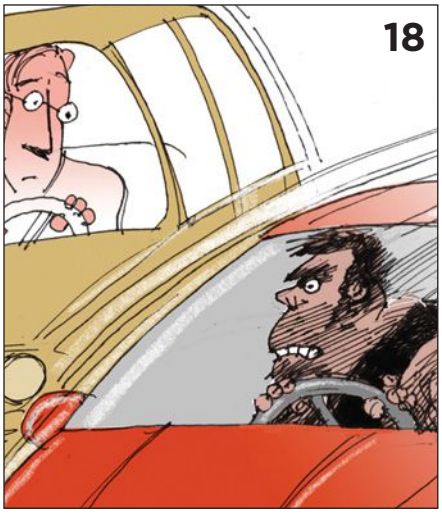

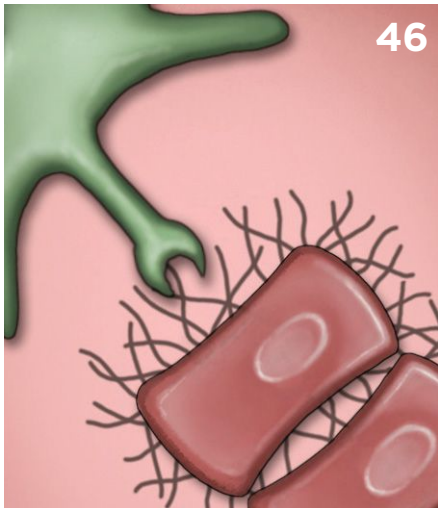

CORRECTION:
"Cellular Teamwork" (*The Scientist*, July 2016) incorrectly stated that erythroblasts' failure to mature can lead to hemolytic anemia. Erythroblast maturation failure leads to aplastic anemia.

The Scientist regrets the error.

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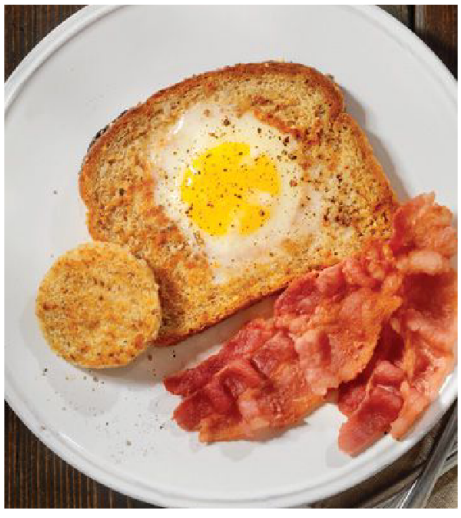

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
Bacterial Baddies
Scientist to Watch and MIT researcher Cullen Buie talks about his quest to devise a method for quickly determining the pathogenicity of microbes.

VIDEO
The Death of Diets
Book author and neuroscientist Sandra Aamodt discusses her struggle with being overweight and the science behind breaking the cycle of gain and loss.

VIDEO
Guppie Porn
Biologist Carin Bondar delivers a TED talk about the wilder side of sex.

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

Coming in September

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

Focus on senses beyond the usual five

- Proprioception
- Human sensory receptors in odd places
- Animals' sense of gravity, electric and magnetic fields, heat, and water flow
- Time perception
- Polarization sensing in tropical bees

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Contributors

While an undergraduate at Pomona College in Claremont, California, **Katherine Pollard** was encouraged by a professor to pursue mathematics alongside her chosen subject of anthropology. She double majored before graduating in 1995. "I was hoping to find some application for it—not just math for math's sake," Pollard says. "That's how I ended up in bioinformatics." After graduation, she spent three years overseas working in epidemiology in the U.K., Australia, Indonesia, and New Zealand, before moving to the University of California (UC), Berkeley, for a PhD in biostatistics. "I was thinking I was going to use my math for public-health purposes," she says. But the release of the human genome sequence in 2001 prompted an interest in the emerging field of genomics. Dividing her time between dissertation writing and internships at local biotechs, Pollard researched methods for analyzing DNA microarrays, and after graduating in 2003, took up a postdoc at UC Santa Cruz, where new genomes were being analyzed. "Given my anthropology background, I became very interested in the chimpanzee genome," she says. Joining UC Davis in 2005, Pollard set up a lab to research genomic differences between chimps and humans, discovering hundreds of fast-evolving areas of the human genome. Now a professor at the Gladstone Institutes in San Francisco, Pollard studies how these "human accelerated regions" might distinguish us from our nonhuman relatives.

Pollard explores the significance of these regions in "Uniquely Human" (page 24).

As a child walking along the chalk roads of western Kansas, **John Hawks** loved finding fossilized shells and shark teeth. That affection bloomed into a lifelong fervor. As an undergraduate at Kansas State University, he helped teach a laboratory course in biological anthropology, which included studying primate and human skeletons. Hawks was enthralled—especially with the bones of the past. "I loved the hominid fossils and the discoveries of the Leakeys," he says. "These bones were part of our ancestors, our relatives." He graduated in 1994 with a BA in French, English, and anthropology. He then moved to the University of Michigan, where he began to use genetics to investigate how natural selection has shaped human genes, earning his PhD in 1999. Three years later, he joined the faculty of the University of Wisconsin–Madison. He now has a popular blog—"I think of it as a diary," he says—that shares his thoughts on anthropology research with hundreds of thousands of people around the world. More recently, Hawks has co-led research in the Rising Star cave system in South Africa, a project supported by National Geographic, with University of Witwatersrand paleoanthropologist Lee Berger. In 2015, they unveiled a discovery among the cave fossils of a new ancient hominid: *Homo naledi*.

But Hawks isn't stuck in the past. He also examines human evolution's present. "People are still surprised to find out humans are still evolving," he says. Hawks explains the ways in which our species has been, and continues to be, under natural selection in "The Ever-Changing Human" (page 32).

Lydia Pyne has never been able to choose between history and anthropology, so she's kept up both. After double majoring in the two subjects at Arizona State University in 2002, Pyne obtained a master's degree in anthropology in 2004, and a PhD in the history and philosophy of science in 2008. "I really loved the narrative structure of writing history to talk about the processes of science," she says. "But I also loved the hands-on history of archaeology and paleoanthropology. I felt that writing about the intellectual history of archaeology and paleoanthropology gave me the opportunity to put together these two subjects." After finishing her PhD, Pyne coauthored a book on the Pleistocene epoch and human origins with her father, environmental historian Stephen Pyne, called *The Last, Lost World* (2012). This first book left her wanting to write another, and she began research on why some scientific discoveries—in particular, fossils—become famous. Drawing on personal experience from excavations at archeological sites from South Africa to the American Southwest, Pyne tackled this question in her most recent book, *Seven Skeletons*—a project that has allowed her to draw on her interdisciplinary background.

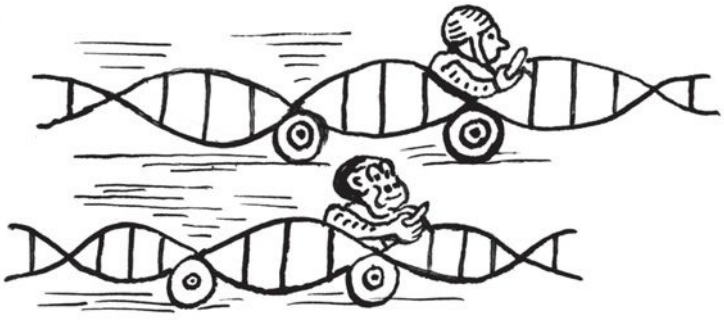
Pyne discusses the factors influencing a fossil's fame in "A Paleolithic Patriarch" (page 64).

CHRIS GOODFELLOW, GLADSTONE INSTITUTES; RUSS CREECH; MOLLY BREWER

The Human Kind

Some thoughts on going to the Galápagos

BY MARY BETH ABERLIN



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"As I was walking along I met two large tortoises, each of which must have weighed at least two hundred pounds: one was eating a piece of cactus, and as I approached, it stared at me and slowly stalked away; the other gave a deep hiss, and drew in its head. These huge reptiles, surrounded by the black lava, the leafless shrubs, and large cacti, seemed to my fancy like some antediluvian animals." Thus Charles Darwin described the September 1835 scene that greeted him on Chatham Island (now San Cristóbal), the first of the Galápagos archipelago he visited.

As this issue of *The Scientist* is rolling off the printing press, I will be in the Galápagos, excited that I might be walking in some of Darwin's footsteps, hoping to witness something close to what he saw. Most fittingly, *Beagle* is the name of the small boat on which I will tour the islands.

Darwin's visit to the different volcanic cones that form the Galápagos Islands was pivotal to the development of his theory of evolution by natural selection. Yet he cogitated for more than 20 years before publishing *On the Origin of Species* in 1859. His HMS *Beagle* research journals came out the following year. And it took another 12 years before he released *The Descent of Man,* his thoughts on the subject of human evolution. In the book's introduction, Darwin describes his fear that addressing the topic would "only add to the prejudices against my views."

Since Darwin's time, there has been no dearth of scholarship about human evolution, and our August issue offers two features devoted to the subject. In "Uniquely Human" (page 24), Katherine Pollard describes her quest to identify the function of short regions of the human genome that differ from our closest living relative, the chimpanzee, with whom we share 99 percent of our DNA. These so-called human accelerated regions are conserved among much of the animal kingdom, but have evolved dramatically in human ancestors after they split from other primate lineages. Many seem to function as genetic enhancers regulating gene expression during embryonic development in uniquely human parts of the brain and body.

John Hawks writes about the evidence for ongoing evolution in modern human populations in "The Ever-Changing Human" (page 32). Combining public-health data with genomic studies will enable researchers to discover more ways humans are evolving, beyond the well-known examples of skin and eye color, lactose tolerance, and blood differences in response to malaria risk. "New evidence of how the human genome has changed over the last several thousand years points to series of massive critical evolutionary changes, setting some aspects of our biology clearly apart from that of our forebears. And we are no doubt continuing to evolve today," says Hawks.

But are we evolving quickly enough to keep pace with our advancing society? João Pedro de Magalhães ("Our Inner Caveman," page 18) argues that our globally connected modern world is still ruled by behaviors forged during the time of our early human ancestors, when "harsh conditions fostered cooperation within small groups, often made up mostly of one's relatives, thus favoring strong social bonds." Pundits from many disciplines have commented on how the survival instincts driving tribal social behavior get activated in today's populous, multicultural settings, and these ideas certainly seem relevant to the confusion and dismay caused by the Brexit vote, the US presidential race, and a spate of violent attacks around the world.

Also on the subject of evolution with interesting implications for human behavior is a Thought Experiment (page 20) by Jacqueline Dillard and David Westneat that describes the close evolutionary connection between monogamy and cooperation.

Every marine iguana, blue-footed booby, and little finch I encounter on my visit (a dream trip for any life scientist) will hammer home the long arc of evolution, from Darwin's "tangled bank" to the continued shaping of our human selves. ■

Editor-in-Chief
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Speaking of Science

Being in the E.U. gives us access to ideas, people and to investment in science. That, combined with mobility [of EU scientists], gives us increased collaboration, increased transfer of people, ideas, and science—all of which history has shown us drives science.

—Nobel laureate **Paul Nurse**, director of the Francis Crick Institute and immediate past president of the Royal Society, speaking four months before UK citizens voted to leave the European Union in the historic "Brexit" referendum (*BBC News*, February 26)

For science to thrive it must have access to the single market, and we do need free movement. We could negotiate that outside the E.U., which will probably end up costing more money and we would have little influence [in deciding research priorities]. Or perhaps we should just reconsider this entire mess and see if there is something that can be done to reconsider this once the dust has settled.

—**Paul Nurse**, on how the June 23 "Brexit" vote to leave the European Union could impact scientific research in the U.K. (*BBC News*, June 29)

Convergence has grown from a little seedling to a sprouting plant, but to become a great tree and orchard yielding fruit for decades into the future, it needs to be nourished, expanded, and cultivated now. Students need to be educated, collaborations need to be encouraged, and resources need to be committed to make sure convergence thrives.

—Nobel laureate **Phillip Sharp**, on the need to merge historically disparate disciplines, such as physics, computer science, mathematics, and the life sciences in order to achieve long-standing goals in biomedicine (*MIT News*, June 23)

By bringing together doctors and data like never before, precision medicine aims to deliver the right treatments in the right dosage at the right time—every time. It helps target the causes of a condition rather than just the symptoms. This is one of the greatest opportunities we've ever seen for new medical breakthroughs, but it only works if we collect enough information first.

—President **Barack Obama**, in a *Boston Globe* opinion piece about his administration's efforts to make personalized medicine a broader reality (July 7)

Earth needs a virtual country: #Rationalia, with a one-line Constitution: All policy shall be based on the weight of evidence.

—Astrophysicist **Neil deGrasse Tyson**, in a recent tweet (June 29)

Tyson is a very smart man, but this is a very stupid tweet, and a very stupid idea. It is even, we might say, unreasonable and without sufficient evidence. Of course imagining a society in which all actors behave logically sounds appealing. But employing logic to consider the concept reveals that there could be no such thing.

—**Jeffrey Guhin**, a University of California, Los Angeles, sociologist, criticizing Neil deGrasse Tyson's controversial #Rationalia tweet (*Slate*, July 5)

Notebook

AUGUST 2016

EVAN CANTWELL/GEORGE MASON UNIVERSITY

LYME LAB: Temple Douglas, then a high school student, works with former George Mason University grad student Davide Tamburro during the Aspiring Scientist Summer Internship Program.

Stepping into the Lyme-Light

Toward the end of her 2009 summer internship at a George Mason University proteomics lab, high school student Temple Douglas wondered if she could use her research to turn a family problem into a clinical breakthrough. Lyme disease, a scourge in her native Virginia, had previously struck both her mother and brother, so the family was aware of the drawbacks of the single available diagnostic test. The current blood test probes for antibodies developed against the disease-causing bacterium *Borrelia burgdorferi*, which only appear several weeks after the onset of symptoms such as the telltale reddish oval or "bull's-eye" rash that appears in three-fourths of cases. What if the hydrogel nanoparticles Douglas had been using to concentrate cancer biomarkers could directly trap *B. burgdorferi* proteins from patients and enable early-stage detection?

"Those bacteria have to be shedding proteins, and those proteins are probably secreted in the urine," Douglas remembers thinking. So she continued to work in Alessandra Luchini's lab throughout the fall and the following summer, fine-tuning the particles and testing their affinity for Lyme-causing bacterial proteins in urine. Her hard work paid off with a published paper (*Biomaterials*, 32:1157-66, 2011), and clinical trials of the test, launched in 2012.

Douglas joined Luchini's lab, her first real research experience, through George Mason's Aspiring Scientists Summer Internship Program (ASSIP). "Our goal . . . was to give [high school and college students] hands-on experiments in the lab so that they could experience the agony and the ecstasy of science," says program cofounder and George Mason researcher Lance Liotta. Prior to Douglas's arrival, Luchini's group had developed particles with a hydrogel exterior that sheltered a core of chemical dyes. The dyes bind with high affinity to

small proteins, concentrating the proteins from a more dilute solution of blood or urine by as much as 2,000-fold. At the same time, the hydrogel lattice prevents the proteins' degradation and excludes larger molecules. Douglas optimized the particles for Lyme by testing the affinity of different dyes for *B. burgdorferi* proteins spiked into synthetic and then real human urine.

It's so rewarding to see that my research materialized into something useful.

— Temple Douglas, Virginia Tech

The proof of concept of her system came from a source close to home: Douglas's grandparents sent her a urine sample from their Lyme-infected dog Jack, and after treating the urine with her concentrating nanoparticles, she was "super excited" to detect the bacterial proteins she was looking for. That eureka moment is exactly what Liotta hopes students will get out of ASSIP: if making an original discovery "doesn't hook them on a career in science, I don't know what will," he says. It certainly worked for Douglas, who went on to graduate from Princeton University, writing her senior thesis on the theoretical physics of cancer.

Meanwhile, Luchini's group partnered with George Mason spinout company Ceres Nanosciences to further develop the Lyme diagnostic for the clinic. Ceres had begun commercializing the hydrogel nanoparticles, which it named Nanotrap, in 2009, and had already collaborated with the Luchini team on an anti-doping urine test for human growth hormone. "We recognized that [Nanotrap] had a really powerful set of capabilities and appeal across a lot of different diagnostic needs," especially for noninvasive tests based on urine or saliva, says Ceres CEO Ross Dunlap.

The Lyme test involves adding Nanotrap particles to urine, where they concentrate *B. burgdorferi* outer surface protein A (OspA), and then eluting OspA from the particles and assessing its presence with standard immunoassays (*J Transl Med*, 13:346, 2015). The protein, which infected animals shed in their urine, is both important for early infection and highly conserved across *Borrelia* species, according to Luchini, and can be detected in human patients' urine after concentration with Ceres's nanoparticles.

Yet Gary Wormser, a Lyme disease researcher at New York Medical College, is skeptical about OspA, which he says is expressed in the tick but often not in the human host. Still, he is interested in collaborating with Luchini's team, especially if the sample volume required for the test is reduced—he is reluctant to part with urine samples from his own Lyme research collection to provide the 40 mL of urine currently needed per patient for the Nanotrap diagnostic. He agrees, however, that standard serological tests are deficient. Anti-*Borrelia* antibodies appear weeks after the onset of symptoms, but once made, the antibodies can linger in the body for decades, complicating the diagnosis of a new infection. "What we need is a test for active Lyme disease," he says.

Many doctors and researchers seem to agree, given that the clinical trial of Ceres's reference test, which is open to any U.S. physician, has already attracted interest from "almost every state," Dunlap says. The company is also developing a point-of-care test that it hopes the FDA will approve in two to three years. Meanwhile, Ceres and Luchini are both pursuing other diagnostic opportunities using the concentrating nanoparticles, for diseases such as malaria, congenital and HIV-coinfected Chagas disease, and traumatic brain injury.

Luchini credits Douglas, "an extremely brilliant young lady," with providing the lab with a refreshing new perspective. "[The Lyme diagnostic] is a beautiful example of . . . bringing an idea dear to the student all the way to patient benefit," she says. For her part, Douglas is pleased to have made a difference, even though she doesn't have a financial stake in the products resulting from her work: "It's so rewarding to see that my research materialized into something useful," she says.

Now a biomedical engineering graduate student at Virginia Tech, Douglas has returned to cancer research—exploring methods to separate metastatic cells from less-invasive populations based on their

varying levels of cell polarizability. "It's nice to apply what I know to a [biological] system that, from a physical point of view, is not very well characterized, and try to see what I can do," she says.

—Jenny Rood

Mind Mending

As a psychiatrist at Western University in London, Ontario, Lena Palaniyappan regularly sees patients with schizophrenia, the chronic mental disorder that drastically affects how a person thinks, feels, and behaves. The disorder can be devastating, often involving hallucinations and delusions. But one thing Palaniyappan and other mental health professionals have noticed is that, unlike those with degenerative neurological disorders such as Alzheimer's disease, Huntington's, or Parkinson's, sometimes schizophrenia patients eventually start to improve.

"In the clinic we do actually see patients with schizophrenia having a very relentless progress in early years," Palaniyappan says. "But a lot of them do get better over the years, or they don't progress as [quickly]." So far, most research has focused on the neurological decline associated with schizophrenia—typically involving a loss of brain tissue. Palaniyappan and his colleagues wondered whether there might be "something happening in the brain [that] helps them come to a state of stability."

To get at this question, he and his colleagues performed MRI scans to assess the cortical thickness of 98 schizophrenia patients at various stages of illness. Sure enough, the researchers noted that, while patients who were less than two years removed from their diagnosis had significantly thinner tissue than healthy controls, those patients who'd had the disease for longer tended to show less deviation in some brain regions, suggesting some sort of cortical amelioration (*Psychol Med*, doi:10.1017/S0033291716000994, 2016). "Some brain regions are regaining or normalizing while other brain regions continue to show deficits," Palaniyappan says.

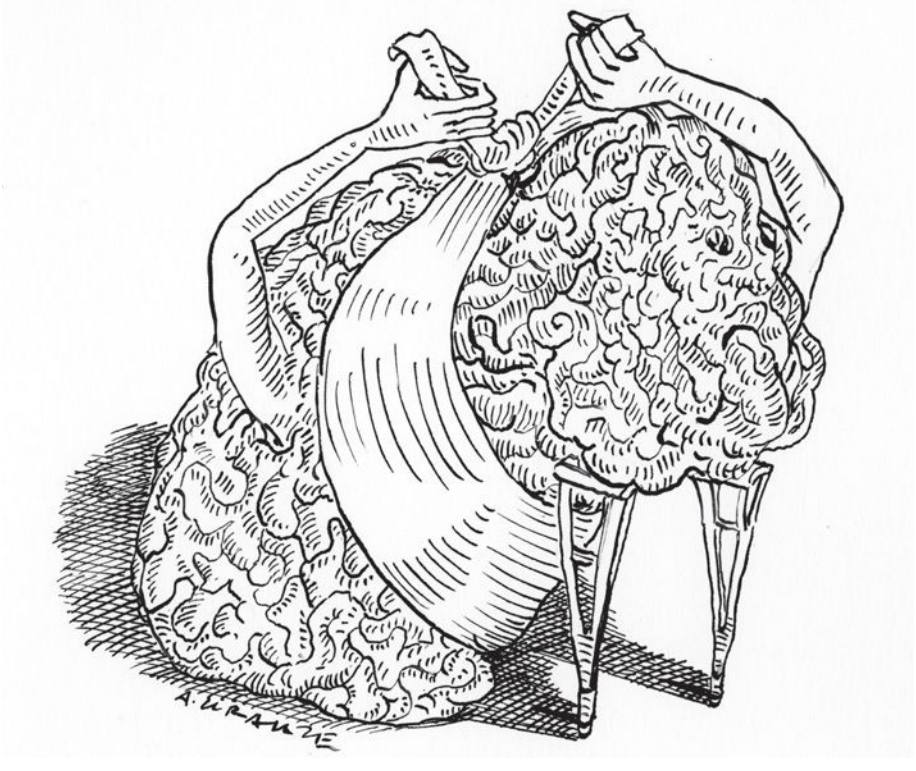
"We know very well now that the brain is plastic and changes over time, but we didn't know if this could happen as a repairing mechanism or a compensatory mechanism in schizophrenia," says Antonio Vita, a professor of psychiatry at the University of Brescia in Italy. "This is a strong suggestion that this could happen."

Palaniyappan cautioned, however, that he and his colleagues were simply looking at a snapshot of schizophrenia patients and correlating cortical thickness with duration of illness. To better understand how the brain changes over time in this disease, it will be necessary to follow individual patients, taking multiple brain scans over the course of their illness, he says. Moreover, he adds, "the improvement in brain tissue in these patients does not directly relate to getting better in terms of symptoms. . . . If it is compensation, it's not fully efficient."

"It's very preliminary," agrees Georgia State University's Jessica Turner, who was not involved in the research. "These guys were very careful in what they did, but until you do it again and again and again, it's a little bit tricky." And while 100 subjects used to be considered "a huge data set," she says, with effect sizes as small as they are in schizophrenia, researchers need to look at thousands to "really get a sense what's going on."

Last year, Turner and her colleagues in the ENIGMA (Enhancing Neuro Imaging Genetics through Meta Analysis) group organized a study across 15 centers worldwide. Participating researchers used standardized methods to assess the subcortical brain structures of a total of 2,028 schizophrenia patients and 2,540 healthy controls (*Mol Psychiatry*, 21:547-53, 2016). And earlier this year at the annual meeting of the Society of Biological Psychiatry, Turner's team presented results they're now writing up on the cortical thickness and surface area of schizophrenia patients, using data from more than 30 centers in Australia, Korea, Japan, South Africa, Europe, and the U.S. "The goal is replicability," says Turner. "Can we find consistent results that are not dependent on a particular data set or a particular subset of subjects?"

And for the most part, the answer is yes. Although the effect sizes from individual study sites are small—so small that



ANDRZEJ KRAUZE

most groups working independently probably would not have published their results, Turner says—collectively, the data tell a consistent story. "Almost all of them agree that almost all the brain regions are thinner in the cases than in the controls," she says. But when the ENIGMA group looked for evidence that schizophrenia patients regain brain mass as their disease goes on, they didn't find it, though the data do suggest that some areas of the brain begin to thin at a slower rate.

Palaniyappan is anxious to understand if the reparatory effect he and his colleagues observed is replicable, and if so, to study what factors might be contributing. All of Palaniyappan's study subjects were on antipsychotics at the time of their MRI scans, he notes, so whether the same changes would have been seen without treatment remains unclear. "But we never used to think that the schizophrenic brain can recover," he says. "[Our study] is an indication the brain is making some attempts, with help or without help."

—Jef Akst

Eye Contact

Just the briefest eye contact can heighten empathetic feelings, giving people a sense of being drawn together. But patients who suffer from autism, even in its most high-functioning forms, often have trouble establishing this sort of a social connection with other people. Researchers are delving into what's going on behind the eyes when these magical moments occur, and the hormones and neural substrates involved may offer hope of helping people with autism.

University of Cambridge neuroscientist Bonnie Auyeung and colleagues gave oxytocin—a compound commonly referred to as the "love hormone," as it's been found to play roles in maternal and romantic bonding—to both normal men and those with a high-functioning form of autism also called Asperger's syndrome. The scientists then tracked the eye movements of the study subjects and found that, compared with controls, those who received oxytocin via nasal spray showed increases in the number of fixations—pauses of about 300 milliseconds—on the eye region of an interviewer's face and in the fraction of time spent looking at this region during a brief interview (*Translational Psychiatry*, doi:10.1038/tp.2014.146, 2015).

Oxytocin, a neuropeptide hormone secreted by the pituitary gland, has long been known to activate receptors in the uterus and mammary glands, facilitating labor and milk letdown. But research on the neural effects of oxytocin has been accelerated by the availability of a nasal spray formulation of the hormone, which can deliver it more directly to the brain, also rich with oxytocin receptors. Auyeung adds that her study used a unique experimental setup. "Other studies have shown that [oxytocin] increases looking at the eye region when presented with a picture of a face," Auyeung says. "The new part is that we are using a live interaction."

James Rilling, a social cognitive neuroscientist who directs the Laboratory for Darwinian Neuroscience at Emory University and was not involved in this research, says that the results are promising. "The ability of oxytocin to get the men to look at the eye region of the face more is probably very important because we receive so many social cues from the eye region," he says. "If you're not attending to those social cues, you miss a lot, and then in turn you miss the opportunity to learn a lot about appropriate social behavior." If oxytocin could normalize this ability for those with autism, Rilling adds, it would "give more opportunity to work on social skill building."

Takahiro Koike and colleagues at Japan's National Institute for Physiological Sciences have used functional magnetic resonance imaging (fMRI) to suggest one clue to the neural mechanism stimulated by eye contact. In people gazing into each other's eyes, the researchers found that both subjects showed enhanced neural synchronization with one another in a region on the side of the right frontal lobe, the right inferior frontal gyrus, which is associated with social communication and empathy (*NeuroImage*, 125:401-12, 2016).

In 2013, Elisabeth von dem Hagen, then of the Medical Research Council in Cam-

LOCKING EYES: The path an observer's eyes take as they scan an image of a human face is superimposed over that image. Dots indicate pauses or fixations in eye movement, and lines show the rapid jumps or saccadic eye movements between those points.

bridge, U.K., and colleagues showed pictures with either averted or direct gazes to males with autism spectrum conditions and found that, compared with controls, these subjects responded to direct gaze with reduced activity in the brain's medial frontal cortex, temporoparietal junction, posterior superior temporal sulcus region, and amygdala—regions that have been described as part of a "social brain" system, key to inferring others' states of mind (*Cerebral Cortex*, 24:1485-92).

Recent animal experiments shed further light on the links between eye contact and behavior. Sébastien Ballesta and Jean-René Duhamel of France's Centre National de la Recherche Scientifique (CNRS) found that when they offered macaques the option to either reward or punish partners, they saw differences in how the animals gazed at their fellow monkey. When one of the monkeys decided to provide a juice treat to its partner, it tended to engage in more eye contact. But if the animal chose to deliver an unpleasant puff of air into the face of its partner, it usually turned away or blinked (*PNAS*, 112:15516-21, 2015).

