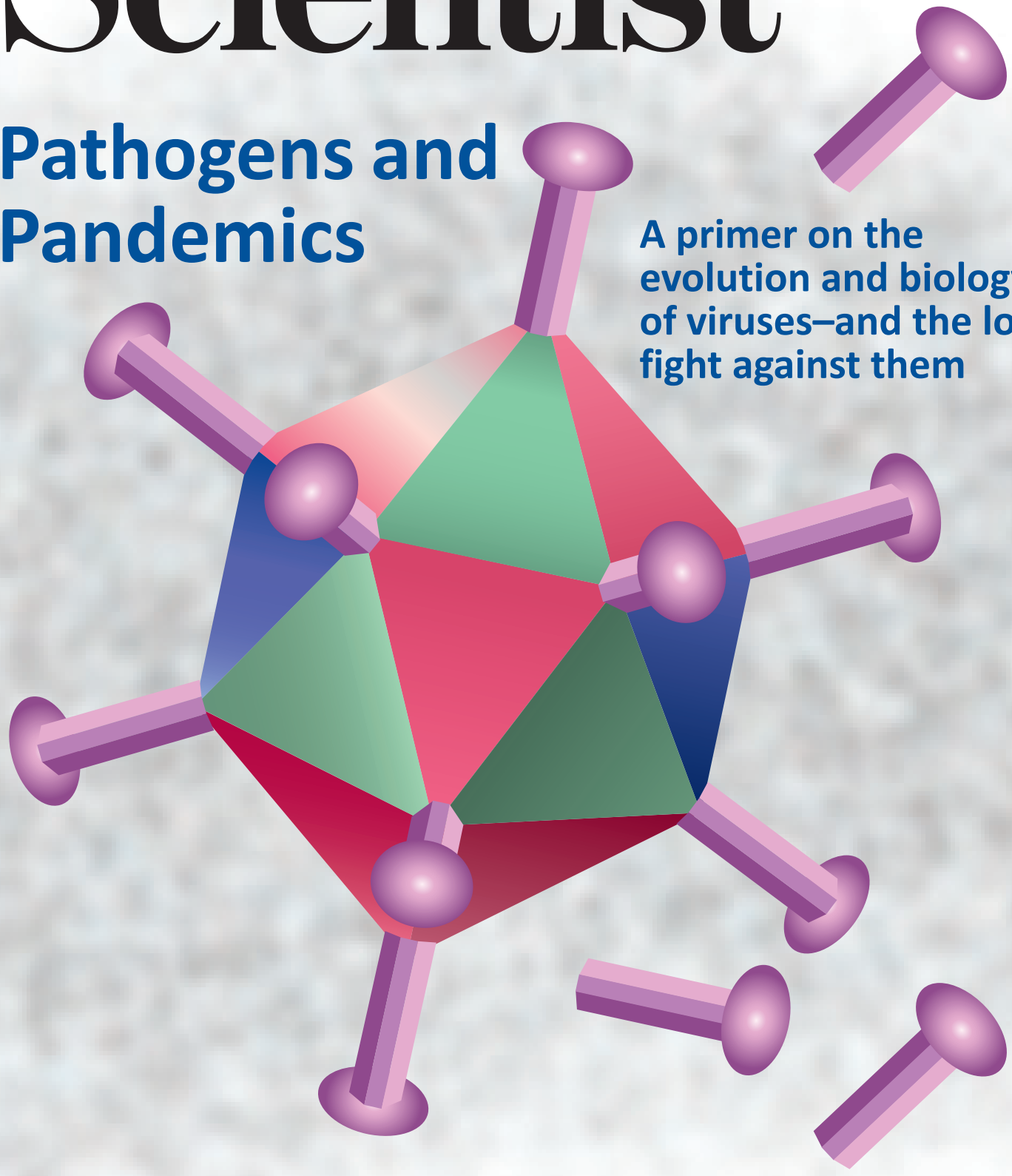
SPECIAL COLLECTION

SARS-CoV-2: The Latest Addition to a Long-Running Cast of Viral Characters

AMERICAN Scientist

Pathogens and Pandemics

A primer on the evolution and biology of viruses–and the long fight against them



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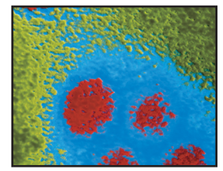

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Introductory Letter

Fenella Saunders, Editor-in-Chief

Illustration Credits

Tom Dunne: cover pages 36–40; 44–48
Emma Skurnick: pages 29–32

Welcome to *American Scientist*!

Thank you for supporting science communication with your subscription to *American Scientist*. In times when access to reliable science information holds particularly great importance for personal and public health, your support allows the magazine to bring you cutting-edge reports directly from experts on the front lines of research. Articles in *American Scientist* are written by scientists and engineers about their own peer-reviewed research, tailored to provide readers with an understandable and enjoyable account not only of the authors' work, but also of their research journeys.

As a subscriber, you have access to digital online versions of past issues of *American Scientist* on our website. PDFs of past articles are available to subscribers at a reduced rate (and are free to download for Sigma Xi members). *American Scientist* celebrated its centennial year of publication in 2012, so there's a long history of past articles to draw on.

For new subscribers, it can be daunting knowing where to start in such a vast library. With that in mind, we've put together a core collection of articles relating to emerging diseases and virus research, which are particularly relevant to the current pandemic surrounding the SARS-CoV-2 virus and COVID-19. These articles have been selected to provide a good background about virology, epidemiology, and infectious disease monitoring. We've dug back into the archive a bit, to give you a sense of the history of how scientists have been watching for emerging diseases over the past few decades. The original publication date is indicated on the first page of each article.

If you are looking for additional information, *American Scientist* has blogs that are being updated with new articles about SARS-CoV-2 and COVID-19. We've also put together a blog that links to additional past articles and multimedia content related to virology and pandemics, more than could fit into this collection. Go to our website at www.americanscientist.org for this information.

We hope that you find this collection useful and informative, and we hope that you will continue to be a subscriber to *American Scientist* for years to come!

Fenella Saunders
Editor-in-Chief

What Might Happen to COVID-19 Over Time?

The novel coronavirus is unlikely to go away completely after its first outbreak. People are only beginning to grapple with what comes next.

To deal with the global pandemic of a novel coronavirus, people all over the world have scrambled to enact social distancing so as to reduce the speed with which the virus is spreading—key to reducing the strain on health care systems. But even as different countries have met that threat with varying degrees of success, they need to prepare for the aftermath. There is a real possibility that this outbreak is not the last COVID-19 curve we'll need to flatten.

Epidemiologist Stephen Kissler of Harvard University and his coauthors published a paper in *Science* on April 14 that explores potential scenarios. "There's a good chance that we'll see some further waves of infection following this first one," he says. Two crucial variables will affect the timing and number of resurgences: how well the virus is transmitted during warmer months, and how long immunity lasts in people who have recovered from the viral infection.

"It's unlikely that the outbreak will just die out over the summer, as some have suggested," Kissler says. His team's study indicates that *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2, the virus causing coronavirus disease 2019, or COVID-19) can spread at any time of year because there are so many susceptible people. However, if the virus becomes less transmissible over the summer months—which has been the case for some other coronaviruses and the flu—that could help reduce the rate of infection. For ongoing or emerging outbreaks, delaying the spread as the weather warms will be a boon.

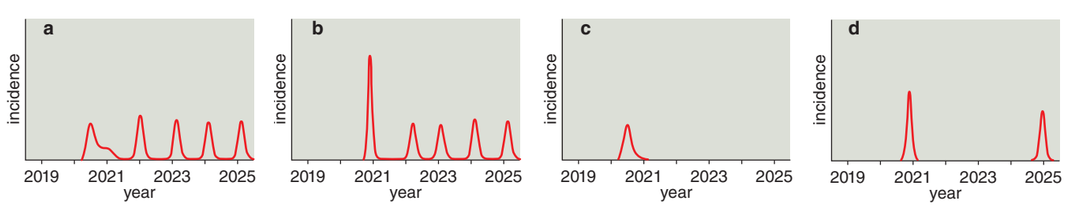
How long immunity lasts will strongly affect when we might see a resurgence in infections—from months to years after the end of the current surge. If immunity is short-lived, as is the case for seasonal flu and colds caused by other coronaviruses, then there could be another wave of SARS-CoV-2 infection in the fall. The previous influenza pandemic—the swine flu H1N1 in 2009—had a second wave of infection after a winter-spring outbreak, Kissler points out. "There was still transmission over the summer, but it was lower. Then we saw a resurgence in the autumn," he explains. Regarding SARS-CoV-2, he says, "We may have to do these social distancing measures again in the fall as the transmissibility rises again."

If immunity lasts years, as was the case for the SARS-CoV-1 outbreak in 2003, or if there is some cross-immunity between this novel virus and other related coronaviruses already circulating within the general population, then it could be two or more years before a resurgence occurs. By then, there may be effective treatments or vaccines in place. In general, though, immunity tends to last longer when viruses incite a stronger immune response. Considering that people can have SARS-CoV-2 without symptoms, the extended-immunity scenario seems unlikely.

Kissler and his coauthors show that if immunity to SARS-CoV-2 is not permanent, it will probably enter into regular circulation among humans. That could mean people will need to deal with one or more resurgences over the next year, or that this disease could become another seasonal disease like the flu and various common colds.

Epidemiologists, including Kissler and his team, are actively studying the length of immunity to and the seasonality of SARS-CoV-2. The progression of COVID-19 infections over the summer will reveal the virus's seasonality. Epidemiologists will need more time to determine how quickly immunity wanes. "We're still months to even a year away from knowing for sure how the immunity is going to look," Kissler says. In the meantime, epidemiologists will monitor viral outbreaks, to home in on remaining chains of transmission of SARS-CoV-2. Deciding where to implement testing for infection continues to be a pressing issue.

"These outbreaks can spread so explosively," Kissler notes. That's why social distancing has been important, and will continue to be so in any upcoming outbreaks. In the longer term, we will need to constantly adjust to new information and prepare to change our behavior permanently. "This virus may be with us for a very long time," Kissler says, "and we need to think about what we can do to structure our own lives and even our society for the reality of this and of whatever other outbreaks come our way in the future."—*Katie L. Burke*



Modeled scenarios of novel coronavirus outbreaks suggest the possibility of resurgence. The selected scenarios are (*a*) no cross-immunity with other coronaviruses, 40-week immunity to SARS-CoV-2, and establishment of sustained transmission in late March; (*b*) no cross-immunity, 40-week immunity, and establishment in early September; (*c*) no cross-immunity, permanent immunity, and establishment in late March; and (*d*) some cross-immunity, two-year immunity, and establishment in mid-August. (Adapted from Kissler, S. M., et al. 2020.)

Extinction Spillover

Mammalian carnivore declines can affect public health.

When the American black-footed ferret nearly went extinct in the 1970s, it did not go down alone. Largely unnoticed, the parasites that live off the ferret found themselves deprived of a key host. A breeding program rebuilt the wild population of ferrets to about 1,000, but one of its parasites—a louse—may not have survived the ferret's near extinction. Parasites on an endangered mammal generally are considered a threat to the host's health. However, Nyeema Harris, a postdoc at the University of California at Berkeley, says fewer parasite species may not be good in the grand scheme and could even pose a health threat.

Mammals, particularly carnivores, share parasites with people: Nearly one-third of the diseases hosted by North American carnivores are *zoonotic*, meaning they infect both humans and wildlife. Carnivores host more than half of the 125 emerging zoonotic diseases, according to a 2001 study led by University of Edinburgh's Sarah Cleaveland.

Although loss of a parasite that infects humans could be beneficial, Harris knew that the loss of one disease decreases overall disease diversity, which influences likelihood of transmission to humans. A parasite may respond to a declining host in three ways, Harris notes: It might (1) go extinct with the host, (2) adapt to a completely novel host, or (3) begin feeding on a more abundant alternate host. Parasites that are highly specialized to a particular host are prone to extinction, giving an advantage to generalists that can hitch rides on various species. As a result, the loss of one host species could change parasite distributions.

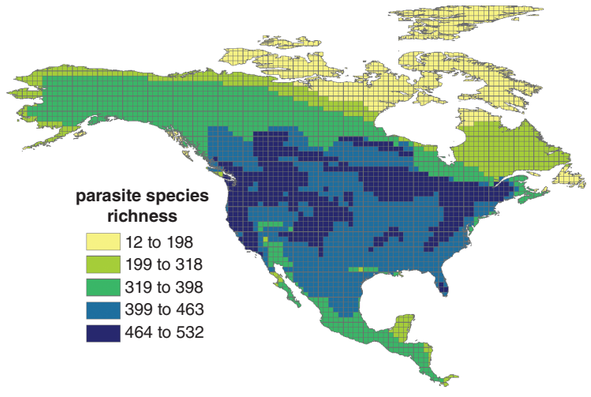
Such change could present a new risk for people. "Certainly for zoonotic diseases, where both humans and wildlife species are potential hosts, the loss of wildlife hosts could be particularly problematic," Harris says, "because the parasites exploiting animal resources may be forced to increase use of humans." If the number of wild hosts declines, the parasites (and diseases they transmit) can end up in humans instead. For instance, research has shown that the incidence of Lyme disease is higher in places with fewer wild species that host ticks.

In a study recently published in the *Proceedings of the Royal Society B* with Rob Dunn of North Carolina State University, Harris mapped the distribution of 29 species of carnivores in North America, with more than 1,300 parasite species. Harris used a broad interpretation of the term *parasite*, including those seen with the naked eye, such as intestinal worms, and those not, such as bacteria. She excluded parasites outside the body, such as lice and ticks, because they vector but do not cause zoonotic disease. Harris wanted to know how patterns of zoonotic disease could change if some carnivores went extinct, so she simulated random species losses across the map. In their model, parasites never evolved to infect a novel host, so they remained only if they could infect another included carnivore. Harris and Dunn's study is a step toward a broader simulation of more complex host–parasite dynamics, including parasites hosted by noncarnivorous species.

In the model, the proportion of diseases that were zoonotic increased as more carnivores were lost overall. Moreover, the local distributions of zoonotic disease, as well as the extent of human exposure, varied substantially, depending on which carnivores remained. Areas that lost wide-ranging carnivores such as red foxes and coyotes experienced a decreased diversity of zoonotic disease. Such decrease is associated with greater risk of transmission to people, because the zoonotic diseases that are left—like the parasites that survive when their preferred hosts die out—tend to be the generalists that can find another, more available host. Although red foxes and coyotes are not threatened, the findings suggest that local losses of carnivores, which commonly occur, may increase zoonotic disease risk in humans.

Although losing wide-ranging carnivores affects zoonotic parasite distribution in the model, the loss of carnivores of conservation concern—such as the gray wolf—does not. These carnivores have more limited distribution and tend to host fewer specialist parasites and parasite species overall. Because the loss of specialists increases generalist zoonotic parasites, more limited carnivores do not strongly affect zoonotic disease. No one knows whether the diversity of parasites, particularly specialists, has declined in the carnivore species that have become rarer. If it has, then those endangered creatures could affect zoonotic disease risk not shown in the model.

"If you are a parasite, or any organism consuming or relying on another organism, you want to associate with the most abundant resource in the landscape," Harris says. Unfortunately, the mammals that are most abundant in the landscape are often livestock, pets—or humans. —*Katie L. Burke*



In Harris and Dunn's model, as carnivores are lost from grid cells on the map, so are their parasites. Loss of wide-ranging carnivores causes declines in local parasite diversity. The map shows parasite species richness with all 29 carnivores present. (Image from N. Harris and R. Dunn, *Ecology Letters* 13:1411.)

First Person: Anna Marie Skalka

Recognized internationally for her contributions to the field of virology, Anna Marie Skalka has conducted research to understand viruses' many functions—both harmful and helpful—and has described their evolutionary role in our species. She is recipient of the 2018 Sigma Xi William Procter Prize for Scientific Achievement, an annual lifetime achievement award established in 1950 by an heir of one of the founders of the Procter and Gamble Company. Previous awardees include engineer Vannevar Bush, physicist Leon Lederman, and behaviorist Jane Goodall, among many others. American Scientist*'s digital managing editor, Robert Frederick, spoke with Skalka about her work and big data's emerging role in the field of virology.*

Over your career, you've seen a lot of changes to the field of virology. Have there been any surprises?

Oh yes! When the first human genome was made available in the early 2000s, I was very surprised by the fact that 8 percent of our genome is actually retroviral sequences—that's really a shocking number. That's more genetic information than is contained in all of the exons that encode all the proteins in our bodies, which is only about 1 percent of our genome.

In some cases these retroviral sequences have been co-opted for our benefit. They've been conserved. One of the most striking examples is in the formation of the mammalian placenta. I don't know how many people know that the formation of the placenta depends on a retroviral protein. It's the protein that makes the envelope of the virus and it's been co-opted in human genetics to make the placenta. If we didn't have that protein, we might be laying eggs, like chickens.

Some of the tools that have become available during your career include all the ways science is being done with computers. How have you seen for yourself computer tools being employed in virology?

I went for a sabbatical at the Institute for Advanced Study in Princeton, to a department called the Simons Center for Systems Biology, which is chaired by Arnie Levine. His idea was to put biologists together with people who did bioinformatics. I worked with Vladimir Belyi, who is a genomics person, but a physicist, really. Together we decided to look to see whether there were any other viral genes in vertebrate DNAs.

What we did was to take all of the vertebrate sequences that were known at the time, in 2009, so there were about 48 vertebrate sequences of various species known. We chose to look first for viral genes from viruses that have RNA genomes but are not retroviruses. There are 16 different families of these. So Vladimir ran all of their genomes against all of the vertebrate genomes, and what we found was really astounding: There were, I think, something like 80 genes of these various viruses, in the sequences of about 16 of these vertebrate species.

The most interesting part is that there are 16 RNA viruses, but the genes were only from two viral families: They were filoviruses (the genus that includes Ebola virus and Marburg virus), and Bornaviruses, which include Borna disease virus. Then, when we looked at phylogenetic trees, we discovered that these viral sequences were integrated something like 40 million years ago, most of them. And more surprisingly, some of these sequences were conserved throughout evolution. And the proteins were actually made in some of the species that have these genes in them.

What is it about these two viral families—with Ebola and Borna—that makes them so special that their genes ended up in vertebrates?

They're both very highly pathogenic. We all know about Ebola virus, which causes a terrible hemorrhagic disease. I think the fatality rate is 50 to 90 percent. Borna virus is equally fatal for the species it infects—horses, cloven-hoofed animals, some dogs, and so forth. There it's 80 percent fatal. So we decided maybe this conservation has something to do with the pathology.

Well, we found a very interesting correlation: Those species that were natural hosts to these Borna viruses, none of them contained any genes from these viruses in their DNA. But those species that were not natural hosts contain the genes. And then we thought, "Maybe these genes are some sort of genetic immunization against infection with the virus." That was just an idea that we put out at the time, but since then it has actually been verified for squirrels. Now, the squirrels have a Borna virus disease gene in them, put in there something like 10 million years ago, so it's pretty new. It turns out that if you express that gene in cells and then infect them with Borna virus, the Borna virus will not grow.

It's really been fascinating. Actually, I think it contributed to a whole new field called *paleovirology*. There's even a journal now for it. And it was a real acknowledgement that, really, viruses are us. We are viruses walking around, for better or for worse.

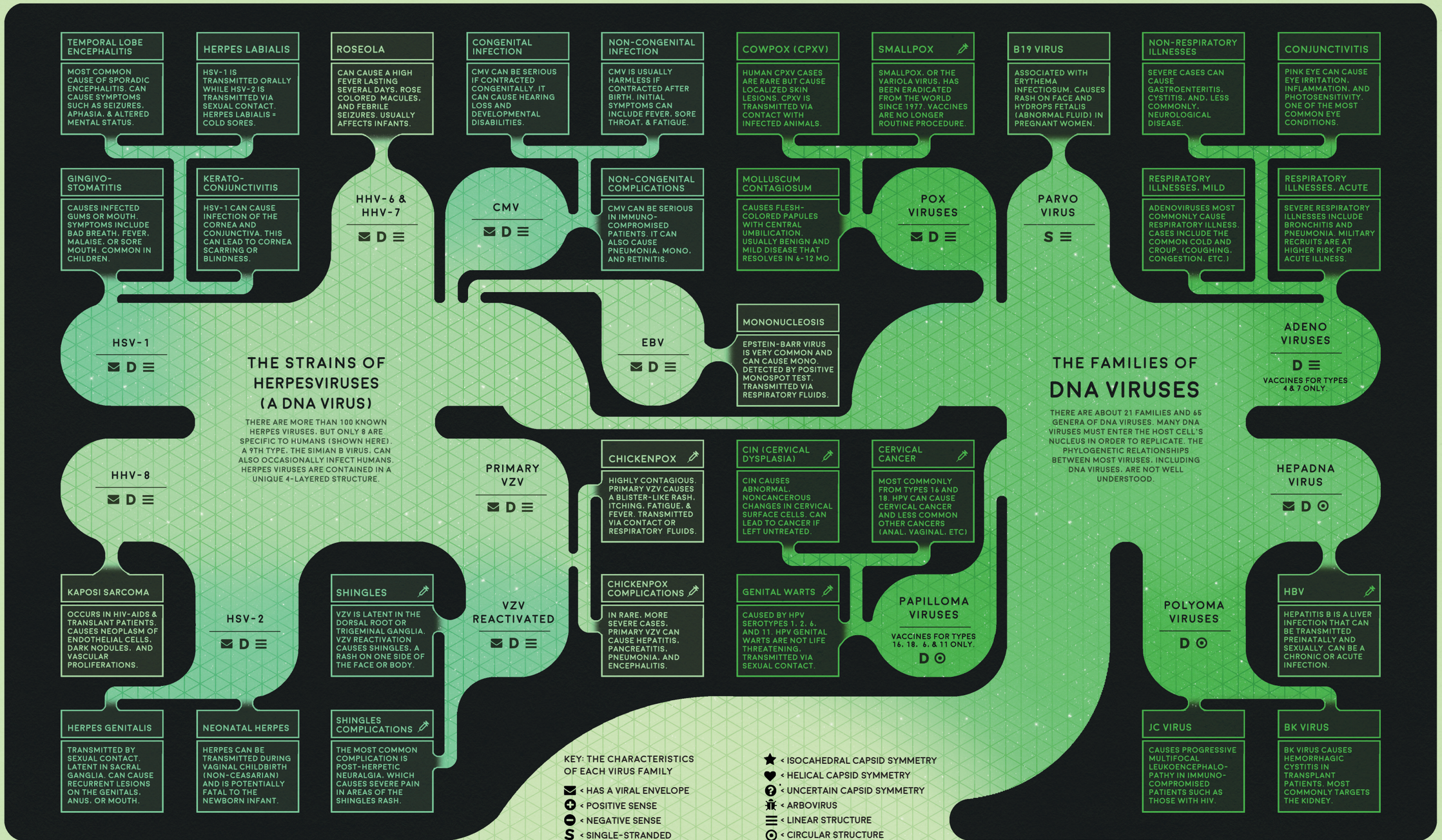
So by trying to defeat some viruses we could be unintentionally—but potentially—hurting human health?

Well, yes. Because viruses, they have two facets. Some of them are bad and some of them are good. And this genetic exchange, of which they are mediators, is important to evolution. And you can see that some of the genes that are useful are conserved. And I think if you didn't have this exchange, you would put a damper on some beneficial aspects of evolution.

A podcast based on the full interview is available online.

VIRUSES AND VACCINES:

A Basic Flowchart of Viral Families



TYPES OF VIRAL VACCINES

VIRAL VACCINES INCLUDE LIVE ATTENUATED VACCINES, KILLED VIRUSES (RABIES, INFLUENZA, POLIO, AND HEPATITIS A), AND SUBUNIT VACCINES (HBV AND HPV).
LIVE ATTENUATED VACCINES ARE DANGEROUS TO GIVE TO IMMUNOCOMPROMISED PATIENTS.

VIRAL REPLICATION

DNA VIRUSES ALL REPLICATE IN THE NUCLEUS EXCEPT FOR THE POX VIRUS, WHICH REPLICATES IN THE CYTOPLASM.
RNA VIRUSES ALL REPLICATE IN THE CYTOPLASM EXCEPT FOR THE INFLUENZA VIRUS AND RETROVIRUSES, WHICH REPLICATE IN THE NUCLEUS.

(CONTINUED ON NEXT SPREAD)

VIRUSES AND VACCINES:

A Basic Flowchart of Viral Families

THIS CHART SHOWS SOME OF THE MOST COMMON HUMAN VIRUSES. VIRUSES ARE OBLIGATE, INTRACELLULAR PARASITES THAT REQUIRE A HOST CELL FOR REPRODUCTION. VIRUSES REPLICATE BY ENTERING INTO A HOST CELL AND PRODUCING VIRAL PROTEINS THAT HELP REPLICATE THE VIRAL GENOME. THE NEW GENERATION OF VIRIONS EXITS THE CELL AND SPREADS TO NEW CELLS.

SINGLE-STRANDED RNA VIRUSES CAN BE CLASSIFIED AS POSITIVE SENSE, NEGATIVE SENSE, OR AMBISENSE. POSITIVE SENSE VIRUSES HAVE A GENOME WITH THE SAME MRNA SEQUENCE THAT IS REQUIRED TO MAKE VIRAL PROTEINS. NEGATIVE SENSE VIRUSES HAVE A GENOME THAT IS COMPLEMENTARY TO THE MRNA SEQUENCE. AMBISENSE VIRUSES ARE POSITIVE SENSE IN SOME GENOME SECTIONS, AND NEGATIVE SENSE IN OTHER SECTIONS.

THE FAMILIES OF RNA VIRUSES

FLAVI VIRUSES

ST. LOUIS ENCEPHALITIS
TRANSMITTED VIA MOSQUITO. CAUSES FEVER, HEADACHE, FATIGUE, VOMITING. ENCEPHALITIS IS MORE COMMON IN OLDER ADULTS.

DENGUE
TRANSMITTED BY AEDES MOSQUITOES. CAN CAUSE HIGH FEVER, HEADACHE, JOINT AND BONE PAIN, RASH, AND BLEEDING OF GUMS & NOSE.

YELLOW FEVER
TRANSMITTED BY AEDES MOSQUITOS. CAN CAUSE HIGH FEVER, JAUNDICE, BLACK VOMIT, AND COUNCILMAN BODIES ON LIVER BIOPSY.

WEST NILE VIRUS
CAN CAUSE MYALGIA, FATIGUE, TRANSIENT MACULOPAPULAR RASH, MENINGITIS, ENCEPHALITIS, OR ACUTE FLACCID PARALYSIS POSSIBLE.

HCV
HEPATITIS C IS TRANSMITTED VIA INFECTED BLOOD. IT IS A CHRONIC INFECTION THAT CAUSES LIVER CIRRHOSIS AND CARCINOMAS.

ZIKA
TRANSMITTED BY AEDES MOSQUITOES. MILD SYMPTOMS IN ADULTS. CAN CAUSE SEVERE BIRTH DEFECTS IF INFECTED DURING PREGNANCY.

RETRO VIRUSES

HTLV T-CELL LEUKEMIA
HTLV CAN CAUSE ADULT T-CELL LEUKEMIA AND HTLV-I-ASSOCIATED MYELOPATHY / TROPICAL SPASTIC PARAPARESIS.

HIV - AIDS
HIV IS DIAGNOSED WITH ELISA, THEN A WESTERN BLOT ASSAY. HIGH VIRAL LOAD ASSOCIATED WITH A POOR PROGNOSIS.

REO VIRUSES

COLORADO TICK FEVER
CAN CAUSE CHILLS, FEVER, HEADACHE, FATIGUE, BODY ACHE. SEVERE CASES CAN CAUSE CNS ILLNESSES SUCH AS STIFF NECK AND CONFUSION.

ROTAVIRUS
CAUSES INFANTILE GASTROENTERITIS AND ACUTE DIARRHEA. DESTROYS VILLI TO CAUSE DECREASED NA+ AND K+ ABSORPTION.

ORTHOMYXO VIRUSES

INFLUENZA
PATIENTS ARE AT RISK FOR FATAL BACTERIAL SUPERINFECTION. CONTAINS HEMAGGLUTININ & NEURAMINIDASE VIRION ANTIGENS.

DELTA VIRUS

HDV
HEPATITIS D IS A "DEFECTIVE VIRUS" IN THAT IT REQUIRES HBV TO REPLICATE. HDV SUPERINFECTION OR COINFECTION CAUSES WORSE PROGNOSIS.

RHABDO VIRUSES

RSV
CAUSES RESPIRATORY TRACT INFECTIONS IN INFANTS, SUCH AS BRONCHIOLITIS AND PNEUMONIA. CONTAINS SURFACE FUSION PROTEIN F.

MEASLES
CAN CAUSE COUGH, CORYZA, FEVER, CONJUNCTIVITIS, RASH, AND KOPLIK SPOTS. RASH STARTS AT HEAD AND SPREADS DOWNWARD.

MUMPS
CAUSES INFLAMMATION OF PAROTID GLANDS AND TESTES. CAN ALSO CAUSE STERILITY AFTER PUBERTY, AND ASEPTIC MENINGITIS.

RABIES
LONG INCUBATION PERIOD. CAUSES FEVER & MALAISE, THEN PHOTOPHOBIA & HYPERSALIVATION, THEN PARALYSIS & COMA, THEN DEATH.

PARAMYXO VIRUSES

PARAINFLUENZA
CAN CAUSE CROUP, UPPER RESPIRATORY TRACT ILLNESSES, BRONCHITIS, PNEUMONIA, AND BRONCHIOLITIS. 4 TYPES (HP1V-1 TO 4)

FILO VIRUSES

EBOLA
HIGH MORTALITY RATE. PATIENTS FIRST PRESENT WITH DIARRHEA, FEVER, MYALGIA, VOMITING, AND ABRUPT ONSET OF FLU-LIKE SYMPTOMS.

CORONA VIRUSES

SARS-COV-2
SARS-COV-2 IS THE VIRUS RESPONSIBLE FOR COVID-19 (PREVIOUSLY CALLED THE 2019 NOVEL CORONAVIRUS).

SARS
SARS CAUSES HIGH FEVER, HEDACHE, COUGH, AND PNEUMONIA. SINCE 2004 THERE HAVE NOT BEEN ANY REPORTED CASES OF SARS.

PICORNA VIRUSES

POLIOVIRUS
POLIO IS VERY INFECTIOUS AND CAUSES PARALYSIS AND MENINGITIS. MILD SYMPTOMS CAN INCLUDE FEVER, FATIGUE, & NAUSEA.

ECHOVIRUS
CAN CAUSE GASTROINTESTINAL INFECTION, MOUTH SORES, AND SKIN RASHES. SERIOUS CASES CAN CAUSE VIRAL MENINGITIS.

RHINOVIRUS
CAUSE OF THE COMMON COLD. >100 SEROLOGIC TYPES. THE ONLY PICORNA VIRUS THAT IS NOT AN ENTEROVIRUS. DESTROYED BY ACID.

COXSACKIEVIRUS
CAUSES HAND, FOOT AND MOUTH DISEASE. USUALLY AFFECTS CHILDREN <5 YRS OLD. SYMPTOMS INCLUDE FEVER, MOUTH SORES, AND A SKIN RASH.

