Synthetic Biology and Endangered Species

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Should scientists genetically rewire nature to save species and habitats?

Ecologists warn of an ongoing extinction crisis. More than 1 million species are at risk of extinction, and millions more are in dangerous decline. Ecosystems under accelerating stress—such as some coral reefs—from climate change and other impacts could disappear within decades. Now a growing number of conservation scientists, conservation organizations, government agencies, and ethicists are debating the potential benefits and dangers of synthetic biology (tools and methods applied at the genetic scale) to help species and habitats survive human-driven onslaughts.

Synthetic biology is rewriting the rules of nature with advances in genetic engineering, gene editing, and gene drives (an inheritable form of gene editing). For decades, agricultural technology companies have developed and implemented genetic tools to control pests and improve crops and domesticated animals.

These black-footed ferret kits were raised at the National Black-Footed Ferret Conservation Center in Colorado so that they could be reintroduced into the wild. Photograph: Kimberly Fraser/US Fish and Wildlife Service, CC BY-SA 2.0.

Now, conservation science has test cases as well. A genetically engineered American chestnut tree (*Castanea dentata*) is growing in research fields in New York State. A wheat-derived single strand of DNA has been added to the tree's 40,000 genes, blocking a fatal acid produced by an invasive fungus (*Cryphonectria parasitica*). This tree is the first transgenic organism in the pipeline for regulatory approval in the United States with a conservation goal. But opponents argue that a transgenic tree in the wild could have unknown effects on forest ecosystems.

Scientists are also testing cloning technologies to revive a rare species. In 2021, a black-footed ferret (*Mustela nigripes*) was successfully cloned. She will be partnered with a captive black-footed ferret. The goal is to increase genetic fitness and improve reproduction rates among captive and reintroduced black-footed ferret populations.

The tools and methods of synthetic biology could be the future of a new precision conservation. These biotechnologies could offer scientists unprecedented accuracy, speed, and efficiency to help species adapt to disrupted habitats and control invasive pests and pathogens. Conservation biotechnology is an extension of what humans have done for thousands of years—though far more aggressively in recent decades—in shaping the evolution of wild and domesticated species.

BioScience 72: 610–617.
https://doi.org/10.1093/biosci/biac040

Humans must take the reins of nature more firmly in hand so that threatened species and ecosystems can survive, according to Beth Shapiro, a paleogeneticist at the University of California, Santa Cruz. "Disagreement about whether biodiversity would be better served by leaving nature alone or intervening more aggressively has deep roots in biodiversity conservation," Shapiro writes in her 2021 book *Life as We Made It: How 50,000 Years of Human Innovation Refined—and Redefined—Nature*. But there "are those of us who believe that it is too late to imagine any portion of the planet not impacted by us, and instead, we must embrace our role as planetary curators."

Critics, though, say that humans are going too far, that science should not interfere so deeply in nature. Wildlife and their habitats are immensely complex. Synthetic biology could lead to disastrous unintended consequences. Synbio (synthetic biological) species or their offspring, therefore, should not be allowed in natural places, even if the goal is to save them, these critics say.

Allowing biotech life into the wild could threaten how conservationists and the public think about nature, according to Eric Katz, retired professor of philosophy from the New Jersey Institute of Technology and a distinguished senior fellow at the Institute for Philosophy and Public Policy at George Mason University. Conservationists have long argued that natural places are fundamentally different from domesticated and other human-dominated ones. Wild places where cloned or artificially modified species are inserted would no longer be considered natural and could lose the special status that protects them.

Conservation biotech is blurring language long used to encourage or justify saving wild species and ecosystems, argues Katz. "It could change the very meaning of *nature* and *conservation* and *preservation*," he says. "If science and tech can create anything we want, what is the purpose of traditional conservation and preservation practices?"

American chestnut test case

Over the first four decades of the twentieth century, an estimated 4 billion American chestnut trees died across their range from Maine to Mississippi. Their killer was a pathogen accidentally introduced in shipments of Chinese chestnut (*Castanea mollissima*), an ornamental tree that had evolved blight protection. American chestnuts were once giants, rising 80–100 feet, with massive trunks. It was a key species in the ecology of North American forests. In some forests, American chestnuts were responsible for up to 45% of the canopy. The annual fall of nutritious nuts was a crucial food source for Indigenous peoples. Today, the American chestnut is functionally extinct, and most survivors are stunted, rising only about 15 feet, and unable to bear fruit.