A behavior that seems so simple and natural to many of us can be a troubling psychosocial event to individuals suffering from autism. But as researchers dig into the neural, hormonal, and behavioral drivers at play behind eye contact, there may be hope that some of the neurological and behavioral symptoms of autism spectrum conditions may be alleviated (See "Parsing the Spectrum," page 38).

—Robert Lavine

Following Your Nose

Narrow, flat, hooked, button, straight, or none of the above, the human nose comes in myriad shapes and sizes. But no matter how noses look, they all share at least one common function: to warm and humidify air on its way to the lungs. Like a wind tunnel, the nasal passages cause turbulence in inspired air, allowing it to touch the inner walls of the nose and draw moisture and heat from our mucosa and blood vessels.

The shape of the human nose has been sculpted in part by climate. "A lot of it depends on the environment that your ancestors grew up in," says Lauren Butaric, a biological anthropologist at Des Moines University. "What you see in the cartilaginous structure, which matches up with the internal structure, is that individuals from cold, dry environments tend to have tall and narrow nasal cavities." In the wide noses often seen in individuals with tropical ancestry, Butaric adds, the air flow is much smoother, traveling straight back with less warming and humidification.

Butaric and her colleagues recently determined that in Alaskan Inuit and Siberian Buryat populations, a longer, narrower nasal cavity is associated with large maxillary sinuses, and in sub-Saharan African populations, the wider nasal cavity is accompanied by smaller maxillary sinuses (*Am J Phys Anthropol*, 160:483-97, 2016). The sinuses function as a sort of "buffer" to accommodate changes in the nasal cavity and other structures of the face, Butaric's team concluded, both during an individual's development and over evolutionary time.

The relationship between sinus and nasal cavity shape has historically created a paradox for anthropologists when it comes to archaic human species. "The sexy topic is Neanderthals," says Butaric. Many scientists have assumed these ancient populations were more adapted to cold weather than *Homo sapiens* are, even those *H. sapiens* at high latitudes, because the Neanderthals were associated with colder and drier conditions during Earth's glacial periods. Accordingly, a typical Neanderthal's sinuses were thought to be larger than a modern-day human's would be if the human was otherwise a similar size. However, instead of a long, narrow external nose structure, the Neanderthal face most likely had a relatively wide nose, judging from bone morphology.

"[Neanderthals] look like they should be adapted to being in warm and wet environments, not cold, dry ones," says Todd Rae, an anthropologist at the University of Roehampton in the U.K. Delving into the internal facial structures, Rae and colleagues used CT scans to compare Neanderthal skulls to *H. sapiens* fossils from Lithuanian archaeological sites, testing the assumption that the Neanderthal sinuses

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Since the late 1800s, the field of immuno-oncology has been exploring the relationship between the immune system and the development, progression, and treatment of cancer. With over a century's worth of discoveries as the guide, the past decade has seen an explosion in the translation of this research into new treatment modalities, often with unprecedented success. Advancements in genomic technologies, like next-generation sequencing (NGS), are helping advance our understanding of interactions between the immune system and cancer, guiding therapeutic development.

IMMUNO-ONCOLOGY: ADVANCING BIOLOGICAL THERAPY

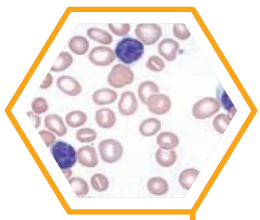
1900

1890
Demonstration of transferred immunity[1]

1893
"Coley's Toxins" used to treat inoperable tumor

1909
Immune surveillance hypothesis proposed by Ehrlich[2]

1934
National Cancer Institute founded by an act of Congress



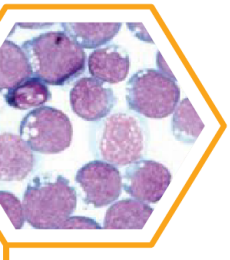
1942
Freund's adjuvant used to bolster immune response

1951
First evidence of viral transmission of cancer[3]

1953
The nonprofit Cancer Research Institute established

1957
Interferon discovered

1959
Bacillus Calmette-Guéri (BCG) treats bladder car



1964
Epstein-Barr Virus (EBV) linked to cancer in humans

1970
Leukapheresis, a method for isolating leukocytes, is invented

1982
First successful treatment of lymphoma with mAb[4]

1983
SCID mice lacking immune systems used as research tools

1993
Mouse strain engineered to generate fully humanized mAbs

2001
Immune surveillance hypothesis reborn

2002
Adoptive T-cell therapy shown to cause tumor regression[5]

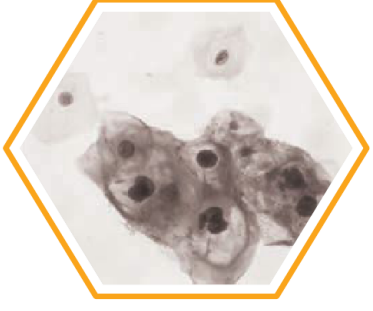
2011
Approval of ipilimumab

2012
First pediatric use of T-cell therapy

2014
Mutational burden shown to correlate with CTLA-4 response[6]

1968
Nude mouse became a major lab research tool

1975
Monoclonal antibodies (mAbs) generated in vitro with hybridomas



2006
Preventive cervical cancer vaccine released

2016
Approval of atezolizumab

cer

1. Behring E, Kitasato S. Ueber das Zustandekommen der Diptherie-Immunität und der Tetanus-Immunität bei Thieren. *Deutsche Medizineische Wochenschrift.*1890. 2. Ehrlich P. Über den jetzigen stand der karzinomforschung. *Ned Tijdschr Geneeskd.*1909. 3. Gross L. "Spontaneous" leukemia developing in C3H mice following inoculation in infancy, with AK-leukemic extracts, or AK-embryos. *Proc Soc Exp Biol Med.* 1951; 76:27-32. 4. Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med.*1982; 306:517-522. 5. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science.*2002; 298:850-854. 6. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med.*2014; 371:2189-99.

BIOLOGICAL THERAPIES

Monoclonal Antibodies (mAbs): mAbs are engineered antibodies with specificity for a single tumor-related epitope. They can be used to target the delivery of chemotherapeutic agents, disrupt growth signaling, and promote the killing and clearance of tumor cells.

Immune Stimulation: Immune stimulation generates a nonspecific increase in the overall functioning of the immune system, including its native anticancer activities. Agents used for immune stimulation include cytokines, toll-like receptor agonists, and attenuated or modified bacteria, like Bacillus Calmette-Guérin (BCG).

Vaccine Immunotherapy: Vaccines train the immune system to quickly address known threats, and cancer vaccines are no different. Vaccines already exist for HPV-induced cervical cancer and HBV-induced liver cancer, and new vaccines are being tested that target tumor cell components and pre-activated T cells.

Oncolytic Viruses: Oncolytic viruses find and infect tumor cells, effectively hijacking their replication machinery and causing the cell to explode. Cell lysis also generates a local immune response against cell components. As an added bonus, some oncolytic viruses can also encode for an immune booster, like GM-CSF.

Immunomodulatory Agents: Some cancer cells express cell-surface proteins that can shut down an immune cell's response, improving the odds that a cancer cell will be able to grow into a tumor. By short circuiting the checkpoint, the immune response continues, killing the aberrant cell.

Adoptive T-cell Transfer: In some patients, tumor-infiltrating lymphocytes (TIL) are isolated from their tumor and expanded. In others, chimeric antigen receptor T cells (CAR-T) are leukapheresed and engineered to express new surface antigens. In both cases, the T-cells are returned to the patient to begin attacking their tumors.

2020

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Biomarker Identification: NGS is an essential tool in the discovery of new, reliable biomarkers to track the patient's response to therapies, monitoring cancer progression, response, or remission.

Tumor Immunogenicity Determination: Neoantigen prediction, or the classification of relevant tumor-specific mutations, relies heavily on NGS and bioinformatics to learn and predict what signatures may spark an immune response.

Tumor Response: A tumor's response to a therapy is multidimensional, and multiplexed NGS enables the creation of a complex web of interconnections between facets of a tumor's response to treatment, including mutational load, epigenetics, microenvironment, and the microbiome.

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Advances in genomic sequencing and data analysis promise to revolutionize health care through precision medicine. Identifying individual variations in genes and correlating them to known outcomes and phenotypes allows for disease susceptibility, prediction of response to medications, preventive care, and therapeutic treatment to be more precisely tailored to the individual than ever before. Thanks to technological advances and efficiencies of scale, scientists and clinicians have an increasingly wide array of tools for uncovering genetic variants in patients.

Projects such as the U.S. federal government's Precision Medicine Initiative and the 100,000 Genomes Project (UK), the latter powered by Illumina sequencing technology, are building the evidence base to guide the clinical use of genomic data.

Oncology is at the forefront of precision medicine; already, a number of therapies are assigned based on the molecular and genomic qualities of the cancer. We anticipate a day where precisely tailored therapies based on an individual's unique genetic information will be standard-of-care. We need to ensure that the proper educational efforts in genomic medicine are in place today, in anticipation of the reimbursement and guideline setting activities required in the near future.

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*Data calculations on file. Illumina, Inc., 2015.

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were relatively larger. Their data showed that the Neanderthal sinuses were not, in fact, any larger than a human's would be if the entire skull was scaled to the same size.

"That suggested to us that [Neanderthals were] not especially cold-adapted," says Rae. Expanding this reasoning to the external nose, Rae hypothesized that the wide-nosed Neanderthal face wasn't an adaptation to the cold at all, but was driven by some other underlying factor (*J Hum Evol*, 60:234-39, 2011).

Butaric suggests that the Neanderthals, with larger bodies and more muscle mass, might have needed larger noses to inhale appropriate amounts of oxygen, regardless of where they lived. In modern humans, males generally have larger noses and nasal passages than females do, which may be due to a higher oxygen demand (*Am J Phys Anthropol*, 160:52-61, 2016). Another possible explanation, Rae proposes, is that the Neanderthals may have simply avoided the extremely cold areas during times that would have provided enough selection pressure to mold their noses into a narrow shape.

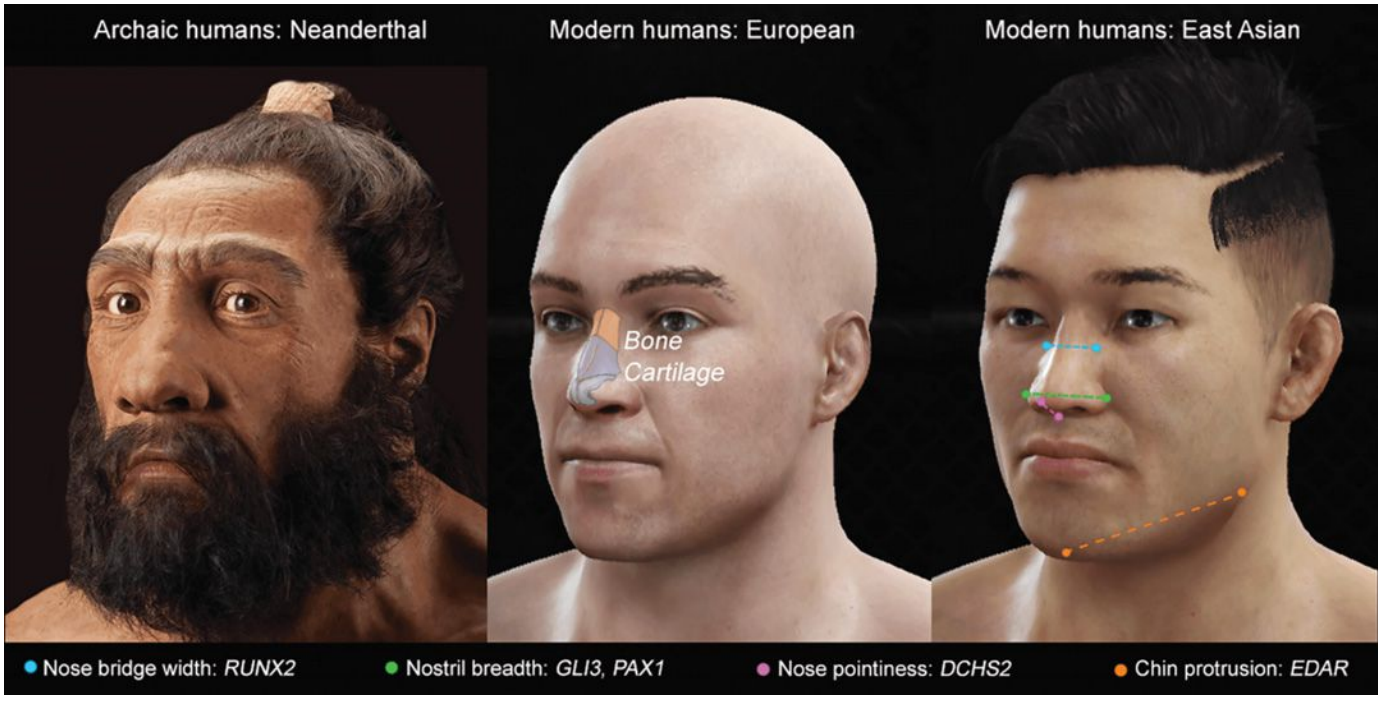
In human evolution, weather isn't everything, either. We've mostly shaped the world around us to avoid the selection pressure of extremely cold environments. Work on cranial morphology suggests that much of the skull variation in today's human populations is explainable more by distance from Africa than by adaptation to the local environment (*Am J Phys Anthropol*, 141:76-82, 2010). "We think it might just be drift—that simply the further you get, the more a population will start to develop differences from copying errors in DNA," says Rae. "You're going to get the narrow nose in places where it's really cold, but [also] where it's relatively temperate; the differences are literally random."

Although these nasal differences in temperate zones may not be driven by environmental variables, geographically related traits are still evident. "There is wide variation across continents, and that sort of tells you that there are underlying genetic reasons," says Kaustubh Adhikari, a population geneticist at University College London. Adhikari and his colleagues recently published a paper exploring the genetic variations associated with differences in external nose shape (*Nat Commun*, 7:11616, 2016).

Earlier studies had uncovered a few genes that play a role in sculpting our noses, but much of the work was done in homogeneous European or North American populations with small morphological differences. Adhikari's team, however, collected genetic samples and facial photographs from a cohort of more than 6,000 Latin Americans across five countries. "Latin America is a genetic melting pot," explains Adhikari. "You have the Native Americans, who are close to East Asians; you have Europeans, and you have Africans—and you have all of these just on one continent. And the admixture is very recent."

Adhikari and his colleagues detected five genes that controlled some aspect of nose structure. All five genes affect bone or cartilage differentiation and craniofacial development, and three have previously been identified as differing between modern humans and extinct species such as Neanderthals and Denisovans—both of which had slightly different nose shapes than *H. sapiens*. "It's not the complete story," says Adhikari, "but it's a little piece of it."

—Karen Zusi



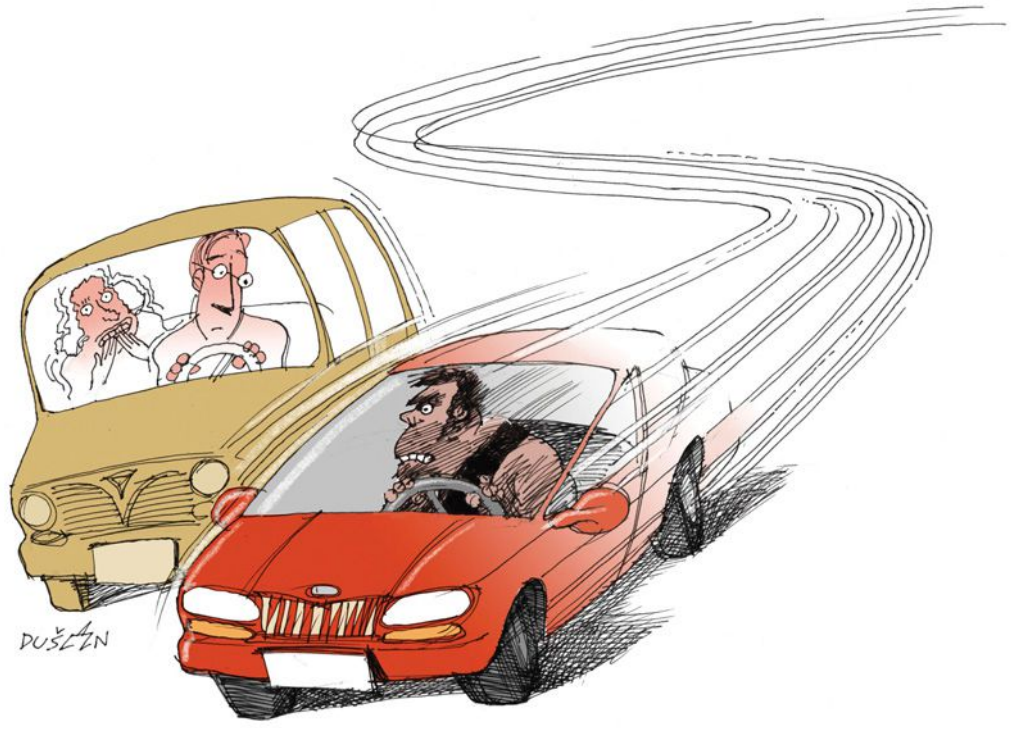
THE NOSE KNOWS: Nose shapes in human ancestors and different populations of modern humans are molded by genes and the environment.

COURTESY OF KAUSTUBH ADHIKARI

Our Inner Caveman

The modern human brain evolved in social and environmental settings very unlike today's. Despite our cultural and technological progress, tribal instincts remain.

BY JOÃO PEDRO DE MAGALHÃES



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We live in a much different world from that in which we evolved. Until very recently, survival for all humans was difficult. Harsh conditions fostered cooperation within small groups, often made up mostly of one's relatives, thus favoring strong social bonds. Over the last few centuries, and especially in the last few decades, however, the invention of rapid communication systems that span vast distances and a flood of affordable commercial conveniences allow us to interact with a huge number of people over our lifetimes, but those interactions are typically much more cursory. Even though our rational, educated minds can adapt to different environments, our basic hardwired instincts are slower to evolve. Biologically, the human brain that went to the Moon is the same that hunted mammoths in the last Ice Age. And tribal instincts, emotions, and attitudes—crucial for our ancestors' survival and reproduction since they first roamed the African savannah—still influence many of our decisions and actions today, often in detrimental ways.

For ancient humans, being accepted into the tribe was essential for survival; interactions with strangers were rare. Every social interaction could therefore have important consequences for one's role in the tribe, and this tribal structure instilled a rigid social hierarchy. An innate respect for such hierarchy, which can manifest as obedience to authority figures, was demonstrated by the classic experiments of Stanley Milgram. When participants were instructed by a scientist to administer electric shocks to another person (an accomplice of the scientist), 65 percent fully complied, even though they had been led to believe the shocks were up to 450 volts and extremely painful (*J Abnorm Soc Psychol,* 67:371-78, 1963). Scientists have also shown that we tend to vote for political candidates who are taller and more attractive, which could be a reflection of our instinct to look up to physically healthy and strong males (*Soc Sci Quart,* 92:1215-35, 2011; *Psychol Sci,* 24:2429-36, 2013). Even children have been shown to pick election winners 64 percent of the time when shown pictures of candidates (*Science,* 323:1183, 2009). Such ideals are not always appropriate in the modern world, where physical performance no longer dictates life success.

Tribal hierarchies may have also contributed to feelings of extreme anger over seemingly trivial disputes. In a tribe, individuals—males in particular—needed to be assertive to gain access to food and to women. Today, when dealing with complete strangers whom we perceive as imposing, our aggressive instincts can take over, and someone gets run off the road or shot.

The tribal setting also instilled many divisive behaviors, such as patriotism and xenophobia, based on imagined differences between groups of people. Experiments have shown that complete strangers arbitrarily split into groups will develop discrimination behaviors (*Sci Am,* 223:96-102, 1970). Scientists have argued that prejudice is a hardwired instinct for protecting the tribe from outsiders who could harm the group by spreading disease, stealing, competing for resources, or by violent behavior (*J Pers Soc Psychol,* 88:770-89, 2005). Given that immigration fears recently propelled UK citizens to vote to leave the European Union and that racial differences will likely play an important role in the next US presidential election, these "caveman" instincts may still feature prominently in our modern society.

For those lower on the social ladder, fitting in with the ancestral group may have been the safest strategy. Fear of embarrassment, disapproval, and rejection is another human tendency that is likely rooted in this tribal need for belonging. In one classic social psychology experiment by Solomon Asch, 35 percent of college students gave a clearly incorrect answer when judging the length of straight lines, merely because four other students (Asch's accomplices) had given the same answer (in *Groups, Leadership and Men,* pp. 177–90, Carnegie Press, 1951). This instinct to seek social acceptance may explain why most people are uncomfortable speaking in public. Fear of public embarrassment and disapproval, while once beneficial in a small group where acceptance was essential to one's survival, can surface today even when dealing with complete strangers we will never meet again. The deep-rooted drive to fit in, a legacy of our tribal past, is already exploited by modern commercial markets. In our information-rich, decision-overloaded environment, companies take advantage of our instinctive behaviors by using celebrity endorsements or claims of popularity to promote products.

Biologically, the human brain that went to the Moon is the same that hunted mammoths in the last Ice Age.

Perhaps the most obvious trace of humans' primordial past is our persistent shortsightedness. We respond quickly to clear and present dangers, but not so rapidly to unclear and future ones. Far more people die of type 2 diabetes than from terrorism; far more people die of skin cancer than from shark bites. But terrorism and shark attacks could kill you tomorrow, so they garner much more of our attention. Short-termism is also why convincing people to act on issues like global warming—which will most significantly affect those in the future, with poorly defined consequences—is so difficult.

Our tribal-era instincts are still very much a part of who we are. Studying the social and physical environments that shaped human evolution, then, could help us better understand the modern human psyche. Acknowledging our own tribal instincts can also help us overcome these obsolete behaviors in our daily lives. ■

João Pedro de Magalhães is a biologist in the Integrative Genomics of Ageing Group, part of the Institute of Ageing and Chronic Disease at the University of Liverpool, U.K.

An Evolutionary Union

Why does cooperation evolve most often in monogamous animals?

BY JACQUELINE R. DILLARD AND DAVID F. WESTNEAT

From packs of wolves cooperating in prey capture to millions of worker ants toiling their entire lives for the benefit of their sisters and queen, cooperative behaviors pervade the animal kingdom. But such selflessness poses a major evolutionary quandary: How could natural selection favor traits that increase the reproduction of others at an apparent cost to the individuals that express them?

William D. Hamilton provided an initial solution to this problem in 1964 with his theory of kin selection. Hamilton predicted that costly altruistic behaviors could be favored if the individuals receiving help were close relatives of the helper. This is because, from the gene's-eye view, close relatives have a greater likelihood of also possessing an allele for altruism than unrelated individuals. In fact, the probability that any two individuals share a particular allele is equivalent to their relatedness to each other. By this logic, helpers should be twice as willing to help a full sibling as a cousin, because the sibling is twice as likely to carry the altruist allele.

Indeed, helpers in most cooperative groups are offspring of the breeding pair that stay and help care for siblings rather than leave to breed on their own. Recently, scientists have suggested that genetic monogamy (exclusive reproduction within a pair) plays an integral role in the evolution of helping by ensuring that relatedness between siblings is maximized. When mating is monogamous, helpers are full siblings of newborns, meaning they are as related to these siblings as they would be to their own offspring. Thus, from the perspective of the gene, a helper's full siblings are as valuable to the spread of the helping allele as the helper's own offspring; both have a 50 percent chance of carrying the allele. In support of this hypothesis, recent studies have shown genetic monogamy as the ancestral state in evolutionary lineages exhibiting cooperation in a variety of animal taxa, including birds, mammals, and some social insects.

New perspectives

The evolutionary correlation between monogamy and cooperation, however, is also consistent with several alternative, yet previously unexplored, hypotheses. Monogamy and cooperation are similar in many ways. Natural selection favors monogamy, for example, when there are few alternative mating opportunities or when there are substantial benefits to providing biparental care. Similarly, young adult offspring are selected to remain with their families as helpers when independent breeding is difficult or when the benefits of caring for siblings are great. The ecological and demographic factors that reduce mating opportunities for parents, such as limited breeding territories, are thus likely to do the same for helpers. Similarly, factors that increase the value of offspring care, such as high risk of juvenile predation or starvation, could select for cooperation in defense or provisioning by both parents and helpers. Because conditions favoring monogamy may be less extreme than conditions favoring cooperation, monogamy may emerge first. In this case, how-

Monogamy and cooperation could interact synergistically, with ancestral social monogamy shaping the social environment in ways that could select for further cooperation.

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ever, the relationship between monogamy and cooperation is consequential, rather than causal.

Social monogamy, in which breeding pairs cooperate but are not necessarily genetically monogamous, is also strikingly similar in form and function to cooperation among other individuals, and adaptations for cooperation between monogamous parents might predispose lineages to more advanced forms of cooperation among other individuals. Adaptations that characterize social monogamy, such as social tolerance, pair bonding, and even increased cognitive capacity in the case of social birds and mammals, are also important in creating and maintaining social interactions that extend beyond the pair. Socially monogamous lineages that express fine-tuned social intelligence, cooperation between social partners, and biparental care of offspring might thus be primed to transition to more-complex social organization under the right circumstances.

Finally, monogamy and cooperation could interact synergistically, with ancestral social monogamy shaping the social environment in ways that could select for further cooperation. For example, if breeding space is limited, the likelihood of young adult offspring successfully competing for breeding opportunities should be much lower in populations where socially monogamous pairs jointly defend territories than in those where singletons occupy and defend breeding space. Here, pair coordination exacerbates preexisting limitations to breeding, further encouraging offspring to stay and help rather than disperse and attempt to breed. Conversely, offspring that stay and help gain higher kin-selected benefits if their parents stay monogamous, and so they may create conditions that reduce either the benefits of or the opportunities for promiscuity.

Synthesis

These additional explanations for the coevolution of monogamy and cooperation are not mutually exclusive with the action of kin selection. Consider the situation where some ecological condition, such as an abundance of predators, selects for both biparental and cooperative care. In this scenario, the risk of predation not only selects for offspring help by increasing the benefits of cooperative defense, but also promotes monogamy by selecting for cooperation and social bonding between parents, leading to increased relatedness between siblings. Previously, researchers considered the increased fitness benefits to relatives and the degree of relatedness between these individuals to be separate, modular components in the determination of offspring help. Now, however, it is clear that these components are likely to be inextricably linked in nature.

This newfound appreciation for the complex coevolutionary dynamics between mating systems and social systems is in line with the emerging field of systems biology. This relatively new area of research is dedicated to shedding light on a variety of complex biological processes, from biochemical interactions that occur within and between cells to ecological interactions that affect demographics and ecosystems. The essence of the approach is that many aspects of the natural world are more than the sum of their parts, whether because some parts interact in unexpected ways or because the phenomenon cuts across multiple temporal or spatial scales. Thus, social creatures such as wolves and ants might engage in their ostensibly selfless behaviors because of a series of ecological influences on both mating decisions and cooperation, as well as feedback between the two that modulates the selection acting on each. Teasing apart exactly how these social patterns arose will take an open mind and keen observation for complex and unexpected evolutionary dynamics. ■

Jacqueline R. Dillard is a PhD student at the University of Kentucky, where she investigates the coordinated evolution of mating and social systems in insects and birds. She works in the lab of David F. Westneat, who studies the behavioral ecology of mating and social behavior, primarily in birds.

THE DIFFERENCE OF ONE SIMPLE SORT

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Search for Switches

An unbiased screen identifies bacterial riboswitches—built-in self-regulators of mRNA transcription.