HAV
HEPATITIS A IS TRANSMITTED FECAL-ORALLY. IT INFECTS THE LIVER BUT DOES NOT CAUSE CHRONIC INFECTION. NO CARRIERS.

CALICI VIRUSES

NOROVIRUS
CAN CAUSE ACUTE GASTROENTERITIS. ACUTE-ONSET VOMITING, NAUSEA, AND DIARRHEA. SYMPTOMS USUALLY LAST 24-72 HOURS.

HEPE VIRUS

HEV
HEPATITIS E HAS A HIGH MORTALITY IN PREGNANT WOMEN. IT IS TRANSMITTED FECAL-ORALLY AND HAS A SHORT INCUBATION PERIOD.

TOGA VIRUSES

RUBELLA
CAUSES FEVER, ARTHRALGIAS, LYMPHADENOPATHY, AND A RASH. MILD IN CHILDREN BUT SERIOUS IN CONGENITAL CASES.

EASTERN EQUINE ENCEPHALITIS
SEVERE CASES CAN PRESENT WITH HIGH FEVER, CHILLS, HEADACHE AND VOMITING, THEN DISORIENTATION, SEIZURES, AND COMA.

WESTERN EQUINE ENCEPHALITIS
USUALLY CAUSES ENCEPHALITIS ONLY IN INFANTS AND THE ELDERLY. TRANSMITTED VIA INFECTED CULEX MOSQUITO BITES.

CRIMEAN-CONGO FEVER
CCHF IS TRANSMITTED VIA TICKS, LIVESTOCK, AND BODILY FLUIDS. MORTALITY RATE IS ~30%. WITH DEATH OCCURRING IN THE 2ND WEEK.

RNA VIRUSES INCLUDE SINGLE AND DOUBLE STRANDED GENOMES. AS WELL AS RETROVIRUSES (A SINGLE STRANDED VIRUS THAT REQUIRES REVERSE TRANSCRIPTION TO REPLICATE). THE FIRST RNA VIRUS TO BE DISCOVERED WAS THE TMV VIRUS WHICH CAUSES TOBACCO MOSAIC DISEASE IN TOBACCO PLANTS.

ARENA VIRUSES

LCMV
PRIMARY HOST IS THE COMMON MOUSE. CAN CAUSE MENINGITIS AND ENCEPHALITIS. FETAL INFECTION CAUSES DEATH OR DEFECTS.

BUNYA VIRUSES

LASSA FEVER ENCEPHALITIS
SEVERE SYMPTOMS INCLUDE BLEEDING, VOMITING, SHOCK, DEATH, RESPIRATORY DISTRESS, AND FACIAL SWELLING. OCCURS IN WEST AFRICA.

CALIFORNIA ENCEPHALITIS
TRANSMITTED VIA MOSQUITO. SEVERE CASES CAN CAUSE NEUROINVASIVE DISEASE. ENCEPHALITIS, COMA, SEIZURES, PARALYSIS.

SANDFLY & RIFT VALLEY FEVERS
COMMONLY SEEN IN LIVESTOCK AND TRANSMITTED VIA MOSQUITO. CAUSES OCULAR DISEASE, ENCEPHALITIS, AND HEMORRHAGIC FEVER.

HANTAVIRUS
MICE ARE VIRUS RESERVOIRS. SEVERE CASES CAN CAUSE FLUID IN LUNGS, COUGHING, FEVER, FATIGUE, HEADACHES, NAUSEA, & DIARRHEA.

Perspective

Zika Goes Viral

While the Zika virus has its moment, few people are discussing the problems underlying the worldwide increase in emerging infectious diseases.

Robert L. Dorit

Most epidemics begin quietly. Beginning in 2015, health workers in Brazil noted an increase in the number of cases of a relatively mild and nondescript infection, likely caused by a mosquito-borne virus known as Zika. On May 7, 2015, the Pan American Health Organization and the World Health Organization (WHO) issued an epidemiological alert that calmly stated, "Currently, the public health authorities of Brazil are investigating a possible transmission of the Zika virus in the northeast of the country." This outbreak seemed little more than one more skirmish in our ongoing war with emerging infectious diseases.

By the fall of 2015, however, scientists began to realize that the Zika outbreak was more than a small-stakes epidemic. This viral outbreak was one with potentially horrific consequences, as children born with abnormally small heads suddenly began to appear with alarming frequency in the northeast provinces of Brazil—almost exactly 38 weeks after reported increases in the incidence of Zika infections. In the Brazilian state of Bahia, the risk of giving birth to a microcephalic child had gone from a background rate of 0.02 percent to anywhere between 0.88 and 13.2 percent for women infected with the Zika virus in the first trimester of pregnancy.

As I write this, more than 406,755 suspected cases of the Zika infection have been reported in the Americas, and 56,685 of them have been confirmed. This virus, until recently the largely ignored and underachieving cousin of the far better-known Dengue fever, yellow fever, and West Nile fever viruses, has suddenly gained a terrible prominence. In the wake of this disease, thousands of devastated parents now care for newborns with severe neurological damage.

Yet Zika will not necessarily remain in the spotlight for long. An argument can be made that epidemics are now subject to the Warhol Effect—every infectious agent is bound to have its 15 minutes of fame. Our short attention spans, as well as our thirst for drama and the amplifying effects of modern media, conspire to make every emerging disease a short-term *cause célèbre* that is forgotten all too quickly.

For those of us who spend our professional lives thinking about infectious disease, the Warhol Effect has profound implications: The thousands of cases of Zika recorded to date draw public attention (and funding) away from prevalent, deadly diseases that people have simply grown weary of hearing about. Some 214 million cases of malaria were reported in 2015. Taken together, tuberculosis, HIV, malaria, diarrheal disease, and pneumonia kill one in five people worldwide, but they are no longer news. For the unaffected, these diseases have become part of a mundane, if grim, backdrop. If the problems underlying all these diseases were better addressed, lives could be saved.

Nevertheless, Zika does deserve attention. Not only is the human cost of this disease profound and, for many, lifelong, this outbreak reveals new weaknesses and strengths in our ongoing standoff with infectious agents. At the same time, this outbreak underscores the importance of viewing ourselves as global citizens. At a time when globalization is viewed with increasing distrust and transnational cooperation is portrayed as inimical to national interests, outbreaks remind us that we are in this together. This Zika outbreak is important in and of itself, but it is also part of a larger pattern that is sometimes hidden. Increasingly frequent disease outbreaks around the globe require a concerted response. Rather than careening from crisis to crisis, closing borders, and blaming migrants, we need ongoing support for international efforts and institutions that monitor and respond coherently to infectious disease.

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Why Zika, Why Now?

Following its identification in 1947, Zika had only occasionally been detected in Africa (or elsewhere): Fewer than 20 cases were reported before 1981. The actual number of cases was certainly higher, but a virus that causes mild and fairly generic clinical symptoms is likely to be ignored and its numbers underestimated. More detailed and focused surveys of selected populations in Africa reveal the presence of anti-Zika antibodies in 38 percent of individuals sampled, suggesting widespread undiagnosed and possibly asymptomatic exposure to it.

The virus spread while we, the host, barely noticed. That changed in the following decades. In 2007 on the tiny Micronesian island of Yap, 108 cases of Zika infection were reported, and 73 percent of residents above the age of three were exposed to the virus. In 2013 the virus struck French Polynesia, affecting as many as 32,000 people. A year later a visitor, likely coming from the Pacific islands to Brazil, acted as an unsuspecting courier for a virus not previously seen in the Americas.

This outbreak is still in its early phase; the epidemic has not yet peaked. The virus has spread far from its original epicenter in northeast Brazil, moving southward into other South American countries and inexorably northward toward the United States. Since 2015, 47 countries have reported Zika transmission for the first time. The magnitude of this event begs for an explanation. What is different this time? What changes have turned this unprepossessing virus into a global villain?

The answers are not straightforward. Epidemiology has its troika—host, agent, and vector—and all three are in motion. Over the past two decades, the human population has unwittingly reconfigured itself to optimize the spread of infectious diseases. The global population continues to grow at more than 1.1 percent per year, expanding the pool of susceptible hosts. More than half of the human population now lives in cities, increasing the probability of transmission for any communicable disease. Every day, on average, more than 8 million people are in the air, and millions more are on the move, transporting potential pathogens quickly around the globe. A more propitious set of conditions for epidemic spread would be hard to imagine. To date, 934 possible cases of Zika in the United States can be traced directly to travelers arriving from countries with a high incidence of the infection.

Lianne Milton

In 2015 Brazilian health workers noted an increase in the number of cases of Zika viral infections, thought to be fairly mild, followed nine months later by an alarming spike in babies born with microcephaly, the result of abnormal brain development. Above, medical doctor Danielle Cruz gives Luhandra, a two-month-old baby born with microcephaly, a check-up at a hospital in Recife, Brazil, the city with the most documented cases of the neurological disorder.

But these trends are not new, nor are they specific to Zika. Although increasing population and travel certainly account, at least in part, for the increasing frequency of emerging infectious diseases, these variables alone cannot explain this outbreak. The virus, too, is evolving rapidly as it adapts to changing circumstances. Like other viruses that preserve their genetic information in the form of RNA, it has made constant change its calling card. In these RNA viruses, the machinery responsible for copying and transmitting genetic information from one generation to the next is notoriously inaccurate.

In contrast to species that have evolved painstaking fidelity when replicating their genomes, RNA viruses pass on to the next generation genetic information riddled with mistakes, many costly. But with such sloppiness comes the promise of innovation: Every viral particle, slightly different from its parent, is an evolutionary experiment. A virus and its offspring are not so much a close-knit family as a swarm of related particles exploring the environment. The strategy has proven fiendishly effective for many viruses, allowing them to evolve rapidly in response to the biology of their hosts and vectors. Not surprisingly, the original Zika strain has diversified into hundreds of distinct isolates whose history records the inexorable march of this virus across the globe.

We cannot yet pinpoint the exact changes in the viral genome that have transformed the comparatively benign Zika virus of the 1950s into the aggressive one of the 2010s. In the thousands of generations that have elapsed since its discovery, this virus has refined its ability to home in on neural stem cells—the progenitors of the fetal brain—by exploiting an abundant receptor on the cell surface to enter into and subsequently hijack the machinery of these cells. The Zika virus has also evolved the capacity to cloak itself in maternal antibodies to cross the placental barrier during pregnancy.

We are not yet certain about what precise information encoded in the Zika genome makes such acts of subterfuge possible, but a suite of mutations holds a partial answer. More recently, the possibility that Zika can be sexually transmitted has come into focus. The case for this route of transmission is still largely circumstantial. A small number of Zika cases in individuals who do not live and had not travelled to areas where the virus and its mosquito vector are present could be explained only by sexual contact with a partner who had recently travelled to a Zika-endemic region.

Furthermore, significant concentrations of active Zika virus have now been found in the seminal fluid of infected men. The virus is exploiting the privileged status of the male reproductive tract, where immune scrutiny is reduced to ensure the survival of sperm cells. By congregating in this protected region, the virus may be evolving a new route of transmission and in the process freeing itself from dependence on a mosquito vector. It is too early to say just how significant this new mode of transmission will be. But for the most part, pathogens, and especially viruses, that have hitched their fate to the persistence and prevalence of sexual intercourse have done chillingly well.

Spreading Vectors

Still, the startling evolvability of this virus cannot fully explain the causes of the current epidemic. The last mem-

ber of the troika, the mosquito, is also in the midst of a dramatic transformation. Mosquitoes are the bush pilots of infectious disease. They transport all manner of pathogens from one host to another, often oblivious of their cargo. Mosquitoes seem to derive little evolutionary profit from this dirty job: The pathogens have simply evolved to hijack this efficient route of transmission. Unbeknownst to the mosquito drawing the blood meal from its host, it is also a transient but indispensable refuge for bloodborne pathogens. The Zika virus, unceremoniously ingested in the course of a normal feeding, migrates over the next 10 days from the mosquito's gut into its circulatory system and eventually to its salivary glands, ready to be injected into a new host as soon as the mosquito bites again.

Two invasive species of mosquito, *Aedes aegypti* and *Aedes albopictus*, appear to be primarily responsible for the spread of this virus in the Americas. These two species differ in their ecology and habits. *A. aegypti* is primarily diurnal, feeds outdoors, and ventures far from its birthplace. In contrast, *A. albopictus* feeds indoors, primarily in the mornings and evenings. Together, they have evolved to take full advantage of ecological opportunities provided by human habitation and can feed on us the entire time we are awake. These mosquitoes are also sip feeders: Preferring more tapas than sit-down meals, they will feed on multiple hosts, taking only small sips of blood from each of them. Each of these meals, in turn, facilitates the spread of the virus to multiple hosts.

The Zika virus exploits the vectors' ecology to increase its transmission. Perhaps most alarming of all, the geographic range of both mosquito species is rapidly expanding. Driven by a combination of increased urbanization, poor sanitation, and climate change, these mosquitoes are on the move. Confined to West Africa until the 15th century, and more recently found mainly in a tropical band around the equator, *A. aegypti* has now established a beachhead as far north as Virginia. Over the past 75 years, *A. albopictus* has spread from its original range in the South Asian subcontinent to the New World, where it is found from Patagonia to Massachusetts. The risk of Zika infections moves with the mosquitoes. In the continental United States the prospect of Zika infections has gone from prediction to reality in the past months. In response to the epidemic, much attention has focused on the eradication of mosquitoes in areas where Zika has gained a foothold, as well as in regions where densities of potential vectors are high.

Although conventional approaches to mosquito eradication, including draining bodies of standing water and using insecticides, have proven somewhat effective in reducing mosquito populations, complete eradication by these means appears unrealistic. Both mosquitoes have adapted to the opportunities provided by human habits, laying their eggs in even the tiniest pool of standing water. Ambitious projects use the latest genetic tools to create mosquitoes that sow the seeds of their own destruction or to deliberately infect mosquitoes with *Wolbachia*, a bacterium that prevents Zika from colonizing the mosquito. Vaccines against Zika have been developed in record time and are now in clinical trials. Taken together, these breakthroughs will help people regain the upper hand, but such developments will take time to come into use. Our collective immunity and ingenuity will eventually make the virus recede, for now, back into its wildlife reservoir. But the human population will still remain ripe for future infections.

Epidemiology is not for the faint of heart. Public health authorities must strike a balance between premature action and overly cautious delay.

High-Stakes Epidemiology

How could we not have seen this, or other important outbreaks of the past several decades, coming? After all, the infectious agents responsible for recent outbreaks were for the most part already known. The factors underlying epidemics—population density, poverty, globalization—have long been identified and are increasingly well understood. Yet we seem constantly caught unaware, unable to predict, and slow to respond. What aren't we seeing?

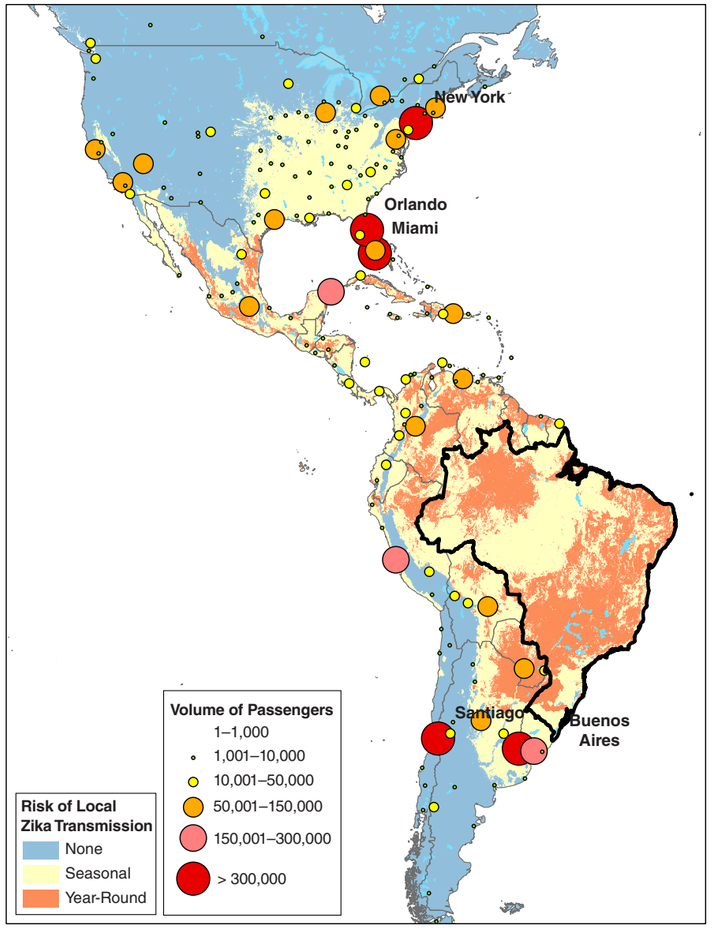
Part of the answer requires recognition that epidemiology is high-stakes science. All scientists are trained to be cautious in the interpretation of data, circumspect in any extrapolation, and careful not to let conclusions outpace evidence. These practices underlie the resounding successes of science but require persistence, support, and, above all, time. Public health authorities responding to an outbreak do not have the luxury of waiting until all the facts are in. Instead, they must strike a balance between premature action and overly cautious delay. Respond too quickly, and resources are squandered for no reason. Respond too cautiously, and infections and deaths that could have been prevented aren't. Epidemiology is not for the faint of heart.

On February 1 of this year, just weeks after the rise in microcephaly cases in Brazil had come into focus, the WHO declared Zika a public health emergency of international concern. This official designation had important regulatory, fiscal, and policy consequences and was intended to focus the attention and efforts of the international community on this emerging threat. The WHO had chosen to sound the alarm well before the scope of the outbreak was understood and before the link between Zika and neurological defects was clear. Yet the costs of waiting for definitive proof were simply too high: The caution and conservatism inculcated into every scientist had to be tempered with the dangers of delay. Their decision was sound in part because in this field *proof* means something very particular.

Events associated in time and space are the currency of epidemiology. Proof in this field, for ethical and practical reasons, seldom comes from direct experimentation. Under such circumstances correlation here *is* indirect evidence of causation. Proof that Zika causes microcephaly will not be definitively established in the conventional sense for quite some time, although evidence for the link is mounting. The molecular, cellular, and developmental evidence relating the virus to its devastating consequences will take painstaking work to accumulate. Until then, the power of statistical inference needs to be brought to bear: Epidemiologists could not explain the coupled rise of Zika and microcephaly except by arguing, at least for now, that the virus is the cause of the neurological defects.

Epidemiology challenges the misconception that the capacity for exact prediction is the hallmark of any real science. Like cosmology and evolutionary biology, epidemiology operates in a realm where history and contingency play fundamental roles. To be sure, this field strives to be predictive: Epidemiologists try to anticipate the severity of an infectious outbreak, pinpoint the location of the next emergent disease, and gauge the potential impact of public health interventions. We do so by using the past as a way to anticipate the future. But extrapolation is a dodgy game when dealing with phenomena involving so many components.

Every player in a disease outbreak—host, vector, reservoir, infectious agents—is constantly changing. No two outbreaks can be exactly the same. The Zika virus, first isolated from a rhesus monkey in Uganda in 1947, has evolved dramatically since then. The two previous Zika outbreaks (on Yap and in French Polynesia) differed from the current one in their severity and consequences. Every outbreak results from a confluence of events that will never be repeated. Epidemiology's power to predict in detail is thus necessarily constrained. In exchange, epidemiologists mine these unique events for their regularities, deepening our understanding of the ongoing dance between humans and their pathogens.



The coupled spread of vectors and hosts creates ideal conditions for an emergent infectious disease. This map reveals the joint effect of human movement out of Brazil—the current epicenter of the Zika outbreak—and the expanding range of the virus's mosquito vectors. As a result, large portions of South, Central, and North America are now potentially at seasonal or year-round risk for local Zika transmission. (Courtesy of Kamran Khan, St. Michael's Hospital, Toronto, Canada.)

We Are Now the Resistance

People are again locked in combat with an emergent disease that, at least for now, appears to have the upper hand. These early skirmishes have revealed a virus capable of exploiting every opportunity we have provided. The consequences of a Zika infection vary considerably: for some, just a slight fever and rash; for others, temporary paralysis and other severe neurological effects; and for pregnant women, the prospect of a microcephalic newborn. It seems cruel to imagine that these are all incidental consequences of a virus shaped by evolution.

The struggle between pathogens and hosts is as old as life itself and has resulted in an unending series of fragile truces. In that sense, the Zika outbreak is just one more in a long line of engagements. But such struggles are becoming more common: New infectious diseases are emerging (or reemerging) more frequently than ever before. Something is changing in our relationship to pathogens. Throughout human history, most infectious disease outbreaks were local. Concentrated in one or at most a few small human populations, infections would quickly run their course, leaving only death and immune survivors in their wake. All that has changed now.

As hosts, vectors, and pathogens move quickly around the globe, we would do better to imagine the human population as a single body. Infection in any part of this body politic threatens the whole. Likewise, an effective response to this new reality transforms our surveillance, containment, and treatment efforts into a planetwide immune response. In a globalized world, eternal vigilance is the price of health.

Daunting as the problem of emergent infections might seem, we are far from powerless before it. New technologies, from mobile phones to Internet searches, now serve as early warning systems for outbreaks. Novel diagnostic technologies make it possible for epidemiologists to pinpoint infectious agents more quickly and with greater resolution than ever before. The very interconnectedness that helps spread infection also serves to recruit citizens and experts in efforts to monitor the earliest stages of an outbreak. The earlier we detect one, the better is the chance of containment. Through our cooperative community-level and international efforts, we become more than passive victims. We are now the resistance.

(A reference list for this article is available at http://www.americanscientist.org.)

The Bright Side of the Black Death

The bubonic plague left its mark on the human population of Europe, showing that what doesn't kill you makes you stronger.

Pat Shipman

The Black Death was so extreme that it's surprising even to scientists who are familiar with the general details. The epidemic killed 30 to 50 percent of the entire population of Europe. Between 75 and 200 million people died in a few years' time, starting in 1348 when the plague reached London. The pandemic moved fast: It often killed a host within days of their first developing the high fever, the telltale rash, and the repellent buboes or swellings in the armpits and groin, which turned black and burst, expelling pus and bacteria. The disease spread through families, houses, villages, towns, and cities with terrifying speed and staggering mortality. This tragedy launched a socioeconomic and evolutionary transformation in Europe that changed the course of history.

So many were struck down and so rapidly, that it was long thought that the Black Death killed indiscriminately. Certainly the disease took men, women, and children, rich and poor. But was it a selective form of death? Anthropologist Sharon DeWitte, who is currently at University of South Carolina, felt the answer could be obtained by studying skeletal remains of plague victims and comparing them to other medieval skeletons buried in normal, nonplague cemeteries, and she tackled that question for her dissertation work at Pennsylvania State University. (Disclosure: I was at Penn State at the time, but I did not serve on DeWitte's PhD committee.) Because of its severity and the existence of documentary as well as biological evidence, the Black Plague looked like a perfect case to investigate the influence of pandemic disease on human populations.

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Origin and Spread

When the plague hit in the mid-1300s, no one knew what caused this dreadful pestilence. Some took it as divine punishment for the world's wicked ways, possibly the end of the world. Others blamed Jews, foreigners, travelers, and lepers, who were shunned and turned away where once they had been welcomed or at least accepted. Some towns barricaded themselves in, afraid to let anyone in who was not already there and equally afraid to let anyone out. Mothers abandoned husbands and children—and vice versa—for fear of catching the contagion. Few other than those in religious orders dared to nurse the sick. Sometimes houses were burned to the ground with the inhabitants inside if they were known to be ill. Ordinary parish burial grounds were insufficient to hold the massive numbers of dead, and new plague cemeteries were opened.

The social and economic havoc created by the plague was almost beyond imagining, yet it is now being paralleled in many ways by the impact of the Ebola virus epidemic. Whole villages die within a few weeks, and fear spreads even faster than the infectious agent.

With hindsight, the pandemic can be traced to the Mongol Empire, which in addition to conquering with its vast army enormous areas of Asia, opened and ensured the safety of the Silk Road for trade. This Pax Mongolica facilitated relatively rapid, long-distance transport, both of people and diseases, as airplanes and railroads do today with sufferers of the Ebola virus. Where travel is highly restricted and populations are small, deadly diseases tend to burn themselves out fairly quickly. But where the disease can be spread easily to new areas, with a new supply of victims, efforts to contain such pandemics are far more difficult.

In medieval times, where the Mongol army went, so went the plague; where the sailing ships carried trade and traders, the pestilence arrived in new regions. According to a contemporary account by Gabriel de' Mussi, a turning point came when the Mongols besieged the trading city of Kaffa in Crimea between 1346 and 1349. Because the army remained in one place for so long, the Black Death had time to spread from man to man or from rat-carried fleas to humans. In the end, the army deliberately hurled the rotting corpses of the dead over the city walls, infecting those inside, poisoning wells, and causing a sickening stench. This strategy has been cited as the first biological warfare. Those that escaped Kaffa fled by ship to Sicily, Genoa, and Venice in 1347 and 1348, carrying the disease with them. Plague ships soon reached busy ports in France, Spain, and Norway, offloading their deadly cargo at each stop.

The plague arrived in England on or about May 8, 1348, at Melcombe Regis, traveling on a ship that had left Bordeaux a few weeks earlier. The epidemic is mentioned in historical documents shortly before June 24 of that year. The disease reached London in August 1348 and reached epidemic proportions by the end of September. From there, it spread into the countryside. By 1351, the pandemic had died down, but whole villages and their fields were empty of inhabitants; cottages and houses sat vacant; many crops had rotted in the field for lack of labor to harvest them; stock animals died, unfed and unwatered, for lack of human care. There were simply not enough serfs and peasants left in England to do the work. The aristocrats also died in droves. The medieval economic system broke down because of the rapid drop in population.

Wikimedia Commons

Pieter Brueghel the Elder's painting *The Triumph of Death* of the mid-1500s depicts the social upheaval brought on by the bubonic plague in medieval Europe, beginning in the 1300s. The skeletons represent death in its inevitability and creativity of means.

Plague struck in three forms, which made its identification problematic until recent times. All three have been traced to the bacterium *Yersinia pestis* by extracting genetic material from bones and dental pulp of victims buried in plague cemeteries and then comparing it to the genotype of modern samples of the bacterium. Although bubonic plague may be carried by black rats and their infected fleas to new areas, it also was transmitted from human to human as a respiratory form and from humans to other humans who handled infectious tissues. Without modern antibiotic treatment, the plague kills 72 to 100 percent of those who contract it.

What Doesn't Kill You

Today, the tragedy of the Black Death offers a unique opportunity to study past human health and the social and genetic consequences of pandemics. DeWitte explains, "I got started on plague because I knew I wanted to study health in the past using skeletal material, but I wanted to do it in the most rigorous way possible, which requires large sample sizes. The Black Death provided the best possible case study because of the existence of exclusive Black Death cemeteries—large samples of people who died within a short period of time from a single known cause of death."

For her PhD thesis, DeWitte studied 490 skeletons from a plague burial in England, the East Smithfield Black Death cemetery, and compared the results with those from a study of 291 skeletons from two medieval but normal and nonepidemic cemeteries of Viborg and Odense in Denmark. For each individual, DeWitte established age at death from skeletal indicators of maturity and sex. She also documented the location and frequency of various bony or dental lesions that indicated poor health, malnutrition, or other causes of "frailty." She found that older individuals and those with significant signs of frailty were at higher risk of dying of the plague than their peers.

"By targeting frail people of all ages, and killing them by the hundreds of

Sharon DeWitte (top left) studied bones from burials before and after the first plague epidemic in the mid-1300s and showed that people were healthier and lived longer afterward. For example, the tibia bone at top right shows periosteal lesions, excess growths of bone that can occur in response to trauma or infection. The bottom panel shows the roof of the eye socket with porous lesions called *cribra orbitalia* that form during childhood in response to anemia. Such lesions were more common and numerous in bodies buried before the plague than afterward. (Photographs courtesy of DeWitte.)

thousands within an extremely short period of time, the Black Death might have represented a strong force of natural selection," DeWitte observes. The disease apparently removed the weakest individuals on a very broad scale over much of Europe, whether their frailty was due to poor immune systems, prior disease, or malnutrition.

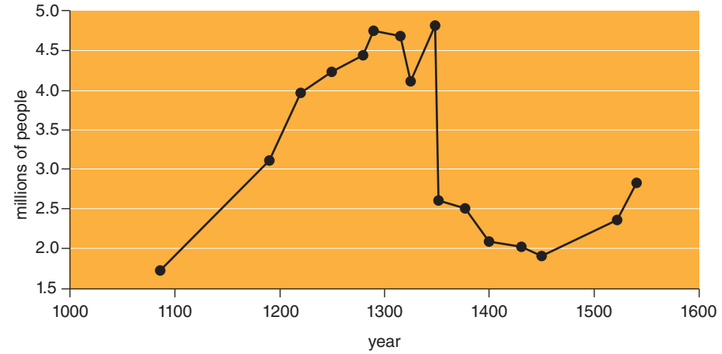
"We know the Black Death marked the beginning or, at the very least, an acceleration of a huge economic and sociological shift in Europe," says DeWitte. It took 200 years for population levels to recover. In the meantime, the medieval system of serfdom collapsed, because labor was more valuable when there were fewer laborers. Despite the dearth of workers, there was more land, more food, and more money for ordinary people. "You might see this as a benefit to the laboring classes," she says.

DeWitte's more recent studies explore the long-lasting biological impact. When the plague returned in 1361—and again in 1368, 1375, 1382, and 1390—DeWitte looked for signs that the population might have been healthier and better fed: Were they taller with fewer signs of lesions? She looked at 464 skeletons from cemeteries used before the Black Death and 133 skeletons from another that was in use from just after the plague hit until 1538, gathering comparable data to her previous work.

Looking simply at age at death, DeWitte found that a higher proportion of people lived to older ages after the Black Death. That is, if you or your parents or grandparents lived through the first plague epidemic, you were more likely to survive until you reached age 50 or above. Descendants of plague survivors were more likely to live through their reproductive years, thus passing on whatever genetic advantages enabled their ancestors to survive the plague.

DeWitte's results do not agree with several documentary studies of population well-being during this time, such as one based on wills by Jens Röhrkasten of University of Birmingham in England. These documents showed spikes in mortality associated with plague epidemics in 1361, 1368, 1375, 1382, and 1390. However, wills and other documents tend to provide evidence only on well-to-do men, excluding married or unmarried women, children, servants, apprentices, laborers, and paupers. The sheer number of wills recorded in various counties amply demonstrates the appalling wave of death, but Röhrkasten's data give only part of the story of the aftermath of the plague.

DeWitte argues that her bioarchaeological data provide information about a much broader sweep of medieval society. Despite repeated plague outbreaks and other episodes of crisis mortality, such as famines or volcanic eruptions, her study indicates that the general population enjoyed a period of at least 200 years during which mortality and survival overall improved compared to conditions before and during the Black Death.



The medieval population of England dropped precipitously when the plague reached London in 1348. The effects on the population were felt for centuries afterward. (Figure adapted from S. Broadberry, B. Campbell, and B. van Leeuwen 2010, English Medieval Population: Reconciling Time Series and Cross Sectional Evidence, http://bit.ly/1ATjCFy.)

