The ability to quickly alter the code of life has given us unprecedented power over the natural world. The question is: Should we use it?

Calla Vanderberg enters the world at Inova Women’s Hospital in Falls Church, Virginia. As with all newborns here, seven of her genes involved in drug metabolism will be analyzed. Physicians in the future will be able to tailor medicines to her unique genetic profile.
Mosquito larvae in the laboratory of Anthony James at the University of California, Irvine pay witness to how a dreaded disease might be stopped. Both are *Anopheles stephensi*, a major carrier of the malaria parasite in urban Asia. Using a technique called CRISPR, James has edited a gene in the larva on the right so that the
insect cannot transmit the parasite. A fluorescent protein signals that the experiment has worked. Released in the wild, mosquitoes engineered with CRISPR and a tool called gene drive could eventually replace the wild mosquitoes that carry the disease. But too much uncertainty still exists to put such science into practice.
Zhou Yin of the Yunnan Key Laboratory of Primate Biomedical Research in Kunming, China, shows off a young long-tailed macaque raised from a CRISPR-modified embryo. Dozens of other organisms—including chickens and cattle, mushrooms and wheat, catfish and koi—have been engineered with CRISPR to carry specific genetic traits. Many more will follow.
If you took a glance around Anthony James’s office, it wouldn’t be hard to guess what he does for a living. The walls are covered with drawings of mosquitoes. Mosquito books line the shelves.

Hanging next to his desk is a banner with renderings of one particular species—*Aedes aegypti*—in every stage of development, from egg to pupa to fully grown, enlarged to sizes that would even make fans of *Jurassic Park* blanch. His license plates have a single word on them: AEDES.

“I have been obsessed with mosquitoes for 30 years,” says James, a molecular geneticist at the University of California, Irvine.

There are approximately 3,500 species of mosquito, but James pays attention to just a few, each of which ranks among the deadliest creatures on Earth. They include *Anopheles gambiae*, which transmits the malaria parasite that kills hundreds of thousands of people each year. For much of his career, however, James has focused on *Aedes*. Historians believe the mosquito arrived in the New World on slave ships from Africa in the 17th century, bringing with it yellow fever, which has killed millions of people. Today the mosquito also carries dengue fever, which infects as many as 400 million people a year, as well as such increasingly threatening pathogens as chikungunya, West Nile virus, and Zika.

In a widening outbreak that began last year in Brazil, Zika appears to have caused a variety of neurological disorders, including a rare defect called microcephaly, where babies are born with abnormally small heads and underdeveloped brains.

The goal of James’s lab, and of his career, has been to find a way to manipulate mosquito genes so that the insects can no longer spread such diseases. Until recently, it has been a long, lonely, and largely theoretical road. But by combining a revolutionary new technology called CRISPR-Cas9 with a natural system known as a gene drive, theory is rapidly becoming reality.

CRISPR places an entirely new kind of power into human hands. For the first time, scientists can quickly and precisely alter, delete, and rearrange the DNA of nearly any living organism, including us. In the past three years, the technology has transformed biology. Working with animal models, researchers in laboratories around the world have already used CRISPR to
Cow blood engorges an exposed mosquito’s gut in Anthony James's lab. Versions of the species carrying Zika and dengue fever can be manipulated with CRISPR so that they give birth to sterile offspring.

DAVID LIITSCWAGER
Scientists used conventional genetic engineering to add genetic material from two other fish species to create the AquAdvantage Atlantic salmon (top), which can reach market size twice as fast as its natural counterpart. The fish consumes less feed and can be raised in isolation close to cities, reducing transportation
costs and emissions, and eliminating any chance of escape into the wild. While the FDA has approved the fish as entirely safe for consumption, doubts over the safety of transgenic foods persist. In the future, CRISPR-engineered foods, which do not combine genes from different organisms, might find quicker acceptance.
No discovery of the past century holds more promise—or raises more troubling ethical questions.

CRISPR—Cas9 has two components. The first is an enzyme—Cas9—that functions as a cellular scalpel to cut DNA. (In nature, bacteria use it to sever and disarm the genetic code of invading viruses.) The other consists of an RNA guide that leads the scalpel to the precise nucleotides—the chemical letters of DNA—it has been sent to cut. (Researchers rarely include the term “Cas9” in conversation, or the inelegant terminology that CRISPR stands for: “clustered regularly interspaced short palindromic repeats.”)

