TheScientist
JANUARY 2015 | WWW.THE-SCIENTIST.COM
EXPLORING LIFE, INSPIRING INNOVATION

SOCIAL CODES

HOW IDENTICAL GENOMES PROGRAM THE BEHAVIOR OF INSECT CASTES

STRESS DISRUPTS GENE EXPRESSION

TRANSPOSONS AND IMMUNITY

MAKING PROTEINS GLOW MORE BRIGHTLY

PLUS
ASSESSING RESEARCHER PRODUCTIVITY

CYTATION 5
imaging reader

DO | WHAT YOU NEVER THOUGHT POSSIBLE

Discoveries await.

The fact is, Cytation™ 5 Multi-Mode Reader represents years of experience perfecting microplate instrument technologies. From our 3rd generation variable bandwidth monochromator system and advanced filter detection to our best-in-class 4-Zone™ incubation and laser-based alpha excitation, every feature has been refined to achieve optimum performance in all key modes. Upgrade to include our fully automated imaging system and you've got the the most sophisticated multi-mode reader on the market today.

Find out what is possible, visit **www.cytation5.com**

Think Possible

BioTek®

www.biotek.com

Panasonic

Life Science

cellIQ™

INTELLIGENT CO_2 INCUBATOR

Smart

TOUCH SCREEN INTERFACE

2 min

CO_2 RECOVERY

8x Faster

DECONTAMINATION

Rethink cell growth.

Empower your cell culture through the most advanced user interface and technology. Optimize your stem cell's environment with a new intelligent touch screen for precise CO_2 recovery, uniform temperature control, and rapid decontamination.

Discovery powered by precision™

us.panasonic-healthcare.com/celliqseries

NEW ENGLAND BioLabs® Inc.

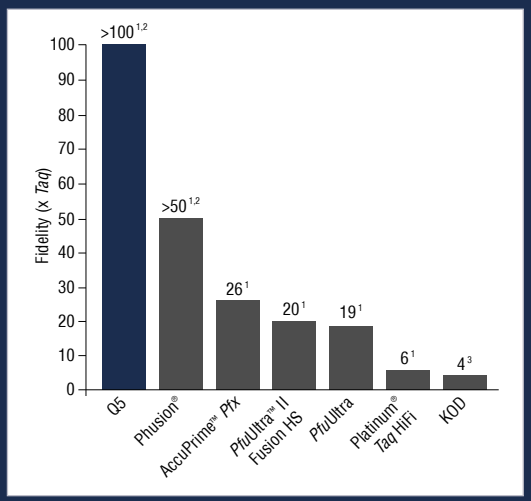
Fidelity amplified.

Q5® High-Fidelity DNA Polymerase

Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (>100X higher than *Taq*), PCR with Q5 DNA Polymerase results in ultra-low error rates. Its unique buffer system provides superior performance for a broad range of amplicons, regardless of GC content. Q5 DNA Polymerase represents the finest in fidelity.

Request a free sample* at www.Q5PCR.com

The highest fidelity amplification available



[1] *PCR-based mutation screening in lacZ (NEB), lacI (Agilent) or rpsL (Life)*

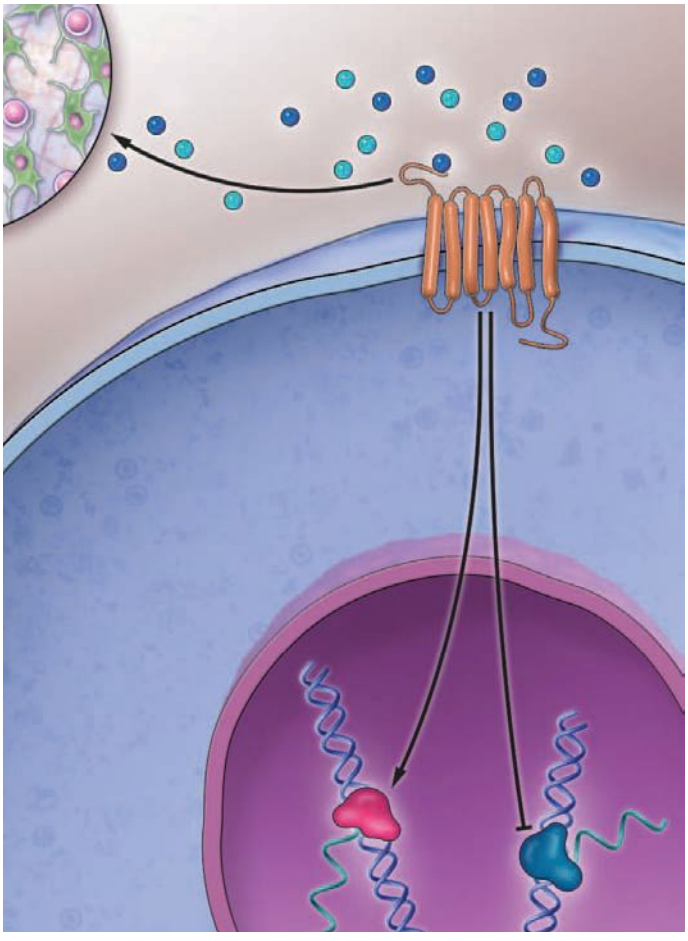
[2] *Due to the very low frequency of misincorporation events being measured, the error rate of high-fidelity enzymes like Q5 is difficult to measure in a statistically significant manner. Although measurements from assays done side-by-side with Taq yield Q5 fidelity values from 100-200 X Taq, we report ">100X Taq" as a conservative value.*

[3] *Takagi et al (1997) Appl. Env. Microbiol. 63, 4504-4510.*

* While supplies last, offer valid in the US only. Limit one sample per customer.

Contents

THE SCIENTIST | WWW.THE-SCIENTIST.COM | VOLUME 29 NUMBER 1



Features

ON THE COVER: © YUTTASAK JANNARONG/SHUTTERSTOCK

BioResearch

Lonza

It All Stems from Here

Simplify Your Reprogramming, Proliferation and Differentiation

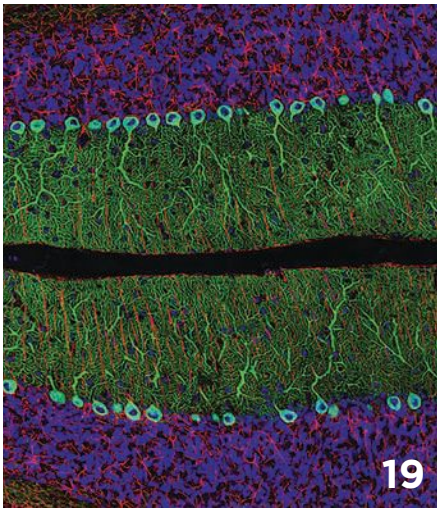
Take the trial and error out of your stem cell workflow with optimized toolkits from Lonza.
Whether you work with iPSC or adult stem cells, your direct route to biologically relevant results starts here.

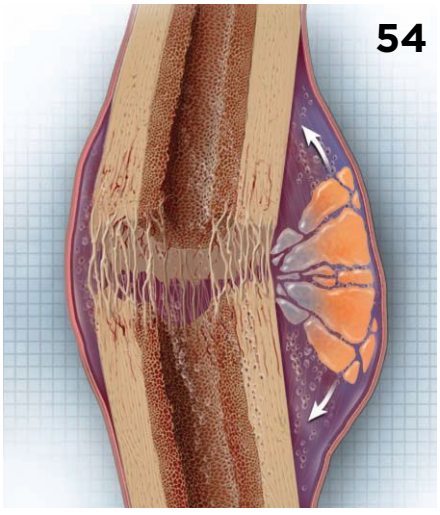
- NEW! L7™ Reprogramming and Culture System – robust system for generation and maintenance of hPSCs
- Nucleofector™ Technology – non-viral transfection for reliable reprogramming and genome editing
- Clonetics™ Primary Cells and Poietics™ Stem Cells – extensive range available

Streamline your stem cell workflow today.

www.lonza.com/stem

Department Contents



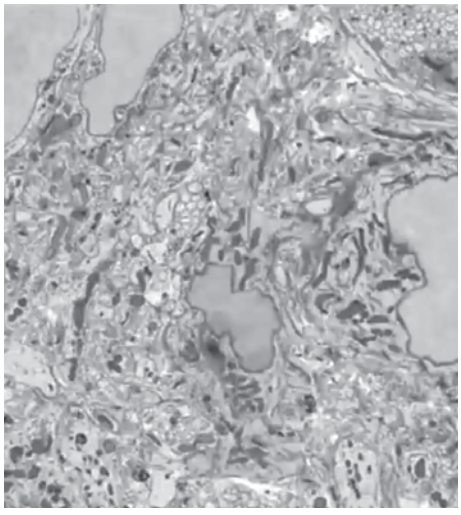


CORRECTION:

In "Lurking in the Shadows" (*The Scientist*, December 2014), Jon Epstein's affiliation was incorrectly stated. He is a veterinary epidemiologist at EcoHealth Alliance.

The Scientist regrets the error.

Online Contents



THIS MONTH AT WWW.THE-SCIENTIST.COM:

VIDEO
Dairy Engineering
Watch how DuPont uses CRISPR to keep dairy products safe from bacteriophages.

VIDEO
Rat Race
Neuroscientist Anthony Zador explains why he uses rats to understand auditory attention in the brain.

VIDEO
Micro Masterpiece
See some of the micrography of Tom Deerinck, a perennial front-runner in Nikon's Small World competition.

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

Coming in February

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

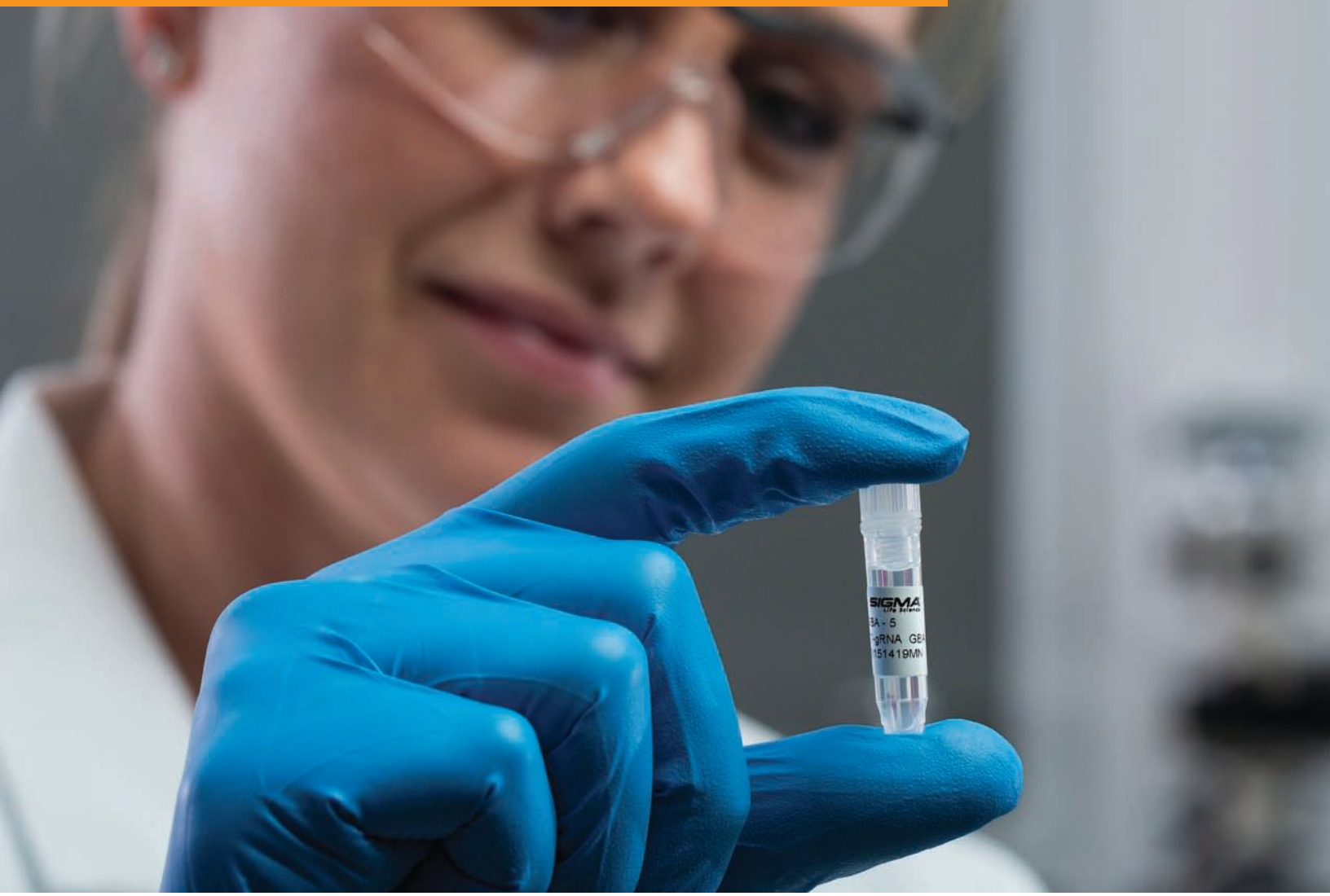
- Noncoding RNAs and persistent viral infection
- Energy management by extremophiles
- Innate immune system memory
- Computer modeling of drug interactions
- Single-cell epigenetics

AND MUCH MORE

PDPHOTO.ORG/WIKIMEDIA COMMONS

#CRISPRresults

@SigmaAldrich



RAPID, RELIABLE AND REPEATABLE GENE EDITING WITH SIGMA® CRISPR PRODUCTS.

- Lentivirus and plasmid formats available
- Fluorophones included for monitoring transfection efficiency
- Positive and negative CRISPR controls for accurate results
- Products you need for gene editing experiments that work first time

Remember to order your supplementary cell culture, serum and reagents. For more information and to buy online, visit **sigma.com/CRISPRresults**

SIGMA® | ALDRICH® | SUPELCO® | SAFC®

SIGMA-ALDRICH®

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

415 Madison Avenue,
Suite 1508,
New York, NY
10017
E-mail: info@the-scientist.com

POSTMASTER: Send address changes to *The Scientist,* PO Box 2015, Skokie, Illinois 60076. Canada Publications Agreement #40641071 *The Scientist* is indexed in Current Contents, Science Citation Index, BasicBIOS IS, and other databases. Articles published in *The Scientist* reflect the views of their authors and are not the official views of the publication, its editorial staff, or its ownership. *The Scientist* is a registered trademark of LabX Media Group Inc. *The Scientist*® (ISSN 0890-3670) is published monthly.

Advertising Office: *The Scientist,* 415 Madison Avenue, Suite 1508, New York, NY 10017. Periodical Postage Paid at New York, NY, and at additional mailing offices.

SUBSCRIPTION RATES & SERVICES
In the United States & Canada individual subscriptions: $39.95. Rest of the world: air cargo add $25.

For assistance with a new or existing subscription please contact us at:

Phone: 847.763.9519
Fax: 847.763.-9674
E-mail: thescientist@halldata.com
Mail: The Scientist, PO Box 2015 , Skokie, Illinois 60076

For institutional subscription rates and services visit www.the-scientist.com/info/subscribe or e-mail institutions@the-scientist.com

LIST RENTALS
Contact Statlistics, Jennifer Felling at 203-778-8700 or j.felling@statlistics.com

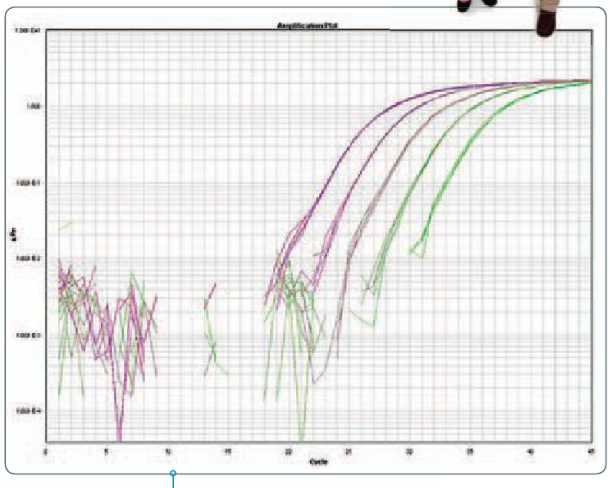
REPRINTS
Contact Lee Denton at ldenton@labx.com

PERMISSIONS
For photocopy and reprint permissions, contact Copyright Clearance Center at www.copyright.com

MAXimize reproducibility. Minimize costs.

pipetmaX™

Advance your science and increase the pace of your experiments by allowing PIPETMAX to do the routine pipetting tasks. Focus on what is really important to your research – scientific analysis and publications.



Standard Amplification Curves for COX gene. Standard cDNA samples were serially diluted 5-fold and amplified in 5 replicates (dilutions left to right: 5x, 25x, 125x, 625x and 3125x).

Accomplish more by automating your sample prep protocols for:

- qPCR
- PCR
- NGS library prep
- ELISA
- most tip-based protocols

GILSON®

instruments • accessories • software • solutions ▶ Meet MAX at **www.pipetmax.com**
Visit www.gilson.com to download Speaking Volumes, our interactive publication for your tablet!

BOUNDARY-BREAKING
ACOUSTIC FOCUSING CYTOMETRY

Introducing the Attune® NxT Acoustic Focusing Cytometer—a high-performance package that's flexible enough for any lab

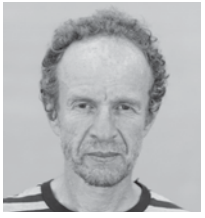
The Attune® NxT Acoustic Focusing Cytometer combines unmatched precision, speed, and sensitivity, in a modular design that can grow with you. With up to 4 lasers and 14 colors, the Attune® NxT cytometer is designed to accommodate most experimental design needs and lab budgets. Boundaries, beware.

See the next big thing in flow cytometry at
lifetechnologies.com/attune

life technologies

A **Thermo Fisher Scientific** Brand

Contributors



As a middle school student in Moscow, Russia, **Eugene Koonin** read the work of pioneering scientists such as Emile Zuckerkandl and Linus Pauling, who together in 1962 founded the field of molecular evolution through a prescient analysis of just a few snippets of protein sequences. "I had the good fortune to read these papers quite early," he says. "I just thought that this was the right way to study life, although at the time . . . the available data were completely inadequate." Koonin completed a PhD at Moscow State University, where he investigated the replication mechanisms of RNA viruses. "This introduced me to the world of viruses and other mobile genetic elements that remains incredibly fascinating to this day," he says. While working at the Institutes of Poliomyelitis and Microbiology of the USSR Academy of Medical Sciences, Koonin began to apply computational tools to unravel microbial evolution. Now a senior investigator at the US National Center for Biotechnology Information, Koonin uses comparative genomics to elucidate evolutionary trends, with a focus on the role of horizontal gene transfer and parasite-host arms races.

A single microbiology lecture at Vilnius University sparked **Mart Krupovic**'s attraction to viruses and his desire to study their origins. He pursued a PhD with Dennis Bamford at the University of Helsinki in Finland, where he studied the molecular underpinnings of virus-host interactions and worked on characterizing viral diversity. "All of these things are equally exciting for me, but I always try to look at them from an evolutionary perspective," he says. As a research scientist in the Molecular Biology of the Gene in Extremophiles unit at the Institut Pasteur in Paris, he is examining other mobile genetic elements, "trying to see what part viruses occupy within this gigantic mobilome." During a three-month stint in Koonin's lab in early 2014, Krupovic and Koonin discovered a transposon origin for a component of bacterial and archaeal CRISPR-Cas antiviral defense systems.

In their feature, "A Movable Defense," on page 46, Koonin and Krupovic detail this finding and the involvement of other genetic transfers in the evolution of immune defense.

Seirian Sumner studied the behavior of Malaysian wasps as a PhD student at University College London (See "Seirian Sumner: Wasp Whisperer," *The Scientist*, August 2011). "I found myself lying on the floor of the rainforest, with a wasp nest a few centimeters from my face, yelling out the colour codes that we'd painted our wasps with," she writes in an e-mail describing her first field season. "I was covered in mud, who knows how many creepy crawlies, and suddenly realised what a peculiar thing I'd got myself into!" As a research fellow at London's Institute of Zoology, Sumner continued to explore the molecular foundations of social behavior in wasps and other insects and cofounded Soapbox Science, an annual public-outreach event focused on female scientists. Now a senior lecturer in behavioral biology at the University of Bristol, she is thrilled by the capabilities of next-generation sequencing to take the field of sociogenomics far beyond the honeybee.

While in high school in Berkshire, England, **Claire Asher** "fell in love with the logic" of biology, and later became fascinated by cooperative behavior in animals. Asher earned a PhD from the University of Leeds, where she investigated the social structure of the Brazilian dinosaur ant *Dinoponera quadriceps*. Working with Sumner and William Hughes (now at the University of Sussex), she showed that "high-ranking workers—the ones that have a chance of taking over the colony—basically don't do any work, and they certainly don't do any of the difficult or dangerous jobs in the colony." This finding seems "eerily reminiscent of humans," Asher says. She also performed transcriptome sequencing on ants of different behavioral castes to analyze the genetic bases of their roles. In the midst of her graduate studies, Asher created a science blog, *Curious Meerkat*, and she now works as a freelance writer and a knowledge-transfer specialist in the Centre for Biodiversity and Environment Research at University College London.

Sumner and Asher describe how omics techniques are transforming researchers' views of eusocial insects in "The Genetics of Society" (page 39).

YURI WOLF; COURTESY OF MART KRUPOVIC; COURTESY OF SEIRIAN SUMNER; LYNNE SHONE

Molecular Probes®

Exhibit the brightest cell images using Alexa Fluor® secondary antibodies

With over 30,000 citations, it's no secret Alexa Fluor® dye–labeled secondary antibodies are the leader in fluorescence. Consistently dependable and available in 20 different colors, their brilliant hues have helped scientists visualize their research for nearly two decades.

Find out more about Alexa Fluor® secondary antibodies at
lifetechnologies.com/alexafluor

life technologies

A Thermo Fisher Scientific Brand

Performance Art

Regulation of genome expression orchestrates the behavior of insect castes and the human response to social stress.

BY MARY BETH ABERLIN

ANDRZEJ KRAUZE

Soldier ants and worker bees made Darwin scratch his head. How to explain the comportment of insects that live in social groups—catering to the colony's queen, nursing her eggs and larvae, foraging for food, defending the nest—yet never getting to have their own offspring? In this month's cover story, "The Genetics of Society" (page 39), Claire Asher and Seirian Sumner describe the growing field of sociogenomics, which uses information gathered from genome sequences, epigenetic patterns, and transcriptome and proteome analyses to suss out how a seemingly identical genome can account for observed forms of caste-specific behavior. It's a story of rapid gene evolution and differential gene expression. "The organization and collective decision making of eusocial insects is even yielding new insights into human behavior and what it means to be part of a society," write the authors. Darwin would have loved this.

Although human behavior is much less typecast than that of bees, ants, and termites, our species' reactions to social situations are also proving to be a matter of differential gene expression. It's been known for quite a while that social adversity, physical abuse, loneliness, and even grief are associated with increased susceptibility to disease. In "Stress Fractures" (page 32) you'll learn about alterations in the expression of immune-system genes that occur as a result of psychological stress, and about the generation of immune cells that can "hide out in the spleen and reemerge months and possibly even years later in response to subsequent stressors, potentially explaining how experiences of social adversity early in life can shape one's inflammatory landscape as an adult." Author Daniel Cossins also reports on new evidence that certain stress-induced epigenetic modifications are heritable. The jury is still out on that one, as well as on new research reporting that microRNAs might play a role in passing down stress-influenced phenotypes.

One type of stress that affects all organisms is pathogenic infection. The well-tuned vertebrate immune system employs two strategies to deal with invaders: an immediate and nonspecific innate response, and an adaptive response tailored to the particular pathogen. Prokaryotes facing down an invader were thought to mount nothing more than a fairly simple innate immune response. Eugene Koonin and Mart Krupovic ("A Movable Defense," page 46) recount the pivotal role of mobile genetic elements such as transposons in the establishment of adaptive immunity in bacteria and archaea. A snippet of an invader's DNA is inserted into a specific region of a host's genome, the CRISPR cassette, with the help of the Cas1 enzyme; subsequent infection with the same parasite activates the CRISPR-Cas system to produce small RNAs called CRISPR targeting RNAs (crRNAs) that direct the destruction of the invader's genome. In other words, prokaryotes can remember a particular infection and mount a specific counterattack upon reinvasion. What's more, this knowledge is passed on to subsequent generations. From extensive comparative genomic studies, Koonin and Krupovic have found that a family of transposons they call casposons lies at the base of the phylogenetic tree in which Cas1 is found: these mobile genetic elements are apparently early ancestors of prokaryote adaptive immunity.

CRISPR-Cas systems—all the rage these days for precision genome editing—are ancient prokaryotic defense mechanisms. But did you know that cheese and yogurt manufacturers rely on the immune systems of bacterial starter cultures to attack viruses that can ruin dairy products? Associate Editor Kerry Grens describes the vaccination of bacterial strains to ramp up their CRISPR-Cas systems, making them better pathogen destroyers and saving businesses a ton of money in the process (page 20).

Further insights into the mechanisms of group living and defense against outsiders lie in wait, whether they're buried deep in the genomes of people suffering social stress, in the epigenetic programs of eusocial ant species, or at the bottom of your yogurt cup. This issue celebrates the scientists who are unlocking the secrets of our very social world. ■

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

Speaking of Science

We know researchers are already sharing content, often in hidden corners of the Internet or using clumsy, time-consuming practices.

—**Timo Hannay**, managing director of Digital Science, which is owned by Macmillan Publishers, in a statement announcing a move by the publisher to make the contents of all journals under Nature Publishing Group freely readable online to users sent a link to a paper by a subscriber (December 2)

To me, this smacks of public relations, not open access. With access mandates on the march around the world, this appears to be more about getting ahead of the coming reality in scientific publishing. Now that the funders call the tune and the funders want the articles on the web at no charge, these articles are going to be open anyway.

—Open-access advocate and senior fellow at the Ewing Marion Kauffman Foundation in Kansas City, Missouri, **John Wilbanks**, in a comment about the announcement of the new Macmillan Publishers policy that all Nature Publishing Group journals will be free to read online if subscribers share a link (*Nature News*, December 2)

The development of full artificial intelligence could spell the end of the human race. Once humans develop artificial intelligence, it would take off on its own and redesign itself at an ever increasing rate. Humans, who are limited by slow biological evolution, couldn't compete and would be superseded.

—University of Cambridge theoretical physicist **Stephen Hawking**, talking to the BBC about the dangers of developing artificial intelligence (December 2)

We are drowning in information, while starving for wisdom. The world henceforth will be run by synthesizers, people able to put together the right information at the right time, think critically about it, and make important choices wisely.

—Harvard biologist **E.O. Wilson**, in his 1998 book *Consilience: The Unity of Knowledge*

© ISTOCKPHOTO.COM/ABLUECUP

Because I was an "unperson" I was fired from the boards of companies, so I have no income, apart from my academic income.

—**James Watson**, Chancellor Emeritus of Cold Spring Harbor Laboratory, and codiscoverer of the double-helical structure of DNA, describing his partial motivation for auctioning off his 1962 Nobel Prize medal, which sold on December 4 for $4.1 million (*Financial Times*, November 28)

In my opinion, a situation in which an outstanding scientist has to sell a medal recognizing his achievements is unacceptable. James Watson is one of the greatest biologists in the history of mankind, and his award for the discovery of DNA structure must belong to him.

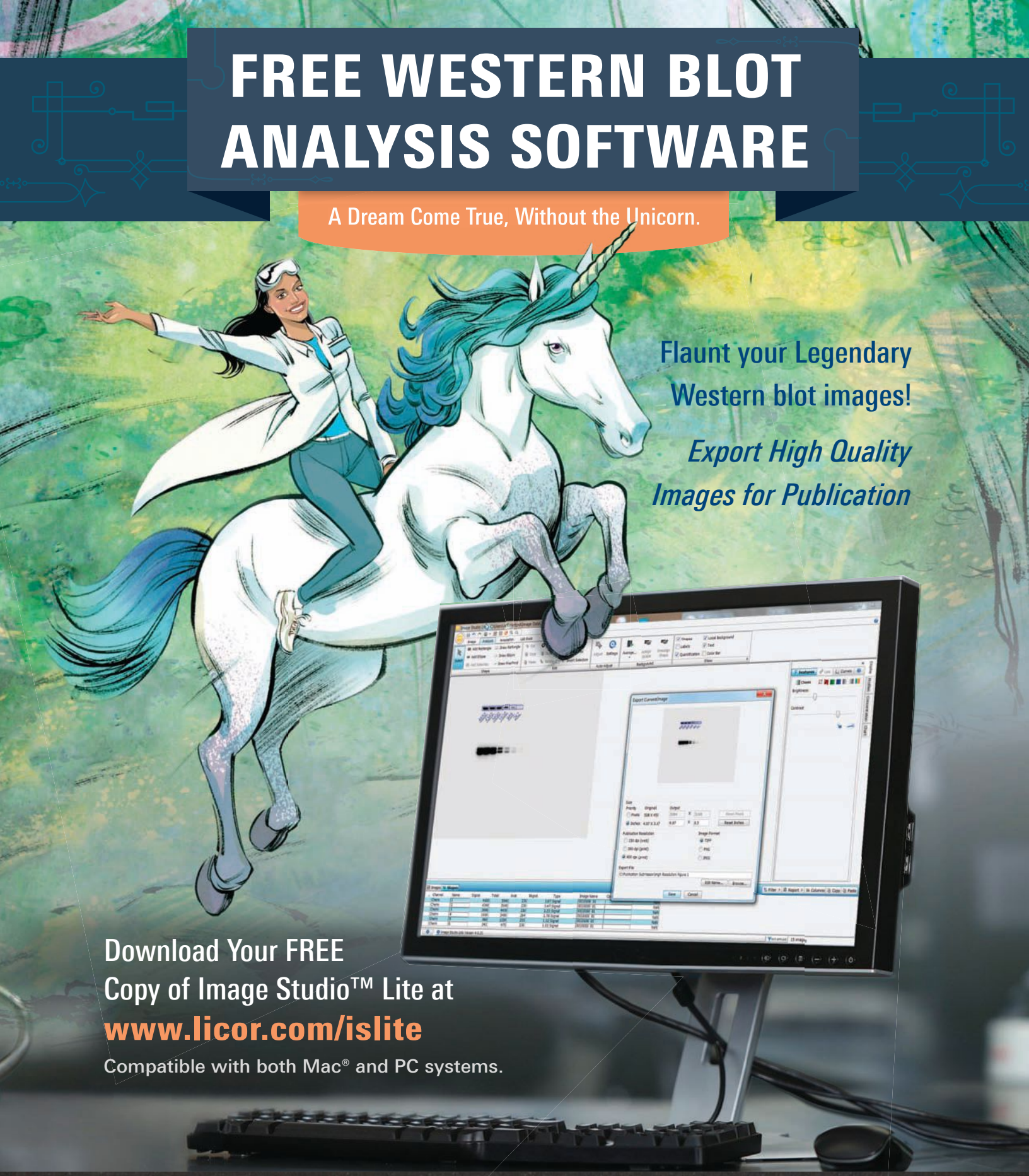
—Russian entrepreneur **Alisher Usmanov**, speaking to *The New York Times* about his decision to return James Watson's 1962 Nobel Prize medal after paying more than $4.1 million for it at auction (December 9)

The idea that stress plays a role in illness is becoming more and more well defined. It happens both directly and indirectly, directly through mechanisms that we partially understand but not fully, and indirectly through changes in behavior.

—**Dean Ornish**, University of California, San Francisco, professor of medicine and founder of the university's Preventive Medicine Research Institute, describing science's emerging understanding of the physiological impacts of emotional stress (December 2008)

FREE WESTERN BLOT ANALYSIS SOFTWARE

A Dream Come True, Without the Unicorn.



Flaunt your Legendary Western blot images!

Export High Quality Images for Publication

Download Your FREE Copy of Image Studio™ Lite at

www.licor.com/islite

Compatible with both Mac® and PC systems.

LI-COR®

eppendorf

Fast Forward

The Eppendorf ep*Motion*® Series: Enjoy easy-to-use and flexible automation

The new Eppendorf ep*Motion* liquid handling workstations are the right choice for your highly reproducible and accurate pipetting results. Discover new features, more speed and worktable positions and the new intuitive software assistant concept.