Did such a strong selective event produce changes that can be seen today? The first suggestion, made in 1998, was that plague survivors had stronger immune systems, which might be expressed as a relatively higher incidence among Europeans of a genetic allele known as *CCR5-Δ32*. It has been identified as conveying resistance to the modern pandemic HIV/AIDS.

However, the link between *CCR5-Δ32* and the plague is more tenuous than it at first seemed. The areas that suffered most profoundly from the Black Death correspond only loosely with the modern distribution of the *CCR5-Δ32* gene. Other scientists suggest that smallpox epidemics are a better candidate for the causal agent behind the *CCR5-Δ32* distribution.

Two genetically distinct but adjacent populations offered a rare opportunity to document the plague's evolutionary effects.

A recent study has pointed to another possibility. A team led by Jaume Bertranpetita and Mihai G. Netea decided to investigate genetic differences between Romanian Europeans and people who lived in the same area of Romania but were of Rroma ancestry (traditionally identified as Gypsies). Linguistic and genetic documentation indicates that the Rroma migrated from northwest India between the 5th and 11th centuries, when they began to settle in Romania. Over the centuries, the Rroma remained largely isolated from Romanians of European descent, although they lived in the same region.

Thus the infectious agents that potentially shaped the Rroma genomes during the last millennium—including the plague—were shared with the European Romanians, but their initial genetic backgrounds were different. Because India did not suffer from the Black Death, the immune-related genes put under positive selection by exposure to the disease should be similar among European Romanians and Rroma but different from those among Indians in Gujarati, home of the populations ancestral to the Rroma. Because Netea is himself Romanian, he realized that these two genetically distinct but adjacent populations offered a rare opportunity to document the plague's evolutionary effects.

The team took DNA samples from 100 European Romanians, 100 Rroma, and 500 individuals from northwest India. The team assayed almost 200,000 small genetic changes known as *single nucleotide polymorphisms (SNPs)*, which are often used as a proxy for genomic differences among cohorts of people. Then they analyzed the results looking for genes under positive selective pressure among the Rroma and European Romanians but not shared by the northwest Indians. Four genes on chromosome 4 met these criteria. Three of the four genes in this cluster are so-called *TLR* genes involved in recognizing pathogens such as bacteria and initiating an immune, anti-inflammatory response.

Of course, the Rroma and European Romanians were subjected to other infectious agents during the last millennium, including smallpox, leprosy, and tuberculosis. Netea finds that the plague was "very likely" to have caused the genetic differences he found. "These other infections are also possible causes," he explains, "but in my opinion somewhat less likely than plague: The geographic distributions of these diseases are more general than plague." He also observes that the role of *TLR* genes in mounting an immune response to a virus, such as that which causes smallpox, is not as well established as their role in combating bacterial infections. His team is currently obtaining complete genotypes for the Indian, Romanian, and Rroma samples and conducting a parallel study focusing on adjacent but distinct African populations.

By synthesizing information from documentary sources, bioarchaeological studies, and genomic studies, researchers are slowly but surely figuring out the awful mysteries of the Black Death. As we meet with new and as-yet-untreatable diseases, like Ebola, insights from the past may help ease the future.

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Harnessing the Web to Track the Next Outbreak

Innovations in data science and disease surveillance are changing the way we respond to public health threats.

Aranka Anema, Carly R. Winokur, Chi Bahk, Sumiko Mekaru, Nicholas Preston, and John S. Brownstein

On March 14, 2014, a French news site reported a strange fever in Macenta, a small village in Guinea. The story described a "new disease whose name is unknown" that caused victims to bleed from their orifices, and had already "killed eight people and contaminated several others." The date of this first media report preceded official government reports on the emerging Ebola outbreak by almost 10 days. Eventually, the virus infected more than 28,000 people and killed more than 11,300, devastating West Africa and inciting fear around the world. Digital surveillance, enabled by recent advances in big data and related technologies, can help governments respond more quickly to public health threats. The online disease alert system HealthMap picked up on that first media report and relayed it to the public the same day.

This knowledge is critical in a world where factors such as climate change, population growth, urbanization, and global travel fuel the spread of infectious diseases and pandemics, and are exacerbated by forces such as the antivaccine movement and increasing antimicrobial resistance.

Traditional Disease Monitoring

Public health surveillance relies on systematically and continuously collecting, analyzing, and disseminating information as quickly as possible to guide disease prevention and control. These tracking systems serve a central role in informing the decisions of public health officials, particularly when it comes to tackling communicable illnesses. Standard disease monitoring has relied on reports from traditional sources, such as health ministries and health-care agencies. Such monitoring uses two types of surveillance, *passive* and *sentinel*, both of which have limitations. Passive surveillance, which involves a national health system gathering information from health-care providers and laboratories, often suffers from incomplete and delayed reporting, particularly in resource-poor settings. Sentinel surveillance is a more laborious approach, which involves monitoring the rate of specific diseases in a small cohort of people to estimate trends in the general population.

As a result, traditional public health approaches are often delayed by the communication chain, as data make the journey from patient, to health practitioner, to confirmatory laboratories, to local administrative bodies within regional and national ministries of health, before finally reaching authoritative world bodies such as the World Health Organization (WHO). The process can take days, weeks, or months, during which time a disease can spread globally. One report showed that in 1996, it could take as long as 167 days from an outbreak's start to its discovery. Newer, electronic surveillance methods allow data to go directly from citizens to public health agencies in near real time.

Big Data and Technology

The proliferation of big data in public health has created new opportunities for understanding and visualizing complex global health risks. Digital epidemiology has transformed the public health community's ability to detect diseases, risks, patterns, and outcomes, by aggregating and filtering online and mobile data from news, social media, blogs, and other informal sources. These applications complement traditional sentinel surveillance methods by providing near real-time, geospatial data about emerging risks, often visualized using interactive maps and dashboards.

Web-based electronic information sources have the potential to play an important role in early event detection and in increasing public awareness of a situation. The current, highly local information they produce can help identify events that may go unreported in the public health system. Food poisoning is a great example, because those sickened by food often do not visit a doctor. Although someone ill might tweet or take to Yelp warning others to avoid a restaurant, there is often no formal reporting. Web sources such as these are potentially useful for detection of food safety events. The challenge remains, however, in distinguishing which reports are actually relevant from the large volume of unrelated chatter on the Internet.

Over the past decade, several Web-based early-warning systems have emerged that collect disease-specific data from informal sources: The Medical Information System (MedISys) mines the Internet for public health hazards; ProMED-mail enables public health experts to report and disseminate disease risk information; and HealthMap, BioCaster, and EpiSPIDER crawl Web-based news and other resources to visualize emerging infectious disease outbreaks through mapping interfaces. The use of news media and other nontraditional sources of surveillance data can facilitate early detection of outbreaks and increase public awareness of health concerns prior to their formal recognition. Combined, these systems enhance both the timeliness and sensitivity of early disease detection. One study that gathered data from 1996 to 2006 found a lag between informal communication of an outbreak and official reporting by WHO Disease Outbreak News of 16 days, an interminable amount of time for a highly communicable disease to circulate undetected by health authorities.

In 2006, our team developed HealthMap, an online global disease alert system, with researchers at Boston Children's Hospital and Harvard Medical School. The aim of the website is to provide a comprehensive, real-time overview of infectious disease activity by geographic location for a diverse audience, from public health officials to international travelers.

The system incorporates automation and expert review to aggregate reports on new and ongoing infectious disease outbreaks in more than 15 languages. It processes information from more than 200,000 disparate sources, including international online news aggregators, eyewitness reports (approved alerts created by community members through the site's mobile app), expert-curated discussions, and validated official reports (such as ones from ministries of health websites).

The software captures the inherently geospatial nature of disease, along with time, case counts, and notable characteristics of the outbreak. Relevant articles are filtered from noise using a computer algorithm that analyzes text patterns, known as *natural language processing* (NLP). Human experts curate information, correcting misclassified alerts (false positives, for example, an automatically generated report of a disease outbreak that is not actually disease related) or changing meta tags automatically assigned by the system before this information is displayed on the HealthMap dashboard. These misclassifications may occur when the system detects words that would typically be disease related: (Justin) Bieber *Fever*, or an *outbreak* of crime. In turn, analyst corrections are used to improve the automated processes.

Following automated processing, alerts are posted directly to the public map and partner pages. The system detects common, seasonal, or endemic conditions, as well as outbreak and epidemic situations. Global health authorities such as the WHO and the U.S. Department of Defense, as well as major news outlets, have credited the software with early-warning detection of new and recurrent infectious disease outbreaks.

Diseases of Direct Contact

Novel data sources and technologies are improving public health surveillance for a variety of different infectious diseases, particularly vector-borne diseases (transmitted through direct contact, such as mosquito, tick, or flea bites), zoonotic diseases (transmitted through animals, such as bird flu), and foodborne diseases. In addition to earlier detection of outbreaks, this monitoring can help policymakers, especially in resource-strapped nations, make decisions on the best course of response based on the potential spread of an infectious disease, saving time and lives. Retrospective reviews of data from past outbreaks offer insights into how fast a disease

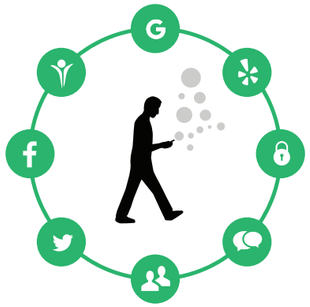
Julian Simmonds/Telegraph Media Group Limited 2014

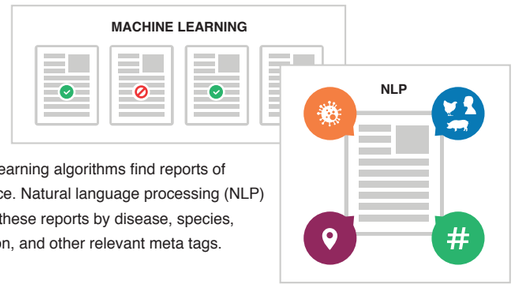
Pedestrians in Monrovia, Liberia, walk past a mural of ways to stem the spread of the Ebola virus. The 2014 outbreak in West Africa was the most widespread and deadliest since the virus was first discovered in 1976. Technology can help to report outbreaks faster, which can reduce their severity.

Aranka Anema, Carly R. Winokur, Chi Bahk, Sumiko Mekaru, and Nicholas Preston are at Booz Allen Hamilton in Boston. John S. Brownstein is at the department of pediatrics at Harvard Medical School and the Children's Hospital Informatics Program at the Harvard-MIT Division of Health Sciences and Technology. Email for Anema: aranka@epidemico.com

1 Acquire

- online news
- social media
- blogs
- official government surveillance reports
- eyewitness reports captured by mobile apps
- expert curated discussion
- patient/physician forums
- specific RSS feeds





2 Filter

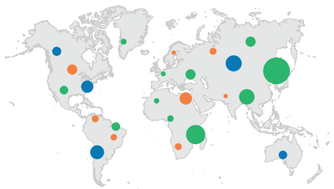
Machine learning algorithms find reports of significance. Natural language processing (NLP) classifies these reports by disease, species, geolocation, and other relevant meta tags.

3 Curate

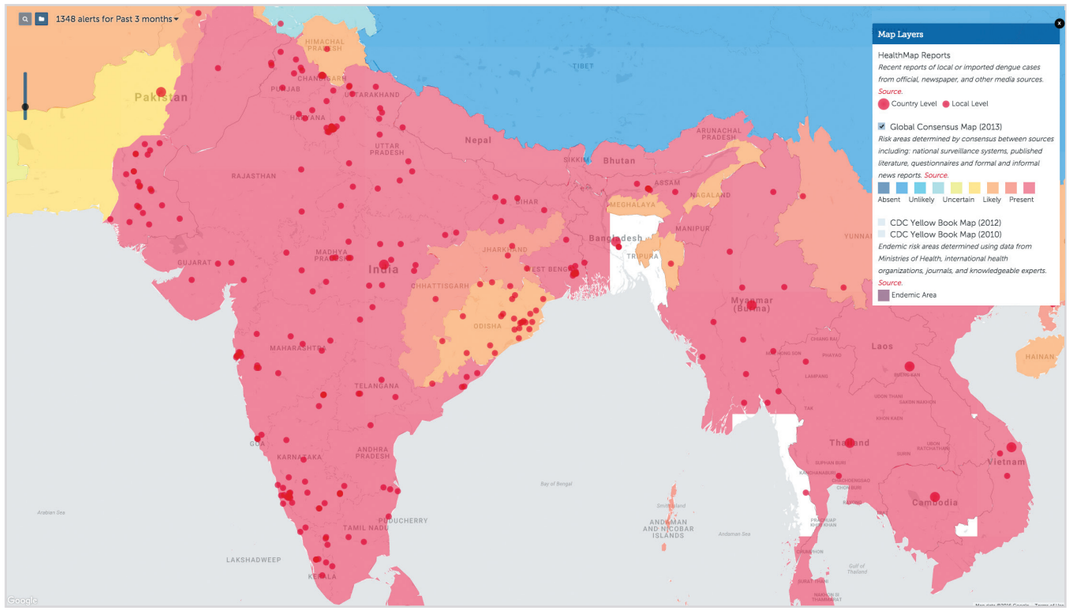
Human curation by public health experts corrects automated tags by removing false positives, further training the classifier and improving NLP dictionaries.

4 Export

Aggregate and granular data are visualized in an interactive, Web-based dashboard.



A software program called HealthMap uses computer automation and expert review to aggregate reports on new and ongoing infectious disease outbreaks in more than 15 languages.



In this Dengue Viral Global Consensus Outbreak Map, high-risk dengue-endemic areas, as defined by the *CDC Health Information for International Travel (Yellow Book)* and HealthMap alerts, are highlighted throughout India and part of Southeast Asia. As many as 400 million people are infected every year with the mosquito-borne virus. (Unless otherwise indicated, images are courtesy of the authors.)

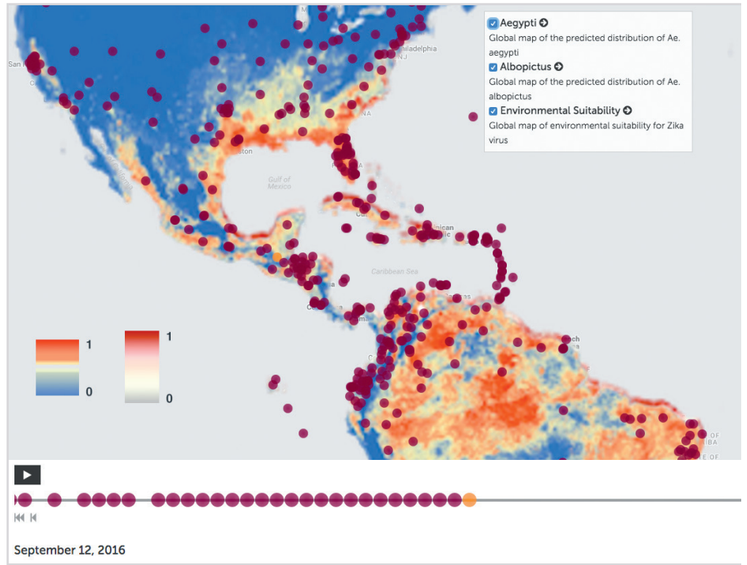
spreads and helps identify other factors that might have been instrumental in the outbreak, such as travel patterns or the lack of sufficient health care.

The need for better understanding of vector-borne diseases has been driving innovative methods of early detection of several notable illnesses, such as dengue, malaria, and Zika. According to the WHO, more than 17 percent of all infectious diseases are vector-borne, and account for more than one million deaths per year.

Dengue hemorrhagic fever is one of the most widespread vector-borne diseases. It is endemic in more than 100 countries, notably in Southeast Asia, the Americas, and the Western Pacific Islands, and it affects an estimated 2.5 billion people. Our group collaborated with Google to develop Dengue Trends, an application for real-time detection of dengue activity based on Google search queries. In a study published in 2011 in *PloS Neglected Tropical Diseases*, our team evaluated whether such searches are a viable data source for the early detection and monitoring of dengue epidemics. Specifically, we examined queries aggregated from Bolivia, Brazil, India, Indonesia, and Singapore, and found that they provided information nearly in real time, as compared to official sources.

Additionally, we have evaluated the use of such unofficial data sources for tracking the recent geographic expansion of dengue across Latin America, and have compared unofficial reports against the areas that the U.S. Centers for Disease Control and Prevention (CDC) has declared endemic. We found that disease data from online media, when used in combination with traditional case reporting, not only improves the timeliness of outbreak discovery and knowledge dissemination but also provides value for public health decision making and forecasting models. Models using dengue-related queries from Google searches adequately estimated true dengue activity measured by the WHO and ministries of health. Our system additionally contributes to DengueMap, part of the CDC's online dengue information resource, and produces its own Dengue Viral Global Consensus Outbreak Map to highlight geographic areas with endemic risk of dengue.

Another vector-borne disease with a high global burden is malaria. The

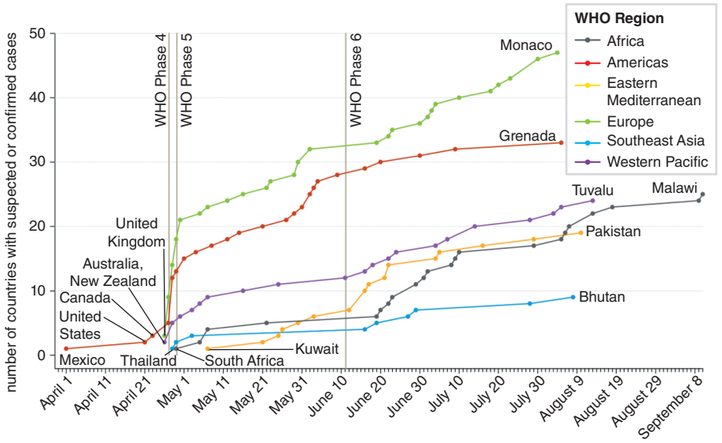


Cases of Zika infection were mapped prospectively, creating an outbreak timeline (*dots*). The results were overlaid with maps showing the low (0) to high (1) predicted distribution of *Aedes aegypti* and *Aedes albopictus*, the mosquito vectors of the Zika virus, and the associated low (0) to high (1) environmental suitability for the spread of Zika globally.

world's population—approximately 3.2 billion people—is at risk of contracting the illness. It is a leading cause of death and disease in developing countries, where young children and pregnant women are the most vulnerable. Over the past 15 years, malaria incidence has fallen by 37 percent around the world, and mortality has dropped by 60 percent. In an effort to reduce the global incidence of malaria cases by a further 90 percent, the World Health Assembly adopted what it calls a "Global Technical Strategy for Malaria 2016–2030."

The proliferation of digital data and the elimination of traditional hierarchical communication barriers have accelerated responses to outbreaks.

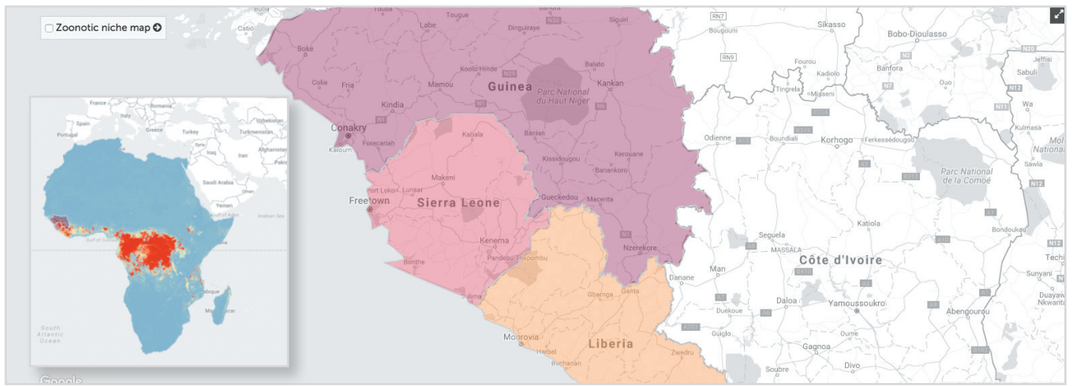
One of the challenges to eliminating malaria is the lack of health care infrastructure to effectively identify and treat infected individuals, underscoring the importance of digital surveillance. Leapfrog technology gy skip intermediate steps and adopt modern technology, such as jumping to cell phones rather than installing land lines) and mobile devices have shown the potential for enhancing the coverage, timeliness, and transparency of public health reporting. Our project experimented with the use of micromonetary incentives to increase public reports of malaria illness in urban centers of India. Self-reports about malaria diagnosis status and related information were solicited online via Amazon's Mechanical Turk, a market in which anyone can post micro-tasks and responders ("Turkers") receive a stated fee for completed tasks. The study found that the prevalence of self-reported diagnoses of malaria were comparable to official prevalence



The timeline shows informal reporting of suspected and confirmed cases of the H1N1 subtype of influenza by WHO region. During the 2009 H1N1 swine flu pandemic, HealthMap detected the earliest informal report from a local Mexican news source, enabling near real-time tracking of this pandemic as it spread globally from Mexico, incurring an estimated 18,000 deaths.

demonstrated the first use of harnessing micromonetary incentives and online reporting for public health surveillance, and highlighted the effective use of online systems such as Mechanical Turk to complement and even enhance traditional survey methods. To further advance digital surveillance for malaria detection, the HealthMap project has built models from Google search queries to estimate malaria activity trends in Thailand, and contributed to malaria forecasting models for endemic countries, such as Uganda.

In May 2015, locally acquired cases of the frightening Zika virus were confirmed in Brazil. There, a surge in infants born with microcephaly (small heads and underdeveloped brains) and other neurological disorders prompted the WHO to declare these Zika-related disorders a Public Health Emergency of International Concern. Early in the outbreak, an international team of researchers used HealthMap data to build models projecting the international spread of Zika virus from Brazil, drawing on digital disease, climatic, and traveler data. Results published in the journal *The Lancet* indicated that Zika had the potential to rapidly spread across Latin America and the Caribbean, with seasonal transmission in many parts of the United States and year-round transmission in certain areas, including parts of Florida and Texas. The HealthMap project has been tracking the Zika outbreak through a prospective timeline, and interpreting incidence data alongside maps of the distribution of *Aedes aegypti* and *Aedes albopictus*, the principal mosquito vectors of the Zika virus. Health authorities and the public can use the application to track new cases. As there is currently no vaccine or treatment available, seeing where Zika cases are in near real time can aid in the public's decision making on such topics as travel or family planning.

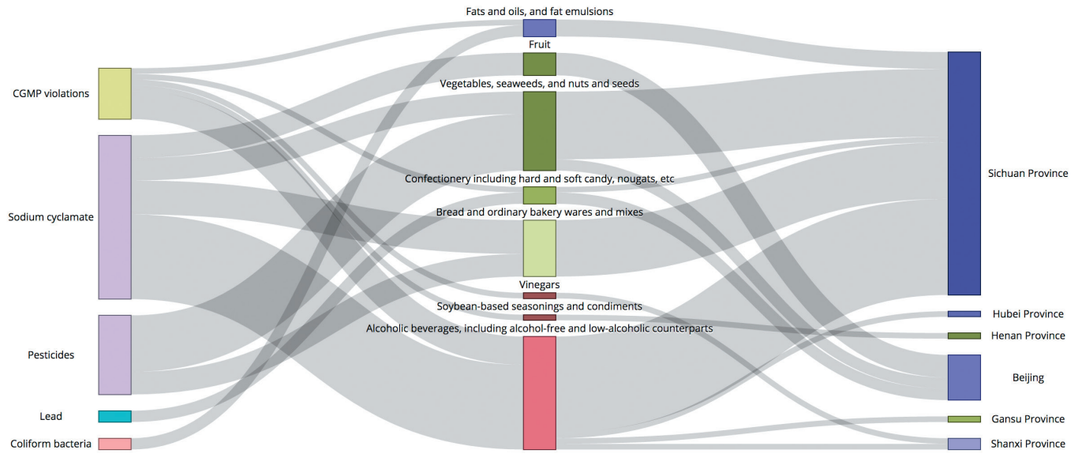


A zoonotic niche map for Ebola shows the incidence of the disease in West Africa (*red dots, inset*) combined with the projected environmental suitability for future outbreaks of the zoonotic virus (*shades of yellow and red*). Ongoing surveillance of zoonotic niches informs public health risk regarding the potential for transmission from animal hosts to humans.

Animals Harboring Diseases

According to the CDC, 6 out of every 10 infectious diseases in humans are spread from animals. Such zoonotic diseases make up the majority of emerging infectious diseases, and their prevalence has been associated with a variety of factors, including climate change and the encroachment of human settlements and agriculture on natural ecosystems.

In 2009, the pandemic influenza A (H1N1) outbreak demonstrated the importance of new digital methods for zoonotic disease tracking and response. On April 1, 2009, HealthMap flagged a news story from Mexico detailing a mysterious respiratory illness in Veracruz that killed two people. In collaboration with the *New England Journal of Medicine*'s H1N1 Influenza Center, our group created an interactive map of worldwide cases, posted frequent Twitter updates on the outbreak, and rapidly disseminated breaking news alerts to users (an estimated one million people used



A *Sankey diagram*—in which the width of the arrows is a proportional representation of the flow quantity—shows the association between food contaminant, food category, and geographic location of products coming from China to the United States. It was produced by the SupplyChainMap application, which monitors products under U.S. Food and Drug Administration regulation (food, medical products, cosmetics, dietary supplements, pet food, veterinary medicines, and so on), focusing on cross-border imports from high-risk countries.

HealthMap to monitor H1N1 activity). During the two major waves of the H1N1 pandemic, HealthMap collected more than 87,000 reports from both informal and official sources. We also tracked the rise in the number of countries with informal reports of suspected or confirmed cases.

In March 2013, avian influenza A (H7N9), a subtype of influenza viruses previously detected only in birds, emerged for the first time in humans in China. During this outbreak, HealthMap reported a steady increase in H7N9 cases throughout the country (all associated with exposure to live poultry or potentially contaminated environments), including 38 cases and 10 deaths. Notably, when a Chinese hospital employee shared a picture of the medical record of a patient with H7N9 on the Chinese social media website Sina Weibo, the action was credited with accelerating the government's acknowledgement of new cases.

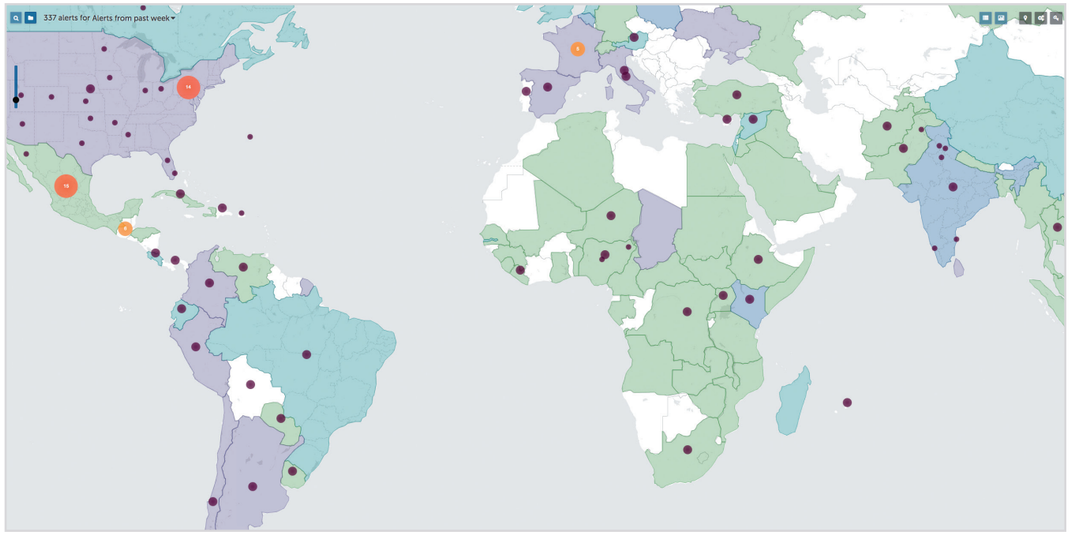
More recently, our work on zoonotic diseases has focused on Ebola, the hemorrhagic fever that swept through West Africa in 2014. Within six months of the outbreak, HealthMap aggregated, classified, and visualized more than 13,000 alerts. The project has since explored in West Africa the correlation between the incidence of digital Ebola reports and reported acts of aggravation (such as riots) or, conversely, with positive public health actions. This qualitative analysis indicated that local aggravating events and regional interventions, as reported in real time by media outlets, were effective proxy measures of changes in Ebola incidence. Further, the software has examined the velocity of the spread of the virus and embedded predictive modeling into the platform. Responding to diseases such as Ebola that spread by human contact requires the cooperation of the public. Patients presenting with symptoms need to be quickly isolated, and burials should be done safely. If the affected population is better informed, public health personnel could focus less on disease control and more on treating those already affected.

In addition to the anti-vaccine movement, the growth of antimicrobial resistance has emerged as a major global threat to public health.

Foodborne Diseases

Diseases can also spread to humans from food tainted with viruses, bacteria, or parasites. The CDC attributes 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually to food-based pathogens and unspecified agents, but many foodborne illness outbreaks often go unreported through official channels. Though the news-making *E. coli* outbreak at the Chipotle restaurant chain caused 55 people to fall ill in 2016, many foodborne cases do not result in a health care interaction, meaning there are no formal reports from health care providers to track.

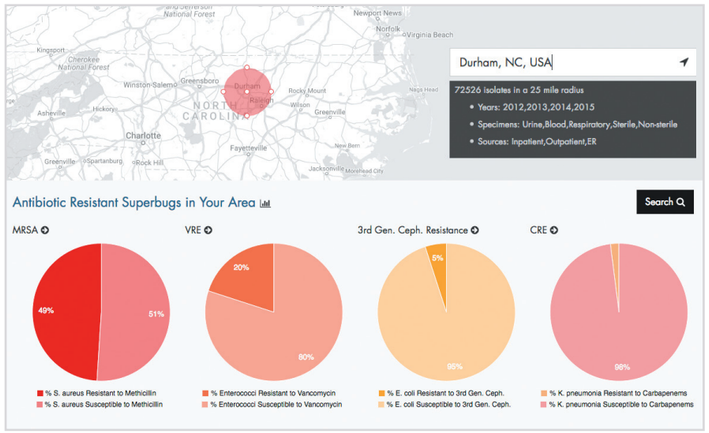
To address this problem, our group tested whether restaurant reviews on Yelp.com (a publicly available business review site) could support foodborne illness surveillance efforts. We obtained reviews from 5,824 food services businesses from 2005 to 2012, and compared digital reports of foodborne illness episodes to official outbreak reports from the CDC. We saw a very similar distribution of foodborne illness reports by implicated foods between CDC and Yelp reports. These findings suggest that social media can provide information on foodborne illnesses, as well as implicated foods and locations, and could complement tra-



This map shows global conversations in mainstream and social media about vaccines, based on data from May 2012 to November 2014. The pins on the map point to vaccination-related articles from more than 100,000 sources, including online news, blogs, expert-curated discussions, and validated official reports. The software producing this map, called Vaccine Sentimeter, provides vital information about public sentiment on vaccines, which can help health-care providers more effectively manage vaccination programs.

ditional foodborne disease reporting. Health authorities such as the Chicago Department of Public Health have adopted the use of social media mining for similar signal detection.