BY RUTH WILLIAMS

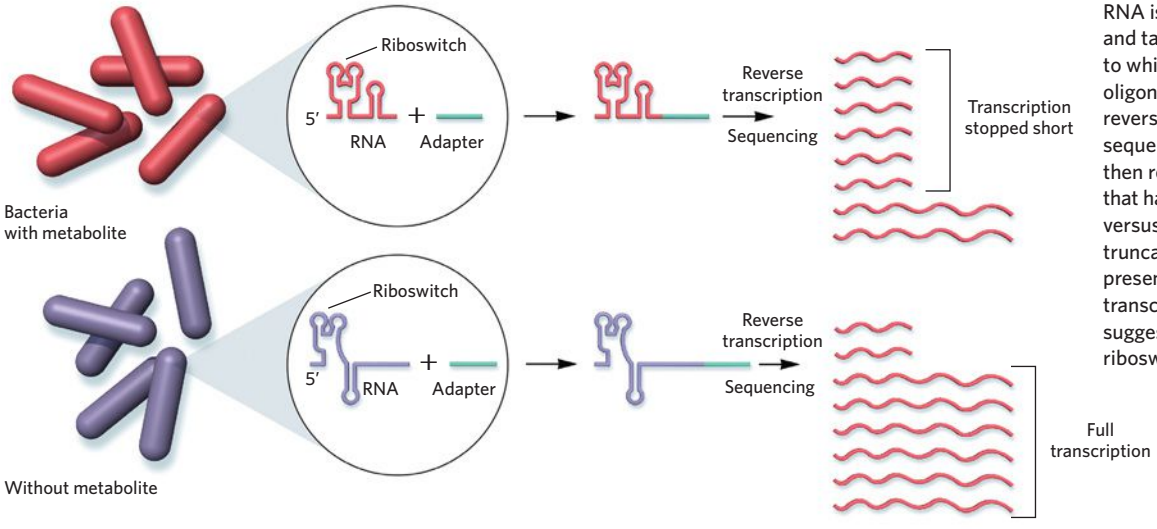
Nature has evolved a staggering array of mechanisms for regulating gene expression, but few are so simple and elegant as the riboswitch. These RNA elements sit within the 5′ noncoding regions of bacterial messenger RNAs (mRNA) and regulate an mRNA's own transcription or translation, depending on the switch's conformation. In the case of a transcription-regulating riboswitch, for example, association of the switch with a particular ligand, such as a metabolite, can alter the switch's structure and in turn terminate transcription.

Since the first riboswitches were identified in the early 2000s, "most of the known riboswitches have been discovered pretty much one by one by sequence comparisons," says RNA expert Thomas Hermann of the University of California, San Diego. But while that approach works well for conserved riboswitches, it fails to identify those that are species-specific, Hermann says.

Term-seq, a new technique developed by molecular geneticist Rotem Sorek of the Weizmann Institute of Science in Rehovot, Israel, finds novel candidate riboswitches without the need for sequence comparisons. Adapter sequences are first ligated to the 3′ ends of bacterial RNAs and then used to initiate genome-wide deep sequencing. If a transcription-regulating riboswitch is present in a given RNA, then sequencing will reveal telltale, prematurely terminated versions of the transcript.

Using term-seq, Sorek and his team successfully identified 49 out of 53 (92 percent) of the known riboswitches present in *Bacillus subtilis* and identified a further 18 new candidate regulators. By treating *B. subtilis* and other bacteria with antibiotics and then performing term-seq, Sorek's team also found a number of antibiotic-resistance genes under riboswitch control.

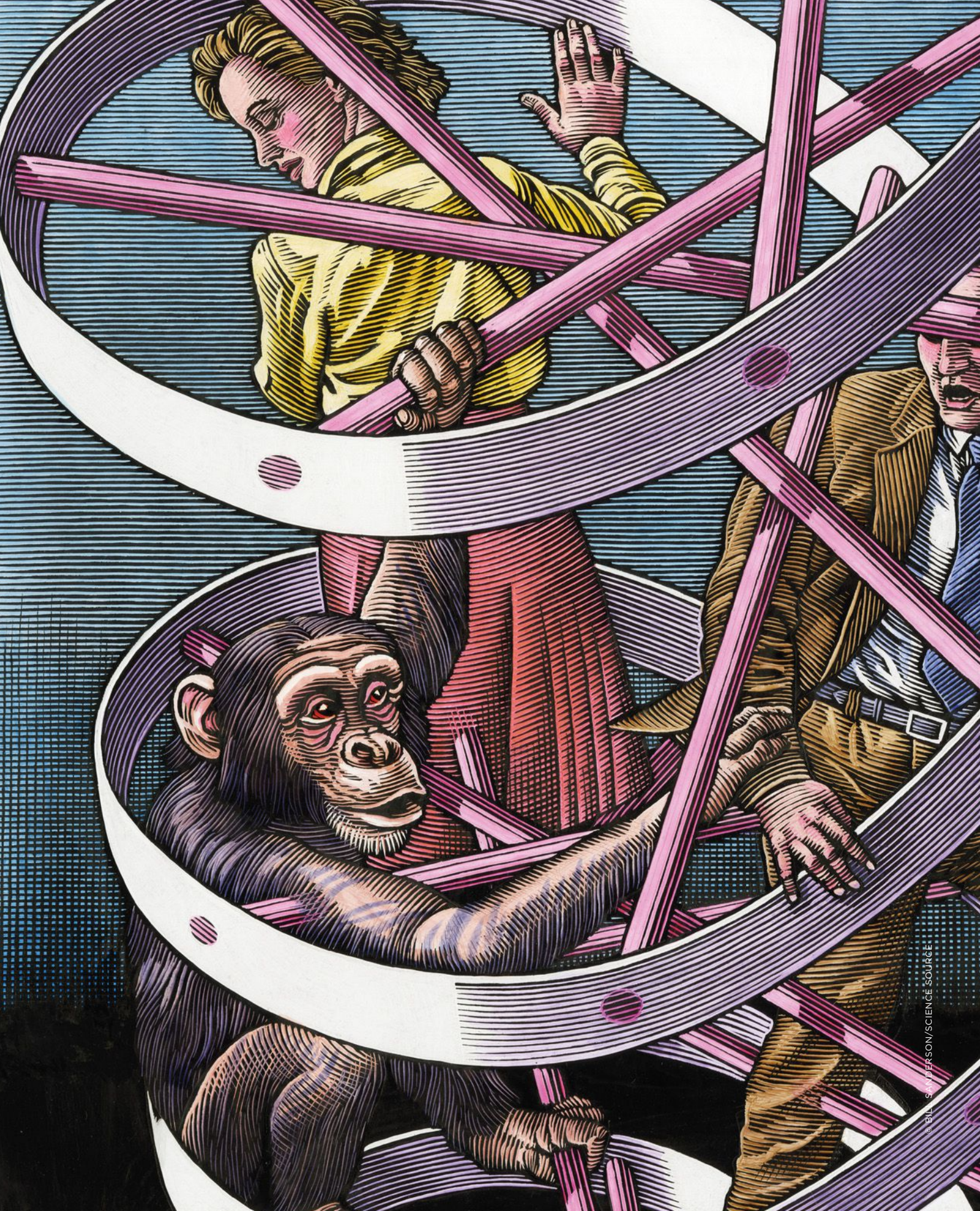
The method is limited to identifying riboswitches that regulate transcription rather than translation, says Hermann, but "since we're going from no method to a method that works in [most] cases, it's already pretty good." (*Science*, 352:aad9822, 2016) ■

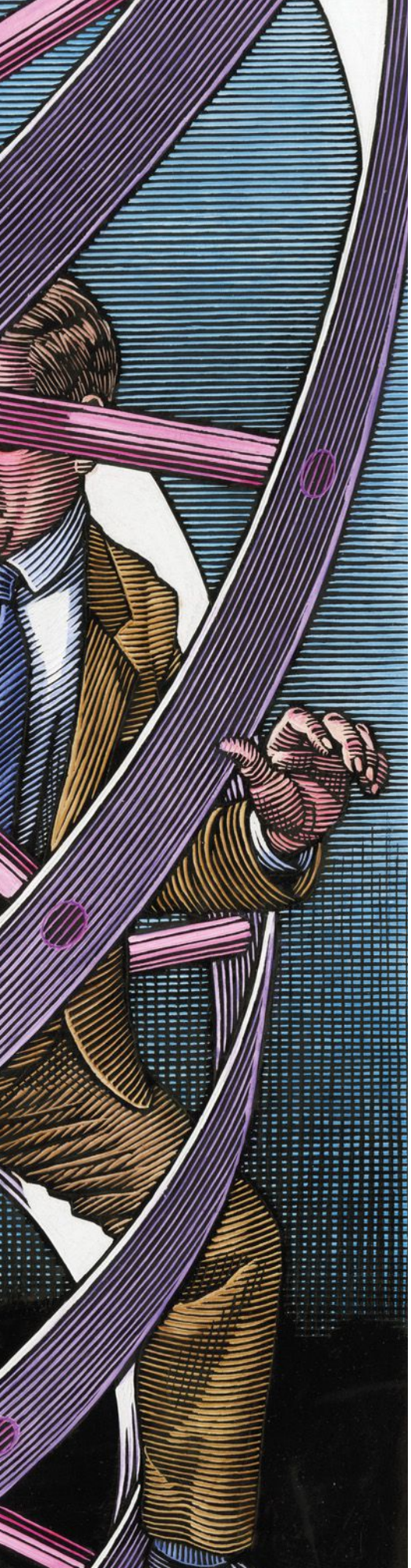


REVEALING RIBOSWITCHES: RNA is isolated from bacteria and tagged with a 3′ adapter, to which a complimentary oligonucleotide binds for reverse transcription and sequencing. Sequence analysis then reveals those transcripts that have been fully transcribed versus those prematurely truncated. The reproducible presence of similarly truncated transcripts for a given gene suggests the presence of a riboswitch in the mRNA.

AT A GLANCE

RIBOSWITCH DISCOVERY METHOD	TECHNIQUE	HYPOTHESIS DRIVEN?	EXPERIMENTAL VALIDATION REQUIRED?	RIBOSWITCH TYPES
Comparative analyses	Transcript sequences screened for similarity to existing riboswitches	Yes	Yes	Transcription and translation regulators
Term-seq	Presence of prematurely terminated transcripts identified during deep sequencing of 3′ adapter-ligated RNAs	No. Unbiased search of whole transcriptome	Yes	Transcription regulators only





Uniquely Human

Fast-evolving regions of the human genome differentiate our species from all other mammals.

BY KATHERINE S. POLLARD

When the first human genome sequence was published in 2001,[1] I was a graduate student working as the statistics expert on a team of scientists. Hailing from academia and biotechnology, we aimed to discover differences in gene expression levels between tumors and healthy cells. Like many others, I had high hopes for what we could do with this enormous text file of more than 3 billion As, Cs, Ts, and Gs. Ambitious visions of a precise wiring diagram for human cells and imminent cures for disease were commonplace among my classmates and professors. But I was most excited about a different use of the data, and I found myself counting the months until the genome of a chimpanzee would be sequenced.

Chimps are our closest living relatives on the tree of life. While their biology is largely similar to ours, we have many striking differences, ranging from digestive enzymes to spoken language. Humans also suffer from an array of diseases that do not afflict chimpanzees or are less severe in them, including autism, schizophrenia, Alzheimer's disease, diabetes, atherosclerosis, AIDS, rheumatoid arthritis, and certain cancers. I had long been fascinated with hominin fossils and the way the bones morphed into different forms over evolutionary time. But those skeletons cannot tell us much about the history of our immune system or our cognitive abilities. So I started brainstorming about how to extend the statistical approaches we were using for cancer research to compare human and chimpanzee DNA. My immodest goal was to identify the genetic basis for all the traits that make humans unique.

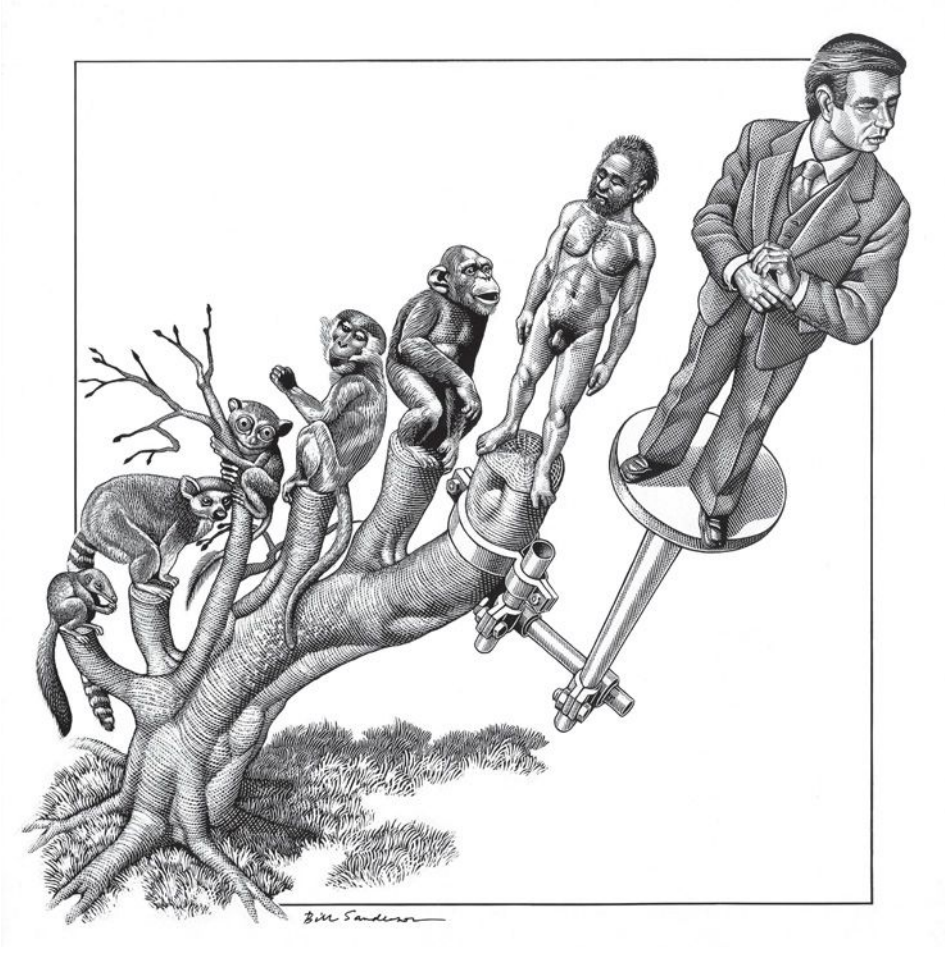
The chimp genome was published in 2005,[2] when I was a postdoc at the University of California, Santa Cruz, and those of 12 other vertebrates followed shortly thereafter. At the same time, computational scientists were busy developing algorithms to scan DNA for similar regions across multiple species. Such sequence conservation suggests that these areas are responsible for critical functions. I took these comparative genomic scans to the next level by writing a computer program to identify DNA sequences that are conserved in other animals but have changed rapidly in humans since we evolved from our common ancestor with chimpanzees. This evolutionary signature predicts a loss or

modification of function in humans. My colleagues and I used this two-part pattern to define the fastest-evolving regions of the human genome, known as human accelerated regions (HARs). We published the first 202 HARs in 2006.[3]

An exciting but daunting pattern emerged: only a handful of HARs were in genes. In fact, we had no idea what the vast majority of these putatively functional and uniquely human DNA sequences did, let alone their role in human evolution. HARs are short—on average just 227 base pairs long, much smaller than a gene. They looked like what we called "junk DNA" at that time and would not have been at the top of anyone's list of genomic regions to study, if not for their compelling conservation across most animals and notable differences in humans.

Thanks to innovations in sequencing technology that have produced a cornucopia of genomes, plus some tweaks to the computational methods by different labs, the combined list of identified HARs now includes nearly 3,000 genome segments.[4] But the original trend still holds; nearly all HARs are outside genes, some quite far away from any gene in the genome.

So what were HARs doing that made their sequences so immutable throughout mammalian evolution? How did the multiple human mutations in each HAR change its function? Ten years in, my group, now based at the Gladstone Institutes in San Francisco, and others continue to investigate these questions, in hopes of better understanding what makes humans different from all other species.



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Uniquely human gene regulators

Ignoring human DNA for a moment, HAR regions are some of the most conserved sequences in the genomes of mammals. Some of them are nearly identical between chimpanzee and platypus, for example. This close identity suggests that the information encoded in these sequences is critical, and that changes to the sequences will alter their important instructions. This makes the human mutations in HARs truly unexpected.

It is tempting to speculate that these mutations destroy or change gene regulatory functions, altering when and where genes turn on. The first two HARs to be functionally characterized support this idea.

Humans suffer from an array of diseases that do not afflict chimpanzees or are less severe in them.

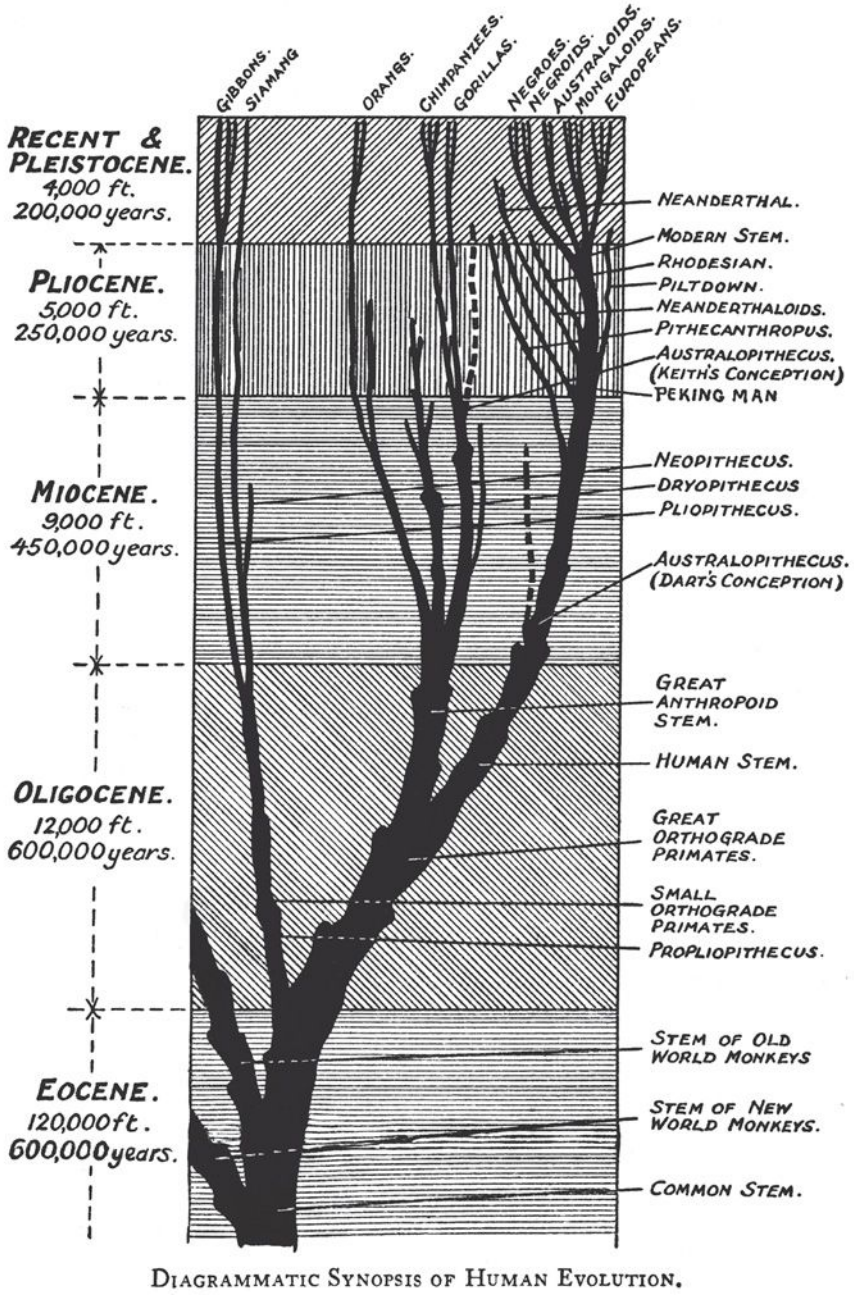
HAR1 does not code for a protein but for a long RNA, a type of molecule that guides proteins or modulates their expression.[5] We predicted that the HAR1 RNA could fold into a three-dimensional structure because its conserved sequence has palindromic regions that pair up to form a series of interconnected "stems" that look like ladders—think of an untwisted DNA double helix. This computational prediction was confirmed by RNA structure–probing experiments using human and chimpanzee HAR1 RNAs synthesized in vitro to identify stems. By labeling HAR1 molecules in human and macaque embryos, we discovered that the RNAs functioned in neurons during patterning and layout of the cortex,[6] a brain structure that expanded greatly in size during human evolution.[7] Exactly which genes HAR1 is regulating remains to be determined.

HAR2 (also known as *HACNS1*) encodes neither a protein nor an RNA. Rather, *HAR2* functions as an enhancer, a DNA sequence that works to increase or decrease the level of a gene's expression.[8] An enhancer can be located thousands of

base pairs away from the gene it regulates. The gene gets activated when it comes into physical proximity with its enhancer. Studies in mice revealed that human *HAR2* is active in several embryonic tissues, including those that give rise to the wrist and thumb, structures that morphed in our ancestors after their split from a common ancestor with chimpanzees. Once again, the genes that are subject to *HAR2* regulation are still unclear, although GBX2, a transcription factor that controls proper expression of genes involved in embryo morphogenesis, is one promising candidate.

Building on these initial discoveries, researchers have revealed the role of other HARs in gene regulation thanks to advances in techniques that measure gene expression at the single-cell level, track where proteins bind to DNA, and assess other epigenetic properties of the genome. (See "Scaling to Singles," *The Scientist*, May 2016; "Silencing Surprise," *The Scientist,* June 2015.) Integrating this new information into computational models, my colleagues and I predicted that about 5 percent of HARs function as noncoding RNAs, while most are enhancers that control gene expression during embryonic development.[9]

To more concretely test this hypothesis, my team has begun examining the function of nearly 100 of the fastest-evolving HARs, many of which we suspected to have enhancer activity. We inject fertilized mouse or fish eggs with a reporter construct that contains the chimp HAR sequence in front of a gene that will label any cells of the embryo in which the HAR functions as an enhancer. So far, two-thirds of HARs tested for enhancer activity turned on a gene during development.[4] For 26 HAR enhancers, we repeated the experiment with the human sequences. Eight HARs showed differences in their enhancer activity when the human mutations were present.[4] These differences modify how genes were expressed in the developing limb (*HAR2, 2xHAR114*), eye (*HAR25*), and central nervous system (*2xHAR142, 2xHAR238, 2xHAR164, 2xHAR170, ANC516/HARE5*).[4,10] Because relatively few time points have been examined, it is likely that an even higher percentage of the tested HARs are active enhancers at some point during embryonic development or in adult tissues, possibly with human-chimp differences.



THE HUMAN TREE: This diagram from 1931 illustrates the evolution of primates from a common ancestor. Later discoveries revealed many of the specifics here to be wrong, but the overall branching of different primate groups continues to inform the study of human biology today.

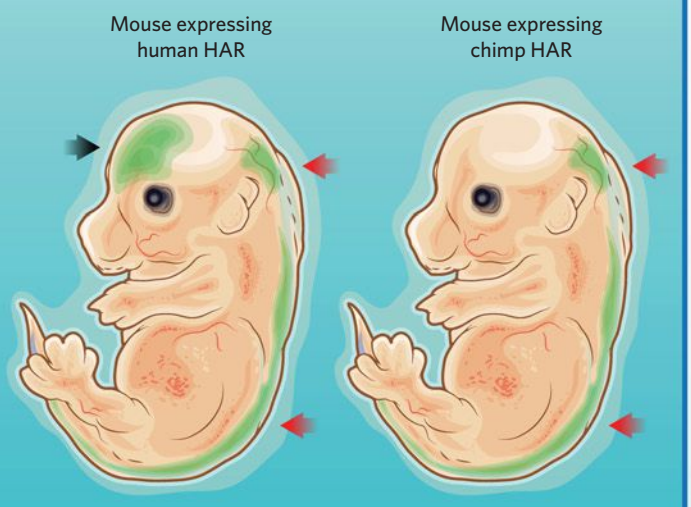
Many HARs are located near genes that control fundamental developmental processes,[9] so their altered regulatory function could have profound effects on human biology. Supporting this, the human version of one HAR enhancer (*ANC516/HARE5*) is active earlier in development and in a larger region of the brain compared to the chimp HAR. Human *HARE5* increases expression of its target gene, *Frizzled 8*, affecting the size and development of the brain in mice.[10]

These experiments demonstrate that HARs may have changed key developmental programs over the course of human evolution. The *HARE5* study is the closest researchers have come to showing that a HAR sequence affects an organ that is important to human evolution. It is possible that human mutations in HARs could

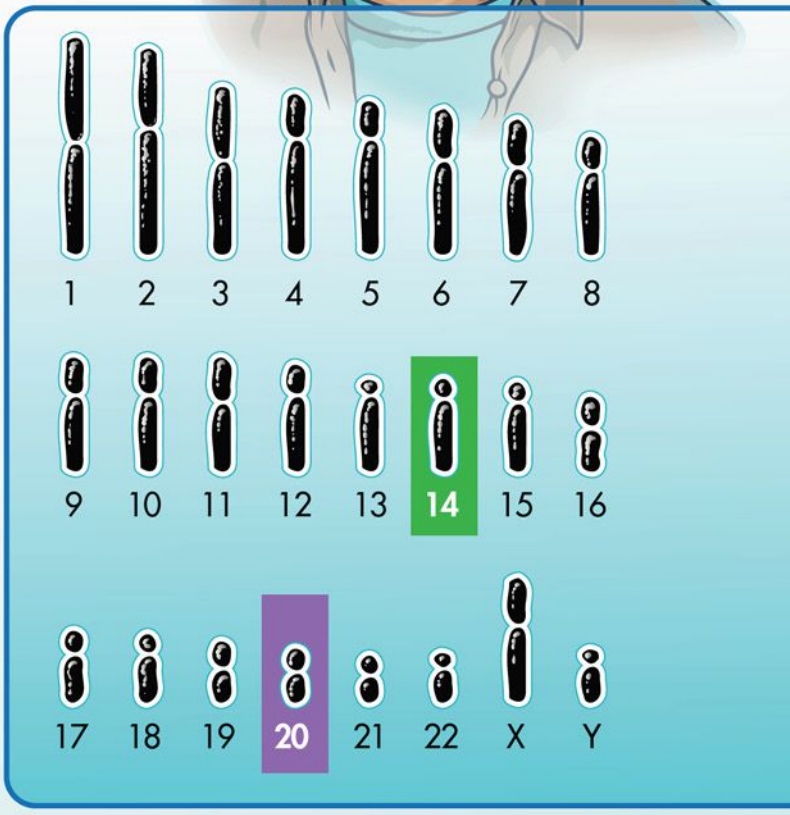
UNDERSTANDING HUMAN ACCELERATED REGIONS

Sections of the genome that are largely conserved across mammals and even the entire animal kingdom, but differ in humans, are known as human accelerated regions (HARs). Deciphering their function may prove key to understanding what sets humans apart from other organisms. For example, *2xHAR.142* and *2xHAR.114*, like many other HARs, function as enhancers, which increase or decrease the level of a gene's expression.

What happens when the reporter gene *lacZ* is put under control of *2xHAR.142*?



Both the chimpanzee and human forms of *2xHAR.142* enhance the expression of *lacZ* in the hindbrain and spinal cord (red arrows), but only the human sequence drives expression in the developing cortex (black arrow), a brain region that grew disproportionately in size after humans split from chimps.



2xHAR.142 is located on chromosome 14 within an intron of *NPAS3*, a gene whose introns contain more than a dozen other HARs.