Scientists first tried to bring back American chestnuts by crossing them with blight-resistant Chinese chestnuts, but that did not result in a thriving hybrid. Other techniques to bring back the American chestnut were considered too slow or ineffective. But researchers finally identified a possible solution, inserting blight-fighting DNA from wheat into American chestnuts. The project is led by William Powell, director of the American Chestnut Research and Restoration Project at the State University of New York College of Environmental Science and Forestry (ESF). These American chestnut trees—known as Darling 58—have been planted in regulated fields. To strengthen Darling trees, Powell's lab and The American Chestnut Foundation (TACF) have crossed them with wild American chestnuts. By 2023, the Darling tree could be fully approved by the Environmental Protection Agency, the US Department of Agriculture, and, because the nut is a food, the Food and Drug Administration.

Restoring the American chestnut to its original range would require public and private mobilizations to plant, propagate, and maintain millions of transgenic seedlings. But that process is many years away. If approved, Darling trees will be distributed to long-term research plots and relatively small-scale public horticultural sites. Plantings will be monitored by citizen scientists under guidance of TACF, a nonprofit organization that supports and partners the restoration. Later, Darling trees would be distributed to the wider public.

In 2021, the National Association of State Foresters declared its support for genetically modifying native trees to fight off destructive invasive pests if conventional measures fail. Numerous groups and individuals have asked for transgenic chestnut seedlings to plant, including the Eastern Band of Cherokee Indians in North Carolina, which seeks to grow them on tribal land. Other native communities have given similar nods to the Darling tree. The Sierra Club has offered its blessing, but only if Indigenous people continue to support it.

But some environmental activists are opposed. Ann Petermann, director of the Global Justice Ecology Project, a US-based environmental organization, says that it is wrong to alter the gene pool of wild trees. "We could be looking at forests that aren't natural or wild but filled with genetically engineered versions of formerly wild trees."

The Darling tree is not a moneymaker; there are no plans to patent it. But the transgenic chestnut project is being used by industry as a Trojan horse, allowing corporations to gain broader public acceptance of transgenic forestry products, according to Petermann. If transgenic American chestnuts are growing wild in forests, Petermann says, regulators could be more likely to approve genetically engineered trees for commercial uses such as hybrid poplar cultivars in field trials supported by industry. Leading companies in commercial forestry do support the Darling project, among them Bayer (formerly Monsanto) and ArborGen, which are among the largest suppliers of pine seedlings for forestry projects in the United States.

Critics of Darling trees worry about their potential negative impacts on native plants, animals, and microbes.

The natural range of the American chestnut (Castanea dentata) extended from Mississippi to southern Ontario and as far northeast as Maine.

Once ranging from Maine to Mississippi, the American chestnut was a key forest species felled by an invasive fungus, but scientists are exploring genetic engineering and other techniques to restore it. Photograph: The American Chestnut Foundation.

Students at SUNY's College of Environmental Science and Forestry perform stem inoculations on young transgenic American chestnut trees to test if they show enhanced resistance to the blight fungus. Photograph: The American Chestnut Foundation.

An American chestnut in Kentucky towers over a man. While these trees have been functionally gone from forests for nearly a century, some isolated mature ones have survived. Photograph: The American Chestnut Foundation.

Tests by ESF researchers and collaborators on Darling trees, however, have found no harm to native frogs, insects, or germination of other trees' seeds. Independent tests have not found nutritional differences in transgenic nuts that could harm wildlife or humans. Reviving the American chestnut could have benefits for pollinators, stream invertebrates, and wildlife consumers of nuts.

Saving the black-footed ferret?

During the spring breeding season of 2022, the cloned black-footed ferret named Elizabeth Ann was expected to pair up with an experienced suitor in a conservation center outside of Fort Collins, Colorado. Choosing the right mate was important. The black-footed ferret is perhaps the rarest mammal in North America, and Elizabeth Ann is far too valuable to risk injury from an aggressive male.