The U.S. Food and Drug Administration (FDA) has also incorporated digital surveillance into its efforts. In January 2011, President Barack Obama signed into law the FDA Food Safety Modernization Act, a sweeping reform that emphasizes the need to enhance surveillance and prevention efforts. In collaboration with the FDA's Office of International Programs, we used HealthMap's core technology as the foundation for a new application that can identify, map, and describe potential contamination in the food supply chain. SupplyChainMap monitors online news and social media for early-warning signals of microbial, chemical, and fungal food contamination among global food suppliers. The application uses automated text processing to tag information regarding location, contaminant type, food group, and company or brand name. Events are categorized as concerning food safety, food fraud, food quality, or food defense. The tool monitors the food supply chain from China to the United States and detects consumer-reported food safety and quality events prior to an outbreak. Preliminary analyses have demonstrated that the system generates timely information regarding food contamination and can effectively trace digitally reported events to commercial trade risks. Dynamic analytics, such as a *Sankey diagram* (a flow diagram that depicts movement from source to destination), allow users to explore the relationship between implicated products, contaminants, and source location; they allow regulatory agencies to decide which manufacturers and importers need inspection and what products require further scrutiny.



An interactive map can show viewers what antibiotic-resistant superbugs are specifically near them. Here, mentions of antibiotic resistance in Durham, North Carolina, are displayed, categorized by four major subtypes: methicillin-resistant *Staphylococcus* (MRSA), vancomycin-resistant enterococcus (VRE), third-generation cephalosporin resistance, and carbapenem-resistant Enterobacteriaceae (CRE).

Other Threats to Public Health

Researchers are increasingly recognizing the value of social media for assessing health-related behaviors and sentiments relevant to disease control. Vaccine hesitancy is a well-known issue in public health and is a driver of vaccine-preventable disease. When a certain amount of the population is not protected against vaccine-preventable, communicable illnesses such as whooping cough and measles, diseases once subdued by modern medicine can reemerge.

To keep abreast of vaccine-hesitant conversations online and enable proactive and targeted communication by public health professionals, we adapted our technology so that it uses vaccine-specific search taxonomy and categorizes content by sentiment toward more than 30 vaccines. This software, called Vaccine Sentimeter, enables researchers to follow specific events associated with changes in public sentiment toward vaccines and analyzes online conversations on the topic. For example, when the American TV show *Katie* featured potential severe side effects of the human papillomavirus vaccine in an episode, data showed that the immediate reaction on mainstream and social media was critical of the show, citing lack of scientific information and balanced information, indicating support for the vaccine. This immediate vaccine-positive reaction waned quickly, however, while vaccine-negative reactions persisted on social media.

In addition to the antivaccine movement, the growth of antimicrobial resistance has emerged as a major global threat to public health. The danger is growing in every region of the world, and in some cases has rendered useless antibiotics once considered to be very strong and effective. In the United States, the CDC reports an estimated 2 million illnesses and 23,000 deaths are attributed to antibiotic-resistant bacteria or fungi each year. With few replacements to existing drugs on the horizon, some scientists warn we could be entering a postantibiotic era.

Our group has delved into this topic, using online open-source data and applying a search taxonomy that includes not only mentions of drug resistance cases but also specific pathogens and mechanisms of resistance. Further, public hospital *antibiograms*, lab tests performed to determine the sensitivity of isolated bacterial strains to drugs, have been manually collected and entered into the system. Aggregating these data, the system displays rates of antibiotic resistance for various drugs and pathogens, specific to user-selected areas.

Challenges of Disease Surveillance

The use of informal data sources for digital surveillance, though game-changing, presents several challenges. The sheer volume of Web-based information is daunting, and can make it difficult to pluck a signal from noise. These data are unstructured, requiring computational methods such as machine learning and natural language processing to make sense of the data. Additionally, there is the potential for false reports, which can include misinformation, disinformation, or reporting bias. All of these challenges are a good reminder that HealthMap data complements traditional public health data. Although digital surveillance can make the public a stakeholder in outbreaks, it can also complicate risk communication. Nonetheless, the value of news and social media for digital disease detection is undisputable.

Important questions remain about how to systematically ensure patient confidentiality in an environment where social media users post their information publicly. Our group takes care to present data in aggregate or otherwise de-identify public data as well as offer opt-out options in our social media data aggregation. The industry as a whole, along with academia and government, will need to establish more guidelines and policies as the field moves forward.

Based on our 10-year experience with HealthMap, we anticipate that digital surveillance will produce even more comprehensive views and interactive analyses of distilled data and insights to further advance population health. Maps with a multitude of juxtaposed data layers—including weather, geography, land use, and endemic diseases—will give the military insight into how risky it is to drop a paratrooper on the ground, for example, or tell an emergency-response worker whether it is wise to enter a questionable zone. As a changing world and overwhelming data make for a more complicated picture of public health, it is our hope that a data-driven approach will create a clearer picture of health threats, and help everyone better manage their own risk and exposure to disease.

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Influenza

The world is teetering on the edge of a pandemic that could kill a large fraction of the human population

Robert G. Webster
Elizabeth Jane Walker

It has all the makings of a cheesy Hollywood horror flick: A shape-shifting killer travels the globe, leaving millions of corpses in its wake, and the world's medical community can't stop the carnage. It's a sophomoric idea for a movie script, but that's exactly what unfolded during the waning months of the first World War, late in 1918, and through much of 1919. Within 10 months the influenza virus affected the lives of up to 500 million people across the globe and killed at least 20 to 40 million—more than twice the number who died on the battlefields of World War I. Many epidemiologists believe that a similar scenario will happen again. But this time it will be worse.

This is not hyperbole. In 1997 the world came perilously close to another global epidemic of the "flu." If this particular virus had attained the ability to spread from person to person, the pandemic might have taken the lives of a third of the human population. As it was, only six people died—and all of them had contracted the virus from chickens sold in Hong Kong poultry markets. The only thing that saved us was the quick thinking of scientists who convinced health authorities to slaughter more than a million domesticated fowl in the city's markets. The avian virus turned out to be a new strain—one that the human population had never seen before. These deadly new strains arise a few times every century, and the next one may arrive any day now.

Most of us are reminded of influenza every autumn when the medical community invites the public to receive the annual "flu shot," or when we succumb to a mild form of the disease during the winter months. Symptoms typically include fever, chills, sore throat, a lack of energy, muscular pain, headaches, nasal congestion and a suppressed appetite. But the flu can quickly escalate, prompting bronchitis, secondary infections, pneumonia, heart failure and, in many cases, death. Infants, the elderly and people with suppressed immune systems are at highest risk of dying from the flu. People who have serious conditions such as lung or cardiovascular disease are also in danger. The exception to these risk factors occurred in the 1918 "Spanish flu" pandemic, when almost half of the people who died were between the ages of 20 and 40. It's still not clear why previously healthy people in this age bracket had such high mortality rates.

Lesser influenza pandemics took place in 1957 (the "Asian flu") and 1968 (the "Hong Kong flu"). There were also flu "scares" in 1976 (the "swine flu") and in 1977 (the "Russian flu"). Precisely how and when the influenza virus will develop into an extremely pathogenic form is beyond our current ability to predict. We understand the virus's structure, how it enters the cells of the human body and how it evades detection by the host's immune system, but knowing these things is not enough to stop another pandemic. The issues extend beyond science into the realms of international and local politics, national budgets, and deeply entrenched cultural traditions. The purpose of this article is not to instill fear, but to educate—the more people there are who understand the problems, the more chance we will have to contain the next outbreak.

In-Flew-Enza

Influenza is spread from person to person by coughs and sneezes, but the virus doesn't begin its journey in a human host. Instead, wild aquatic birds such as ducks and shore birds perpetuate the influenza viruses that cause human pandemics. Although these birds carry the genes for influenza in their intestines, they usually don't become sick from the virus. And because they can migrate thousands of miles, the healthy birds can spread the virus across the globe even before the microbe makes contact with the human population.

As it happens, the form of the virus found in wild birds doesn't replicate well in human beings, and so it must first move to an intermediate host—usually domestic fowl or swine—that drinks water contaminated by the feces of aquatic birds. Horses, whales, seals and mink are also periodically infected with influenza. Although the intermediate hosts can sicken and die from the infection, swine can live long enough to serve as "mixing vessels" for the genes of avian, porcine and human forms of influenza. This occurs because swine have receptors for both avian viruses and human viruses.

Swine have probably played an important role in the history of the human disease. These animals appear to serve as living laboratories where the avian and mammalian influenza viruses can come together and share their genes (a reassortment of RNA segments) and create new strains of flu. When a strain of virus migrates into the human population, it changes into a disease-causing microbe that replicates in the respiratory tract. A sneeze or a cough spreads the virus in a contagious aerosol mist that is rich in virus particles.

Most pandemics originate in China, where birds, pigs and people live in close proximity. Hong Kong's 1997 "bird flu" was an avian influenza virus that probably attained virulence through reassortment of genes from geese, quail and teal. Many bird species were housed together in the Hong Kong poultry markets, and this was an ideal environment for reassortment. This strain of influenza killed thousands of chickens before it moved to human beings. Eighteen people were infected—all through direct transmission from chickens, not from contact with other people. In this instance, the outbreak was curtailed before the virus could mutate into a form that could spread from person to person. Scientists had known since 1972 that the influenza virus originated in aquatic birds, but the 1997 epidemic was the first case to document influenza's direct transference from poultry to people.

Anatomy of a Killer

Influenza viruses are members of the Orthomyxoviridae family, and they fall into one of four genera—A B, C and thogotovirus, which is a tick-borne virus. Type C influenza does not seem to cause serious disease. The type B virus, recently isolated from seals in Holland, often creates regional epidemics in human populations. But type A influenzas have avian lineages, and these are the viruses that cause human pandemics.

The influenza virus contains eight separate RNA segments that encode genes for at least 10 proteins. This unusual genetic structure explains why reassortment happens so often. If two different viruses infect the same cell, an exchange of gene segments can easily take place, yielding up to 256 (or 2^8) different offspring.

Type A influenzas are categorized by the structural variations of two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which protrude from the surface of the virus. HA's job is to attach the influenza virus to the sialic acid receptors on the surface of the human cell. After binding, the flu virus penetrates the host cell; there, viral RNA strands move into the cell's nucleus. The viral RNA strands encode messenger RNA and ultimately produce new

Figure 1. Hong Kong poultry markets saw the slaughter of more than one million birds in 1997 to prevent the spread of the "bird flu" in the human population. The 1997 outbreak of the H5N1 influenza virus was the first direct evidence that avian influenza viruses could be transmitted to human beings. Eighteen people were infected and six died, suggesting a high rate of virulence. The extent of the outbreak was curtailed by the destruction of the birds. At press time, H5N1 has reappeared in Hong Kong poultry farms, live-bird markets and, most alarmingly, in the area's free-flying wild birds. The virus is causing significant mortality in aquatic birds in Kowloon Park. But to date there is no evidence that this virus has been transmitted to people. Here author Robert Webster (left) briefly pauses with a colleague in a wholesale poultry market in 1997 on the day that all of the live poultry in Hong Kong were destroyed. (Image courtesy of the authors.)

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virus particles. The task of NA is simple but important: It enables the newly created virus to separate from the host cell and travel freely from one cell to another through the respiratory tract.

Scientists have identified 15 HA and 9 NA subtypes, all of which are found in avian hosts. Epidemics occur when the HA or the NA proteins mutate. The subtypes of type A viruses are named according to the particular variants of the HA and NA molecules they contain—such as H1N1, the culprit in the 1918 holocaust and the 1976 "swine flu" scare, or H5N1, the "bird flu" of 1997.

Influenza's unpredictability springs from its ability to alter its HA and NA surface proteins and so avoid identification by the host's immune system. When a person is infected with the flu, the immune system produces antibodies and cell-mediated responses against all of the virus's gene products (antigens). If the person later encounters the same virus, his antibodies will bind to it and prevent an infection. However, the virus can alter antigenic sites—points on the HA and NA molecules where the antibodies would normally bind—by the process of antigenic drift. In DNA-based genomes a proof-reading enzyme carefully scrutinizes the process of copying a strand of DNA, catching and correcting any mistakes made during replication. But, like other RNA-based viruses, the influenza virus lacks a proofreader, so mistakes made during replication go uncorrected and the virus can mutate swiftly. The mutations can change the antigenic sites in such a way that the host's antibodies no longer recognize the virus.

The HA and NA molecules are particularly important in antigenic drift. As genetic point mutations are gradually accumulated by the viral genome, and the HA or NA genes and proteins have undergone several minor changes, the host's antibodies no longer recognize them, and the person may sicken again. Type B influenza strains use this process to alter the amino acid structure of these proteins and so evade the human immune system.

Every 20 to 30 years or so, the type A influenza virus undergoes an antigenic shift. If antigenic drift were compared to a shudder, antigenic shift would be likened to an earthquake. Antigenic shift engenders a much more immediate and dramatic change in the HA glycoprotein. During antigenic shift, genes from other influenza subtypes can completely replace the HA and NA proteins with new ones that the host has never experienced. When human immune systems cannot recognize the new virus, a pandemic ensues.

Scrutinizing the Shift

Influenza was first described by Hippocrates as early as 412 B.C., and the tiny virus has spent the succeeding centuries shifting, drifting and wreaking havoc. Humanity has been seeking ways to eliminate the threat since the first pandemic was recorded in 1580. Although the Spanish flu happened nearly a century ago, the extreme pathogenicity of the H1N1 1918 influenza virus is still not understood. Virologists have traveled the world to obtain samples of the virus so that they could unlock the secrets of its virulence, even exhuming victims from the Alaskan and Norwegian permafrost. Jeffery Taubenberger and his colleagues at the Armed Forces Institute of Pathology studied bodies and fragments of lung samples that had been stored in paraffin blocks since 1918. Through sequence and phylogenetic analysis of RNA fragments taken from the lung tissues, they determined that the virus was avian in origin but was closely related to a strain of influenza that is known to infect swine. Ongoing studies of the total genome sequence may someday uncover reasons for the potency of this strain of influenza. If they can understand the genome of the virus and the genome of the host, scientists may be one step closer to pinpointing which viruses in the wild will cross over to human beings.

When the H5N1 virus leapt from poultry to people in 1997, scientists from the World Health Organization (WHO) immediately began to investi-

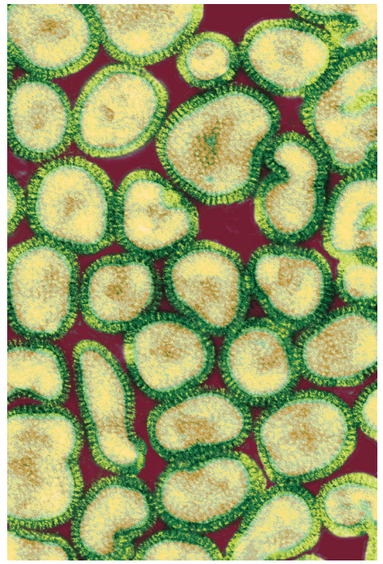
I had a little bird …

Nearly 500,000 people died of the flu in the United States from 1918 to 1919. In many cities, public gatherings were prohibited, livestock markets were closed and coffins were in short supply. American troops unwittingly participated in biological warfare as they carried the "Spanish flu" to the battlefields of Europe during the first World War. Forty percent of the healthy young Americans who shipped out for Europe succumbed to influenza rather than the flying bullets on the battlefield. In time, the enormity of this event was lost to our cultural memory.

National Museum of Health and Medicine

An army hospital at Camp Funston, Kansas, is filled with soldiers suffering from the influenza epidemic near the end of World War I.

After the 1918 influenza epidemic in the United States, little girls jumped rope to a new rhyme.



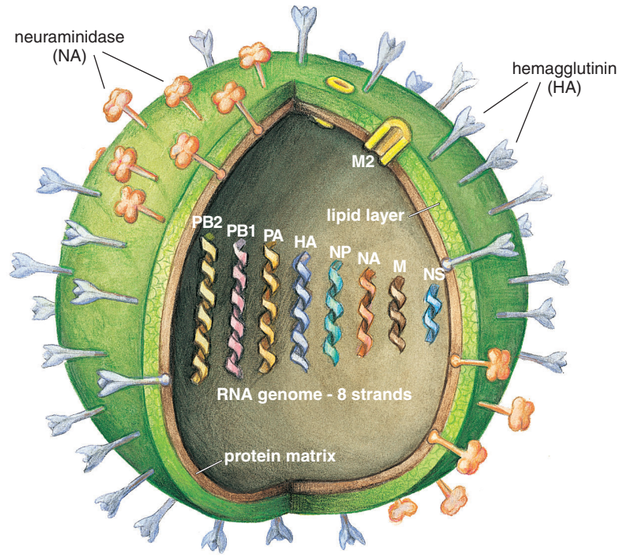


Figure 2. Type A influenza viruses—which are responsible for human pandemics—are spheroidal particles (left), about a tenth of a micrometer across. The virus is characterized by a relatively simple structure (right): an internal nucleocapsid, containing the viral genome, and a surrounding envelope consisting of an inner matrix protein, a lipid bilayer and external surface proteins. The genome consists of eight single-stranded RNA segments that code for 10 proteins: PB2, PB1, PA, HA (hemagglutinin), NP (nucleoprotein), NA (neuraminidase), M1 (matrix protein), M2 (ion-channel protein), and two nonstructural proteins, NS1 and NS2. Subtypes of the type A virus are classified according to structural variants for the two surface proteins: hemagglutinin (15 variants—H1 to H15) and neuraminidase (9 variants—N1 to N9). An ion-channel protein (M2), embedded in the lipid bilayer, is a target for the antiviral drugs amantadine and rimantadine, which inhibit the protein's function. (False-color micrograph courtesy of Gopal Murti, St. Jude Children's Research Hospital.)

gate the phenomenon. Postmortem examinations of two victims revealed unusually high levels of cytokines—proteins such as interferon and tumor necrosis factor-alpha (TNF-α)—that regulate the intensity and duration of the immune response. These cytokines are the first line of defense against viruses. They are part of the innate immune response, a nonspecific response that will target any pathogen and does not require a previous exposure to a virus (unlike the production of antibodies, which does require the exposure to viral antigens). Studies of human macrophage cultures, by Malik Peiris and his colleagues in Hong Kong, show that the H5N1 virus causes an exaggerated response of cytokines (such as TNF-α), and this could result in a toxic-shock-like syndrome (including fever, chills, vomiting and headache), which ultimately results in death. Although the cytokines can sometimes inhibit its proliferation, the virus may develop strategies to subvert this innate immune response.

This is what happened in Hong Kong. The H5N1 virus found a way to circumvent the effects of the infection-fighting cytokines. Sang Seo, Erich Hoffmann and author Robert Webster at St. Jude Children's Research Hospital used reverse genetics—the opposite of the traditional gene-to-protein direction of genetic analysis—to identify a gene that played a crucial role in the transformation of the influenza virus. The new technology offers many tantalizing opportunities: It might drastically reduce the time required for vaccine production, and it might help scientists gain insights into viral pathogenicity.

We removed the so-called nonstructural (NS) gene from H5N1 and inserted it into a previously benign strain of flu. Experiments showed that the newly transformed virus was considerably more virulent in swine. Pigs infected with a virus that carried the NS gene experienced much more severe and prolonged fever, weight loss and viremia than pigs that were not infected with a virus containing that gene. This suggests that the product of the NS gene, the NS1 protein, plays a crucial role in limiting the antiviral effects of the cytokines. According to Adolfo García-Sastre and Peter Palese of the Mount Sinai School of Medicine, and their colleagues, the NS1 protein seems to do this by downregulating the expression of genes involved in the molecular pathway that signals the release of the cytokines.

Gene sequencing reveals that a single point mutation occurred in the NS gene of the Hong Kong virus. This changed the identity of an amino acid—glutamic acid at position 92 in the NS1 protein—which produced a version of the protein that was much more effective at downregulating the activation of the cytokines than the normal version. This made the Hong Kong virus much more virulent than other influenza viruses—a remarkable consequence for such a tiny alteration. These discoveries might help us to understand the extreme pathogenicity of the 1918 influenza virus, and they perhaps suggest new targets for drug development.

In 2001 a new variety of the H5N1 virus surfaced in the live poultry markets of Hong Kong, but this time the fowl were slaughtered before people

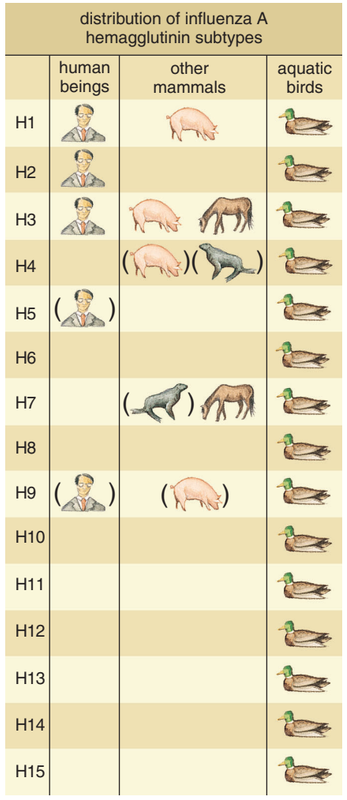


Figure 3. Hemagglutinin subtypes—H1 to H15—have been found in various combinations among human beings, mammals and birds. All 15 subtypes are present in avian species, mostly aquatic birds. Five subtypes have been found in human beings, but the H5 and H9 subtypes have not established lineages in our species. Similarly, H4 and H7 have been isolated in seals, and H4 and H9 have been isolated in swine, but these subtypes have not yet established lineages in these animals. Aquatic birds usually don't become sick from the virus, and they are believed to be the living repositories for all subtypes of the type A viruses. (Adapted from a graphic by the International Influenza Education Panel.)

could become infected. Yet another genotype of H5N1 appeared in 2002. This evidence indicates that viruses similar to the 1997 strain are still circulating in the bird population of Southeast China. They are reassorting and making new versions of H5N1—not the same virus that surfaced in 1997, but different mutations that retain the same HA and NA configurations.

Prevention and Treatment

The changeable nature of the influenza virus ensures that it can escape immune surveillance and circumvent the body's defense mechanisms. Moreover, the influenza vaccine that protected humans against infection last year may be ineffectual this year. Scientists at more than 100 WHO laboratories are constantly collecting and analyzing the influenza viruses that circulate in the human population worldwide. After isolating the viruses for antigenic and molecular analysis, WHO scientists annually identify two type A strains and one type B strain that are most likely to cause epidemics during the coming season. Vaccine manufacturers then incorporate all three strains into the vaccine composition that will be used for that year. The resulting flu shots protect individuals only from the targeted strains—not from unexpected viruses that may arise after the WHO determination has been made.

Years ago, influenza vaccines were impure, whole-virus vaccines that caused the recipients to run fever and display other flu symptoms. Most pharmaceutical companies today split the virus into subunit vaccines, which contain only specific viral protein units. To create the vaccine, technicians grow the vaccine virus in fertile hens' eggs. The virus is then inactivated (so that it cannot cause infection) and purified. Because these vaccines are not made of live viruses, but only purified portions of those viruses, immunization promotes immunity but does not cause infection. The human immune system then creates antibodies that attack viruses containing those proteins.

Several other types of vaccines are also in development. Some evidence suggests that weakened live-virus vaccines may prompt a more protracted immune response than subunit vaccines. Recent clinical trials by Robert Belshe of St. Louis University School of Medicine and William Gruber of Vanderbilt University have indicated that a new nasal spray containing such a live-virus vaccine is safe and effective in both children and adults. DNA vaccines and vaccines created through the use of reverse genetics may also prove useful someday.

The classic way to create a seed virus for production of an influenza vaccine is to generate a virus that contains six genes from a high-yield virus such as H1N1 and two genes (HA and NA) from circulating strains. This method of creating a seed virus is cumbersome and time-consuming. Recently, however, scientists at St. Jude discovered how to generate the high-yield virus using eight plasmids (laboratory-made molecules of double-stranded DNA, which is made from viral RNA). This eight-plasmid system allows for the rapid generation of reassortment influenza A viruses, which can be used as master virus seeds for the manufacture of vaccines.

Another goal is to find ways to produce vaccines more quickly. When a pandemic occurs, pharmaceutical companies must manufacture a vaccine as quickly as possible, while they incorporate procedures to ensure that the drugs are both safe and effective. The time required to produce, test and distribute a new flu vaccine ranges between seven and eight months, so it's virtually impossible to produce an adequate amount of vaccine during a pandemic. In 1976, laboring in the shadow of an expected "swine flu" pandemic, American drug manufacturers produced 150 million doses of vaccine—enough for the entire U.S. population. Given today's increased population and stringent regulatory processes, vaccine production might take much longer. A number of groups are seeking ways to drastically reduce that production time.

Flu outbreaks generally pose the most serious threat to people who are very young, elderly, immunosuppressed or chronically ill. In the United States, vaccination is suggested for people who are at least 50 years old or deemed to be at high risk for infection. Canada is a little more progressive—in Ontario, vaccinations are available at no charge for citizens older than 6 months. People infected with the flu have a high risk of dying from bacterial pneumonia. Pneumococcal pneumonia kills thousands of elderly people in the United States each year. During an influenza pandemic, this mortality rate skyrockets. Vaccines are now available to protect people against almost all of the bacteria that cause pneumococcal pneumonia and other pneumococcal diseases.

For years, antiviral drugs such as amantadine and rimantadine occupied the front line of influenza treatment. These drugs obstruct the function of an ion-channel protein called M2. When taken during exposure to influenza, these M2 inhibitors may help prevent infection, and if infection has already

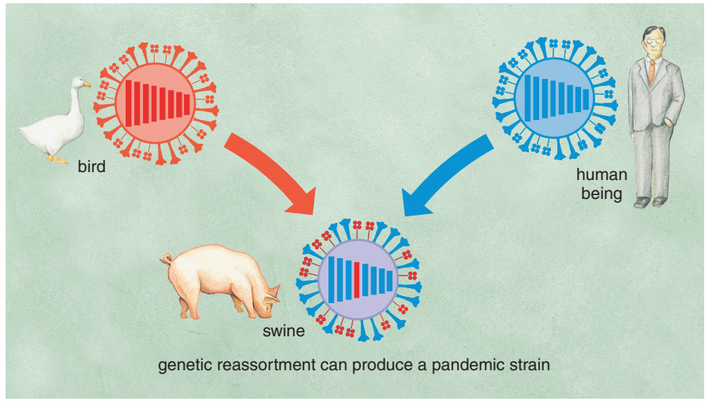


Figure 4. Swine serve as "mixing vessels" for the genes of avian, porcine and human forms of the influenza virus. In the host pig, the avian and mammalian viruses can share (reassort) their genes and so create new strains of flu. Swine have probably played an important role in the history of human influenza epidemics.

taken hold, their early administration may reduce the severity and the duration of the symptoms. But because type B influenzas do not possess M2 molecules, the drugs are effective only against type A influenza. More important, all strains of influenza quickly acquire resistance to these drugs.

Two families of antiviral drugs have been developed that are less prone to resistance, have fewer adverse side effects than M2 drugs, and are effective against types A and B influenza. These antineuraminidase drugs hobble the NA glycoprotein on the surface of the influenza virus. When NA is inhibited, the virus is unable to release itself from the host cell to spread infection—it simply gets stuck and dies. If administered soon after the initial infection, NA inhibitors such as zanamivir and oseltamivir can effectively prevent viral replication.

Preparing for a Pandemic

When the H1N1 virus crossed the globe in 1918–19, physicians watched helplessly as their patients succumbed quickly to pneumonia and other complications of influenza. The suffering patients had no access to antibiotics, vaccines or antivirals. Today we live in a world where air travel is common. A tourist in Hong Kong can spread the virus around the globe within hours. Whether a pandemic comes about as a result of natural forces or bioterrorism, the world is currently unprepared for the onslaught.

Unfortunately, another pandemic is inevitable. Historically, pandemics sweep the globe several times every century. Thanks to the efforts of the World Health Organization, scientists

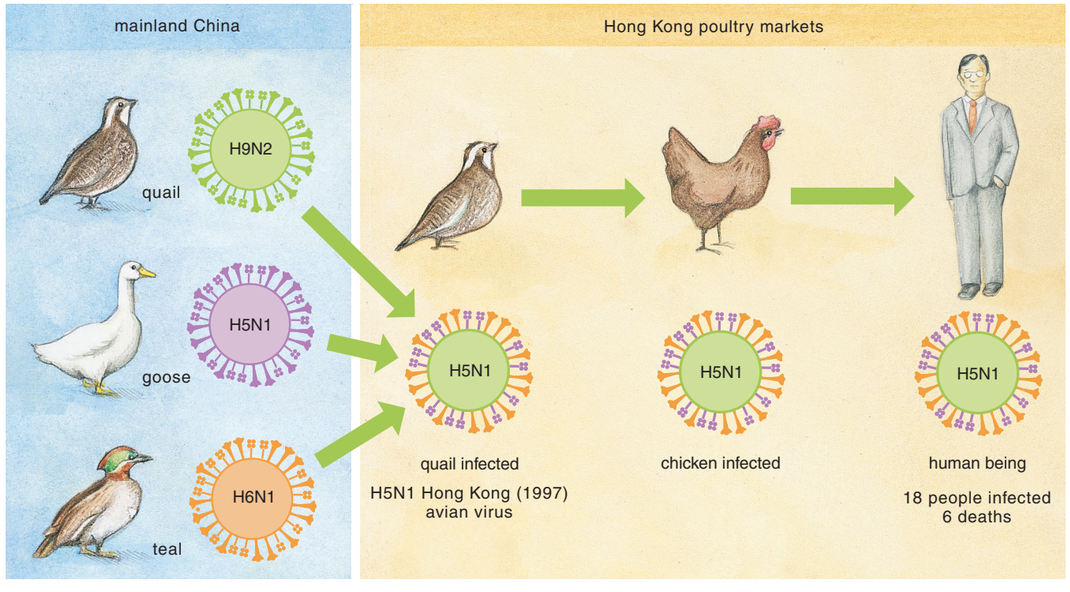


Figure 5. Hong Kong's deadly H5N1 (1997) virus is believed to be a product of three viral strains found respectively in quail, geese and teal from mainland China. The birds are housed together in Hong Kong's poultry markets, which provides an optimal environment for genetic reassortment. Surveillance studies in the poultry markets have shown that quail are susceptible to all tested strains of influenza viruses, and that they can serve as an intermediate host between ducks and chickens. Quail may have been very important in the genesis of the 1997 H5N1 influenza virus, which killed six people. To reduce the probability that other H5N1 flu viruses will emerge, quail are now banned from the live chicken markets in Hong Kong. The H5N1 virus re-emerged in Hong Kong's poultry markets in 2001, necessitating the slaughter of all poultry for the second time in four years. Poultry markets appear to play an important role in the emergence of reassortant influenza viruses.