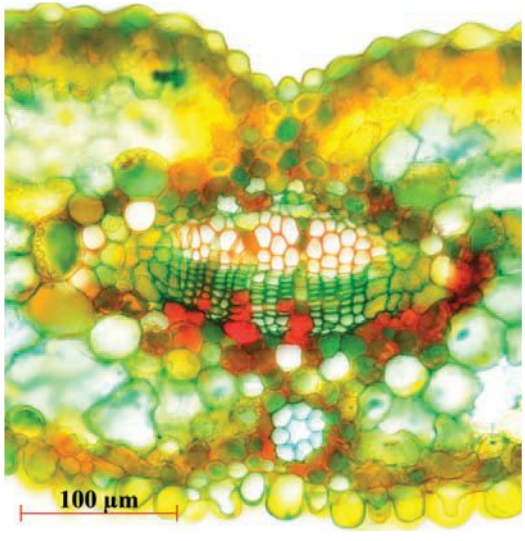
> Available as ThermoMixer®, vacuum manifold or magnetic separation options
> User guided, touchable software assistants for easy application programming
> UV light decontamination and HEPA air filter option

www.eppendorfna.com/automation • 800-645-3050

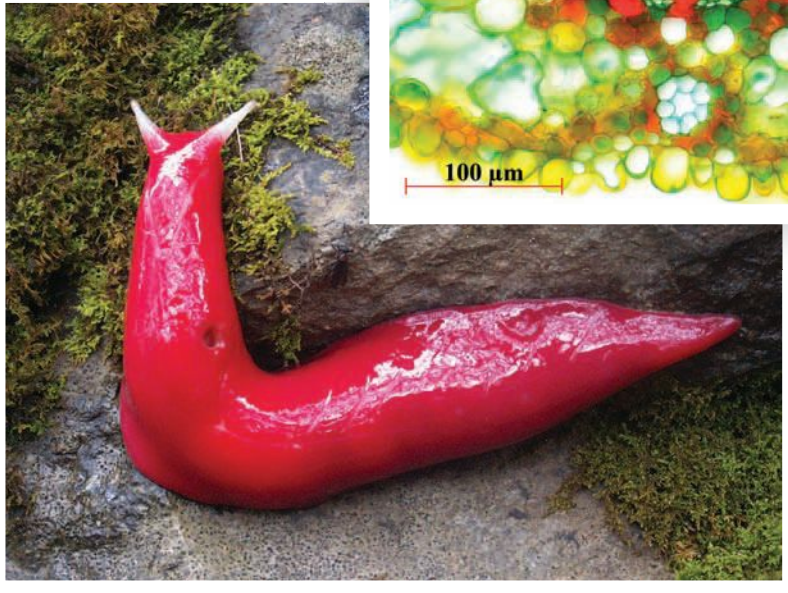
Caught on Camera

Selected Images of the Day from www.the-scientist.com

FANTASTIC FEEDERS
Bdelloid rotifers are microscopic freshwater animals with diverse jaw forms, seen here in a scanning electron micrograph.
Posted: *November 10, 2014*



BOTANICAL TRANSPORT
A cross section of a vascular bundle in the leaf of a dawn redwood (*Metasequoia glyptostroboides*)
Posted: *November 7, 2014*



PINK AND SLIMY
The giant pink slug (*Triboniophorus* aff. *graeffei*), recently determined to be a distinct species, can grow up to 8 inches long and lives only on Mount Kaputar in eastern Australia.
Posted: *November 24, 2014*

ROTIFERS: *PLOS BIOLOGY*, DIEGO FONTANETO; LEAF: WIKIMEDIA, ROLF-DIETER MUELLER; SLUG: PHOTO BY MICHAEL MURPHY

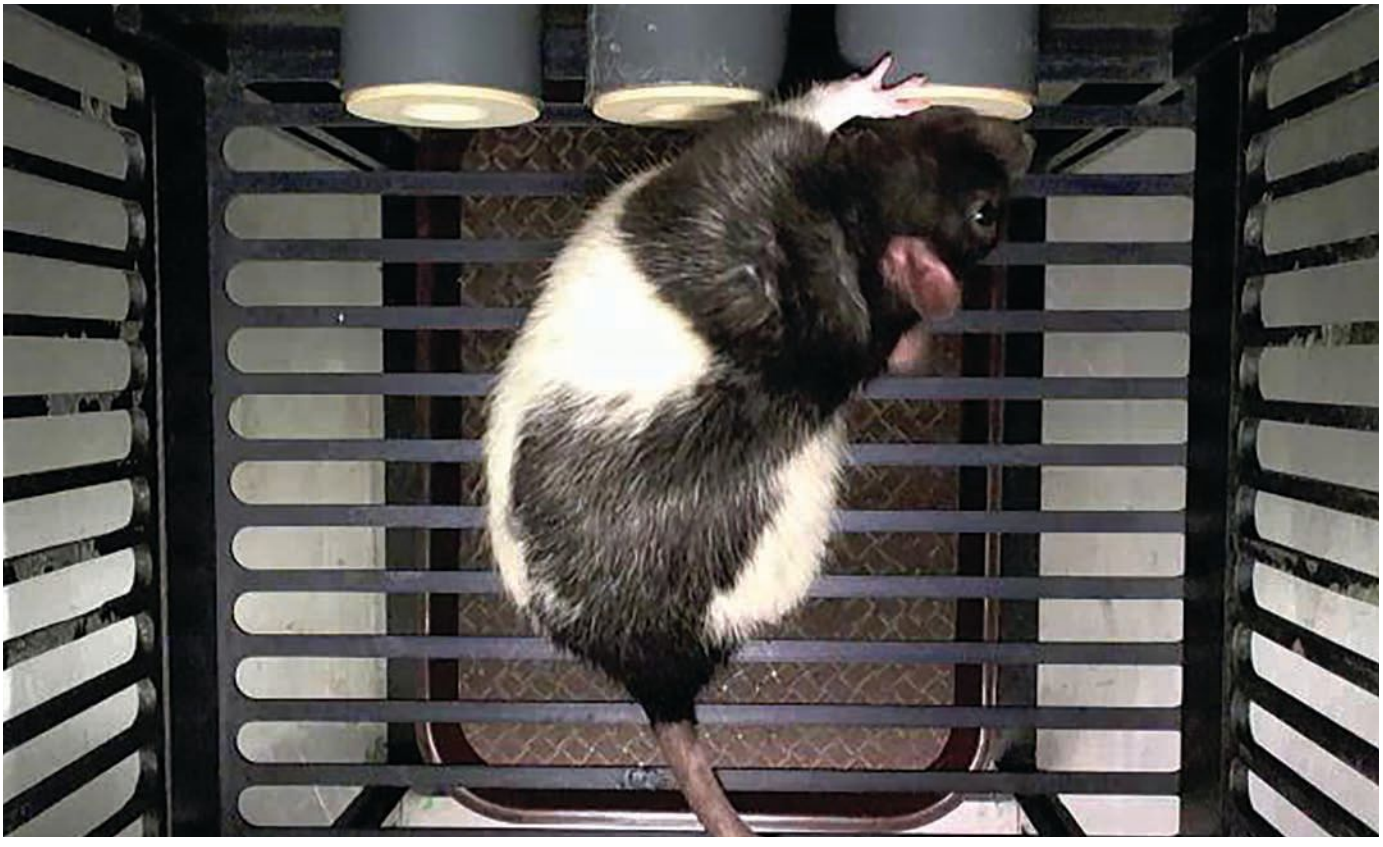
TheScientist

REGISTER
FOR FREE
TO RECEIVE
OUR DAILY
E-NEWSLETTER

The Scientist's website features award-winning life science news coverage you won't find in the magazine, as well as features, profiles, scientist-written opinions, and spectacular videos, slide shows, and infographics.

www.the-scientist.com

Notebook



SANTIAGO JARAMILLO

RAT RACE: Viewed from above its enclosure, a lab rat pokes its nose into a device designed to test its decision-making abilities.

May the Best Rodent Win

Rats are smart, and mice are dumb. For more than a century, this was the prevailing dogma among scientists who study how brains make choices based on sensory inputs—the type of researchers who train rodents to run mazes in order to uncover mechanisms of long-term memory, problem solving, and other cognitive tasks.

When Anthony Zador set up his lab 15 years ago at Cold Spring Harbor Laboratory in New York, he and his colleagues began developing "tricks to train rats." After several years, he says, "we were pretty confident that [rats] can process sensory stimuli and make decisions about them—that they have attentional processes." At the same time, researchers were generating a wealth of genetic and molecular tools in mice, allowing the visualization and manipulation of specific neural circuits and subtypes.

Myths about mice are being dispelled little by little.

— Matteo Carandini, University College London

As a postdoc working with Zador, Santiago Jaramillo was eager to take advantage of these resources to examine auditory processing in mice. "The question at that point was: Well, now that we have all these great tools to study the neurobiology, do the mice actually have the behavioral repertoire that we want to be able to study cognition?" recalls Jaramillo, now an assistant professor at the University of Oregon.

"The rumor was that it's very difficult to train mice, and that you could never train them as well and effectively as you can train rats," says Zador. But his group's experience establishing rats as a behavioral model, even in the face of skepticism from primate researchers, suggested that stubborn scientists, rather than dim-witted animals, might underlie such assumptions.

So Jaramillo and Zador set out to compare the decision-making abilities of rats

and mice in a head-to-head contest (*Front Syst Neurosci*, 8:173, 2014). In a "flexible categorization task," they trained both mice and rats to discriminate between high- and low-frequency sounds by poking their noses into ports on the right or left side, respectively, of a three-port chamber. The animals received a water reward for correct choices. Although mice took longer to train than rats on average, both rodent species successfully adapted to changes in the boundary between high and low frequencies.

"It was surprising that they actually performed equally well," says Jaramillo. "I wasn't expecting that the behavior [of the mice] would be pretty much what we achieved with the rats." The shorter training times for rats, he says, could be "because of us as experimenters, and our larger experience working with rats."

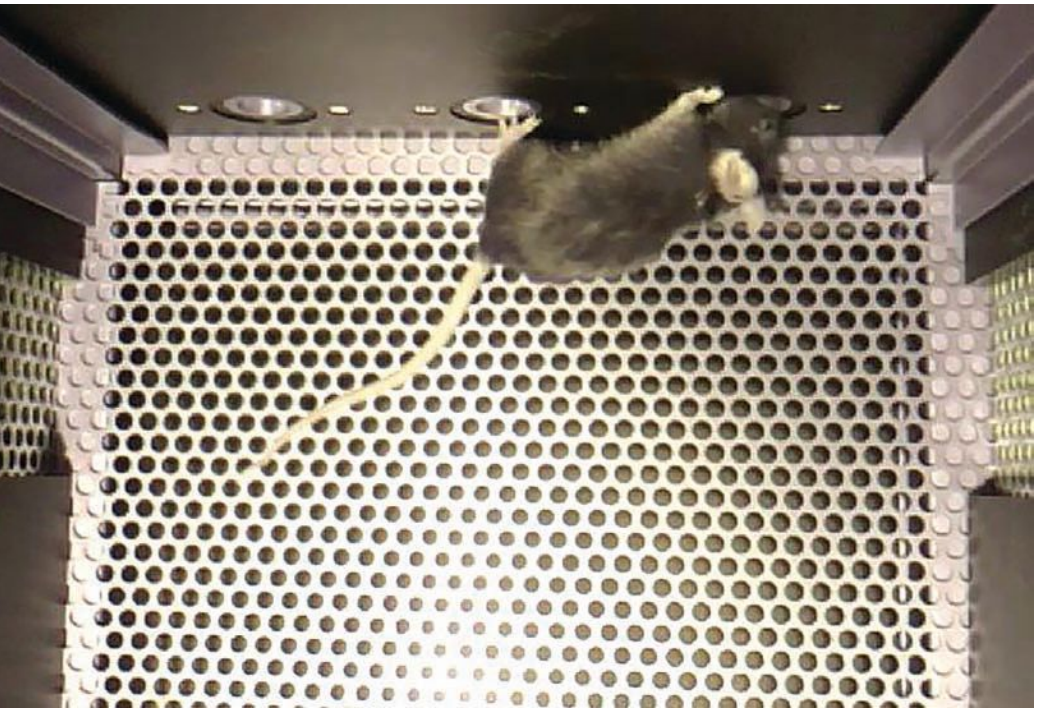
Thanks to the recent profusion of transgenic mouse lines, mice offer broader possibilities than rats when it comes to techniques such as optogenetics, which gives researchers the ability to control subtypes of neurons using particular wavelengths of light. (See "The Birth of Optogenetics," *The Scientist*, July 2011.) For example, transgenic mice engineered to produce a photosensitive opsin protein in a specific subset of auditory cortex neurons enabled Jaramillo's research group to decipher how neurons represent auditory signals.

On the other hand, viral-vector approaches to light-sensitive protein expression are constantly improving, making optogenetics increasingly accessible in rats, says Jeffrey Erlich, who recently established his own research group at New York University Shanghai. He plans to use both rats and mice to investigate the effects of stress on economic decisions. "With mice we can knock out specific receptors, and we can really target the molecular pathway between stress and economic decisions," says Erlich. "With the rats, I think it's going to be easier to do the electrophysiology, and there are certain things, I think, that rats are still better at."

For one, rats are more patient than mice: they are able to wait for several seconds while sensory stimuli are presented to them. And rats' larger size makes them more amenable to experiments that use head-mounted probes to record their neural responses while they move about freely. (For cautionary notes about using mice in behavioral studies, see "Mouse Traps," *The Scientist*, November 2014.)

Still, mice may have the edge when it comes to studies of visual processing, according to Matteo Carandini of University College London. "It's really important to control the sensory environment, so you know exactly what sensory stimuli you're giving to your animal," he says. "Because we study vision, we need to make sure that we know where the eyes are pointing." Carandini and his team conduct many of their experiments in mice with their heads fixed—a setup that can be "disastrous" in bigger, stronger rats, he says.

"We never see it as a fight" between mouse and rat models of cognition, says Jaramillo, who points out that the ultimate goal of this research is to shed light on attention and decision-making in humans. Even so, self-described "mouse supremacist" Carandini rejoices as "myths about mice . . . are being dispelled little by little." And while "there is nothing stopping the field from developing the same tools in rats that have been developed in mice," he says, "the question is, do we need to?" —Molly Sharlach



SANTIAGO JARAMILLO

MOUSING AROUND: Mice performed almost as well as rats at similar decision-making tasks, according to Santiago Jaramillo.

There's CRISPR in Your Yogurt

Two years ago, a genome-editing tool referred to as CRISPR (clustered regularly interspaced short palindromic repeats) burst onto the scene and swept through laboratories faster than you can say "adaptive immunity." Bacteria and archaea evolved CRISPR eons before clever researchers harnessed the system to make very precise changes to pretty much any sequence in just about any genome.

But life scientists weren't the first to get hip to CRISPR's potential. For nearly a decade, cheese and yogurt makers have been relying on CRISPR to pro-

That was an eye-opening moment when we first thought of the link between CRISPR sequencing content and phage resistance.

—Rodolphe Barrangou, North Carolina State University

ANDRZEJ KRAUZE

duce starter cultures that are better able to fend off bacteriophage attacks. "It's a very efficient way to get rid of viruses for bacteria," says Martin Kullen, the global R&D technology leader of Health and Protection at DuPont Nutrition & Health. "CRISPR's been an important part of our solution to avoid food waste."

Phage infection of starter cultures is a widespread and significant problem in the dairy-product business, one that's been around as long as people have been making cheese. Patrick Derkx, senior director of innovation at Denmark-based Chr. Hansen, one of the world's largest culture suppliers, estimates that the quality of about two percent of cheese production worldwide suffers from phage attacks. Infection can also slow the acidification of milk starter cultures, thereby reducing creameries' capacity by up to about 10 percent, Derkx estimates.

In the early 2000s, Philippe Horvath and Rodolphe Barrangou of Danisco (later acquired by DuPont) and their colleagues were first introduced to CRISPR while sequencing *Streptococcus thermophilus*, a workhorse of yogurt and cheese production. Initially, says Barrangou, they had no idea of the purpose of the CRISPR sequences. But as his group sequenced different strains of the bacteria, they began to realize that CRISPR might be related to phage infection and subsequent immune defense. "That was an eye-opening moment when we first thought of the link between CRISPR sequencing content and phage resistance," says Barrangou, who joined the faculty of North Carolina State University in 2013.

Within a couple of years the team concluded that CRISPR sequences indeed confer phage resistance (*Science*, 315:1709-12, 2007). The bacterial genome integrates a sequence of the viral genome, called a spacer, upon infection; that sequence later serves as a guide for destroying any matching DNA, so that subsequent viral infections are fended off. (See "A Movable Defense" on page 46.) Bacteria use this system naturally, but the scientists wanted to harness it to immunize cultures.

DuPont filed a number of patents on its technique, so the details are under wraps, but essentially the dairy culture developers expose select bacteria to particular viruses and collect the bacterial strains that manage to survive attack. Such an approach—challenging a bacterial population with phages and selecting for resistant cells—is nothing new. Derkx says it's a classic strategy that's been employed for more than 25 years. But DuPont's spin on it is to identify whether the bacteria have acquired new CRISPR spacers. "CRISPR is not the only mechanism to resist phages. It is one that is used efficiently," says Horvath. After isolating those "CRISPRized" cells, the food scientists grow up a new culture and repeat, challenging the resistant strains over and over again with phage to increase the breadth of the CRISPR-encoded resistance. "The trick really is to use a diversity of phages that are specific to those strains to make them resistant" to a broad range of viruses, says Horvath. Already, DuPont has 6,000 phages in its collection to immunize bacteria, and the list is growing.

DuPont began vaccinating bacterial strains in 2007. In 2012 the company announced the first commercial application of CRISPR-enhanced cultures for making pizza cheese. It dubbed the blend of *S. thermophilus* strains CHOOZIT SWIFT. Barrangou says that all of DuPont's *S. thermophilus* cultures are now optimized using the company's CRISPR technique, and given DuPont's share of the dairy culture market—about 50 percent, by his estimate—chances are good that you and I, and all our fellow dairy lovers, have eaten CRISPRized food.

But like any vaccination strategy, this one is imperfect. Although Barrangou says it's a rare event, so-called CRISPR-escape mutant phages can outwit immunization through genetic mutation, altering their DNA so that it is no longer a match for the sequence adopted by the vaccinated bacteria. "This is probably where CRISPRs have a big disadvantage," says Derkx.

There are a number of other ways to create phage-resistant strains. For instance, Derkx says, isolating naturally

occurring bacterial mutants that prevent phages from binding to cell surface receptors is a particularly robust approach. Chr. Hansen has a few of these receptor mutant cultures on the market, says Derkx. "We haven't seen any phages that can overcome this resistance."

It's important to note that all the genetic modification to generate phage resistance is done by good old-fashion biology, and not by recombinant DNA technology. Barrangou calls the CRISPR-enhanced dairy cultures "non-GMO genetically modified organisms." It's not a lack of know-how or even of desire—customizing bacterial genomes could really ramp up immunity or provide any number of desirable traits in crops or livestock—but rather a general public distaste for GMO that keeps CRISPR, in the genome-editing sense, off the plate in the food sciences. Barrangou says that the concept has preceded acceptance. "Until regulatory pathways and the general public are more receptive to GMOs, people will have to keep working that way."

—Kerry Grens

Micro Master

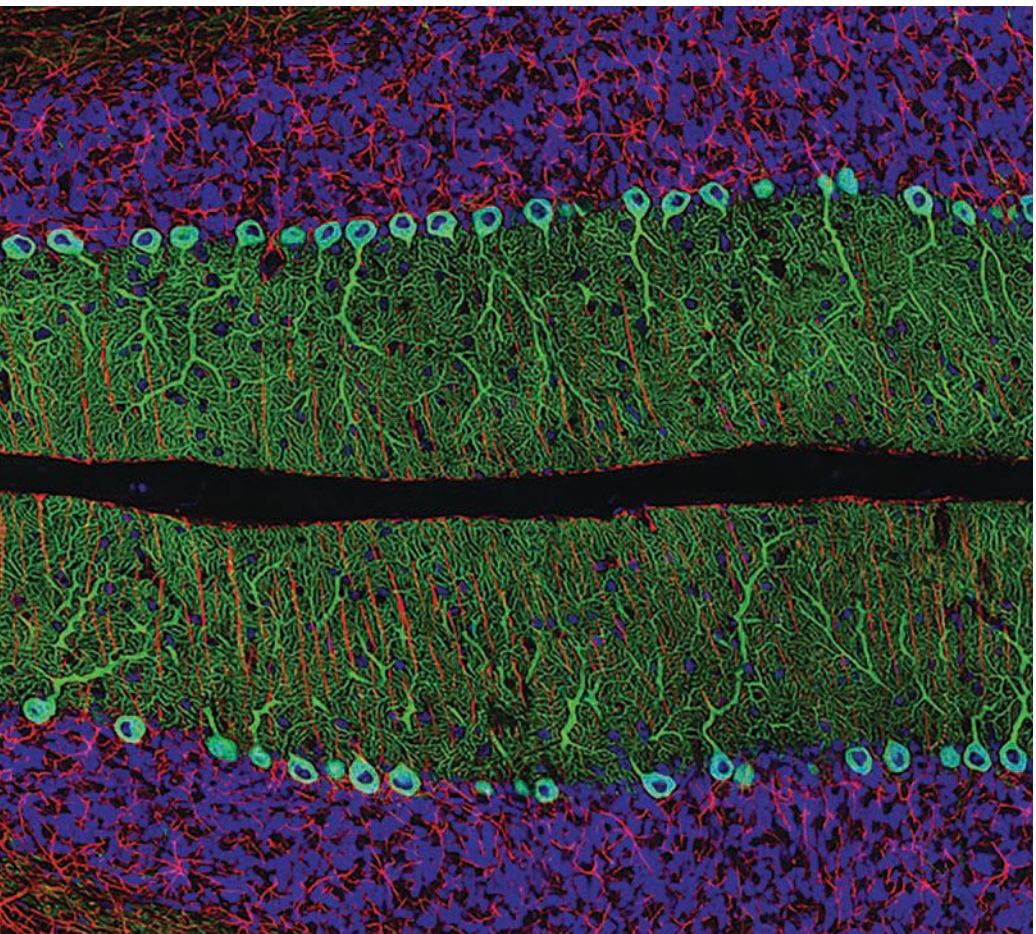
Last October, 40 stories high in 7 World Trade Center, Thomas Deerinck was among the first to check out the winners of the 2014 Nikon Small World Photomicrography Competition, lined up around the perimeter of a room whose front windows looked out onto a panoramic view of New York City. It was the photo contest's 40th anniversary, so the location was fitting. "It was pretty spectacular," Deerinck recalls. A slide show of winning entries from the photo competition's four decades featured one of his own works: a 2002 snapshot of a slice of rat cerebellum.

Deerinck has been a microscopist for nearly as long as Nikon has been running the Small World competition. He works at the National Center for Microscopy and Imaging Research (NCMIR), a National Institutes of Health–funded facility at the University of California, San Diego. The 56-year-old is constantly involved in a wide range of research projects that involve taking photographs of the microscopic world. And Deerinck's good at his job. In addition to his win in 2002, the first year he entered, he has placed in the Nikon Small World top 20 five times. "Tom Deerinck is what I consider kind of a rock star in the scientific imaging world," says Nikon communications manager Eric Flem.

Deerinck got drawn into microscopy when a researcher from a local college came to his high school in Stockton, California, to speak about a new program focused on training electron microscopists. "Once she started showing pictures from the scanning electron microscope, I was hooked," Deerinck says. He graduated from the two-year program at San Joaquin Delta College in 1978 and was immediately hired by UCSD's Mark Ellisman, future director of the NCMIR.

In the decades that followed, Deerinck had a front-row seat for the explosion in microscopic imaging technologies that has revolutionized diverse fields of study. First, Deerinck recalls, there was confocal microscopy in the mid-1980s, which allowed researchers to visualize microscopic structures in 3-D. "Especially since we do a lot of neuroscience, it was very vital to be able to take crisp, in-focus images of three-dimensional objects," he says. After that, Winfried Denk developed two-photon microscopy. Then came green fluorescent protein (GFP) and other glowing molecular tags. "And all of this kind of then spurred the whole super-resolution movement that's really in vogue right now," Deerinck says. The 2014 Nobel Prize in Chemistry went to Eric Betzig, Stefan Hell, and William Moerner for their contributions to the development of super-resolved fluorescence microscopy.

As microscopy has evolved, the Nikon Small World competition has received a significant uptick in the number of



TOP NOTCH: Deerinck won first place in the 2002 Nikon Small World Photomicrography Competition for this image of a rat cerebellum, captured using a confocal scope and fluorescent proteins.

THOMAS J. DEERINCK/NIKON SMALL WORLD

BERTHOLD TECHNOLOGIES

Certain configurations of this product are not available for sale in the U.S.A.

Mithras²

Monochromator Multimode Reader*

- double monochromators for excitation & emission
- all measurement technologies
- all microplate formats
- up to 4 reagent injectors
- filters RFID coded

Detect and Identify

www.berthold.com/bio

entries—from fewer than 100 submissions in the late '90s to some 2,000 each year for the past few years. "It's grown immensely over the years," Flem says. "It really acts as kind of an echo of what's happening in the scientific community, and to a certain degree even in the artistic community." And it's not just the number of entries that has changed, but the type as well, Flem adds. Since the switch to digital, "the number of biological images just started exponentially increasing; now that's really the majority of it."

Tom is probably the most successful here in terms of producing not only great science but spectacular art. He's a master.

—Mark Ellisman, National Center for Microscopy and Imaging Research

Deerinck has noticed that changing imaging technologies have also drawn a do-it-yourself crowd to the Small World competition. At the unveiling of this year's winners in New York City, Deerinck was impressed by the entries of so-called amateurs, including the winner: Rogelio Moreno of Panama, a computer system programmer and self-taught microscopist. The third-place micrograph also caught Deerinck's attention: a photo of jumping-spider eyes captured by 18-year-old Noah Fram-Schwartz. "This kid had to wait four years to be old enough to enter the competition because he was only fourteen when he started doing microscopy," says Deerinck. "I hate to call them amateurs. Anyone who can take a beautiful picture, that's the proof." (Another of Fram-Schwartz's images—an ant eye—got an honorable mention.)

Deerinck says the Nikon Small World competition has not only been great for his career, but sharing his photos in this way has also been good publicity for biomedical research in general. "We do research; we don't think about art or competitions like this," he says, "but it turns out it's actually very useful for research because it kind of gets [it] out into the public eye." In this same vein, Deerinck has helped put together microscopy exhibits at the San Diego and Washington Dulles airports.

Some of his current scientific projects include collaborating with Nobel Laureate Roger Tsien at the University of California, San Diego, to design new GFP-like probes to localize proteins and macromolecules in the cell. With other collaborators, Deerinck is using a 3-D imaging method called serial block-face scanning electron microscopy to automatically scan multiple layers of brain tissue and piece those images together into three-dimensional brain maps. At any given time, the NCMIR could be assisting researchers on as many as 100 different projects, Deerinck says, and it's part of the reason he loves his job. "I see the kids come through, and they get their PhDs, and they go on to become scientists, and the further they progress in their career, the less they are in the laboratory. All I was interested in, and all I am interested in, is being in the laboratory and actually doing all the hands-on work. That's what I get to do."

And there's no reason not to make a little art along the way. "Tom is probably the most successful here in terms of producing not only great science but spectacular art," Ellisman says. "He's a master."

—Jef Akst

Taming Bushmeat

Amid the bustle of a traditional produce market in southern China's Guangxi province, a small menagerie surrounded New York-based disease ecologist Peter Daszak: sacks of toads, piles of salamanders, snakes, alligators, nocturnal mammals called civets, herons, and more. "There were hundreds of different species," he recalls. "The diversity was incredible."

Used in traditional cuisine and for medicine, the array of meats is in constant demand, and is frequently procured by trapping these animals in the wild. Earlier this year, Daszak and colleagues of his from EcoHealth Alliance were in the region hunting cryptic diversity: pathogens in these animals that are primed to enter human populations when the meat is handled or consumed.

In late 2002, a mysterious virus—the cause of an atypical pneumonia that would later be named severe acute respiratory syndrome (SARS)—emerged in the human population, likely from an animal source in southern China. The previously unknown coronavirus sickened more than 8,000 people and killed more than 700 in more than 35 countries across the globe. Researchers tracking the epidemic found that a high proportion of the earliest cases occurred in people who had handled wild animals used as food. None were farmers, but seven were chefs at restaurants where several animal species were slaughtered on the premises; one sold snakes at a produce market; and another purchased meat at such markets for restaurants. The SARS coronavirus or viruses very closely related to it were also found in palm civets, raccoon dogs, bats, and other wild mammals (*Emerg Infect Dis*, doi:10.3201/eid1006.030852, 2004).

To better understand the disease risk posed by eating or handling wild-caught animals, Daszak and other EcoHealth Alliance researchers have recently embarked on a project in Guangxi, Guangdong, and several other provinces in China. The group aims to identify pathogens in blood, fecal, and other samples from wild animals in the region and estimate the risks they pose to human health. In the process, they are also working with farmers who have begun to captive-breed wildlife.

Observing the constant demand for wild species—and the potential profits to be had—several small farms in the region have begun rearing animals that were traditionally wild-caught. The efforts are profitable, according to Shangzheng Wei, a farmer in Guangxi who rears bamboo rats, porcupines, civ-

ALEKSEI CHMURA

GAME FARM: Shangzheng Wei farms wild animals in Guangxi, China, where he houses nutria in outdoor enclosures.

ets, and nutria. "I started because I felt it a good opportunity to make money," Wei told *The Scientist* in a translated e-mail. "I saw there was a local demand, but little supply [from the wild]."

Dwindling wild populations—largely a result of overhunting by people—may recover if farming efforts increase, Daszak suggests. The pangolin, a scaly mammal prized as meat and medicine, is now listed as critically endangered by the International Union for Conservation of Nature. "They used to be quite common [in this region] but are rare now," says Daszak. "They've basically been eaten almost to extinction."

Breeding traditionally wild-caught species offers potential human health benefits as well; preliminary results from Daszak's team indicate that farm-bred animals carry fewer viruses than the same species caught in the wild.

But whether farming such animals decreases threats to wild populations is unclear. A 2010 study conducted in Vietnam found that commercial porcupine farming did wild populations more harm than good, as restaurants continued to buy lower-priced wild meat, and many farmers sought their breeding stocks from the wild, rather than from other farms (*Biol Conserv*, doi:10.1016/j.biocon.2010.07.030).

Rearing rare species can also be a risky business. Whether caretakers on farms might acquire microbes as a result of prolonged exposure to the animals is uncertain, and another question Daszak's team aims to address. In addition, farmers must experiment to learn the animals' optimal diet, breeding and living conditions, and how to cope with an ailing nutria or civet. For example, wild bamboo rats eat, well, bamboo. But breeders have found the animals fare better on a mix of other foods, including beans. "Raising these animals is unique—different from domestic animals—and I had to find my own way by trial and error," says Wei. "There were no experts in raising them."

Although selling the animals remains the biggest challenge to farmers attempting to establish themselves as sources for these foods, Wei and other farmers have gradually built informal cooperatives to share information and exchange animals to prevent inbreeding. "I enjoy making a study of the problems [and successes] I encountered in the breeding process [over the years]," says Wei. Now, he has also written a book about his experiences "to help other people who want to raise bamboo rats and other wild animals."

One hurdle the EcoHealth Alliance team is unlikely to face is people's preferences for the source of their meat. People don't seem to take into account whether an animal is wild-caught or farm-raised when they eat favorite foods at restaurants, according to epidemiologist Maureen Miller of EcoHealth Alliance.

After a visit to Wei's farm, Miller and others dined at a restaurant that specializes in wildlife. They ate a rare mountain frog "that tasted very much like a [commonly found species of] frog," according to Miller, and bamboo rats from Wei's farm—cleaned and prepared on-site for the visitors. "[People] have a taste for certain animals that are wildlife," Miller adds. "If it's prepared the way they like, [they are unlikely] to ask about the source. What they want is simply a good, high-quality meal."

—Jyoti Madhusoodanan

Assessing Research Productivity

A new way of evaluating academics' research output using easily obtained data

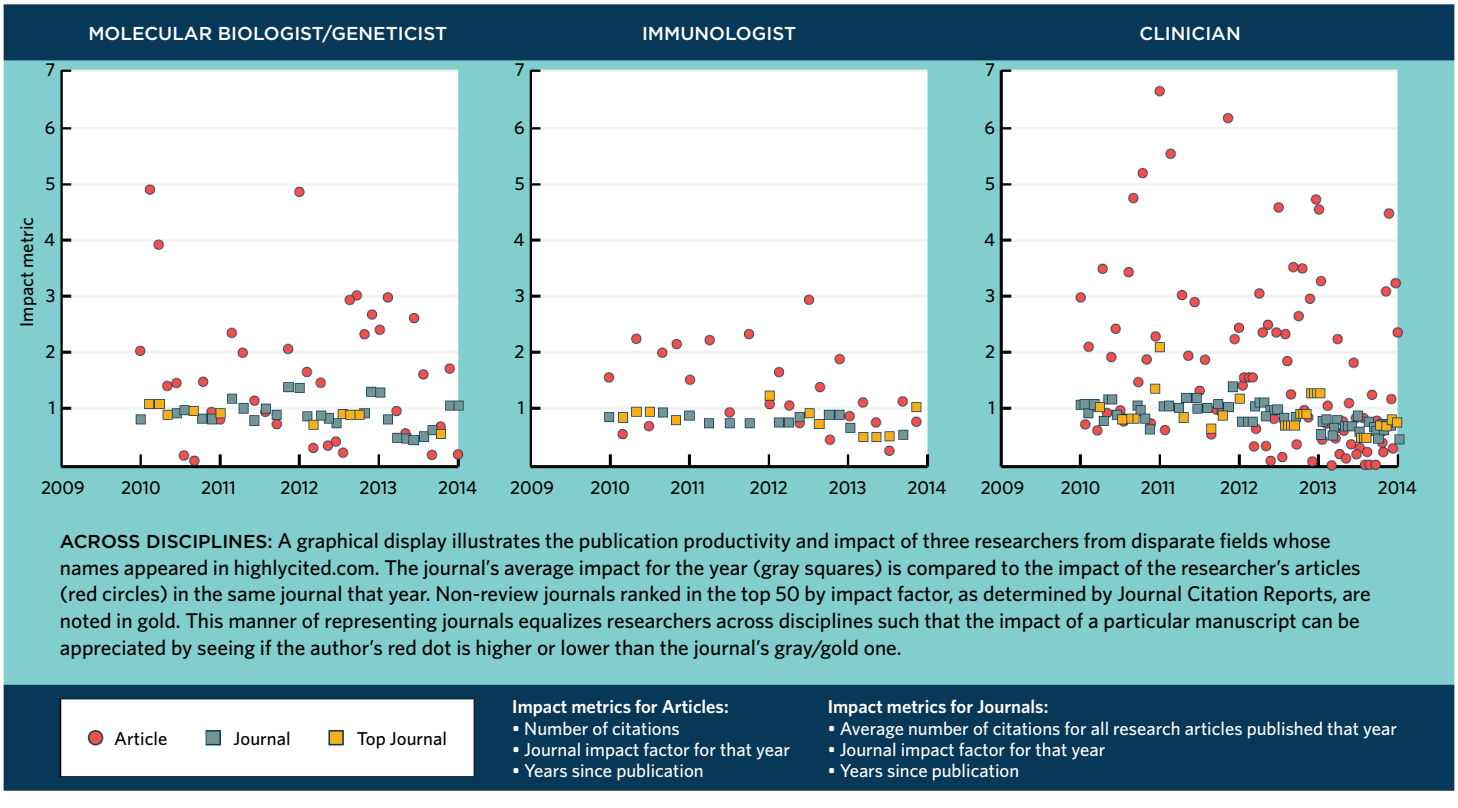
BY USHMA S. NEILL, CRAIG B. THOMPSON, AND DONNA S. GIBSON

It can often be difficult to gauge researcher productivity and impact, but these measures of effectiveness are important for academic institutions and funding sources to consider in allocating limited scientific resources and funding. Much as in the lab, where it is important for the results to be repeatable, developing an algorithm or an impartial process to appraise individual faculty research performance over multiple disciplines can deliver valuable insights for long-term strategic planning. Unfortunately, the development of such evaluation practices remains at an embryonic stage.

Several methods have been proposed to assess productivity and impact, but none can be used in isolation. Beyond assigning a number to an investigator—such as the h-index, the number of a researcher's publications that have received at least that same number of citations, or a collaboration index, which takes into account a researcher's relative contributions to his or her publications—there are additional sources of data that should be considered. At our institution, Memorial Sloan Kettering Cancer Center (MSKCC) in New York City, there is an emphasis on letters of recommendation received from external expert peers, funding longevity, excellence in teaching and mentoring, and the depth of a faculty member's CV. For clinicians, additional assessments of patient load and satisfaction are also taken into consideration by our internal committees evaluating promotions and tenure. Other noted evaluation factors include the number of reviews and editorials an individual has been invited to author; frequency of appearance as first, middle, or senior author in collaborations; the number of different journals in which the researcher has published; media coverage of his or her work; and the number of published but never-cited articles.

Much as in the lab, where it is important for the results to be repeatable, we need an impartial process to appraise individual faculty research performance.

Here we propose a new bibliometric method to assess the body of a researcher's published work, based on relevant information collected from the Scopus database and Journal Citation Reports

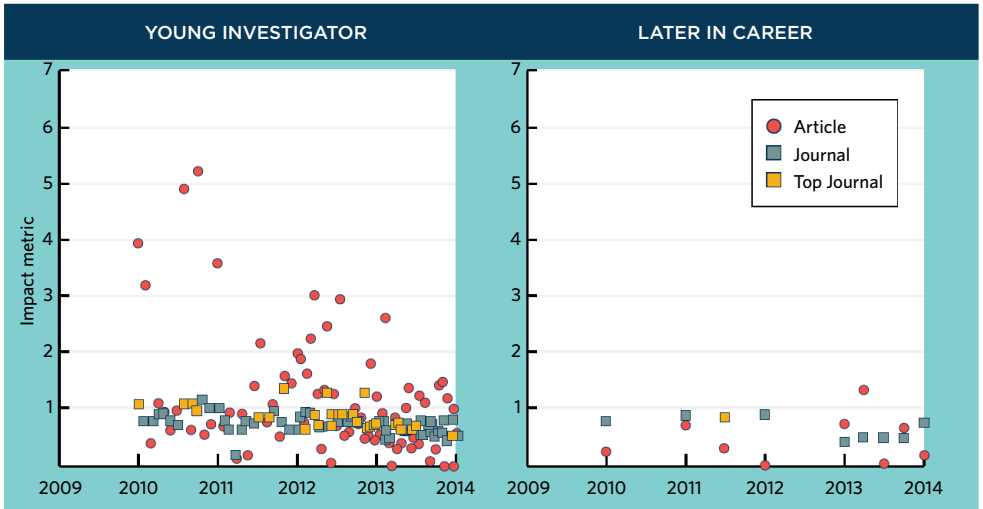


ACROSS DISCIPLINES: A graphical display illustrates the publication productivity and impact of three researchers from disparate fields whose names appeared in highlycited.com. The journal's average impact for the year (gray squares) is compared to the impact of the researcher's articles (red circles) in the same journal that year. Non-review journals ranked in the top 50 by impact factor, as determined by Journal Citation Reports, are noted in gold. This manner of representing journals equalizes researchers across disciplines such that the impact of a particular manuscript can be appreciated by seeing if the author's red dot is higher or lower than the journal's gray/gold one.