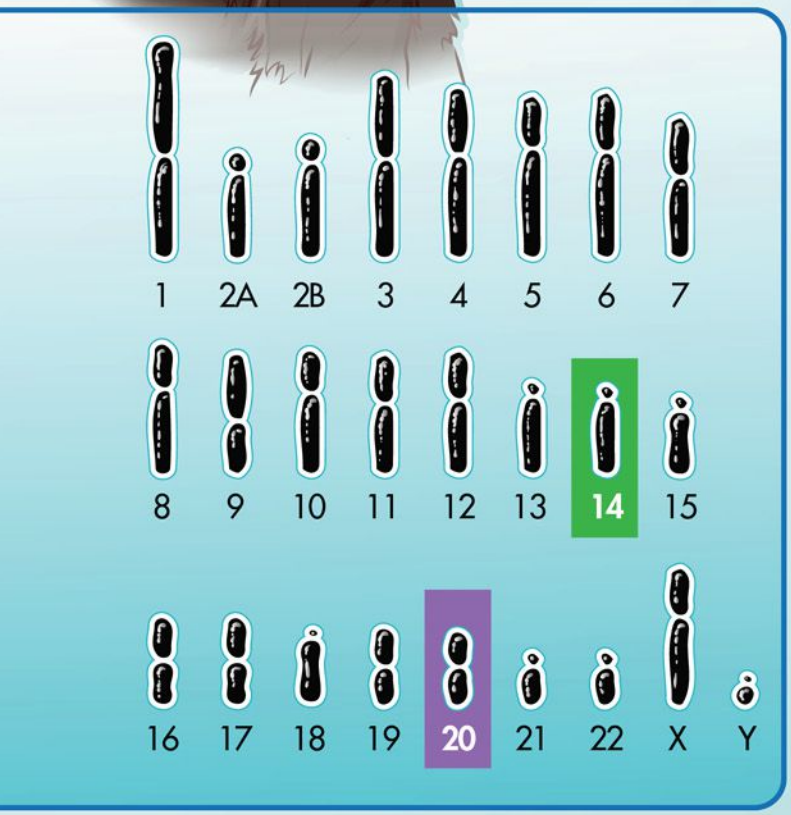
HUMAN

...GCGTAGAATGAAGAATTCAGAATCAATGTACTCCCCATTTCATAG
ACGTGCTATTAGTAACGAT...

CHIMP

...GCACAGAATGAAGAATTCAGAATCAATGTACTCCCCAGTTCATAG
ATATGCTATTAGTAATAAT...

CHIMP



2xHAR.114 is located on chromosome 20 in between the genes *FOXS1* and *MYLK2*.

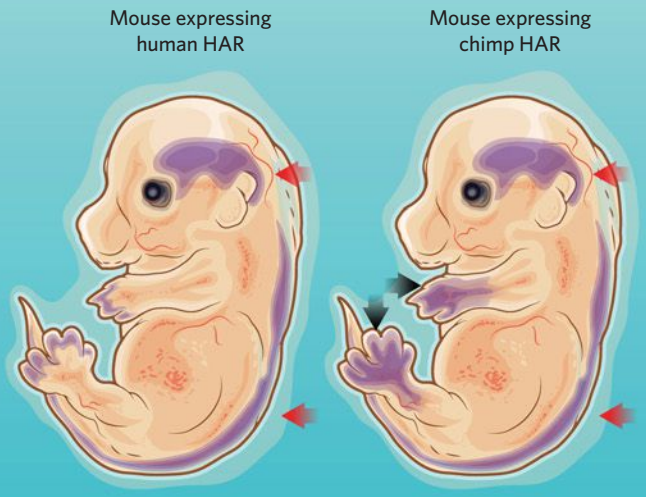
HAR FACTS

Location: Typically outside genes, though some HARs are found in gene introns

Size: Just 227 base pairs long, on average

Function: Most HARs studied so far are enhancers, sequences of DNA that increase or decrease the expression of a gene.

What happens when the reporter gene *lacZ* is put under control of *2xHAR.114*?



Both the chimpanzee and the human *2xHAR.114* sequences drive expression of *lacZ* in the spinal cord and developing brain (red arrows), but the chimpanzee sequence drives expression to a more extensive region in the limb buds (black arrows). Human hands are weaker than chimps' but they are better at fine motor control.

HUMAN

...CACAGGTCTGGAAGCCACTAAGCCCACATCTGGTTTGGATTACA
TCAGGGCTGGTGACACTGCCTTTCCCTTTCTGGGTCCAGTGGCCTTG
TATTCCACTGGCCACCTTGGACCCAATAA...

CHIMP

...CATGGGTCTGGAAGCCACTAAGGCCACATCTGGTTTGGATTACAT
CAGGGCTGGTGACGCTGCCTTTTCCTGTCTGGGTCCAGTGGCCTTGT
ATTCCACTGGCCACCTTGGACCCAACAA...

influence human traits such as fine motor skills, spoken language, and cognition. But linking HAR mutations to organismal innovations is hard, given the obvious limitations on testing the effects of genetic changes in humans or apes. Establishing these connections is our biggest challenge going forward.

Emergence of HARs

The most recent common ancestor of humans and chimps probably lived about 6 million years ago. The fossil record shows that our two species have changed continually in different ways since then. Knowing when a HAR mutated during human evolution could help researchers link it to traits that changed at the same time. Conversely, as we elucidate which biological processes are affected by HAR mutations, the ages of the mutations could help date the emergence of traits that are hard to discern from fossils.

Estimating when a HAR evolved is challenging because these calculations rely on comparisons with genomes from hominins that split off from our ancestors at different times in the past. Without these molecular signposts along the human lineage, it is hard to say if a HAR evolved right after the human-chimpanzee split or only a few generations ago. But ancient-DNA sequencing is beginning to shed some light on the issue.[11] For example, by comparing a human HAR sequence with the HAR sequence of an archaic hominin, researchers can estimate if the HAR mutated before, after, or during the time period of our common ancestor.[12] This approach has revealed that the rate at which HAR mutations emerged was slightly higher before we split from Neanderthals and Denisovans.[3,13] As a result, most HAR mutations are millions of years old and shared with these extinct hominins (but not with chimpanzees).

Some HARs have evolved much more recently, however. About 10 percent of mutations in HARs are polymorphic, meaning that only a subset of people carry the mutated sequences, while others have the DNA sequence seen in chimps.[4] These polymorphic changes in HARs happened relatively recently in human evolution—they are unlikely to be more than 1 million years old. But such newer HAR mutations are found in people around the globe, indicating that they predate the long-distance human migrations that began about 60,000 years ago.

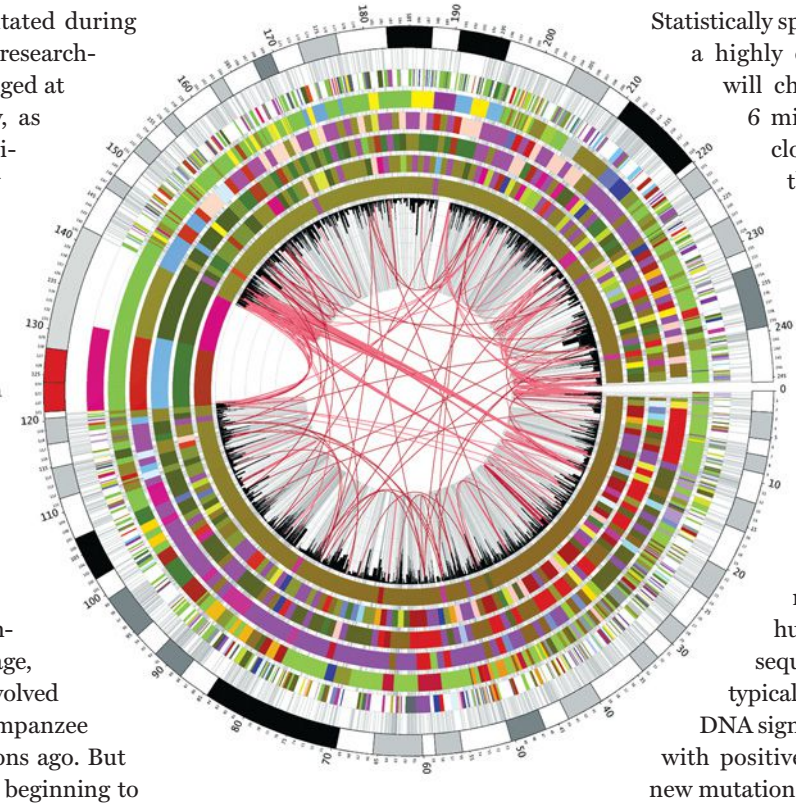
As more human genomes from different populations are sequenced, it will be exciting to see if any traits are associated with carrying the mutated versus ancestral version of polymorphic HARs. This approach has already revealed medically relevant traits linked to Neanderthal ancestry in other parts of the human genome.[14] For example, blood tends to clot more quickly in those of us with the Neanderthal DNA in one such region, while another Neanderthal sequence is associated with depression.

Statistically speaking, the probability that a highly conserved DNA sequence will change multiple times over 6 million years of evolution is close to zero.

Forces that created HARs

Statistically speaking, the probability that a highly conserved DNA sequence will change multiple times over 6 million years of evolution is close to zero—that is, unless the forces that have been selecting against mutations in its sequence suddenly change. *HAR2*, for example, appears to turn on a gene involved in human limb development thanks to the loss of sequences that keep it switched off in the embryos of other species.[15]

Most HARs carry more differences between human and chimp DNA than sequences that change through typical mutational processes. This DNA signature of HARs is associated with positive selection that favors the new mutations in humans. Processes that increase the rate of mutations in localized regions of the genome (e.g., enzyme-induced mutagenesis) or drive the spread of new mutations for nonadaptive reasons (e.g., GC-biased gene conversion) can mimic positive selection, however. It's



SPECIES COMPARISON: This circular genome map shows shared genetic material between humans (outer ring) and (from inner ring outwards) chimpanzee, mouse, rat, dog, chicken, and zebrafish chromosomes. The colors form a heat map, the pattern of which represents hot spots of shared genetic material.

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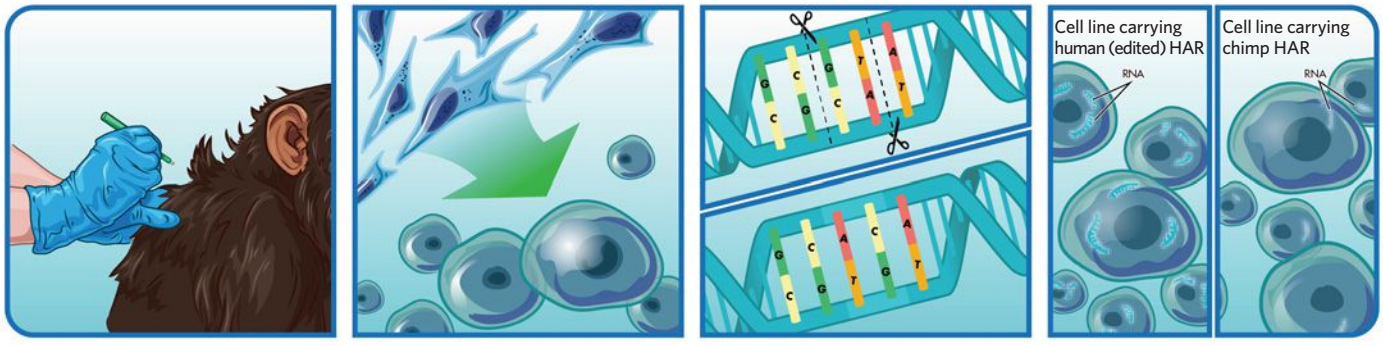
NAILING DOWN HAR FUNCTION

A remaining challenge in the study of human accelerated regions (HARs) is establishing their specific functions during development and other biological processes. But modern stem cell technologies could provide the answer.



Take skin biopsy from a nonhuman primate.

Reprogram skin fibroblasts into brain, heart, or liver cells.

Edit the DNA of a specific chimp HAR to match the human sequence.

Test the cells for differences in gene expression.

likely that a multitude of forces combined to spur the evolution of these uniquely human DNA sequences.

Researchers have come a long way toward illuminating the functions of HARs and their potential roles in human evolution, but we are still far from understanding their specific functions in development and other processes. One of the major challenges that we face is establishing causality. Fortunately, emerging technology has made it possible to create brain, heart, and liver cells from a primate skin biopsy[16] and edit the DNA of these cells in the laboratory. These advances allow researchers to test whether specific human mutations alter the ability of HARs to activate genes in human or primate cells.[17] Additionally, because enhancer activity can now be assayed with high-throughput genomic techniques, it is conceivable to move from testing HARs one by one to investigating thousands of them in parallel. These exciting breakthroughs promise to accelerate research on HAR function and the evolutionary forces that shaped HARs.

High-performance computing and algorithm development will continue to be critical to HAR research. My analysis that discovered the original 202 HARs would still be running today if I had implemented it on a single desktop computer rather than a 1,000-node computer cluster. Instead of waiting for the program to end, we spent the past decade showing that HARs are key regulators of embryonic development. This is a huge step forward from HARs being viewed as bizarre junk DNA of unknown function. Looking ahead to when all of our genomes have been analyzed and tools exist for precise editing of HARs in human cells, it seems possible to figure out what happened when each of these evolutionarily conserved sequences suddenly mutated in humans. ■

Katherine S. Pollard is a biostatistician at the Gladstone Institutes in San Francisco, California.

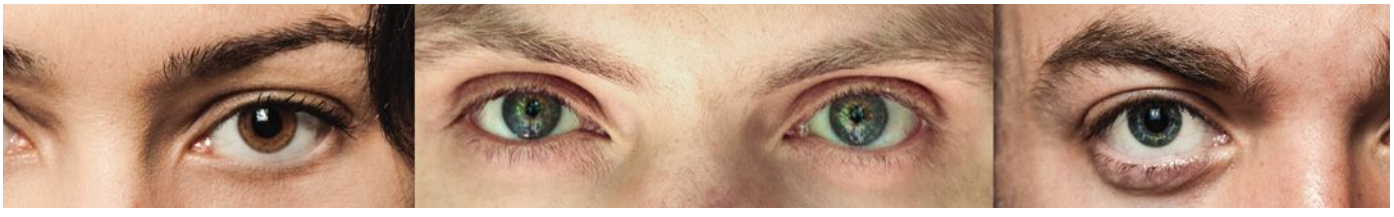
References

1. International Human Genome Sequencing Consortium, "Initial sequencing and analysis of the human genome," *Nature,* 409:860-921, 2001.
2. The Chimpanzee Sequencing and Analysis Consortium, "Initial sequence of the chimpanzee genome and comparison with the human genome," *Nature,* 437:69-87, 2005.
3. K.S. Pollard et al., "Forces shaping the fastest evolving regions in the human genome," *PLOS Genet,* 2:e168, 2006.
4. M.J. Hubisz, K.S. Pollard, "Exploring the genesis and functions of Human Accelerated Regions sheds light on their role in human evolution," *Curr Opin Genet Dev,* 29:15-21, 2014.
5. K.S. Pollard et al., "An RNA gene expressed during cortical development evolved rapidly in humans," *Nature,* 443:167-72, 2006.
6. A. Beniaminov et al., "Distinctive structures between chimpanzee and human in a brain noncoding RNA," *RNA,* 14:1270-75, 2008.
7. S. Herculano-Houzel, "The remarkable, yet not extraordinary, human brain as a scaled-up primate brain and its associated cost," *PNAS,* 109(Suppl 1):10661-68, 2012.
8. S. Prabhakar et al., "Human-specific gain of function in a developmental enhancer," *Science,* 321:1346-50, 2008.
9. J.A. Capra et al., "Many human accelerated regions are developmental enhancers," *Philos Trans R Soc Lond B Biol Sci,* 368:20130025, 2013.
10. J.L. Boyd et al., "Human-chimpanzee differences in a *FZD8* enhancer alter cell-cycle dynamics in the developing neocortex," *Curr Biol,* 25:772-79, 2015.
11. J. Krause, S. Pääbo, "Genetic time travel," *Genetics,* 203:9-12, 2016.
12. R.E. Green et al., "A draft sequence of the Neandertal genome," *Science,* 328:710-22, 2010.
13. K.S. Pollard et al., "Analysis of human accelerated DNA regions using archaic hominin genomes," *PLOS ONE,* 7:e32877, 2012.
14. C.N. Simonti et al., "The phenotypic legacy of admixture between modern humans and Neandertals," *Science,* 351:737-41, 2016.
15. K. Sumiyama, N. Saitou, "Loss-of-function mutation in a repressor module of human-specifically activated enhancer HACNS1," *Mol Biol Evol,* 28:3005-07, 2011.
16. I. Gallego Romero et al., "A panel of induced pluripotent stem cells from chimpanzees: A resource for comparative functional genomics," *eLife,* 4:e07103, 2015.
17. S. Weyer, S. Pääbo, "Functional analyses of transcription factor binding sites that differ between present-day and archaic humans," *Mol Biol Evol,* 33:316-22, 2016.

The Ever-Changing Human

The emergence of blood abnormalities, an adult ability to digest milk, and changes in our physical appearance point to the continued evolution of the human race.

BY JOHN HAWKS



Natural selection is tricky to catch in action. As Darwin put it, "A grain in the balance will determine which individual shall live and which shall die." The grain in the balance—the slightly increased chance that organisms carrying one gene variant will fail in the struggle for existence—is the cost of selection. It is almost invisible, only becoming statistically evident when viewed across thousands of individuals, who may display only subtle differences in the affected character.

In the human population, the toll of natural selection is hidden within millions of deaths and births around the world every year. Everyone dies, many tragically young. And while obvious patterns sometimes emerge from early deaths—certain diseases, traffic accidents, drug overdoses—these are often challenging to connect to the action of genes. Likewise, only by comparing the genes of parents with those of childless people, and the genes of large families with those of small families, can we begin to understand how natural selection is acting on births.

Six years ago, Yale University's Stephen Stearns and colleagues took advantage of a long-running study in Framingham, Massachusetts, to assess whether the effects of natural selection could be discerned among the people in the multigenerational study population. Over the last seven decades, public-health researchers have been monitoring the residents of Framingham, noting their vital statistics as well as blood sugar and cholesterol levels to understand the factors that lead to heart disease. As the initial group of research subjects got older, the study started to include their children, and then their grandchildren. The records provide a unique view of the health of a segment of the American population since 1948.

When Stearns and his coworkers analyzed the data, they found lots of evidence that selection was occurring, albeit with many curious patterns. Shorter women had more children than taller women, and heavier women had more children than lighter women. For men, height and weight weren't as correlated with fecundity. High or low blood-sugar readings in both men and women were associated with fewer offspring, and the age at which individuals had their first child also seemed to influence lifetime reproduction—people who had their first child younger ended up with larger families.[1]

The results left scientists frustrated. To what extent are these traits—stature and age at first birth, for example—heritable?

What other factors are shaping the population? Age at first birth is surely influenced by cultural factors that can confound the attempt to tease out the contribution of genes.

To get at those kinds of details, we need to combine records of traits with a look at the genes themselves. That kind of research is just now becoming possible.

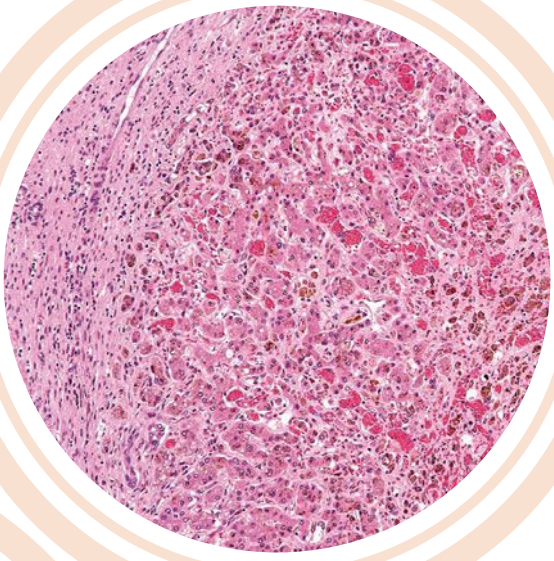
Last month, for example, Harvard University's Jonathan Beauchamp published a study in which he compared known gene variants with relative lifetime reproductive success (rLRS)—a proxy for the number of biological offspring an individual has—in people of European descent living in the U.S. and enrolled in the Health and Retirement Study. In this cohort, Beauchamp found evidence that evolution may have selected against educational attainment, while favoring a higher age at menarche for women. Although he notes that cultural and environmental factors may have overridden the effects of natural selection, he makes the case that humans do continue to evolve.[2]

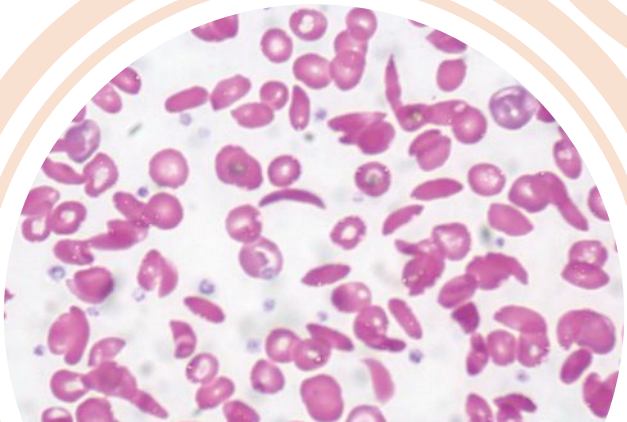
In the blood

The first solid evidence of natural selection in recent human populations was found in blood. Type B blood is common across central Asia, but much rarer in other places. Newly identified blood types outside the ABO system have also been found, and each has a distinctive geographical distribution. One of the most extreme is the Duffy blood type, which has three different versions, or alleles, just like the ABO system. One of these types, Duffy "null," occurs in up to 95 percent of people in sub-Saharan Africa, but is very rare among people whose ancestry comes from other parts of the world.

In addition to blood type, researchers have investigated the evolution of blood disorders and abnormalities. One of the most interesting is a deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD), which helps maintain red blood cells. An insufficient level of this enzyme occasionally causes extreme, even lethal health problems, but is better known for causing a reaction to fava beans in people suffering from the deficiency. Other blood peculiarities include the sickle cell trait, reduced production of hemoglobin (alpha thalassemia), hemolytic anemia (ovalocytosis), and abnormal hemoglobin types (hemoglobin C and hemoglobin E). By examining the frequencies of these conditions, researchers have found that these blood variations coincide with regions where malaria has been common throughout history. Further work revealed how small changes to hemoglobin can impede the malaria parasite's ability to break into red blood cells. The Duffy null allele, too, helped carriers to resist malaria.[3] The *FYA* and *FYB* versions of the gene both result in molecules on the surfaces of red blood cells that function in inflammatory reactions but also provide an avenue of attack for the malaria species *Plasmodium vivax*.[4] People who lack these molecules may avoid *P. vivax* infection.

These variations were not without consequences, however. While one sickle cell allele is protective against malaria, most people who carry two copies die young, usually without reproducing. It's no surprise, then, that malaria-free areas have extremely low rates of the sickle cell trait and other red blood cell variations.





RESISTANT TO MALARIA: Blood disorders and abnormalities such as the sickle cell trait (below) can impede the malaria parasite's ability to infect red blood cells and are more frequent in regions of the world where malaria was once common. But while these blood differences provided protection against the parasite, they are also associated with health risks, such as cirrhosis of the liver (right).

ED UTHMAN/WIKIMEDIA COMMONS; NEPHRON/WIKIMEDIA COMMONS

Milk digestion

While the distribution of blood types and abnormalities was the first evolutionary pattern identified among recent human populations, perhaps the most famous is people's ability to digest milk beyond infancy. Around 30 percent of the calories in milk from humans and all other mammals come from a sugar called lactose, and to make use of the energy stored in lactose, the digestive system must be able to break it down into its two chemical subunits, galactose and glucose. This chemical reaction is catalyzed by the enzyme lactase, the gene for which is shared across all mammals. In most species, however, lactase is only expressed in young prior to weaning, leaving adults unable to digest lactose.

AGRICULTURAL ADAPTATIONS: As human populations began to domesticate animals and consume their milk, they evolved the persistent expression of the lactase gene, which breaks down lactose and is usually only expressed in young animals.

Pre-agricultural humans followed, and many modern humans still follow, this same pattern of lactase expression in infancy only. Regular consumption of milk by an adult can sometimes spur a minimal amount of lactase production, but drinking a large amount of milk or other lactose-containing dairy products can cause severe digestive distress. People from China often have trouble digesting milk, as do many people from southern Europe. Yet in northern Europe and parts of sub-Saharan Africa, more than 95 percent of people produce the lactase enzyme throughout their lives and can thus digest milk as adults without difficulty. A smaller fraction of adults in other populations, such as those in the western half of Eurasia and other parts of sub-Saharan Africa, also have this persistence of lactase.

THE ADVANTAGE OF LACTASE PERSISTENCE WAS ENORMOUS, PERHAPS THE STRONGEST KNOWN FOR ANY RECENT HUMAN TRAIT.

The persistence is not due to any change to the enzyme itself, but to the short patches of DNA outside the gene that regulate its activity. People from Ireland to India share one mutational change that prompts lactase persistence. In Arabia and sub-Saharan Africa there are four others. At least five times, ancient humans had a chance mutation that spurred lactase activity in adults and began to spread through the population. Not surprisingly, these populations live in precisely the areas where people domesticated cattle, sheep, goats, and camels for the purpose of consistent milk production. That domestication happened only within the last 10,000 years, and cattle became common in sub-Saharan Africa and northern Europe much later than this, placing an upper time limit on these genetic changes.

Lactase persistence is one of the most profound changes in recent human populations, and was one of the first to be investigated by scientists working with DNA directly from ancient skeletal remains, first by Joachim Burger of Johannes Gutenberg University in Germany and colleagues, and later by many others. Before 7,000 years ago, the ancient peoples of Europe lived only by hunting, fishing, and gathering; they did not farm or keep domesticated animals. Gene sequences from the remains of these people have never produced any evidence of lactase persistence. Only well after people began to keep cattle—as evidenced by milk residues found in pottery from early farming and herding contexts in Europe and west Asia—did mutations promoting lactase persistence arise. (See "What's Old Is New Again," *The Scientist*, June 2015.)

Once it appeared within these ancient populations, the numbers of people with lactase persistence grew by up to 10 percent per generation. Its advantage was enormous, perhaps the strongest known for any recent human trait. This kind of evolutionary advantage likely resulted from increases in fertility. Women on calorie-restricted diets have lower fertility, and they take longer after the birth of a child to conceive again. If lactase-persistent women could use the extra energy from milk to begin their reproductive lives a couple of years earlier, or could space their children a few months closer together, it would create a huge reproductive advantage.

Indeed, the frequency of lactase persistence has continued to climb substantially in some places even within the past 2,000 years. Just this spring, Stanford University's Yair Field and colleagues reported on a new study that sampled more than 3,000 human genomes from the United Kingdom to look at the effects of selection on genes. They found that lactase persistence is the largest single change within the British popu-

lation since Roman times, increasing in frequency more than any other allele across the genome.[5]

Not so simple

The lactase example connects human populations and their cultural innovations. But in one important respect it is misleading: it is much too easy to understand. Unlike lactase persistence, most human traits are not the product of a single gene. Rather, they are influenced by many genes, and studying selection on such traits has proven very difficult.

Skin color is a classic example. One of the largest and most obvious physiological differences between populations, skin color is influenced by more than two dozen genes in a pathway that produces the pigment melanin and regulates the amount of this pigment in different tissues. Changes to these genes interrupt the generation of the dark pigment eumelanin, leaving skin with larger amounts of the reddish pigment pheomelanin, leading to various skin tones and patterns of coloration, such as freckles. Despite its complex genetics, skin color shows consistent patterns of evolution across the globe. People whose ancestors lived in the tropics tend to be dark-skinned, while those who lived further north and south tend to be lighter. One of the revelations of the last 15 years is just how recent this pattern really is. According to analyses of ancient DNA, people who lived in northern Europe only 10,000 years ago would not have had the extremely light skin of today's people in that region.

Other types of human coloration are also evolving. In their recent study, Field and colleagues found several genes related to hair and eye pigmentation that had markedly increased in the ancestors of modern Brits. These traits include one associated with blue eyes and two that are found in people with blond hair. Britain has experienced extensive immigration since Roman times, including the arrivals of Vikings, Anglo-Saxons, and Normans, but the genetic changes seen in this population are not due merely to migration; they mark the increase of particular genes above and beyond the contributions of immigrants. The British have become blonder over recent millennia.

Stature is another complex trait that has continued to evolve in recent years. Northern Europeans are a bit taller than southern Europeans, and looking at the genes that differ between them, Field and his colleagues found that the height differences were driven by natural selection for taller stature in the north over the last 2,000 years. This trend is not seen worldwide, however. The Framingham population and other studies in the U.S. have found that shorter women have had a reproductive advantage during the last few decades. On the other hand, a study from one sub-Saharan African nation, Gambia, showed a pattern more in line with the changes seen in Britain's population: taller women had more children.[6] For men the story is even more mixed. Dutch and Polish men have been under weak selection for taller height over the last several decades, but in other countries a man's stature seems to make no difference to his lifetime reproduction.[7]

The skeletons of ancient people likewise show physical changes over the past several thousand years. Heads changed shape, becoming broader and a bit smaller over time in many parts of the world. We do not yet know which genes might be connected to such changes, just as we do not know many of the genes that might drive earlier reproduction. As we learn more about the genetics of human biology, studying the pattern of natural selection in genes may help us to uncover the biology of such traits.