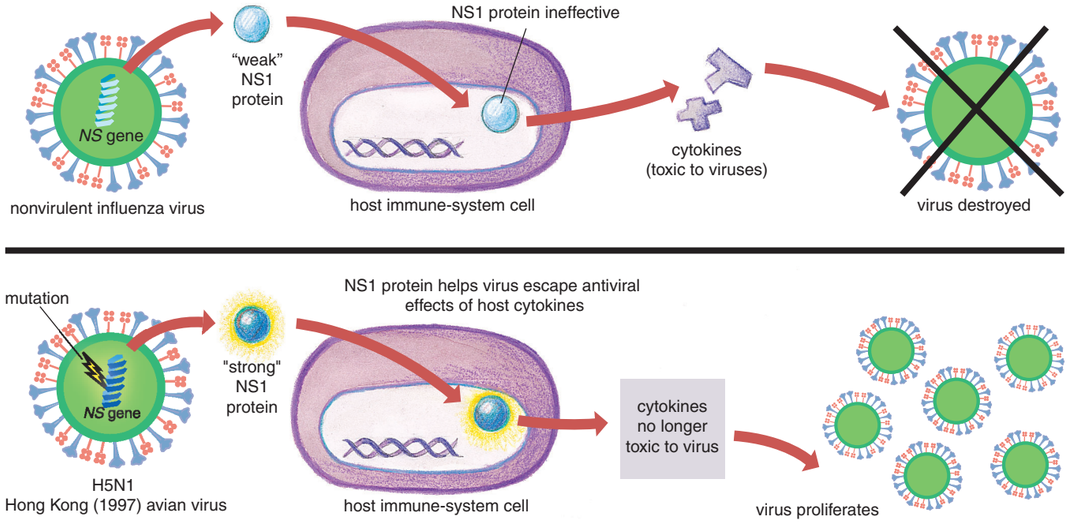


Figure 6. Variants of the NS1 protein may help the influenza virus escape the host's immune system. Nonvirulent forms of the influenza virus produce a "weak" NS1 protein, which doesn't appear to protect the virus against immune-system molecules, such as cytokines, produced by the host (top). However, the H5N1 (1997) avian virus that killed six people in Hong Kong appears to have been especially lethal because a point mutation in the NS gene produced a "strong" version of the NS1 protein (bottom) that helped the virus elude the antiviral effects of cytokines.

are conducting surveillance studies of the influenza virus at the animal-human interface. That surveillance probably prevented a worldwide catastrophe in 1997. Virologists in thousands of laboratories are trying to predict the virus's movements. By learning, for example, how one mutation in the "bird flu" helped that virus circumvent cytokine responses, they are a step closer to understanding influenza's evolutionary processes, and to developing drugs to combat the virus's effect. But even the most sophisticated methods and the latest discoveries offer no guarantee of predicting the next pandemic.

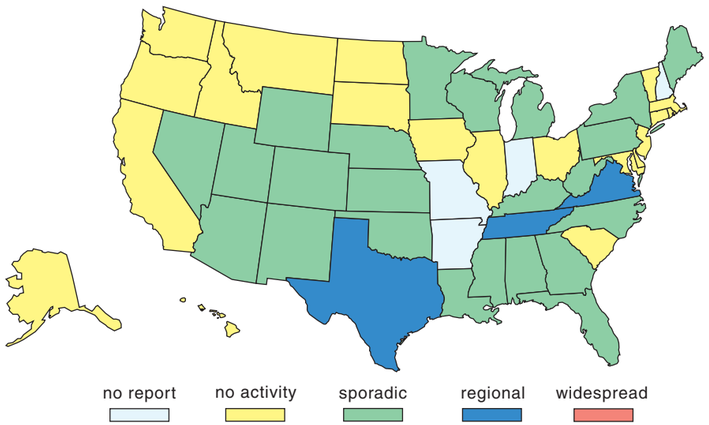


Figure 7. Incidence of influenza cases is monitored in the United States on a weekly basis from October through May as part of a global surveillance of the virus. Among other things, the surveillance tracks the type of flu circulating, whether there are changes in the virus and the impact the flu has on the number of deaths. In early January, the Centers for Disease Control and Prevention (CDC) in Atlanta reported that the incidence of flu-related deaths in the U.S. has increased in the past three decades largely because the aging population is more vulnerable to the disease. Here the influenza activity for the week ending on December 28, 2002 is shown for each state as reported by epidemiologists to the CDC. (See www.cdc.gov/ncidod/diseases/flu/weekly.htm)

Asia—particularly Hong Kong—has been identified as the epicenter for influenza pandemics. After the 2001 outbreak of H5N1 in Hong Kong, a new regulation was installed: All poultry must be removed from the markets on a specific day each month to minimize the chance of viral replication. A better solution to the problem would be to replace the live poultry markets with markets selling frozen or refrigerated meat. But the poultry markets are an integral part of the Hong Kong economy and its culture, so they aren't likely to be eliminated in the near future. Similar live-poultry markets in New York City should also be closed. Because of cultural mores, politics and entrenched traditions, however, the Hong Kong and New York markets will likely remain open until another pandemic erupts, forcing the issue.

When a virus does manage to evade the scientific community's gatekeepers, it may travel the world in a matter of hours. Fewer than a dozen companies worldwide currently manufacture flu vaccine (in the U.S. there are only two companies making vaccine), and even though the influenza outbreaks of the past two years have been relatively mild, these companies have had difficulty meeting the demands for vaccine. Subunit vaccines take months to create, so vaccine manufacturers will be incapable of producing enough vaccines to subvert the progress of a pandemic. M2 inhibitors such as amantadine and rimantadine may be useful if the virus does not acquire resistance to their effects. The NA inhibitors offer the most promise for treatment options in the event of a pandemic, but they are expensive and in short supply. For NA inhibitors to be effective, they must be administered soon after the initial infection. Drug companies require a year and a half to produce adequate quantities of antivirals. Unless production and stockpiling of drugs begins well in advance of a pandemic, adequate supplies of antivirals will not be available.

WHO and the developed nations of the world have created pandemic plans that specify ways to prepare for a world crisis. In the United States, the plan includes measures for improving surveillance systems and increasing the breadth of the country's vaccination programs. The plan also supports research into detection of new strains and the creation of new vaccines and antivirals. The national pandemic plan addresses such topics as communication systems, as well as medical readiness and how community services will be maintained. One way to prepare for the inevitable pandemic is to vaccinate as many people as possible during the interpandemic years. No-cost, universal vaccine programs such as the one in Ontario offer perhaps the best way to increase the capacity to make a vaccine in a crisis. The nations of the world must develop these plans.

If a pandemic happened today, hospital facilities would be overwhelmed and understaffed because many medical personnel would be afflicted with the disease. Vaccine production would be slow because many drug-company employees would also be victims. Critical community services would be immobilized. Reserves of existing vaccines, M2 inhibitors and NA inhibitors would be quickly depleted, leaving most people vulnerable to infection. The nations of the world spend untold billions on military equipment, stockpiling bombs and other weapons. But governments have not invested a fraction of that amount into stockpiling drugs for defense against influenza. The scientific community has a responsibility to convince nations to stockpile NA inhibitors and promote vaccine production. The cost to developed nations would be minuscule, compared with the social and economic disaster that will occur during a full-scale pandemic.

Acknowledgments

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Survival of the Fittest Molecule

Biochemists harness a novel form of evolution to sculpt new compounds for the fight against dengue fever, cancer and other modern plagues

Robert G. Webster
Elizabeth Jane Walker

Over billions of years, life has evolved into a spectacular diversity of forms—more than a million species presently exist. For each, the source of its uniqueness is the particular constellation of proteins found within its cells. Yet in the midst of this diversity, the similarities between living things are profound. For example, although the fruit fly genome encodes about 14,000 different proteins, and humans have two to three times that number, many proteins are still recognizably similar in sequence and task, reflecting their common ancestry. In fact, when scientists have put human disease genes into flies, they often cause the same symptoms in the insects as they do in people. Furthermore, addition of a normal human gene can sometimes compensate for the deletion of the same gene from the fly.

The differences that do exist between equivalent genes in flies and humans are the result of 900 million or so years of DNA mutations—the amount of time that has passed since the divergence of arthropods (including fruit flies) and chordates (including human beings). Some of these mutations also changed the sequence of amino acids in the proteins encoded by these genes. Out of these modified proteins, a small fraction worked in a superior or novel way and gave some advantage to their bearers; ultimately, organisms with the mutation left more descendants than those without it. In other words, mutation provides the raw material for natural selection.

Evolution also works over shorter timescales. Anatomically modern humans are probably less than 170,000 years old (see "We Are All Africans," page 496), yet the genetic diversity among living Homo sapiens runs to several million mutations, also known as polymorphisms, which form the basis for a vast number of combinations, each with a potentially different set of properties. Because preserving a low mutation rate is important for complex organisms, the principal source of functional genetic diversity is recombination between sister chromosomes, a process that creates new combinations from existing point mutations. These rearrangements are not necessarily beneficial—many are neutral or harmful—but the process is endlessly iterative, creating in each generation novel combinations of mostly old mutations. Indeed, recombination followed by natural selection is the foremost mechanism of organic evolution.

Now biomolecular engineers seeking to craft proteins that will perform certain functions have co-opted this evolutionary strategy. Pharmacologists and industrial biochemists are patently interested in tailoring proteins to individual needs, and developing the tools to create these molecular thoroughbreds is crucial. In this article, we discuss the importance of custom-built proteins and describe some of our own breakthroughs that have dramatically increased the speed and efficiency of their creation.

Biotech Promise

Proteins make and maintain life, so they play an understandably central role in biomedical science. Many human maladies come from disease genes that cause specific protein changes, including hemophilia, muscular dystrophy and sickle cell anemia. While some of these altered-protein diseases remain difficult to correct, others can be treated. In the case of classic hemophilia, replacing the blood-clotting factor VIII protein that is missing or reduced in people with the disease can lessen the chronic bleeding problems. The original source for this purified protein was plasma from human blood, but in 1984 the normal version of the factor VIII gene was inserted into a microbial host. These transformed bacteria were designed to produce massive quantities of the human factor VIII protein, and in 1990 a large clinical trial demonstrated the success of the new drug. The recombinant protein was safer as well as more efficient to produce: Bioengineered factor VIII freed patients from the risks associated with blood borne diseases such as HIV and hepatitis.

For all its promise, the biotechnology revolution is still relatively young. The first therapeutic use of genetically engineered human proteins was only 25 years ago, after recombinant insulin was produced in bacteria. Such therapies have exploded since then, aided by the increasing sophistication of tools for manipulating DNA. The main advance was the brilliantly simple use of a combination of two well-studied classes of bacterial enzymes: restriction endonucleases and ligases. These enzymes cut and rejoin, respectively, pieces of DNA in a sequence-specific, origin-independent manner. This allowed molecular biologists to create hybrid DNA molecules from different species, meaning that genes could be copied, or cloned, endlessly by putting them into fast-dividing bacteria. Likewise, the proteins encoded by DNA could be made in quantities that were impossible to get from the original sources.

Modern molecular technologies now provide unprecedented access to the enormous diversity of natural proteins. However, these proteins tend to be very dependent on the cellular context in which they evolved. When they are removed from the original cellular milieu and produced and assayed in a different environment, the proteins are apt to perform poorly or not at all.

Improving Nature's Design

In light of this inherent limitation, an important goal of biotechnology is to re-optimize molecules to preserve or enhance their function. For example, some industrial enzymes need to be modified for expression at extremely high levels, yet retain their function under harsh physical conditions. In the pharmaceutical industry some human proteins may require a different receptor-binding property or circulatory half-life to survive therapeutic administration. Another example comes from our own lab, where we are optimizing a viral vaccine to induce a strong and broadly protective immune response. This is a challenge because viruses have evolved specifically to evade the immune systems of their hosts.

At present two different but complementary strategies are being pursued for the optimization and redesign of proteins. They are generally known as rational design and directed evolution.

Rational design, also known as computer modeling, attempts to modify or create molecules for specific applications by predicting which amino acid sequence will produce a protein with the desired properties. Using a combination of x-ray crystallography, three-dimensional structure determination and computer-simulated amino acid interactions, biochemists can often predict which amino acid substitutions are the best candidates to elicit the desired change in a protein.

Unfortunately, the task of accurately modeling protein function is Herculean—there are a staggering number of interdependent variables that influence protein function. Cells go through

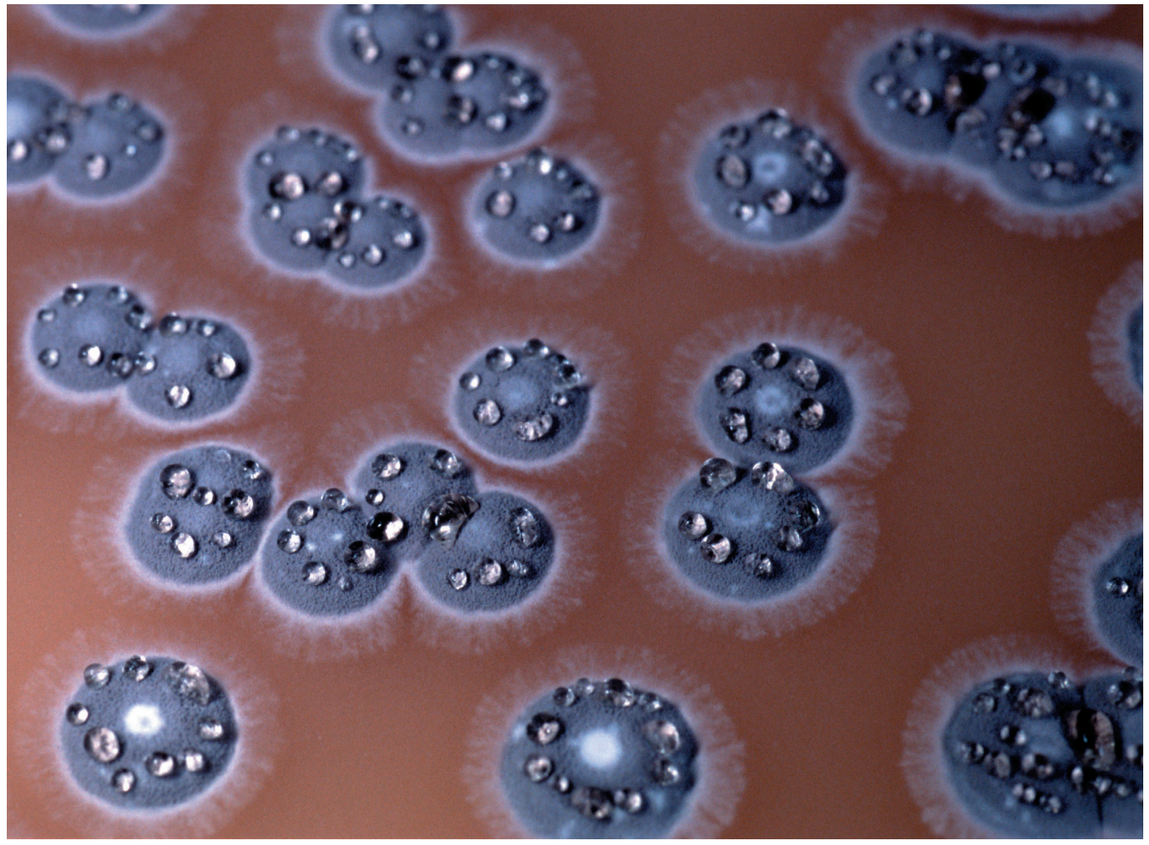

Figure 1. A strain of the bacteria Streptomyces ambofaciens produces the antibiotic polyketide spiramycin, seen as beads of clear liquid on the surface of each blue colony. In this article, the authors discuss their development of strategies to optimize the function of proteins and entire bacterial genomes by mimicking in vitro the natural process of DNA recombination—an approach that they term "DNA breeding." (Photograph courtesy of Tobias Kieser, John Innes Centre, Norwich, U.K.)

Willem "Pim" Stemmer invented DNA breeding and co-founded Maxygen Incorporated in 1997 to develop the technology. Currently, Maxygen and its subsidiaries, Codexis and Verdia, are focused on applying this technique to pharmaceuticals, vaccines, chemicals and agriculture, and Pim has co-founded a new company called Avidia Research Institute to focus on antibody-like molecules. Brett Holland is an assistant professor of biology at California State University in Sacramento, where he teaches evolution and genetics. His research interests include sexual selection and sexually antagonistic coevolution. Dr. Stemmer's address is Avidia, 2450 Bayshore Drive, Mountain View, CA 94043. Internet: pim.stemmer@avidia.org

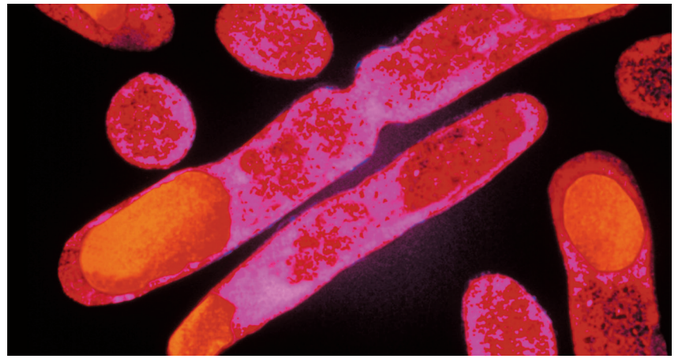

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Figure 2. Insulin was the first recombinant protein produced by genetically engineered bacteria for therapeutic use in humans beings. This pseudocolored transmission electron micrograph shows Escherichia coli bacteria carrying the human insulin gene; sites of protein expression are orange.

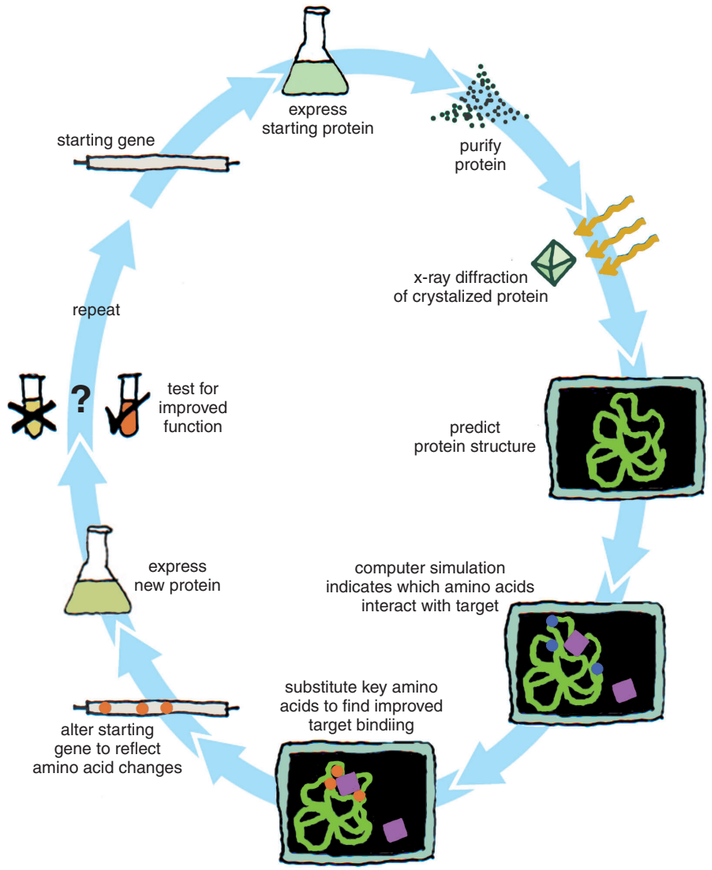

Figure 3. Rational protein design is one strategy for tailoring proteins to specific needs. To optimize a known protein, the starting gene product is purified and crystallized, and the three-dimensional structure of the protein is deduced using x-ray diffraction. A computer model predicts the forces between amino acids and identifies key sites that interact with a target molecule. Based on an understanding of the physical principles that govern these interactions, specific amino acid changes can be tested that may improve protein function.

many steps between DNA and an active protein—including but not limited to RNA and peptide synthesis, post-translational modification, subcellular targeting and intermolecular binding—and each step is regulated by multiple mechanisms. Protein folding and stability alone are sensitive to dozens, if not hundreds, of internal and external factors, and the consideration of any additional properties, such as activity in the presence of organic solvents, complicates matters further. Even without calculating such interactions between molecules, modeling the forces exerted by amino acids within the protein is an enormous undertaking—an average-size polypeptide has 300 amino acids, and each interaction is influenced by changes in the solution composition.

With all of these variables, computer models are understandably constrained by their requirement for massive computational power. The steady rise of microprocessor speed suggests that this approach may be increasingly fruitful in the future, but it will remain limited by the detail with which we can describe a protein's interactions with thousands of other molecules in the cell.

Directed evolution is an alternative to rational design that does not rely so heavily on future technology. Over the past decade, a much smaller number of scientists, including us, have followed this alternate approach with increasing success. Rather than trying to modify existing proteins or design new proteins by computer simulation of physical principles, we harness natural selection at the molecular level and direct the evolution of proteins that are customized to meet specifications set by medicine, agriculture and industry.

Evolution In a Tube

The most powerful form of directed evolution, called DNA breeding, is a modern derivative of classical breeding, which is familiar to anyone who has supervised the reproduction of plants or animals. The strategies are the same: Select promising parents, breed them to create a diverse pool of genetic variants and select those offspring that have the best combination of desirable traits. Our task resembles that of early man who domesticated the dog 14,000 years ago. Starting with the wolf Canis lupus—a magnificent animal but a poor domestic companion—prehistoric humans selectively bred those individuals with favorable traits and shaped hundreds of dog breeds in a relatively short time. Some dogs were even bred to perform highly specialized roles, such as sheepherding in border collies—a prime example of why this technique is powerful. Herding behavior is so far removed from the wolf's original behavioral genetic program that it could never have been designed rationally, even with the most sophisticated models and the most powerful computers imaginable. By contrast, directed evolution does not require a priori knowledge of how a system works. Its great advantage lies in the ability to modify a complex property without knowing every detail of its mechanism.

DNA breeding makes use of established biochemical techniques to apply strong selective pressure to molecules rather than whole animals. And because the evolution takes place in test tubes rather than in kennels, the entire process is notably faster.

The basic advance of DNA breeding is the recombination of diverse genetic material into novel and potentially more productive forms. This strategy can be applied at many levels, with the first requirement being some reservoir of genetic variation. In the simplest form of DNA breeding, which uses only a single gene, the functional diversity that is normally provided by a natural population needs to be generated in the laboratory. To do this, thousands of copies of the gene are randomly mutated and a small number of improved variants are identified by protein expression and screening. These selected clones are chopped into fragments of random length with a restriction enzyme, reassembled into full-length sequences and amplified using the polymerase chain reaction, or PCR, creating a much larger number of new combinations. This fragmentation-religation process creates novel combinations of the original set of mutations, while preserving the order of the pieces. The exponential nature of PCR generates an enormous number of new sequences in only a few hours.

Generating molecular diversity is far easier than evaluating the performance of the new molecules. Hundreds of unique progeny produced by PCR must be analyzed to find the small fraction that can perform the desired function. The method used to screen these candidates depends on the function of the protein. If binding a specific molecular target, like a receptor or ligand is critical, then the target can be immobilized on a solid support. A solution containing the pool of new molecules is passed over the target, and molecules that bind it are retained as nonbinding proteins wash away. To cull the strongest-binding proteins, the immobilized target is washed several times with increasingly stringent solutions until only a small number of molecules remain. Other screening methods are used if some catalytic ability is sought: Reaction rates can be evaluated through chemically linked indicator molecules that change color as the reaction proceeds. With this technique, automated optical assays can screen thousands of reactions per hour for the color intensity within each sample.

Once the population of mutated compounds has been winnowed to a scant number of contenders, positive mutations and a few remaining negative mutations from the selected candidates are refashioned into new combinations. This generates an entirely new but closely related pool of molecules to analyze for the targeted trait. Thus, the iterative process of diversity generation and selection is repeated until we achieve the desired quality or combination of qualities.

DNA Family Breeding

Directed evolution through random mutation of a single starting sequence can result in many-fold improvements in activity and substrate specificity within a few generations. However, this strategy has the disadvantage of having to examine an extremely high number of candidates to find the rare improvements that are needed as the input for DNA breeding.

A more potent variation of the DNA breeding strategy goes by the term multi-gene shuffling or DNA family breeding; it refers to the recombination of multiple equivalent genes from related species rather than random mu-

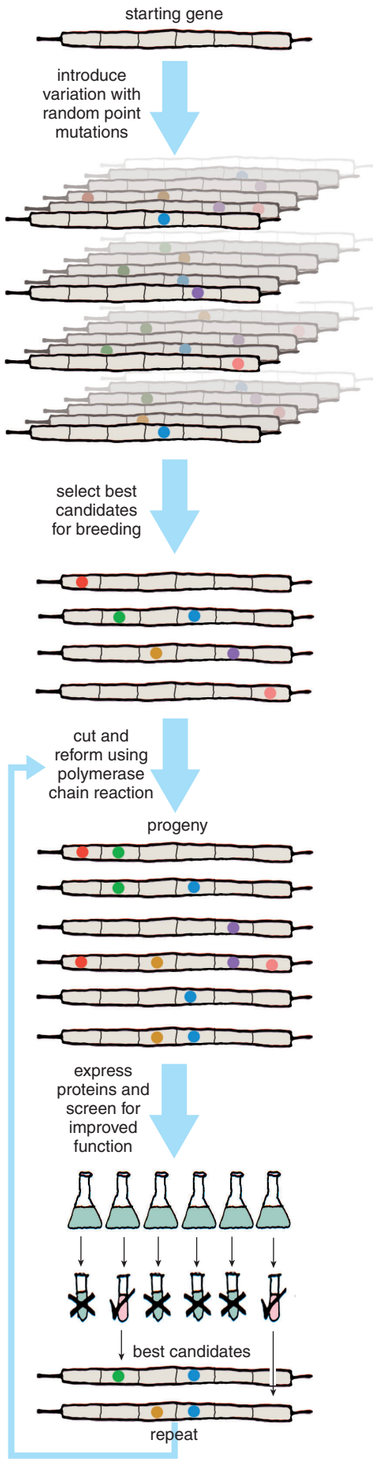

Figure 4. Single-gene breeding starts with the generation of sequence diversity through random mutagenesis. After an initial screen to select promising mutants from among thousands of clones, the best candidates can be "bred" by cutting and recombining the parental sequences to yield "progeny." A second screen identifies new molecules with even further improvements in function.

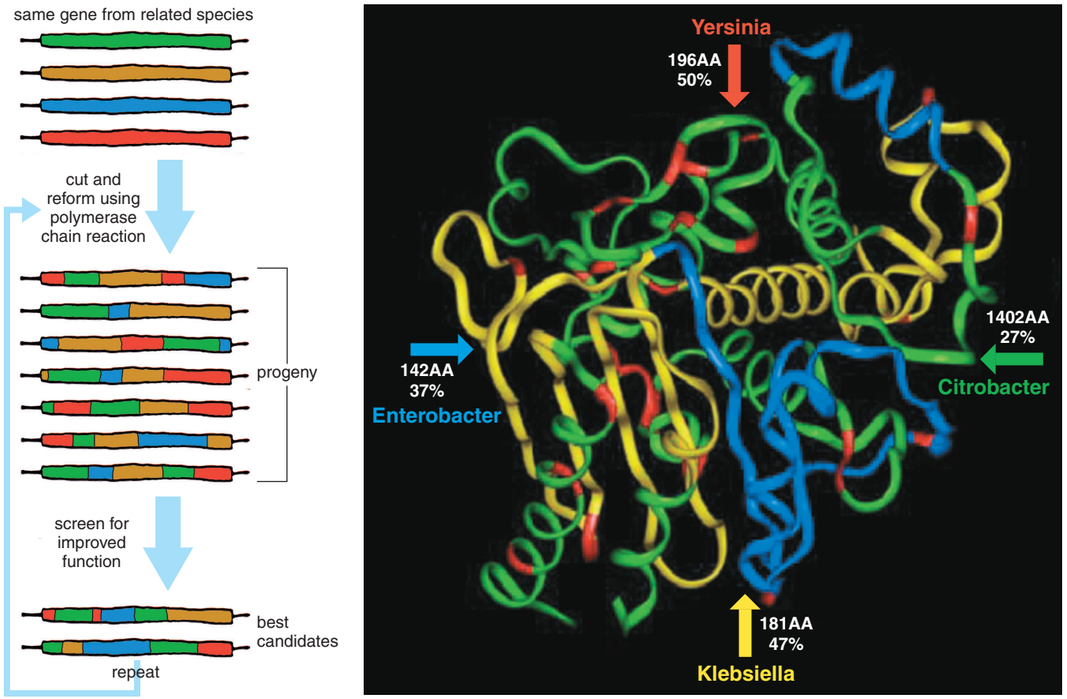

Figure 5. In DNA family breeding, the initial sequence diversity is provided by recombining the same gene from different species (left). Similar to single-gene breeding, the parental DNA is shuffled into new arrangements while preserving the order of the pieces. An example of this technique is the recombinant beta-lactamase enzyme shown above, which is a mosaic made from portions of the same gene from four distantly related microbes. This particular shuffled protein is 270 times more efficient than the best parental sequence. (Courtesy of the authors.)

tagenesis of a single gene. This approach takes advantage of the fact that while extensive novelty is provided by the cross-species interchange, most of the deleterious mutations have long ago been removed by natural selection. The reassortment of old, proven mutations yields a higher frequency of functional progeny sequences, and because multi-gene shuffling starts with more than one parental sequence, it accesses a broader range of progenitor combinations. These attributes make the process more efficient, minimizing loss-of-function mutations so that fewer progeny molecules need to be screened to discover superior performers. In our lab, we observed that this variation of DNA breeding yields striking improvements in complex properties even with just a few hundred progeny. Because screening is the most laborious step in directed evolution, DNA family breeding is a major advance.

This kind of directed evolution is not only faster—it is also remarkably powerful. This can be seen in some of our earlier work with the technique, which we presented in 1998. In that study, we compared random mutagenesis of single genes to multi-gene shuffling of an identical set of related sequences. We chose to focus on bacterial genes that encode the beta-lactamase enzyme, which are clinically relevant because these proteins inactivate the antibiotic penicillin. The starting genes came from four distantly related microbes and differed in sequence by 58 to 69 percent. We found that the best clone generated by random mutagenesis showed an eight-fold increase in beta-lactamase catalytic activity. However, when the same four genes were shuffled, the best clone was 33 times better than the random mutation champion—in other words, a 270-fold increase in the rate of catalysis.

Widespread Shuffling

We can also broaden the technology by applying the concept of DNA breeding to entire genomes rather than single genes; the advantages of such genome shuffling are proportional to the size of the genome. One example of this technique used Streptomyces bacteria, which are valuable as natural sources of the antibiotic tylosin. Understandably, drug makers would like to find strains that show higher tylosin expression to increase production efficiency. Using a classic approach based on random point mutation and screening, a team of scientists developed Streptomyces variants with a six-fold increase in tylosin production. This achievement required 20 years of mutation and selection, and the cumulative screening of more than 1,000,000 mutants. Our lab performed a similar search using genome shuffling, and we found the same improvement—but it was achieved in a single year after screening only 24,000 mutants.

This technique is applied more frequently to mimic protein evolution in prokaryotes, which have continuous genes. We have developed other methods specifically suited to evolve eukaryotic proteins, which are encoded by many short stretches of DNA separated by long noncoding spans. We can mimic eukaryotic protein evolution by focusing on those parts of the genome that actually encode proteins.