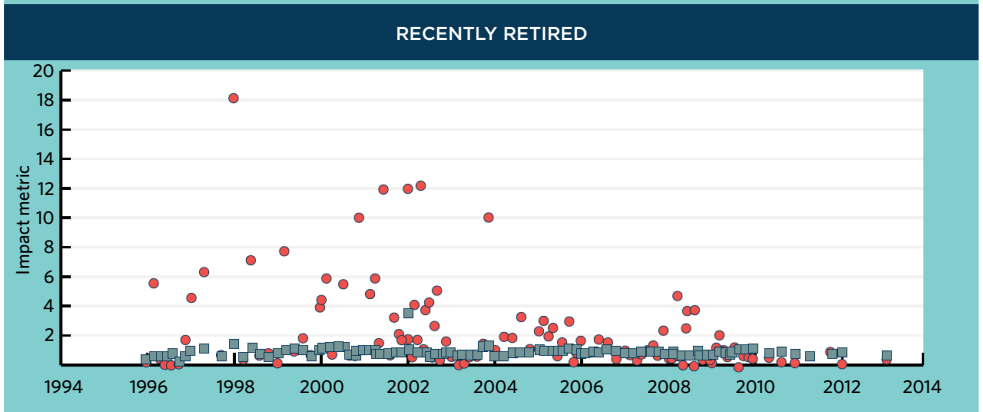
(JCR). This method does not require intricate programming, and it yields a graphical representation of data to visualize the publication output of researchers from disparate backgrounds at different stages in their careers. We used Scopus to assess citations of research articles published between 2009 and 2014 by five different researchers, and by one retired researcher over the course of his career since 1996, a time during which this individual was a full professor and chair of his department. These six researchers included molecular biologists, an immunologist, an imaging expert, and a clinician, demonstrating that this apparatus could level the playing field across diverse disciplines. (See graphs on previous page.)

The metric we used calculates the impact of a research article as its number of citations divided by the publishing journal's impact factor for that year, divided by the number of years since the article was published. The higher the number, the greater the work's impact. This value is plotted together with the average impact of all research articles the journal published in that same year (average number of citations for all research articles published that year divided by the journal impact factor for that year divided by the number of years since publication). Publications in journals that rank in the top 50 by impact factor (not including reviews-only journals) are also noted.

By developing such a graph for each scientist being evaluated, we get a snapshot of his or her research productivity. Across disciplines, the graphs allow comparison of total output (number of dots) as well as impact, providing answers to the questions: Are the scientists' manuscripts being cited more than their peers' in the same journal (red dots above gray)? How many of each researcher's papers were published in leading scientific journals (gold squares)? The method also allows evaluation of early-career scientists and those who are further along in their careers. (See graphs at right, top.) For young researchers, evaluators can easily see if their trajectory is moving upward; for later-stage scientists, the graphs can give a sense of the productivity of their lab as a whole. This can, in turn, reveal whether their laboratory output matches their allocated institutional resources. While the impact factor may be a flawed measurement, using it as a normalization tool helps to remove the influence of the journal, and one can visualize whether the scientific community reacts to a finding and integrates it into scientific knowledge. This strategy also allows for long-term evaluations, making it easy to appreciate the productivity of an individual, in both impact and volume, over the course of his or her career. (See graph above.)



ACROSS AGES: This method of visualizing researchers' productivity can be a useful tool for comparing scientists at different points in their career.



LONG-TERM ANALYSIS: A nearly 20-year stretch (1996–2014) is shown for a newly retired faculty member after a productive research career. Note that this individual did not publish any articles in top 50 non-review journals as determined by Journal Citation Reports impact factors. Although this researcher published several papers before 1996, Scopus has limited reliability for citations prior to that year; therefore the analysis excluded these data.

Assessing research performance is an important part of any evaluation process. While no bibliometric indicators alone can give a picture of collaboration, impact, and productivity, this method may help to buttress other measures of scientific success. ■

Ushma S. Neill is director of the Office of the President at Memorial Sloan Kettering Cancer Center (MSKCC). Craig B. Thompson is the president and CEO of MSKCC, and Donna S. Gibson is director of library services at the center.

Funding Research in Africa

The ongoing Ebola epidemic in West Africa is drawing more money to study the virus, but what about funding for African science in general?

BY PAULA PARK

The Ebola outbreak in Sierra Leone, Guinea, and Liberia has laid bare the dearth of basic research funding for scientists in many African countries, which cannot afford the high cost of building laboratories and purchasing equipment needed to study such diseases.

Since 2010, the National Institutes of Health (NIH) has provided $300 million in grants to researchers in just 17 of 54 African countries, for example, with around $200 million going to South Africa alone, according to the NIH RePort funding database.

Some donors and scientists predict the heightened awareness may result in expanded funding on not just Ebola, but also diverse neglected diseases in Africa. “Ebola has brought in very sharp relief the extent of our ignorance in Africa and the Western world” about emerging infectious diseases, said Steve Kayizzi-Mugerwa, director of development research at the African Development Bank, which collects contributions from African governments and donors to fund development across the continent. “It’s a very serious epidemic, but everything has a silver lining,” he told *The Scientist* at the recent African Economic Conference in Addis Ababa, Ethiopia.

So far, however, there have been no signs that funding for African research in general will surge, and some worry that increased funding for Ebola in the midst of the epidemic will actually draw funds away from other research programs.

Since August, the UK government has pledged around £200 million ($318 million), including £3 million ($4.8 million) for research into how Ebola spreads. In the U.S., the government has committed $350 million in aid and research. The National Institute of Allergy and Infectious Diseases (NIAID), for example, spent $42.5 million on Ebola vaccines and treatment research in fiscal year 2013, which ended October 31, according to James Meegan, director of global research at the agency. Meegan added that the agency has proposed spending $44 million in fiscal 2014, though that may increase as NIAID finances further research on Ebola vaccines. In addition to shifting funds, NIAID is tasking virologists from Africa to set aside research on other hemorrhagic disease research programs to work on Ebola, Meegan said.

While the funding is welcomed by the scientific community, Julien Potet, a policy advisor for Doctors Without Borders, wonders what it will mean for other areas of research. Funding is already stretched to research such neglected diseases as African trypanosomiasis (sleeping sickness), malaria, and filarial diseases, such as elephantiasis, he said;

© ISTOCKPHOTO.COM/LCSWART

treatments for Ebola and other new diseases should be additional. “We have to take a look and make sure it’s not being taken from funding that is already pledged for some other research,” Potet told *The Scientist*.

It is too early to know whether new concern over Ebola will cut into research on other diseases, but it likely will do so, agreed Ambrose Talisuna, senior clinical research fellow at the Kemri-Wellcome Trust Research Programme in Kenya and head of malaria drug resistance surveillance for East Africa. The Gates Foundation, however, continues its commitment to eradicating malaria, announcing in November 2014 that it was pledging another $500 million to combating the disease.

Not all funding for Ebola and other diseases that affect the African population is going to researchers on the continent, said

Erica Ollmann Saphire, an investigator at the La Jolla-based Scripps Research Institute. Ollmann Saphire works with teams of researchers on two NIH-funded hemorrhagic disease projects, and just a handful are physically in Africa, for example. And the lion's share of funds that the Gates Foundation devotes to research into malaria are spent in developed countries outside Africa where drug development systems are already in place.

"Most of the African researchers are mainly focusing on getting resources, publishing their papers in collaboration, but are not in the position to lead the research that will solve the continent's problems," said Solomon Nwaka, acting executive director of the African Network for Drugs and Diagnostics Innovation (ANDI), an international organization conceived by the World Health Organization and launched with seed money from the European Union. "The problem is resources. There is no pan-African research funding agency."

Ebola has brought in very sharp relief the extent of our ignorance in Africa and the Western world about emerging infectious diseases.

—Steve Kayizzi-Mugerwa, African Development Bank

In August 2014, the U.K.'s Department of International Development and the Wellcome Foundation unveiled a £40 million ($64 million) five-year research project called the Developing Excellence in Leadership, Training and Science Initiative for African Scientists (DELTAS). The program is intended to fund training for junior scientists in Africa and to help support senior researchers across all areas of health research, said Val Snewin, Wellcome's international activities manager. Infectious diseases have declined in significance to many researchers in mid-income African countries, as concern has grown over diseases of the West such as diabetes, heart disease, and hypertension, said Snewin. DELTAS may fund some of these areas, she said.

The funding for the DELTAS program was already in the pipeline when the Ebola outbreak started, however, so whether the continent will see more general research funding initiatives like this in the wake of the epidemic remains to be seen. For now, the research community welcomes the funding dedicated to Ebola, which until this point has been limited, Talisuna said. But broader funding initiatives are also needed. Timely responses to these diseases require "top-quality scientists and top-quality infrastructure," he said. "[Donors and governments] should fund these institutions, not just for research, but to train quality scientists in Africa." ■

Paula Park is a freelance writer living in Ghana, Africa, and a former editor at The Scientist. *A version of this article appeared at www.the-scientist.com on November 12.*

On "Funding Research in Africa"

A letter to *The Scientist*

BY FRANCIS S. COLLINS AND JEREMY FARRAR

We are writing to comment on your November 12 article entitled "Funding Research in Africa" by Paula Park. The article appropriately identified a very real problem—the dearth of basic-research funding for scientists in many African countries. While the US National Institutes of Health (NIH), the Wellcome Trust, and others fund a considerable amount of research in Africa, many of those awards are made to non-African institutions and scientists, who in turn subcontract for collaborations with their African colleagues. However, Park apparently did not appreciate that the proportion of awards made directly to African scientists and institutions is steadily increasing, and now accounts for about 40 percent of Wellcome Trust and 63 percent of NIH funding for research in Africa.

As a prominent example of this increasing focus on direct funding to African investigators, the NIH and the Wellcome Trust recently established a collaborative program, called Human Health and Heredity in Africa (H3Africa). H3Africa directly funds African scientists at African institutions to conduct research on the genomic and environmental bases of health problems of importance in Africa. Support is being provided for basic research, improved infrastructure, and training. One of the long-term goals of H3Africa is to increase the ability of African scientists to compete for international research funding.

NIH and the Wellcome Trust have, together, committed the equivalent of at least $76 million to H3Africa for the period 2012–2016, and both agencies will consider extending the program for an additional five years pending the outcome of rigorous peer review processes at each agency. The current H3Africa awards were made after an open competition, and each agency reviewed the applications according to its normal peer review process.

As the research areas of the grant applications were determined entirely by the applicants, it is interesting to note that, while some H3Africa grants address infectious diseases (e.g., trypanosomiasis, tuberculosis, pediatric HIV/AIDS, and fevers of unknown origin), the majority are actually directed at noncommunicable diseases (NCDs). NCDs are, as Park noted, of increasing importance in Africa. Among the NCDs currently under study in H3Africa projects are type 2 diabetes, rheumatic heart disease, cardiometabolic disease, stroke, schizophrenia, and kidney disease. Infrastructure compo-

nents of H3Africa include a continental bioinformatics network, called H3ABioNet, and biorepositories, which are also located on the continent. All projects within H3Africa are organized as research consortia to promote intra-African collaboration and the sharing of experience among investigators and trainees.

The Wellcome Trust is also investing significantly in programs that will develop a cohort of outstanding researchers in sub-Saharan Africa. One example is the £30 million ($47 million) African Institutions Initiative, established in 2008, in which all the funds for more than 50 African institutions are going directly to Africa. More recently, the Wellcome Trust has supported, with an initial commitment of £40 million ($62.6 million) over five years, the Developing Excellence in Leadership Training and Science (DELTAS) Africa initiative, which aims to facilitate collaboration with other funders of African research.

In addition, the Wellcome Trust and the UK Department of International Development have cofunded two programs, at £10 million ($15.6 million) each, to support national health research funding organizations in Kenya and Malawi. These programs are free to set and manage their own research funding priorities.

Another NIH-funded, African-led global health program—the Medical Education Partnership Initiative (MEPI)—works in partnership with the President's Emergency Plan for AIDS Relief (PEPFAR) to provide $130 million in direct support over a five-year period to 34 African medical schools in 12 countries. Strengthening and expanding the clinical research workforce in Africa are among the aims of MEPI.

The NIH and Wellcome Trust are very encouraged by the early results of these direct funding programs, and urge other funding agencies to consider joining us in our efforts to increase the capacity of African scientists performing world-class research in their own countries. Of course, in the long term, the success of scientific research in Africa will depend on the willingness and ability of African governments to invest in research. Such investments will lead to a sustainable African workforce and infrastructure that can address the health concerns of all Africans. ■

Francis S. Collins is the director of the US National Institutes of Health. Jeremy Farrar is the director of the Wellcome Trust. This letter first appeared at www.the-scientist.com on November 28.

Grab 'n' Glow

Engineered proteins can tether multiple fluorescent molecules to give a brighter signal—and that's not all.

BY RUTH WILLIAMS

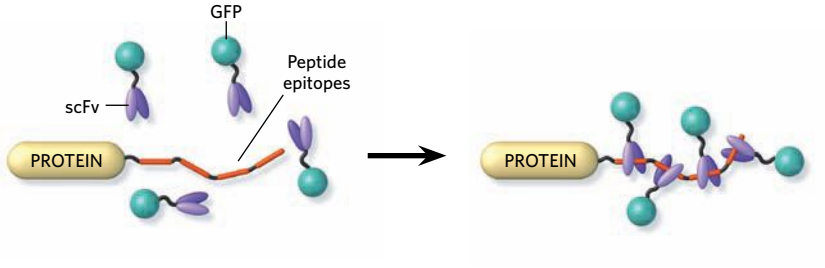
A common way to visualize single DNA or RNA molecules in cells is to insert into the target molecule multiple copies of a sequence to which a fluorescently tagged protein can bind. The more copies of the sequence, the more bound fluorescent proteins, the brighter the signal. Marvin Tanenbaum, a postdoc in Ronald Vale's lab at the University of California, San Francisco, thought to himself, "Why don't we do this for proteins?" So he did.

Prior to Tanenbaum's technique, called SunTag, the main way to visualize a protein in cells was to recode its sequence to contain a fluorescent domain—such as green fluorescent protein (GFP). Proteins with a single GFP domain were often too dim for some types of fluorescent microscopy, however, and adding more GFP domains didn't always work—the proteins became unfeasibly large.

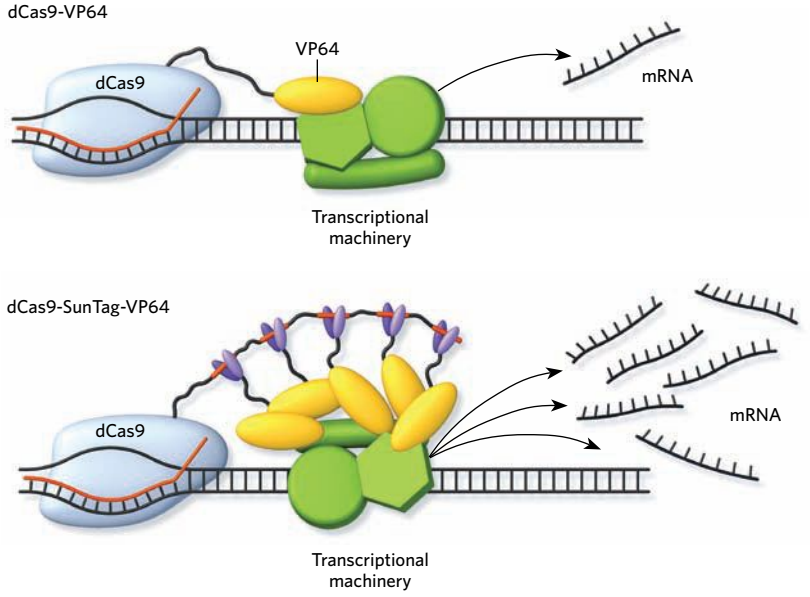
With SunTag, proteins of interest are instead recoded to contain multiple copies (up to 24) of a tiny peptide epitope. The protein is then coexpressed with GFP-tagged single-chain antibodies that recognize the epitope and grab on.

"It's a relatively simple idea—that's the beauty of it," says Robert Singer of Albert Einstein College of Medicine in New York City. "But it wasn't simple to effect," he says, referring to the rigorous tweaking and testing Tanenbaum and his colleagues had to do. "They clearly put a lot of effort into optimizing the system."

But it was worth it, because not only can SunTag be used to make proteins glow more brightly, it can also locally attract other types of proteins to the epitope. For example, Tanenbaum has used it to recruit multiple transcription factors to a DNA-binding protein to ramp up expression of a gene. (*Cell,* 159:635-46, 2014)



The SunTag approach allows researchers to add numerous tags to a single protein by genetically engineering the protein to include repeats of a small peptide epitope (orange). The epitopes then recruit a single-chain antibody, scFv, which has the indicator tag—in this case, green fluorescent protein (GFP)—attached.



SunTag can also be applied to enhance transcription. For instance, engineering a nuclease-dead version of the enzyme Cas9, called dCas9, which can locate a gene's promoter region, to include a string of epitopes allows it to carry multiple copies of the transcriptional activator VP64. These additional copies ramp up transcription of the gene.

GEORGE RETSECK, REDRAWN FROM *CELL*, 159:635-46, 2014

AT A GLANCE

TECHNIQUE	HOW IT WORKS	SINGLE-MOLECULE IMAGING?	POSSIBLE FUSION PROTEINS	MULTIPLE COLORS?
Fluorescent fusion proteins	A recoded protein contains a fluorescent domain such as GFP.	Unlikely with regular fluorescence microscopes as signal is too dim	Practically any, assuming correct function of fusion protein can be confirmed	Yes. Many different fluorescent fusion proteins can be expressed in the same cell.
SunTag	A recoded protein contains multiple copies of a small epitope that binds fluorescent fusion antibodies.	Yes. The fluorescent signal is approximately 20 times brighter than a single GFP.	Best with proteins that stably associate with subcellular structures; must confirm correct function of fusion protein	Not yet

Stress Fractures

Social adversity shapes humans' immune systems—and probably their susceptibility to disease—by altering the expression of large groups of genes.

BY DANIEL COSSINS

In 2005, Steve Cole began to peer inside the cells of lonely people, training his sights on the activity of their genomes. Cole, a psychologist turned molecular biologist at the University of California, Los Angeles, was interested in how psychological stressors such as chronic social isolation could be bad for our health, increasing our susceptibility to certain diseases. Research had already implicated stress hormones, which are produced at higher-than-average levels in people who feel lonely for long stretches. But Cole wanted to know what was going in the genes, and not just one or two. He suspected that the expression of large collections of genes might be disrupted in people who consistently reported feeling isolated. "I had an abiding mistrust of one-gene answers because genes generally work in coordinated networks in cells," he says.

Cole teamed up with University of Chicago social psychologist John Cacioppo, who had already been tracking 166 healthy middle-aged adults for three years, periodically asking them how socially isolated they felt and gathering all manner of biomedical, psychological, social, and economic data. Cole and Cacioppo took blood samples from 153 of the study subjects and focused on the eight most socially secure people and the six loneliest, who had scored highest on the UCLA Loneliness Scale for the past three years. When Cole ran these 14 subjects' white blood cells through a microarray analysis, he spotted more than 200 genes that were expressed differently between the two groups. Many of the genes dialed up in lonely individuals were involved in inflammation, while the down-regulated genes tended to be associated with antiviral response, antibody production, and restraint of inflammatory responses.[1]

It was a tiny sample, but the implications of the study, published in 2007, were great: loneliness, it seems, shapes one's health by controlling the "dimmer switch" for whole networks of immune-related genes. Indeed, this overexpression of pro-inflammatory genes and suppression of anti-inflammatory and antiviral genes might explain why lonely people are more likely to succumb to a variety of diseases, and why HIV ravages socially isolated people more quickly than their more connected peers. "It was gratifying to see the story [of how loneliness affects health] move beyond the genotype to include the functional aspect of the genome," says Cacioppo.

Cole and others have since produced evidence for similar gene-expression shifts in people experiencing various types of social stress, from facing the death of a loved one or intentional rejection by close friends to low socioeconomic status and physical abuse during childhood. Across such diverse experiences, Cole spotted the same pattern. "You see the same general increase in inflammatory gene expression and decrease in antiviral gene expression," he says. "It's pretty consistent."

Social stress seems to reach deep into cellular control centers to shape key aspects of the immune system—and, as a result, can impair one's ability to avoid or fight off disease and psychiatric disorders. Now researchers are trying to pin down the molecular mechanisms by which psychological stress is translated into gene-expression changes, and to develop a more precise understanding of the conditions under which stress-mediated gene regulation affects health.

"The emphasis so far has been on demonstrating that the relationship [between stress and disease] exists," says Jenny Tung of Duke University who studies gene-behavior interactions in non-human primates. "Now it's about understanding which pathways are important and what kinds of social adversity have the greatest effect in which types of people."

Worried sick

Forty years ago, few scientists accepted the idea that psychological states could affect physiology and potentially influence disease. Then a string of studies in the 1980s revealed that the nervous and immune systems are in communication, and that their conversations can impact health. In 1984, for example, research-

ers found that the innate immune system's virus- and tumor-fighting natural killer cells were less active in medical students during exam time.[2] In addition, large epidemiological studies revealed that chronic social stressors correspond with marked disparities in death rates and susceptibility to disease. Among British civil servants, for instance, employment grade—a proxy for socioeconomic status—correlated with big differences in mortality from coronary heart disease, despite universal access to health care.[3]

More recent studies of British civil servants have demonstrated that chronic work stress increases susceptibility to heart disease, type 2 diabetes, depression, and more. These days it's widely accepted that stress does influence disease risk. Figuring out the mechanisms underlying this link, however, has been tricky: How do feelings induced by our social environment feed into our physiology and, ultimately, our health?

Researchers have demonstrated that the brain responds to psychological stress in the same way that it reacts to physical threats: by activating the hypothalamic-pituitary-adrenal (HPA) axis and "fight or flight" responses via the sympathetic nervous system (SNS). These two pathways control the release of stress hormones, such as cortisol, which help regulate inflammation. But scientists are also now starting to understand the effects of stress at a deeper level—that of individual genes and networks of genes that work in concert. "There is a sense that something has to be going on at the level of genome regulation," says Tung. "But insight has come pretty recently in terms of how long we've been trying to solve the puzzle."

Since Cole and Cacioppo's 2007 study, the evidence for a correlation between social stress and widespread proinflammatory gene expression has been stacking up. In 2011, the two researchers replicated their loneliness results in an expanded study of 93 people, detailing the types of white blood cells, or leukocytes, that exhibited the most pronounced differences in gene expression.[4] Meanwhile, collaborating with Northwestern University psychologist Greg Miller, Cole demonstrated similar transcriptional signatures in people caring for family members with brain cancer and people experiencing long-term interpersonal troubles.[5,6]

The altered expression of immune-related genes resulting from social adversity can linger much longer than the adversity itself. Cole and Miller have discovered, for instance, that healthy adults who experienced low socioeconomic status during childhood carried the same skewed expression profile, while participants who had grown up in higher-income homes had normal levels of expression.[7] The disparities were independent of the subjects' current socioeconomic status, lifestyle, or perceived stress.

Cole proposes that this pattern of upregulated proinflammatory genes and downregulated anti-inflammatory and antiviral genes represents a conserved transcriptional response to adversity—a phenomenon that likely evolved as an adaptive response to accelerate wound healing and to limit bacterial infection in the face of myriad physical threats. In the modern developed world, however, where people tend to live comfortably indoors, chronic inflammation in response to social stress is unnecessary. Worse, it appears to contribute to a long list of maladies.

Greg Gibson, director of the Center for Integrative Genomics at Georgia Tech in Atlanta, says this sort of work is important. While most genomics researchers focus on identifying single genes involved in disease risk, he points out, Cole and others are "trying to understand the bigger picture in terms of how whole suites of genes are coordinately regulated . . . and how all sorts of environmental and behavioral factors are involved."

There is a sense that something has to be going on at the level of genome regulation. But insight has come pretty recently in terms of how long we've been trying to solve the puzzle.

—Jenny Tung, Duke University

Studying humans, though, it's difficult to establish a direct causal connection. For starters, it's not possible to experimentally manipulate social stress in people over long time spans. Moreover, human lives are so complex that it's extremely hard to rule out confounding factors, such as diet and exercise or access to health care, particularly with the relatively small sample sizes used so far.

A handful of animal studies have yielded results that support the idea that stress can influence widespread gene expression. Turning to captive rhesus macaques, for example, Tung—along with her postdoc advisor Yoav Gilad of the University of Chicago and other colleagues—found that when mid-ranking females were randomly assigned to a low rank in a new group, their white blood cells dialed up inflammatory genes. Even more strikingly, gene-expression data alone were sufficient to predict dominance rank with 80 percent accuracy.[8] "That gestures toward causality," says Tung.

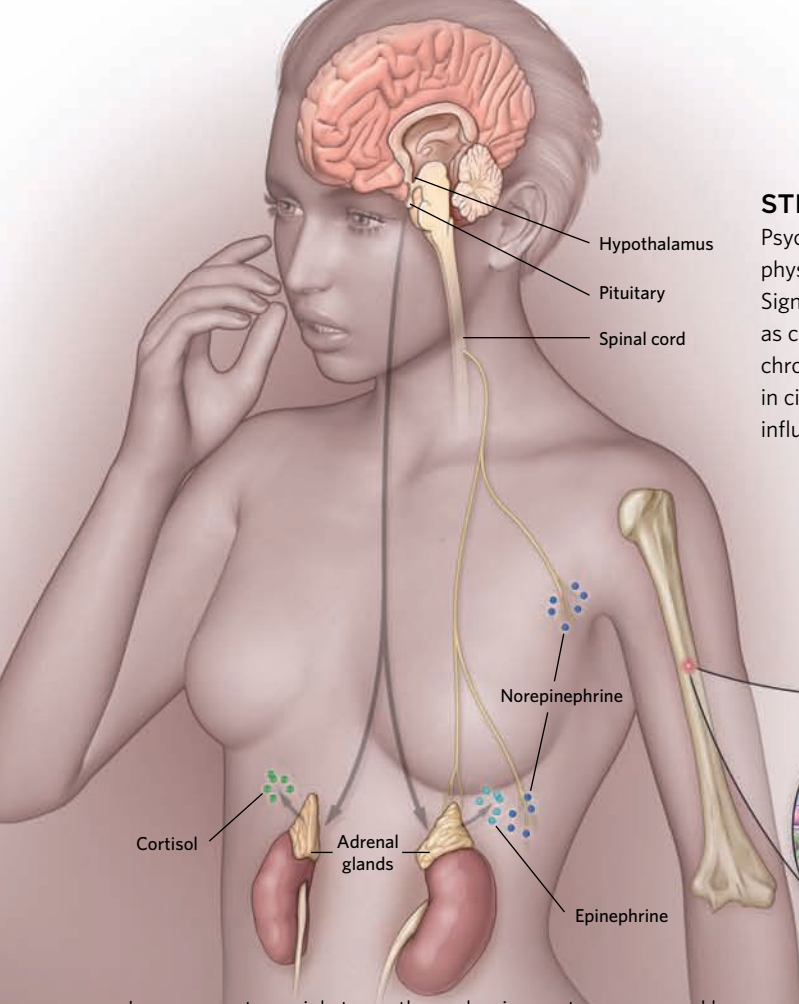
Feelings to physiology

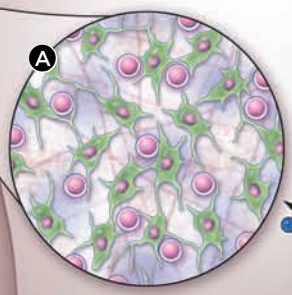
To understand how stress is translated into gene-expression changes, Cole and his colleagues performed bioinformatics analyses to identify transcription factors that act on the promoter regions of genes dialed up or down in circulating leukocytes of stressed people, monkeys, and mice. Their results suggested that transcriptional shifts in some genes are the result of increased activity of NF-κB transcription factors, a family of DNA-binding proteins known to increase inflammatory gene expression. Cortisol released via the HPA axis in response to acute stress puts the body on high alert, stimulating increased glucose production. Cortisol also plays a key role in shutting down the stress response by binding to glucocorticoid receptors, which inhibits NF-κB activity and thereby restrains inflammation. Under chronic stress, however, the glucocorticoid receptor is somehow desensitized, resulting in unrestricted proinflammatory gene expression. (See illustration on opposite page.)

STRESS IN THE BODY

Psychological stress resulting from our social environment causes the same physiological reaction that occurs in response to the threat of physical attack. Signals processed in the brain trigger the release of stress hormones such as cortisol and epinephrine to put the body on high alert. When stress is chronic, changes in the expression of large groups of immune-related genes in circulating white blood cells can cause ramped-up inflammation that influences susceptibility to disease.

Social stress causes the sympathetic nervous system to release the neurotransmitter norepinephrine directly from nerve fibers and the hormone epinephrine via the adrenal gland. Both neurotransmitters bind beta-adrenergic receptors, leading to increased inflammation and reduced viral defenses:





Beta-adrenergic binding during chronic stress leads to increased production of a particular type of white blood cell, an immature proinflammatory monocyte A.

Epinephrine and norepinephrine

Beta-adrenergic receptor

In response to social stress, the endocrine system, governed by the hypothalamus-pituitary-adrenal (HPA) axis, releases cortisol, which binds glucocorticoid receptors in white blood cells to mediate the stress response:

Cortisol

WHITE BLOOD CELLS

Under acute stress, cortisol puts the body on alert, then curtails the stress response by binding glucocorticoid receptors in white blood cells 1.

1 Glucocorticoid receptor

NUCLEUS

2 NF-κB

Cortisol-bound glucocorticoid receptors inhibit the activity of NF-κB transcription factors that otherwise promote the expression of proinflammatory genes 2.

When stress is chronic, however, the receptors somehow become desensitized, resulting in unrestricted expression of proinflammatory genes 3.

3 Proinflammatory cytokines

The binding of beta-adrenergic receptors also leads to enhanced activity of transcription factors, such as the cyclic AMP response element-binding protein (CREB), that increase the expression of genes encoding proinflammatory cytokines . . . B

NUCLEUS

CREB

IRF

B

C

Proinflammatory cytokines

. . . and inhibition of interferon response factor (IRF) transcription factors, which suppress the expression of genes involved in antiviral and antitumor responses C.

Meanwhile, the SNS responds to perceived threats by releasing the neurotransmitter norepinephrine directly from nerve fibers and the hormone epinephrine via the adrenal gland. Epinephrine and norepinephrine bind beta-adrenergic receptors, leading to the activation of transcription factors such as the cyclic AMP response element-binding protein (CREB), which upregulates genes encoding proinflammatory cytokines. So stress, it seems, serves up a one-two punch, with signals from the HPA axis failing to restrain inflammation, while messages coming though the SNS ramp it up. In addition, epinephrine and norepinephrine binding of beta-adrenergic receptors inhibits interferon response factor (IRF) transcription factors, thereby suppressing genes involved in antiviral responses.

The beta-adrenergic pathway could also account for more lasting changes in immune-cell growth and development. In a 2013 study, Cole, Miller, and others demonstrated that blood taken from mice and humans exposed to chronic stress contained significantly higher levels of immature proinflammatory monocytes than controls.[9] Mediated by norepinephrine released from nerve fibers, social stress appears to alter the process of immune-cell production. Last year, another group observed the same phenomenon in mice and in 29 doctors working in an intensive care unit.[10]

"The sympathetic nervous system advises blood stem cells to lighten up on making big, smart lymphocytes [that fight viruses and restrain inflammation] and instead tells them to invest in first-line defense cells like monocytes" that ramp up inflamma-

SURVIVORS: Young prisoners at Auschwitz upon the concentration camp's liberation in January 1945

UNITED STATES HOLOCAUST MEMORIAL MUSEUM/WIKIMEDIA COMMONS

ECHOES OF TRAUMA

People exposed to severe social stress in early life are more likely to suffer behavioral and psychiatric disorders during adulthood than people who grew up stress-free. That much is well established. More contentious is the idea that those effects can be passed down through the generations.

The most extreme examples come from people with posttraumatic stress disorder (PTSD). The children and grandchildren of people who survived the Nazi death camps, for instance, have a greater-than-average risk of suffering symptoms associated with PTSD. Numerous epidemiological studies have shown that the offspring of people who experienced other forms of early-life stress, such as neglect or abuse, are also more likely to develop behavioral and psychiatric problems, despite not having themselves been exposed to childhood stress.

"It's something that has been observed for many years, but it has not been explained from a mechanistic perspective," says Isabelle Mansuy, a neurobiologist at the University of Zurich.

The observed next-generation problems could be the result of genetic predisposition, or of having been raised by traumatized parents. In the past few years, however, researchers have begun to produce experimental evidence in support of another explanation: that stress-induced epigenetic alterations in the genome can be inherited.

In 2010, for example, Mansuy and colleagues showed that male mouse pups that were repeatedly and unpredictably separated from their mothers showed symptoms of depression later in life, and that their offspring displayed the same behavioral traits despite having been reared normally. Examining the genomes of the mice, the researchers found that repeated maternal separation altered DNA methylation in the promoter region of several genes associated with social behavior in the germline of stressed males. They then identified similar methylation profiles in the brains of those males' offspring, suggesting that the epigenetic effects of stress are transmitted to offspring via sperm.[1]

Many researchers aren't buying it, though. Critics insist that the process of epigenetic reprogramming that takes place in sperm and eggs wipes the slate clean of any epigenetic marks. But Mansuy says it's possible for some marks to survive. "Yes, there is massive reprogramming [in gametes], but no one ever showed that the entire genome is reprogrammed." Indeed, in 2013, Jamie Hackett and colleagues at the University of Cambridge in the U.K. demonstrated that a small number of methylated genes escape multiple rounds of reprogramming in the primordial germ cells of mice.[2] Still, Mansuy says, "we don't know exactly how [epigenetic inheritance] works, and clearly there is still a lot to be discovered."

tion, says Cole. "So social stress doesn't just change which genes are active in the existing complement of cells, but has the capacity to change the cellular composition." These proinflammatory cells can hide out in the spleen and reemerge months and possibly even years later in response to subsequent stressors, potentially explaining how experiences of social adversity early in life can shape one's inflammatory landscape as an adult.

Other possible mediators of stress-induced gene expression changes are epigenetic processes, such as DNA methylation. "Epigenetic modifications are attractive because we know they *can* respond to the environment, they *can* effect downstream gene regulation, and they *can* be stable over time," says Tung. "There is a good case to look into it."

This idea stems from an oft-cited 2004 study showing that maternal neglect in young rodents is correlated with increased DNA methylation of the promoter region of the glucocorticoid receptor gene in the hippocampus, suppressing the receptor's expression.[11] With fewer receptors to bind to, cortisol fails to shut down the stress response, which continues to pump out more cortisol. Thus, this epigenetic modification appears to account for higher baseline cortisol levels and greater secretion in response to subsequent stress.

Last year, Seth Pollack, a psychologist at the University of Wisconsin–Madison, and colleagues found that children who suffered neglect or abuse had increased methylation at several sites on the same gene, compared with children who were not maltreated.[12] To

A 2014 study from Mansuy's lab pointed to the possibility that changes in gene expression induced by exposure to early-life traumatic stress might be mediated by noncoding microRNAs (miRNAs). Newborn male mice exposed to chronic stress showed depressive-like behaviors, and altered levels of miRNAs in the blood; in the hypothalamus and hippocampus, two regions of the brain involved in the stress response; and in sperm, which carry thousands of different RNAs. The offspring of these mice displayed comparable behaviors and had similar changes in the levels of miRNAs in the brain and the blood, despite not having experienced trauma themselves. To nail down a causal connection, Mansuy and colleagues injected all RNAs extracted from the sperm of traumatized males into fertilized eggs from wild-type females. Sure enough, when the resulting pups grew up, they displayed the same depressive behavioral traits as the mice subjected to trauma.[3] "It's the first evidence that traumatic stress can alter noncoding RNAs, and that these RNAs can act as mediators of the expression and transmission of the phenotypes," says Mansuy.