Only a few of the recent evolutionary changes are obvious to us. Most are well-hidden, driven by genetic pathways we are still discovering. The record of ancient DNA from Europe is at the moment far more detailed than elsewhere in the world, but this is changing rapidly as ancient DNA samples from the Americas, Ethiopia, India, China, and other areas are coming online. We are already learning about the ancestry of these peoples from single genomes. Soon we will be able to look at past gene frequencies to map the history of adaptations that shaped their recent evolutionary history.

Evolving into the future

If there is one common theme in all this recent selection, it is that much of the human diversity we see around us today arose very recently. More than 90 percent of the heritage of every living human comes from sub-Saharan Africa sometime around 100,000 years ago. Fifteen years ago, many geneticists saw this recent common ancestry as evidence that human evolution had mostly drawn to a close. After diverging from our common chimpanzee and bonobo ancestors some 7 million years ago, hominins underwent massive changes in body size, diet, behavior, and brain size. Huge evolutionary innovations marked the beginning of upright walking, tool

HUMANS ACROSS THE GLOBE HAVE BEEN LIVING UNDER VERY DIFFERENT SELECTIVE PRESSURES SINCE OUR SUB-SAHARAN ROOTS, AND THE CULTURAL DIFFERENCES THAT HAVE EMERGED APPEAR TO HAVE ACCELERATED SOME KINDS OF EVOLUTIONARY CHANGES.

use, culture, and language. And those changes all happened before 100,000 years ago. (See "Uniquely Human" on page 24.)

With such a dramatic picture from the fossil record, it is understandable that many scientists assumed that the final phases of human prehistory were fairly boring, at least from the Darwinian point of view. Across most of the genome, humans everywhere in the world are very similar to one another, much more so than to chimpanzees or most other kinds of primates. Modern humans vary profoundly in cultures and languages, but those differences are mostly learned, not coded in our genes.

Nevertheless, humans across the globe have been living under very different selective pressures since our sub-Saharan roots. And, in fact, the cultural differences that have emerged appear to have accelerated some kinds of evolutionary changes. The domestication of animals led to the invention of dairying, for example, a new dietary niche in which lactase persistence provided a huge advantage. Clearing tropical lands for planting domesticated crops and keeping water in pots changed human ecology in more-disturbing ways, making new habitats for mosquito species that afflict human populations with yellow fever and malaria and spurring protective changes in red blood cell morphology. Moving into new ecosystems also demanded new adaptations from the growing human population, from lighter pigmentation at high latitudes to maintain vitamin D production to improved oxygen metabolism in peoples living at high altitude.

Natural selection is fickle. Behavior that ensured survival in our ancestors' environment may not be as advantageous under modern conditions. (See "Our Inner Caveman" on page 18.) New evidence of how the human genome has changed over the last several thousand years points to a series of massive critical evolutionary changes, setting some aspects of our biology clearly apart from that of our forebears. And we are no doubt continuing to evolve today. ■

John Hawks is a paleoanthropologist and professor at the University of Wisconsin–Madison.

References

1. S.G. Byars et al., "Natural selection in a contemporary human population," *PNAS*, 107:1787-92, 2010.
2. J.P. Beauchamp, "Genetic evidence for natural selection in humans in the contemporary United States," *PNAS*, doi:10.1073/pnas.1600398113, 2016.
3. L.H. Miller et al., "The resistance factor to *Plasmodium vivax* in blacks: The Duffy-blood-group genotype, *FyFy*," *N Engl J Med*, 295:302-04, 1976.
4. J.D. Batchelor et al., "Red blood cell invasion by *Plasmodium vivax*: Structural basis for DBP engagement of DARC," *PLOS Pathog*, 10:e1003869, 2014.
5. Y. Field et al., "Detection of human adaptation during the past 2,000 years," *BioRxiv*, doi:10.1101/052084, 2016.
6. A. Courtiol et al., "The Demographic transition influences variance in fitness and selection on height and BMI in rural Gambia," *Curr Biol*, 23:884-89, 2013.
7. G. Stulp, L. Barrett, "Evolutionary perspectives on human height variation," *Biol Rev*, 91:206-34, 2016.

Parsing the Spectrum

By looking within—at genes, neurons, and the developing brain—scientists are piecing together a new understanding of autism.

BY MEGAN SCUDELLARI

In late 2013, psychologist Raphael Bernier welcomed a 12-year-old girl and her parents into his office at the University of Washington (UW) in Seattle. The girl had been diagnosed with autism spectrum disorder, and Bernier had invited the family in to discuss the results of a genetic analysis his collaborator, geneticist Evan Eichler, had performed in search of the cause.

As they chatted, Bernier noticed the girl's wide-set eyes, which had a slight downward slant. Her head was unusually large, featuring a prominent forehead. The mother described how her daughter had gastrointestinal issues and sometimes wouldn't sleep for two to three days at a time. The girl's presentation was interesting, Bernier recalls, but he didn't think too much of it—until a week later, when he met an eight-year-old boy with similarly wide-set eyes and a large head.

Bernier did a double take. The "kiddos," as he calls children who come to see him, could have been siblings. According to the boy's parents, he also suffered from gastrointestinal and sleep problems. The similarities between the unrelated children were remarkable, especially for a disorder so notoriously complex that it has been said, "If you've met one child with autism, you've met one child with autism." But Bernier knew that the patients shared another similarity that might explain the apparent coincidence: both harbored a mutation in a gene known as *chromodomain helicase DNA binding protein 8* (*CHD8*).

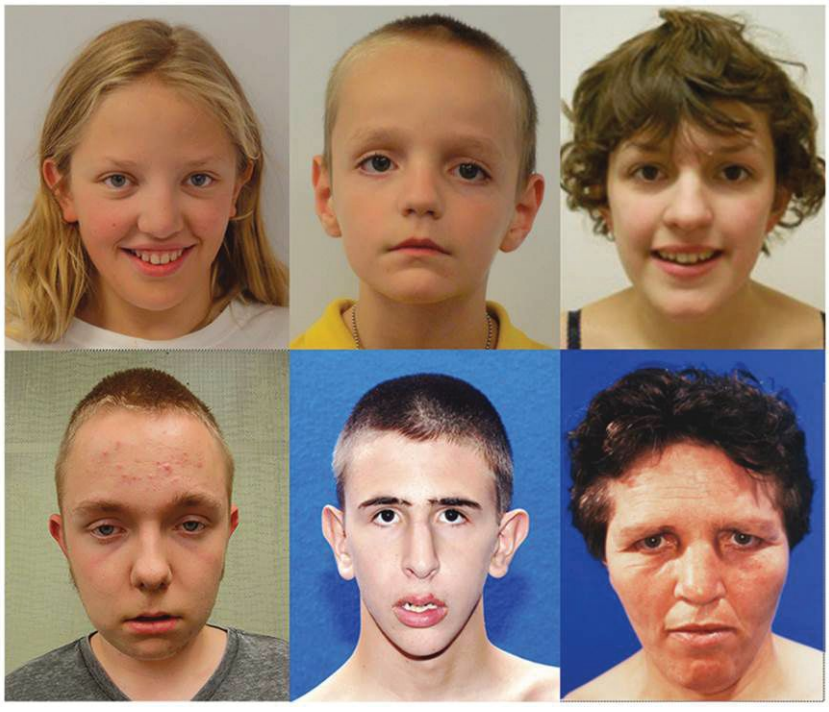
CHD8 produces a protein that regulates chromatin—the conglomeration of tightly packed DNA and proteins in the nucleus—during fetal development. A year earlier, Bernier and Eichler had screened the genomes of 2,000 children for mutations in genes suspected to be involved in autism. Nine of those 2,000 had disruptive mutations in *CHD8*, and Bernier had now met two of them.

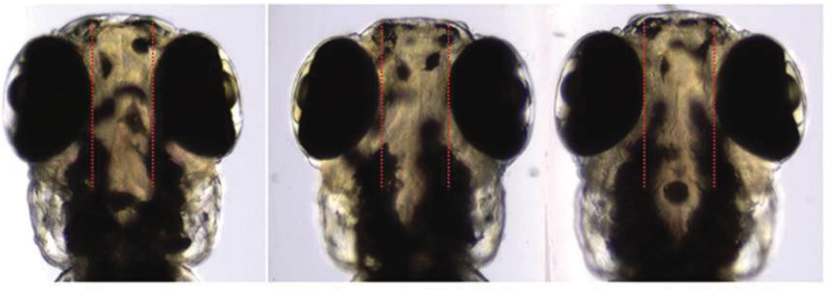
Bernier began inviting others with *CHD8* mutations from around the world to his lab. To date, he has met or reviewed records from 25 such children. They all present with similar physical appearances and symptoms.[1] Mutated *CHD8* is now one of dozens of recognized genetic subtypes of autism. Bernier suspects there are many more.

A large proportion of autism research begins with and is centered upon external presentations of the disorder, primarily behavioral manifestations such as social communication difficulties and repetitive behaviors. Such measures are crude, however, and symptoms often present in different combinations. To better define the disorder, Bernier, Eichler, and others are instead looking for molecular, cellular, and anatomical indicators of autism, including single genetic mutations as well as the behavior of 10-week-old neurons cultured from patients' skin cells and the folding of brain tissue in two-year-old children.

> It's cancer all over again. The victories we've had over cancer weren't for the disease in its entirety, but gradually picking apart subtypes of the disease and developing therapies for those.
>
> —Evan Eichler, University of Washington

This inside-out approach has confirmed a long-held suspicion that autism is not a single biological disorder. The causes and types of autism are as multitudinous as the symptoms, and any successful treatments will likely be as varied. "It's cancer all over again," says Eichler. "The victories we've had over cancer weren't for the disease in its entirety, but gradually picking apart subtypes of the disease and developing therapies for those."





CONTROL *CHD8* MUTANTS

FAMILIAR PHENOTYPES: Mutations in the *CHD8* gene for chromatin-regulating protein (possessed by all patients shown here) is associated with distinct physical features, including large heads (macrocephaly) and wide-set eyes, as well as sleep and gastrointestinal (GI) problems. In a zebrafish model of the mutation, the fish developed large heads (denoted here as increased distance between the eyes, as compared with the control) and poor GI function.

Matchmakers

When Bernier joined the UW faculty in 2008, the vast majority of autism cases were considered "idiopathic," of unknown origins. Despite the fact that autism is a highly heritable disease, no smoking gun had been found in the genome. The prevailing theory was that autism is caused by unfortunate combinations of common mutations.

Eichler, a geneticist who joined UW in 2004, did not subscribe to that theory. In 2006, his lab was in the midst of publishing a dozen papers linking copy number variations (CNV)—large deletions or duplications in the genome—to neurodevelopmental disorders, including about 7 percent to 8 percent of autism cases. Eichler and his colleagues found that patients with particular CNVs often presented with similar phenotypes. "We didn't set out to do this, but we started to define new syndromes," which are referred to by CNV position, such as 17q21 (on the long arm of chromosome 17), says Eichler.

At the time, it was prohibitively expensive to look for mutations at the level of individual genes. But that was about to change. Just as next-generation sequencing was coming onto the scene, Eichler met Bernier, who was studying neural

CELL, 158:263–76, 2014

mechanisms underlying autism. In 2008, at an all-day UW meeting, the chair of genome sciences suggested Bernier reach out to Eichler. "He said, 'I bet you'll get along well,'" Bernier recalls.

That was an understatement. "It was love at first sight," says Eichler with a laugh. Now close friends, they embarked on a joint research endeavor to identify genotypes associated with autism. The majority of past studies—and many current ones—began with a rigorous phenotyping of a child with the disorder, followed by a genetic analysis. Eichler, after his experience with CNVs, opted to put genetics first and foremost. For each subject, he began with a deep dive into the genome.

Eichler's team performed exome sequencing on 677 DNA samples from 209 families who had donated blood to the Simons Simplex Collection, where each family has one child with autism. Most of the mutations he identified were spontaneous—not found in either parent—and they disrupted the function of proteins in similar biological pathways, such as synapse function or chromatin remodeling.[2] But hundreds of different genes were affected, meaning few children had any mutated genes in common.

The researchers then performed targeted sequencing on 44 candidate genes in 2,446 children with autism. This time, thanks to the larger pool of participants, the scientists began to identify clusters of children with the same mutated genes. For example, disruptive mutations in any of six genes—*CHD8, DYRK1A, GRIN2B, TBR1, PTEN,* and *TBL1XR1*—appeared to be responsible for 1 percent of sporadic cases of autism.[3]

Bernier began inviting these children to his lab for two to three days of phenotyping, from brain scans to cognitive tests. When combined with genetic screens of idiopathic autism patients from collaborating physicians around the world, defined subgroups began to emerge. For example, children with mutations in *DYRK1A* have unusually small heads and skull deformities, in addition to behavioral traits characteristic of autism. Individuals with a 16p11.2 deletion are engaged but socially awkward, and have high rates of psychiatric disorders.[4] And *CHD8* mutations, as Bernier had noticed, are linked with wide-set eyes, large heads, and sleep and gastrointestinal problems. To study *CHD8* further, Eichler worked with geneticists at Duke University to create a zebrafish model that carried a mutated version of the gene; the fish developed large heads and poor GI function.

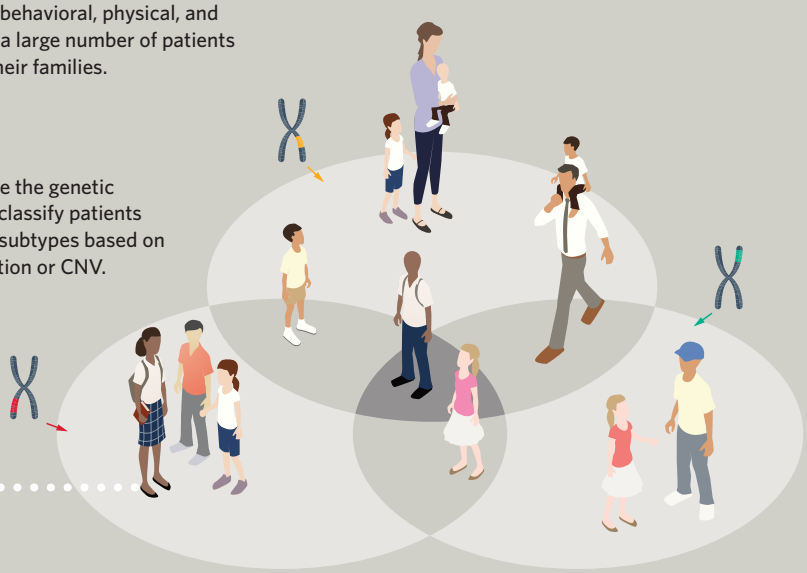
Bernier and Eichler are currently three years into a grant from the National Institutes of Health to track down and fly in individuals from around the world to compare phenotypes to genetic profiles and continue to identify such subtypes. In 2011, the two estimated that more than 800 genes are involved in autism. Today, there are about 100 that have been confidently linked to the disorder, says Eichler, and another several hundred have been flagged as candidates. In 2014, the team collaborated with two other labs—led by Michael Wigler at Cold Spring Harbor Laboratory and Matthew State, then at Yale—to determine that about 30 percent of "idiopathic" cases appear to be due to a large CNV deletion or duplication or a

PERSONALIZING AUTISM TREATMENT

More than 800 genes are suspected to be involved in autism, and researchers today attribute 30 percent of cases to known copy number variations (CNV)—large deletions or duplications in the genome—or spontaneous genetic mutations in a protein-coding gene. For the other 70 percent of cases, the causes remain unknown.

As researchers learn more about the underlying causes of this diverse disorder, they are beginning to think about developing personalized treatments for patients with specific genetic subtypes of autism. While a drug for a single subtype may only be applicable to less than half of 1 percent of patients, such an approach might increase the chances of finding a successful treatment for larger groups of patients.

Physicians gather behavioral, physical, and genetic data from a large number of patients with autism and their families.



Researchers use the genetic information to classify patients into molecular subtypes based on a genetic mutation or CNV.

Often, phenotypic data and gene expression data will be added to the genetic data to more accurately group patients.

If a treatment proves effective for a particular subtype, doctors can then classify new patients based on biomarkers for that group.

known, spontaneous genetic mutation in a protein-coding gene.[5] Wigler suspects that another 20 percent of such cases are attributable to mutations carried by unaffected mothers—who are protected by as-yet-unexplained factors—that cause autism in their sons.[6]

Wigler has spent the last five years studying spontaneous, rare mutations that may play causal roles in autism, and recently narrowed a list of genetic suspects to a proposed set of 200 "vulnerable" genes, as he calls them. He found these vulnerable genes have fewer mutations than typical human genes, suggesting the genes are protected by evolution due to adaptive disadvantages when they are mutated.[7] A surprising proportion of them are expressed in the developing brain in utero but then sharply reduced in expression after birth. Many of the genes, like *CHD8*, affect chromatin structure, while others code for proteins involved in receptor-signaling pathways.

"There's no sore thumb in the group," says Wigler. "I would say, by and large, the brain has a set of genes that are critical for its highest level of development, and some of those genes are [required] to have a healthy brain."

Functional studies are ongoing, and there are many other autism-linked genotypes yet to be discovered, but already the work is having a "magical" impact on families, says Eichler. "Once these families are linked by a common genetic etiology, they become a little society of themselves, sharing practical life experiences and how to cope with their kids' disabilities."

Beyond the genome

Of course, not all forms of autism appear to be caused by genetic abnormalities. Even the most hard-core geneticists in the field—Eichler, Bernier, and Wigler included—are quick to note that genetics does not and will not explain every autism case. Numerous nongenetic factors, such as environmental conditions, can affect the developing brain. For example, premature infants who experience a brain bleed have a 30-fold higher incidence of autism than the general population. To identify other contributors to autism, researchers are working toward a better understanding of neural development. And thanks to advances in induced pluripotent stem cell (iPSC) technology, scientists can now grow entire brain-like structures (organoids) derived from cells of patients with autism.

Last year, Yale University's Flora Vaccarino and colleagues reprogrammed skin cells from boys with autism who also had large heads—a condition known as macrocephaly, a relatively common phenotype in autism patients—into iPSCs, then differentiated the cells into neurons. Under special culturing conditions, the cells developed into 3-D organoids—mini brains—that mimic forebrain development at about 10 to 16 weeks post-conception, says Vaccarino. The researchers also created organoids using the cells of the boys' healthy fathers, and compared them to those structures derived from the boys' cells. (See "Mini Brains Model Autism," *The Scientist*, July 16, 2015.)

Vaccarino initially expected to study dozens of families before finding statistically significant results. But after just four father-son pairs, she had found that the boys' cells grew significantly faster than their fathers' cells, and that they overexpressed genes involved in brain cell proliferation, inhibitory neuron fate, and synapse assembly.[8] These differences are likely not a result of macrocephaly, Vaccarino notes, as the fathers had large heads as well, and because the differences were directly correlated to the behavioral symptoms of the children. Vaccarino suggests that the neurological differences might thus be causally related to autism, but she cautions that more families are needed to draw definitive conclusions. Her team also plans to study cells from patients with autism and normal head sizes.

At the level of the whole organ, researchers have revealed a biological indicator of autism buried in the deepest folds of the brain. Researchers used to believe cortical folds in the brain are set before birth, but Christine Deruelle at the Institut de Neurosciences de la Timone in Marseille, France, and colleagues recently used MRI imaging to show that sulcal pits—the deepest part of each fold of the cerebral cortex—continue to deepen after birth, and that the number of sulcal pits increases when a child is about 2 years old, a common age of onset for autism. Comparing the brains of boys with autism, aged 2 to 10, with boys suffering from a development disorder or typically developing boys, the researchers found that certain

WHAT'S IN A GENE?

Many of the genes involved in autism affect chromatin structure, while others produce proteins involved in receptor-signaling pathways, synapse development, axon targeting, and neuron motility.

Functional category	Exemplar genes
Chromatin structure	*CHD8, TBL1XR1*
Signaling pathways	*DYRK1A, SCN2A, PTEN, CTNND2, GRIN2B*
Synapse development	*RAS, SHANK2/3, RhoA/B, MAPK3, SynGAP1*
Axon targeting	*TBR1, NRXN1, NLGN3*
Actin network	*MYH11, FLNA, CYFIP1*
Neuron motility	*DCC, WNT*

sulcal pits were shallower among children with autism. In a small region of the brain involved in language and communication called Broca's area, the sulcal pits in the autism patients appeared to be wasting away—although, paradoxically, the deeper the pit, the more impaired a child's social communications skills were.[9] "It's like the maturation of this small part of the brain was disrupted," says Deruelle.

Such retrospective studies cannot distinguish between causes and consequences of the disorder. Yet a prospective study that begins following children before they develop symptoms would require a prohibitively large number of participants to ensure that a sizable quantity of autism patients are enrolled, considering that only 1 in 45 to 68 children in the U.S. develops the disorder each year. That is, unless a scientist studies a population of children at higher risk for autism.

Mustafa Sahin, a neurologist at Harvard Medical School and Boston Children's Hospital, works with children suffering from a rare genetic disorder called tuberous sclerosis (TSC), characterized by the growth of benign tumors in the brain and other vital organs. Over the years, Sahin has found that about 50 percent of children with TSC develop autism. And because TSC is typically diagnosed in utero or as an early newborn, these kids constitute a population that researchers could follow to study the onset of autism, Sahin reasoned.

In an initial brain imaging study in 2013, his team found that the children with TSC who develop autism have less connectivity, and less-organized connectivity, in their brains.[10] His team is now doing the prospective study he's long hoped for—scanning the brains of 150 infants born with TSC and following them from birth to age 3 to try to determine why some develop autism and others don't. He also plans to test therapies to see if it's possible to prevent the development of autism in this highly susceptible population. In the first year of life, for example, TSC patients often develop epilepsy—another disorder marked by abnormalities in brain activity—so Sahin and colleagues will begin to treat the newborns with an epilepsy medication, and monitor how that affects the onset of autism.

Once a few treatments are discovered, autism research will once again follow in the footsteps of cancer research, where a drug approved for one cancer type subsequently proved beneficial in others.

"So far, studies done in humans have focused on relatively old individuals affected with autism, and they have not been that successful," says Sahin. "Here, we have the capability of doing an intervention in children who don't have autism yet, [to] see if we can prevent it."

Starting small

Prevention is a lofty goal when there are yet few treatments for autism. But as more details about the diverse disorder emerge, the potential for personalized treatments is gaining traction. Bernier and Eichler, for example, are planning a clinical trial of autism patients with a mutation in *SCN2A*, which encodes a sodium channel. They hope to test the effectiveness of a US Food and Drug Administration–approved medication that has successfully treated mice with sodium channel disruptions. Meanwhile, at Boston Children's Hospital, Sahin is running a clinical trial studying biomarkers in children with autism and a *PTEN* mutation that disables a tumor suppressor in the body and has been linked to macrocephaly and language and social difficulties.

Bernier admits that a drug for one genetic subtype will likely be applicable to less than half of 1 percent of individuals with autism. But it's something, he says. "If we can have a meaningful impact in a small group, that's fine. Let's just get started somewhere."

And, perhaps, once a few treatments are discovered, autism research will once again follow in the footsteps of cancer research, where a drug approved for one cancer type subsequently proved beneficial in others, says Sahin. "We don't have one form of autism we can treat yet, but hopefully one day soon we will have one or two forms. Then, we'll see if others can benefit from those treatments." ■

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References

1. R. Bernier et al., "Disruptive CHD8 mutations define a subtype of autism early in development," *Cell*, 158:263-76, 2014.
2. B.J. O'Roak et al., "Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations," *Nature*, 485:246-50, 2012.
3. B.J. O'Roak et al., "Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders," *Science*, 338:1619-22, 2012.
4. E. Hanson et al., "The cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically ascertained population," *Biol Psychiatry*, 77:785-93, 2015.
5. I. Iossifov et al., "The contribution of *de novo* coding mutations to autism spectrum disorder," *Nature*, 515:216-21, 2014.
6. X. Zhao et al. "A unified genetic theory for sporadic and inherited autism," *PNAS*, 104:12831-36, 2007.
7. I. Iossifov et al., "Low load for disruptive mutations in autism genes and their biased transmission," *PNAS*, 112:E5600-07, 2015.
8. J. Mariani et al., "FOXG1-dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders," *Cell*, 162:375-90, 2015.
9. L. Brun et al., "Localized misfolding within Broca's area as a distinctive feature of autistic disorder," *Biol Psychiatry Cogn Neurosci Neuroimaging*, 1:160-68, 2016.
10. W.W. Lewis et al., "Impaired language pathways in tuberous sclerosis complex patients with autism spectrum disorders," *Cereb Cortex*, 23:1526-32, 2013.

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2016 TOP 10 INNOVATIONS

The Literature

IMMUNOLOGY

Special Forces

THE PAPER

J. Wang, P. Kubes, "A reservoir of mature cavity macrophages that can rapidly invade visceral organs to affect tissue repair," *Cell*, 165:668-78, 2016.

The immune system is best known for fighting infections and targeting anything it senses as foreign. But it also serves a less-appreciated, but crucial, duty: swooping in when the body's own cells are injured or dying.

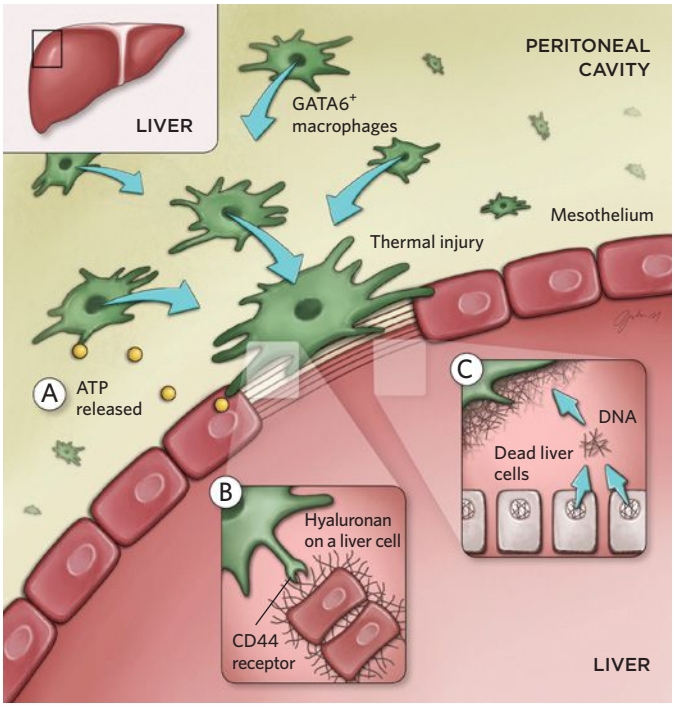
University of Calgary immunologist Paul Kubes has been working toward understanding this lesser-known role of immune cell function. Working in mice, he and postdoc Jing Wang burned a tiny spot on the surface of the liver and used fluorescence microscopy to observe what happened next. Many of the expected cells, such as platelets and neutrophils, showed up at the wound, but there was also a surprise arrival: a cell type that "seemed to be doing very, very important things in allowing for healing . . . and we had no idea where this cell was coming from," says Kubes.

The cells, which arrived within the hour, expressed general markers of macrophages, immune cells known for engulfing foreign cells and debris, but their source was unclear. They couldn't be the liver's resident macrophages, as those are stationary, and the injury had obliterated local cells. Nor could they be derived from macrophage precursors called monocytes, because that process of recruitment and differentiation takes days.

Rather, the cells expressed GATA6, a marker specific to large macrophages from the peritoneum, the body cavity that surrounds visceral organs such as the liver. The result revealed a job no one had known these cells were performing. "It shows that the peritoneal cavity . . . actually contains macrophages which themselves translocate into damaged tissues and therein actually help drive the repair process," says Steve Jenkins, who studies the cells at the University of Edinburgh.

When Kubes and his colleagues transferred GFP-labeled peritoneal macrophages into a mouse with liver damage, the glowing green cells flocked to the injury. When the labeled macrophages were injected into the bloodstream, however, they did not reach the wound, indicating that the peritoneal macrophages took a nonvascular path.