To understand how the technique works, it's helpful to look at how eukaryotic proteins are made. A single protein often resembles a string with many beads, in which each bead performs a specific task, such as binding a target. These structurally and functionally autonomous regions, or domains, work in combination to execute the overall function of the protein. One or more DNA segments called exons, which make up only about 1 percent of the human genome, encode each domain. Between the exons are large spans of intervening, noncoding introns, which get distinguished from exons during RNA splicing. Tracts of so-called junk DNA, mostly short repeated sequences that do not encode any protein information, separate the genes; these regions make up 75 percent of the human genome.

Exons can move around the genome using a variety of mechanisms. This form of mutation based on mobile, functional modules is termed exon shuffling. It is predisposed to be far more useful, in frequency and magnitude, than random changes in sequence because exons or groups of exons frequently encode autonomous functional domains.

Last year, we published a method for in vitro exon shuffling, once again mimicking a natural evolutionary process, by PCR-based recombination of multiple exons. This technique promises to be especially useful in the creation of new therapeutic proteins because the starting variation can come entirely from human genes, rather than from nonhuman species or random mutations. We expect this will minimize the risks of triggering an immune response to the therapeutic protein, because the constituent exons will be familiar to human immune systems on the prowl for foreign invaders.

Breeding Molecular Medicines

Technological advances are important, but ultimately they are only tools used to accomplish larger goals. Today, virtually any human protein, such as a cytokine, growth factor, antibody or enzyme, can be cloned, expressed, purified and rendered administrable as a potential therapeutic. The completion of the human genome project has enabled scientists to identify hundreds of new proteins with the potential to treat disease. However, because ideal drug properties are generally different from those of the native protein, most of these pharmaceutical candidates will require optimization to meet specific therapeutic goals. DNA breeding is ideally suited for this purpose. It can

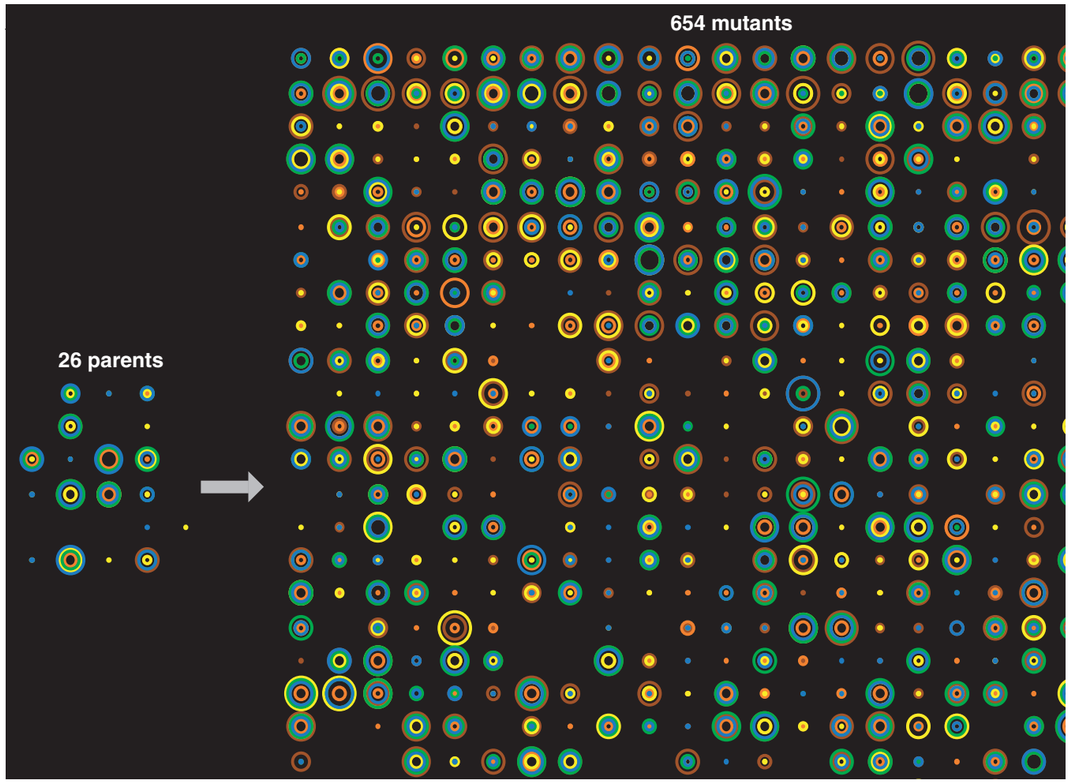

Figure 6. DNA family breeding of the subtilisin gene illustrates the potential for rapid improvement in protein function. Subtilisin is a protein-degrading enzyme used in laundry detergent and the most highly engineered enzyme in existence. Twenty-six subtilisin genes from various Bacillus microbes were shuffled to yield 654 progeny that were screened for enzyme activity at five commercially relevant conditions. Each clone is shown as a set of concentric circles, with the size of the circle indicating the activity of the enzyme and each assay condition represented by a different color. Several progeny showed simultaneous improvement in multiple properties over the best parental sequences.

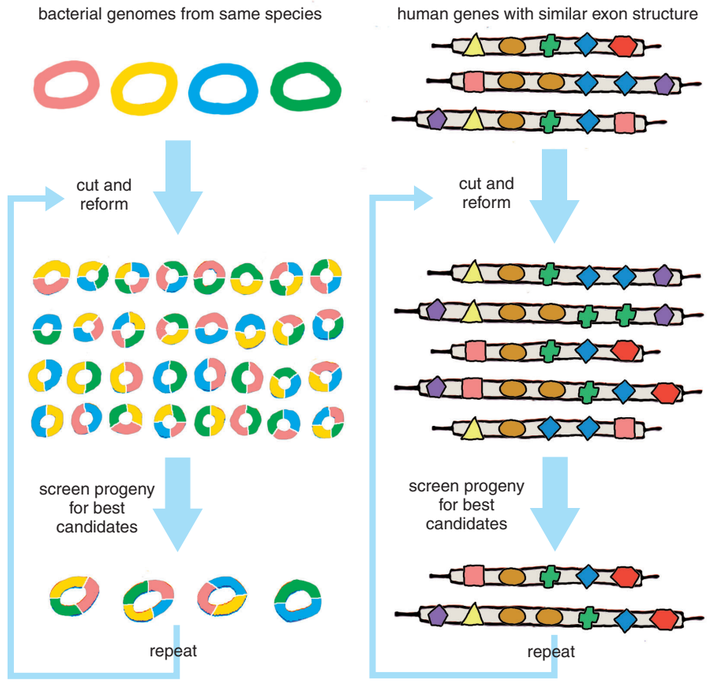

Figure 7. Genome shuffling (left) and exon shuffling (right) are two other applications of the DNA breeding technique. Genome shuffling is modeled after the natural evolution of prokaryotic organisms, whereas exon shuffling mimics eukaryotic protein evolution. In both cases the progeny are more likely to have useful properties because the breeding step starts with "proven diversity"—natural selection having already removed most deleterious point mutants.

enhance desirable biological activities such as binding activity, receptor specificity, circulation half-life, expression level and stability, and reduce undesirable side effects such as immune-system rejection and toxicity.

One example of the potential for directed evolution to improve existing drug therapies comes from our work with interferon alpha, variants of which are encoded by more than 20 genes in humans. Interferons are a group of cytokine proteins that have a broad spectrum of anticancer and antiviral activities, and human interferon alpha is already a billion-dollar product used to treat these conditions. We sought to optimize interferons to combat specific types of human cancer or viruses using DNA breeding. With a starting pool of 20 interferon alpha genes, we screened 1,700 shuffled clones with a total of only 68 antiviral assays and found several progeny proteins with improved activity on mouse cells. The best of these showed specific activity 135,000 times greater than the existing drug. After the second round of shuffling and screening, we isolated clones with a 285,000-fold increase in potency compared with the product now available. DNA sequencing showed that the three best progeny each consisted of segments from up to five parental interferons without any amino acid point mutations—an important advantage because the lack of point mutations decreases the likelihood of an immune response, a common problem with traditional mutants. In studies using live mice, animals that were given the novel interferons were fully protected against viral infection, whereas mice given the most potent native interferon enjoyed only partial protection.

Another project focuses on our aim to fine tune immune responses by modifying the T-cell surface protein CD80. This protein is crucial to the rapid immune response that targets foreign antigens, such as pathogens, while ignoring native proteins. The decision to attack or remain quiescent is mediated by the interaction of CD80 with two receptors with very different effects. Binding to one of them increases the immune response, which we sought to bolster in order to improve the response to vaccines and to fight cancer and infection. In contrast, when CD80 binds to the other receptor the immune response is reduced, which would be useful in the treatment of autoimmune diseases and to create tolerance for organ transplants. We strove to increase the selectivity of these binding events by shuffling CD80 genes from seven species—human, orangutan, rhesus monkey, baboon, cat, cow and rabbit—and screening the progeny for preferential binding to one receptor but not the other. The strategy was successful, and the two distinct types of CD80 variants have behaved as expected in a variety of in vitro assays. One of these new molecules is being evaluated further in monkeys.

A third area of research in our lab is the development of a vaccine for dengue fever, a viral disease transmitted by mosquitoes. Dengue was originally a tropical disease, but it is rapidly spreading throughout the world and has entered the United States. While surviving a bout of the disease does confer immunity, there are four viral variants or serotypes, and they each elicit a distinct immune response. Survivors that are reinfected with a different serotype are at increased risk of developing an often-fatal disease called dengue hemorrhagic fever, so dengue vaccines must protect against all four serotypes simultaneously. Our approach to this dilemma was to create a single-protein vaccine: Genes encoding the antigens from all four variants were shuffled, and the resulting protein progeny was tested for binding to antibodies that recognize each of the four. The best vaccine candidates were complex combinations of the key sites from all four parental serotypes. These proteins were evaluated in mice for their vaccine potential, and several induced antibody responses that cross-neutralized all four dengue serotypes—completely preventing viral infection. Candidate vaccines are now progressing to trials in primates.

We haven't yet reached the end of the potential offered by directed evolution. The application of the technique to vaccine development is especially promising: By shuffling sequences from multiple viral subtypes into a single protein, we might be able to conquer influenza, human papilloma, herpes simplex 1 and 2, foot and mouth, and even bacterial infections such as those caused by the ulcer-inducing Helicobacter pylori. This approach may even help reverse the traditional advantage conferred by the overwhelming number of pathogens that assault us. With a world of microbes to select from, evolution exerts exceptional pressure to generate substrains and varieties that threaten to evade immunization efforts and antimicrobial drugs. Yet maybe an immune system primed with a shuffled, multistrain inoculant would be prepared to meet the summed diversity of an entire slice of the pathogenic spectrum.

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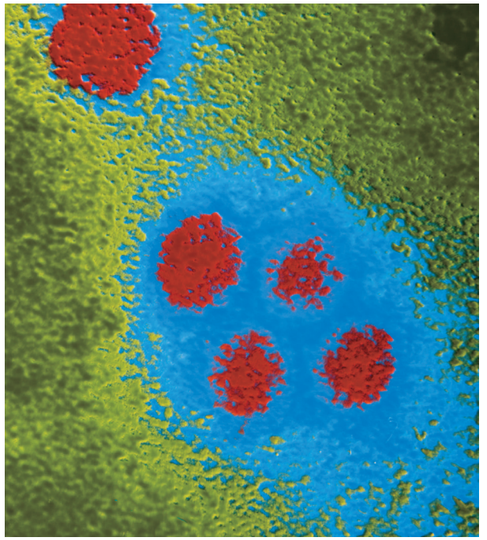

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Figure 8. Dengue fever is a viral disease that is carried by the mosquito Aedes aegypti (left), a species that feeds during the day and prefers human beings. Dengue and dengue hemorrhagic fever can be caused by any of four distinct viral varieties, or serotypes, and this makes the effort to develop an effective vaccine especially suited to the application of DNA breeding. Shuffling the viral antigens into a single protein inoculant appears promising: Early results in mice suggest that several of the vaccine candidates prevent infection from all four serotypes. At right, a pseudocolored transmission electron micrograph illustrates dengue virus particles (red circles) within a host cell.

As we demonstrated in 2001, laboratory-directed evolution can be used to rapidly predict the future course of natural evolution, as observed in antibiotic-resistant pathogens from clinical samples. Of course, this advantage is temporary—the shuffling technique cannot anticipate all future infectious agents. Like the Red Queen from Lewis Carroll's Alice in Wonderland, medical technology and the human immune system must constantly run in order to stay in place. But for the first time we have the ability to affect the treadmill itself, evolving our medical and immune system defenses at a faster rate than that of its challengers.

DNA breeding is explicitly modeled on natural evolution—both are blindly opportunistic, and this is an indisputable strength of both processes. Virtually any combination of sequences may be used in a shuffling reaction, and as true, bottom-up, rational protein engineering continues to progress, it too will be incorporated into directed-evolution methodologies to increase further the fraction of useful progeny. We are optimistic that directed evolution will exponentially catalyze development of the next generation of useful proteins to flow from biotechnology's fertile cornucopia.

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Cheating Viruses and Game Theory

The theory of games can explain how viruses evolve when they compete against one another in a test of evolutionary fitness

Paul E. Turner

The 19th-century circus showman P. T. Barnum is reputed to have coined the phrase "There's a sucker born every minute"—although Barnum denied the saying was his, and it has been variously attributed by biographers. In any event, whoever did voice this cynical view of human gullibility could not have predicted that the terms "cheaters" and "suckers" would describe individuals in the world of microorganisms as well. However, my colleagues and I have been studying interactions between viruses, and it seems that strategies for taking advantage of the other fellow are just another way for the viruses, too, to make a "living."

The temptation to cheat appears to be a universal fact of life. In the struggle to survive and reproduce that drives evolution, selfish individuals may be favored over cooperators because they are more energy efficient. By definition, cheaters expend relatively little energy in a task because they specialize in taking advantage of others—"suckers"—whose efforts they co-opt to their own advantage. In certain animal species some males exert tremendous energy maintaining and defending territories to attract females. Meanwhile, the population may contain subordinate "sneaker" males that are uninterested in territory but linger at the boundaries and specialize in surreptitious copulations. This strategy is very successful for maintaining a subpopulation of sneakers, but it's unlikely that the population will evolve to contain only cheaters because territorial males are most attractive to female mates.

In general, cheaters are highly successful when they are rare because they frequently encounter suckers. The benefits of cheating wane as more individuals in the population opt to cheat. In the parlance of evolutionary biology, the success of cheaters should be governed by *frequency-dependent selection*. That is, some cost should be associated with a cheating strategy so that selfish individuals are at an advantage when they are rare, but disadvantaged when they are common.

Game theory is a useful approach for mathematically predicting which strategy, if any, will dominate such a contest. Social scientists use game theory to predict which behaviors will spread through a population, especially in contests involving classic strategies such as "hawk" versus "dove," and "cooperator" versus "cheater." One of the most intriguing results of this approach is a mathematical proof demonstrating that cheating can take over a population, even though deceit can be considered an irrational behavior because it is punishable.

My colleagues and I have applied game theory to the experimental evolution of viruses in the laboratory. This is a field that is relatively new, but is proving to be powerful for testing fundamental questions in evolutionary biology. It's an approach with many advantages: Viruses are easy to culture. They have rapid generation times and large population sizes. In addition, an array of modern tools makes them easy subjects for manipulation and study. Although the experiments are conducted in the laboratory, evolution proceeds by natural selection because the laboratory habitat dictates which genetic variants are favored to contribute their genes to the next generation. This is very different from artificial selection, such as dog breeding, where the experimenter determines the variants that will reproduce. Perhaps most important, microorganisms can be stored in a freezer indefinitely, creating a "fossil record" that permits direct comparisons between the genetic makeup of an ancestral population and that of its evolved descendants. Our experiments suggest that yes, perhaps at this moment, there may be cheaters among the viruses vying for survival within and near your own cells. But in the long run, such crimes don't always pay.

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Figure 1. Cheaters often win in the simulations used in game theory, a branch of mathematics that analyzes competitive interactions between individuals. Regrettably too common among human beings, cheating has also been found among many other animal species and, perhaps surprisingly, among viruses. The cheating viruses were discovered in laboratory experiments by the author. In *Le Tricheur* ("The Cheat"), the 17th-century French painter Georges de La Tour depicts a card game with at least one deceitful player.

Viruses in Conflict

We experience viruses mainly through the symptoms, such as fever and fatigue, that signal that our body is defending against an invader's attempt to commandeer the normal activities of the body's cells. Viruses are parasites that rely on the genetic machinery of a host organism to make copies of themselves. At any particular moment an infected individual can harbor several species of viruses or even genetic variants (genotypes) of the same species. So the host serves as an ecosystem for potential interactions between the viruses. These interactions may be indirect—for example, when the host's immune system takes action against one species of virus, while simultaneously affecting other viruses. A host's fever may be a generalized response to a specific infection, but the high temperature can impair the growth of all viruses within the body.

When viruses interact directly, their effects on each other are more difficult to detect because they take place within an individual cell. When a virus enters a cell, it hijacks the host's metabolism, instructing it to make the bits and pieces needed to assemble other viral particles. When more than one virus infects a cell, the metabolic products are freely accessible to any of the co-infecting viruses. In a process called *complementation*, one virus provides a useful product that cannot be made by another virus. If the viruses provide each other with useful resources, the interaction is of mutual benefit. Consider the co-infection of a cell by two mutant viruses, which differ by having inactivated genes at different locations in the genome. The common resource pool allows the viruses to use each other's protein products. The co-infection rescues the mutants, allowing them to reproduce when they couldn't otherwise do so.

Such mutually beneficial interactions between viruses are either rare or extremely difficult to detect. More often, viruses seem to experience a conflict of interest over the resource pool. When this happens one virus can selfishly usurp resources to the detriment of other virus species or genotypes.

In a form of complementation known as *phenotypic mixing*, a virus acquires certain observable (phenotypic) traits from another virus. This phenomenon frequently involves a conflict over proteins used to create the viral *capsid*, a shell that protects the genetic material of the virus. Phenotypic mixing allows a virus to acquire capsid proteins from the resource pool of a different virus. This is critical because some proteins on the capsid dictate whether a virus can attach to a particular host cell and thrive.

An interaction between two plant viruses illustrates how this strategy can be important in the transmission of a virus. Luteoviruses infect nearly all the crops that people grow for food or fiber. These viruses can easily travel between plants by hitching a ride with the tiny plant-sucking insects called aphids. Umbraviruses also infect crop plants, but they are unable to hook up with the aphid vector. This situation changes if luteoviruses and umbraviruses happen to co-infect the same plant. The umbraviruses steal some of the capsid proteins from the luteovirus resource pool, attach themselves to the aphids and so move on to new host plants. Meanwhile, the hapless luteoviruses experience a net loss in the capsid proteins they need to assemble their progeny.

Complementation can also be a factor in conflicts when one virus usurps an enzyme needed for replication from another virus. The best known examples involve an ordinary virus and a defective form, typically a virus with a "shortened" genome, one that lacks one or more essential genes. This phenomenon was first described in laboratory experiments involving polioviruses. When these viruses are grown at high densities there is strong selection pressure for them to lose genes (become defective) because shortened viruses replicate much faster than viruses with genomes of normal length.

Figure 2. Cheating as an evolutionary strategy can be studied by measuring the reproductive success of animals that engage in covert copulations or fertilizations, such as these bluegill sunfish *(Lepomis macrochirus)*. Here a male bluegill *(right)* gains access to a nesting female *(center)* by mimicking her appearance, and so cuckolds a so-called "parental" male *(left)* who courts the female and cares for the offspring. Both breeding tactics used by the males are successful, and so neither can displace the other in the population. Cheating viruses use tactics very different from the mimicking male fish, but it is possible to conduct experiments that show how cheating affects their reproductive success and thus the viruses' evolutionary fitness. (Image courtesy of Brian D. Neff, University of Western Ontario.)

The defective viruses interfere with the reproductive success of the ordinary viruses by using their gene products, so that the ordinary viruses take on the role of helpers. Because viruses reproduce exponentially, even a slight advantage in replication rate can result in drastic differences in the relative success of the viruses. The problem faced by the "defective-interfering" viruses is that they are entirely dependent on helper viruses to provide key proteins.

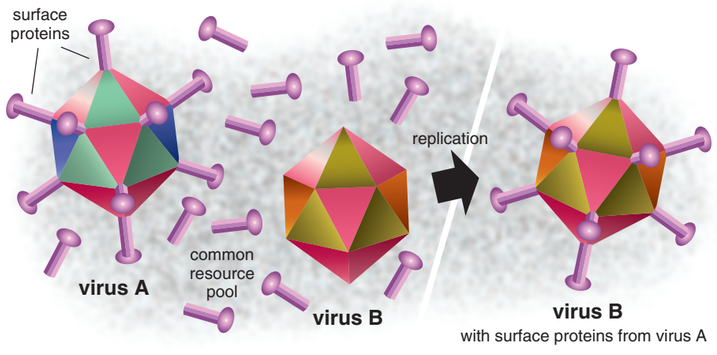


Figure 3. Viruses compete for resources when they co-infect the same cell. In some instances, one virus may provide a useful product for another virus, a phenomenon called complementation. In this hypothetical example, virus A carries a gene that codes for a valuable surface protein—for example, one that allows it to infect other cell types. Although virus B lacks the gene for this protein, it can steal the protein from the common resource pool inside the host cell. Virus B gains the use of the protein, whereas the offspring of virus A now experience a shortage of the protein. A form of complementation called phenotypic mixing takes place when one virus acquires observable (phenotypic) traits from another virus, as shown here. Complementation may be involved when viruses cheat.

If the replication advantage of the defective-interfering viruses drives the helpers to extinction, both strains will die.

Most virologists view defective-interfering viruses as an unfortunate nuisance, one that may compromise their research goals—as when, for example, such a virus contaminates the purity of a commercial vaccine. To a microbial ecologist, however, the defective-interfering viruses are especially intriguing because they are parasites on parasites, or hyper-parasites, something rarely seen elsewhere in biology.

This naturally raises the question of whether defective-interfering viruses are merely laboratory artifacts. Some recent evidence suggests that a related phenomenon may not be uncommon outside the laboratory. Natural hyperparasitism is seen among viruses that infect farm animals and crops. The majority of the defective viruses identified from these systems are *satellite* viruses; they are usually unrelated to their helpers. By contrast, defective-interfering viruses have recognizable genetic similarities to the helpers from which they evolved by losing certain genes. Defective-interfering viruses may be rare in nature because the helper viruses appear to evolve a resistance to being parasitized by closely related viruses. For some reason satellite viruses are more easily able to circumvent any resistance put up by the helpers.

Defective viruses are not known to play a widespread role in human disease. A notable exception is the hepatitis delta virus, a satellite virus associated with its helper, the hepatitis-B virus. Together these two viruses cause unusually severe liver damage in cases of chronic active hepatitis. It is not clear why defective viruses are not commonly implicated in human disease, but it is conceivable that other examples have yet to be discovered by the medical community.

Although hyperparasitic viruses may evolve readily, they can easily wind up as evolutionary dead ends because of their strict reliance on helper viruses. It would be a daunting task to study the relative success of parasitic viruses in nature because there are so many uncontrolled variables in field studies. However, virus interactions can be examined under controlled laboratory conditions in experiments that measure relative growth rates. This approach can be augmented with mathematical models that explore how easily parasitic viruses arise and whether they can persist. As it happens, game theorists have had a long-standing interest in the success of parasites and other cheaters, so there are many mathematical models to choose from.

Cheaters Sometimes Prosper

To understand how game theory might be applied to virus interactions, consider a game involving cooperators and cheaters, known as the "prisoner's dilemma." This scenario, which has been used to explore philosophical, political, economic and biological questions for half a century, involves two felons who are separately interrogated about a crime they committed. In one version of the game, an understanding between the prisoners is assumed: They can cooperate in denying the crime in the hope that they'll both be let off the hook. The interrogator, however, offers two choices to each prisoner. If both stay silent (cooperate), each will receive a light one-year jail sentence. If both confess, each goes to prison for 10 years. However, if one confesses—in other words, cheats—the cooperator will receive a 20-year sentence while the cheater goes free. So what's a prisoner to do?

Game theory holds that in such a dilemma, it always pays the individual to cheat because cheating, even though it risks a long sentence, offers the only possibility of obtaining the best payoff—freedom. The result is intriguing because it explains how a potential, but uncertain, reward can drive individuals to behave in a way that is collectively irrational: When both prisoners follow their individual interests, both lose.

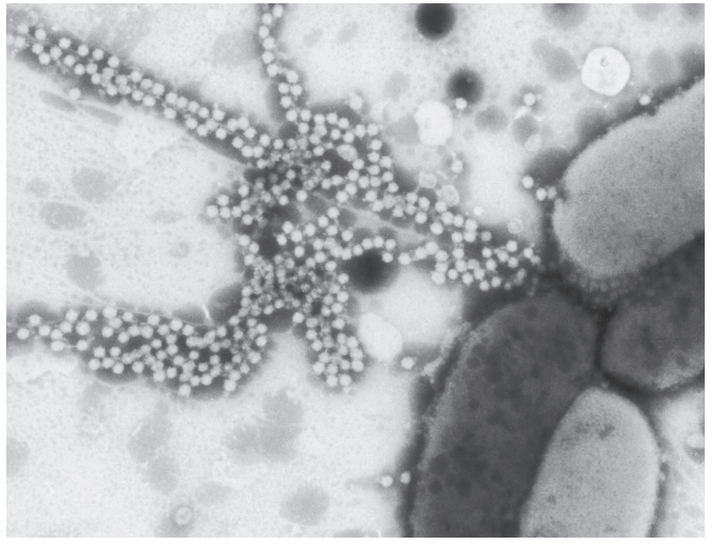
When the differing strategies are associated with different underlying genetics, game theory can be applied to the study of evolution. Popularized by the late British biologist John Maynard Smith, evolutionary game theory comes into play when an individual's reproductive success, or *fitness*, is frequency dependent. Consider a predator that preferentially feeds on the most common type of organism in a prey population because these individuals provide an easy "search image." Prey types with a rare appearance, perhaps sporting an uncommon fur color, will have a higher fitness because they escape the predator's notice. This advantage will wane as their type becomes more common in the population and therefore more obvious to the predator.

Evolutionary game theory weighs the costs and benefits in terms of fitness associated with different strategies, and so predicts the evolutionary fate of the different types. This is done by creating a 2×2 matrix that contains all pairwise interactions between two different strategies. Each entry within the matrix consists of the fitness payoff to one strategist when it interacts with the other. The matrix reveals the relative success of the strategies in the contest so long as the mathematical fitness values can be calculated. When

	cooperator	cheater
cooperator	reward	sucker's payoff
cheater	temptation to cheat	punishment

Figure 4. Payoff matrix for a contest between a "cheater" and a "cooperator" shows the outcome of each pairwise interaction for one individual *(left side of matrix)* who encounters another *(top of matrix)*. A cooperator who meets another cooperator is rewarded, whereas a cooperator who meets a cheater receives the "sucker's payoff," typically a loss of some useful resource. A cheater gains this valuable resource when interacting with a cooperator and so is tempted to cheat. When a cheater meets another cheater, nothing is gained, and the two are usually punished in some way. The relative success of cheaters and cooperators can be determined if the respective costs and benefits can be quantified for each of the interactions. When these strategies are associated with different underlying genetics, the payoff matrix can predict whether one tactic will displace another over the course of evolution.

a population evolves to contain only individuals with a single strategy, it is defined as an "evolutionary stable strategy." If two strategies are unable to displace one another, as hinted at



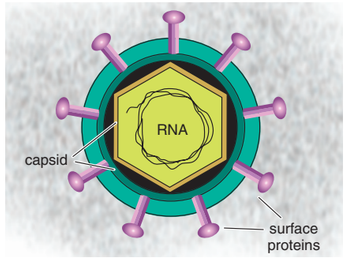
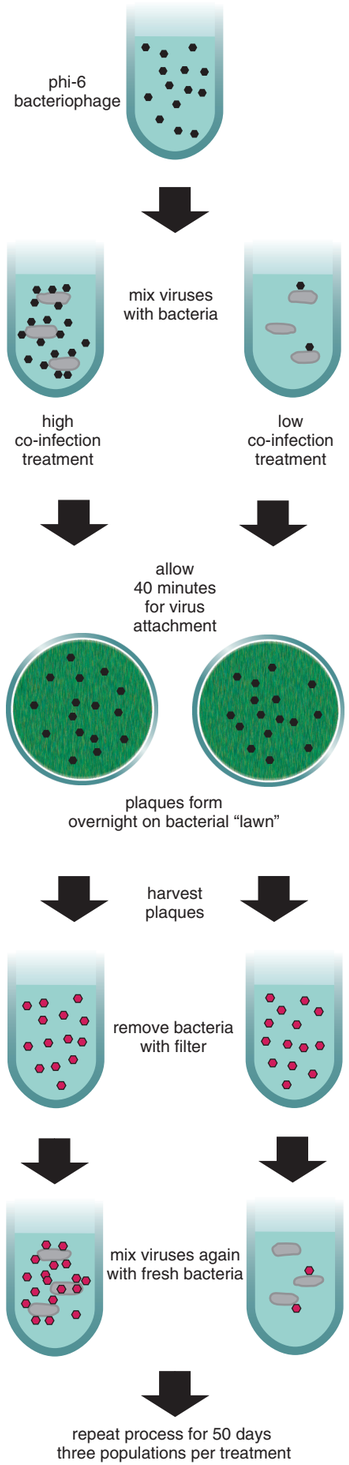


Figure 5. Viral particles of the bacteriophage phi-6 *(left, small circles)*, which grow on *Pseudomonas phaseolicola* bacteria *(larger lozenge shapes)* in the laboratory, are the subjects of experiments in evolutionary game theory. Each phi-6 particle *(above)* consists of the genetic material RNA, which is housed in a protein shell (or capsid) and a lipid membrane. The phi-6 bacteriophages may compete against one another for the capsid proteins in the common resource pool inside the bacterial host. (Electron micrograph courtesy of Dennis Bamford, University of Helsinki.)



in the predator-prey example above, both strategies will coexist indefinitely; this is defined as a "mixed evolutionary stable strategy."

In the prisoner's dilemma, evolutionary game theory suggests that cheaters should take over the population—selfishness turns out to be the evolutionary stable strategy. The result is striking because it is somewhat counter to Darwin's theory of evolution by natural selection. Darwinism holds that differential performance allows the fittest individuals to produce more offspring, which steers the population to become better adapted to its environment over time. The prisoner's dilemma indicates that cheaters can successfully displace cooperators, while simultaneously *lowering* the average fitness of the population. The prisoner's dilemma is very easy to prove mathematically, but it took laboratory experiments on viruses to demonstrate that the strategy may take place in a biological population.

Experiments in Evolution

My colleague Lin Chao, of the University of California, San Diego, and I designed a series of experiments to explore the evolution of behavioral interactions between viruses. In this case, the players in our experimental game were bacteriophages, or "phages," viruses that infect bacteria. Phages are not typically thought to exhibit behavior, but they have proved to be very useful to test models of conflicting behavioral strategies in evolutionary game theory. Such tests would be difficult if not impossible to do with higher organisms.

We employed the services of a phage called phi-6, an RNA virus (one that has RNA instead of DNA as its genetic material) in the family *Cystoviridae*, which attacks legume-infecting bacteria. In the laboratory, the virus is typically grown on *Pseudomonas phaseolicola* bacteria, which are easily cultured on agar plates. By combining the phage and bacterial populations in different ratios it is simple to control whether a virus will infect a bacterial cell on its own or co-infect the same cell with other viruses.