The researchers don't know how trauma alters miRNAs in sperm, though Mansuy suspects there may be components in the blood that somehow modulate epigenetic factors in testes or sperm cells that dial up the production of miRNAs in the resulting offspring.

Recent work in humans also hints at the epigenetic inheritance of trauma. In August 2014, a team led by Rachel Yehuda at Mount Sinai School of Medicine in New York City investigated 80 adults with at least one parent who had survived the Holocaust and suffered PTSD. The researchers found that in children with a father who survived the Holocaust and a mother who did not suffer traumatic experiences, the promoter region of a gene that encodes a glucocorticoid receptor—which binds cortisol to control stress response—had higher levels of methylation than controls. If both parents were survivors, their offspring had lower-than-control methylation in this promoter region.[4]

Children of people who experienced early-life stress are more likely to develop behavioral and psychiatric problems, despite not having been exposed to childhood stress themselves.

And in another study published last year, scientists from the University of Geneva and colleagues in Rwanda compared the children of 25 Tutsi women who experienced the 1994 genocide firsthand during pregnancy and 25 Tutsi women who were pregnant during the same period but did not witness the violence. The researchers found that children born to women who had experienced the brutality, just like their moms, had lower cortisol levels than controls, and higher levels of methylation on a gene known to play a role in regulating the stress hormone.[5]

"This is a relatively new field, and there is a great deal still to learn about germline epigenetic changes," says John Krystal, a neurobiologist at Yale University and editor of *Biological Psychiatry*. Ultimately, though, "it may be helpful in guiding the development of pharmacologic or behavioral therapies aimed at . . . preventing relatively small adjustment issues in young people from developing into more serious or long-standing threats to mental and physical health."

"The clinical relevance won't be immediate," says Mansuy, "but [for now] I think it's important for psychiatrists to take into account that patients may have inherited nongenetic marks that may make them more susceptible to psychiatric problems."

1. T.B. Franklin et al., "Epigenetic transmission of the impact of early stress across generations," *Biol Psychiatry*, 68:408-15, 2010.
2. J.A. Hackett et al., "Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine," *Science*, 339:448-52, 2012.
3. K. Gapp et al., "Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice," *Nat Neurosci*, 17:667-69, 2014.
4. R. Yehuda et al., "Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring," *Am J Psychiatry*, 171:872-80, 2014.
5. N. Perroud et al., "The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis," *World J Biol Psychia*, 15:334-45, 2014.

date, researchers have charted stress-induced epigenetic modifications in several genes of mice, monkeys, and humans. In Tung's rhesus macaque study, high- and low-status females had different methylation patterns around the very genes whose expression changed in response to the animals' social demotion. And in 2012, Northwestern's Miller teamed up with Michael Kobor, who runs an epigenetics lab at the University of British Columbia in Vancouver, and showed a correlation between early-life poverty and DNA methylation across the genome in the white blood cells of adults.[13] Some studies even suggest that stress-induced epigenetic modifications can be passed down to offspring, though that idea is controversial. (See "Echoes of trauma" on page 36.)

But researchers casting around for methylation on more than a handful of genes have come up empty, leading Cole to suggest that epigenetics "doesn't seem to play a significant role in the dynamics we see in leukocytes." Even where epigenetic marks do appear, he adds, it's hard to know how much of an effect they have on gene expression. But Kobor thinks it's possible that methylation at one or two key sites could indirectly affect the expression of multiple genes. "You could imagine, hypothetically speaking, that methylation of the promoter of a gene that makes a key transcriptional regulator would have effects downstream, potentially regulating the expression of groups of genes."

Relax and reverse?

Although several studies point to the enduring effects of social stress on gene regulation, molecular evidence is also emerging to suggest that those transcriptional changes are reversible. In a handful of small randomized trials, Cole and his collaborators have demonstrated that it's possible to reverse stress-related gene expression by taking steps to reduce anxiety. In a 2012 study involving 200 women with early-stage breast cancer, Cole and University of Miami psychologist Michael Antoni found that a 10-week stress-management course reversed anxiety-related upregulation of proinflammatory gene expression. Women who attended a one-day educational seminar, on the other hand, retained the gene expression alterations associated with adversity.[14]

"You do get detectable changes in transcription profiles over time" as a result of stress management, says Cole, who admits he was surprised to find evidence in subsequent trials that meditation could elicit a similar change in proinflammatory gene expression in the cells of chronically lonely people and people caring for relatives with dementia. Again, the sample sizes are small, but Cole is confident. "From any one study I'd be very queasy," he says, "but we now have several studies finding similar results." Tung's macaque study provides further evidence that gene expression can be mediated by taking steps to reduce stress: when monkeys who had been relegated to lower social ranks were later promoted, their immune–gene expression profiles tracked the upturn in social status.[8]

Given the evidence that stress affects the activity of genes known to be important in disease risk and progression, researchers are already starting to think about implementing formal strategies to mitigate the ill effects of anxiety. "This is an enormous public health issue," says Georgia Tech's Gibson. "What can we do to reverse adverse gene expression profiles that result from social stress?"

Stress management is one option; drugs that block the pathways involved in stress-induced expression shifts are another. The University of Chicago's Cacioppo, for example, is investigating compounds that could hold off the biological effects of loneliness in conjunction with cognitive behavioral therapy. "I think there could be value in pharmacological interventions, but we have to test them," says Cacioppo.

And although scientists still need to forge a more precise and nuanced understanding of the conditions under which stress-induced changes in gene regulation are important for disease, Cole doesn't think it's too early to start thinking about clinical applications. "There is one crowd who won't believe it at all until you have a full molecular mechanism; we're working diligently on that," says Cole. But in the meantime, "I don't think you have to foreswear all consideration of how this stuff might be bent to good purposes." ■

Daniel Cossins, a former associate editor of The Scientist, *is a freelance writer living in London.*

References

1. S.W. Cole et al., "Social regulation of gene expression in human leukocytes," *Genome Biol,* 8:R189, 2007.
2. J.K. Kiecolt-Glaser et al., "Psychosocial modifiers of immunocompetence in medical students," *Psychosom Med,* 46:7-14, 1984.
3. M.G. Marmot et al., "Inequalities in death: specific explanations of a general pattern," *Lancet,* 1:1003-06, 1984.
4. S.W. Cole et al., "Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes," *PNAS,* 108:3080-85, 2011.
5. G.E. Miller et al., "A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-κB signaling," *Biol Psychiatry*, 64:266-72, 2008.
6. G.E. Miller et al., "Chronic interpersonal stress predicts activation of pro- and anti-inflammatory signaling pathways 6 months later," *Psychosom Med*, 71:57-62, 2009
7. G.E Miller et al., "Low early-life social class leaves a biological residue manifested by decreased glucocorticoid and increased proinflammatory signaling," *PNAS*, 106:14716-21, 2009.
8. J. Tung et al., "Social environment is associated with gene regulatory variation in the rhesus macaque immune system," *PNAS,* 109:6490-95, 2012.
9. N.D. Powell et al., "Social stress upregulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoiesis," *PNAS,* 110:16574-79, 2013.
10. T. Heidt et al., "Chronic variable stress activates hematopoietic stem cells," *Nat Med,* 20:754-58, 2014.
11. I.C.G. Weaver et al, "Epigenetic programming by maternal behavior," *Nat Neurosci*, 7:847-54, 2004.
12. S.E. Romens et al., "Associations between early life stress and gene methylation in children," *Child Dev*, doi:10/1111/cdev.12270, 2014.
13. L.L. Lam et al., "Factors underlying variable DNA methylation in a human community cohort," *PNAS*, 109:17253-60, 2012.
14. M.H. Antoni, "Cognitive-behavioral stress management reverses anxiety-related leukocyte transcriptional dynamics," *Biol Psychiatry*, 71:366-72, 2012.

SOCIAL LIVING: These South American social wasps (*Synoeca cyanea*) live in a distinctive mud nest in Morretes, Brazil.

The Genetics of Society

Researchers aim to unravel the molecular mechanisms by which a single genotype gives rise to diverse castes in eusocial organisms.

BY CLAIRE ASHER AND SEIRIAN SUMNER

Eusocial insects are among the most successful living creatures on Earth. Found in terrestrial ecosystems across the globe (on every continent except Antarctica), the world's ants alone weigh more than all vertebrates put together. Bees are key pollinators of major crops as well as many other ecologically important plants. Termites construct thermoregulating homes that can dominate the landscape, and that are inspiring new energy-efficient skyscraper designs. The organization and collective decision making of eusocial insects is even yielding new insights into human behavior and what it means to be part of a society. But one of the biggest unanswered questions in our understanding of these complex insect groups is how a single genome can produce such diverse and contrasting physical and behavioral forms, from egg layers, provisioners, and caretakers to soldiers.

In a eusocial colony, reproduction is dominated by one or a few individuals adapted to egg laying, while their offspring—colony workers—display physical and behavioral adaptations that help them perform their subordinate roles. These phenotypic adaptations can be extreme. A leafcutter ant queen is 10 times larger than her smallest workers, for example. (See photograph on opposite page.) And some carpenter ant species have evolved a "kamikaze" caste, born with a self-destruct button that causes the insect to explode upon colony attack, killing itself and covering the invading animals in toxic chemicals. Remarkably, differences in the behavior and morphology of insect castes are usually generated through differences in the expression of identical sets of genes. (There are a few cases of genetically determined castes, but this is the exception, not the rule.)

We are now entering a new era of research into eusocial insects. For the first time, scientists are investigating the molecules that underlie eusocial behavior at a depth that was previously unimaginable. New, affordable sequencing technologies enable scientists to examine how genes across the entire genome are regulated to generate different caste phenotypes, the roles of DNA methylation and microRNAs in this differential expression, and what proteins are synthesized as a result. This burgeoning area of research, dubbed "sociogenomics" in 2005 by Gene E. Robinson,[1] is revolutionizing our understanding of the evolution of eusociality from a solitary wasp-like ancestor to the million-strong colonies we see today. New work is yielding insights into how genomes interact dynamically with the physical and social environment to produce highly adapted, specialized castes with remarkable phenotypic innovations. These findings are, in turn, illuminating the importance of gene regulation and epigenetics in controlling behavioral plasticity across the animal kingdom.

The extreme altruism exhibited by eusocial insects was one of the most perplexing traits that Darwin encountered when developing his theory of natural selection.

The birth of eusociality

The extreme altruism exhibited by eusocial insects was one of the most perplexing traits that Darwin encountered when developing his theory of natural selection. How can nature select for a worker phenotype, which exists solely to help others reproduce, when it does not have any offspring of its own? We now understand that worker behavior can evolve because workers still pass on their genes through the related offspring they help raise. This has allowed eusociality to evolve multiple times throughout biological history: 10 times in the Hymenoptera (the ants, bees, and wasps), and an additional 22 times in termites, aphids, thrips, and snapping shrimps. It has even appeared a couple of times in mammals, with independent origins in two species of mole rat. (See "Underground Supermodels," *The Scientist*, June 2012.)

Each eusocial lineage evolved from a solitary ancestor—a species in which a single genome produced a single adult phenotype, as is the case for the majority of insects alive today. Based on the morphology of both extant and extinct species, it was long believed that bees represented the most ancestral of the hymenopteran lineages. However, recent high-throughput sequencing of transcriptomes indicates that wasps may in fact be the more ancient group, with bees and ants having diverged from the wasp lineage around 145 million years ago.[2,3] The first eusocial societies were simple, much like some of today's halictid bees and *Polistes* paper wasps, whose behavioral castes look identical. Since then the order Hymenoptera has diverged into more than 14,000 eusocial species spanning almost every level of social organization, including the much more complex societies of honeybees, ants, and others. Collectively, these insects provide glimpses into the evolution of eusociality. (See illustration on page 42.)

So how did we get from a solitary ancestor to a species with diverse specialized phenotypes? A long-standing hypothesis, proposed by the eminent social insect biologist Mary Jane West-Eberhard, goes like this: the solitary ancestor lived as a single mother; she laid eggs and foraged alone to provide food

GIANT MOTHER: A queen Texas leafcutter ant (*Atta texana*) is many times larger than her worker daughters.

© ALEX WILD

for her growing brood. Once mature, her offspring would leave the nest to forage and reproduce, also on their own. This is how most insects still make a living. One of the first steps on the road to eusociality was for these offspring to stay behind at the nest for some time into adulthood, where they helped their mother raise their younger siblings. As these helpers evolved to specialize in particular roles, characteristics and behaviors that were once enacted sequentially by the solitary female slowly became decoupled. Reproductive traits were the exclusive responsibility of a newly evolved phenotype, the "queen," and behaviors such as foraging were now performed by another new phenotype—the "worker."

The hypothesis that social castes arose from the decoupling of once-solitary behaviors is compelling in its simplicity and its conformity with a well-established theory on the molecular mechanisms of evolution. Like the HOX cluster, a relatively small set of genes that underpins multicellular development in almost all life on Earth, a genetic toolkit for social behavior could have enabled the evolution of eusocial systems via an uncoupling of the genes regulating different solitary behaviors. If so, we expect to find suites of the same "toolkit" genes regulating caste-specific morphology and behavior across multiple independent evolutionary iterations of eusocial life. These genes may have been predisposed to a role in eusocial behavior, perhaps because of their key role in provisioning or in physiological activity.

Although this mechanism is supported by behavioral data, we have previously lacked the molecular tools to help us test the importance of phenotypic uncoupling in caste evolution. The genome sequences of 11 eusocial hymenopteran species have now been published, and these data are further accompanied by caste-specific transcriptomic and proteomic analyses. Together, these resources are unveiling the gene-level dynamics that underlie eusocial behavior. Methylome and microRNA sequencing have also begun to reveal the regulatory factors involved in mediating caste-biased differential gene expression.

Make new genes, but keep the old

To some extent, recent sociogenomic studies have confirmed the existence of common genes underlying queen and worker phenotypes across social species. For example, a gene associated with roving behaviors in fruit flies and nematodes, in which the animals go looking for food, is also associated with foraging behavior in honeybees, ants, and bumblebees, which represent multiple independent origins of eusociality. Moreover, recent investigations of division of labor in eusocial insects with simpler societies have highlighted many of the same toolkit genes associated with castes found in the highly eusocial honeybee.

Some of these "old" genes have adopted new functions in certain species. The

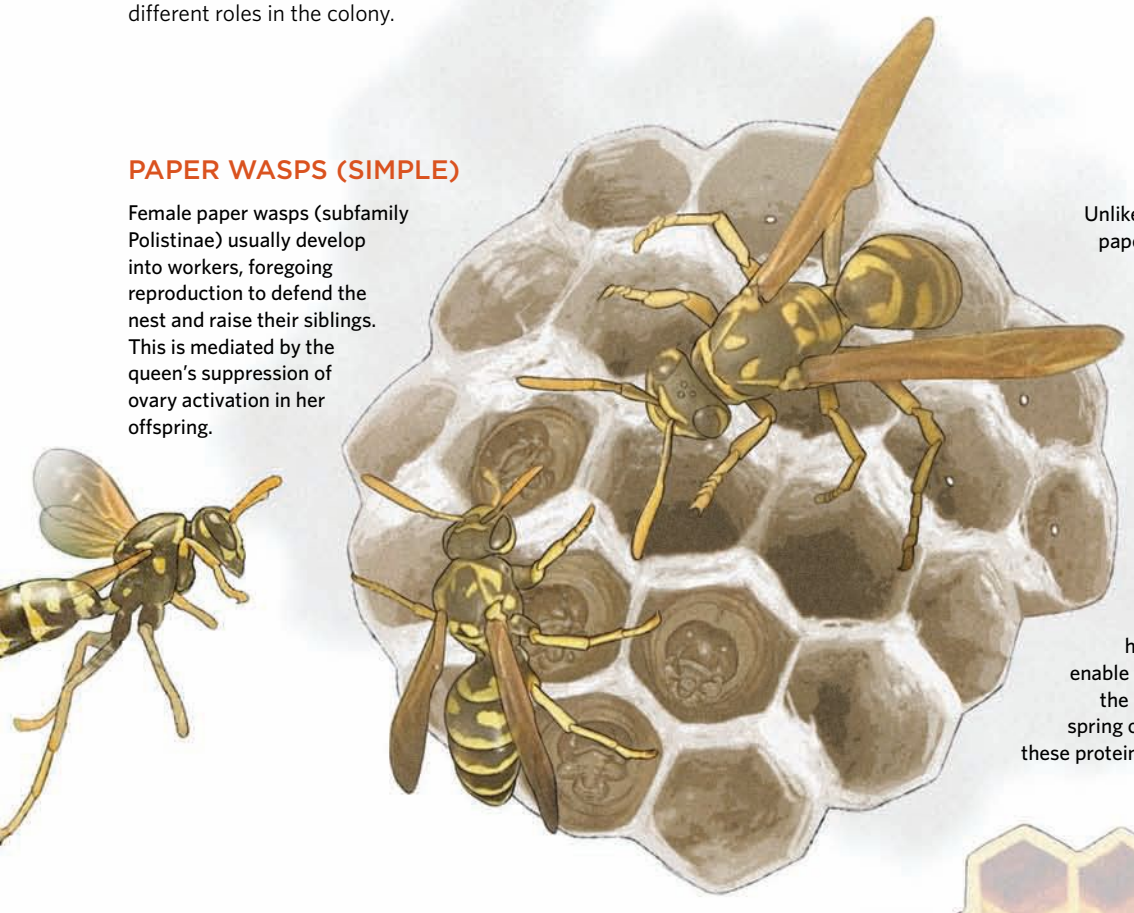
SOCIETY LIFE

Eusociality has evolved multiple times in the history of life. But not all eusocial societies are the same. They range from simple, with castes differentiated by behavior only, to complex, with phenotypically diverse queens and workers, and sometimes multiple worker castes that play different roles in the colony.

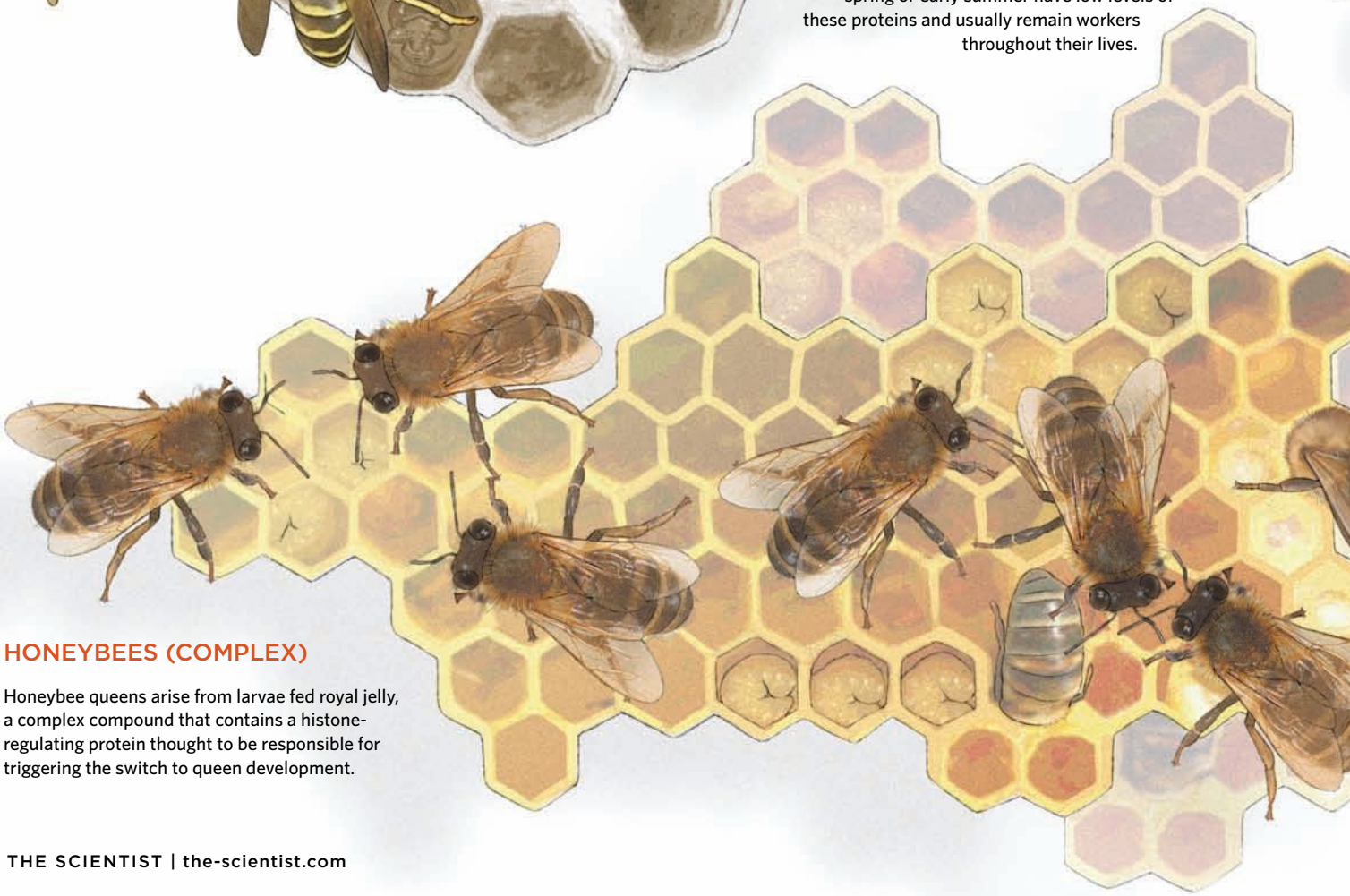
PAPER WASPS (SIMPLE)

Female paper wasps (subfamily Polistinae) usually develop into workers, foregoing reproduction to defend the nest and raise their siblings. This is mediated by the queen's suppression of ovary activation in her offspring.

Unlike other eusocial species with fixed castes, paper wasp workers can switch to be a queen if the opportunity arises to found a new nest or supersede the current queen (her mother) in her own nest. Future queens show higher expression of several genes involved in caste determination in other eusocial insects that have more visible distinctions between castes.



In temperate species, larvae that develop toward the end of the summer have high levels of a group of proteins that enable them to survive the winter and reproduce the next year, while larvae that develop in the spring or early summer have low levels of these proteins and usually remain workers throughout their lives.

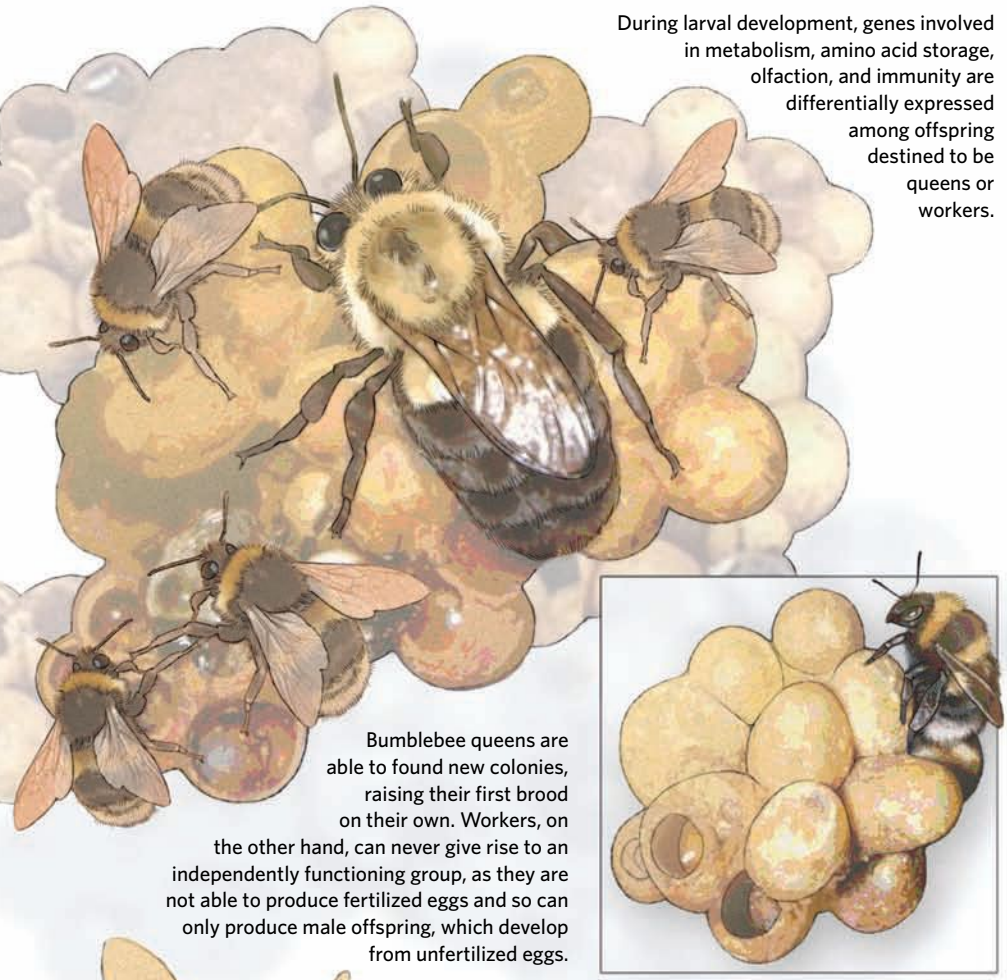


HONEYBEES (COMPLEX)

Honeybee queens arise from larvae fed royal jelly, a complex compound that contains a histone-regulating protein thought to be responsible for triggering the switch to queen development.

BUMBLEBEES (INTERMEDIATE)

During larval development, genes involved in metabolism, amino acid storage, olfaction, and immunity are differentially expressed among offspring destined to be queens or workers.



Bumblebee queens are able to found new colonies, raising their first brood on their own. Workers, on the other hand, can never give rise to an independently functioning group, as they are not able to produce fertilized eggs and so can only produce male offspring, which develop from unfertilized eggs.

© CATHERINE DELPHIA

Honeybee workers care for the brood, forage, or defend the nest, transitioning through different tasks as they age. This transition is controlled by a mutually inhibitory relationship between two proteins, vitellogenin and juvenile hormone. Vitellogenin also influences whether honeybee workers specialize in pollen or nectar foraging.

ancestral function of juvenile hormone (JH), for example, was to produce yolk for egg development. And in all eusocial insects studied to date, JH is upregulated in queens, suggesting they retain the gene's ancestral function. However, JH has also evolved a new function—regulating foraging behavior in workers of several eusocial species. In honeybee workers, JH regulates the fine-scale, age-based transition from nursing (as a young worker) to foraging (as an older worker). Recent studies have also shown that the hormone forms a functional link between insulin signaling pathways and the insect neuroendocrine system, which allows foraging and brood-rearing behavior to be modulated by the nutritional and energetic needs of both the individual and the colony.

Sociogenomic analyses are also unearthing surprises. Across three independent origins of eusociality in bees, two-thirds of genes that show recent rapid evolution were linked to the level of eusociality—complex or simple.[4] Such genes include novel, or taxonomically restricted, genes—those that have evolved uniquely in a single taxonomic group, and so, to date, lack any sequence similarity with any known organism outside the sequenced group. In the honeybee gene set, for example, more than 250 genes are "orphans," meaning they are unique to honeybees, or are restricted to the Hymenoptera; of these, 58 percent are expressed differently in queens and workers or in different worker castes.[5] More than 40 percent of worker-biased genes in the rock ant *Temnothorax longispinosus*,[6] and 75 percent of caste-biased genes in the paper wasp *Polistes canadensis*,[2] are novel.

Thus, a core sociality toolkit appears to have been augmented by the de novo birth of novel genes and gene families and rapid evolution of ancestral genes to generate queen and worker phenotypes in eusocial insects. But our understanding of the role of orphan genes is largely dependent on the available sequence data. As more species are sequenced over the coming years, our picture of the importance of new, old, and modified genes in eusocial evolution will become clearer.

From genotype to phenotype

Differential expression of shared genes is just one small step in the link from genes to physical form. Sociogenomics research is now starting to focus on dissecting the mechanisms that regulate gene expression and determine the resulting proteome, and ultimately, the phenotype.

The role of microRNAs and epigenetic processes, such as DNA methylation and posttranslational histone modification, in suppressing or activating genes during development has long been recognized in model organisms such as *Drosophila* and mice. Such mechanisms may also regulate caste differentiation and behavioral plasticity in eusocial insects.[7] A functional DNA methylation system appears to operate in eusocial bees, wasps, ants, and termites, whose genomes encode the key DNA methyltransferases DNMT1 and DNMT3.[8] These methyltransferases tag specific genes with methyl groups, resulting in their reduced transcription.

Researchers first suspected a role for DNA methylation in eusocial insects in 2008, when Robert Kucharski of Australian National University and colleagues used RNA interference (RNAi) to knock down DNMT3 in honeybee worker larvae, which as adults went on to develop ovaries, like a queen.[9] A more recent study found that honeybee DNA methylation levels changed with gene expression during the transition from nursing to foraging, and back again.[10] A similar role for DNA methylation in regulating caste fate has since been suggested for bumblebees, where chemical inhibition of DNMT3 promotes reproduction by workers in colonies with no queens.[11] These findings suggest that the role of DNA methylation may be much more dynamic and unstable in insects than in mammals, changing with age, developmental stage, and social environment.

The methods, applicability, and affordability of omics technologies are improving at breakneck speed, giving us the tools we need to uncover the molecular secrets behind the complex lives of eusocial insects.

HIGH-DENSITY LIVING: Southern wood ants (*Formica rufa*) live in colonies with up to 100 or more queens and 100,000 to 400,000 workers.

© ANDREY PAVLOV/SHUTTERSTOCK

Several lines of evidence now suggest that histones, the proteins responsible for the tight packaging of DNA into chromatin, also play important roles in regulating caste-biased gene expression in eusocial insects. One recent study by Astrid Spannhoff and colleagues at the University of Texas in Austin identified a histone-regulating protein as a key ingredient in royal jelly, which worker bees secrete to nourish hive larvae and to trigger the switch from worker to queen in select larvae as needed.[12]

Understanding the regulation of gene transcription is a major piece of the puzzle. But a lot can happen between transcription and protein production, and a new challenge in sociogenomics is to connect the dots among transcription, translation, and protein products. Regulatory elements called microRNAs are known to mediate cell fate and posttranscriptional gene regulation, and have been found to show caste-specific expression in honeybees and ants.[13,14] However, we still lack a deep understanding of exactly how microRNAs influence caste and behavior in eusocial insects.

Natural selection acts on the phenotype, not directly on genes; the proteome is the closest molecular representation of the phenotype, and perhaps the key to understanding the evolution of eusociality. So far, proteomics studies on eusocial insects are few and far between, but recent large-scale mapping of the proteome of the honeybee worker brain has revealed proteins that are differentially expressed in nursing and foraging individuals.[15] Researchers must now begin to embrace cutting-edge bioinformatics methods that allow dual analysis of transcriptomes and proteomes in the same individuals. A coordinated analysis of transcription, gene regulation, and protein production, alongside carefully assayed behavioral repertoires, will bring us closer to understanding the emergence of social diversity from a single genome.

FABIO BRAMBILLA/WIKIMEDIA COMMONS

SIMPLE SOCIETIES: Worker paper wasps (*Polistes dominula*) defend the nest and raise their siblings, while the morphologically similar queen lays the eggs.

The future of sociogenomics

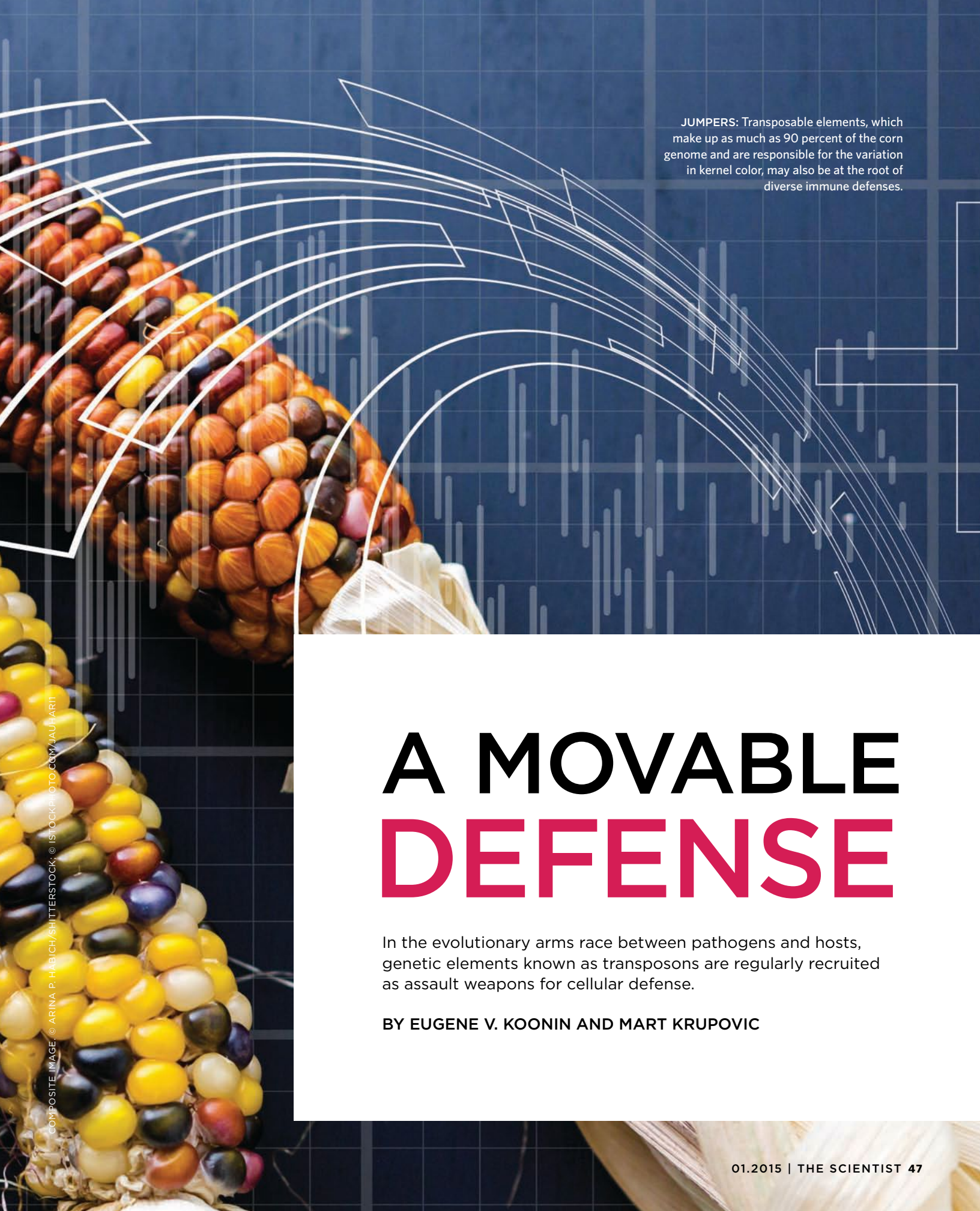
Sociogenomics is young, but the field is exploding. The methods, applicability, and affordability of omics technologies are improving at breakneck speed, giving us the tools we need to uncover the molecular secrets behind the complex lives of eusocial insects. It is now possible to study any species, and most importantly, to study them in their natural habitat. This is especially important for studying simple societies, such as those of stenogastrine hover wasps and allodapine bees, where worker behavior depends so much on the ecological constraints of the environment.