In the 1970s, scientists thought the black-footed ferret was extinct in the wild. In 1981, however, a colony of survivors was discovered. The little colony thrived for a while before sylvatic plague, caused by the same bacterium that causes plague in humans, decimated the population. The US Fish and Wildlife Service (USFWS) captured the surviving 18 ferrets for a captive breeding program. Only seven of those animals managed to reproduce. Today, about 250–350 ferrets live in captivity, and 300 more live on reintroduction sites in the wild—all descended from the seven founders, except for Elizabeth Ann.

The cloning of Elizabeth Ann is the brainchild of Revive & Restore, a biotech conservation organization based in Sausalito, California. In 2012, the futurist Stewart Brand and his wife, Ryan Phelan, created Revive & Restore to support research on advanced technologies that could be used to protect endangered species and perhaps revive extinct species. In 2018, the organization and its partners received a first-of-its-kind endangered species recovery permit from the USFWS to attempt a genetic rescue of the black-footed ferret.

Elizabeth Ann is a genetic replica of a black-footed ferret named Willa that died in captivity 35 years ago and never reproduced. Willa was a surviving member of a lost colony of a different lineage than the founders. Fortunately, a cell culture from Willa's tissues had been stored in a freezer. Decades later, embryos were created from that cell culture and implanted in three domestic ferrets (*Mustela putorius*). One surrogate mother gave birth to Elizabeth Ann. The ferret cloning was done by a consortium that included the USFWS, ViaGen (a Texas-based pet and horse cloning company), Revive & Restore, the Association of Zoos and Aquariums, and the San Diego Zoo.

Elizabeth Ann is the first native endangered animal species in North America to be cloned—and the first to reach sexual maturity. If Elizabeth Ann breeds in captivity, she could introduce her much-needed genetic fitness into the remnant population.

But was creating Elizabeth Ann the right thing to do? "You *can*

Elizabeth Ann, the first cloned US endangered species, is a black-footed ferret, although her siblings and surrogate mother are domestic ferrets. Photograph (left): US Fish and Wildlife Service National Black-footed Ferret Conservation Center. Photograph (right) Cassie, with the USFWS National Black-footed Ferret Conservation Center.

do genetic cloning," says Samantha Wisely, a conservation geneticist at the University of Florida and a consultant on the Elizabeth Ann cloning project. "But *should* you do genetic cloning? Every incorporation of new biotechnology into conservation should have that question asked."

Wisely collaborated on a 2021 study in *Biological Conservation* on the ethics of the black-footed ferret cloning project with Ronald Sandler, a philosopher and director of Northeastern University's Ethics Institute. The team asked questions such as whether cloning was the best tool available for a conservation purpose, whether it could be done responsibly in this case, and whether there was support from the public and within the conservation community for the project.

"New technologies are moving forward in a lot of different ways in lots of different spaces," says Sandler. "The question is whether there is an ability to make good decisions about which technologies, in what context, and under what conditions they should be used. We should look not only at how the technologies work but also at things like who controls them, who shoulders the costs, and who are the beneficiaries."

Cloning, for instance, was not only the best tool in this situation but the only tool available to introduce genetic fitness into the endangered population, according to Wisely. There were no other individuals to add to the genetic pool beyond the original seven founders.

Sandler and his colleagues concluded that the cloning project was done responsibly. "The partners have a track record of thinking through issues in a thoughtful, collaborative way," he says. "The initial point of the project isn't to modify the ferrets; it's just to increase the genetic diversity of the population in the context of a captive breeding and release program. So this project fits into the standard, familiar conservation practices and paradigms." The Sandler team did recommend that the cloning project should more fully inform local landowners and other stakeholders about its progress.

But what makes the Elizabeth Ann project important is not just that she is the first clone of an endangered species native to North America. It is that cloning could make other powerful technologies more feasible as the logical next step in restoring wild populations of the black-footed ferret. Those technologies would almost certainly be more controversial than cloning because they would alter the genome of the animal instead of duplicating it.

Gene editing and gene drives

Elizabeth Ann lacks a critical feature that her progeny would need in the wild. Now Revive & Restore researchers and partners are exploring the use of CRISPR and other advanced tools to immunize black-footed ferrets and future generations against sylvatic plague, the invasive pathogen from Europe that infects their primary food source, the prairie dog.