Whatever the route, the actions of these peritoneal macrophages are important for healing. Once at the liver, the macrophages dismantled the nuclei of dead cells, releasing DNA into the injured area, which could possibly protect the area from infection by trapping microbes, Kubes says. And the wounded areas in mice whose peritoneal macrophages had been depleted regrew blood vessels more slowly than in mice whose macrophages were intact.



TO THE RESCUE: Upon liver injury, GATA6$^+$ peritoneal macrophages sense ATP released from the wound and migrate toward it (A). At the injury site, macrophage-liver interaction is mediated by binding of macrophages' CD44 to the carbohydrate hyaluronan exposed on the injured tissue (B). The macrophages degrade the nuclei of the dead hepatocytes and a layer of released DNA forms a cover across the wound (C).

To better model human liver damage such as cirrhosis, the researchers treated the mice with the liver toxin carbon tetrachloride, which, unlike the thermal injuries, wreaked internal organ damage. They found that the peritoneal macrophages migrated across the mesothelium, the membrane separating the liver and other internal organs from the body cavity, and into the liver to a depth of several cell layers.

"One of the more important . . . areas this research would go in, then, is actually to find out what the role of peritoneal macrophages would be in chronic, repetitive liver damage," such as that caused by alcohol abuse, says Jenkins, adding that repeated carbon tetrachloride administration in rodents could serve as a model for such hepatic harm. —Ashley P. Taylor

SEA TO LAND: Feral horses on Sable Island, Canada, munch on marram grass that has been enriched with nitrogen from local seal populations.

ECOLOGY

Following the Food

THE PAPER

P.D. McLoughlin et al., "Density-dependent resource selection by a terrestrial herbivore in response to sea-to-land nutrient transfer by seals," *Ecology*, doi:10.1002/ecy.1451, 2016.

ISLAND LIVING

Working on Sable Island, Nova Scotia, population ecologist Philip McLoughlin noticed that many of the local feral horses visited a small spit of land on the island's west coast to eat marram grass and other vegetation. But flicking back through photos of the area, the University of Saskatchewan researcher found that 50 years ago, the spit had been just a strip of sand. "Something had happened since the 1960s to make this an important area for the horses," he says.

SEAL EXPLOSION

One thing the team knew had changed was the number of pupping gray seals in the area. In the 1960s, the population was probably under 1,000, McLoughlin says; now nearly 400,000 seals use the island. The animals could be transferring nutrients from the sea via defecation or their decaying carcasses, McLoughlin reasoned—promoting vegetation growth, and consequently influencing the behavior of the island's largest herbivores.

PICKY EATERS

McLoughlin and colleagues used stable isotope measurements in marram grass to show that seals do indeed enrich vegetation with nitrogen. They then used modeling to demonstrate that horses preferentially selected those areas of nitrogen-enriched vegetation to eat. "It was really neat," says Douglas McCauley of the University of California, Santa Barbara. "It takes the stage with a handful of studies that are wonderful examples of how intimately connected living systems actually are."

THE CIRCLE OF LIFE

McLoughlin now wants to look at how the seals' impact perpetuates. "The next step is to ask what this means for how horses move around, their population dynamics, and how they are distributing these nutrients across the island." —Catherine Offord

TRAINSPOTTING: Railside populations of *Arabidopsis thaliana* exhibit low genomic diversity thanks to selection on an organelle mutation.

GENETICS & GENOMICS

Catching a Lift

THE PAPER

P.J. Flood et al., "Whole-genome hitchhiking on an organelle mutation," *Curr Biol*, doi:10.1016/j.cub.2016.03.027, 2016.

STAYING ALIVE

In the U.K. between 1957 and 1992, herbicides used along hundreds of kilometers of railway track provided strong selection for plants with a chloroplast mutation known to confer resistance. When geneticist Pádraic Flood identified herbicide resistance genes in *Arabidopsis thaliana* sampled in Cornwall—400 km from where they were first identified in 1988—he and his colleagues at the Max Planck Institute for Plant Breeding and Research saw a rare opportunity to study indirect, genome-wide effects of adaptive selection on an organelle.

LUCKY ASSOCIATION

Flood found that the adaptive mutation had arisen just once in railside populations of ***A. thaliana***, and that these populations now had low genetic diversity across the entire genome. "There's been a massive increase [in the frequency of] that nuclear genome—which is not doing anything particularly special—merely because of its association with this organelle that provides resistance to herbicide," Flood explains.

RAIL PASS

The nuclear genome isn't the only thing hitching a ride. Flood found the same genotype all along that 400 km of rail network, implying that trains and their passengers helped spread the herbicide-resistant plants. This spread "will have a lasting consequence," Flood suspects, "because now this nuclear genome and its associated alleles are at a high frequency."

NOT A ONE-OFF?

The scale of the impact is "really quite striking," says Stephen Wright of the University of Toronto, adding that although the concept of nuclear genes hitchhiking on organellar mutations is uncontroversial in self-fertilizing species like *Arabidopsis*, such processes are rarely observed in nature. Flood suspects the phenomenon may be more common than realized, and hopes to investigate it by outcrossing species with the same adaptive chloroplast mutation. —Catherine Offord

SARAH MEDILL; PÁDRAIC FLOOD

PROFILE

Set on Sequencing

At 77, Clyde A. Hutchison III continues to work at the lab bench, trying to piece together a living cell from scratch.

BY ANNA AZVOLINSKY

As an undergraduate at Yale University, Clyde Hutchison III was required by his financial-assistance package to have a part-time job. Planning to major in physics, he had lined up a sophomore-year job with an astrophysicist. But Hutchison returned to school to find that the professor had given the job to someone else. "I was pretty upset, because the people assigning jobs wanted me to work in an accounting office, and I was not interested in that. I pleaded with them to find me a science job." Hutchison got his wish: he was assigned to work in Harold Morowitz's biophysics lab, a placement Hutchison says has directed his career trajectory up to the present day. He worked with Morowitz's postdoc, Carl Woese, who went on to discover Archaea as the third domain of life. "I went from working in the dining hall freshman year to working with Carl Woese, one of the most influential people in biology." With Woese, Hutchison studied chemicals, including L-alanine, that could trigger bacterial spore germination, publishing his first paper in 1958.

"One of the themes that runs through my work is that I like to think small. . . . The smaller the thing you are studying, the more chance you have of trying to understand the whole thing."

While Hutchison always knew that he would major in science and, emulating his chemist father, that he would pursue a PhD and a career as a researcher, it was the influence of the Morowitz lab that pulled him from physics to biology. In 1960, he moved on to Caltech to do PhD in biology.

Here, Hutchison traces his path from studying genomes to synthesizing them, explains why he likes to "think small," and reveals why he still finds himself at the lab bench after all these years.

HUTCHISON HATCHES

Late bloomer. Hutchison was born in 1938 in New York City, where his father, Clyde A. Hutchison Jr., a physical chemist, was a postdoc at Columbia and later returned there to participate in the Manhattan Project, working on isotope separation. Hutchison attended first grade at the Horace Mann School (then a part of Teachers College, Columbia University), where he refused to learn to read. "It was fashionable at the time to not learn phonics, but full words. The teacher would hold up a sign that said 'cat,' but didn't mention the letters, nor that the written language was linked to the spoken one, or that the letters stood for sounds," says Hutchison. The school was permissive, so Hutchison opted to spend reading lessons hiding in forts that he built out of blocks. In 1945, when he moved with his mother and sister to a small town in Ohio following the end of World War II (his father had gone ahead to the University of Chicago to set up his laboratory), his second-grade teacher was shocked to learn that the young Hutchison couldn't read. "That's when the teachers told me about the letters, and I quickly learned how to read."

Drawn to genetics. At Caltech, Hutchison joined the lab of biophysicist Robert Sinsheimer, who was studying the ΦX174 bacteriophage. Hutchison was drawn to the gene mapping that faculty members Robert Edgar and Max Delbrück were doing with mutants of other phages. "I wanted to apply the same approach to ΦX174, which had a relatively small genome, to get a detailed picture of its genes." In Sinsheimer's lab, Hutchison used the genetic techniques he was learning in other labs within the department to help characterize how the phage infects bacteria and to identify and map genes on ΦX174's 5,000-base-pair genome.

Time of his life. Hutchison stayed at Caltech for eight years. "Graduate school is a perfect existence; I wish I was still there! You can work a lot in the lab and you can focus, because you don't have much money so you don't have to waste time spending it." During his time at Caltech, Hutchison collaborated with Marshall Edgell, a Sinsheimer lab postdoc. Sinsheimer recommended both scientists to the University of North Carolina at Chapel Hill, which was recruiting new faculty members. Both Hutchison and Edgell became assistant professors there in 1968 and ran their labs jointly for about 25 years. They had seen a publication by Hamilton Smith on type II restriction enzymes (which would later earn Smith a Nobel Prize) and became early adopters of the enzymes as a tool to study DNA. Along with Edgell, Hutchison's lab showed that the ΦX174 genome could be cut up into specific DNA pieces. His student June Middleton did the first screen for novel restriction enzymes from *Haemophilus aegyptius*, identifying a number of new enzymes including endonuclease Z, now called HaeIII.

HUTCHISON HARVESTS

Maternal heredity. Hutchison next decided to apply restriction enzymes to analyze more-complex mammalian genomes. But to keep things simple, his lab focused on the 16-kilobase mammalian mitochondrial genome. Prior studies had demonstrated that amphibian mitochondrial DNA is

COURTESY OF CLYDE HUTCHISON

CLYDE A. HUTCHISON III

Distinguished Professor, Synthetic Biology Group,
J. Craig Venter Institute, San Diego, CA
Professor Emeritus, University of North Carolina, Chapel Hill

Greatest Hits

- Along with Marshall Edgell, developed a marker rescue assay for specific fragments of the ΦX174 phage genome
- With colleagues, provided the first evidence of the maternal inheritance of mitochondrial DNA in mammals
- Took part in sequencing the first DNA molecule, the genome of the ΦX174 phage
- Codeveloped site-directed mutagenesis with Michael Smith
- Codiscovered L1, the most abundant transposable element in the mammalian genome
- Took part in creating the first synthetic minimal genome

inherited from the mother, and Edgell and Hutchison wanted to test whether the same was true in mammals.

Isolating mitochondrial DNA from the livers of horses, donkeys, mules (a hybrid with a horse mother and a donkey father), and hinnies (a donkey-mother, horse-father hybrid) they showed that the hybrid had the same mitochondrial DNA pattern as the mother. The work, published in 1974, demonstrated the maternal nature of mitochondrial inheritance in mammals.

Sequencing points the way. In 1975, Hutchison spent a yearlong sabbatical in Fred Sanger's lab in Cambridge, England. There, Hutchison learned how to sequence DNA using the method Sanger had recently developed and helped to sequence the genome of ΦX174, the first DNA molecule to be completely sequenced. "At the time, other researchers would say to me, 'Why would you want to sequence DNA?' Some people didn't perceive the DNA sequence thing as interesting." But ΦX174 turned out to have interesting features: a compact genome with overlapping genes. "The protein sizes hadn't added up correctly according to the DNA sizes. I think we never would have figured out the virus's genes using mutational genetics. I think this helped to stimulate the rapid development of sequencing. But maybe that's just an egocentric view of the history."

Ahead of the pack. While in Sanger's lab, Hutchison met Michael Smith, a University of British Columbia researcher also there on sabbatical. Smith had been working on methods to enzymatically link nucleotides together to form oligonucleotides before automated machines began to churn out DNA oligos. Hutchison and Smith collaborated to develop site-directed mutagenesis of the ΦX174 genome using small oligonucleotide primers synthesized in Smith's lab that could introduce base pair substitutions. Smith shared the 1993 Nobel Prize in chemistry for helping to develop the method. Hutchison also began to sequence murine hemoglobin genes, again, because the task was "relatively small and approachable," says Hutchison. "Coming back from Sanger's lab, we were in a unique position. Our lab was initially the first in the U.S. to use Sanger's sequencing method, and then it quickly caught on and everyone was doing it." Within the beta globin cluster, Hutchison and Edgell's lab discovered a transposable, repetitive element in the mammalian genome called long interspersed repetitive element one (L1). L1—the most abundant transposable element in the mammalian genome—encoded a gene that looked like reverse transcriptase, which synthesizes a DNA molecule from RNA. The repeated element was later established as a retrotransposon.

Minimal genome. Focusing on small genomes led Hutchison, in 1990, to begin work with *Mycoplasma genitalium*, which has the smallest known genome for an independently replicating cell. With graduate student Scott Peterson, Hutchison and colleagues used a random sequencing approach, which placed markers on the physical map of the *M. genitalium* genome akin to shotgun whole-genome sequencing. "We didn't have the wherewithal to get the whole sequence, but we got a few short sequences, and this meant we could identify genes by comparing sequences with other genes and make estimates of the fraction of the genome that coded for proteins." The paper caught the eye of Hamilton Smith, who was then collaborating with Craig Venter to sequence the *Haemophilus influenzae* genome. The *H. influenzae* sequence was published in 1995, the first full cellular genomic sequence to be determined. Smith called Hutchison, and thus began the initial collaboration among the three scientists. That same year, the trio published the complete sequence of *M. genitalium* using whole-genome random sequencing and assembly. Hutchison then took a sabbatical year at Venter's Institute for Genomic Research (TIGR) in Rockville, Maryland. At TIGR, Hutchison developed a global transposon mutagenesis method to see whether disruption of each of *M. genitalium*'s genes could still result in a viable organism. The study, published in 1999, suggested that as many as 350 of the organisms' 480 protein-coding genes are essential.

What retirement? Since 1996, Hutchison has collaborated with TIGR, spending part of his time doing bench work there. "As I was approaching a normal retirement age, I thought it would be interesting to work at TIGR since I had been at UNC for a long time." Hutchison moved to TIGR in 2005; the following year it was merged with several other organizations to form the J. Craig Venter Institute (JCVI), headquartered in Rockville. In 2007, Hutchison moved to a new JCVI campus in La Jolla, California.

Synthetic biology beginnings. "It was during those experiments identifying nonessential genes in mycoplasma that Craig, Ham, and I started thinking about synthesizing genomes and about synthesizing the 5,000-base-pair ΦX174 genome as a test case," says Hutchison. They reported the construction of that synthetic genome in 2003. "Those experiments took a while because the mutation rates in the chemically synthesized DNA pieces were too high, so we kept getting inaccurate assembly." Their synthetic viral genome, however, was not the first: Eckard Wimmer's lab, in 2002, had made a synthetic poliovirus. Hutchison and his colleagues subsequently synthesized the much larger (half a million base pairs) genome of *M. genitalium* in 2008. "For that, it was key that Gwynedd Benders figured out how to clone these genomes as extra chromosomes in a yeast cell."

Putting it all together. In 2010, Hutchison, along with Daniel Gibson and others, synthesized the 1.1 million–base-pair genome of the bacterium *M. mycoides* from scratch, assembled it inside a yeast cell, and then transplanted the genome into a related bacterium, *M. capricolum*. Called JCVI-syn1.0, it was the first cell controlled by a synthetic genome and the culmination of the team's work since 1995. "What was key, besides cloning these genomes as yeast extra chromosomes, was that one of the postdocs, Carol Lartigue, had learned how to transplant the genome of one mycoplasma species into another. We switched to *M. mycoides* because we couldn't figure out how to transplant the *M. genitalium* genome."

Down to the basics. This year, Hutchison and his colleagues built on this JCVI-syn1.0 cell using global transposon mutagenesis to create an *M. genitalium* cell with a minimal genome. They worked with eight overlapping DNA segments, delineating which genes were essential, nonessential, or what the team called quasi-essential (the organism could live without them, but their absence significantly slowed its growth). "It took three cycles and many years. The genome we ended up with is about 100 genes bigger than the number of known essential genes, and we don't have a clue as to what 149 of these genes do, which is surprising."

End goal. "To me, a minimal genome is less important than having one where we know what all of the parts do. If we have that, we can make a computer model of how the cell works. Having a computer model based on a living cell will be a satisfactory explanation of how gene function predicts cell behavior and what happens if you add or take away genes or change the environment," Hutchison says.

HUTCHISON HERE AND NOW

Music spurts. Hutchison took piano lessons in elementary school but quit after five years. In his forties, he began to take jazz piano lessons and now plays once a week at a bar restaurant in La Jolla as "Clyde and Mac." "Mac is a MacBook Air that plays bass and drums."

Predictions. It's been 17 years since Hutchison's 1999 work on the minimal mycoplasma genome. "In the 1999 paper, we had discussed the building of a synthetic genome, and it was Craig's encouragement to put the sentence at the end of the paper that synthesizing a genome from scratch was the way to test this. It seemed a bit out there to me at the time, but it's happened! To me, 17 years seems like a short time."

Think small. "One of the themes that runs through my work is that I like to think small. The reason is that the smaller the thing you are studying, the more chance you have of trying to understand the whole thing."

Hands-on. "I've always thought it was important to keep doing laboratory experiments. It is hard to make time for it between writing grants and mentoring and other obligations, but I've always thought it was important because, first, I do like doing it, and second, you can easily lose touch with what is reasonable to do in the lab and what is reasonable to expect from someone else if you aren't doing it yourself." ■

Cullen Buie: Electrifying Bacteriology

Associate Professor, Department of Mechanical Engineering, MIT. Age: 34

BY ANDY EXTANCE

Frustrated by microbes that refused to grow during a 10-day visit to his Australian collaborators' lab in 2012, Cullen Buie attended an emergency lunchtime meeting that ended up reshaping his research. The MIT engineer had wanted to use his team's 3-D microfluidics tool to determine the effectiveness of bacteria for use in microbial fuel cells by analyzing the properties of their surface membranes. But the recalcitrant microbes left him "twiddling his thumbs." Buie's University of Queensland collaborators suggested giving him different *E. coli* strains to analyze. "They said, 'Maybe you can distinguish pathogens from nonpathogens in your devices,'" Buie recalls. "We saw huge differences in their responses." That result started his transition from fuel cells to a deeper focus on microbial physiology.

This detour brought Buie closer to his original intention to major in premed as an undergraduate, as his older sister had. But that early plan had changed before he even started college at Ohio State University, when he visited the university's office of minority affairs, which suggested that an engineering major could either lead to a later medical degree or provide an alternative career. Then, Buie discovered an engineering summer school with college-level classes offering scholarship money to students who did well. "I signed up for the program on Friday, and it started on Sunday," he says.

Deciding to stick with engineering, Buie did his PhD on microfluidic electro-osmotic pumps for fuel cells with Juan Santiago at Stanford University, finishing in 2009. He then landed a faculty job at MIT, but first took a postdoctoral post with Liwei Lin at the University of California, Berkeley, studying microbial fuel cells. There Buie used his PhD experience to manipulate bacteria, specializing in dielectrophoresis, Lin explains.

Buie continued to explore how electrical fields influence bacterial outer cellular envelopes, designing more-sensitive tools.[1] Then, six months after he took up his MIT post, Buie's 35-year-old sister died of sepsis. "It was mind-boggling to me that in 2010 people still died of bacterial infections," Buie says.

After his Australian epiphany, Buie realized that his research might help prevent the same thing from happening to others. Going beyond *E. coli,* Buie and his MIT and Queensland colleagues showed that dielectrophoresis could distinguish phenotypes of particularly virulent *Pseudomonas* strains.[2]

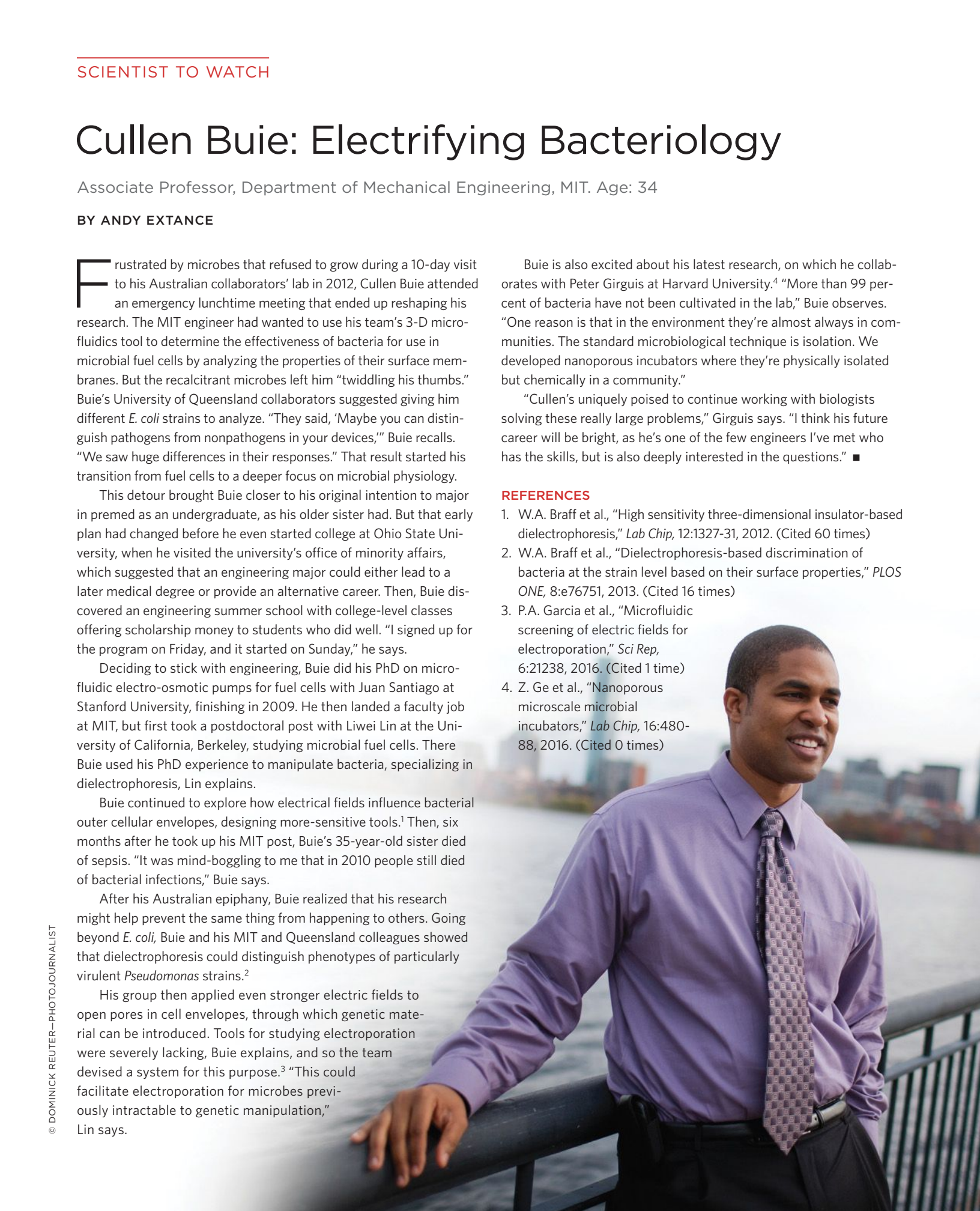
His group then applied even stronger electric fields to open pores in cell envelopes, through which genetic material can be introduced. Tools for studying electroporation were severely lacking, Buie explains, and so the team devised a system for this purpose.[3] "This could facilitate electroporation for microbes previously intractable to genetic manipulation," Lin says.

Buie is also excited about his latest research, on which he collaborates with Peter Girguis at Harvard University.[4] "More than 99 percent of bacteria have not been cultivated in the lab," Buie observes. "One reason is that in the environment they're almost always in communities. The standard microbiological technique is isolation. We developed nanoporous incubators where they're physically isolated but chemically in a community."

"Cullen's uniquely poised to continue working with biologists solving these really large problems," Girguis says. "I think his future career will be bright, as he's one of the few engineers I've met who has the skills, but is also deeply interested in the questions." ■

REFERENCES

1. W.A. Braff et al., "High sensitivity three-dimensional insulator-based dielectrophoresis," *Lab Chip,* 12:1327-31, 2012. (Cited 60 times)
2. W.A. Braff et al., "Dielectrophoresis-based discrimination of bacteria at the strain level based on their surface properties," *PLOS ONE,* 8:e76751, 2013. (Cited 16 times)
3. P.A. Garcia et al., "Microfluidic screening of electric fields for electroporation," *Sci Rep,* 6:21238, 2016. (Cited 1 time)
4. Z. Ge et al., "Nanoporous microscale microbial incubators," *Lab Chip,* 16:480-88, 2016. (Cited 0 times)



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Lane Patrol

A look at some image analysis software

BY AMBER DANCE

When you're looking at bands on a Western blot, it's often obvious which lane has a lot of protein or a little. But it's not always easy to tell with the naked eye precisely how two bands compare. Plus, looks can deceive. For example, if a lane widened or a band developed a "smile" as the gel ran, a protein-heavy band might look faint.

That's where imaging software can help, by putting numbers on the density of a band. Just draw a box around your band and the program will tell you the pixel density. Some programs do much more, such as quantifying the number of colonies on a petri dish or the intensity of fluorescent signals in a 96-well plate. "It's just an improvement on the eyeball," says Jeff Silk, president of Silk Scientific, in Orem, Utah, which sells gel analysis software.

Researchers have plenty of options for software to bolster their own vision, ranging from freebies that work with any type of image to expensive programs or ones that are specific to their imaging machinery. Unlike Photoshop or other general image-editing software, these dedicated programs can often automatically detect where each lane starts and ends or sum up multiple bands. They can also winnow away background signal, which arises from a variety of causes such as nonspecific binding of antibodies to the entire blot or overexposure when imaging.

One important factor to consider is what file types the software accepts, says John Wiktorowicz, a professor at the University of Texas Medical Branch at Galveston. Some use a proprietary format linked to the company's imaging system, while many accept generic formats such as JPEG or BMP. Wiktorowicz, director of proteomics for the biomolecular resource facility at the university, prefers to work with TIFFs. "It leaves the values intact," he explains. Other formats such as JPEG compress the file size and could result in loss of crucial information. "I would never use JPEGs for any quantitative study," says Wiktorowicz.

Any imaging software should save raw data files, and record any modifications users make to an image. "When it comes time to publish, you need that information," says Mark Chen, a graduate student at Duke University.

For certain users, such as those who produce pharmaceuticals, a complete data trail is required by the Food and Drug Administration, as laid down in the Code of Federal Regulations Title 21, Part 11 (CFR 21 Part 11). This is particularly important for drug production and quality control, and some clinical labs may want this added layer of record keeping and password security, says Raymond Miller, a product manager at Bio-Rad in Hercules, California.

The simplest choice, Chen says, is to work with the software that comes with your imager. For researchers who want more options, *The Scientist* profiles five imaging programs.

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IMAGEJ

imagej.net/Welcome

The public domain ImageJ software platform, developed at the National Institutes of Health and augmented by various users, is loved by some and hated by others. ImageJ fan Corentin Cras-Méneur, an assistant professor at the University of Michigan Medical School in Ann Arbor, appreciates the flexibility. "It's one shop for everything," he says. "With ImageJ I would analyze Western blots, I would do some quantifications of fluorescent microscopy, I would control the microscope . . . anything you can think of."

For most users, standard ImageJ should be sufficient to analyze bands on a gel or Western, Cras-Méneur says. But for those who want more, many plug-ins are available; coders versed in the Java language can also create their own plug-ins or macros. For example, Cras-Méneur uses a plug-in that analyzes how background signal varies across an image and subtracts it from bands accordingly, instead of assuming the background is uniform.

PROS

- With a variety of plug-ins available, ImageJ is flexible.
- It supports stacked images, such as pictures collected from a series of different levels using a confocal microscope.

CONS

- ImageJ wasn't specifically designed for gel or blot analysis, and can be intimidating to new users. While many common questions are answered in online forums, there's no tech support line to call if you have a specific query. "It's a lot easier if you have someone around you who has been using it," says Cras-Méneur.
- Letitia Jones, a postdoc at the University of Rochester Medical Center in New York, disliked the fact that ImageJ typically uses a single box size for all bands on a gel, even if some bands stretched or smiled. She found that, depending on the box drawn by individual users, the results varied quite a bit. "Two people can have totally different conclusions," says Jones. She prefers Image Studio (see below) because it allows her to customize the box to each band.
- Juan Pablo de Rivero Vaccari, an assistant professor at the University of Miami Miller School of Medicine, says ImageJ's results didn't match what he saw with his own eyes—he could tell a band was darker, but the numbers coming out of the software didn't back him up. He switched to another program, UN-SCAN-IT gel (see below).