Understanding the molecular basis of queen and worker caste formation and maintenance is only the start. The next steps will focus on what, if any, molecular changes accompanied major transitions in eusocial evolution, such as workers' loss of the ability to mate, and the honeybee queen's loss of the ability to found a nest on her own. The next few years will also see the scientific community studying a broader taxonomic spread, to capture the extent to which molecular processes vary within different eusocial lineages and across different levels of societal complexity. Sociogenomics provides an exciting common ground for ecologists, evolutionary genomicists, and developmental biologists to study broad-scale macroevolutionary patterns and behaviors in the fine-scale detail of gene regulation. When disparate disciplines of biology are united, new ideas, new hypotheses, and a deeper understanding of the natural world invariably emerge. ■

Claire Asher works in knowledge transfer at the Centre for Biodiversity and Environment Research, University College London, and is also a freelance science writer who writes the Curious Meerkat *blog (www.curiousmeerkat.co.uk). She recently completed a PhD studying the social and sociogenomic controls of behavior in simple ant societies. Seirian Sumner is a senior lecturer in behavioral biology at the School of Biological Sciences, University of Bristol. Her work specializes in exploiting molecular tools to address questions of how and why eusocial behavior evolves.*

References

1. G.E. Robinson et al., "Sociogenomics: social life in molecular terms," *Nat Rev Genet*, 6:257-70, 2005.
2. P.G. Ferreira et al., "Transcriptome analyses of primitively eusocial wasps reveal novel insights into the evolution of sociality and the origin of alternative phenotypes." *Genome Biol*, 14:R20, 2013.
3. B.R. Johnson et al., "Phylogenomics resolves evolutionary relationships among ants, bees, and wasps," *Curr Biol*, 23:2058-62, 2013.
4. S.H. Woodard et al., "Genes involved in convergent evolution of eusociality in bees," *PNAS*, 108:7472-77 2011.
5. B.R. Johnson, N.D. Tsutsui, "Taxonomically restricted genes are associated with the evolution of sociality in the honey bee," *BMC Genomics*, 12:164, 2011.
6. B. Feldmeyer et al., "Gene expression patterns associated with caste and reproductive status in ants: worker-specific genes are more derived than queen-specific ones," *Mol Ecol*, 23:151-61, 2014.
7. S. Patalano et al., "Shifting behaviour: epigenetic reprogramming in eusocial insects," *Curr Opin Cell Biol*, 24:367-73, 2012.
8. H. Yan et al., "Eusocial insects as emerging models for behavioural epigenetics," *Nat Rev Genet*, 15:677-88, 2014.
9. R. Kucharski et al., "Nutritional control of reproductive status in honeybees via DNA methylation," *Science*, 319:1827-30, 2008.
10. B.R. Herb et al., "Reversible switching between epigenetic states in honeybee behavioral subcastes," *Nat Neurosci*, 15:1371-73, 2012.
11. H.E. Amarasinghe et al., "Methylation and worker reproduction in the bumble-bee (*Bombus terrestris*)," *Proc Roy Soc B*, 281:20132502, 2014.
12. A. Spannhoff et al., "Histone deacetylase inhibitor activity in royal jelly might facilitate caste switching in bees," *EMBO Rep*, 12:238-43, 2011.
13. D.F. Simola et al., "Social insect genomes exhibit dramatic evolution in gene composition and regulation while preserving regulatory features linked to sociality," *Genome Res*, 23:1235-47, 2013.
14. J.K. Greenberg et al., "Behavioral plasticity in honey bees is associated with differences in brain microRNA transcriptome," *Genes Brain Behav*, 11:660-70, 2012.
15. L.G. Hernández et al., "Worker honeybee brain proteome," *J Proteome Res*, 11:1485-93, 2012.



JUMPERS: Transposable elements, which make up as much as 90 percent of the corn genome and are responsible for the variation in kernel color, may also be at the root of diverse immune defenses.

COMPOSITE IMAGE: © ARINA P. HABICH/SHUTTERSTOCK; © ISTOCKPHOTO.COM/JAUHARI11

A MOVABLE DEFENSE

In the evolutionary arms race between pathogens and hosts, genetic elements known as transposons are regularly recruited as assault weapons for cellular defense.

BY EUGENE V. KOONIN AND MART KRUPOVIC

Researchers now recognize that genetic material, once simplified into neat organismal packages, is not limited to individuals or even species. Viruses that pack genetic material into stable infectious particles can incorporate some or all of their genes into their hosts' genomes, allowing remnants of infection to remain even after the viruses themselves have moved on. On a smaller scale, naked genetic elements such as bacterial plasmids and transposons, or jumping genes, often shuttle around and between genomes. It seems that the entire history of life is an incessant game of tug-of-war between such mobile genetic elements (MGEs) and their cellular hosts.

MGEs pervade the biosphere. In all studied habitats, from the oceans to soil to the human intestine, the number of detectable virus particles, primarily bacteriophages, exceeds the number of cells at least tenfold, and maybe much more. Furthermore, MGEs and their remnants constitute large portions of many organisms' genomes—as much as two-thirds of the human genome and up to 90 percent in plants such as corn.

Despite their ubiquity and prevalence in diverse genomes, MGEs have traditionally been considered nonfunctional junk DNA. Starting in the middle of the 20th century, through the pioneering work of Barbara McClintock in plants, and over the following decades in a widening range of organisms, researchers began to uncover clues that MGE sequences are recruited for a variety of cellular functions, in particular for the regulation of gene expression. More-recent work reveals that many organisms also use MGEs for a more specialized and sophisticated function, one that capitalizes on the ability of these elements to move around genomes, modifying the DNA sequence in the process. Transposons seem to have been pivotal contributors to the evolution of adaptive immunity both in vertebrates and in microbes, which were only recently discovered to actually have a form of adaptive immunity—namely, the CRISPR-Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated genes) system that has triggered the development of a new generation of genome-manipulation tools.

Transposons seem to have been pivotal contributors to the evolution of adaptive immunity both in vertebrates and in microbes.

Multiple defense systems have evolved in nearly all cellular organisms, from bacteria to mammals. Taking a closer look at these systems, we find that the evolution of these defense mechanisms depended, in large part, on MGEs—those same ele-

MOBILE DNA: A false-color transmission electron micrograph of a transposon, a segment of DNA that can move around chromosomes and genomes

© PROFESSOR STANLEY N. COHEN/SCIENCE SOURCE

ments that are themselves targets of host immune defense.

Layers of defense

As cheaters in the game of life, stealing resources from their hosts, parasites have the potential to cause the collapse of entire communities, killing their hosts before moving on or dying themselves. But hosts are far from defenseless. The diversity and sophistication of immune systems are striking: their functions range from immediate and nonspecific innate responses to exquisitely choreographed adaptive responses that result in lifelong immune memory after an initial pathogen attack.[1]

Over the last two decades or so, it has become clear that nearly all organisms possess multiple mechanisms of innate immunity.[2] Toll-like receptors (TLRs), common to most animals, recognize conserved molecules from microbial pathogens and activate the appropriate components of the immune system upon invasion. Even more widespread and ancient is RNA interference (RNAi), a powerful defense system that employs RNA guides, known as small interfering RNAs (siRNAs), to destroy invading nucleic acids, primarily those of RNA viruses. Conceptually, the biological function of siRNAs is analogous to that of TLRs: an innate immune response to a broad class of pathogens.

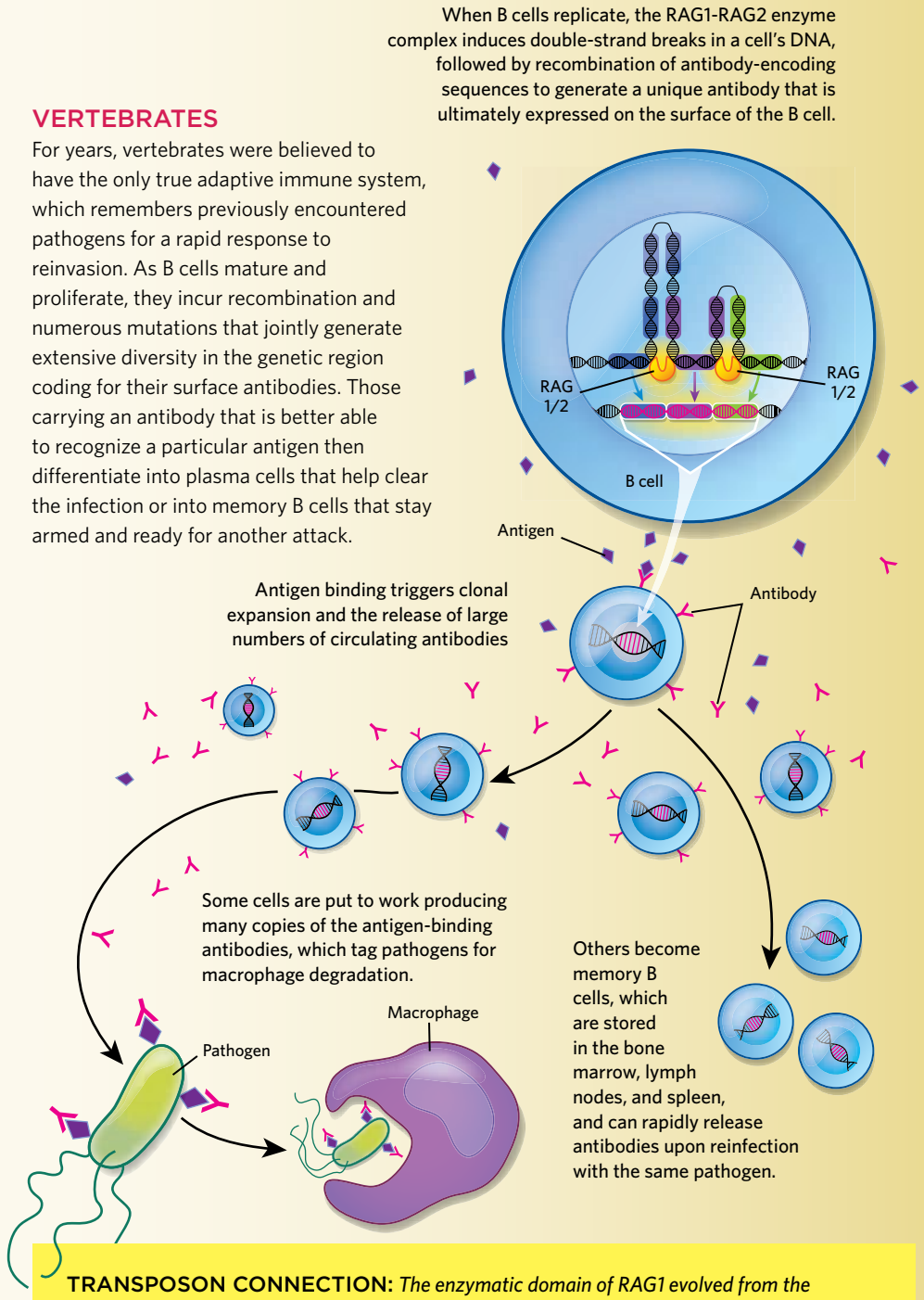
Prokaryotes possess their own suite of innate immune mechanisms, including endonucleases that cleave invader DNA at specific sites and enzymes called methylases that modify those same sites in the prokaryotes' own genetic material to shield it from cleavage, a strategy known as restriction modification (RM).[3] If overwhelmed by pathogens, many prokaryotic cells will undergo programmed cell death or go into dormancy, thereby preventing the spread of the pathogen within the organism or population. In particular, infected bacterial or archaeal cells can activate toxin-antitoxin (TA) systems to induce dormancy or cell death. Normally, the toxin protein is complexed with the antitoxin and thus inactivated. However, under stress, the antitoxin is degraded, unleashing the toxin to harm the cell.

TRAVELING SOLDIERS

Mobile genetic elements (MGEs) can insert into new genomes, thereby spreading their functionality to different species and even different kingdoms of life. While MGEs are evolutionarily selfish, it turns out that many organisms have co-opted such elements for their own defense. In many cases it is unclear in which context, cellular or MGE, a defense mechanism first arose.

VERTEBRATES

For years, vertebrates were believed to have the only true adaptive immune system, which remembers previously encountered pathogens for a rapid response to reinvasion. As B cells mature and proliferate, they incur recombination and numerous mutations that jointly generate extensive diversity in the genetic region coding for their surface antibodies. Those carrying an antibody that is better able to recognize a particular antigen then differentiate into plasma cells that help clear the infection or into memory B cells that stay armed and ready for another attack.



TRANSPOSON CONNECTION: *The enzymatic domain of RAG1 evolved from the recombinases (also known as integrases or transposases) of animal transposons known as Transibs. The recombination signal sequences of the antibody genes, which are required for recombination and are recognized by the RAG1-RAG2 recombinase, also appear to have evolved via Transib insertion.*

DISRUPTING COLOR: The variations in color seen in this dahlia "flower" (actually a cluster of small individual flowers, or florets) can be caused by transposon-induced mutations.

Many viruses that infect microbes also encode RM and TA modules.[4] These viruses are, in effect, a distinct variety of MGEs that sometimes have highly complex genomes. Viruses use RM systems for the very same purpose as their prokaryotic hosts: the methylase modifies the viral genome, whereas the endonucleases degrade any unmodified genomes in the host cell, thereby providing nucleotides for the synthesis of new copies of the viral genome. And the TA system can ensure retention of a plasmid or virus within the cell. The toxin and antitoxin proteins dramatically differ in their vulnerability to proteolytic enzymes that are always present in the cell: the toxin is stable whereas the antitoxin is labile. This does not matter as long as both proteins are continuously produced. However, if both genes are lost (for example, during cell division), the antitoxin rapidly degrades, and the remaining amount of the toxin is sufficient to halt the biosynthetic activity of the cell and hence kill it or at least render it dormant. A plasmid or virus that carries a TA module within its genome thus implants a self-destructing mechanism in its host that is activated if the MGE is lost. (See illustration on page 53.)

When an MGE inserts into the host genome, it inevitably modifies that genome, typically using an MGE-encoded recombinase (also known as integrase or transposase) as a breaking-and-entering tool. Speaking in deliberately anthropomorphic terms, the MGEs do so for their own selfish purposes, to ensure their propagation within the host genome. However, given the ubiquity of MGEs across cellular life forms, it seems extremely unlikely that host organisms would not recruit at least some of these naturally evolved genome manipulation tools in order to exploit their remarkable capacities for their own purposes. Immune memory that involves genome manipulation is arguably the most obvious utility of these tools, and in retrospect, it is not surprising that unrelated transposons and their recombinases appear to have made key contributions to the origin of both animal and prokaryotic forms of adaptive immunity.

Guns for hire

Until recently, prokaryotes had been thought to entirely lack the sort of adaptive immunity that dominates defense against parasites in vertebrates. This view has been overturned in the most dramatic fashion by the discovery of the CRISPR-Cas, RNAi-based defense systems found to be present in most archaea and many bacteria studied to date.[5] In 2005, Francisco Mójica of the University of Alicante in Spain and colleagues,[6] and independently, Dusko Ehrlich of the Pasteur Institute in Paris,[7] discovered that some of the unique sequences inserted between CRISPR, known as spacers, were identical to pieces of bacteriophage or plasmid genomes. Combined with a detailed analysis of the predicted functions of Cas proteins, this discovery led one of us (Koonin) and his team to propose in 2006 that CRISPR-Cas functioned as a form of prokaryotic adaptive immunity, with memory of past infections stored in the genome within the CRISPR "cassettes"—clusters of short direct repeats, interspersed with similar-size nonrepetitive spacers, derived from various MGEs—and to develop a detailed hypothesis about the mechanism of such immunity.[8]

When a mobile genetic element (MGE) inserts into the host genome, it inevitably modifies that genome, typically using an MGE-encoded recombinase as a breaking-and-entering tool.

Subsequent experiments from Philippe Horvath's and Rodolphe Barrangou's groups at Danisco Corporation,[9] along with several other studies that followed in rapid succession, supported this hypothesis. (See "There's CRISPR in Your Yogurt," page 20.) It has been shown that CRISPR-Cas indeed functions by incorporating fragments of foreign bacteriophage or plasmid DNA into CRISPR cassettes, then using the transcripts of these unique spacers as guide RNAs to recognize and cleave the genomes of repeat invaders. (See illustration on opposite page.) A key feature of CRISPR-Cas systems is their ability to transmit extremely

efficient, specific immunity across many thousands of generations. Thus, CRISPR-Cas is not only a bona fide adaptive immunity system, but also a genuine machine of Lamarckian evolution, whereby an environmental challenge—a virus or plasmid, in this case—directly causes a specific change in the genome that results in an adaptation that is passed on to subsequent generations.[10]

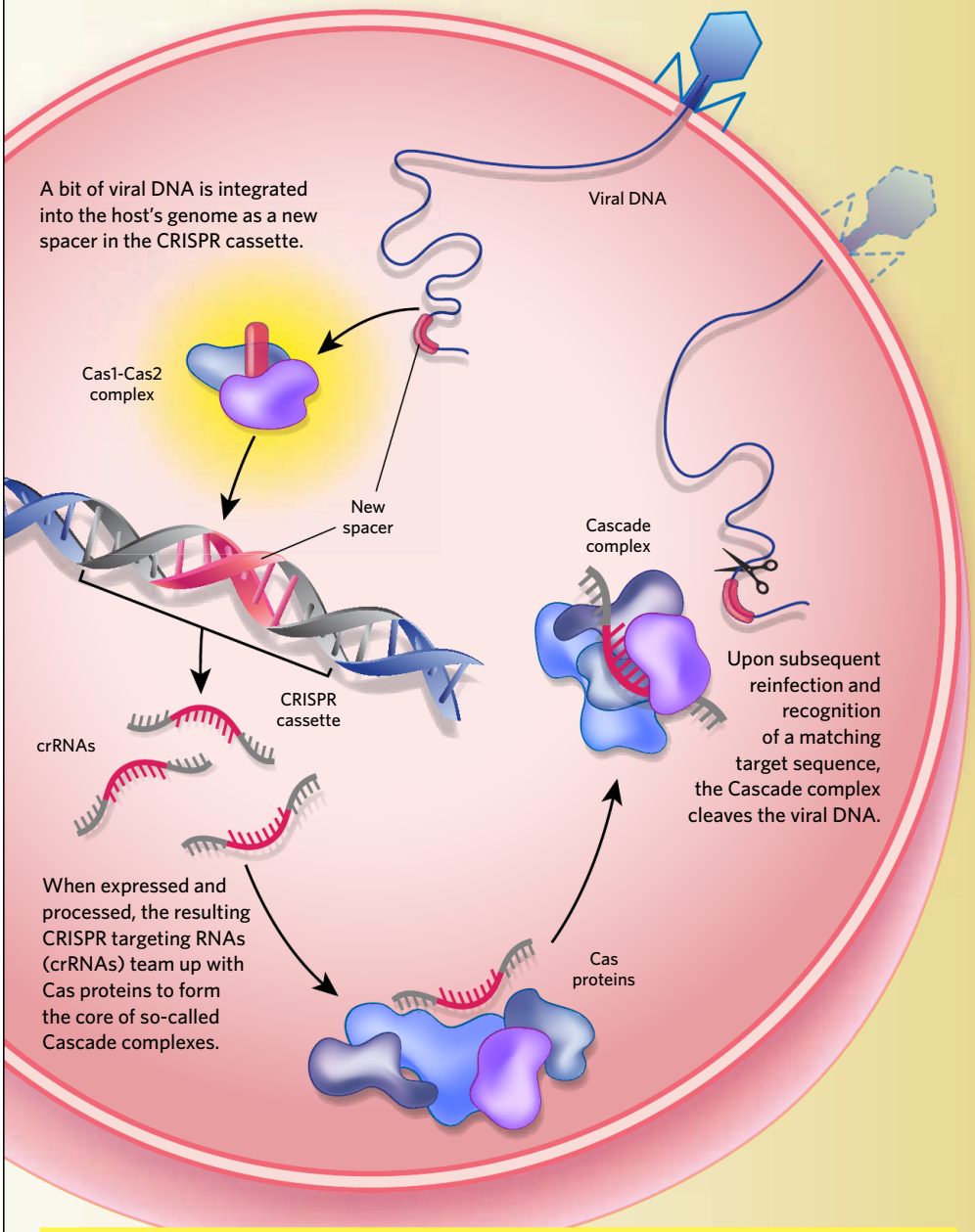
A torrent of comparative genomic, structural, and experimental studies has characterized the extremely diverse CRISPR-Cas systems according to the suites of Cas proteins involved in CRISPR transcript processing and target recognition.[5,11] While Type I and Type III systems employ elaborate protein complexes that consist of multiple Cas proteins, Type II systems perform all the necessary reactions with a single large protein known as Cas9. These findings opened the door for straightforward development of a new generation of genome editing. Cas9-based tools are already used by numerous laboratories all over the world for genome engineering that is much faster, more flexible, and more versatile than any methodology that was available in the pre-CRISPR era.[12]

And it seems that humans are not the only species to have stolen a page from the CRISPR book: viruses have done the same. For example, a bacteriophage that infects pathogenic *Vibrio cholera* carries its own adaptable CRISPR-Cas system and deploys it against another MGE that resides within the host genome.[13] Upon phage infection, that rival MGE, called a phage inducible chromosomal island-like element (PLE), excises itself from the cellular genome and inhibits phage production. But at the same time, the bacteriophage-encoded CRISPR-Cas system targets PLE for destruction, ensuring successful phage propagation.

Consequently, in prokaryotes, all defense systems appear to be guns for hire that work for the highest bidder. Sometimes it is impossible to know with any certainty in which context, cellular or MGE, different defense mechanisms first emerged.

PROKARYOTES

It turns out that prokaryotes can also remember previous pathogens they've encountered in what can be considered an analogous system to vertebrate adaptive immunity. The CRISPR-Cas system allows microbes to insert spacers, short bits of plasmid or virus DNA, into the CRISPR cassettes, clusters of spacers interspersed with short direct repeats. The expression of these sequences can be processed by the cell and used to guide the targeted destruction of plasmid or viral DNA.



TRANSPOSON CONNECTION: *The function of Cas1, the key enzyme of CRISPR-Cas that is responsible for the acquisition of foreign DNA as spacers within CRISPR units, resembles the activity of MGE recombinases. In the recently discovered casposons, a distinct group of transposons, a Cas1 homolog is predicted to function as a recombinase.*

Transposon origins of adaptive immunity

Recent evidence from our groups supports an MGE origin of the CRISPR-Cas systems. The function of Cas1—the key enzyme of CRISPR-Cas that is responsible for the acquisition of foreign DNA and its insertion into spacers within CRISPR cassettes—bears an uncanny resemblance to the recombinase activity of diverse MGEs, even though Cas1 does not belong to any of the known recombinase families. As a virtually ubiquitous component of CRISPR-Cas systems, Cas1 was likely central to the emergence of CRISPR-Cas immunity.

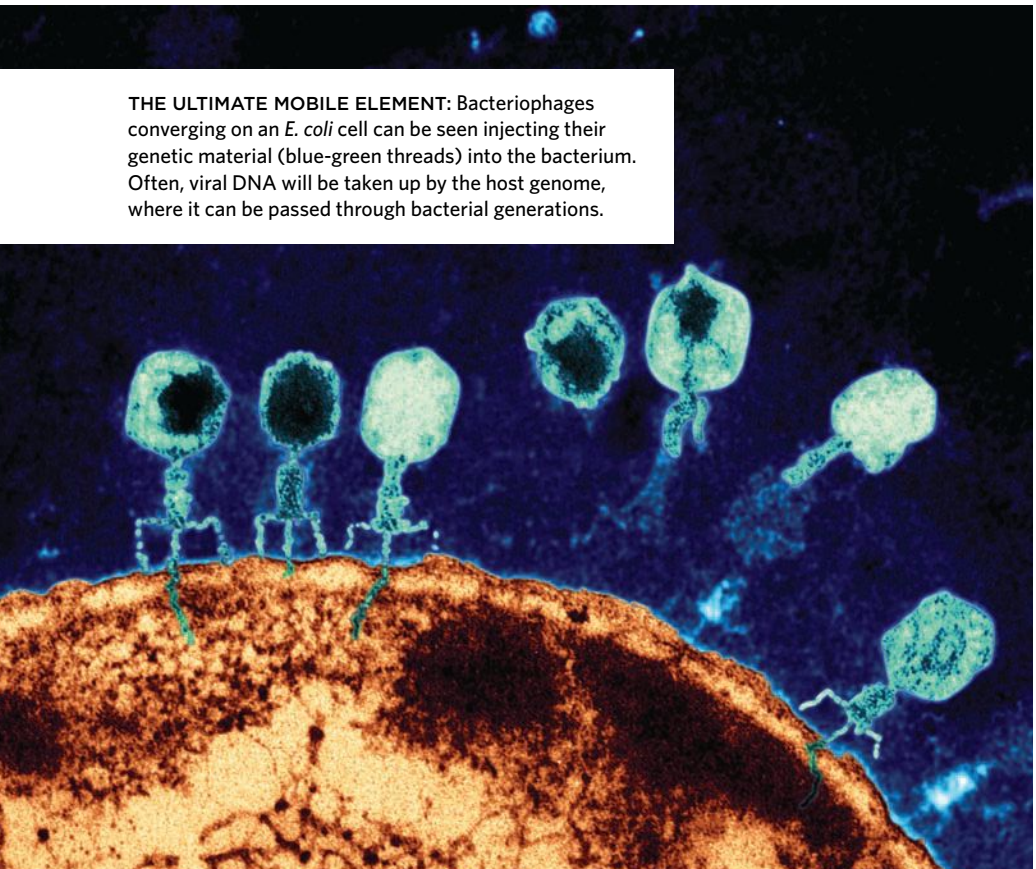
During a recent exploration of archaeal DNA dark matter—clusters of uncharacterized genes in sequenced genomes—we unexpectedly discovered a novel superfamily of transposon-like MGEs that could hold the key to the origin of Cas1.[14] These previously unnoticed transposons contain inverted repeats at both ends, just like many other transposons, but their gene content is unusual. The new transposon superfamily is present in both archaeal and bacterial genomes and is highly polymorphic (different members contain from 6 to about 20 genes), with only two genes shared by all identified representatives. One of these conserved genes encodes a DNA polymerase, indicating that these transposons supply the key protein for their own replication. While diverse eukaryotes harbor self-synthesizing transposons of the Polinton or Maverick families, this is the first example in prokaryotes. But it was the second conserved protein that held the biggest surprise: it was none other than a homolog of Cas1, the key protein of the CRISPR-Cas systems.

We dubbed this new transposon family Casposons and naturally proposed that, in this context, Cas1 functions as a recombinase. In the phylogenetic tree of Cas1, the casposons occupy a basal position, suggesting that they played a key role in the origin of prokaryotic adaptive immunity.

In vertebrates, adaptive immunity acts in a completely different manner than in prokaryotes and is based on the acquisition of pathogen-specific T- and B-lymphocyte antigen receptors during the lifetime of the organism. The vast repertoire of immunoglobulin receptors is generated from a small number of genes via dedicated diversification processes known as V (variable), D (diversity), and J (joining) segment (V(D)J) recombination and hypermutation. (See illustration on page 49.) In a striking analogy to CRISPR-Cas, vertebrate adaptive immunity also seems to have a transposon at its origin. V(D)J recombination is mediated by the RAG1-RAG2 recombinase complex. The recombinase domain of RAG1 derives from the recombinases of a distinct group of animal transposons known as Transibs.[15] The recombination signal sequences of the immunoglobulin genes, which are recognized by the RAG1-RAG2 recombinase and are necessary for bringing together the V, D, and J gene segments, also appear to have evolved via Transib insertion.

The two independent origins of adaptive immune systems in prokaryotes and eukaryotes involving unrelated MGEs show that, in the battle for survival, organisms welcome all useful molecular inventions irrespective of who the original inventor was. Indeed, the origin of CRISPR-Cas systems from prokaryotic casposons and vertebrate V(D)J recombination from Transib transposons might appear paradoxical given that MGEs are primary targets of immune systems. However, considering the omnipresence and diversity of MGEs, it seems likely that even more Lamarckian-type mechanisms have, throughout the history of life, directed genomic changes in the name of host defense.[16]

Moreover, the genome-engineering capacity of immune systems provides almost unlimited potential for the development of experimental tools for genome manipulation and other applications. The utility of antibodies as tools for protein detection and of RM enzymes for specific fragmentation of DNA molecules has been central to the progress of biology for decades. Recently, CRISPR-Cas systems have been added to that toolkit as, arguably, the most promising of the new generation of molecular biological methods. It is difficult to predict what opportunities for genome engineering could be hidden within still unknown or poorly characterized defense systems. ■



THE ULTIMATE MOBILE ELEMENT: Bacteriophages converging on an *E. coli* cell can be seen injecting their genetic material (blue-green threads) into the bacterium. Often, viral DNA will be taken up by the host genome, where it can be passed through bacterial generations.

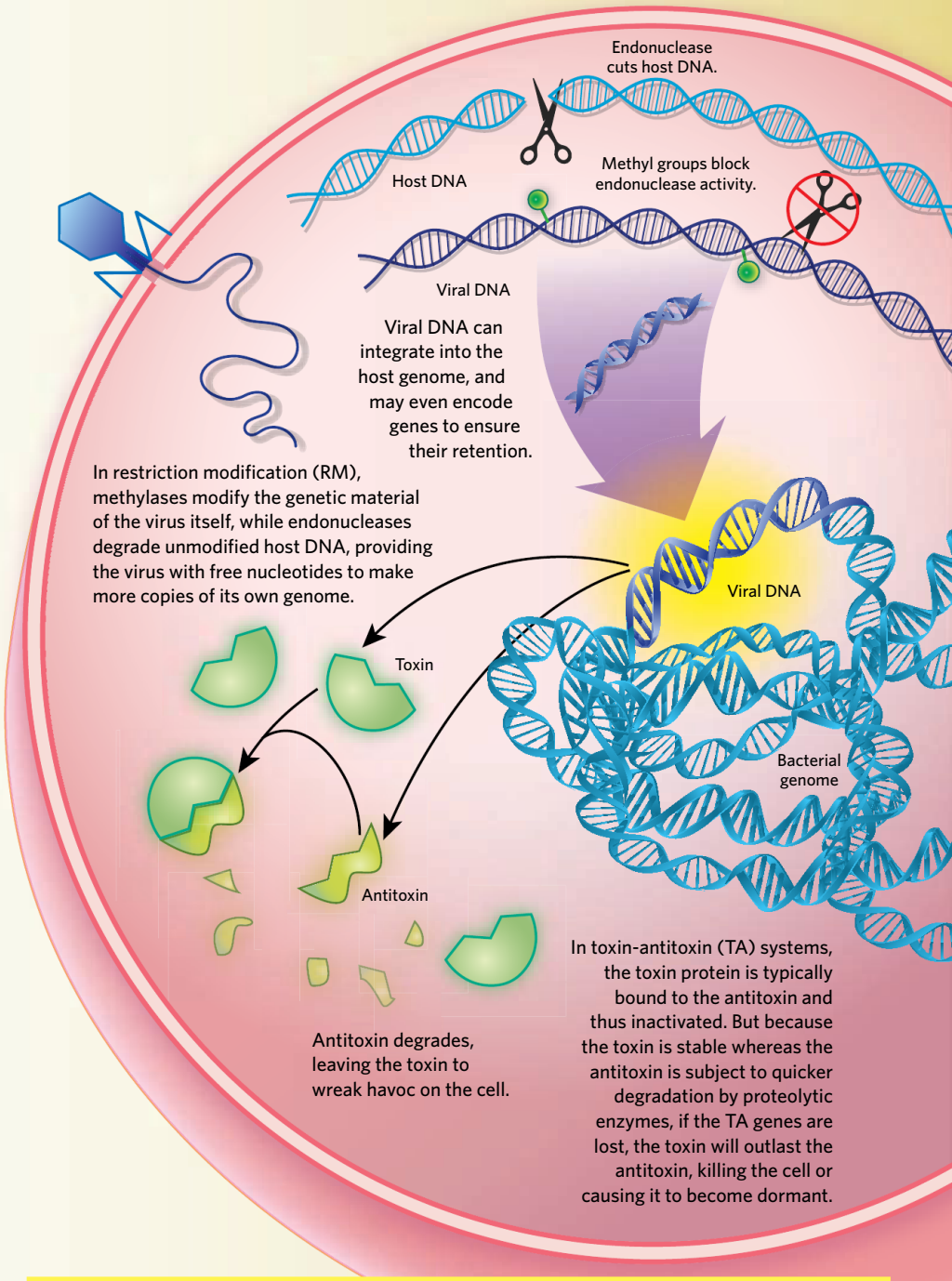
Eugene V. Koonin is a group leader at the National Library of Medicine's National Center for Biotechnology Information in Bethesda, Maryland. Mart Krupovic is a research scientist at the Institut Pasteur in Paris, France.

References

1. T. Boehm, "Design principles of adaptive immune systems," *Nat Rev Immunol*, 11:307-17, 2011.
2. R. Medzhitov, "Approaching the asymptote: 20 years later," *Immunity*, 30:766-75, 2009.
3. K.S. Makarova et al., "Comparative genomics of defense systems in archaea and bacteria," *Nucleic Acids Res*, 41:4360-77, 2013.
4. J.E. Samson et al., "Revenge of the phages: defeating bacterial defences," *Nat Rev Microbiol*, 11:675-87, 2013.
5. R. Barrangou, L.A. Marraffini, "CRISPR-Cas systems: Prokaryotes upgrade to adaptive immunity," *Mol Cell*, 54:234-44, 2014.
6. F.J. Mójica et al., "Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements," *J Mol Evol*, 60:174-82, 2005.
7. A. Bolotin et al., "Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin," *Microbiology*, 151:2551-61, 2005.
8. K.S. Makarova et al., "A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action," *Biol Direct*, 1:7, 2006.
9. R. Barrangou et al., "CRISPR provides acquired resistance against viruses in prokaryotes," *Science*, 315:1709-12, 2007.
10. E.V. Koonin, Y.I. Wolf, "Is evolution Darwinian or/and Lamarckian?" *Biol Direct*, 4:42, 2009.
11. K.S. Makarova et al., "Evolution and classification of the CRISPR-Cas systems," *Nat Rev Microbiol*, 9:467-77, 2011.
12. H. Kim, J.S. Kim, "A guide to genome engineering with programmable nucleases," *Nat Rev Genet*, 15:321-34, 2014.
13. K.D. Seed et al., "A bacteriophage encodes its own CRISPR/Cas adaptive response to evade host innate immunity," *Nature*, 494:489-91, 2013.
14. M. Krupovic et al., "Casposons: a new superfamily of self-synthesizing DNA transposons at the origin of prokaryotic CRISPR-Cas immunity," *BMC Biology*, 12:36, 2014.
15. V.V. Kapitonov, J. Jurka, "RAG1 core and V(D)J recombination signal sequences were derived from Transib transposons," *PLOS Biol*, 3:e181, 2005.
16. E.V. Koonin, M. Krupovic, "Evolution of adaptive immunity from transposable elements combined with innate immune systems," *Nature Rev Genet*, doi:10.1038/nrg3859, December 9, 2014.

VIRUSES

Viruses also carry around counter-defense genes to ensure their own survival. Many viruses infecting prokaryotes use defense mechanisms similar to those of their hosts, such as the expression of restriction-modification and toxin-antitoxin systems.



TRANSPOSON CONNECTION: *Viruses, themselves a form of mobile genetic element, can carry counter-defense gene complexes between hosts, where the virus's genetic elements can integrate into the host genome, thus mediating the spread of immune factors among species.*

The Literature

PHYSIOLOGY

Straighten Out

THE PAPER

C. Rot et al., "A mechanical jack-like mechanism drives spontaneous fracture healing in neonatal mice," *Dev Cell*, 31:159-70, 2014.

When people break a bone, they usually go to an orthopedist to straighten out any misaligned pieces so that the bone does not heal crookedly. But doctors have long observed that when infants get fractures—even if they receive minimal medical intervention—their bones heal reasonably straight.