The domestic ferret, originally from Europe, has developed natural immunity to the plague. Researchers hope to identify DNA in domestic ferrets that codes for immunity. If that is successful, scientists might be able to CRISPR edit immunizing DNA into the population of black-footed ferrets.

CRISPR is a gene-modifying suite of techniques. (The acronym stands for clustered regularly interspaced palindromic repeats.) CRISPR is the fastest, most inexpensive, and most precise way to manipulate the code of life. Researchers have CRISPR-designed pigs that resist swine fever, cows that do not grow horns, and mosquitoes that cannot procreate. CRISPR editing, though, has risks. Gene editing could cause unintentional mutations in the target genome, allowing unexpected traits to emerge. But those changes—both intentional and unintentional—might not be lasting.

An individual's unique trait and its underlying causal DNA typically have a 50% chance of being passed on to the next generation. So a unique trait—whether natural or CRISPR designed—usually does not spread far very quickly. But a natural or synthetic gene drive theoretically could be used to hack the inheritance system, increasing a unique trait's inheritance chances. An organism modified by both a gene drive and CRISPR-modified DNA could result in nearly all offspring's inheriting both the target genetic trait and capacity to pass on that trait to next generations. A gene drive could steer the targeted trait throughout a population immensely faster than by natural selection.

National Park Service landscape ecologist Tanya Shenk releasing black-footed ferrets. Photograph: National Park Service.

Most gene drive experiments have been done successfully on laboratory insects. No synthetic gene drive has been released into the wild. But biotech companies and other groups are testing them in hopes of fighting agricultural pests and controlling disease vectors for public health. Now, a CRISPR-modified gene drive has been shown to be feasible in mice, according to a December 2021 *Nature Protocols* study conducted by Kimberly L. Cooper of the University of California, San Diego, and her colleagues. If remaining technical hurdles can be overcome, the researchers note, gene drives eventually could be used in strategies to eliminate rodent pests that have overwhelmed island ecology.

The Genetic Biocontrol of Invasive Rodents program is targeting research efforts on gene drives and other biotechnologies for this purpose. This international group of university researchers, governments, and nongovernmental organizations managed by the nonprofit Island Conservation is exploring the use of gene drives that could cause all offspring of invasive rodents to be one sex so they could not reproduce and would eventually die out.

Gene drives in conservation do present risks, though. Some gene drives could be unstoppable once they are released. They could change species, populations, and ecosystems in unintended ways. Scientists are exploring gene drives that would be limited to a set number of generations. Gene drives would be programmed to die out when their task is done. Kevin Esvelt, a biologist at the Massachusetts Institute of Technology, was the first scientist to figure out how CRISPR-based gene drives could rapidly spread a trait through a population, and now he is exploring safer gene drives that could disappear over time. He and other scientists in the gene-drive community have called for improved regulations of gene-drive field trials in a policy paper published in 18 December 2020 issue of *Science*.

Many of today's conservation practices and paradigms could shift as immensely powerful genomic technologies become more available, shaping species and ecosystems to meet challenges of climate change and other human-driven impacts.

For decades, conservation has been nostalgic, repairing human impacts and returning species assemblages to places where they had once thrived but were lost. "That approach might not always be possible anymore given that nature is changing so dramatically," says Sandler. "It's becoming more and more difficult for conservation to be backward looking. These disruptive technologies could have transformative impacts on standard conservation practices, structures, philosophies, goals, and norms."

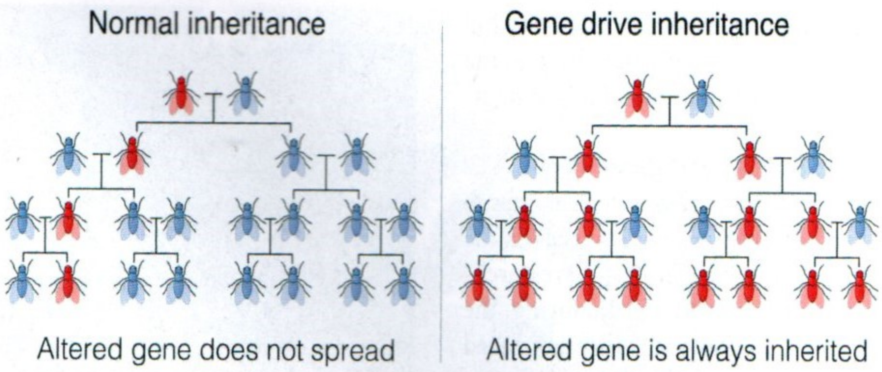
Facing forward?