The guide’s accuracy is uncanny; scientists can dispatch a synthetic replacement part to any location in a genome made of billions of nucleotides. When it reaches its destination, the Cas9 enzyme snips out the unwanted DNA sequence. To patch the break, the cell inserts the chain of nucleotides that has been delivered in the CRISPR package.

By the time the Zika outbreak in Puerto Rico comes to an end, the U.S. Centers for Disease Control and Prevention estimates that, based on patterns of other mosquito-borne illnesses, at least a quarter of the 3.5 million people in Puerto Rico may contract Zika. That means thousands of pregnant women are likely to become infected. Currently the only truly effective response to Zika would involve bathing the island in insecticide. James and others say that editing mosquitoes with CRISPR—and using a gene drive to make those changes permanent—offers a far better approach.

Gene drives have the power to override the traditional rules of inheritance. Ordinarily the progeny of any sexually reproductive animal receives one copy of a gene from each parent. Some genes, however, are “selfish”: Evolution has bestowed on them a better than 50 percent chance of being inherited. Theoretically, scientists could combine CRISPR with a gene drive to alter the genetic code of a species by attaching a
desired DNA sequence onto such a favored gene before releasing the animals to mate naturally. Together the tools could force almost any genetic trait through a population.

Last year, in a study published in the Proceedings of the National Academy of Sciences, James used CRISPR to engineer a version of Anopheles mosquitoes that makes them incapable of spreading the malaria parasite. “We added a small package of genes that allows the mosquitoes to function as they always have,” he explained. “Except for one slight change.” That change prevents the deadly parasite from being transmitted by the mosquitoes.

“I’d been laboring in obscurity for decades. Not anymore, though—the phone hasn’t stopped ringing for weeks,” James said, nodding at a sheaf of messages on his desk.

Combating the Ae. aegypti mosquito, which carries so many different pathogens, would require a slightly different approach. “What you would need to do,” he told me, “is engineer a gene drive that makes the insects sterile. It doesn’t make sense to build a mosquito resistant to Zika if it could still transmit dengue and other diseases.”

To fight off dengue, James and his colleagues have designed CRISPR packages that could simply delete a natural gene from the wild parent and replace it with a version that would confer sterility in the offspring. If enough of those mosquitoes were released to mate, in a few generations (which typically last just two or three weeks each) entire species would carry the engineered version.

James is acutely aware that releasing a mutation designed to spread quickly through a wild population could have unanticipated consequences that might not be easy to reverse. “There are certainly risks associated with releasing insects that you have edited in a lab,” he said. “But I believe the dangers of not doing it are far greater.”

IT HAS BEEN MORE THAN 40 years since scientists discovered how to cut nucleotides from the genes of one organism and paste them into the genes of another to introduce desired traits. Molecular biologists were thrilled by the possibilities this practice, referred to as recombinant DNA, opened for their research. From the start, however, scientists also realized that if they could transfer DNA between species, they might inadvertently shift viruses and other pathogens too. That could cause unanticipated diseases, for which there would be no natural protection, treatment, or cure.

This possibility frightened no one more than the scientists themselves. In 1975, molecular biologists from around the world gathered at the Asilomar Conference Grounds, along California’s central coast, to discuss the challenges presented by this new technology. The group emerged from the meeting having agreed to a series of safeguards, including levels of laboratory security that escalated along with the potential risks posed by the experiments.

It soon became clear that the protections seemed to work and that the possible benefits were enormous. Genetic engineering began to improve the lives of millions. Diabetics, for example, could count on steady supplies of genetically engineered insulin, made in the lab by placing human insulin genes into bacteria and then growing it in giant vats. Genetically engineered crops, yielding more and resisting herbicides and insects, began to transform much of the world’s agricultural landscape.

Yet while genetically engineered medicine has been widely accepted, crops produced in a similar fashion have not, despite scores of studies showing that such products are no more dangerous to eat than any other food. As the furor over the labeling of GMOs (genetically modified organisms) demonstrates, it doesn’t matter whether a product is safe if people refuse to eat it.