Figure 6. Different strains of phi-6 bacteriophages *(dots)* can be created in the laboratory by controlling the number of bacteriophage particles that can co-infect a single bacterial cell *(lozenges)*. Three populations were grown on a bacterial "lawn" at phage-bacterium ratios that allowed at least two or three bacteriophages to enter each host cell *(left protocol)*, whereas three other populations were allowed to evolve in ratios where no more than one phage entered a bacterial cell *(right protocol)*. After 50 days, or 250 phage generations, each population was allowed to compete against an ancestral strain *(see Figure 7)*.

Chao and I created six laboratory populations of phi-6 growing on the *P. phaseolicola* bacteria. Three populations were allowed to evolve at phage-host ratios that resulted in strictly single infections. The other three populations were grown at ratios that allowed co-infection with an average of about two or three viruses entering each bacterium. We let the viruses grow for 50 days, which corresponds to about 250 generations of phage evolution. By comparison, a similar experiment using a human population would take 5,000 years (assuming about 20 years per generation).

After 250 viral generations had elapsed, the evolved populations were each placed into an agar-plate "arena" to compete against the revived ancestor that had been stored in the freezer. This allowed us to gauge changes in viral fitness, which we defined by measuring the growth rate of the virus on the bacteria. If both viruses grew equally well, the fitness of an evolved virus relative to its ancestor was said to equal one. However, if evolution had either improved or worsened the virus's ability to grow, then the fitness was respectively either greater or less than one.

A conspicuous result of the study was that the viruses cultured in the co-infecting populations had much higher fitnesses during co-infection, than during single infections. This result is consistent with the possibility that evolution under co-infection had selected for cheater viruses—genotypes that could efficiently use the products of other viruses in the resource pool, but that were less efficient on their own. The evolved viruses also had the ability to infect the bacteria and replicate on their own, indicating that the cheaters were not simply defective-interfering viruses that had lost key genes. Because the ancestral virus did not show any fitness advantage during co-infection with other virus genotypes, we defined the ancestral strategy as cooperation.

Game-Theory Solutions

The evolution of cheater viruses containing a full set of genes provided a unique opportunity to examine whether the phage was caught in the prisoner's dilemma. We needed to confirm two key predictions. First, the fitness of the cheaters relative to the ancestral cooperator had to be frequency dependent—sensitive to the ratio between cheaters and cooperators—because the model predicts that cheaters will show their greatest fitness advantage when they are rare relative to the cooperators. Second, the cheaters had to displace the ancestral cooperators completely and take over the population. If these two criteria were met, and the takeover by cheaters resulted in a decline in the average fitness of the population, then the results would be consistent with the prisoner's dilemma.

We set up a series of competitions between an evolved cheater and the ancestral cooperator. To test for frequency-dependent fitness, the two strains were represented at different initial frequencies (ranging from 0.1 to 0.9) for each competition and the numbers were sufficiently high to allow co-infection. After allowing the strains to compete for five generations, we found that, indeed, the fitness of the cheater decreased sharply as its initial frequency increased. In other words, when cheaters were rare, they generally were involved in co-infections with cooperators (rather than with other cheaters), and so they gained a large fitness advantage through their ability to usurp the components in the resource pool. However, when the cheaters were common, they tended to co-infect cells with other cheaters and so could not profit from their selfish behavior.

The same experiment also supported the second prediction: The cheaters must takeover the population. The fitness of the evolved cheaters relative to the ancestral cooperator was always greater than one at all of the initial ratios. This global advantage predicts that the evolved cheater will always displace the ancestral cooperator. The strong competitive advantage allows the cheaters to increase their numbers rapidly when they are initially rare. Even though the cost of cheating increases as the number of cheaters increases, the cooperators that interacted with the cheaters always had the lowest fitness in the system. For this reason, nothing could prevent the cheaters from taking over the population. Our study was the first to demonstrate the evolution of irrational, selfish behavior in a biological system *(see "Estimating the Payoffs," next page)*.

Interactions between selfish defective-interfering viruses and cooperative helper viruses can also be explained using game theory. Imagine a population composed entirely of cooperative helpers growing in an environment where co-infection is common. If a mutant defective-interfering virus enters the population, it has a very large fitness advantage because it is surrounded by cooperators that provide essential gene products. So defective-interfering

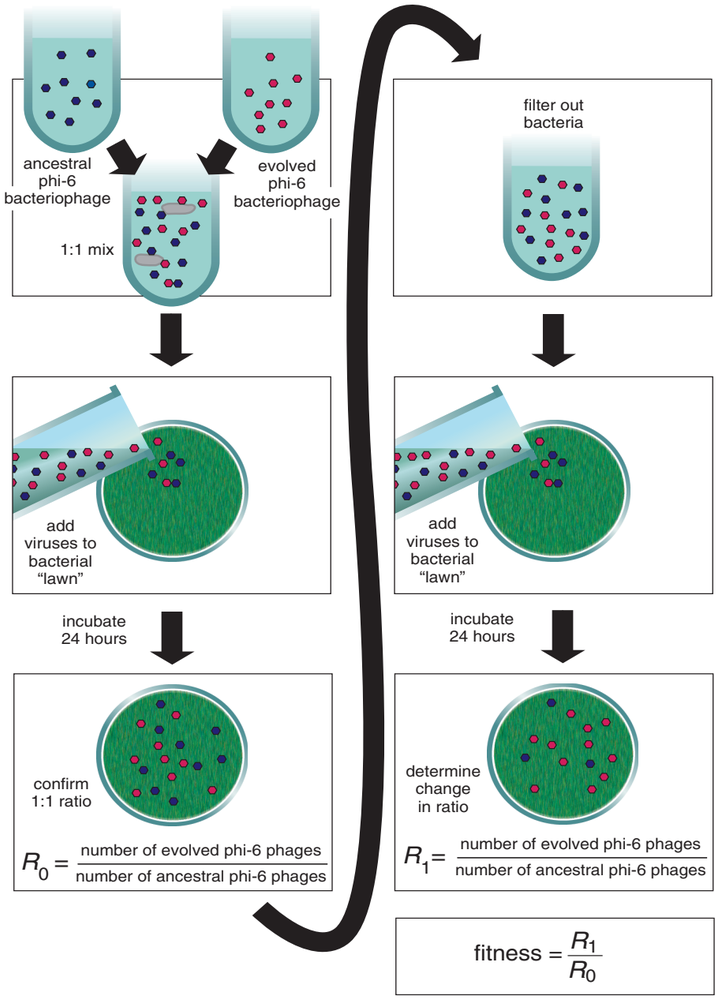
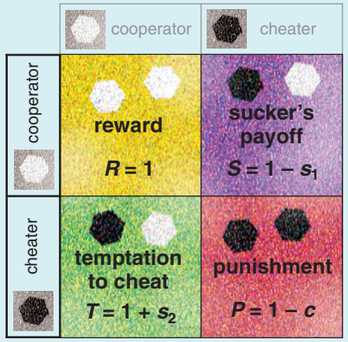


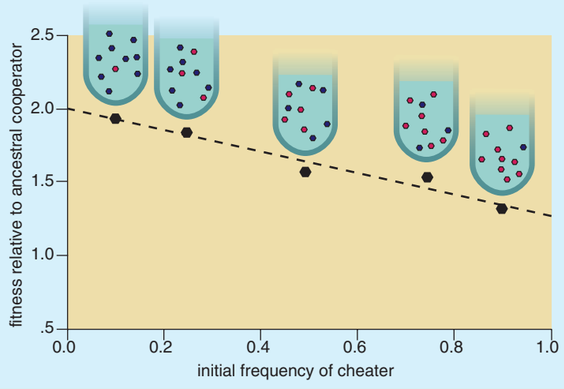
Figure 7. The fitness of an evolved strain of phi-6 bacteriophages relative to the ancestral phi-6 bacteriophages can be determined by having the two strains compete for bacterial hosts. The ability of the two populations to establish themselves and reproduce on a bacterial "lawn" is first established by confirming that they maintain a 1:1 ratio after a 24-hour incubation period *(left column)*. When the phages are again counted after a second 24-hour incubation period, the change in the ratios of the populations (R_1 vs. R_0) is a measure of their ability to compete against each other. In the author's experiments, bacteriophages that evolved under conditions of high co-infection had a higher fitness during co-infection than during single infections. This result is consistent with the possibility that evolution had selected for "cheating" bacteriophages—strains that could usurp the products of other bacteriophages ("cooperators") from the resource pool, but were less efficient on their own. The evolutionary costs and benefits of the interactions between cheaters and cooperators can be calculated with further experiments (see "Estimating the Payoffs," *next page*).

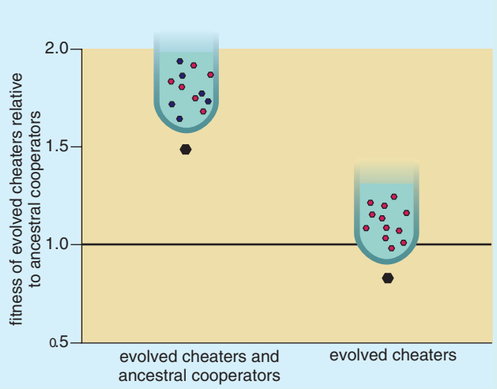
Estimating the Payoffs

Experiments between cheating and cooperating viruses allow scientists to estimate the fitness payoffs for each of the strategies in a 2×2 matrix *(right)*. In the prisoner's dilemma, when two cooperators interact the reward is defined as $R = 1$. When a cheater meets a cooperator, the temptation to cheat, T, must exceed the reward for cooperating by some value, say s_2, so that $T = 1 + s_2$. So the fitness of the cheater relative to the cooperator is T/R, when the cheaters are rare. The value of s_2 can be determined from experimental data. My colleagues and I set the equation, $T/R = (1 + s_2)/1$, equal to the left y-intercept of the regression line for the data *(see graph below, left)*. The y-intercept represents the case where a cheater is very rare and therefore guaranteed of interacting with cooperators. So an individual cheater receives the maximum benefit: $T/R = (1 + s_2)/1 = 1.99$, and $s_2 = 0.99$.

When two cheaters meet, there is a loss of fitness, and the punishment for cheating is defined as $P = 1 - c$. A cooperator also loses fitness when interacting with a cheater and receives the "sucker's payoff," $S = 1 - s_1$. Here we set $P/S = (1 - c)/(1 - s_1)$ equal to the right y-intercept of the regression line, which represents the case when there is the greatest frequency of cheaters. Because our results found that the right y-intercept was a number greater than one, P had to be a value greater than S. However, the ratio cannot be solved because of two unknown variables, c and s_1. We needed to devise an additional experiment to directly estimate P or S. We did this by measuring the growth rates of the cheaters and cooperators when co-infecting cells on their own. This experiment mimics the situation when the cheater viruses take over the virus population. We found that the growth rate of the cheaters was 83 percent of the cooperators' growth rate. So $P = 1 - c = 0.83$. We substitute the value $c = 0.17$ in the equation for P/S. It was then trivial to estimate the parameter s_1, so we could fill in the payoff matrix. Because the cheater ultimately replaces the cooperator, while lowering the fitness of the population *(see graph below, right)*, the results are consistent with the prisoner's dilemma.







viruses become increasingly common in the population. However, as the selfish individuals increase in relative frequency, their fitness will decline because there are fewer cooperators present. If the defective-interfering viruses take over, their fitness falls to zero because they cannot reproduce on their own. In this case, the strategy of the defective-interfering viruses can only persist through a mixed evolutionary stable strategy involving a helper virus. Evolutionary game theorists call this the "chicken game."

It is still not clear how the selfish phi-6 genotypes can so efficiently sequester products from the resource pool to the detriment of their cooperative ancestor. Evidence from other experiments with phi-6 suggest that complementation may be involved. When the ancestral phi-6 strain is allowed to co-infect the same cell with various less fit mutants of the virus, a greater-than-expected number of mutants appear among the offspring. This suggests that complementation can take place passively whenever multiple phi-6 genotypes co-infect the same cell. The evolution of cheating viruses may occur because their prolonged exposure to co-infection results in a strong selection for the virus to become more efficient at complementation—a trait that was already present in the ancestor. This idea assumes that complementation is not always entirely passive and so can be improved through selection. One possibility is that the cheaters may be poor at producing capsid proteins, which could explain their low productivity when they infect cells on their own. However, they may be very efficient at stealing entry into capsids produced by cooperators during co-infection. It may be that cheaters have evolved mechanisms that recognize attachment and entry into viral capsids.

Outside the Laboratory

Laboratory studies of cheating viruses and bacteria may seem esoteric, but they have much to offer for understanding the ecology and evolution of microorganisms in nature and in medical and commercial settings. Very little is known about the interactions between microorganisms in the wild. In fact, the vast majority of microbial species in nature have yet to be described. Cheating has been observed in laboratory experiments among viruses, bacteria and slime molds, and it seems likely that we will discover cheaters in natural communities of these microorganisms as well.

Human beings have a long history of using bacteria and yeasts for the production and flavoring of foods and beverages, including cheese, bread, wine and beer. More recently, we have purposefully cultured microorganisms to create vaccines, which are often weakened or inactivated microbes that are administered to elicit an immune response. Vaccines are widely available for combating infectious diseases such as polio, measles and mumps, and there are now efforts to develop vaccines for other diseases such as AIDS and malaria. Similar strategies are used in agriculture, where vaccines are administered to prevent diseases in livestock, and crops are sprayed with viruses or bacteria to combat plant diseases or to target insects that destroy crops. The research described in this article suggests that industrial producers of microorganisms should be wary of contamination by cheaters, which may compromise the desired tastes of foods and beverages, or the effectiveness of vaccines and biological pesticides.

On the other hand, cheating viruses and bacteria may provide desirable and exciting new avenues for the application of microorganisms. For example, scientists are now trying to determine whether defective HIV strains can interfere with the ability of ordinary HIV to replicate and spread within the body and so prevent or delay the onset of AIDS in HIV-infected individuals.

Biologists still need to determine the conditions that promote or hinder the growth of microbial cheaters. Mathematical models such as evolutionary game theory will be valuable for predicting their long-term success. As we continue to discover the intriguing interactions between microorganisms that foster the evolution of cheating strategies, we will in turn provide opportunities for exchanges between scientists interested in evolutionary biology and those working in basic and applied aspects of microbiology.

A Dilemma?

Following our study, other scientists suggested that certain populations of yeast and bacteria may also evolve according to the prisoner's dilemma. Some yeast cells forgo the production of a sugar-digesting enzyme, opting to steal sugar that was digested by cooperators. And certain bacterial mutants cheat by ignoring a chemical signal to stop growing, while others in the population enter a stationary phase. But not everyone agrees that the prisoner's dilemma is the best way to describe interactions between microorganisms. Microbes obviously lack the complex behavior of "higher" life forms, and so it's been suggested that mathematical models that are not based on animal behavior may be more accurate to describe these phenomena.

An alternative interpretation involves "producers" and "scroungers." A producer expends energy generating opportunities to exploit resources that are essential to survival and reproduction, whereas a scrounger takes advantage of these opportunities, usurping the resources that producers extract from the environment. In this view, the ancestral phi-6 phage is the producer, whereas the descendant viruses that evolved under co-infection are scroungers. The limited resources could be replication enzymes or other proteins essential for the production of progeny. When scroungers are rare, they frequently encounter producers, so they have many opportunities to grab the resources. The scroungers have an advantage and should increase when they are rare.

The producer/scrounger analogy assumes there is a cost associated with scrounging. This may be simply the increased competition between scroungers when they become common. If the cost of scrounging is not too great, the scrounging strategy will replace the producer strategy in the population. However, if each producer retains a sizable fraction of the resources it creates, despite a high frequency of scroungers, the producers will increase when they are rare and drive the two strategies into a mixed equilibrium in the population.

The cooperator/cheater and the producer/scrounger conflicts obviously have many similarities—most notably, both analogies deal with the parasitism of one virus by another. One difference may be that scroungers broadly excel at indirectly competing for rare resources, whereas cheaters narrowly specialize in directly competing with their particular helper virus. So, examining frequency-dependent fitness relative to a variety of viral genotypes might resolve whether one conflict is a better descriptor than the other. In either case, there's always a cheater and a sucker involved. A showman aware of these viruses and their prodigious rate of replication might have quipped instead, "There's a sucker born every microsecond."

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The Emergence of New Diseases

Lessons learned from the emergence of new diseases and the resurgence of old ones may help us prepare for future epidemics

Richard Levins, Tamara Awerbuch, Uwe Brinkmann, Irina Eckardt, Paul Epstein, Najwa Makhoul, Cristina Albuquerque de Possas, Charles Puccia, Andrew Spielman and Mary E. Wilson

As recently as 25 years ago, the threat of plague seemed old-fashioned, even medieval. Death from infectious disease was thought to be the result of poor hygiene and a lack of good antibiotics and vaccines, problems that by the mid-1970s had been largely overcome in the United States and most other industrialized nations. Medical practitioners were confident that infectious disease would represent a vanishingly small percentage of their concern. Wrote one prominent biologist in 1975, "During the last 150 years the Western world has virtually eliminated death due to infectious disease."

At the time, his optimism seemed justified. Smallpox had nearly been eradicated; tuberculosis and polio were on the decline and, with the exception of malaria, so were all of the other major infectious health threats of the 20th century. Scientists believed that, thanks to improved hygiene and sanitation, immunizations and antibiotics, all remaining infections of human beings and domestic animals would soon be eradicated.

Of course, skepticism was expressed even then. Agents of disease ranging from bacteria to insects had started to show resistance to the drugs and chemicals that had once so successfully killed them. And the optimistic projections were not consistent with what scientists knew to be true about the remarkable malleability of pathogens. For example, scrapie, a relatively mild disease of sheep, could somehow be transmitted to cattle, where it is devastating. Plants were also known to become afflicted with new diseases as old ones were eliminated. But the enthusiasm of the medical profession in general was not dampened by these examples, which after all, came from other disciplines and seemed too remote from the urgencies of medical practice.

Then came Lyme disease (1975), Legionnaire's disease (1978), toxic-shock syndrome (1978) and, more recently, AIDS (1981), chronic-fatigue syndrome (1985), and hantavirus (1993). The seventh cholera pandemic began in Indonesia in 1961, spread to Africa in the 1970s and reached South America in 1991, and now a new variant has emerged. Malaria reemerged in regions where it had been eliminated. Dengue and yellow fever are spreading. The incidence of tuberculosis started to climb in countries that had previously reported declines. Diphtheria reemerged in adults in the former Soviet Union. Suddenly, the proclamation of freedom from infection seemed, at best, premature.

These days, scientists no longer predict that the history of human infection will progress steadily toward the total elimination of infectious disease. More likely, the pattern will be one of disease turnover. With a new acceptance that infectious diseases will always be part of the human experience comes the realization that scientists will have to adopt a new approach to understanding the patterns of disease evolution. Rather than place sole confidence in measures we would use to fight infectious diseases after they arise, we, the members of the Harvard Working Group on New and Resurgent Diseases, are trying to identify the factors that encourage the emergence and spread of new diseases. To do that, we integrate complex social, epidemiological, ecological and evolutionary processes to understand how events in these various dimensions interact under changing circumstances to produce radically new health problems. In exploring potential threats to human health, we examine recent trends as part of epidemiological history and explore the progression of human diseases, as well as those of plants and animals. In order to anticipate new disease problems, including diseases that have not yet emerged, we have to examine the patterns of existing diseases and vectors and also look at the gaps in epidemiology. We apply current concepts and reexamine the conceptual framework that guides our present strategy of disease control. It is one of our principles that the emergence of new diseases can not be fully understood without understanding the social context in which they emerge.

What Is a New Disease?

At the start of our work we had to make two major decisions. First we had to define when a disease would be considered "new." Toward that end we identified ways in which a new disease may be recognized. A disease is recognized as new when its symptoms are distinct from any disease that has come before, or when a previously tolerated condition becomes unacceptable, as was the case with chronic exhaustion. A disease also becomes recognized when a previously marginal population gains a public voice, which is what happened with black-lung disease. Diseases that are slow to develop may be newly identified in a population whose life span is increasing. In addition, conditions are identified as new when a local disease becomes widespread, a rare disease becomes common or a mild disease becomes severe. Diseases also become recognized when cases cluster in a locality or social group, which was the situation with Legionnaire's disease. Sometimes a disease is identified as new when a new human population is medically examined or when improved diagnostic techniques allow a unique infectious agent to be seen.

We also had to settle on a definition of infectious disease. We agreed that it would be defined as a disease in which infection is brought about by one or more kinds of parasite invading a susceptible animal. These parasites, commonly called pathogens (literally, the origin of suffering) can be microorganisms such as bacteria and viruses, or they can be multicellular organisms, such as worms. In spite of their diverse classifications, they all contribute to disease by a similar mechanism. They all carry out part of their life cycle inside another ani-

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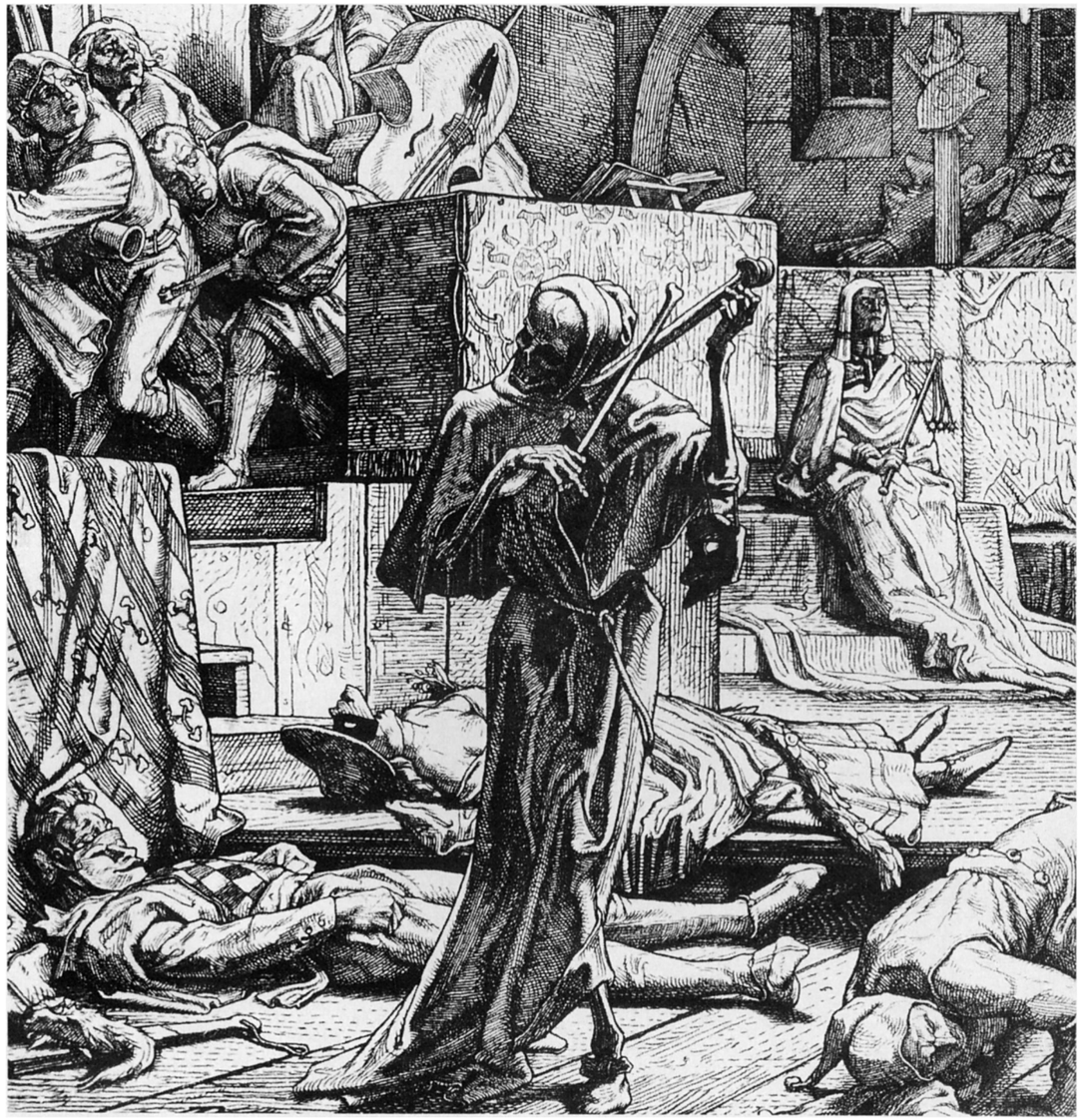
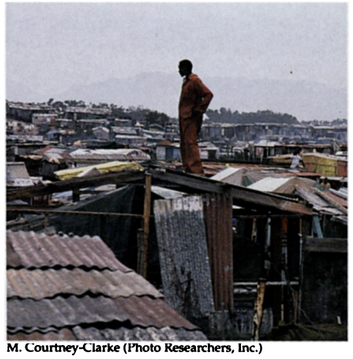


Figure 1. -Macabre images of plague-as-killer, such as this one from an 1845 wood carving by European artist Alfred Rethel, seemed, by the mid 1970s, old-fashioned. The near-elimination of smallpox and many other infections made many scientists confident that death from infectious disease was a thing of the past. Then came the emergence of new diseases and the resurgence of some old ones, and many scientists began to reassess the importance of infection in the future of human medical history.

Recent New Diseases		
viruses: newly identified agents		
1957 India	Kyasanur Forest	severe systemic infection and fever; transmitted by tick
1959 Uganda	O'nyong–nyong	explosive outbreaks of acute illness with fever and severe joint pain; transmitted by mosquito
1983	HIV-1	Acquired Immunodeficiency Syndrome
1989	Hepatitis C virus	transfusion-related and sporadic hepatitis
1990	Hepatitis E virus	acute hepatitis water-borne epidemics and sporadic hepatitis
1991	Venezuelan hemorrhagic fever Guanarito virus	outbreaks of severe hemorrhagic illness
viruses: old agent, new location		
1992–1993 Kenya	Yellow fever	severe hepatitis and hemorrhagic fever; mosquito-borne virus
1993 southwestern U.S.	Hantavirus	new syndrome with pulmonary distress
rickettsial diseases: newly identified agent		
1986	*Ehrlichia chaffeenis*	moderate to severe systemic infection with fever, headache, low white-blood-cell count
bacterial diseases: newly identified agents		
1975	*Borrelia burgdorferi*	Lyme disease manifestations include arthritis and skin rashes
1976	*Legionella pneumophila*	Legionnaire's disease typically severe pneumonia. water-associated bacterium
1978	*Staphylococcus aureus*	toxic-shock syndrome; profound shock, kidney failure
1983	*Afipia felis*	cat-scratch disease; mild infection with enlarged, tender lymph nodes usually; acquired from cat
1992	*Vibrio cholerae* 0139	new variant of cholera

Figure 2. In spite of antibiotics and vaccines, a number of new diseases have emerged in the past 40 years. Here, a partial list demonstrates that the future of human health history will be one of disease turnover. New diseases come about through a complex interaction of biological, social, economic, evolutionary and ecological factors.



M. Courtney-Clarke (Photo Researchers, Inc.)

Alan D. Carey (Photo Researchers, Inc.)

Rapid urbanization is one of the socioeconomic factors that puts people in contact with an unknown vector or pathogen

Creating new habitats—for example, by bulldozing forests—permits rare or remote microorganisms to become abundant and gain access to people

mal—the host—where, for a time, they live, eat and reproduce. Disease is often a by-product of these activities. The parasite may produce a chemical that is toxic to the host, or it may damage the cells it infects. In addition, the host's own response may damage tissues.

But the parasite is actually only one of many factors that contribute to disease. Many parasites require an accomplice to facilitate their spread from host to host. Often this accomplice, called a vector, is an insect. Frequently the vector bites an infected animal and ingests some of its blood. It then bites a second animal and deposits into that animal's tissues some of the parasite-ridden blood from the first. Sometimes insect vectors themselves require carriers. Rodents, in addition to harboring pathogens, can also serve as hosts to fleas, ticks or other insects in which the pathogens reside. An animal's contact with parasites and vectors will obviously be an important factor in determining whether it will become sick. A potential host's general state of health and nutrition as well as its genetic predisposition to control infection will also determine the outcome of an encounter with a pathogen. Furthermore, social processes shape pathways of infection and disease. In the end, it is likely that the classification of diseases into infectious, environmental, psychosomatic, autoimmune, genetic and degenerative will prove to be applicable only to a sample of cases where one factor overwhelms all others. The more accurate viewpoint will encompass full complexity of this network of factors that leads to recognizable disease.

Recognizing New Diseases
• a previously tolerated condition becomes unacceptable (chronic exhaustion)
• a marginal population acquires public voice (black lung)
• increase of life span allows a slow disease to develop
• a local disease becomes widespread
• a rare disease becomes common
• a mild disease becomes severe
• symptoms are clearly distinctive
• contagion is high and latency short, so that cases cluster in a locality or social group
• health service examines a new population
• diagnostic techniques permit visualization of a new pathogen

Figure 3. Criteria for recognizing a disease as "new" include changing attitudes as well as changes in the organisms responsible for disease.

Identifying the Pathogen

Most bacteria are not pathogens, most arthropods are not disease vectors and most mammals are not a source of human disease. What, then, are the characteristics of a successful pathogen or vector, and where should we look for them?

The potential for a nonpathogenic species to become a pathogen can be assessed by examining the present distribution of diseases, symptoms and virulence across groups of pathogens. We call this field systematic epidemiology and focus on ecological and social processes that influence disease emergence.

Systematic epidemiology asks, for example, questions about the range of hosts a particular pathogen can infect as well as the types of pathogen a particular host can support. It also explores unique and shared characteristics among related species of pathogen, symptom variability for the same pathogen in different hosts, modes of transmission and the epidemiological potential for different species groups.

For a sample of 247 infections, we note that, relative to other pathogens, fungi are less common in serious primary human infections, but are prominent pathogens of fish and plants. We also note that viruses depend much more on arthropod vectors than other groups, while fungi typically require no vectors at all. Viruses are smaller and more fragile than fungal spores, which probably accounts for their reliance on vectors.