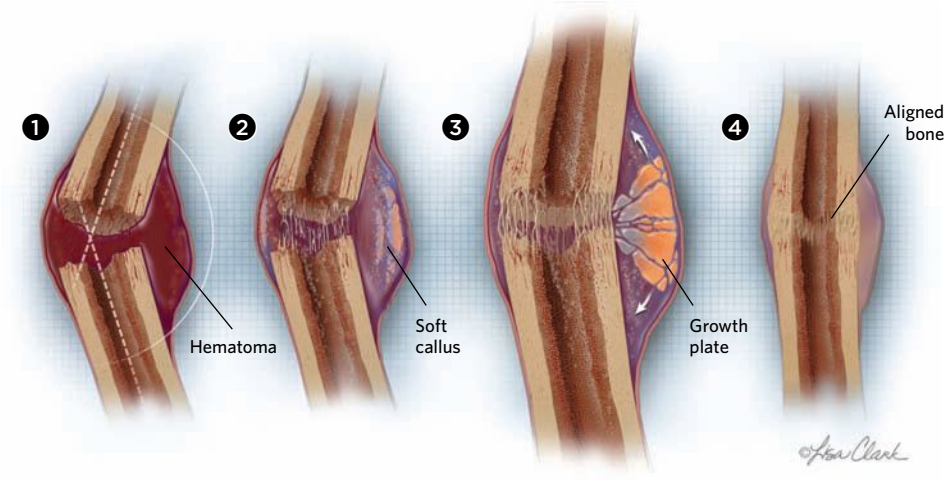
The assumption had been that fractures in infants at first heal crookedly and then are reshaped through bone remodeling, a lifelong process by which old or damaged bone is resorbed and replaced. But researchers at the Weizmann Institute of Science in Rehovot, Israel, showed that mouse bone fragments realign themselves before fusing back together.

To better understand the healing process, the researchers broke bones in the front legs of newborn mice. They then allowed the mice to move about freely and took periodic computed tomography (CT) scans as the fractures healed. Within 28 days, fractures with broken-bone angles less than 40 degrees had completely realigned themselves, while more severely misaligned fractures didn't heal perfectly straight but significantly improved.

The researchers also labeled the bones' surfaces with fluorescent markers, finding the colorful coatings remained intact during realignment, indicating realignment was happening by physical movements of the bones, rather than by remodeling of their surfaces.

When a fracture occurs, a fibrocartilaginous material called soft callus, made up of osteoblasts, chondrocytes, and fibroblast-like cells, forms around the break. Staining tissue from the area of the fracture, the researchers noticed that the soft callus was asymmetrical, with an excess of chondrocytes on the concave side of the angled break. Moreover, when they analyzed chondrocyte gene expression in the callus, they saw an enrichment of markers characteristic of bidirectional bone growth plates—like those that help a developing skull expand—on the concave side of the fracture. Unlike most growth plates, which form new bone only in one direction, the plate in the fracture's bend appeared to be moving in two opposite directions. The researchers hypothesize that the forces thus generated push the bone fragments into alignment.

Realignment was "a very fast process," says coauthor Elazar Zelzer. "It basically happened before the fractured bones were united and ossified." Only once the bones were aligned did the soft callus connecting them fully harden.

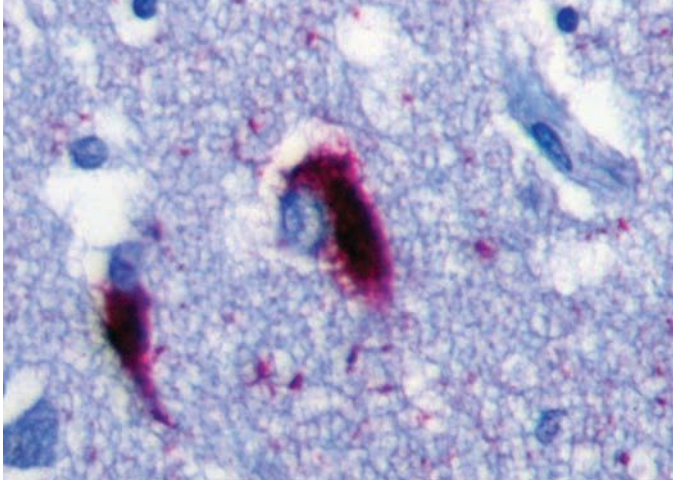


SPONTANEOUS STRAIGHTENING: After a fracture, blood rushes to the site of injury, forming a hematoma ❶. Next to form is the soft callus (purple), flexible tissue containing osteoblasts, chondrocytes, and other types of cells that surrounds the bone fragments ❷. A bidirectional growth plate on the concave side of the fracture promotes bone growth (orange) in opposing directions, generating a force that brings the bone fragments into alignment ❸. The soft callus ossifies into solid bone ❹.

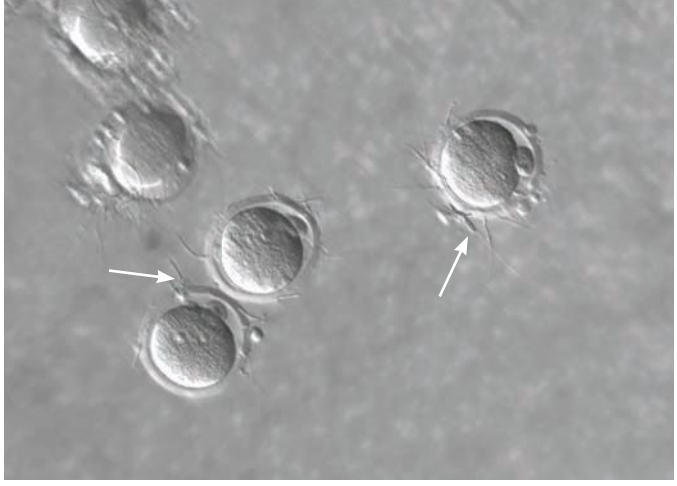
When the researchers paralyzed mice's muscles by injecting them with Botox, the bones healed crookedly, and the growth plates did not appear. Zelzer says he does not know why having functional muscles is important for realignment, but he speculates that muscle contractions help cells sense the extent of the fracture and direct growth plate formation.

"It's no doubt that they've identified a bidirectional growth plate," says Jill Helms, a professor at the Stanford University School of Medicine. However, Helms remains skeptical about whether such a growth plate can actually generate enough force to counteract muscle contractions that pull the fractured bone into its angulated position. "That's a question that I guess remains for the next series of experiments," she says.

"If we are able to understand more about our new process," says Zelzer, "we may be able to come up with new ideas about how to improve fracture healing in pathological situations." —Kate Yandell



ALL TIED UP: Neurofibrillary tangles, shown here in magenta, appear to originate from the cleavage of tau by an enzyme called asparagine endopeptidase.



CONTROLLED COUPLING: Mouse sperm (examples indicated by arrows) swim through culture medium to reach egg cells.

NEUROSCIENCE

Tangle Trigger

THE PAPER

Z. Zhang et al., "Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease," *Nature Medicine*, 20:1254-62, 2014.

THE BACKGROUND

Tangles of truncated tau proteins squished inside brain cells are a hallmark of Alzheimer's disease (AD), though their precise origins are mysterious. Aging, the strongest risk factor for AD, is linked to a drop in neurons' pH, hinting that acidosis might influence tau fragmentation. In 2008, Keqiang Ye of Emory University and colleagues discovered that at a pH of 6.0, a lysosomal enzyme called asparagine endopeptidase (AEP) moved into the cytoplasm and cleaved brain proteins. This led them to explore whether AEP also acted on tau.

THE DISCOVERY

Assays of mouse and human brains confirmed that AEP cuts tau at two asparagine locations, N-255 and N-368; corresponding tau fragments were abundant in AD specimens of both species. The enzyme's activity ramped up with age in mice. AEP activity in cultured rat neurons also increased in response to rising doses of amyloid-β peptide, which is a critical precursor to plaque formation in AD. Injecting an AEP-resistant version of tau into rodent brains protected mice from signs of AD.

THE PATHWAY

The results suggest that "AEP cleavage is responsible for the biochemical and pathological defects of tau [in AD]," says Ye. AEP activation by amyloid-β indicates that the enzyme may initiate the formation of tau neurofibrillary tangles in Alzheimer's disease. Ye suggests that AEP might provide "a potential new drug target."

THE FUTURE

The result "looks compelling, and points to potential disease causality," says Charles Glabe of the University of California, Irvine, in an e-mail. But only clinical tests will establish AEP's true significance, he adds.

—Jyoti Madhusoodanan

DEVELOPMENTAL BIOLOGY

Fertility Treatment Fallout

THE PAPER

S.K. Feuer et al., "Sexually dimorphic effect of in vitro fertilization (IVF) on adult mouse fat and liver metabolomes," *Endocrinology*, 155:4554-67, 2014.

THE BACKGROUND

Embryos formed by in vitro fertilization (IVF) experience very different early conditions from naturally conceived embryos. In previous studies, Paolo Rinaudo of the University of California, San Francisco, and colleagues observed that IVF-conceived female mice were more insulin resistant and metabolized glucose poorly compared to normally conceived females, and that IVF mice of both sexes showed altered gene expression, which led them to wonder what might cause such lasting metabolic and transcriptional changes.

THE EXPERIMENTS

Rinaudo's team transferred IVF-conceived and normally conceived blastocysts into recipient mice. Two months later, when the animals were young adults, the researchers examined their liver, muscle, fat, and pancreatic tissues for metabolic parameters.

THE RESULT

Across tissues, the researchers observed many differences in metabolite levels between the IVF and natural cohorts, although they failed to find a distinctive "IVF fingerprint," says Rinaudo. The normal sex disparities in fat metabolites shrank among the IVF mice, while in liver tissue the disparity was exaggerated. Fat tissue from IVF females also showed greater signs of oxidative stress. These alterations in metabolism and in underlying gene expression could play a role in the dysfunctional glucose metabolism and insulin resistance observed previously in mice, Rinaudo suspects.

THE VIEW AHEAD

The results suggest that the genome of IVF-conceived embryos "is utilized in a very different way, which translates into [their later] ability to handle metabolic changes," says reproductive biologist Mark Green of the University of Melbourne in Australia. The big question, says Rinaudo, is "to discover if what happens in animals is true in human children."

—Jyoti Madhusoodanan

PATHO/WIKIMEDIA COMMONS; SKY FEUER, RINAUDO LAB

Why, Oh Y?

A toothpick and a bit of chance shaped David Page's career, which he has dedicated to understanding the mammalian Y chromosome and fetal germ cell development.

BY JEF AKST

After his first year of medical school, David Page spent the summer working in Ray White's lab at the University of Massachusetts Medical School. "My project, using the technology of 1979, was to work toward and ultimately construct a genetic linkage map of the human genome," he recalls. It would take many people many years to complete the task, but what Page found that summer would ultimately drive his entire research career.

"We were picking bits of the human genome absolutely at random from what was then the first library of the human genome, the Maniatis lambda phage library," Page says. "I was literally picking—with a toothpick—lambda phage plaques that contained 15-kilobase segments of the human genome. And it turns out that one of my first toothpickings was of a lambda phage clone that contained a segment of DNA that derived from the human X and Y chromosomes." Page has now spent more than three decades researching the Y chromosome, defending it against hypotheses that it was slowly disappearing, and demonstrating its role both within and now outside the reproductive tract. "[For] every experiment that we've done since, I can trace an unbroken line back to that toothpick."

> "By the late 90s, it was clear that the Y chromosome carried more genes than anyone had given it credit for."

Page has helped clone and sequence the Y chromosomes of humans, rhesus monkeys, mice, and a handful of other species. His group has demonstrated that the human Y carries many genes involved in sperm production, as well as a handful of genes encoding genome regulators that are expressed throughout the body. Along the way, he's also explored the roots of germ-cell biology in mouse embryonic development, searching for molecular decision makers involved in producing eggs or sperm. Here, Page talks about the race to identify the Y's sex-determining gene; how his group won that race, but was ultimately wrong about the answer; and where the rest of the Y chromosome has since led him.

PAGE SENDS OUT PROBES

Rural roots. Page grew up amid farmland on the outskirts of Pennsylvania Dutch country. He loved nature, but he was never really exposed to any scientific research. That all changed when he became the first member of his family to attend college. Swarthmore College was only 90 miles away, but "it was a completely different world," Page says. He became enthralled with the life of academics, and of scientists in particular. "I came to realize that there were people who spent their time thinking about big ideas and how things worked." Early in his freshman year, cell biologist Bob Savage drove him to nearby Haverford College for a seminar on the chemical origins of life. "It wasn't that I was so taken by the subject matter. . . . Just this notion of people traveling around with ideas to share was quite intoxicating for me."

Basement science. After his junior year, Page spent the summer at the National Institutes of Health (NIH), working on the structure of histones in the basement lab of the late biochemist Robert Simpson, a Swarthmore alum. At night, he bunked in the basement of Simpson's house. "Talk about an immersion experience in the life of a scientist," Page says. "I basically became a member of the family for a year." Page enjoyed the experience so much that he enrolled in only a single seminar the following semester at Swarthmore. He continued living in Simpson's basement and working at the NIH—and took the train up to Pennsylvania once a week for his class.

Bookkeeping error. After college, Page joined the Health Sciences and Technology (HST) MD program hosted by both Harvard and MIT. Having worked on chromosomal proteins as an undergrad, he wanted to switch gears and "do something with nucleic acids," he says. At the advice of MIT's David Baltimore, another Swarthmore alum, Page connected with David Botstein, also at MIT, and with White's lab at UMass that first summer when he toothpicked his way to studying sex chromosomes. But figuring out that the phage clone he'd snagged came from the X and the Y took a bit of sleuthing. The variation in the DNA he'd purified from different samples, which came from American Red Cross blood donations, appeared to correlate with sex: in some cases females would have two bands where males and other females had just one, suggesting the snippet of DNA might come from the X chromosome. And there was one band that appeared to come from the Y: it was evident in all of the male samples and in none of the females—except one. "It took me probably six months, but I eventually showed it was a bookkeeping mistake, and that that was actually a male sample that had been mislabeled as female," Page says.

PAGE PROBES DEEPER

Founding fellow. As Page neared the end of his MD program, Botstein mentioned to him that Baltimore was in the process of setting up and staffing the Whitehead Institute. A few months

CHRISTOPHER CHURCHILL, WHITEHEAD INSTITUTE

DAVID C. PAGE

Director, Whitehead Institute
Professor of Biology, MIT
Investigator, Howard Hughes Medical Institute

Greatest Hits

- Identified deletions on the long arm of the Y chromosome that caused the failure of spermatogenesis and resulting infertility.
- Cloned the human Y, making it one of the first two cloned human chromosomes.
- With graduate student Bruce Lahn, helped detail the evolution of the sex chromosomes from a pair of ordinary autosomes.
- With colleagues at Washington University in St. Louis, developed a new sequencing technique, dubbed SHIMS (single-haplotype iterative mapping and sequencing), that allowed them to sequence the human Y chromosome, leading to the identification of large palindromes that render the chromosome susceptible to infertility-causing deletions.
- Overturned the idea that differences in the timing of meiotic initiation determined whether a germ cell becomes an egg or a sperm, demonstrating that mouse ovarian germ cells can become oocytes even in the absence of meiosis.
- In 2014, identified 12 surviving gene pairs that exist in different copies on the X and the Y chromosomes that may explain sex differences in disease susceptibility.

later, Page became the Whitehead's first fellow; the building opened six weeks after he graduated. "I essentially entered the day it opened, and that was 30 years ago. It's an altogether unlikely and irreproducible life story, but makes me very much appreciate the role of chance and unlikely opportunities that come one's way."

Near miss. "I was scared out of my wits," Page says about starting his own lab at the Whitehead with no PhD or postdoctoral training under his belt. He decided to continue studying the human Y chromosome, joining many other researchers in the search for a gene that set male development into motion in the mammalian embryo, which defaults to a female anatomy. "There was a very fierce international competition to chase down the sex-determining gene on the Y chromosome," Page recalls. At one point, he and his group thought they'd struck gold with a gene dubbed *ZFY*, which encodes the zinc finger Y-chromosomal protein. "In late 1987 we published, to enormous fanfare, what we thought was the sex-determining gene on the Y chromosome. It turned out to be a miss, a near miss; [*ZFY*] was the gene next door to *SRY*."

Mapping the Y. A few years later, after *SRY* was correctly identified as the sex-determining gene on the Y by the U.K.'s Peter Goodfellow and Robin Lovell-Badge, molecular biologists began interrogating *SRY* or left the field altogether, Page says. "People went home, found other jobs. . . . Because there was the assumption that there was nothing else there on the Y, that was it." But Page shifted his focus from the short arm of the chromosome, where *SRY* resides, to the long arm. Because the Y chromosome doesn't cross over with the X, it doesn't lend itself to traditional genetic linkage mapping—part of the difficulty the field faced in identifying the sex-determining gene. Instead, Page turned to naturally occurring deletions in the chromosome. Within a few years, he and others began identifying deletions on the long arm that caused spermatogenic failure and resulted in infertility. "By the late 90s, it was clear that the Y chromosome carried more genes than anyone had given it credit for," Page says.

Hall of mirrors. With the advent of PCR, the team continued to refine its maps, and in 1992, Page and his colleagues succeeded in cloning the entire Y, giving them a near-complete physical map of the chromosome. Still, not all of Page's questions about the Y's structure were answered. "It was extremely confusing at first, because it turned out that there were big chunks of DNA that were present multiple times on the long arm of the Y chromosome," Page says. He teamed up with colleagues at Washington University in St.

Louis to sequence the human Y. The task would require the invention of a new sequencing technique, dubbed SHIMS (single-haplotype iterative mapping and sequencing), that allowed for much longer reads of 150,000 to 200,000 base pairs. Page finally identified the source of his confusion: the Y chromosome's long arm, some 42 million bases long, carried a handful of large palindromes, the largest of which spans three million bases of DNA. His team would go on to show that these Y palindromes make the chromosome susceptible to the deletions that cause spermatogenic failure. In 2012, the team found that one particularly harmful deletion on the long arm of the Y crops up anew in one in every 2,400 newborn boys. "This is a spectacularly high rate of new mutation," Page says. "We began to realize that this seemingly chaotic hall of mirrors actually was like a crystal palace."

The evolving Y. As Page's group generated more-detailed maps of the Y, the researchers began to suspect that the chromosome, along with the X, had evolved from what were once ordinary autosomes, an idea originally proposed almost a century earlier. One of the first clues came in the mid-90s when Page's team discovered that a family of genes—dubbed DAZ for "deleted in azoospermia"—had come to the Y by a transposition of an autosomal segment. Over the next few years, the researchers began to uncover pairs of genes that existed in copies on both the X and Y chromosomes and appeared to have been retained from the ancestral autosomal pair, serving as "living fossils" that detailed the differentiation of the sex chromosomes. In 1999, Page and his then graduate student Bruce Lahn published an analysis showing that the X and Y had diverged in four discrete steps, starting some 200 million to 300 million years ago, as bits of the chromosomes lost their ability to cross over. "I would say that paper from 1999 was, for me, and I think for the field, the turning point in recognizing that the X and Y chromosomes had evolved from ordinary autosomes. . . . We now had the smoking gun at a molecular level." (See "Doris Bachtrog: Sex Chromosome Wrangler," page 59.)

Palindrome problems. One particular Y chromosome variant that Page's group studied is what's known as an isodicentric Y or an iso-Y. "It's an anomaly of the Y chromosome in which the entirety of the chromosome becomes a palindrome," Page explains. His group discovered that these chromosomes resulted from aberrant crossing over between two copies of the Y (after the chromosome has replicated during cell division), if the two copies were aligned head-to-toe. Page was struck by the wide range of phenotypes displayed by the people carrying these iso-Y chromosomes: men who were infertile but otherwise healthy; people with ambiguous external genitalia; people who had clearly developed as females, but nonetheless carried iso-Ys in at least some of their blood cells. Page's group reasoned that, because iso-Y chromosomes have two centromeres, they were prone to being lost during cell division. And if the iso-Y is lost, what would remain is an XO genotype—typically associated with Turner syndrome, a developmental disease that afflicts girls. Sure enough, when Page and his colleagues took a closer look at the individuals carrying iso-Ys, "in fact, there were a number of girls and women who had been diagnosed with Turner syndrome but who were carrying this isodicentric Y in some of their cells," Page says. "They were actually mosaics in many cases."

PAGE OVERTURNS

Germ cell meiosis. Early on in his research into the sex chromosomes, Page realized that he ought to also study germ-cell biology. Massive differences in male and female gamete production can be traced back to embryonic development and the initiation of meiosis in the primordial germ cells. In females, these cells initiate meiosis and begin to develop into eggs within just a few days of arriving in the developing ovary. In males, the germ cells reach the developing testis and simply "hunker down" until it's time for sperm production to begin in puberty, Page explains. These differences in the timing of meiotic initiation led to the hypothesis that this is what determines whether a germ cell becomes an egg or a sperm. But in a 2013 *Nature Genetics* paper, Page and colleagues found that even in the absence of meiosis, mouse ovarian germ cells became oocytes that could be ovulated, fertilized by sperm, and even develop into two-cell embryos. "This pretty much blew up the idea that fetal initiation of meiosis represented commitment to the oocyte-like development," Page says.

Sex chromosomes and disease. Page, who never liked the idea that the Y chromosome was headed for extinction, has spent much time defending Y's honor, he says. While the idea of the disappearing Y chromosome has, by now, largely been "laid to rest," he says, "I think that debate has kept us from really considering more broadly the role of the X and Y chromosomes in sexual dimorphism." Specifically, his recent work has led Page to recognize the vast differences between human males and females in the incidence and severity of disease. Autoimmune diseases such as rheumatoid arthritis and lupus, for example, are more common among women, while autism spectrum disorders are more prevalent in boys. "These sex biases in incidence and severity are not the exception; they're actually the rule," Page says. "There's no simple explanation in our anatomies as to why these differences should exist, and I think that ultimately these differences will trace their biological origins back to the X and Y chromosomes."

The roles widen. In a paper published last year, Page and his colleagues compared the Y chromosomes of eight mammals, including the human, other primates, and the mouse, along with the chicken Z, identifying surviving gene pairs that existed in copies on both the X and the Y. On the human Y, they found 12 such surviving genes, and all were global regulators of gene expression, such as chromatin modifiers. "These are powerful, influential players in the lives of our cells," Page says. What's more, the group learned, both the X and Y copies of these genes, which are not identical, are widely expressed throughout the body, and a cell's sex chromosome genotype determined the isoforms it expressed. "We begin to consider the possibility . . . that some fundamental molecular difference between XX and XY cells could potentially contribute to these very different susceptibilities to a wide range of diseases outside the reproductive tract." ■

Doris Bachtrog: Sex Chromosome Wrangler

Associate Professor, Department of Integrative Biology, University of California, Berkeley. Age: 39

BY MOLLY SHARLACH

Growing up on a farm in rural Austria, Doris Bachtrog was "always kind of the black sheep and the outlier in my family," she recalls. "I cared about education; I was very curious." She left home at age 13 to attend a boarding school that specialized in teaching chemical engineering.

Bachtrog earned an undergraduate degree in biology, and made use of her quantitative skill set to pursue a master's thesis in population genetics. She wrote a computer program to identify microsatellites—variable DNA repeats used as genetic markers—in the *Drosophila melanogaster* genome. This first research experience left her "amazed that there was so much still unknown" about the natural world, Bachtrog says.

© PAUL SIMCOCK

One area ripe for investigation with molecular tools was the evolution of sex chromosomes, and *Drosophila miranda* turned out to be a useful model system. Bachtrog and her PhD advisor, University of Edinburgh biologist Brian Charlesworth, examined sequence variation among four genes on the species' so-called neo-X and neo-Y chromosomes, which arose around a million years ago from the fusion of an autosome to the Y chromosome. They found that rates of evolution in the neo-Y reflected the beginnings of gene loss, or degeneration, and in the neo-X showed evidence of incipient dosage compensation, a process that equalizes protein levels between XY males and XX females.[1]

As a postdoctoral fellow in the lab of Andrew Clark at Cornell University, Bachtrog combed DNA sequence data to explore the divergence of two fruit fly species, *D. santomea* and *D. yakuba*, which live at different altitudes on the West African island of São Tomé, but sometimes interbreed. She and her colleagues observed differences in the species' nuclear genes, but discovered surprising similarity in their mitochondrial DNA, highlighting unevenness in evolutionary dynamics across the genomes.[2]

Bachtrog had "a laser focus on the problems that she wanted to pursue," recalls Clark, who says he felt more like her colleague than her mentor.

Bachtrog joined the faculty at the University of California, Berkeley, in 2008. Her colleague Michael Eisen soon convinced her to sequence the *D. miranda* genome. "I had no lab or anything," she says. "Instead of looking for an apartment, I spent two weeks in the lab preparing a genomic library . . . that was my way of jumping into genomics."

After obtaining the genome sequence, says Eisen, "she began to think about ways of studying sex chromosome evolution in a much more macroscopic way." In 2013, Bachtrog and her postdoc Christopher Ellison used the genome sequences of *D. miranda* and related species to demonstrate that transposable elements called helitrons play an important role in the evolution of dosage compensation on the X chromosome.[3]

Bachtrog has also taken full advantage of next-generation sequencing to explore the origins and diversity of sex chromosomes in other species, including snakes and birds.

"She's gone on to make quite a big splash in the field of evolutionary genomics," says Charlesworth. "It's been one of her characteristics that she's not been afraid to get into things which are challenging."

Bachtrog also brings a spirit of adventure to her *Drosophila* field-collecting trips—in the past year, she's visited several nations in Southeast Asia, zipping through the countryside on a motorbike in pursuit of fruit flies.

"Half the time . . . it's some weird trip she's taking, and half the time it's some weird science she's doing," says Eisen. "It's often hard to see the boundary." ■

REFERENCES

1. D. Bachtrog, B. Charlesworth, "Reduced adaptation of a non-recombining neo-Y chromosome," *Nature,* 416:323-26, 2002. (Cited 137 times)
2. D. Bachtrog et al., "Extensive introgression of mitochondrial DNA relative to nuclear genes in the *Drosophila yakuba* species group," *Evolution,* 60:292-302, 2006. (Cited 119 times)
3. C.E. Ellison, D. Bachtrog, "Dosage compensation via transposable element mediated rewiring of a regulatory network," *Science,* 342:846-50, 2013. (Cited 10 times)

Eye on the Fly

Automating *Drosophila* behavior screens gives researchers a break from tedious observation, and enables higher-throughput, more-quantitative experiments than ever before.

BY SARAH C.P. WILLIAMS

The dynamics inside any fruit fly room are as riveting as a reality TV show. Some *Drosophila* strains are bullies, while others are just out to mate; some spend more time chowing down; and some are more dedicated to grooming themselves. A decade ago, studying these complex behavioral dynamics was a tedious task, requiring hours spent watching fuzzy videos of flies being flies, jotting down their every action and the time it occurred.

"The problem was that, not only was this prohibitively time-consuming and mindless, but the behaviors were fairly subjective and people would categorize them differently," says biologist Benjamin de Bivort of Harvard University.

Now, that's all changing. With the plummeting cost and rising quality of high-definition cameras, sensors, and machine-learning programs, biologists are using computers or touchpads to automate the detection of fly behaviors, from grooming to mating—even detecting how often they eat. Today, such methods are so sensitive that they can reveal the individual motion of each of the six legs of a *Drosophila*.

"There's a revolution happening in behavioral neuroscience that comes about because of all these cheap sensors designed for phones and personal electronics," says de Bivort.

These new techniques are giving scientists the tools to integrate quantitative behavioral data into studies of neuroscience, aging, and even metabolism—zeroing in on the neurons responsible for different fly behaviors, for instance, and how neurodegeneration or obesity changes those neurons' activity. "This isn't just a tool to make experiments go faster," says David Anderson, a neurobiologist at Caltech. "We're trying to take a field that's been defined by people sitting in the jungle with a notebook and make it objective and quantitative."

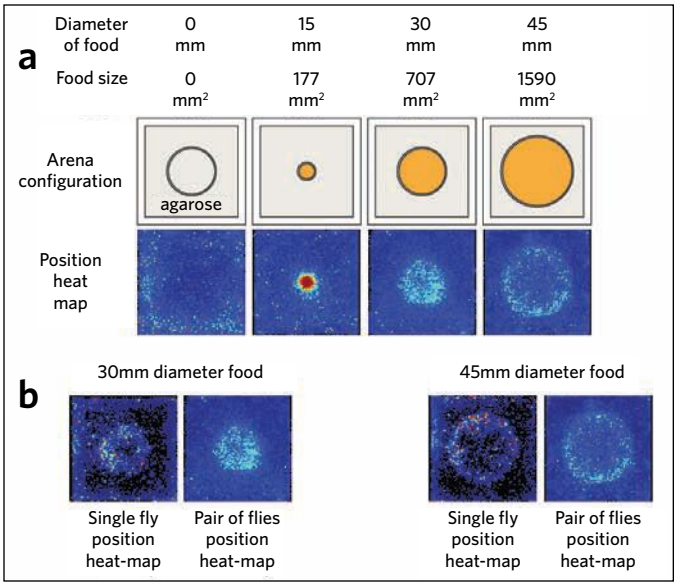
WALK LIKE A FLY, GROOM LIKE A FLY

Some of the first automated screens of fly behavior used computer programs to track the movement of a fruit fly tethered atop a rotating ball. This treadmill contraption kept the animal in a camera's field of view, and the program could detect and quantify the turning of the ball and the fly's motion. But if the fly was standing still, these programs couldn't discern what it was doing—was it flapping its wings? Rubbing its eyes? Cleaning its abdomen? All these unique motions of the animal's legs occur while a fly is stationary.

At Harvard, de Bivort studies individual differences in behavior. To discern the distinctive behavioral quirks displayed by flies from the same genetic strain and raised in the same environments, de Bivort wanted to track more detailed information about a fly's movements than just its walking patterns, so his group has modernized the rotating-ball experiment. They first attached dots of infrared-fluorescent dye to each of a fly's legs. Then, they tethered the fly above a transparent ball. Below the ball, lasers aimed upward to excite the dots, and on each side they set up infrared sensors—scrounged from a type of computer mouse used to play high-performance video games—to detect the movements of the dots.

LOGGING LEG USE: *Drosophila melanogaster* with pieces of fluorescent polymer attached to each of its legs. These dye spots, each only 100 microns across, glow infrared when illuminated with red light and allow real-time tracking of leg position.

DE BIVORT LAB



TERRITORIAL BEHAVIOR: Using an automated screen, scientists can easily determine how much time flies spend on or near patches of food. Not surprisingly, when there's food around, flies are mostly found in the areas nearest the chow. The heat map in (a) shows the feeding behavior of male-male fly pairs exposed to food patches of different sizes. Panel (b) compares the feeding behavior of individual flies with that of two flies that occupy the same arena: the competing flies spend more time patroling the edges of the food patch.

The team developed a computer program that could track the motion of these spots and taught the program to link the motion with movement of the fly's legs. When the animal is rubbing its eyes clean, for instance, it moves both of its front legs. When it's cleaning its abdomen, however, the dots that move will be associated with other combinations of legs (*Nat Commun*, 4:1910, 2013).

What you can learn: Although the technique is still limited to one fly at a time, the so-called LegTracker lets you collect information about fly behavior in much more detail than an individual observer can, and even detect patterns that you didn't set out to study. "The computer can pick up very subtle things, like postural adjustments, that a person wouldn't even notice," explains de Bivort. Already, his team has published results about idiosyncrasies in fly patterns of walking and grooming. In general, he says, most flies spend the same total percentage of time doing certain activities, but how they transition between activities varies: one fly might always go from cleaning its eyes to grooming its abdomen, while another always follows eye cleaning with some walking around.

What it takes: The physical setup is relatively easy to replicate, de Bivort says, although it took trial and error, and lots of jury-rigging, to make it work. "The biggest challenge with the LegTracker is on the human side," says de Bivort. "Getting the dexterity to mark the flies' legs takes a while." For labs that have already been using a ball to follow flies' walking patterns, adding the fluorescent dots and infrared to track each leg can be an easy modification. To set up the LegTracker from scratch would cost about $5,000, de Bivort estimates, with the most expensive components being the laser used to fluoresce the dots and the two cameras that record experiments.

FLY-ON-FLY INTERACTIONS

For some researchers, tethering a fly in place too strongly constrains behaviors of interest. At Caltech, Anderson studies the neurological basis of social behaviors, and he needs to be able to quantify how a fly acts while it interacts with other flies and its environment. What causes one fly to lunge aggressively at another? What makes a fly pace in a circle around food?

Anderson, in collaboration with Caltech machine-vision expert Pietro Perona, developed an arena that is lit from below and sports a video camera to tape the interaction between flies. The technique's real punch comes from the computer program, designed by Perona, which can be taught to recognize and quickly quantify any particular event caught on tape—how often the flies get within a certain distance of each other or how often they lunge, for instance.

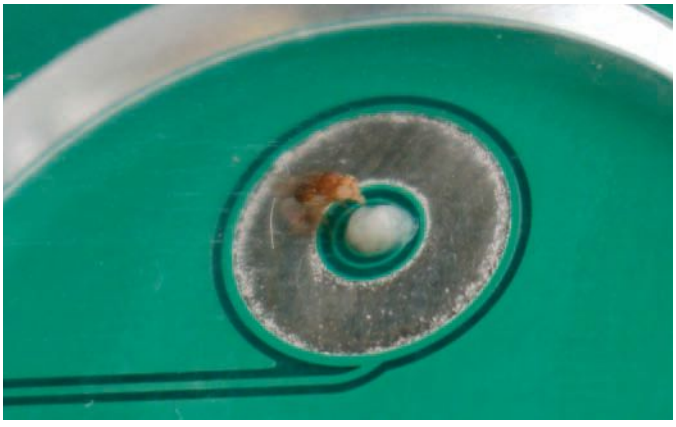
"It allows us to take tens of hours of videotape, which would take a human being a week of nine-to-five work to analyze, and have the computer process it in five minutes," says Anderson. "It's been absolutely transformative for us."

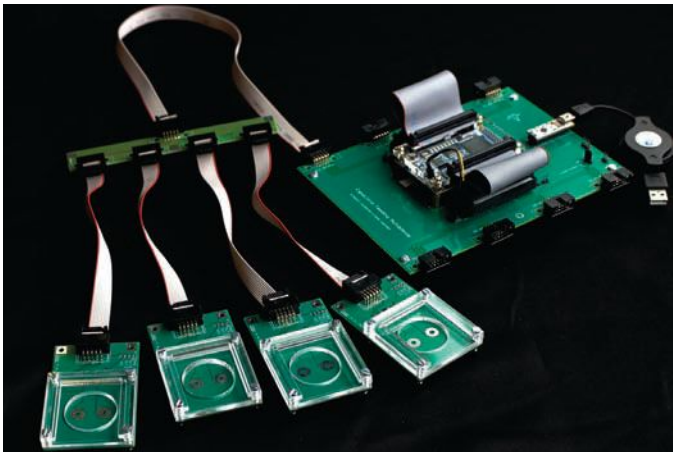
What you can learn: Anderson's group recently used the setup to study how two male flies compete over food, showing that the presence of food increases the frequency of aggression between males (*PLOS ONE*, 9:e105626, 2014). Moreover, by using genetic mutants in the experiments, they found that sugar-sensing neurons are required for this food-mediated aggression. The tracking program can be used to study a broad variety of behaviors, including mating.

What it takes: The setup is much the same as what's been used for decades by human observers. But you'll need to design a program that's customized to your particular arena, and "teaching" the program to recognize a specific movement can be tricky. After being fed videotape with each frame annotated—this is a fly, this is not, this is a lunging fly, this is not—the program can start to analyze fresh data. Anderson recommends that biologists collaborate with computer scientists to develop such a program for their own use. Because no two arenas look the same, a program has to be customized and retrained to work for a particular arena and lighting setup. Anderson's setup is unique to his lab, and he says it costs around $8,000 to build—not including cameras—but some researchers are working toward programs that will be easier to customize. The Janelia Automatic Animal Behavior Annotator (JAABA), for instance—developed by scientists at Howard Hughes Medical Institute's Janelia Farm campus—is a free, open-source machine-learning program that can be used to track some behaviors.