CRISPR may provide a way out of this scientific and cultural quagmire. From the beginning of the recombinant era, the definitions of the word “transgenic” and the term “GMO” have been based on the practice of combining in a laboratory the DNA of species that could never
A worker waits to enter a clean room at China Regenerative Medicine International in Shenzhen, where pig corneas are modified for transplant into humans. Chinese scientists have twice conducted experiments to alter nonviable human embryos with CRISPR. Much work remains before the technique could be applied to viable human embryos that would pass on genetic changes.
The potential for CRISPR research to improve human medicine would be hard to overstate. The technology has already transformed cancer research by making it easier to engineer tumor cells in the laboratory, then test various drugs to see which can stop them from growing. Soon doctors may be able to use CRISPR to treat some diseases directly.

Stem cells taken from people with hemophilia, for example, could be edited outside of the body to correct the genetic flaw that causes the disease, and then the normal cells could be inserted to repopulate a patient's bloodstream.

In the next two years we may see an even more dramatic medical advance. There are 120,000 Americans on waiting lists to receive organ transplants, and there will never be enough for all of them. Thousands of people die every year before reaching the top of the list. Hundreds of thousands never even meet the criteria to be placed on the list.

For years, scientists have searched for a way to use animal organs to ease the donor shortage. Pigs have long been considered the mammal of choice, in part because their organs are similar in size to ours. But a pig's genome is riddled with viruses called PERVs (porcine endogenous retroviruses), which are similar to the virus that causes AIDS and have been shown to be capable of infecting human cells. No regulatory agency would permit transplants with infected organs. And until recently, nobody has been able to rid the pig of its retroviruses.

Now, by using CRISPR to edit the genome in pig organs, researchers seem well on their way to solving that problem. A group led by George Church, a professor at Harvard Medical School and MIT, used the tool to remove all 62 occurrences of PERV genes from a pig's kidney cell. It was the first time that so many cellular changes had been orchestrated into a genome at once.

When the scientists mixed those edited cells with human cells in a laboratory, none of the human cells became infected. The team also modified, in another set of pig cells, 20 genes that are known to cause reactions in the human immune system. That too would be a critical

Without regulation, the tremendous potential of this revolution could be overshadowed by fear.

mate in nature. But scientists hope that using CRISPR to alter DNA could appease the opposition. It gives researchers the ability to redesign specific genes without having to introduce DNA from another species.

Golden rice, for example, is a GMO engineered to contain genes necessary to produce vitamin A in the edible part of the grain—something that doesn't happen naturally in rice plants. Each year up to half a million children in the developing world go blind for lack of vitamin A—but anti-GMO activists have interfered with research and prevented any commercial production of the rice. With CRISPR, scientists could almost certainly achieve the same result simply by altering genes that are already active in rice plants.

Scientists in Japan have used CRISPR to extend the life of tomatoes by turning off genes that control ripening. By deleting all three copies of one wheat gene, Caixia Gao and her team at the Chinese Academy of Sciences in Beijing have created a strain that is resistant to powdery mildew.

Farmers have been adjusting genes in single species—by crossbreeding them—for thousands of years. CRISPR simply offers a more precise way to do the same thing. In some countries, including Germany, Sweden, and Argentina, regulators have made a distinction between GMOs and editing with tools such as CRISPR. There have been signs that the U.S. Food and Drug Administration might follow suit, which could make CRISPR-created products more readily available and easily regulated than any other form of genetically modified food or drug. Whether the public will take advantage of them remains to be seen.

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At Guangzhou General Pharmaceutical Research Institute in China, vet Long Haibin pets Taingou, one of two beagles grown from embryos edited to double muscle mass. Such experiments could eventually improve understanding of muscular dystrophy and other human diseases.
Some bacteria have evolved a powerful system, called CRISPR, to fight viral infections. When a virus strikes, a bacterium captures and stores a short, identifying sequence of the virus’s DNA—a sort of genetic “memory card.” If the same virus attacks future generations of the bacteria, they use the memory card to guide a killer enzyme to the identical sequence in the new invader and cut it away. Scientists have co-opted this natural molecular machinery not only to turn off the action of a gene but also to insert new genetic code into living organisms, including humans. CRISPR has sparked an explosion of research—and a heated ethical debate.

**How to Hack DNA**

Researchers studying how viruses infect bacteria discovered a natural immune system that cuts the invader’s DNA.
Hacking the system
Scientists can begin to understand gene function by turning a gene on and off. To do this, they program CRISPR-Cas9 structures in a lab to snip DNA and disable genes that affect health and crops.