IMAGE STUDIO

www.licor.com/bio/products/software/image_studio_lite

The Image Studio software comes with imaging instruments from LI-COR Biosciences, but any scientist can download the Lite version. It's the same software, minus the ability to control imagers.

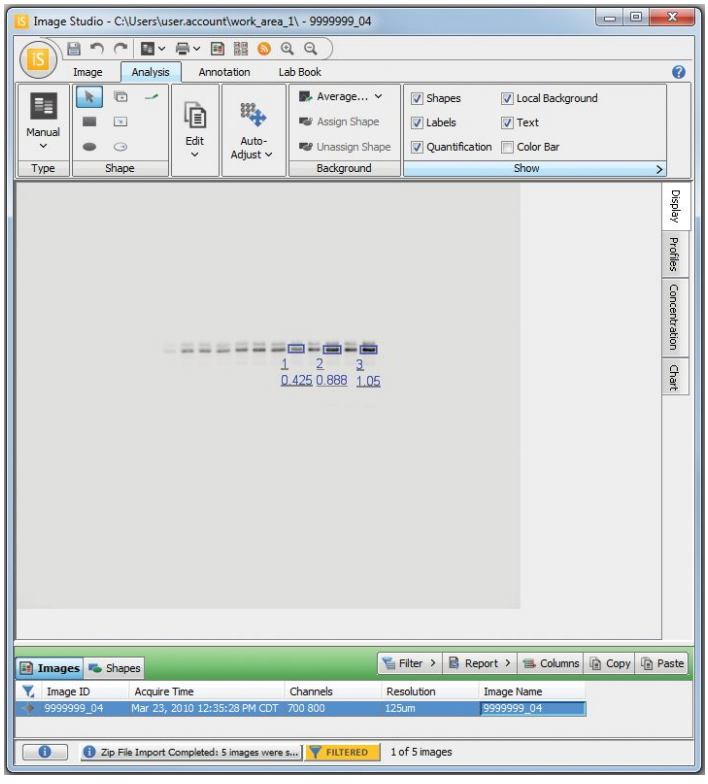
Most users are happy with the Lite version, notes Jeff Harford, senior product marketing manager at LI-COR in Lincoln, Nebraska. However, LI-COR also sells additional, optional features. These include analysis of two-color or in-cell Westerns; a small-animal imaging tool that allows users to draw shapes around objects such as tumors or organs; and a tool to analyze multiwell plates.

PROS

- Chen says the design reminds him of Microsoft Word or Excel, making Image Studio easy to pick up.
- You can sort your files based on parameters such as image date, the type of analysis, or the fluorescent color channels you used. "It's easy to find old images," says Chen.
- You can customize the box around each band, to fit bands that stretched or smiled.

CONS

- The software is primarily focused on a few types of analysis, notes Harford. Scientists who want other functions, such as colony counting or microscopy image analysis, could do it with Image Studio but may prefer to look for software dedicated to their needs.
- Jones notes that many tutorials are video format, while she has a harder time finding the written instructions when she wants them. Harford says written quick-start guides and tutorials are available.



SOME, MORE, MOST: All programs discussed in this article can quantify band weight. LI-COR's Image Studio shown here.

IMAGE LAB

www.bio-rad.com/en-us/product/image-lab-software

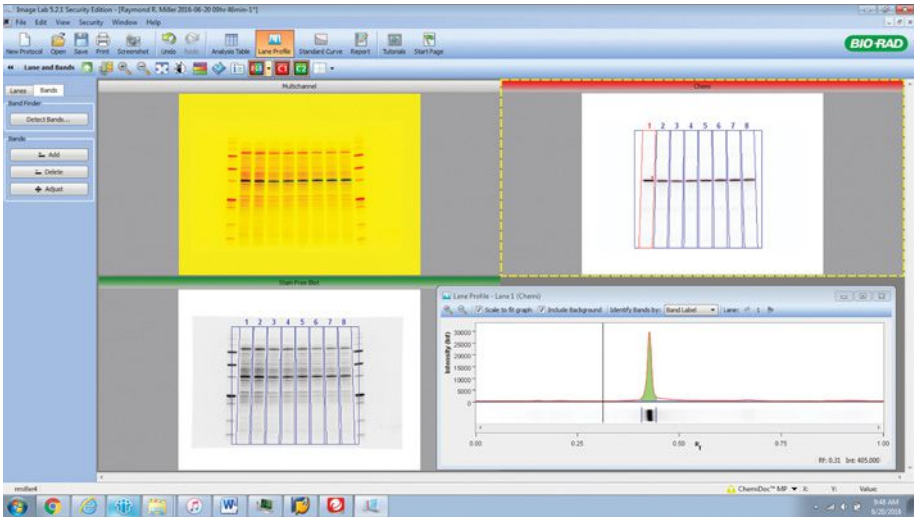
Bio-Rad imagers, such as the Gel Doc or ChemiDoc systems, come with the Image Lab software. Researchers can use the software to control the machine and analyze data right on the spot, or transfer the files to a computer for analysis. The newest version of the software features a touchscreen and controls the ChemiDoc Touch.

Image Lab offers numerous features—so many that some users find it overwhelming. One can annotate bands, compare bands to molecular weight standards, and much more. "We're trying to move away from the concept of just drawing boxes around bands," says Miller.

PROS

- The software makes it easy to program your imager for your needs, automatically filling in parameters such as the filters necessary for a Western blot or a Ponceau stain.
- One can perform "total protein normalization," comparing bands of interest to the total protein in each lane, based on labeling such as Ponceau stain. This is more accurate than the common method of labeling housekeeping proteins such as actin to standardize protein load across lanes, says Miller. The result is a ratio of band intensity to total protein in a lane.
- It automatically subtracts the local background around each band, rather than using a single background value.

COURTESY OF LI-COR BIOSCIENCES



LINEUP: Bio-Rad's Image Lab software can automatically define lanes and graph the density of signal across each, as can UN-SCAN-IT gel and CLIQS.

CONS

- Users can find the abundance of features and lengthy manual intimidating. Chen, who tried Image Lab, recalls, "I felt like somebody needed to train me how to use it."
- It isn't very good at automatic lane detection, though Miller says Bio-Rad plans to improve this feature.
- Image Lab mostly uses Bio-Rad's proprietary .SCN file format, though users can perform some basic analyses with TIFFs, as well as export TIFFs for use in other programs. The company is considering opening it up to more generic formats, says Miller, but adds that users who don't have Bio-Rad imagers won't get much benefit from the software.

UN-SCAN-IT gel

www.silkscientific.com/gel-analysis.htm

UN-SCAN-IT gel was designed to work with any kind of imaging platform—even a regular office scanner or digital camera. It can quantify Westerns, dot blots, gels, and thin-layer chromatography plates. "I like its simplicity," says user de Rivero Vaccari. "I just need a pixel 'thing' and that's it." Scientists typically export the data to other programs such as Microsoft Excel or SigmaPlot for analysis and visualization.

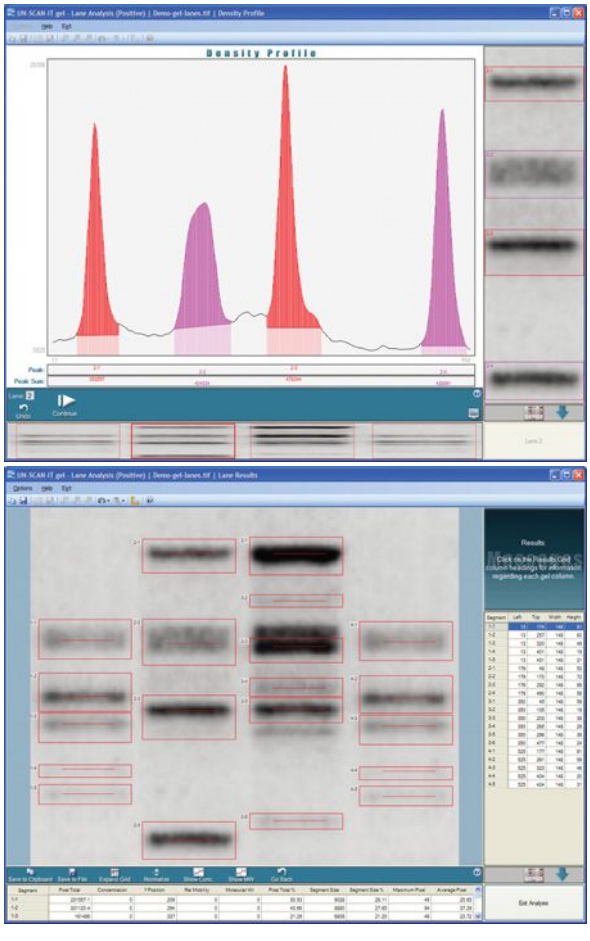
UN-SCAN-IT gel also comes bundled with Silk Scientific's UN-SCAN-IT software, which analyzes hard-copy graphical input—such as a line graph in an old publication or a strip of paper from an analog electrocardiogram—when you don't have the original data. It digitizes the graph and extrapolates the numbers that likely generated it.

PROS

- It's easy to use, says de Rivero Vaccari.
- When you draw a box around a band, the software graphically shows the distribution of pixel intensity. It will automatically suggest the borders of the band, but you can adjust the borders of the box based on the graph. "You can use that scientific intuition," Silk says.
- When two or three bands overlap, the software will help you distinguish them.
- It's made by a small, focused company. "You get me when you call for customer support," says company president Silk.

CONS

- UN-SCAN-IT gel lacks some of the fancy features of some other programs; for example, it can't process 2-D gels.
- De Rivero Vaccari finds it annoying that the software often stretches his original image in the main viewer window, which can make discrete bands look like smears. Silk says the user can choose to stretch the image or maintain the original aspect ratio.



SETTING BOUNDARIES: All programs discussed let you fiddle with band edges based on the image and density plots. Shown here: UN-SCAN-IT gel from Silk Software.

COURTESY OF BIO-RAD; COURTESY OF JEFFREY SILK

CLIQS

totallab.com/cliqs/

Totallab's Core Laboratory Image Quantification Software (CLIQS, a combination of two programs formerly known as Phoretix and Totallab Quant) offers a variety of functions to analyze gels, blots, and microplate arrays, and to count colonies. It can automatically detect lanes and bands, calculate molecular weights based on a standard, and subtract background. It can also perform fairly basic measurements of spots on 2-D gels.

Totallab's family of imaging programs also includes CLIQS 1D Pro, SameSpots, and SpotMap. CLIQS 1D Pro version includes additional features for analysis of one-dimensional gels and plates. For example, it looks for matches between complex band patterns, such as the restriction fragment length polymorphisms used in DNA fingerprinting.

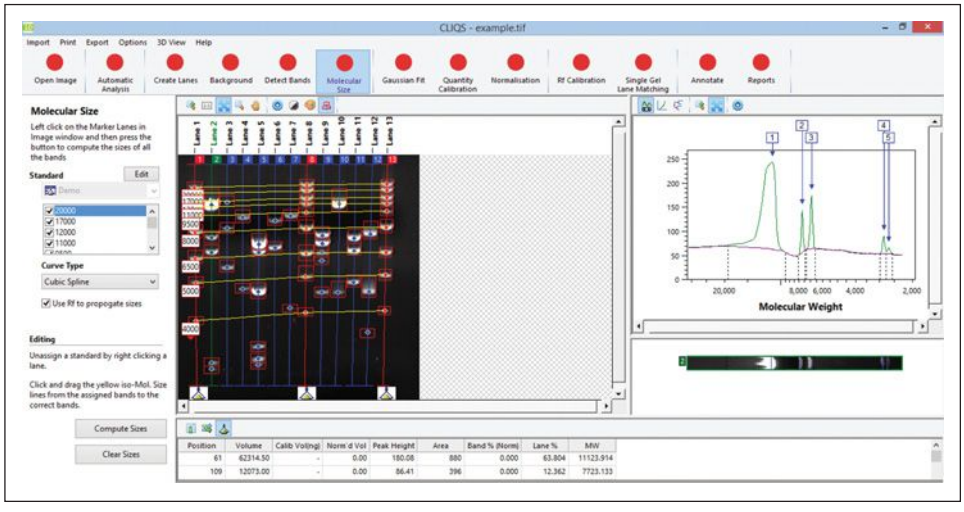
SameSpots and SpotMap are designed for in-depth analyses of 2-D gels. SameSpots allows you to compare multiple gels and match up the spots between them. SpotMap adds the capability to compare those gels to Westerns, and allows more-extensive image editing; you can add or delete spots. It is particularly useful for analysis of residual host cell protein content in biopharmaceutical production.

PROS

- CLIQS is one program with a wide variety of functions that works with any imager.
- It's easy to extract the numerical data on the bands for statistical analyses, says Denis Wafula, a postdoc at the University of Maryland, College Park, who used the program during his PhD at Florida Agricultural and Mechanical University in Tallahassee.
- You can use total protein normalization or housekeeping proteins to control for protein levels.

CONS

- It can be a bit difficult to figure out the program at first, though it's easy once you get going, says Wafula.
- With multiple, overlapping programs available from Totallab, it can be difficult to identify the right choice for your lab's needs, Wafula adds. ■



SIZE IT UP: All programs discussed in this article will compute protein size based on a lane with standard markers. Shown here is CLIQS from Totallab.

COURTESY OF TOTALLAB

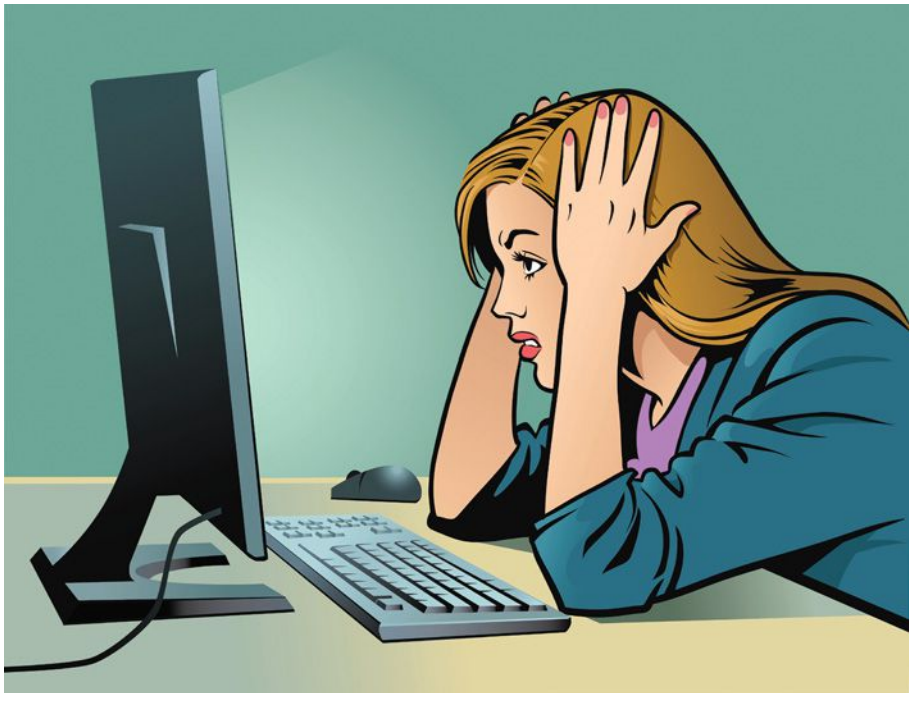
STATS

Program/Company	Cost	Platform	CFR 21 Part 11 compliance (FDA)
ImageJ	Free	Mac/Windows/Linux	No
Image Studio LI-COR	Lite version is free. Keys for small-animal, microplate, or multiplex Westerns cost $750 each; the key for in-cell Westerns is $2,175. Some of those features come with certain LI-COR instruments.	Mac/Windows	For those purchasing LI-COR instruments, it is possible to add a custom, CFR 21 Part 11–ready option to the package, but customers are still responsible for ensuring compliance.
Image Lab Bio-Rad	Although the website lists a price of $1,085, anyone can download the software for free simply by creating a Bio-Rad account.	Mac/Windows	A security license costs $3,705.
UN-SCAN-IT gel Silk Software	$445 (perpetual) or $129 (annual); demo version allows you to try it with a couple of your own images.	Mac/Windows	No
CLIQS Totallab	GBP850; the company can arrange a trial.	Windows	Yes

Taking Command of the Command Line

How to build bioinformatic pipelines using Galaxy

BY JEFFREY M. PERKEL



Huge swaths of modern biomedical science run on high-throughput DNA sequencers. These instruments can pump out data at an astonishing clip, producing gigabytes or more a day. But the end of the sequencing run is not even close to the end of the experiment. Researchers must somehow convert all those As, Cs, Gs, and Ts into knowledge by filtering, assembling, and interpreting the raw data to create a coherent biological picture.

That's the role of bioinformaticians, and for labs lucky enough to have one on staff, data analysis is just an email request away. Many labs, though, aren't so lucky. It's not a lack of tools that is the problem: most popular bioinformatics software is free and open source. But downloading and installing those tools isn't necessarily easy. Nor, for that matter, is using them. (See "Learning Bioinformatics," *The Scientist*, July 2016.)

That's because sequence-analysis tools largely run on the computer "command line," invoked not with a mouse and clickable control elements but via lengthy and bewildering textual instructions that specify, say, which reference genome to use or the minimum size of a sequence match. Often these tools depend on other software to function, and are run in series, with the output of one tool being fed into another in a so-called "pipeline." But as with any method—and make no mistake, says Carole Goble, a professor of computer science at the University of Manchester, U.K., bioinformatics *is* a method—researchers often need to try many variations and permutations to make their analyses work just right. And they must track precisely which data went into each analysis, the location of the data files that were created in the process, and the tools (and version numbers) used, if they are to properly document and repeat their work.

For all these reasons, many biologists are intimidated by bioinformatics. But there are tools to help, including Galaxy.

According to James Taylor, an associate professor of biology and computer science at Johns Hopkins University and one of the project's originators, Galaxy provides a point-and-click web interface alternative to the bioinformatics command line, thus allowing researchers to easily create, run, and troubleshoot analytical pipelines.

"The first goal is really to make complex analysis more accessible," Taylor explains.

And, because Galaxy maintains a detailed record of precisely what analyses each user has run and in what order, the software also fosters reproducibility, making it possible to repeat and share analyses, and/or revisit them at a later date.

The Scientist asked Taylor and other informaticians how researchers can build their own pipelines. Here's what they said.

How do I get started?

Perhaps the easiest way to begin is to create an account at usegalaxy.org. This is a free, public, shared Galaxy "instance," or running copy, that you can use without downloading the software onto your own computer—the equivalent of a public terminal in the library. This instance of Galaxy comes preconfigured with many of the most popular bioinformatics tools. As a shared resource, it is somewhat limited, Taylor admits: "We have to have quotas for the amount of disk space and the number of concurrent analysis jobs someone can have." And some tools simply are not available at usegalaxy.org. (Some 80+ other publicly shared Galaxy servers, each featuring slightly different tool sets, also are available with a guide

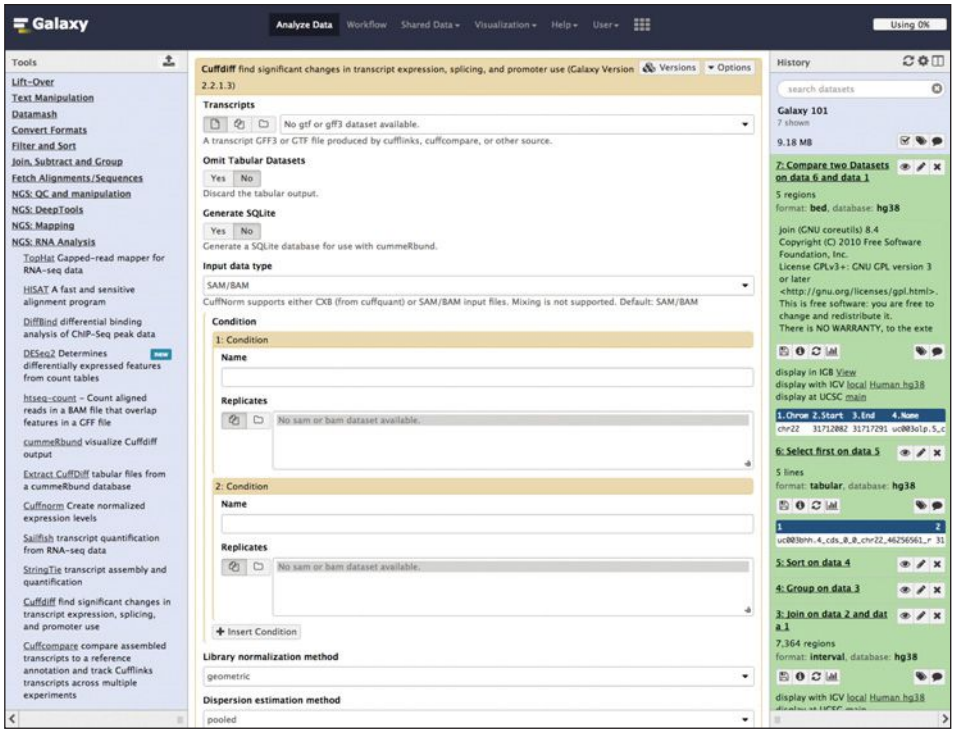
to their capabilities at wiki.galaxyproject.org/PublicGalaxyServers.) Those needing more privacy or specialization can run Galaxy on the Amazon cloud, using the Cloud Launch tool (launch.usegalaxy.org/launch). But, given the highly unpredictable cost of cloud computing, Taylor says, a local installation may be the most economical long-term option, assuming users have the IT support necessary to install and maintain the software (wiki.galaxyproject.org/Admin/GetGalaxy).

How do I upload my data?

However it's run, the main Galaxy interface comprises three panels—a tool menu at left, a history at right, and a tool interface in the center. Users can import data by launching "Get Data" from the tool menu and selecting either a local file or remote resource. "If you have large sequence data sets that are on a server somewhere and accessible, you can fetch them directly from the URL into Galaxy," Taylor says. Alternatively, Galaxy provides an interface to pull data off several remote servers, such as the UCSC Genome Browser (useful for downloading the coordinates of all human exons, for example), BioMart, and various model organism databases.

How do I find a new tool?

If you know the name of the tool you want to use (for instance, the alignment software Bowtie), just enter it in the tool menu search pane; if it's there, it's ready to run as is. Alternatively, assuming users have the necessary authority (that is, they are running a local or cloud-based Galaxy), they can install new tools from the Galaxy Tool Shed (toolshed.g2.bx.psu.edu). With some 3,990 tools currently available, the Tool Shed is a resource for sharing, documenting, and keeping track of different software versions in Galaxy, Taylor says. And that's important, because it's not always enough simply to have the most up-to-date version of a given tool; different versions may use slightly different algorithms, or sport unanticipated bugs.



WORKING WITH GALAXY: The main Galaxy interface includes a toolbox (left) and workflow history (right). The large center panel is where users can view data and configure tools. Shown in the history panel is a portion of the Galaxy 101 tutorial, while the center panel shows the configuration page for the Cuffdiff tool.

"Any tool that goes into the Tool Shed, you can always go back and get that configuration later."

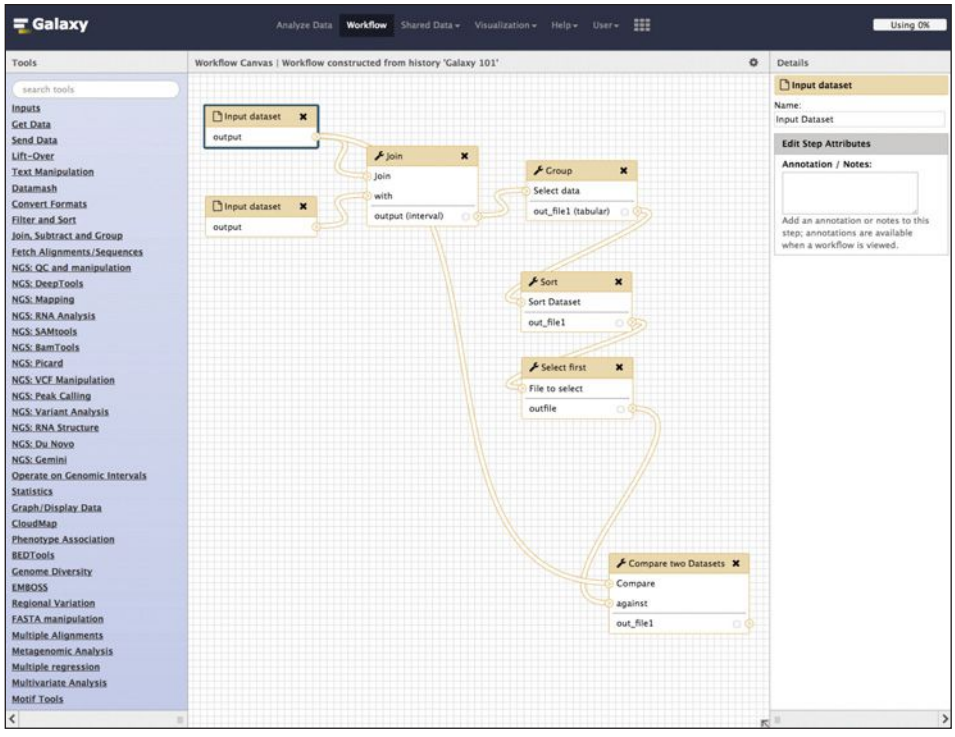
New tools that aren't in the Tool Shed can also be imported; simply create an XML configuration file that describes the tool's parameters, default settings, and instructions for mapping them to graphical elements on the screen. But newbies needn't worry about how to do that, Taylor says: "Typically it's the tool developers who actually will end up writing those config files."

How can I build a new workflow?

Users can build pipelines directly on the "Workflow Canvas," a graphical drag-and-drop interface in which tools are configured and interconnected by linking the output of one to the input of another. Say you want to use Trimmomatic to remove adapter sequences from a set of Illumina reads, map them against a reference genome with BWA, and call variants with FreeBayes. Simply select each application from the tool menu at left to place it on the canvas. Configure its behavior using the configuration panel at the right, and insert it into the workflow by creating connections between the appropriate input and output points. Alternatively, you can extract a workflow from the history pane, if you've already run through the desired steps once. Just select "Extract Workflow" from the History menu to apply those same steps to a new data set.

How can I run a published workflow?

More than 80 Galaxy workflows have been reported in the literature, according to PubMed, for research areas such as genomics (e.g., MGEScan), proteomics (e.g., Galaxy-P), and metabolomics (e.g., Galaxy-M and Workflow4Metabolomics). If the developer has shared that workflow in a public Galaxy instance, users can easily access and launch it via the Shared Data > Workflows menu item. If not, other options are available. Some developers create "virtual machine" implementations or "Docker containers"—preconfigured, ready-to-run software installations that users download and then launch locally or onto a server, for example, in the Amazon



BUILDING A WORKFLOW: The workflow editor canvas provides a graphical interface for creating, configuring, and modifying analytical pipelines. Above, the history shown in the screenshot at left has been converted into a workflow using the "Extract Workflow" menu option.

cloud (details vary, but published papers generally provide instructions). Others make their tools available via the Galaxy Tool Shed or git repository, as is the case with Galaxy-M. Professor Mark Viant and postdoc Ralf Weber, both of the University of Birmingham, U.K., built Galaxy-M in 2015 as a way to share their custom algorithms with the broader metabolomics community (*GigaScience*, 5:10, 2016). "We wanted to take metabolomics and make it more approachable and digestible to biologists," Viant explains, "not just to the analytical chemist or to the computational scientist."

How can I access external web services?

Suppose a user wanted to build a pipeline to align their sequence reads to a reference genome, identify key sequence variants, and then search external databases, such as Ensembl or PubMed, to triage those results. Galaxy alone cannot do that, says Michael Cornell, a clinical bioinformatics scientist at the University of Manchester, U.K. Its "centralized approach" requires administrators to bring resources, such as databases, in-house into their local Galaxy environment, he says.