WATCH WHAT THEY EAT

Carlos Ribeiro, a scientist at the Champalimaud Neuroscience Programme in Lisbon, Portugal, studies how flies translate an internal state—such as nutrient depletion—to a behavior, like eat-





FEEDING BEHAVIOR: Top: By using an ordinary touchpad and placing a fly's food on a separate electrode from the fly itself, researchers in Carlos Ribeiro's group can detect exactly when the fly's proboscis reaches out to touch the food at the center of the circle. Bottom: The attachment of multiple feeding arenas to a single interface enables the acquisition of data from many flies at once.

ing, so he wanted to quantify how often and how much the animals eat. This information isn't just key to understanding which neurons influence feeding behavior and drive hunger, but also what causes metabolic disease, overeating, and obesity. Ribeiro turned to a touchpad like those found in tablet computers, which these days are so sensitive that when a fly walking on the touchpad sticks its proboscis into a tray of food, the pad detects it. "We wanted something which is robust and can go high-throughput and which doesn't require expensive cameras," says Ribeiro. "These sensors are actually very cheap now that they're in all these consumer devices."

With their current setup, Ribeiro's group can connect up to 32 different feeding arenas, each monitoring one fly's eating patterns, to a single computer. By testing flies with genetic mutations or induced metabolic diseases, they hope to uncover factors that drive eating behavior.

What you can learn: Ribeiro's team fluorescently labeled fly food to measure precisely how much the insects eat with each extension of the proboscis. The researchers used this information to calibrate their flyPAD to quantify how often, how long, and how much a fly eats. They also used the technique to study flies' food preferences by quantifying the different food choices made in one sitting (*Nat Commun*, 5:4560, 2014). And the flyPAD's utility extends beyond feeding, Ribeiro says. It could also be adapted to other behaviors, such as egg laying, that require small interactions between an animal and the ground below it. "These kinds of behaviors are very hard to capture quantitatively with a camera," he says.

What it takes: Ribeiro's group is collaborating with other scientists interested in using the flyPAD. Ribeiro will ship the setup to a lab for one-time use, or help you build your own—which will cost around $1,200. Building your own requires devices that most biology labs don't have lying around—special electrodes and capacitance converters—but might be worth the investment if you're studying a behavior that can be tracked using the touchpad.

TAKING FLIGHT

One of the trickiest behaviors to study in flies is flight. It adds an extra dimension to the animals' movement and requires following the bending and flapping of hard-to-see wings. Barry Ganetzky, a geneticist at the University of Wisconsin–Madison, studies how the fly neuromuscular system develops and how it weakens with age or disease. He needed a quick and easy way to screen lots of flies, of different ages or with different genetic mutations, for their ability to fly. The system he and postdoc Daniel Babcock developed, called the Flight Tester, is simple: drop the flies from above into a graduated cylinder after covering its inner wall with sticky flypaper. The better a fly is at flying, the sooner after it's dropped it will start to head sideways—straight into the flypaper. Animals that have totally lost their ability to fly will fall much further down before getting caught (possibly even hitting the bottom). The researchers can then remove the flypaper, snap a photo of it, and use photo analysis software to quantify the density of flies at different heights along the paper. (Ganetzky and Babcock used ImageJ, a free program developed by the NIH.) "Even if you have two or three hundred flies, we can make a graphic demonstration of where they're distributed," says Babcock.

What you can learn: In a February 2014 *Journal of Visualized Experiments* paper, Ganetzky and Babcock reported the use of the Flight Tester to compare wild-type flies with a strain dubbed slowpoke. While the healthy flies landed, on average, at a height of 73 cm in the meter-high graduated cylinder, the slowpoke flies fell much farther, hitting the sticky side around 44 cm. Since then, the lab has used the approach to characterize nine other mutants and has now started analyzing the genetics of these flies.

What it takes: Setting up the Flight Tester is cheap—around $250, Ganetzky estimates—and easy, requiring little more than a graduated cylinder with a funnel on top, sticky flypaper, a camera, and a piece of computer software that's already commonly used in many biology labs. "This system is as simple as possible and has worked really well for everything we want to do right now," he says. ■

PAVEL ITSKOV, RIBEIRO LAB

Picturing Infection

Whole-animal, light-based imaging of infected small mammals

BY KELLY RAE CHI

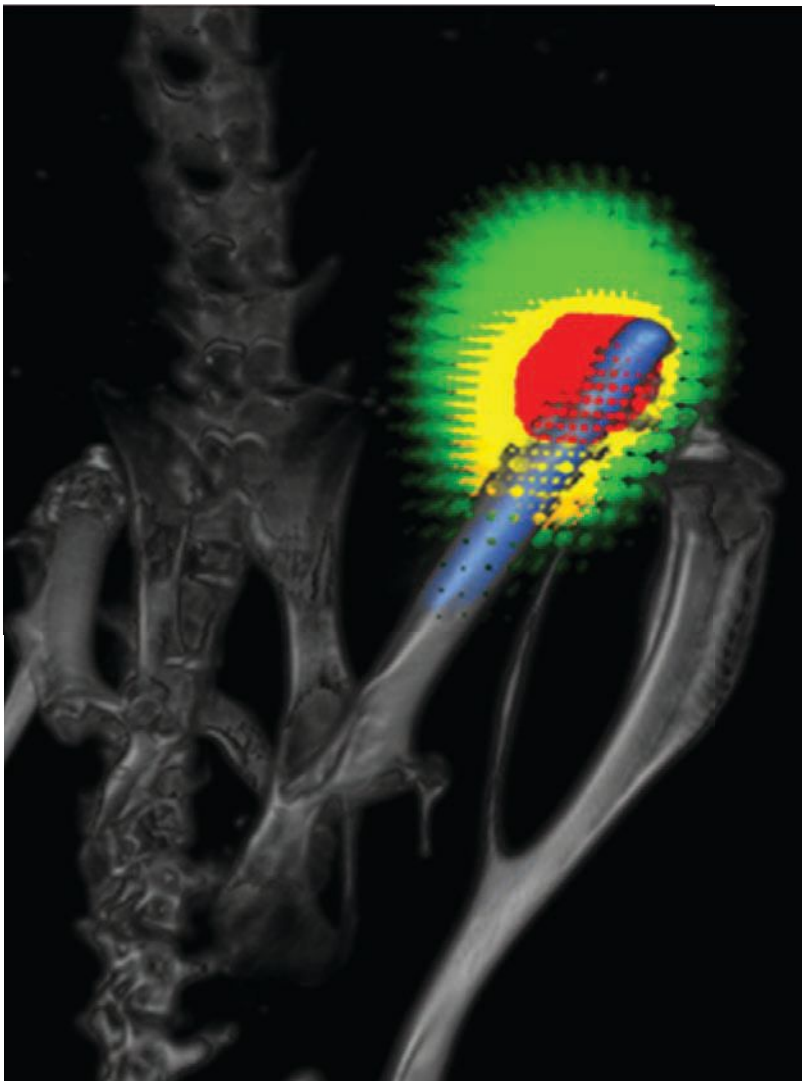
Epidemiologists are sleuths who track infections—what causes them and how they spread through a geographical area over time. Another kind of infection sleuth wants to know whether or how infections spread within the living body—as in the case of medical implants that get infected, or tuberculosis that moves from the lungs to other tissues—and whether infection hot spots will succumb to immune-system attack or to drugs over time.

Used most often in mice and other small rodents, in vivo optical imaging "is one really powerful tool to noninvasively monitor infection over time," says Lloyd Miller, an associate professor of dermatology and orthopedic surgery at Johns Hopkins School of Medicine. Using a mouse orthopedic implant model, his group simultaneously tracks the spread of *Staphylococcus aureus* and the responses of the animals' immune cells, such as neutrophils.

The method is powered by new refinements in fluorescent probes and in bioluminescently labeled pathogens. And a number of instruments now allow researchers to merge light-based imaging with other imaging modalities, such as CT scans. With these tools, infectious-disease researchers can probe deeper into the bodies of small animals, examining the effects of pathogens on their hearts and lungs.

In vivo optical imaging also comes with the major advantage of cutting down on animal use. Researchers who typically euthanize animals at various time points to measure the growth of infection in colony-forming units or to watch the body's unfolding response to it can now keep the animals alive and track infections at multiple points in time.

The Scientist talked to expert users and core-facility directors about whether and how to add in vivo optical bioluminescence or fluorescence imaging to your arsenal. Here's what they told us.



COURTESY OF LLOYD MILLER, MD, PHD

INFECTED IMPLANT: In vivo optical imaging of a titanium bone implant (blue) in a mouse femur combines bioluminescence, fluorescence, and CT imaging. The yellow areas show colocalization of an injected strain of bioluminescent *Staphylococcus aureus* (red) and fluorescently labeled neutrophils (green), early immune responders to infections.

HEAD TO YOUR CORE

If the imaging core facility at your institution has an in vivo optical imager, that should be your first stop. An initial consultation will determine whether your biology agrees with the capabilities of the equipment, says Kelly Stefano Cole, associate director of the Regional Biocontainment Laboratory, a biosafety level 3 (BSL3) facility at the University of Pittsburgh's Center for Vaccine Research. The more specifics you can offer—what you hope to measure, how often, and for how long—the easier it will be for them to help you.

Studying infectious diseases, of course, requires the right level of biosafety. With optical imaging, each core facility does it differently: some have equipment dedicated to a specific pathogen, whereas others are using the same machine to monitor a variety of animal models and pathogens. You may have to place your infected animal in a customized chamber that you can then use within the machines. These boxes help minimize the

risk of pathogen spreading, but they also cut back on the sensitivity of the imagers, Cole says.

Cores offer a range of services, from training the investigator to work on his or her own to assisting with running the equipment, and even in some cases doing the experiments. The higher the biosafety level you're working in, the longer it may take to clean the equipment before and after use. This translates into a higher cost; it is typically around 20 percent higher to use a BSL3 core compared to a BSL2, Cole says. "When you move up the scale to a BSL3, you just need to recognize that things get more complicated and expensive. As long as people understand that going into it, they're okay," she adds.

MACHINERY

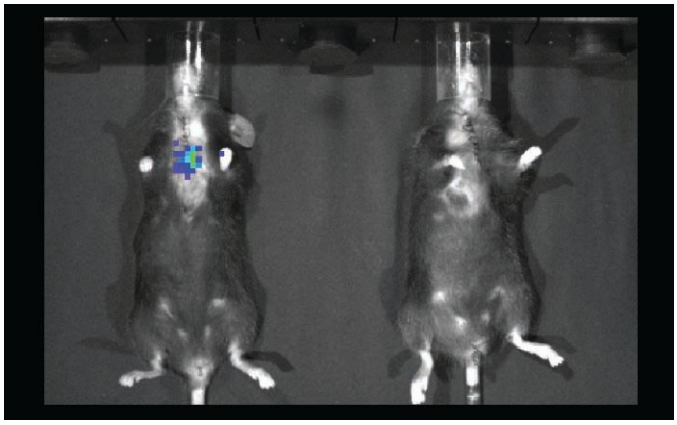
In vivo optical-imaging machines are about the size of a dorm refrigerator—boxes equipped with a laser light and lens, a sensitive camera, and a platform on which you place an anesthetized mouse or other small rodent. The machines take up to several minutes to acquire the light emitted from the animal.

Although 3-D systems are on the market, you're more likely to find a 2-D system at a core facility. Over the past few years, PerkinElmer bought up many of the smaller in vivo optical-imaging vendors, and the company now sells two different lines of IVIS (in vivo imaging systems): the IVIS Lumina Series III (consisting of four models, for 2-D imaging) and the more sensitive IVIS Spectrum Series (three models, for 2-D and 3-D imaging). The instruments range in price from around $100K–$550K.

The 2-D imagers within these two series are popular for rapid, high-throughput screening of animals, especially for drug-efficacy studies, says PerkinElmer's Kevin Francis, whose development of bioluminescently labeled bacteria helped lay the groundwork for in vivo infectious-disease imaging. "If you have a [bioluminescently labeled] infectious disease that you're treating with an antibiotic, you can see whether or not you're efficiently killing that particular disease because the light goes out," he says.

Some of the more sophisticated IVIS models incorporate X-ray or CT scanning, which allows you to do 2-D or 3-D coregistration, lining up your molecular image with an anatomical one. The machines that do 3-D optical imaging, including 3-D bioluminescent and fluorescence tomography, allow you to more precisely locate the signal spatially, and to build images that you can rotate.

But newcomers should realize that in vivo optical imaging is "more than just a pretty picture," says Matthias Nahrendorf of Massachusetts General Hospital's Center for Systems Biology. There's a real art to setting up these studies, which require at least five or six animals for each variable (infection or treatment) that's being assessed. And, though the imaging method is not to the level of measuring single cells, it is semiquantitative and will allow you to measure the area of a region of interest over time.



COURTESY OF YE AND NAHRENDORF

GETTING TO THE HEART OF THE MATTER: This in vivo optical image shows a mouse infected with a bioluminescent strain of *Staphylococcus aureus* in the inner lining of the animal's heart (left). The control mouse is on the right.

CHOOSING BIOLUMINESCENCE

In most cases, you label your infectious agent before introducing it into the animal. One decision you'll make at the outset is whether to work with bioluminescent or fluorescent labels; each comes with its own advantages.

Bioluminescence works by harnessing the natural ability of some organisms, such as fireflies, jellyfish, or bacteria, to glow. The enzyme luciferase from these creatures can be engineered into a variety of bacteria and other pathogens for infectious-disease research. In weighing the pros and cons of bioluminescence versus fluorescence, bioluminescence sometimes wins out because of its higher sensitivity and better light penetration (low background), Nahrendorf says. His group uses bioluminescence to track stem cells or bacterial infections in mice, and the team can easily see both in the heart. (See photograph above.)

Bacterial luciferase is especially popular for generating bioluminescent pathogens, and a variety of these pathogens are commercially available. There are also luciferases that allow you to monitor more-dynamic processes, such as calcium release, in a tissue. "There are a lot of tools now available," for sale and from other researchers who might be willing to share, Francis says. "With academics especially, rather than reengineering everything yourself, it would be better to do a collaboration and get these pathogens in-house to do the studies with."

OPTING FOR FLUORESCENCE

People pick fluorescence over bioluminescence for the former's ability to merge well with other methods, such as microscopy or flow cytometry, after you've harvested tissues from the animals, or in a parallel line of experiments.

Bacteria transfected with fluorescent proteins are not as bright as bioluminescent bacteria—and so are probably not sensitive enough to be detected using whole-animal in vivo optical imaging, Miller says. Researchers instead use transgenic

mice whose host cells or proteins are fluorescently tagged. Or they can inject targeted fluorescent probes that are conjugated to quantum dots, nanoparticles, or dyes to visualize aspects of the host's immune response. Because the latter strategy doesn't require genetic engineering, fluorescence studies also have greater potential for clinical translation.

Fluorescence is better for multiplexing, too. Measuring multiple signals using bioluminescence is more challenging because there are many fewer reporter systems available with nonoverlapping substrate and emission spectra, and signals may take several hours to decay, says Jason Lee, director of the Preclinical Imaging Technology Center, Crump Institute for Molecular Imaging at the University of California, Los Angeles.

Traditional fluorescent reporters, such as green fluorescent protein (GFP), are severely limited in their ability to be detected deep within tissue, however. To help solve this problem, researchers have devised near-infrared fluorescent proteins for in vivo imaging. The DNA constructs for these are available via Addgene for $65 per plasmid.

Even with these improvements, single-cell detection, especially at depths of greater than several millimeters, is not possible using near-infrared probes, says Vladislav Verkhusha of the Albert Einstein College of Medicine, who has developed a variety of fluorescent proteins for in vivo imaging. As with any of these optical imaging methods, especially the 2-D version, you will need to validate that the signal is coming from the expected tissue by dissecting and imaging individual tissues.

MICE ADVICE

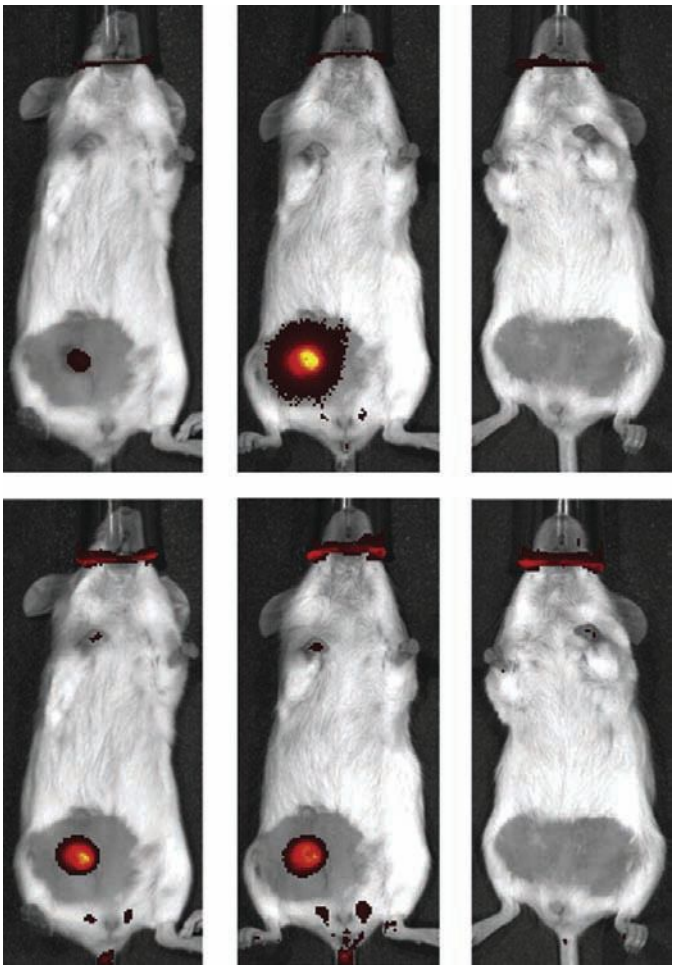
If you want to use mice for your imaging experiments, you're in luck. Although some in vivo optical-imaging instruments can, in theory, handle larger rodents such as ferrets, they can't see as deeply within these larger animals. And with mice, you can screen more than one animal at a time. Some instruments, such as the Spectrum series, routinely screen five mice, and allow you to screen as many as 10 with the right peripherals, says Francis.

There's one easy way to improve your images: go hairless. Because the black fur of typical wild-type mice impedes light penetration, researchers opt for hairless mice or thoroughly remove fur by shaving or applying depilatory creams.

Creating a chronic infectious-disease model can be a bit tricky. It involves striking a balance between injecting the mouse with enough of a pathogen to cause disease that's detectable using the imagers, on the one hand, and on the other, keeping the animal alive for long enough to take images over time. "Imaging is an additional stress [for the animal] because you have to put it under anesthesia with isoflurane. That actually can put some strain on the mouse. You also have to make sure the mouse doesn't get cold," Nahrendorf says.

Says Francis, "Normally the rule of thumb is, if the pathogen is causing disease you should be able to visualize it anywhere in a mouse or a rat. But if you want to use an artificial model where you've got low numbers of colony-forming units, then often you're scavenging for the signal," meaning your scans may take longer.

Many researchers are working out ways to image larger animals. Using near-infrared fluorescent probes with photoacoustic imaging (see "Brainspotting," December 2011) is one method that Verkhusha, Miller, and others say they are excited about for the technique's ability to image at greater depth than purely in vivo fluorescent methods. In photoacoustic imaging, light shined into the animal causes a subtle vibration of fluorochromes that is then picked up using ultrasound. The instruments, sold by VisualSonics and Endra Life Sciences, start at about $250,000. ■



COURTESY OF VLADISLAV VERKHUSHA, *CHEMISTRY & BIOLOGY*, 20: 1078–86, 2013

NEWER PROBES: Researchers continue to generate new fluorescent probes that can report on protein interactions and modifications in deep tissues of whole animals. In the example shown here, a near-infrared reporter iSplit, developed by Vladislav Verkhusha and colleagues, homes in on a specific protein-protein interaction. The top row shows near-infrared fluorescence of iSplit before (left) and after (center) the protein-protein interaction in a mouse tumor model and a control mouse (right). The bottom row shows far-red fluorescence of another probe used in addition to iSplit to gauge tumor size.

The Scientist TOP TEN INNOVATIONS 2014

Congratulations to all our 2014 winners!
To read the full article, please visit bit.ly/Top10Innovations2014

Top Row (L to R): DRAGEN Bio-IT Processor Edico Genome • MiSeqDx Sequencing System Illumina HiSeq X Ten Sequencing System Illumina • IrysChip V2 BioNano Genomics RainDance Technologies RainDrop Digital PCR System

Bottom Row (L to R): TCS SP8 STED 3X Leica Microsystems • exVive3D Liver Organovo HAP1 Knock-Out Cell Lines Haplogen Genomics Immucor PreciseType Human Erythrocyte Antigen Test • Sciencescape

Know Your PIO

Scientists and public information officers share several common goals. Here's how to collaborate effectively.

BY TRACY VENCE

While Nélida Leiva-Eriksson was wrapping up her PhD studies in biochemistry at Lund University in Sweden last year, her advisor suggested that she contact the school's communications department to discuss her work and future plans. The timing was right: she had just published a paper in *Plant & Cell Physiology* on the differential expression of hemoglobin genes during sugar beet (*Beta vulgaris* spp. *vulgaris*) growth. After defending her thesis, Leiva-Eriksson was fielding phone calls from journalists eager to learn more about her work. A university press release issued in November had captured international media attention.

"Now, after everything has calmed down, I think overall [the experience] was very good," says Leiva-Eriksson, who is now at the University of Essex in the U.K. "I got a lot of questions and interest in my work, which I hope will be reflected in resource allocation to this project."

For life-science researchers, speaking with the media is important to help broadcast their research findings and to ensure the accuracy of reports about their discoveries. More personally, accepting interviews with journalists can help scientists become recognized—by colleagues, journal editors, and funding agencies—as experts in their fields. (See "Why Trust a Reporter?" *The Scientist*, September 2010.) But the phone will never ring if no reporters are aware of the work.

When a researcher is genuinely excited about the work, there's usually something there that's pitchable.

—Mika Ono, The Scripps Research Institute

Enter university public information officers (PIOs). As important players in the dissemination of scientific knowledge, they mediate researcher-journalist relations and write and distribute press releases announcing new and noteworthy research happening at their institutions. Many PIOs were once scientists or journalists themselves, and this insider's perspective on both ends of the science-communication spectrum can help the two parties find common ground.

"In some cases there can really be a disconnect between scientific journal articles, which tend to be cautious, and media coverage, which can ride on sensationalism. Sometimes I've been a bit hesitant to speak with journalists because of that," says microbiome researcher Jennifer Fettweis of Virginia Commonwealth

University's Center for the Study of Biological Complexity. "University media-relations departments can help bridge that gap."

Speak up

Scientists outnumber PIOs at every research institution, making it impossible for PIOs to keep up with all the research happening in every lab. While some PIOs are able to periodically check in with the scientists on their beat to find out what they're working on, most often they rely on researchers to reach out when they have something new to share.

There can really be a disconnect between scientific journal articles, which tend to be cautious, and media coverage, which can ride on sensationalism. University media-relations departments can help bridge that gap.

—Jennifer Fettweis, Center for the Study of Biological Complexity, Virginia Commonwealth University

"The most common misconception [about the work of PIOs] is that we actually know what's being published and what's coming up," says Mika Ono, director of communications at Scripps Research Institute in La Jolla, California. "We don't really know. . . . We don't have some sort of central repository of information."

"The single largest obstacle is learning about research findings in a timely way," agrees Matt Shipman, a science writer and editor at North Carolina State University in Raleigh. But convincing researchers to take a proactive approach to sharing their work with a broad audience is not easy, he adds. "No one becomes a professor in order to talk to the public-relations guy; that's not what their focus is on."

Researchers may also be unsure which of their research studies warrants a call to the PIO. Thousands of biomedical research papers are published every day. To be sure, very few of them are newsworthy enough to alert the general media, and for a scientist who has pursued a line of research for several years, it can be difficult to recognize those that are. Journal name recognition can certainly help, and the publishers of *Nature* and *Science* have their own press offices that issue media advisories pertaining to some papers to be printed in their pages. For this reason, it's especially important that researchers also bring papers slated to appear in lesser-known journals to the attention of PIOs, as these are the studies that are more likely to be overlooked by the media.

Keeping up with the science sections of news publications is a great way to get a feel for the types of stories that are covered. Journalists and PIOs apply a variety of filters to determine the newsworthiness of a paper, patent, or other announcement. According to the PIOs *The Scientist* spoke with, applicability is the single biggest factor. Stories covering basic research can indirectly impact policy or public health, for example, or influence how other scientists approach their work. Biomedical work may directly influence clinical practice. "When communicating research you have to focus on the utility—implications or applications," says Earle Holland, who worked in research communications at Ohio State University for 35 years until his retirement in 2012.

"A great story about academic research . . . needs to be able to answer the question: 'So what?'" agrees A'ndrea Messer, a senior science and research information officer at Penn State University. "If it can't do that, it's not a story."

Of course, some stories aren't immediately applicable—they're just interesting, says Shipman. "Maybe it's not a big deal or particularly practical at this point, but if it's really freaking cool, I want to know about it," he says.

Another critical factor is timeliness. Unfortunately, scientists and journalists "have very different concepts of time," Shipman notes. While a paper published a few weeks ago is new in the realm of science, it is likely to be considered stale by reporters covering breaking news.

"In science it takes years to do these studies, and so it's a little hard sometimes to realize that, in the news business, if something has been online for two weeks, it's old news, and no one wants to write about it," says Ono, "even if it took you 10 years to do that paper."

Given the rapid pace of the news cycle, it's best to alert PIOs of upcoming papers before they are published—ideally, as soon as they're accepted. Operating under standard journal publication schedules, "you've got this perfect window between when the paper is accepted and published online," says Shipman. "I ask researchers to notify me as soon as a paper is accepted, and then we can work together to figure out if it's something we want to promote."

And if you're on the fence about a study's potential news impact, ask your PIO. "When a researcher is genuinely excited about the work, there's usually something there that's pitchable," says Ono.

Scope it out

At smaller research institutions, determining the proper PIO to contact is fairly straightforward—look for the person who covers science. Larger institutions tend to employ multiple science writers, so figuring out whom to connect with can require a bit more sleuthing. Rather than blindly e-mailing PIOs, Holland suggests that researchers consult their institution's own news coverage and scan the bylines. Is there one writer who consistently covers stories that align with your own work? He or she is probably your best bet.

Once you've identified the PIO you're likely to work with, send an introductory message explaining your line of research and the current projects going on in your lab. "This [initial exchange] may be an investment in the future," says Holland.

A PIO who takes interest in your research will have questions about it and will schedule a phone interview or a visit to your lab. Based on your conversation, he or she will draft a press release and ask you to review it. This proofreading step gives you a chance to check the facts and ensure that your science is accurately depicted.

"If there is anything in the release that makes the researcher uncomfortable at all, then we need to fix it," says Shipman. "It could be a choice of words or an overemphasis [on something] or a lack of recognition for coauthors. Anything at all—I want to know about it."

An oft-encountered stumbling point, says Messer, is finding common language. PIOs tend to discourage the use of scientific jargon, while researchers might push back on how complex topics are conveyed. "One of the biggest challenges is walking that line between accuracy and accessibility," says Ono. "There is usually a middle ground there; it's a matter of finding it."

Fettweis suggests keeping an open mind when working on the wording of a press release, and notes that it can take some "back-and-forth" to ensure that all parties are happy. "The language we use to communicate with [other] scientists isn't always the language we need to use to communicate with the public," says Fettweis.

"It's important to look at this as a partnership," says Holland. "Only when PIOs understand what research is all about and what the limitations are, and when researchers understand—at least to some extent—what's going on in communications" do meaningful stories emerge, he says. ■

Innovation Renovation

Is the fear of funding and doing fundamental, risky research killing our ability to make breakthroughs?

ROBERTA B. NESS

Sidney Farber is one of America's foremost scientific heroes. The story of this pediatric pathologist, who birthed chemotherapy, is a perfect illustration of a struggle that has become a hallmark of the modern research enterprise: creation vs. caution. In the late 1940s and 1950s, Farber discovered that folate antagonists could help treat certain childhood leukemias and lymphomas, overturning the existing reality that cancer always killed. Farber worked with minimal funding, doggedly pursuing his holy grail of curing leukemia and other pediatric cancers, despite colleagues' skepticism. But he often failed to obtain consent to test drugs that killed many patients and published only the subset of his data that showed the best results. Since then, the economics, sociology, and ethics of scientific research have taken a sizable cautionary turn. Indeed, Farber may not have succeeded in revolutionizing cancer treatment—with the unfortunate tolls paid on the road to the innovation—had he been working today.

Most science funders these days favor caution, seeking evolutionary (rather than revolutionary) advances that are quickly deliverable, tangible, and potentially marketable. The newest cancer drugs prolong life for a few months and make huge profits, but no tranformative treatments have been developed in a generation. Congress has put increasing pressure on funding agencies to attain quick wins. Cash-strapped academic institutions have accelerated this focus by turning more and more toward intellectual property–based revenue. Fundamental research, the engine of transformational progress, is in decline. This is the quandary I dissect in my newest book, *The Creativity Crisis: Reinventing Science to Unleash Possibility*.

Sociologically, science and its practitioners, too, are cautious. Science is hierarchical, insular, and slow to change. Thought leaders and policy makers are loath to disrupt norms based on partial evidence—a necessity, as new discoveries (think the atomic bomb) can harm people. But resistance to disruptive changes in existing paradigms also has less lofty motivations.

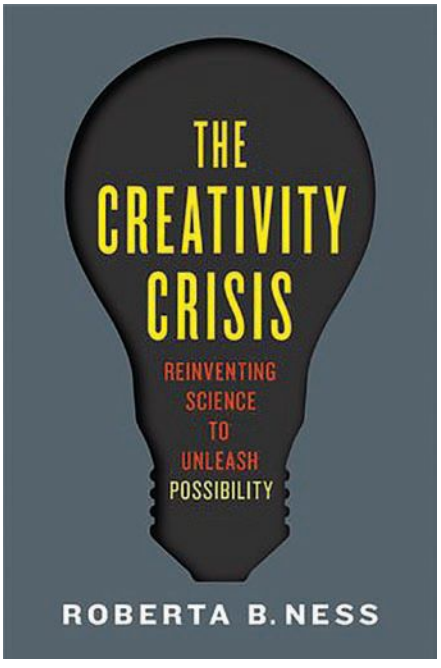
True innovation threatens top scientists who control research agendas—enduring elites who arise as "owners" of ideas. Farber's novel approach to fighting cancer has frightful limits: tumors become resistant and side effects can be life-threatening. Nonetheless, it took two generations for oncology to begin to wean itself from this all-out war wherein the patient sometimes fell victim to the very treatment intended for the tumor.

The newest sociologic revolution, crowdsourcing, is a supremely democratic, Web-based problem-solving tool, which has the power to quickly and efficiently harness group originality. But tradition-bound science has been slow to embrace this approach.

Ethics constitutes a final battlefield between creation and caution. The same scientific breakthrough can be used for good in the hands of one person and evil in the hands of another. Caution is a necessary check on the destructive potential of amoral creation. Yet, when every individual and institution is considered to be a threat, overregulation strangles novelty. Innovation requires risk; society's growing culture of risk aversion limits science's ability to make breakthroughs.

During my lectures to thousands of academic scientists about a systematic method that teaches innovative thinking, I have been repeatedly challenged by an alarming reality check: "Your innovative thinking tools are inspirational. But if I were to put forth really unusual ideas I would never succeed in my career," audience members have told me over and over again. Always voiced by young professionals, this remonstration represents their feeling of being chained by the resource-poor, hidebound, risk-averse environment of the research community. Realistically, the large organizations that employ most scientists must pay attention to their bottom lines, retain traditions, and have rules in order to survive. Science suffers from the ongoing creation vs. caution conflict.

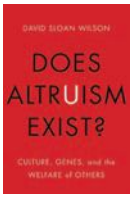
The Creativity Crisis offers concrete proposals that abandon convention and reach for more-optimized approaches to promoting innovation. My challenge to all is to confront the creativity crisis by reinventing our scientific ecosystem. ■



Oxford University Press, January 2015

Roberta B. Ness is vice president for innovation at the University of Texas Health Science Center at Houston and holds the M. David Low Chair in Public Health at the University of Texas School of Public Health. Read an excerpt from The Creativity Crisis *at www.the-scientist.com.*

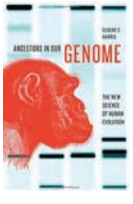
Does Altruism Exist? Culture, Genes, and the Welfare of Others
David Sloan Wilson
Yale University Press, January 2015



Is this the last nail in the selfish gene's coffin? I wouldn't toll the bell quite yet, but biologist David Sloan Wilson does make quite a compelling case for altruism in his latest book, *Does Altruism Exist?* The author, a longtime proponent of the concept of group selection, argues that the social phenomenon does indeed exist and constructs a solid theoretical foundation for how the selfless behavior evolved and operates in social animals, especially humans.

The classic example, eusocial insects, makes an appearance in Wilson's book, but his focus is on altruism as embodied by human behavior. As ever, evolutionary history provides the context for his argument that altruism is a real and adaptive trait, best categorized by the observation of action, not the interpretation of intention. Wilson surveys group-level functional organization—a state that arises from the evolution of altruism—in a variety of human institutions, such as religion and economics. In the book's closing chapters, he even offers a prescription for the salvation of the human race: a concerted effort to behave altruistically. "As far as our selection criteria are concerned," he writes, "we must become planetary altruists."

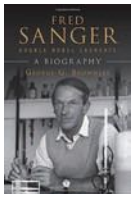
Ancestors in Our Genome: The New Science of Human Evolution
Eugene E. Harris
Oxford University Press, December 2014



After researchers sequenced the human genome in 2001, our closest living branch-mates on the tree of life were soon to follow: chimpanzees in 2005, macaques in 2007, orangutans in 2011, and bonobos and gorillas in 2012. And scientists continue to sequence other primate genomes. In *Ancestors in Our Genome*, molecular anthropologist Eugene Harris shares the most recent insights into human evolution that have attended the decoding of these genomes.

Although we know that humans and other great apes (bonobos, chimpanzees, gorillas, and orangutans) share a large percentage of DNA, there is also a clear split that signals our divergence from a common primate ancestor sometime in the mists of evolutionary history—around 5 million–7 million years ago, by current estimates. Going beyond the paleoanthropological approach that for decades dominated the study of human evolution, Harris employs population genomics to explore questions answerable with the flood of information regarding primate and hominin DNA. Though the book may be a tad rudimentary for the genomicists among us, it gives a good overview of the state of the science regarding the genomics of human evolution.

Fred Sanger—Double Nobel Laureate: A Biography
George G. Brownlee
Cambridge University Press, December 2014



With all the talk of CRISPR and next-gen sequencing, it's all too easy to forget that the modern genomic revolution started with one man: an unassuming British biochemist named Frederick Sanger. The first-ever full biography of the researcher, *Fred Sanger—Double Nobel Laureate*, comes at a time when the revolution Sanger launched is poised to break down yet another barrier and move into the realm of personal genomics. George Brownlee, who was Sanger's PhD student, relates the story of the famed researcher's life from humble Quaker beginnings in the English countryside to his retirement from science in 1983, after developing methods to sequence proteins, RNA, and DNA, netting two Nobel Prizes in the process.

A must-read for any student of science history, *Fred Sanger* is a reminder that transformative science often comes from modest people and serves as an homage to the man whose work launched a million sequencers before his death in 2013. Sanger sequencing may soon be a thing of the past, superseded by faster and more efficient methods, but Sanger's story and legacy, with the help of this book, endures.

Stiffs, Skulls & Skeletons: Medical Photography and Symbolism
Stanley B. Burns and Elizabeth A. Burns
Schiffer Publishing, Ltd., December 2014

The dawn of photography in the 19th century came at a time when science, too, was experiencing a transformational shift. Charles Darwin published *On the Origin of Species* a mere 20 years after French inventor Louis-Jacques-Mandé Daguerre displayed the very first photographs ever produced: daguerreotypes. Ever since, science and photography have marched hand in hand through technological advances and conceptual breakthroughs, each feeding the other. *Stiffs, Skulls & Skeletons*, a compilation of more than 400 rare medical photographs, is a celebration of the early days of this relationship.