Customized genomes
Synthetic DNA sequences can also be engineered in the lab and spliced in at the site of the cut, introducing desired traits into an organism, such as resistance to a parasite.

Unlimited possibilities
With CRISPR, scientists can alter and edit any genome that has been sequenced—quickly, cheaply, and efficiently.

APPLICATIONS FOR CRISPR TECHNOLOGY

Treating Disease
Genome-editing technology is revealing which DNA sequences are involved in diseases such as AIDS.

Altering Ecology
The spread of vector-borne illnesses like malaria could be reduced by introducing disease-resistant genes into wild insect populations.

Transforming Food
CRISPR could be used to develop drought-resistant or otherwise hardier crops. CRISPR mushrooms that don’t brown have already been approved in the U.S.

Editing Humans?
Experiments with nonviable embryos show that much work will have to be done—and many questions answered—before CRISPR can be used to edit humans.
Church has always wanted to find a way to provide transplants for people who aren’t considered healthy enough to receive them. “The closest thing we have to death panels in this country are the decisions made about who gets transplants,” he said. “A lot of these decisions are being made based on what else is wrong with you. Many people are rejected because they have infectious diseases or problems with substance abuse—a whole host of reasons. And the conceit is that these people would not benefit from a transplant. But of course they would benefit. And if you had an abundance of organs, you could do it for everyone.”

The black-footed ferret is one of the most endangered mammals in North America. Twice in the past 50 years, wildlife ecologists assumed that the animals, which were once plentiful throughout the Great Plains, had gone extinct. They came close; every black-footed ferret alive today descends from one of seven

But the ferrets, inbred for generations, lack genetic diversity, which makes it harder for any species to survive.

“The ferrets are a classic example of an entire species that could be saved by genomic technology,” said Ryan Phelan of the group Revive & Restore, which is coordinating efforts to apply genomics to conservation. Working with Oliver Ryder at the San Diego Frozen Zoo, Phelan and her colleagues are attempting to increase the diversity of the ferrets by introducing more variable DNA into their genomes from two specimens preserved 30 years ago.

Phelan’s work can address two immediate and interlocking threats. The first is lack of food: Prairie dogs, the ferrets’ main prey, have been decimated by sylvatic plague, which is caused by the same bacterium that gives rise to bubonic plague in humans. And the plague is also fatal to the ferrets themselves, which become infected by eating prairie dogs that have died of the disease. A vaccine against human plague developed in the 1990s appears to provide lifelong immunity in ferrets. Teams from the Fish and Wildlife Service have captured, vaccinated, and released as many of the ferrets (a few hundred exist in the wild) as they can. But such a ferret-by-ferret approach cannot protect the species.

A more sophisticated solution has been proposed by Kevin Esvelt, an assistant professor
Human blood filters through pig lungs in the lab of Lars Burdorf at the University of Maryland School of Medicine. Thousands of people die every year for lack of transplantable human organs. Scientists are experimenting with CRISPR to rid pig organs of viruses that harm humans. Pig organs have already been successfully transplanted into primates.
at the MIT Media Lab, who developed some of the CRISPR and gene drive technology with Church. Esvelt describes his work as sculpting evolution. “All you need to do is provide resistance,” he explained—by encoding antibodies generated by vaccination and then editing them into the ferrets’ DNA.

Esvelt believes a similar approach could not only help the ferrets resist plague but could also help eradicate Lyme disease, which is caused by a bacterium transmitted by ticks that commonly feed on white-footed mice.

If resistance to Lyme could be edited into the mice’s DNA with CRISPR and spread through the wild population, the disease might be reduced or eliminated with little visible ecological impact. Esvelt and Church, however, both feel strongly that no such experiment should be attempted without public participation and unless the scientists who carry it out have developed a reversal system, a kind of antidote. Should the original edits have unforeseen ecological consequences, they could drive the antidote through a population to cancel them out.

Black-footed ferrets are hardly the only endangered animals that could be saved through a CRISPR gene drive. The avian population of Hawaii is rapidly disappearing, largely because of a type of malaria that infects birds. Before whalers brought mosquitoes in the early 19th century, birds in the Hawaiian Islands had no exposure to the diseases that mosquitoes carry,
and therefore no immunity. Now only 42 of more than a hundred species of birds endemic to Hawaii remain, and three-quarters of those are listed as endangered. The American Bird Conservancy has referred to Hawaii as “the bird extinction capital of the world.” Avian malaria is not the only threat to what remains of Hawaii’s native birds, but if it cannot be stopped—and gene editing seems to be the best way to do that—they will likely all disappear.