Here's where another pipeline tool, Taverna, can come in handy. According to Goble, Taverna (taverna.incubator.apache.org) is more of a power users' tool, sporting "first-class support" for web services. (Web services are interfaces that users query remotely in order to run a simulation or render graphics, for instance.) "Researchers often use Galaxy and Taverna in tandem," she says. Cornell, who lectures on clinical bioinformatics at Manchester to teach clinical scientists the ins and outs of sequence analysis, has helped students build such workflows. In one example, they identify variants in Galaxy, send them first to a web service at the University of Leiden called Mutalyzer to ensure the variants are named according to proper nomenclature, then on to PubMed to identify key literature citations, and finally return the results to Galaxy for further processing. "The two systems are quite complementary," he says. (Some Taverna workflows are publicly available at MyExperiment.org.)

How can I document my workflow?

Once a user has created a workflow, they can download it or share it with others. (Select the workflow from the Workflows menu item and select "Share or Download" to obtain a link.) They can also create an annotated "Galaxy Page," a web document that combines the workflow and plain-text explanations so researchers can document precisely what a workflow does, for instance, as supplementary data to a published paper. "You can actually write up the analysis or whatever and embed workflows and data sets in that description," Taylor says. (Here's one example: usegalaxy.org/u/xjasonx/p/hpgv-2.)

Where can I find more information?

There's no shortage of online documentation for Galaxy (wiki.galaxyproject.org/Learn), including screencasts (vimeo.com/galaxyproject), tutorials (e.g., Galaxy 101: github.com/nekrut/galaxy/wiki/Galaxy101-1), online courses (such as this one at Coursera: www.coursera.org/learn/galaxy-project), lectures available at vimeo.com/album/3456144, and more. There's also an annual Galaxy Community Conference (held June 25–29 this year at Indiana University in Bloomington), offering both scientific and technical talks as well as two days of in-person training.

Should I consult a bioinformatician anyway?

Short answer: yes. Taverna is not intended for newbies. Galaxy is easier by far, and more user-friendly than the command line, but it can be complicated to use nonetheless. Setting up a local Galaxy instance is particularly challenging, but even firing up a virtual machine on Amazon isn't trivial. Pipelines and workflows are methods like any other, Goble says, and they too must be planned and debugged and revisited, not tossed into the mix as an afterthought. Her advice: invest in computational expertise, rather than circumventing it. "Recognize that the bioinformatician is a key component of the scientific team." ■

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Rising from the Cornfields

Iowa's biotechnology sector is growing—and diversifying.

BY JENNY ROOD

In the early 2000s, cystic fibrosis researchers were frustrated with available mouse models of the disease. The mice carried the mutant version of the *cystic fibrosis transmembrane conductance regulator (CFTR)* gene that causes the disease in people, but the animals didn't develop the mucus-coated lungs, coughing, or breathing difficulty observed in human patients. Researchers at the University of Iowa (UI) thought they might have a solution—genetically modified miniature pigs. In September 2008, the team published their results: the mutant pigs had defective transmembrane chloride ion transport, the molecular hallmark of cystic fibrosis that leaves human patients' skin tasting salty. Additionally, the newborn pigs shared human CF infants' defects in the intestine, pancreas, and liver (*Science*, 321:1837-41, 2008). It was later found that, unlike the mice, the animals developed CF-like lung disease within two months of birth (*Sci Trans Med*, 2:29ra31, 2010).

Several factors aligned to make the mutant *CFTR* pigs possible. The pig was already a well-established medical research model, and its genome was being sequenced. It probably also didn't hurt that the scientists were working in the U.S.'s top pork-producing state. Buoyed by their success in cystic fibrosis, the UI researchers thought pig models could help crack other human diseases for which mouse models had been inadequate. In collaboration with John Swart, then an executive director at Boehringer Ingelheim's Iowa operations, they created a startup to commercialize the idea. Exemplar Genetics of Sioux Center, in northwestern Iowa, was born.

Iowa is "a really good place to emerge as a leader in biotech," says Joe Hrdlicka, executive director of the Iowa Biotechnology Association, a nonprofit trade organization for the sector. The state has a competitive advantage in what Swart calls "value-added agriculture," or products that build on primary commodities such as crops and livestock, partly due to the workforce: "You can hire staff who are very familiar with livestock, but who are highly educated and very interested in doing something that they see really makes a difference in the world," he says.

Companies like Exemplar also benefit from private and government investment to keep innovative biotech in the state. Iowa spends 12 percent more than the national average on biosciences research, and UI and Iowa State University (ISU) plow some 67 percent of their research budgets into the sector. Together, these factors are giving rise to varied and innovative biotech ventures—from riffs on Iowa's agricultural origins to new advances in human medicine and diagnostics that are unrelated to pigs and corn.

An agricultural past—and present

Iowa has long been blessed with agricultural bounty, which has made it the leading US producer and exporter of crops such as corn and soybeans. "We have the best soils in the world," says Robert Brown, a biofuels researcher at ISU. The land's productivity has been further boosted by many decades of academic and industry research; DuPont Pioneer, based in the Des Moines suburb of Johnston, recently celebrated 90 years of optimizing seeds to improve crop yields. Originally founded to serve Iowa farm-

ers—many of whom still use its products—the company now develops hybrid and genetically modified seeds for global farming challenges, such as AQUAmax hybrid corn lines for water-limited environments and Plenish soybeans with no trans fats. Most recently, Pioneer scientists have used the CRISPR-Cas9 gene editing system to cut years off the production time of waxy corn hybrids, the raw material for cornstarch.

The company feels connected to its Iowa community, says Jerry Flint, vice president of industry and regulatory affairs: Pioneer collaborates with smaller local firms at its Innovation Center and maintains close ties with nearby ISU in Ames. Specifically, Pioneer is part of the Cultivation Corridor initiative, set up by ISU and more than a dozen industry and civic partners in 2014 to attract investment in the state's agricultural biotechnology businesses. And some of the country's most productive acres fall within a 300-mile radius around the company's main campus, says Flint—placing Pioneer researchers in close proximity to farmers growing the crops they've developed.

Iowan researchers are also branching out from traditional uses of their crops, finding new ways to turn that productive land into valuable commodities. The state's biofuel industry, for example, has flourished thanks to government support, expertise in milling techniques, and an abundance of raw material. Post-harvest corn leftovers known as stover, which serve as a source of cellulose for the production of ethanol, can also be used to generate fossil-fuel alternatives. The Center for Biorenewable Chemicals (CBiRC), a National Science Foundation–supported research center based at ISU, is developing chemical intermediates to replace petroleum-based products. CBiRC unites researchers from across the country and around the world, has partnered with more than 30 industry members, and has spun off ten startups in the center's first eight years of operation.

The Iowa government is also supporting the biomaterials push; the state legislature recently passed the first state-level production tax credit for biobased chemicals other than biofuels. Beyond plastics and polymers, CBiRC Director and ISU professor of chemical and biological engineering Brent Shanks says that some of the high-value chemicals the center makes could serve as antimicrobials, and they are currently testing the insecticidal properties of some of their compounds against mosquitoes carrying the Zika virus.

Iowa spends 12 percent more than the national average on biosciences research, and UI and Iowa State University (ISU) plow some 67 percent of their research budgets into the sector.

Moving towards medicine

These innovative twists on agriculture are also giving rise to companies, such as Exemplar Genetics, that straddle the line between traditional Iowan industries and biomedicine. Ames-based animal vaccine producer Harrisvaccines is another example. Recently acquired by Merck as part of its animal health division, the company was launched in 2005 by then ISU professor Hank Harris and his postdoc Matt Erdman, who identified and sought to develop a vaccine from a wild strain of the devastating porcine reproductive and respiratory syndrome virus (PRRS). They teamed up with AlphaVax, a human vaccine company based in Raleigh, North Carolina, to apply its proprietary alphavirus-based technology to veterinary medicine for the first time.

Because Harris was already based at ISU, it was logical to start labs and manufacturing in Ames, says his son Joel Harris, a postacquisition associate director at Merck Animal Health. There, they also had the advantage of direct contact with the USDA Center for Veterinary Biologics, located just down the road, which sped up the approval process for their new method of creating animal vaccines. Since the initial PRRS vaccine, Harrisvaccines has produced the first pig vaccines on the market for swine flu and porcine epidemic diar-

HERE PIGGIE: Exemplar Genetics of Sioux Center in northwestern Iowa develop lines of human-size "minipigs" that could serve as more-accurate models of human disease.

EXEMPLAR GENETICS

rhea virus, as well as bird vaccines following the 2015 outbreak of avian flu that plagued the state's chicken flocks. "It has almost made us the first responders for new animal diseases," Joel Harris says.

While Harrisvaccines has used technology designed for humans to benefit animal health, Exemplar Genetics has designed animals to study human disease. In the eight years since Exemplar's founding, it has developed pig models of cardiovascular diseases, neurological disorders, cancer, and more. It's also adding services to its portfolio: housing the human-size minipigs for urban researchers who don't have space, for example, and running safety and efficacy trials of new therapeutics using its porcine subjects. Pigs might prove more reliable than rodents for the purpose, says Swart. For example, when Exemplar treated its cardiovascular pig model with Lipitor, it turned out that "they respond almost exactly like humans, and very unlike the mouse," in which the drug had no effect, he notes.

Other Iowa-based companies are not just testing new medicines, but developing them, too. Ames-based NewLink Genetics creates cancer immunotherapies, as well as vaccines for Ebola and Zika, for example, while KemPharm, located just outside of Iowa City, is using a novel prodrug approach—creating inactive compounds that are only metabolized into their active form within the body—to battle opioid abuse. Specifically, KemPharm generates opioid-based compounds that only act in the intestine, and are thus ineffective if snorted or injected.

By launching in Iowa, these and other Iowan companies have taken advantage of one of the nation's lowest costs of doing business: Iowa ranked seventh lowest of the states in 2014. The relatively low cost of living provides an added bonus, notes UI psychiatrist Rob Philibert, company CEO and principal founder of Iowa City–based Behavioral Diagnostics Inc., a company that's developing quantitative tests of tobacco and alcohol use based on the level of DNA methylation at certain genetic loci. "You often get twice the person at a third of the price," he says.

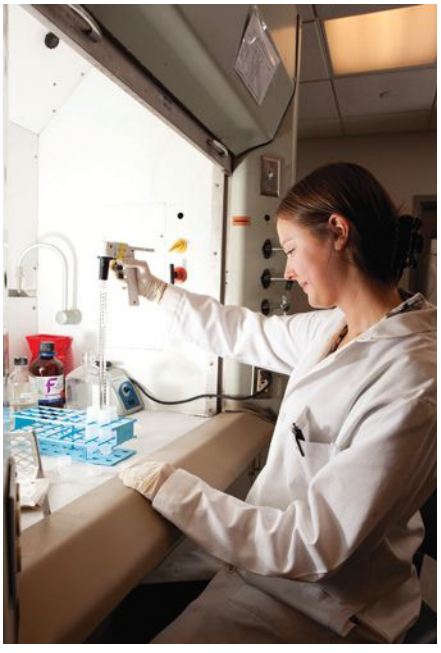
And for his company specifically, everything Philibert and his colleagues need to produce the methylation-based biomarker tests, which he hopes will enhance treatment-program compliance and even curb childhood smoking and alcohol use, is close by. The diagnostic relies on saliva-collection kits made by IBI Scientific in Peosta, outside of Dubuque. The DNA is then bisulfite-sequenced to assess the level of methylation, using primers synthesized by Integrated DNA Technologies (IDT), the world's largest producer of oligonucleotides, which is headquartered in the Iowa City suburb of Coralville. And upcoming clinical trials of the alcohol diagnostic will be carried out in the Quad Cities, 60 miles to the east.

The draw of Iowa for biotech ventures is clear. Companies such as Exemplar Genetics, Harrisvaccines, NewLink, KemPharm, and Behavioral Diagnostics have contributed to the 10 percent growth of the state's drug and pharmaceutical sector since 2001—a stark contrast to the 3.1 percent shrinkage of the industry nationwide in the same time frame. "We've done a lot more innovation than people might give us credit for," says Hrdlicka.

Building a biotech corridor

Several of these companies, including Exemplar Genetics, Behavioral Diagnostics, IDT, and KemPharm are among the 45 businesses in biotech and high tech housed at UI's Research Park in Coralville. Harrisvaccines is located in a similar research park at ISU. Both Swart of Exemplar Genetics and Joel Harris of Merck note the dramatic growth of these university-based clusters of companies over the past decade, and see them as the basis for a successful biotech corridor.

Part of that growth comes from targeted government support in Iowa's research strengths. Iowa Economic Development Authority (IEDA) spokeswoman Tina Hoffman notes that the state government has a history of identifying growth opportunities in niche areas, such as biobased chemicals, and finding ways to support them. "The state is very committed," says CBiRC's Shanks. IEDA also provides a tax credit for research activities, which can help support fledgling companies just getting off the ground. The focus is "trying to find the place where government can come in and, with a little bit of assistance, close some gaps," Hoffman says.



VAX PRODUCTION IN THE MIDWEST: A researcher at Harrisvaccines/Merck Animal Health conducts an RNA-particle production protocol that the company uses to manufacture vaccines for animal diseases.

Where should the government fill in next? Hrdlicka of the Iowa Biotechnology Association suggests that one area ripe for investment might be a common strength shared by the state's agricultural and medical biotechnology industries: the innovative use of genetics. Swart of Exemplar Genetics sees value in those techniques not only at his own company but in Iowa's capacity to create cost-effective green fuels, develop solutions for livestock diseases, and reduce feed waste. With great universities and government investment, he says, "the state is really set up for success." ■

Jenny Rood is a freelance science writer based in Cambridge, Massachusetts.

HARRISVACCINES/MERCK ANIMAL HEALTH

A Paleolithic Patriarch

Thanks to modern research, Neanderthals are being transformed from brutish troglodyte into something more human.

BY LYDIA PYNE

On August 3, 1908, the first near-complete Neanderthal skeleton was discovered in a cave near the village of La Chapelle-aux-Saints in south central France, during a survey of the region's Paleolithic archaeological sites.

For decades prior, prehistorians had collected bits and pieces of curious but not-quite-human fossils from museums and excavations alike—the odd skull here, a scrap of tooth there. In 1863, the mélange of bones was finally given its own species designation, *Homo neanderthalensis*. Forty-five years later, the La Chapelle discovery was the first Neanderthal specimen found in an original archaeological context and the first to be expertly excavated and carefully studied. Because the body was arranged in a flexed, fetal position and carefully placed in the floor of the cave, excavators argued that fossil—nicknamed the Old Man—had been purposefully buried by his Neanderthal contemporaries.

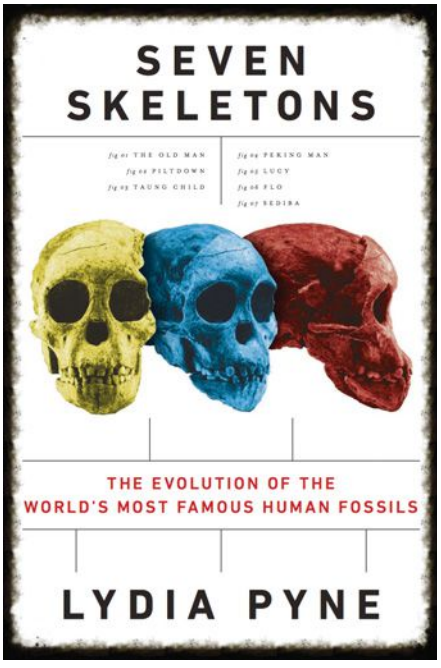
More than any other single individual, the Old Man of La Chapelle has shaped the way that science and popular culture have thought about Neanderthals. But why? What is it about *this* Neanderthal's story that is so special? In short, the Old Man was the right fossil found at the right time. He was—and still is—offered as a key bit of evidence in debates about evolution and human origins. He quickly became a scientific touchstone, an archetype for how science and popular culture create celebrity fossils. I explore the stories of similarly spectacular paleoanthropological finds in my new book *Seven Skeletons: The Evolution of the World's Most Famous Human Fossils*.

Once they had excavated the fossil, the discoverers sent the Old Man remains to Marcellin Boule, an eminent expert in human evolution at the Muséum National d'Histoire Naturelle in Paris, for careful study. Boule spent two years examining the fossil, and his initial analysis of the La Chapelle Neanderthal would shape the perception of our evolutionary cousins for a hundred years—preconceptions that contemporary archaeologists and paleoanthropologists are doing their utmost to counter.

Boule concluded that Neanderthals were sad specimens of nature. He argued that the species was stooped in its posture and stunted in its culture. Boule's conclusions quickly turned into the pop-culture caricature that we tend to associated with the Neanderthal species. The image of a hunched, cave-dwelling lout barely capable of brandishing a club quickly caught the public's imagination in the early 20th century thanks, in no small part, to the portrayal of Neanderthals in museums and in the press. (How could a creature so primitive as a Neanderthal, the logic went, have something as complex as a culture that involved burying the dead?) It was no wonder, Boule's work implied, that the species went extinct, especially compared with the superior *Homo sapiens*.

The conclusions Boule drew from his analysis of the La Chapelle skeleton couldn't have been more wrong.

Today, we're rather used to the idea that Neanderthals had a vibrant culture, but science and society's acceptance of each new piece of the Neanderthal story is an uphill battle, thanks to the Old Man's early days in the public's eye. We now have archaeological evidence that



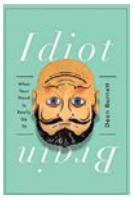
Viking, August 2016

Neanderthals built structures; that they had sophisticated hunting strategies, fire-starting technologies, and art; and, of course, that they buried their dead. Analyses of Neanderthal DNA show us more and more similarities between ourselves and Neanderthals, with every indication that modern humans and Neanderthals interbred in their evolutionary history. Every "human" behavior we can claim to separate ourselves from our Pleistocene relatives, we eventually find in Neanderthals, blurring the line between human and not.

Decades of researchers have studied the Old Man since Boule's original analysis. Every new iteration of the Neanderthal's story humanizes him, turning the fossil from a dim troglodyte into a dignified paleo patriarch. The more we study the Old Man, the more the differences between our species melt away. ■

Lydia Pyne is a writer and research fellow in the Institute for Historical Studies at the University of Texas at Austin. Read an excerpt from Seven Skeletons *at the-scientist.com.*

Idiot Brain: What Your Head Is Really Up To
Dean Burnett
W. W. Norton & Company, July 2016



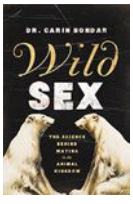
We've all had that feeling: you know the one, where it seems as though your own brain is conspiring against you. Cardiff University neuroscientist Dean Burnett certainly has, and he's devoted his debut book, *Idiot Brain*, to explaining the biology behind insomnia, blackouts, blank stares, and other instances where our most powerful organ acts pretty powerless.

"Bottom line: the brain is fallible," he writes in the book's introduction. "It may be the seat of consciousness and the engine of all human experience, but it's also incredibly messy and disorganized despite these profound roles."

But we can learn something from the brain's occasional hiccups, Burnett explains. Understanding these temporary lapses—such as how we can recognize a face but blank on the name that goes with it—highlights the stark differences between our modern lives and the cauldron in which the human brain was forged millennia ago.

Featuring a healthy dollop of humor (not surprising from Burnett, who dabbles in stand-up comedy), *Idiot Brain* is a refreshing dive into the neuroscience that strives to make sense of all those times when our brains just don't make sense.

Wild Sex: The Science Behind Mating in the Animal Kingdom
Carin Bondar
Pegasus Books, August 2016



Population ecologist Carin Bondar has made a career out of sex. *Wild Sex* in particular. In her latest book, which carries the same title as her web video series, Bondar expounds on her favorite subject: the smorgasbord of biology and behaviors that govern the world of animal copulation.

"The notion of normal when it comes to sex is completely impossible to define, which is an important point to keep in mind," she writes in the introduction to *Wild Sex*. "There is really no regular way that sex can or should happen. The human-derived notion of what happens 'naturally' is about to get blown out of the water, because if we are going to label 'natural' as all the things that happen in nature, we've got to grasp that natural could mean stabbing one's partner in their forehead with a razor-sharp penis, or fertilizing juvenile females before their eggs are even mature."

Bondar's tour of the oddities of animal sex doesn't stop at the piercing penis of the male sea slug. She goes ever deeper into the evolution, equipment, and behaviors that make the topic so titillating.

Why Diets Make Us Fat: The Unintended Consequences of Our Obsession with Weight Loss
Sandra Aamodt
Current, June 2016

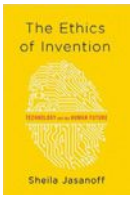
Dieting is big business. Americans, among the most overweight people on Earth, spend inordinate amounts of money, energy, and time every year battling the bulge. This despite the fact that most diets—even those that result in dramatic weight loss—typically end with the dieter gaining back the weight they lost, and then some.

Neuroscientist Sandra Aamodt counts herself as one of millions trapped in a cycle of expanding and contracting waistlines. She relays her personal story, and through it details the biological and psychological perils of dieting, in her latest book, *Why Diets Make Us Fat*.

Aamodt tells of her eventual, and simplistic, success in breaking free from the vicious circle. "As my New Year's resolution in 2010, I vowed to go an entire year without dieting or weighing myself and to exercise every day," she writes. "I was so pleased with the results that I've maintained all three habits ever since. My weight used to bounce around a fifty-pound range as I starved or binged my way through the first half of my adult life, but now it remains stable even though I don't worry about calories anymore."

The key to ditching the practice of dieting is to understand how our brains, running on the prime directive to keep our bodies alive, drive our habits and routines, Aamodt suggests. "Instead of fighting with our brains, we can accept their limitations and arrange the circumstances to create new habits that support our well-being," she writes.

The Ethics of Invention: Technology and the Human Future
Sheila Jasanoff
W. W. Norton & Company, August 2016



Humanity is firmly ensconced in the technology age. But the amazing progress our species has made in the relative blink of an eye is accompanied by thorny ethical dilemmas. In her latest book, *The Ethics of Invention*, Sheila Jasanoff of the Harvard Kennedy School of Government takes aim at the balance between risk and promise in our modern race to innovate.

Biomedicine features prominently in the book. The crux of the matter: modern advances in genetics and genomics carry with them tons of ethical baggage and controversy. The rise of genetically modified organisms is a case in which technology has outpaced "the theories and practices of global governance," Jasanoff writes. And of the privacy and ethical issues surrounding the decoding and manipulation of the human genome, she writes: "The decades following the unraveling of the genetic code witnessed intense and widespread social experimentation with the stuff of life. . . . What emerged very clearly from this ferment is that disentangling the laws of life went hand in hand with creating new entanglements between lives and laws."

Jasanoff reminds us that as we forge ever further into a brave new future, technology expands what we can do, but ethics dictates what we should do. —Bob Grant

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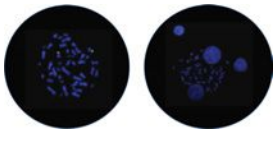

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Myxococcus Micrographs, 1982

BY TRACY VENCE

Starting in the late 1970s, Stanford University's Dale Kaiser worked for years to visualize a certain bacterial phenomenon. Microbiologists had known that, when starved, some soil-dwelling myxobacteria aggregate, forming so-called fruiting bodies full of hardy spores. Yet capturing this behavior in action, Kaiser found, was a challenge.

Working with a strain of *Myxococcus xanthus*, Kaiser and his then graduate student Jerry Kuner had the idea that perhaps the medium was holding them back. They decided to abandon traditional, solid agar—which often contained impurities, compromising starvation and fouling imaging studies—and opted instead to culture the cells in a defined liquid medium.

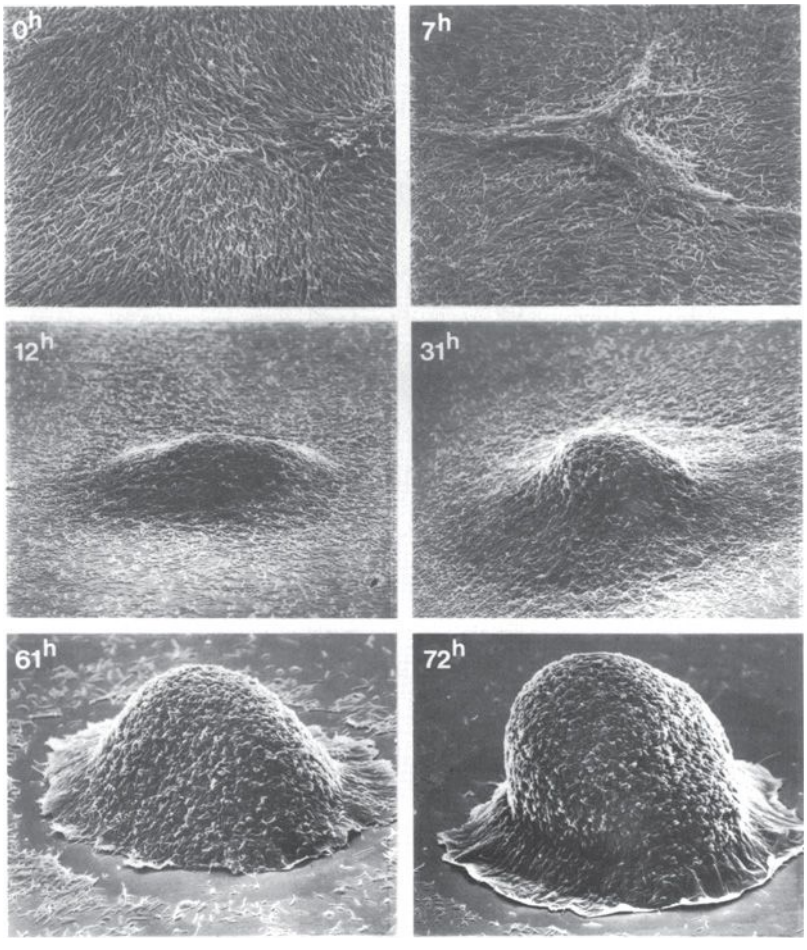
The team at first grew *M. xanthus* cells in a minimally nutritive liquid medium Kaiser and another colleague described in February 1978, called CTT. Overnight, the cells formed a film at the bottom of the dish, so adherent that the researchers could replace the liquid medium with water without disturbing the bacteria, effectively starving the cells, which then aggregated and formed fruiting bodies.

Culturing a less adherent mutant strain of *M. xanthus* atop a cover slip in a dish and, again, replacing leftover CTT liquid medium with water, Kaiser and Kuner saw that the starved cells failed to aggregate. Tinkering with the nutrients they included in the mixture, the team found that one mineral had been missing. Because the cells aggregated with the addition of calcium chloride ($CaCl_2$), but not magnesium chloride ($MgCl_2$), the researchers deduced that calcium ions were required.

"If calcium was added to the medium, the cells would stick to the plastic and form a kind of biofilm," says Kaiser. "And in that biofilm, the cells would aggregate. By 72 hours, the aggregate was about the size of a normal fruiting body."

Kaiser and Kuner removed the cover slips from the dishes, exposed them to fresh aggregation-inducing solution containing a fixative, coated them with gold, and imaged the slides using field-emission scanning electron microscopy. The resulting pictures—compiled in a time-series figure of six micrographs that took up more than three-quarters of a printed page—appeared in the *Journal of Bacteriology* in July 1982 (151:458-61).

"You can take a long time to describe fruiting body formation and how amazing it is, but those pictures really jump out at you, telling the whole story," says Rutgers University's Ann Stock, who penned a perspective on the 1982 paper this February (*J Bacteriol*, 198:602, 2016).



AGGREGATION IN ACTION: Dale Kaiser and Jerry Kuner's panel of six electron micrographs shows aggregation and the formation of a fruiting body by *Myxococcus xanthus* strain DK 1622 cells. The researchers starved these bacteria of nutrients over the course of 72 hours by replacing a liquid growth medium with distilled water. With the addition of a calcium chloride solution, bacteria kept at a constant pH (6.8) aggregated and then formed a fruiting body—a cluster of cells that would later be filled with spores. The resulting time-lapse micrographs remain some of the clearest pictures of fruiting body formation in this gram-negative bacterium.

Other groups have since applied the submerged culture method described by Kaiser and Kuner to further study fruiting body formation in *M. xanthus* and related species. "It looked like we had an in vitro method of forming fruiting bodies," says Kaiser.

The team's images are still reprinted today. Stock says, "These images spoke to me from my many years of listening to *Myxococcus xanthus* talks," starting when she was a graduate student. "It's a pretty interesting organism with its multicellular, social lifestyle. It stands out among the bacteria." ■

J BACTERIOL, 151:458-61, 1982.

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