Stanley Burns, physician and renowned medical photography historian, curates the collection, and provides commentary on the striking images. The book not only contains some early attempts at recording dissection, bone morphology, and X-ray imaging, it captures the Victorian era's fascination with dead bodies and body parts. A cultural, historical, and medical trove of photographic treasures, *Stiffs* would, for my money, make way better waiting-room reading than *Golf Digest*.

—Bob Grant

Live Cell Imaging – The Smart Way

Sized and priced for virtually any lab, the CytoSMART™ System is an easy-to-use, affordable live cell imaging technology. The innovative CytoSMART™ Cloud Functionality allows cell culture monitoring using your computer, smart phone or tablet. Monitor your cell culture anywhere anytime. With the CytoSMART™ System your cell culture results are just a click away.

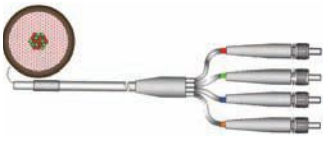
LONZA WALKERSVILLE, INC.
1-800-521-0390
scientific.support@lonza.com
www.lonza.com/cytosmart

Compact Flow Cytometer CytoFLEX

- Delivers high sensitivity and resolution for excellent fluorescence and nanoparticle detection
- Fits in a 16-inch square bench space and weighs just 50 pounds
- Offers up to 21 configurations, from basic setup to a multi-color, multi-laser sophisticated analyzer
- Patent-pending optical design optimizes excitation and light collection efficiency from up to three lasers (405, 488 and 638 nm)

BECKMAN COULTER
www.beckmancoulter.com

Multichannel Fiberoptic System Art Photonics FluoDuoFiber



- Modular platform of the system allows users to change LED and diode lasers for different excitation wavelengths—with correspondent filter changes in PMT channels
- Dual channel system and correspondent fiber probes can be also modified to triple or more channels and can be customized not only for its use in lab, but for a broad variety of another applications

ELECTRO OPTICAL COMPONENTS
www.eoc-inc.com

Protein Detection System for IHC SNAP i.d.® 2.0

- Streamlines immunohistochemistry workflows and significantly decreases slide handling time
- Enables parallel processing of up to 24 tissue slides at a time, reducing process variability
- Applies proven vacuum-driven technology to remove reagents from tissue sections, in seconds
- Intuitive format reduces slide handling and speeds wash steps during blocking, washing, antibody incubation and labeling

EMD MILLIPORE
www.emdmillipore.com

Microsampling Device Neoteryx Mitra (RUO)

- Designed for the collection, transport, storage, and analysis of biological fluids
- Consistently collects 10 µL of fluid in seconds while eliminating the volumetric blood hematocrit bias typically associated with the dried blood spotting technique
- Offered in two formats including an automation-friendly option for high-throughput environments
- Suited for non-experts and requires no specialized lab equipment

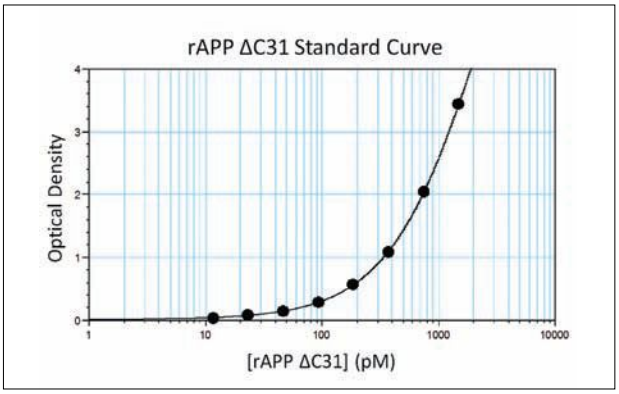
PHENOMENEX
www.phenomenex.com

Scanning Electron Microscope MultiSEM 505

- Features 61 beams working in parallel, and offers a capture speed of 1220 megapixels per second at a pixel size of 4 nm
- High acquisition speed is used for imaging neural tissue in brain research where it is now possible to observe much bigger samples than before
- Designed for continual operation and fitted with the intuitive ZEN Software

ZEISS
www.zeiss.com

ELISA Kit for Monitoring Neuronal Death and Alzheimer's Disease Progression



Enzo Life Sciences has added a new ELISA kit to its expanding range of assays for biomarker detection. This first-to-market, complete immunoassay kit allows the quantitation of APPΔC31 in cell lysate, cerebral spinal fluid (CSF) serum and plasma samples with results in less than two hours. APP ΔC31, is an important amyloid precursor protein fragment with a unique pro-apoptotic mechanism, produced after caspase-mediated cleavage that also releases the cytotoxic C31 fragment (AβPP-C31) after interaction with the amyloid-β protein (Aβ) peptide. C31 and APP ΔC31 modulate death signaling pathways leading to Alzheimer's disease (AD). This ELISA protocol utilizes a sandwich format and includes ready-to-use color-coded reagents and pre-coated microtiter plate with break-a-part strips.

One of the hallmarks of AD is the build-up in the brain of senile plaques of Aβ. Aβ is generated from β-amyloid precursor protein (APP) through sequential cleavages, first by β-secretase and then by γ-secretase complex. APP695 is one of the three major isoforms formed through alternative splicing of mRNA. Clevage of APP695 by caspase at Asp664 results in the release of a 31 amino acid C-terminal peptide (C31) from the remaining larger neo-APP fragment (APP ΔC31) with both of these entities being pro-apoptotic possibly through subsequent γ-secretase cleavage of APP ΔC31 resulting in the production of APP intracellular domain (AICD), another cytotoxic peptide. Recent studies highlight the importance of the APP664 cleavage event and the associated proteins in understanding progression of AD. The sensitivity and specificity of the assay was confirmed by low cross reactivity to isoforms of APP. The utility of the assay in drug screening application was confirmed with caspase inhibitors which reduced the production of APP ΔC31. This robust and sensitive assay for APP ΔC31 is well suited for AD drug screening applications as well as a powerful tool to investigate the role of Aβ in the activation of cellular pro-apoptotic pathways leading to neuronal death.

ENZO LIFE SCIENCES, INC.
10 Executive Blvd
Farmingdale, NY 11735 USA
Phone: Toll Free • Tel: 1-800-942-0430
Fax: 610-941-9252
E-mail: info-usa@enzolifesciences.com
Technical Service:
techserv-usa@enzolifesciences.com
www.enzolifesciences.com

Enzo Life Sciences

High Throughput Screening of Novel Protein Therapeutics

The high throughput expression facility at Molecular Partners (www.molecularpartners.com) has chosen the INTEGRA VIAFLO 96 multichannel pipette to help streamline the discovery and development of a novel class of targeted protein therapeutics termed DARPins.

Andreas Lehmann, an Expert Technical Assistant working in Molecular Partners 96-well high throughput expression facility commented "Our lab is extremely satisfied with our decision to purchase and incorporate INTEGRA's VIAFLO 96 into our development protocols. The main application we are using the system for currently is protein purification using IMAC (Immobilized Metal Affinity Chromatography). The VIAFLO 96 is used for various steps in the process starting from the preparation of bacterial cultures right through to the actual purification. Overall our protocol involves 63 full plate liquid transfers; such a workload would not be feasible with a traditional handheld multichannel pipette. We have found that the VIAFLO 96 is easy to integrate into our standard operating protocols because parameters can be defined: pipetting mode, volume, pipetting speed and pipetting height. We particularly like the repeat dispense function on the VIAFLO 96 as it saves the lab a lot of time and improves the overall reproducibility of tests as all samples processed at the same time in the same way."

Mr. Lehmann added "Being responsible for training of new staff, I also appreciate that the VIAFLO 96 is user friendly and intuitive to use because very little training is required."

The INTEGRA VIAFLO 96 is a handheld 96-channel electronic pipette that has struck a chord with scientists looking for fast, precise and easy simultaneous transfer of 96 samples from microplates without the cost of a fully automated system.

INTEGRA Biosciences Corporation
Hudson, NH 03051 USA
www.integra-biosciences.com

INTEGRA

How can you improve your experimental accuracy and traceability?

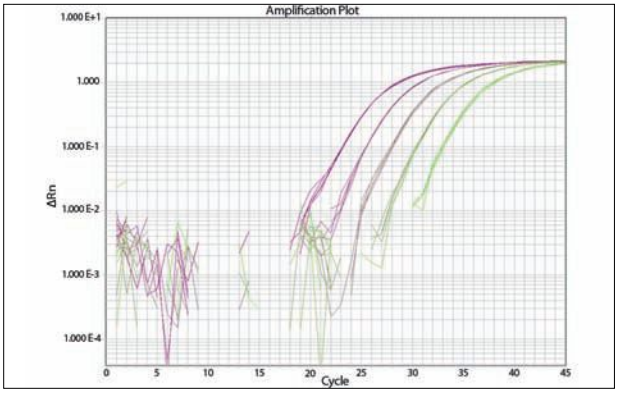


Figure: Standard cDNA samples serially diluted 5-fold and amplified in 5 replicates.

When preparing biological samples, you need a lab assistant you can trust, one that is focused on consistency and reproducible results across 10s and 100s of samples. Advancing your science and increasing the pace of your experiments is your job; being tied to the bench for routine pipetting tasks doesn't have to be! Free up your time for scientific analysis and publications, work with fewer technical replicates and improve experimental accuracy across a breadth of different applications:qPCR, NGS, cell toxicity assays, serial dilutions, and more.

qPCR assays
Numerous technical replicates are often included in the experimental design in order to compensate for user inconsistencies; however, that wastes samples, time and reagents. PIPETMAX® prepares every assay with proven consistency, delivering high precision and accuracy in routine pipetting tasks. The versatility of PIPETMAX allows you to run a multitude of different applications, providing you with an accurate, multi-tasking, experiment-driven assistant allowing you to save bench space and money. PIPETMAX supports many types of labware, devices, and samples with hardware and software built to meet your specific methods.

qPCR Assistant software carries out the qPCR method setup for you, reducing technical variability and eliminating sample cross-contamination... all at your fingertips on your PIPETMAX touch screen tablet!

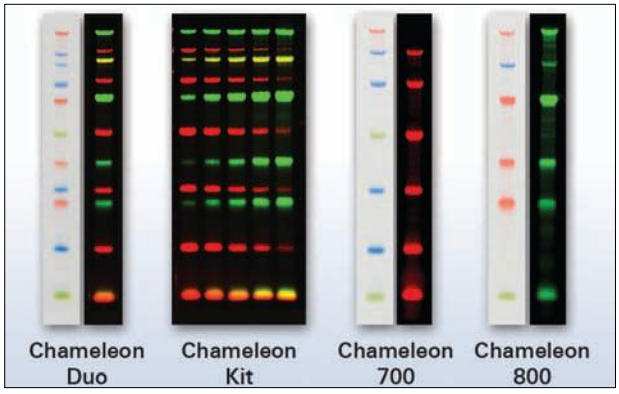
- Maximize qPCR sample prep accuracy and reproducibility
- Eliminate inherent variability
- Eliminate sources of contamination
- Increase data import/export consistency in seconds

Learn more about qPCR experimental accuracy:
www.pipetmax.com

GILSON, INC.
www.gilson.com
3000 Parmenter Street
Middleton, WI 53562
800-445-7661

GILSON®

Capture Sharp, Vibrant Colors in Your Western Applications



Capture the sharp, vibrant colors of Chameleon™ Protein Ladders in your Western blot applications.

These versatile protein markers offer multi-colored, pre-stained proteins for both visual inspection and two-color near-infrared detection.

- Recombinant proteins range from 8 kDa to 260 kDa for broad-range molecular weight determination.

Use the ladders for easy visualization of gel migration and protein size, and to orient your gel or membrane quickly.

From left to right in above image:

Chameleon Duo
Use for two-color near-infrared detection in a pre-mixed format.

Chameleon Kit
Use the kit components to customize fluorescence intensities for two-color near-infrared detection.

Chameleon 700
Use for one-channel analysis in the 700 channel.

Chameleon 800
Use for one-channel analysis in the 800 channel.

Choose your ladder at www.licor.com/choosemyladder

LI-COR BIOSCIENCES
4647 Superior Street
Lincoln, NE 68504, USA
Tel: 402-467-3576
www.licor.com/bio

LI-COR®

Cold Spring Harbor Laboratory
2015 Meetings & Courses

Meetings

Systems Biology: Global Regulation of Gene Expression
January 28 - February 1 abstracts due November 18

Cellular Dynamics & Models
March 3 - March 6 abstracts due January 9

Exercise Science & Health
March 9 - March 12 abstracts due January 9

Systems Biology: Networks
March 17 - March 21 abstracts due January 16

Wiring the Brain
March 24 - March 28 abstracts due January 23

Patenting in the Life Sciences: The Patentability of Self-Replicating Systems
March 30 - April 2

RNA & Oliogonucleotide Therapeutics
April 8 - April 11 abstracts due February 6

Fundamental Immunology and Its Therapeutic Potential
April 14 - April 18 abstracts due January 23

The Ubiquitin Family
April 21 - April 25 abstracts due January 30

Telomeres & Telomerase
April 28 - May 2 abstracts due February 6

Biology and Genomics of Social Insects
May 2 - May 5 abstracts due February 13

The Biology of Genomes
May 5 - May 9 abstracts due February 13

The Biology of Cancer: Microenvironment, Metastasis & Therapeutics
May 12 - May 16 abstracts due February 20

Retroviruses
May 18 - May 23 abstracts due February 27

80th Symposium: 21st Century Genetics - Genes at Work
May 26 - May 31 abstracts due March 6

The Evolution of Sequencing Technology: A Half-Century of Progress
July 16 - July 19

Metabolic Signaling and Disease: From Cell to Organism
August 11 - August 15 abstracts due May 22

Eukaryotic mRNA Processing
August 18 - August 22 abstracts due June 2

Mechanisms of Eukaryotic Transcription
August 25 - August 29 abstracts due June 5

Eukaryotic DNA Replication & Genome Maintenance
September 1 - September 5 abstracts due June 12

Microbial Pathogenesis and Host Response
September 8 - September 12 abstracts due June 19

Cell Death
September 15 - September 19 abstracts due June 26

Genome Engineering: The CRISPR/Cas Revolution
September 24 - September 27 abstracts due July 3

Neurobiology of *Drosophila*
September 29 - October 3 abstracts due July 10

Stem Cell Biology
October 7 - October 11 abstracts due July 17

Probabilistic Modeling in Genomics
October 14 - October 17 abstracts due July 24

Genome Informatics
October 28 - October 31 abstracts due August 14

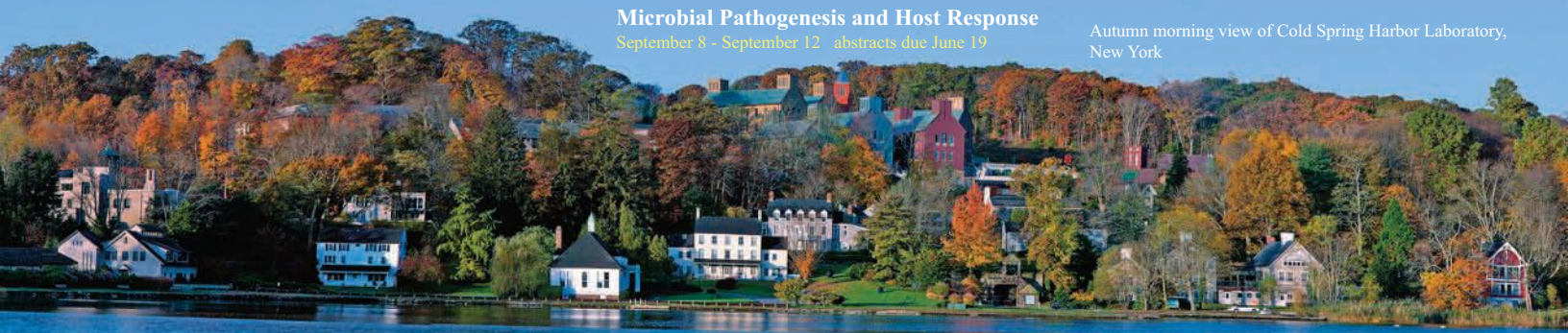
Cell Biology of Yeasts
November 3 - November 7 abstracts due August 21

Single Cell Analyses
November 11 - November 14 abstracts due August 28

Behavior & Neurogenetics of Nonhuman Primates
November 17 - November 20 abstracts due September 4

Plant Genomes & Biotechnology: From Genes to Networks
December 2 - December 5 abstracts due September 18

Rat Genomics & Models
December 9 - December 12 abstracts due September 25



Autumn morning view of Cold Spring Harbor Laboratory, New York

Courses

Workshop on Leadership in Bioscience
March 13 - March 16

Protein Purification & Characterization
April 8 - April 21

Quantitative Imaging: From Cells to Molecules
April 8 - April 21

Cell & Developmental Biology of *Xenopus*: Gene Discovery & Disease
April 9 - April 21

Single Cell Analysis
June 3 - June 16

Advanced Bacterial Genetics
June 3 - June 23

Ion Channels & Synaptic Transmission
June 3 - June 23

Mouse Development, Stem Cells & Cancer
June 3 - June 23

Workshop on Autism Spectrum Disorders
June 4 - June 10

Statistical Methods for Functional Genomics
June 18 - July 1

Workshop on Pancreatic Cancer
June 24 - June 30

***Drosophila* Neurobiology: Genes, Circuits & Behavior**
June 26 - July 16

Frontiers & Techniques in Plant Science
June 26 - July 16

Advanced Techniques in Molecular Neuroscience
June 30 - July 16

Vision: A Platform for Linking Circuits, Perception and Behavior
July 7 - July 20

Proteomics
July 14 - July 27

Eukaryotic Gene Expression
July 21 - August 10

Yeast Genetics & Genomics
July 21 - August 10

Imaging Structure & Function in the Nervous System
July 22 - August 10

Synthetic Biology
July 27 - August 10

Cellular Biology of Addiction
August 4 - August 10

Programming for Biology
October 12 - October 27

X-Ray Methods in Structural Biology
October 12 - October 27

Computational & Comparative Genomics
October 28 - November 3

Antibody Engineering & Phage Display
November 9 - November 22

Advanced Sequencing Technologies & Applications
November 10 - November 22

The Genome Access Course
March 30 - April 1 November 16 - November 18

www.cshl.edu/meetings

Join Keystone Symposia
for our first conference on:

Optogenetics

March 12–16, 2015
Westin Downtown, Denver, Colorado, USA

Scientific Organizers:
Edward S. Boyden, Klaus M. Hahn and Chandra Tucker

Optogenetics is a new and rapidly evolving field that combines light control with genetic engineering, allowing conditional regulation of signaling processes with seconds and microns precision. Join us to learn more about how this exciting technology can impact research in many different areas of biology.

The three-day conference aims to:

- Present the latest and most innovative genetically-encoded tools and technologies for manipulating biological systems with light;
- Promote exchange between those developing light-responsive probes and developers of related tools (e.g., biosensors, hardware) that will maximize the power of optogenetics;
- Foster the exchange of ideas between optogenetic tool developers and users.

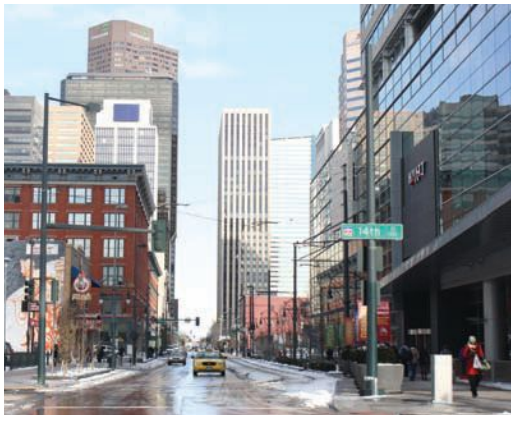
Session Topics:

- Optogenetic Engineering
- Structure and Genomic Diversity of Photoreceptor Proteins
- Optical Control of Biological Processes

CONFIRMED SPEAKERS
(as of December 10, 2014):

Steven G. Boxer
Edward Boyden
Mathieu Coppey
Valentina Emiliani
Viviana Gradinaru
Klaus M. Hahn
Xue Han
Peter Hegemann
Ehud Isacoff
Matthew J. Kennedy
Brian Kuhlman
Michael Z. Lin
J. Keith Moffat
Andreas Moglich
Denise J. Montell
Jared Toettcher
Roger Y. Tsien*
Chandra Tucker
Gane Ka-Shu Wong

**Keynote speaker*



*Discounted registration (saving $150 on the later registration fee) ends **January 13, 2015**. Abstracts can also still be submitted online through this date for publication in the meeting abstract book.*

To see the full program and for additional details, visit **www.keystonesymposia.org/15C5**.

KEYSTONE SYMPOSIA™
on Molecular and Cellular Biology
Accelerating Life Science Discovery

PO Box 1630 | Silverthorne, CO 80498 | 1.800.253.0685 | 1.970.262.1230

Genome Editing Applications

Therapeutic and Biomedical Applications of CRISPRs, ZFNs, TALENs, AAVs and other Genome Engineering Technologies

March 18-19, 2015 · Hilton Boston Back Bay · Boston, MA

Keynote Presentations

Development and Applications of CRISPR-Cas9 for Genome Editing

Feng Zhang, Ph.D.
Investigator, McGovern Institute for Brain Research and Core Faculty Member, **The Broad Institute of MIT and Harvard**

Radical Genome Editing

George Church, Ph.D.
Robert Winthrop Professor of Genetics, **Harvard Medical School** and **MIT Health Sciences and Technology**

Accelerate Your Therapeutics to Market and Improve Drug Discovery Efficiencies

Learn about therapeutic applications of genome editing
in cardiovascular disease, gene therapy, stem cell therapy, CART cell immunotherapy, hemophilia B, Parkinson's disease and other advances in in vivo genome editing

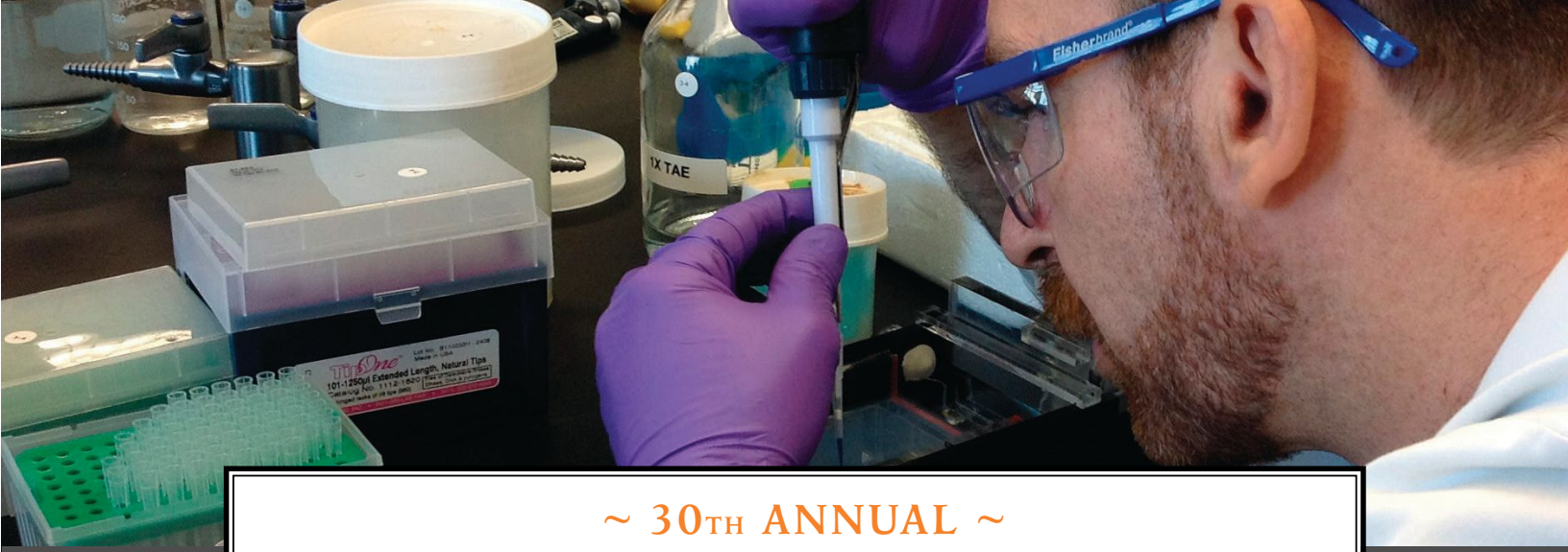
Receive an in-depth update on the latest technology advances and applications
of CRISPR-Cas9 including a discussion of off-target issues, delivery to cells/tissues, improving specificity and multi-genic alterations

Apply genome editing as a research and drug discovery tool
in target identification and validation, genome-scale screening, gene targeting, cell line validation and transgenic animal models

Compare the advantages and disadvantages
of CRISPRs, ZFNs, and TALENs for various applications and discover ways to navigate the IP landscape of genome editing technologies

The Scientist Readers Register Now for 20% off the Standard Rates with Code D15223SCIAD

www.IBCLifeSciences.com/GenomeEditing



~ 30TH ANNUAL ~
MOLECULAR BIOLOGY SUMMER WORKSHOP

Learn Molecular Biology in 2 Weeks!

We are pleased to announce the thirtieth annual Molecular Biology Summer Workshop, sponsored by New England Biolabs in conjunction with Smith College. The workshop is held at Ford Hall, Smith College, Northampton, MA, USA. Well over 4,000 people have graduated from this intensive training program in the past twenty-nine years. This intensive course emphasizes hands-on molecular biology laboratory work and covers a wide variety of topics and techniques. This is the largest and longest running molecular biology course for professionals in the world. No prior experience in molecular biology is required.

when:

two-week session:
July 19 -August 1, 2015

where:

Ford Hall
Smith College
Northampton, MA 01063
USA

Dr. Steven A. Williams, Director

to apply:

website:
http://www.science.smith.edu/neb

email:
biolabs@smith.edu

phone:
(413) 585-3915

Two-week Molecular Biology Session Available

This two-week long course covers in-depth DNA cloning, PCR, DNA sequencing, genomics, next-gen sequencing and bioinformatics. Learn hands-on techniques used in gene expression analysis including microarray analysis, RNAi and quantitative RT-PCR, bioinformatics and genomics and proteomics.

application information: No previous experience in molecular biology is required or expected. Forty participants will be selected from a variety of disciplines and academic backgrounds, including principal investigators, directors of programs, medical doctors, postdoctoral fellows, graduate students, research assistants, sales associates, equipment engineers, etc.

fee: $4300 per participant. This fee includes lab manual, use of all equipment and supplies, and room and board (all rooms are singles).

application deadlines: July 1, 2015.
First come, first served (apply now!). Late applications will be accepted on a space available basis.

payment deadline: Three weeks following receipt of your email acceptance letter.

topics / techniques:

- gene cloning (and library construction)
- gene expression analysis
- PCR and quantitative RT-PCR
- genomics and bioinformatics
- DNA sequencing, including NextGen
- RNAi and siRNA
- RNA-Seq
- and much more — visit our website for a complete list!

NEW ENGLAND BioLabs® Inc.

www.neb.com

The Sex Parts of Plants, 1736

BY KERRY GRENS

Carl Linnaeus's lasting legacy, hands down, is binomial nomenclature. He transformed what was once a clunky system for naming organisms, involving a formal name and a lengthy description, into a simple, two-part title. Such efficiency was wildly popular among taxonomists in the 18th century, and binomial nomenclature has withstood centuries of scientific progress toward understanding the relationships among organisms.

But in the 1730s, the self-proclaimed "prince of botany" made a contribution to taxonomy that, at the time, was just as profound as any of his other achievements. After realizing that floral sex parts varied in number, Linnaeus developed a plant classification system based on their sexual anatomy. The number of stamens (which produce male gametes), their length, and whether the stamens were fused relegated a plant to one of 23 Classes (there was a 24th class, nonflowering plants); the plant's Order was then determined by pistils, the female structures. "I think it was quite enthusiastically taken up because of its simplicity," says Charlie Jarvis, an expert on Linnaeus's botanical nomenclature at the Natural History Museum in London.

Linnaeus's focus on the arrangement of plants' sexual anatomy afforded him the opportunity to make salacious puns, and he took it, referring to stamens as husbands, pistils as wives, and their arrangement as a marriage. The "bridal bed" became a rather crowded place for certain plants, and more sensitive botanists took offense at such indecent descriptions. Although Linnaeus took criticism poorly, "he didn't care" to tone down the language, says James Reveal, a plant systematist at Cornell University.

Linnaeus's plant classification system streamlined a complicated process, and botanists adopted it with zeal, especially in England. But after about a half century, taxonomists began to pick it apart, says Reveal, and by 1830 it was laid to rest for good. The fatal flaw was that the system didn't represent true relatedness between taxa. Legumes, for instance, were split into two groups: those with 10 free stamens were placed in Decandria (Class 10;K in the illustration), while others went into Diadelphia (Class 17 or R) because nine of those stamens are fused into a single tube. However, it was apparent to botanists even then that legumes are more closely related and that the system did not represent natural relationships. "Linnaeus understood this," says Jarvis. "He saw it as a means to an end, but one that in its time was very useful."

The sexual organization system was eventually replaced by more natural systems of classification. In 2007, Birgitta Bremer of the Royal Swedish Academy of Sciences and Stockholm University looked at how well Linnaeus's sexual classification system overlapped with today's most commonly used system, the Angiosperm Phylogeny Group (APG), which is based on genetic analyses. Of Linnaeus's 23 Classes of flowering plants, 22 include unrelated plants according to the APG. Only his Class 15, Tetradynamia (Class P), which includes broccoli, mustard, and cabbage and whose flowers have six stamens, four long and two short, contains plants that all belong to the same family under the APG system, the Brassicaceae. Bremer wrote in her study that "there is little correspondence between the sexual system and the APG-system." But, she added, "this does not mean that the sexual system has been useless or misleading." Nothing else came close to the simplicity Linnaeus introduced to classification. ■

FLORAL SEXUAL ANATOMY: Georg Dionysius Ehret, one of the most esteemed botanical illustrators of the 18th century, drew this example of Carl Linnaeus's plant classification system based on the sexual parts of flowers. Ehret had been working for George Clifford, a wealthy banker, when he met Linnaeus in the mid-1730s. Clifford had hired Ehret to do some artwork based on the plant collection in his gardens and greenhouses, and during this time, in 1736, Ehret also painted this iconic image of floral sex parts (assigning letters to Linnaeus's numbered classes). Charlie Jarvis of the Natural History Museum in London says there was some dispute between Linnaeus and Ehret as to who invented the classification system, but ultimately Linnaeus earned the credit.

WIKIPEDIA

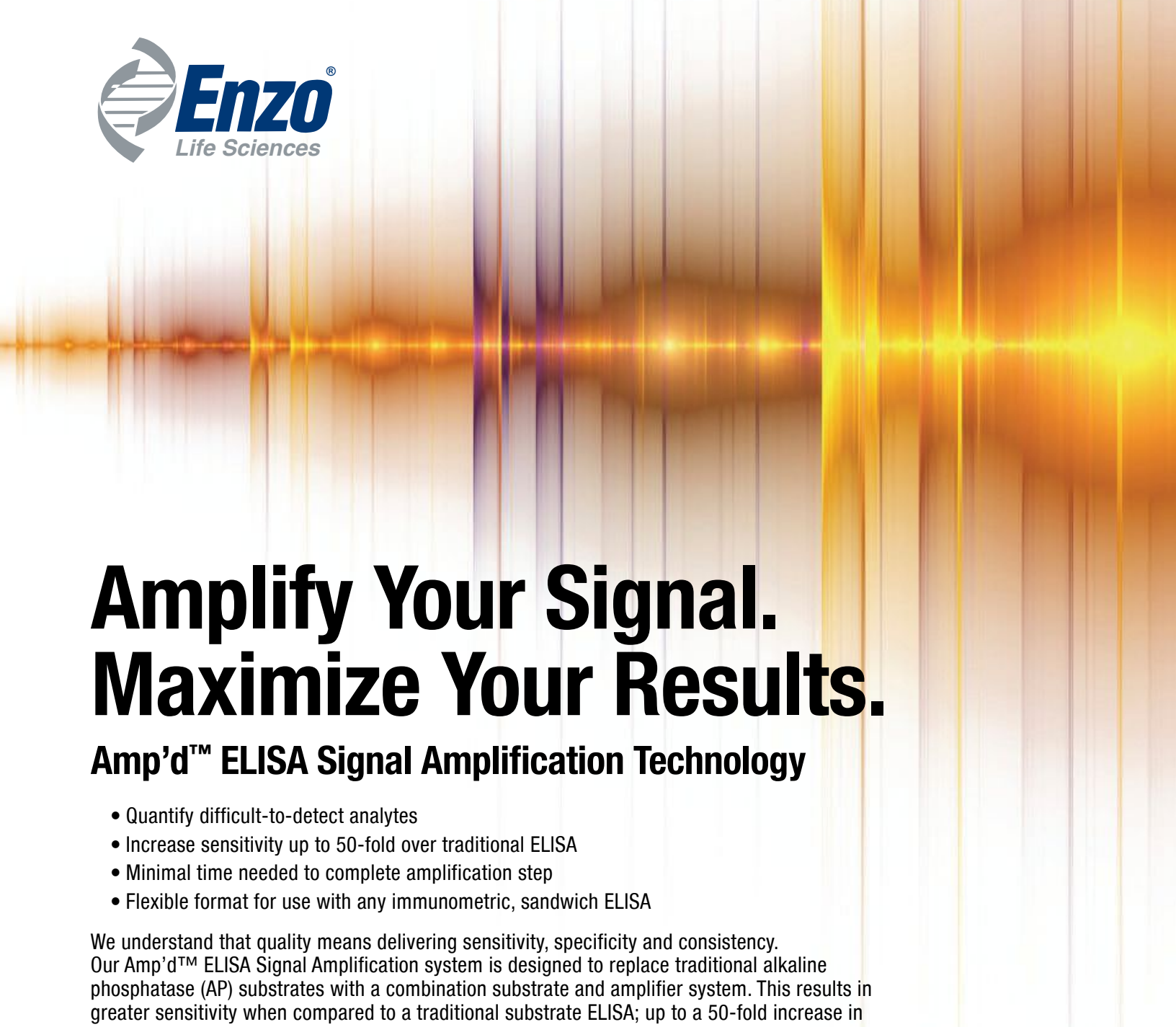
EMD MILLIPORE

Take Control
with dynamic cell culture.

Biology is so much more than DMEM/FBS, 37 °C, 5% CO_2.
It's easy to program automated changes to culture media, gas and temperature, while tracking cell responses, with the flexible, intuitive CellASIC® ONIX Microfluidic Platform. By taking control of this truly *in vivo*-like environment, you'll be able to perform dynamic, time-lapse experiments never before possible.

Watch live cells respond in real time:
www.emdmillipore.com/CellASIC

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany



Amplify Your Signal. Maximize Your Results.

Amp'd™ ELISA Signal Amplification Technology

- Quantify difficult-to-detect analytes
- Increase sensitivity up to 50-fold over traditional ELISA
- Minimal time needed to complete amplification step
- Flexible format for use with any immunometric, sandwich ELISA

We understand that quality means delivering sensitivity, specificity and consistency. Our Amp'd™ ELISA Signal Amplification system is designed to replace traditional alkaline phosphatase (AP) substrates with a combination substrate and amplifier system. This results in greater sensitivity when compared to a traditional substrate ELISA; up to a 50-fold increase in sensitivity over traditional ELISAs while detecting lower concentrations of target in samples.

Amplify your research at:
www.enzoifesciences.com/ampd

Higher Sensitivity | Novel Technology | Optimal Results

scientists ***enabling*** scientists.™

www.enzolifesciences.com