Genetically modified conservation species will not pause at national borders, but regulatory pathways for synthetic biology in conservation vary in different countries around the world. There is no standard international definition for synthetic biology. And there is no internationally agreed-on way of governing synthetic biology.

Since 2010, the Convention on Biological Diversity (CBD) has been discussing the need for international regulations for the use of synthetic biology in conservation. The CBD has not defined *synthetic biology*, because any selected definition would determine which of the existing treaties, accords, and regulations would become paramount.

In June 2020, Revive & Restore brought together 57 conservationists, biologists, restoration specialists, geneticists, ethicists, and social scientists to author an intended consequences statement. It outlines ethical guidelines for conservation biotechnology as efforts advanced to clone the black-footed ferret and genetically engineer the American chestnut tree.

A key message of the statement is that decisions about developing and testing biotech organisms should be made in concert with communities and stakeholders, especially Indigenous and marginalized groups. Local people must be involved in discussing conservation problems and exploring possible solutions. "Science



Researchers are studying gene drives, which are powerful techniques of rapidly driving targeted genetic changes through animal, insect, or plant populations. Illustration: Mariuswalter, CC BY-SA 4.0 International.

In August 2020, scientists announced the birth of a Przewalski's horse foal that had been cloned from 40-year-old frozen cells. Native to the Asian steppe and descended from only 12 founders, all surviving Przewalski's horses live in captivity, as the one shown here in a German game park. The cloned foal's genes could add diversity to the founding gene pool. The project was a collaboration among ViaGen Equine, the San Diego Frozen Zoo, and Revive & Restore.

alone cannot tell us what we should do," note the authors. But the signatories warn that not taking innovative genetic action also has risks that should be considered.

Conservation biotech today is a small-scale sideshow to the main genetic events. Nearly all cutting-edge genetic research projects on free-living organisms (ones that can interact with wild species) are supported in just two areas: protecting or enhancing agricultural products and improving public health by disarming disease vectors such as mosquitoes.

"Synthetic biology investments in agriculture are huge," says Kent H. Redford, an ecologist and principal at Archipelago Consulting, in Portland, Maine, and coauthor of the 2021 book *Strange Natures: Conservation in the Era of Synthetic Biology*. To control farm pests or enhance crop growth, researchers are genetically engineering free-living species such as pink boll worm (*Pectinophora gossypiella*) and screwworm flies (*Cochliomyia hominivorax*). Some microbial species that inhabit insects and plants are being genetically modified as well.

"Agriculture is generally considered to be one of the major impacts on the long-term conservation of biodiversity," says Redford. "But conservation scientists are doing almost no work on the positive and negative dimensions of synthetic biology applications to agriculture in relationship to conservation. We can't continue to pretend that this is not happening. We must not ignore it and become irrelevant."

That is an unpopular stance in some quarters, says Redford. "There are statements from the conservation community such as "'You are playing God, that you have no right to do it, that it ruins nature, and Nature needs to be left alone. We shouldn't talk about this stuff. We should just close down any discussion because it's dangerous.' But the bigger danger is that we're not willing to change. We've spent too long looking out the back window and decrying things that have already passed. We need to face the front and talk about what we do want and don't want before [species losses and ecosystem damages] have already happened. We need to better address the new science and engage larger society to achieve conservation goals that are so important."

Progress in conservation biotech has daunting challenges. Basic genetic information of threatened species is very small compared with that of domesticated species. Conservation scientists usually lack the resources to develop this basic research. And many conservation scientists and conservation groups express wariness of synthetic biology. But the biotech revolution is coming to conservation; innovations will spill over from other disciplines into conservation science. As species become increasingly imperiled and more disappear forever, genetic science could offer solutions, although they have risks. If the public gains confidence in test cases of conservation biotech and in regulations to control them, many natural areas someday might be more fully curated by humans and inhabited by transgenic species such as the Darling chestnut. Words such as *conservation* and *preservation* could start having different connotations than the ones we have known.

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Further reading

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