Jack Newman is a former chief science officer at Amyris, which pioneered development of a synthetic form of artemisinin, the only genuinely effective drug available to treat malaria in humans. Now he focuses much of his attention on eliminating mosquito-borne disease in birds. The only current method of protecting birds from malaria is to kill the mosquitoes by spreading powerful chemicals over an enormous region. Even that is only partially successful.

“In order to kill a mosquito,” Newman says, “the insecticide actually has to touch it.” Many of these insects live and breed deep in the hollows of trees or in the recessed crags of rock faces. To reach them with insecticides almost certainly would require poisoning much of the natural life in Hawaii’s rain forests. But gene editing, which would result in sterile mosquitoes, could help save the birds without destroying their surroundings. “Using genetics to save these species is just an incredibly targeted way to address a variety of environmental ills,” Newman
Spreading the Cure

Most genes in a species have a one-in-two chance of being inherited by each offspring. But with the advent of CRISPR and a controversial technique called engineered gene drive, scientists are beating those odds in the lab. An alteration that makes a mosquito resistant to malaria, for example, can be engineered to be inherited by all its offspring.

California, Berkeley, where she is professor of chemistry and molecular biology. In 2012, Doudna and her French colleague Emmanuelle Charpentier were the first to demonstrate that scientists could use CRISPR to edit purified DNA in lab dishes. “I don’t know that we know enough about the human genome, or maybe any other genome, to fully answer that question. But people will use the technology whether we know enough about it or not.”

The more rapidly science propels humanity forward, the more frightening it seems. This has always been true. Do-it-yourself biology is already a reality; soon it will almost certainly be possible to experiment with a CRISPR kit in the same way that previous generations of garage-based tinkerers played with ham radios or rudimentary computers. It makes sense to be apprehensive about the prospect of amateurs using tools that can alter the fundamental genetics of plants and animals.

Nevertheless, it would be shortsighted to pretend that the possibility for harm (including, and perhaps especially, accidental harm) does not exist with these new molecular tools. The scientists most responsible for advances like CRISPR agree that when we begin to tinker with the genetic heritage of other species, not to mention our own, it may not be easy, or even possible, to turn back.

“What are the unintended consequences of genome editing?” asked Jennifer Doudna, as we spoke in her office at the University of California, Berkeley, where she is professor of chemistry and molecular biology. In 2012, Doudna and her French colleague Emmanuelle Charpentier were the first to demonstrate that scientists could use CRISPR to edit purified DNA in lab dishes. “I don’t know that we know enough about the human genome, or maybe any other genome, to fully answer that question. But people will use the technology whether we know enough about it or not.”

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But the benefits of these tools are also real, and so are the risks of ignoring them. Mosquitoes cause immense agony throughout the world every year, and eradicating malaria or another
The altered gene provides a template to repair the cut, allowing the wild gene to accept the changed sequence. The altered gene can now be passed on through the generations.

With gene drive, CRISPR can cut out a targeted gene in an offspring’s germ-line cell, which holds gene sequences from both its CRISPR-edited parent and its wild parent.

When faced with risks that are hard to evaluate, we have a strong tendency to choose inaction. But with millions of lives at stake, inaction presents its own kind of danger. Last December scientists from around the world met in Washington to discuss the difficult ethics of these choices. More discussions are planned. There will never be simple answers, but without any regulatory guidance—and there is none yet for editing human DNA—the tremendous potential of this revolution could be overshadowed by fear.

“With gene drives and CRISPR we now have a power over species of all kinds that we never thought possible,” says Hank Greely, director of Stanford’s Center for Law and the Biosciences. “The potential good we can do is immense. But we need to acknowledge that we are dealing with a fundamentally new kind of power, and figure out a way to make sure we use it wisely. We are not currently equipped to do that, and we have no time to lose.”

What do you think about editing the DNA of living organisms? Scientists have the tools—but how should they use them, and who should decide? 

Pro-con essays probe the issues at ngm.com/Aug2016.