Will Kastens, a student at the Harvard School of Public Health, prepared a preliminary survey of 412 human infections. Of these, 180 were exclusively human diseases, 118 were primarily hu-

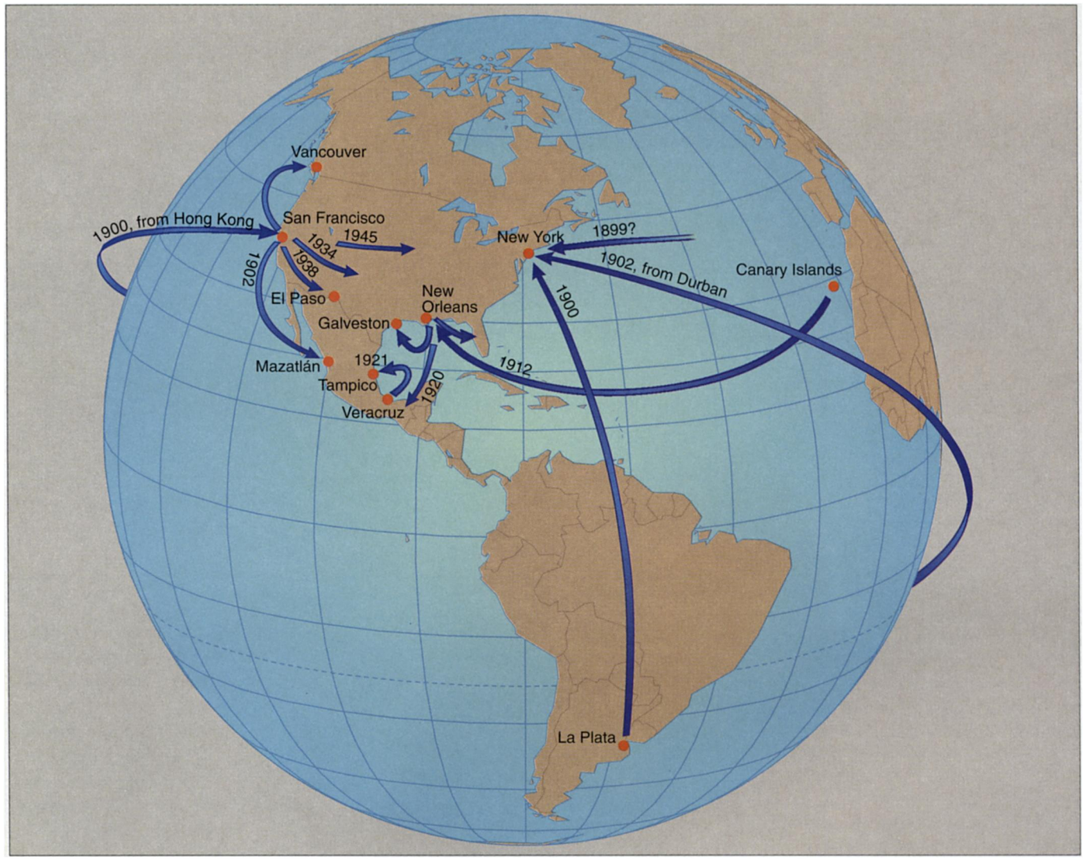


Figure 4. Map of the spread of bubonic plague in the early 20th century demonstrates how modern, rapid transportation can turn a local disease into a worldwide pandemic. When travel time is shorter than the incubation time of the disease, pathogens are more likely to move between countries and even continents. The spread of disease shown on this map would have been improbable during Christopher Columbus's time.

man diseases but could be found in other animals and 62 are principally found in other animals but can occasionally be found in people. The remaining pathogens are not normally pathogens of people or animals. Usually they are free-living microbes that cause disease when a chance encounter places them in contact with people. Of the diseases shared between people and other animals, 35 are widespread among mammals, about a dozen are shared with livestock and domestic animals, and approximately seven are shared with nonhuman primates. A few are shared between people and birds, and a smattering of pathogens can infect fish, people, shellfish and insects. Finally, at least two species of *Vibrio*, the microorganism that causes cholera, can be associated with plankton.

Kastens's data also show some interesting trends with regard to virulence. Those pathogens that mostly affect animals but sometimes invade human populations include a greater proportion with high virulence. We speculate that this is because their evolution is dominated by selection in their nonhuman hosts. On the other hand, diseases that are commonly or exclusively human have evolved in part in the human context, and some of them evolve to be gentler to people. Paul Ewald of Amherst College has suggested that vector-borne diseases tend to be more virulent than those requiring direct contact between people. His argument is that where the mobility of the patient is a requirement for transmission, it is to the pathogen's advantage that the infected person remain mobile, even if this limits the pathogen's rate of reproduction. But these are only tendencies. More detailed examinations of natural selection in pathogens reveal many exceptions to these examples.

Adaptive Potential

Although we give names to different species of pathogens, vectors, reservoirs and their associated diseases, these are not static entities. Pathogens as organisms undergo natural selection both within the host and in the course of transmission between hosts. And a pathogen's success in adapting to conditions within and between hosts will determine, in part, its success in spreading throughout a population.

A pathogen is confronted with three sometimes conflicting demands. It must obtain its nourishment to develop and reproduce, avoid being killed by the body's defenses and find a satisfactory exit to another host. Meeting these demands may require that a pathogen localize to a particular site in the body. For example, the blood is an optimal site for feeding, but it is a site of high immune activity. A pathogen in the central nervous system is relatively secure from destruction by the immune system but has no easy exit. The skin is also relatively safe from the immune system and can be exited fairly easily, but it is not a good site for reproduction.

John Gerlach (Animals Animals)

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A pathogen is introduced to a human population, as when carrier rodent populations increased and exposed people to hantavirus

People moving into a new country may encounter pathogens against which they have no resistance

Some pathogens adopt strategies for dealing with the immune system, so they are freer to choose sites in the body where immune activity is high. The human immunodeficiency virus, which causes AIDS, can remain in the blood because it destroys part of the immune system. Trypanosomes, which cause sleeping sickness, can also remain in the blood because they are adept at changing their protein coat, and in this way dodge detection by the immune system.

From the point of view of the pathogen, the symptoms suffered by the host are merely by-products of the pathogen's life-style. For example, in diarrheal diseases, the most obvious symptom arises when the pathogen exits one host in search of another. The pathogens remaining in the original host invade the gastrointestinal mucosa so they are not whisked away during the diarrheic episode.

Pathogens face other strategic decisions as well. Should they reproduce rapidly and exit quickly, or should they prolong the infection in the face of uncertain success in infecting someone else? The strategy adopted will depend on the relative rates of pathogenic reproduction, contagion possibilities and the danger of strong and effective treatment of the infection.

The role of drugs—antibiotics and antivirals in particular—in directing natural selection in the pathogen makes the intervenor a part of the system being intervened in. The host's behavior in effect becomes part of the selection pressure and affects the characteristics of the pathogen in the next outbreak. For example, if the host uses antibiotics, some of the pathogens may develop resistance to the drugs. During future outbreaks, these drug-resistant pathogens may predominate, and other antibiotics will have to be used to eliminate them.

Vectors, like pathogens, also undergo evolutionary change. Currently, a new biotype (or possibly, sibling species) of the whitefly *Bemisia tabaci*, carrier of bean golden mosaic virus, is spreading at the expense of the previous biotype. The new biotype has a wider range of host plants to feed on and is therefore spreading viruses to new plant species. In this case, a change in host range of the vector makes new species of plants serve as reservoirs for infections of crop plants. Reservoirs can maintain pathogens at low levels in small wild populations without being noticed, until a change in the environment or vector opens up new opportunities.

Pathogens on the Move

To cause a disease, a pathogen must first find a potentially receptive population of hosts. Sometimes the pathogen is required to travel. Various measures have been proposed to indicate the likelihood of a disease invading a population, among them the number of new cases derived from a given case, which epidemiologists refer to as the reproductive rate.

We have identified several key factors affecting the introduction of diseases into new populations. When diseases are carried from one area to another, it is important to establish the travel time needed to reach the new population relative to the rate of progression of the disease. For example, in Columbus's time, crossing the Atlantic was slow compared to the progression of smallpox. Since all carriers of the virus manifest symptoms of the disease, most of the infected travelers would have either become sick and died or recovered before reaching the New World. As a result, smallpox probably did not reach the Americas until several decades after Columbus's voyage. If Columbus were to begin his journey today, the situation might be different. Modern transportation has cut travel time to almost anywhere in the world to a few days at most, less than the average incubation time of many pathogens. Travel time, therefore, presents a less significant barrier to the spread of disease than it once did.

So does travel itself. Populations are much more easily moved than before. Left to their own devices, species spread into new areas very slowly. It has taken just over a century for rodent-borne plague to reach Mississippi from the West Coast. Fire ants spread at a rate of only a couple of miles a year. But man-made transport can speed the process so that pathogens can travel many thousands of miles in a few days. Political and economic oppression and opportunity are prime motivators for large-scale movements of people across countries and continents. The net effect of so much human migration is that diseases once confined to small regions of the globe can potentially spread to many regions.

Large-scale global commerce increases the probability of introducing vectors (often insects) and nonhuman carriers of disease to naive populations—a situation that may have touched off and accelerated the seventh cholera pandemic. For example, a freighter is thought to have transported the cholera vibrio in its ballast water from China to Peruvian coastal waters. Vibrio flourished in al-

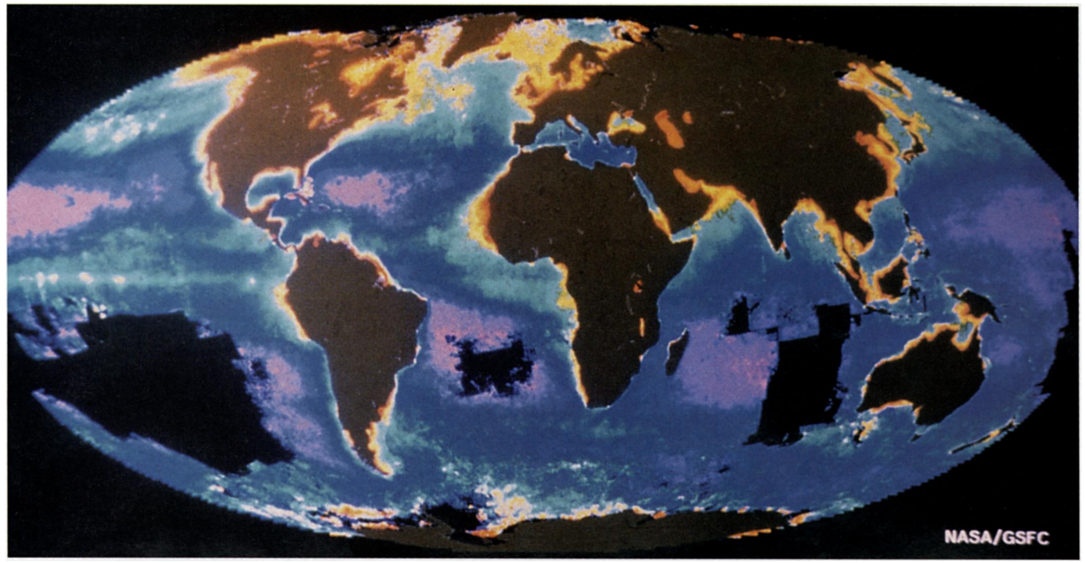


Figure 5. Satellite image shows the global distribution of coastal algal blooms, which are breeding grounds for pathogens, in particular the microorganism that causes cholera. Sewage and fertilizer pouring into marine ecosystems, overharvesting of fish and shellfish and the loss of wetlands, combined with climatic changes, have conspired to cause a worldwide explosion in the number of such blooms. (Photograph courtesy of NASA.)

gal blooms enriched with nitrogen and phosphorus from sewage and fertilizers. Algae are filtered and eaten by molluscs, crustaceans and fish that are, in turn, eaten by people. Once it entered, the infection in Latin America spread rapidly, as social and economic conditions provided a fertile environment for infection. Rapid urbanization, foreign debt and political changes strained the economy made sanitation and public health low priorities and paved the way for the epidemic spread of cholera. As of August 1992, more than 500,000 Latin Americans had become ill, and 5,000 of those people had died.

Changing Ecosystems

Just arriving in a new location does not ensure that a pathogen will take hold there. In fact, most introductions do not result in colonization because the species does not land in a hospitable niche. To successfully colonize new terrain, the invader must find a suitable environment and, if it is a pathogen, a receptive host population.

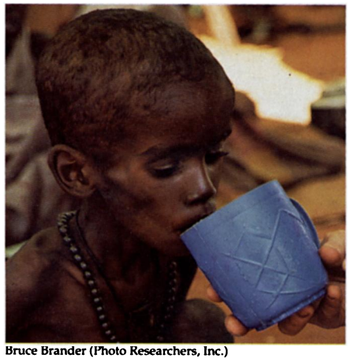
In general, invasion is easiest in regions of low biological diversity, where the invader faces less competition from native species. Oceanic islands are notoriously vulnerable to invasion. They have been known to be devastated by invasions of rats, goats or weeds, because their few native species could not compete.

Also vulnerable to invasion are habitats that have been disturbed by natural events or human activity. These events eliminate predators and competitors and create opportunities for new species to take up residence. For example, the spread of Lyme disease in New England is related to a number of human activities that have dramatically altered the region's ecology. During the past century, the forests were cleared to make way for agriculture. This eliminated from the area both the deer and their predators. The forests returned eventually, as did the deer. But the deer's predators did not. The deer tick, carrier of the infection, could spread, unimpeded, throughout the deer population and into the human populations that came into contact with them.

Vectors of human disease generally thrive in newly established habitats. Piles of used tires around the edges of rapidly growing cities collect water in which the mosquito *Aedes aegypti*, a vector for the organism that causes dengue and yellow fever, reproduces. Irrigation ditches, borrow pits, construction sites, poorly drained water pumps and puddled river bottoms each may serve as breeding sites for the mosquitoes that carry malaria. The pathogens carried by the mosquitoes can feed in these man-made habitats without being diverted to other animals, who are less successful in shuttling the pathogen to human hosts. In this manner, whole new niches have been created beyond the original geographic and ecological range of the vectors.

Of course, the successful spread of a human pathogen requires a vulnerable human population. The vulnerability of a group of people to a pathogen depends, on one hand, how contagious the pathogen is and how quickly it is transmitted from person to person, versus the population's immunity on the other. In this equation, all environmental changes are potentially reflected epidemiologically since conditions can affect the opposing processes of contagion and recovery, acquisition and loss of immunity.

The contagion rate depends on the number of pathogens that leave an infected individual and enter the environment. It also depends on the number that survive in that environment and gain contact to and ultimately infect other people. Each of these steps is complex and combines biological and social factors that are not constant. For example, no two people are equally susceptible to infection. A person's general state of health is as much determined by social, nutritional, age and gender factors as by genetics. Personal habits, such as smoking, sexual practices, alcohol consumption and food avialability and preferences can also contribute to a person's susceptibility to a particular disease.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Malnutrition, as well as immunosuppresive drugs and environmental stressors, makes people vulnerable to infection

Animal pathogens mutate and acquire the ability to infect people; the human immunodeficiency virus may have evolved from a monkey virus

In addition, there is now widespread concern about the potential effects of climatic change on disease. Changes in global temperature would carry with them changes in wind and precipitation patterns, humidity, soil composition and vegetation. All of these affect human activity and movement of populations.

Finally, the environment of a pathogen includes other parasites. In developing countries, it is not uncommon for people to harbor two to four simultaneous infections. Within their shared host, these pathogens may interact in familiar ecological patterns. They may compete for nutrients, or they may alter immune function in such a way as to benefit one while deterring another. They may alter their shared environment by causing fever or by damaging cells. The symptoms of one infection, say, sneezing, may facilitate the spread or mask the symptoms of the other. What this suggests is that the most effective way to deal with disease in the clinic is to consider the entire epidemiological profile, rather than consider one disease at a time.

Hantavirus

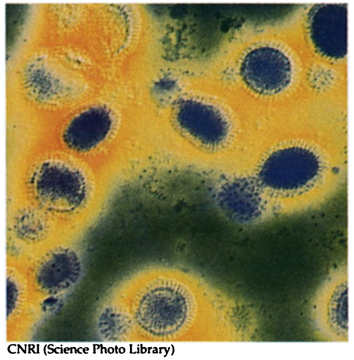
The emergence of a disease within a changing ecosystem was dramatically illustrated when a mysterious illness emerged in the Four Corners region of the southwestern United States earlier this year. In August 1993, a 37-year-old farmer who worked in the Four Corners area sought medical help when an illness he had had for six days took a turn for the worst. At first, the farmer experienced flu-like symptoms, including fever, nausea and vomiting, which progressed to coughing and shortness of breath. An xray showed fluid in both of the farmer's lungs. After 12 hours, he developed acute respiratory distress and died. Several weeks and several cases later, scientists at the Centers for Disease Control in Atlanta linked the mysterious disease to a new strain of hantavirus, viruses that have been associated with hemorrhagic fevers and renal disease in Europe and Asia, but that had not previously been known to cause disease in North America. Where had the virus come from, and why did it suddenly emerge?

That answer came serendipitously from studies conducted by Robert Parmenter and colleagues at the University of New Mexico. Parmenter and his team had been interested in the sudden increase in deer mice, which, as it turns out, are carriers of the hantavirus. Six years of drought ended in the spring of 1992, when heavy rains deluged the area. The abrupt change disturbed the ecological balance in the region, producing an abundance of piñon nuts and grasshoppers, food for the mice. The deer-mouse population flourished, but the drought had virtually eliminated all of the mouse's predators. In the year between May 1992 and May 1993, the deer-mouse population increased ten fold. By October 1993, the deer-mouse population had declined sharply, and the epidemic apparently came to an end. It had taken its toll. Forty-two cases of hantavirus pulmonary syndrome were reported in 15 states, mostly clustered in the Southwest. Twenty-six of those cases were fatal.

One of the lessons to be learned from such case studies is how disruption of stable ecosystems can alter an existing disease and facilitate its spread. For that reason, we are particularly concerned with recent disruptions of marine ecosystems, which are undergoing dramatic changes. Sewage and fertilizer pouring into marine ecosystems, overharvesting of fish and shellfish and the loss of wetlands, combined with climatic changes, have conspired to cause a worldwide ex-


Earl Roberge (Photo Researchers, Inc.)

Pollutants or radiation increase the mutation rate of pathogens



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Influenza virus and other pathogens may evolve toward greater virulence

Although it has not happened yet, bioengineered organisms may contribute to infection in the future

plosion in coastal algal blooms around coastal regions, providing a rich environment for diverse communities of microorganisms. The sea-surface temperatures in these environments are frequently elevated, which shifts organisms towards more toxic species, possibly by increasing their mutation and reproduction rates.

Among the new species that have been identified in these algal blooms is a new variant of the cholera vibrio. Now present in 10 Asian nations, this new variant seems to be distinct from previous forms of cholera, based on immunological tests. Antibodies that recognize other known variants do not recognize this one. This newly emergent, environmentally hardy form of cholera threatens to become the agent of the eighth pandemic. Monitoring algae and other microscopic marine organisms for vibrios offers the opportunity for establishing an early warning system for this new pathogen. Images from remote-sensing satellites can help guide this operation.

Confronting Uncertainty

The ultimate goal of these types of analyses is to anticipate the onset of new diseases and to eliminate the situations that facilitate their spread. In meeting these challenges, however, we confront a high degree of uncertainty. We can make some short-term predictions about new disease outbreaks by recognizing conditions that favor outbreaks of known disease and by anticipating ecological changes associated with human activity. We are developing models based on evolutionary ecology that would allow us to make some longer-range predictions about new disease outbreaks.

Just as organisms adapt rapidly to a new condition, we too can respond rapidly to new disease threats. But to do that, we have to recognize them quickly. An analysis of the factors facilitating or delaying recognition along with improved diagnostic techniques, might allow us to recognize problems sooner than is now possible.

We also must be very general in our response to disease. Our current therapeutic tools—immunization and antibiotics— are highly specific for the diseases they fight and can be developed only after the pathogen has been studied. Other organisms develop very nonspecific resistances that confer protection against a range of threats. For human beings, measures could be taken to boost the body's defenses in general. These would include good nutrition, pollution control, biodiversity for vector control and social arrangements that ensure these measures reach the entire population.

We advocate a mixed strategy, which offers back-up protection in case the first plan turns out not to be the best. A mixed strategy combines the tried-and-true approaches with newer ones. It may rely on some short-range predictions and monitoring, but it encourages longer-range ecological and evolutionary surveillance. It may concentrate on public health measures and seek to create healthful social systems that renegotiate our relations with the rest of nature.

Ultimately, we may have to reevaluate our notion of disease. We must see disease as the outcome of multiple conditions arising from changes not only within cell nuclei, but also around the globe, including changes in climate, economic patterns and communities of species. Any effective analysis of emerging diseases must recognize the study of complexity as perhaps the central general scientific problem of our time.

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MEDICINE

The Shape of Plagues to Come

Robert Dorit

TWENTY-FIRST CENTURY PLAGUE: The Story of SARS. Thomas Abraham. viii + 165 pp. Johns Hopkins University Press, 2005 (first published by Hong Kong University Press in 2004). $24.95.

Many of us have a romanticized view of the living world as a peaceable kingdom in which only the occasional excesses of *Homo sapiens* disturb the balance. But, as Thomas Abraham makes clear in *Twenty-First Century Plague,* the reality is less benign. We, the more than six billion people on this planet, who represent the single largest threat to the world's biological diversity and to Earth's capacity to self-regulate and self-correct, are, ironically, ourselves threatened: We're a vast and vulnerable target for transmissible diseases, low-hanging fruit for any infectious agent able to invade a human host.

For most of our history as a species, we have lived in small, nomadic and relatively isolated assemblages spread thinly on the ground. Yet in the past 600 years (only 30 generations), and particularly in the past 200 years, the human population has increased dramatically in size and density; never before have so many lived in sedentary agglomerations. Combine that trend with the speed and ubiquity of travel over long distances and with the vast global inequities in the distribution of primary and preventive health care, and you have the makings of an epidemiological perfect storm.

Twenty-First Century Plague is a ringside account of the world's recent encounter with the emerging infectious disease SARS (severe acute respiratory syndrome). Engagingly written by an accomplished journalist, the book traces the spread through the human population of a relatively obscure coronavirus, one that previously had infected only animals such as palm civets and ferret badgers. The epidemic began in Guandong Province in China in the fall of 2002 and spread swiftly around the world, infecting more than 8,000 people by the following summer and killing nearly 1 in 10 of them. As the book makes clear, the SARS outbreak was chilling, not so much because of the number of afflicted individuals—which comes nowhere near the toll of HIV or previous influenza outbreaks—but because of what it revealed about our ability to respond to new infectious agents.

How did we do? The verdict is, at best, mixed.

Maddening political, personal and institutional obstacles prevented the sharing of critical information; Abraham is at his best describing the human hurdles to an effective response. In an era in which information could circle the world within seconds, the SARS virus initially outraced vital knowledge about treatment and control. Doctors in Guandong quickly developed basic containment procedures against the spread of SARS, but that information was not conveyed even to health workers struggling with the virus elsewhere in China, let alone to those in the rest of the world. The authorities in the affected countries lost valuable time worrying about the effects that news of the local outbreak might have on their economies and on their personal, national and political reputations. Abraham, who has a keen reporter's eye, moves beyond the press releases extolling international collaboration and preparedness to expose the dissembling and the politicking.

But he also finds a number of heroes: the country doctors and health workers who rushed into affected areas knowing full well that the virus had already infected and killed some of their coworkers; the epidemiologists who traced an outbreak in Hong Kong; the scientists who managed to identify the virus within weeks of the first reported case outside China. Many laboratories coordinated their efforts so that the sequencing of SARS could be completed by mid-April 2003. The epidemic flamed out, first in Vietnam and eventually around the world, and by early July the World Health Organization declared it contained.

In retrospect, this new disease was checked relatively quickly. The success can be attributed in part to the national and international agencies charged with detecting and responding to outbreaks, which reacted courageously but inconsistently. Their efforts were frequently obstructed by individuals and ministries outside the health system. But we also caught a lucky, and unusual, break: SARS, although potentially lethal, is not highly transmissible.

Abraham rightly urges us to demand that the lessons gleaned from the SARS outbreak be incorporated into plans for epidemic surveillance and response. We can be virtually certain that the system will be tested again and again—not by deliberate acts of bioterrorism, where most of our attention is currently focused, but by emerging diseases. Scientists around the world are keeping a wary eye on a new strain of bird influenza capable of moving from its avian hosts into the human population. Other less well-known infectious agents are making similar tentative excursions into our species, probing for weaknesses in our immune defenses. SARS may presage new and more dangerous battles in the long evolutionary struggle between *Homo sapiens* and its pathogens. Somewhere on the planet, a new organism is evolving to exploit six billion specimens of ready prey.

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Ecological Dependency

Katie L. Burke

SPILLOVER: Animal Infections and the Next Human Pandemic. David Quammen. 587 pp. W. W. Norton and Company, 2012. $28.95.

In his first book since the 2008 essay collection *Natural Acts: A Sidelong View of Science and Nature*, David Quammen looks at the natural world from yet another angle: the search for the next human pandemic, what epidemiologists call "the next big one." His quest leads him around the world to study a variety of suspect zoonoses—animal-hosted pathogens that infect humans. Quammen interweaves his narrative with facts about zoonotic spillover—when a pathogen moves from its host species into another species—discussing a wide range of viruses (Hendra, Ebola, SARS, herpes B, HIV, Nipah and Marburg), bacteria (Q fever, Lyme disease and psittacosis) and malarial protists (a varied cast of *Plasmodium* species). Such diseases are difficult to study, he emphasizes, because of their complex ecological and evolutionary contexts and the mystery and misinformation that surround them. Quammen hunts for the generalities connecting high-profile zoonoses, which biologists have identified in order to better predict what the next big one will be like.

Like many readers, I have long enjoyed Quammen's work, but I picked up this book with trepidation: The cover, which features a blurry photograph of a primate with red teeth bared, suggested the contents might contribute to the already excessive hype that surrounds many of these pathogens. But the image is misleading: *Spillover* is not sensationalist. Quammen notes:

> I don't say these things about the ineradicability of zoonoses to render you hopeless and depressed. Nor am I trying to be scary for the sake of scariness. The purpose of this book is not to make you more worried. The purpose of this book is to make you more smart. That's what most distinguishes humans from, say, tent caterpillars and gypsy moths. Unlike them, we can be pretty smart.

In addition to the difficulty of describing human and animal suffering, or perhaps because of it, public perception of zoonotic diseases is fraught with misunderstanding. Quammen recognizes this problem; he spends pages debunking myths about Ebola that were most notoriously popularized by Richard Preston's *The Hot Zone*. (Quammen confesses to gobbling up this popular book when it came out in 1994—as did I.)

Although the zoonoses covered in *Spillover* are gruesome, the number of deaths they cause is generally small in comparison to that of other human diseases. Quammen acknowledges this difference but makes a compelling case for the need to study these "anomalies":

> Given the global scorecard of morbidity and mortality caused by old-fashioned and nonzoonotic infectious diseases . . . why divert attention to these boutique infections, these anomalies, that spill out of bats or monkeys or who knows where to claim a few dozen or a few hundred people now and then? *Why?* Isn't it misguided to summon concern over a few scientifically intriguing diseases, some of them new but of relatively small impact, while boring old diseases continue to punish humanity? . . . It's a fair question but there are good answers. . . . The bluntest is this: AIDS.

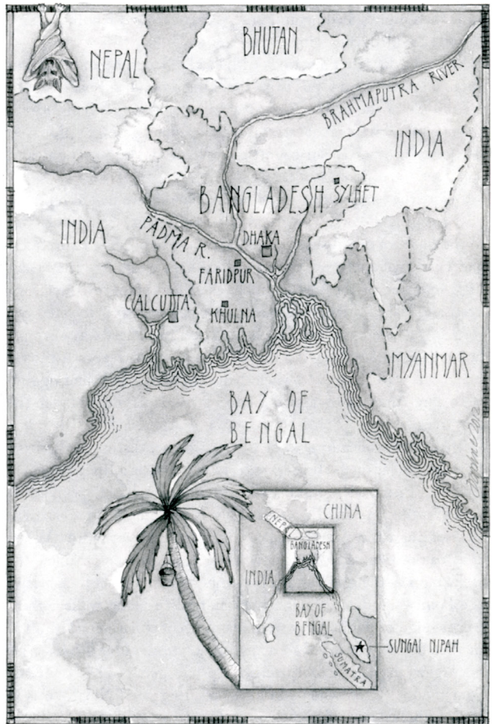
A book that covers AIDS, Ebola, and other notoriously disturbing ailments is not for the faint of heart. Quammen gets down to business in the first chapter, describing "horse heads lying around, severed limbs, blood and other fluids flowing down the gutter, suspect organs and tissues going into bags." Those who aren't comfortable reading anecdotes that regularly mention vomit and diarrhea, or mass killings of animals harboring zoonotic infections, might do better to read one of Quammen's earlier books instead. But if you can set aside your queasiness, you'll be rewarded by Quammen's prose.

He stresses that understanding ecology is essential to understanding enigmatic and puzzling new epidemics: "Ecological circumstance provides opportunity for spillover. Evolution seizes opportunity, explores possibilities, and helps convert spillovers to pandemics." Thankfully, at the mention of ecology, Quammen does not resort to circle-of-life campiness; instead he details the ecological complexities that influence the incidence of outbreaks, including forest fragmentation, biodiversity loss, and increased contact between forest systems and high densities of people and livestock. In one of my favorite parts of the book, he reports a conversation with Rick Ostfeld, an expert on Lyme disease from the Cary Institute of Ecosystem Studies:

> Some people take "All life is connected" to be the central truth of ecology, Ostfeld added. It's not. It's just a vague truism. The real point of the science is understanding which creatures are more intimately connected than others, and how, and to what result when change or disturbance occurs.

The narratives included in *Spillover* are not simply disturbing or scary. They often incorporate the fun and quirkiness of scientific research, not to mention the passion and effort behind it. The book includes plenty of lines like this one: "Picture two guys in a dark stone room, wearing headlamps, high-fiving in nitrile gloves." Studying potential epidemics is exciting but risky work, and Quammen follows researchers into caves harboring cobras, onto roofs decked out with mist nets for catching bats, deep into African jungles to tranquilize gorillas, onto Bangladeshi temple grounds full of semi-tame macaques and onto rat farms in China (where he samples the fare).

Paraphrasing an unnamed American field biologist, Quammen writes, "If you take too many risks, you don't get home. If you take too few, you don't get the data." In the face of dicey field excursions, understanding mathematical models and crunching statistics are the least of these researchers' worries. In addition to stories of fieldwork, though, Quammen tackles some of the most difficult concepts to understand in infectious disease biology. With verve and clarity, he details mathematical epidemiological models, the life cycle of the malaria-causing *Plasmodium* and the relation between transmission and virulence. An example: "The first rule of a successful parasite is slightly more complicated than Don't kill your host. It's more complicated even than Don't burn your bridges until after you've crossed them. The first rule of a successful parasite is $\beta N / (a + b + n)$." Although he doesn't spell out the meaning of each variable, he translates the formula into plain English, so readers will come away knowing this model says that the infection rate is based on changes in population density, as well as transmission rate and virulence. In addition to new diseases, Quammen touches on many of the examples often included in standard disease ecology or epidemiology courses—for example, myxomatosis in nonnative rabbits of Australia, and mutation rates in RNA versus DNA viruses. I highly recommend this book to readers who are interested in biology, and as a source of supporting discussion material for a course on infectious disease.



The Nipah virus, first identified in northern Malaysia in 1998 among pig farmers, reemerged in Bangladesh in 2000. Outbreaks there were eventually traced to date-palm *(Phoenix sylvestris)* sap, which both humans and flying foxes *(Pteropus giganteus)* enjoy drinking. The latter, which are a species of bat, are carriers of the virus. From *Spillover*.

Quammen concludes with an answer to the question of when, where and what the next big disease will be: "It depends." More specifically, as he puts it in a discussion of work by ecologists Roy Anderson and Robert May, "It depends on the specifics of the linkage between transmission and virulence. . . . It depends on ecology and evolution." That makes a pretty good answer to any question about the future of human kind.

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