discovered *G. extinctus*, wrote in a blog post. These conditions, scientists believe, enabled the evolution of species found nowhere else.

Yet it wasn't long before paradise was lost. Roads and agriculture crept across western Ecuador and untold tracts of forest were bulldozed to make way for cacao, coffee, and banana plantations on the fertile slopes. In 1991, two influential biologists, Cal Dodson and Alwyn Gentry of the Missouri Botanical Garden, published a seminal paper describing the habitat destruction in the region, focusing on the Centinela Ridge. The lush jungle had largely vanished, the biologists wrote based on their frequent travel to the area, and "an undetermined number of … species are now apparently extinct." Even more shocking was how quickly it happened: Just 13 years before Centinela's ruin, "there seemed little reason for concern."

This tiny, rare plant, *Amalophyllon miraculum*, persists in remnant patches of cloud forest.

Biologists enshrined Centinela as a cautionary tale about the threat of rapid deforestation. In his 1992 book, *The Diversity of Life*, esteemed Harvard University biologist E. O. Wilson coined the term "Centinelan extinction." It has since been applied to similar examples of habitat loss and presumed species wipeouts, such as arthropod communities that live in the canopy of old growth forests in British Columbia.

Meanwhile, conservation biologists turned their attention to other places deemed salvageable. "We said, 'OK, it's been studied, so let's move to the next thing,'" Gonzalo Rivas-Torres, an ecologist at San Francisco de Quito University who was a part of the team that rediscovered *G. extinctus*, says of Centinela. "We thought there was not much more we could do."

Yet over the years, scientists found hints that some species feared extinct may have persisted after all. Plants that were thought to be unique to Centinela were found elsewhere. Botanists who visited the isolated ridge also stumbled upon patches of forest healthy and large enough to host packs of howler monkeys.

This led a 10-person team in 2021 to revisit Centinela for a closer look. For 3 days, the researchers navigated the tangled network of rural roads by truck, looking for remnants of primary forests. They located more than 20 strips of jungle along ravines or slopes too steep to farm, as well as a 50-hectare forested fragment. *G. extinctus* was growing in three of the sites.

Since then, Clark and others have returned to the area for five more expeditions. In March 2022, he met a landowner while photographing a rare caoba tree in bloom. The man led Clark to the back of his property, to "a small island of forest surrounded by agriculture." There, he spotted the new species: *A. miraculum*, a member of a genus that is common elsewhere in South America but not in western Ecuador. To find one clinging to a mossy rock in a forest that had supposedly been destroyed decades ago was shocking, he says.

Carmen Ulloa Ulloa, a senior curator at the Missouri Botanical Garden who is not involved in the research, says the findings "emphasize how important those small remnants really are. They still harbor these novelties that we need to protect."

The research doesn't overturn the account of massive deforestation in the region that was reported in the 1991 paper, Clark notes. "It was a very impactful paper in raising awareness about rapid deforestation."

Still, it's unfortunate scientists didn't continue to track the area's biodiversity, Schaefer says. "Maybe they had lost hope." (It didn't help, he adds, that Gentry died 2 years after the famous paper was published when his plane crashed in the Ecuadorian mountains.)

With Centinela back in focus, scientists and conservationists are making up for lost time. Schaefer's land trust is in the process of purchasing tracts of land containing primary cloud forest. Scientists have started their own campaign, called Viva Centinela, to promote research and the preservation of the area's unique biodiversity. And two full-time botanists are working to document the species that persist.

If their work is successful, the forest that was once the poster child for deforestation and loss could someday become a symbol for conservation and resilience. ■

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BIOLOGY

# Two teams supercharge gene spread in plants

## First synthetic "gene drive" for plants could help tame weeds—or transform them

*By* **Erik Stokstad**

More than a decade ago, a research group used the genome editor CRISPR to put evolution on fast forward, spurring a gene to spread throughout a population of lab-reared fruit flies many times faster than it normally could in nature. Mosquitoes with CRISPR-based "gene drives" came soon after, then mice a few years later—advances that brought a fraught mix of technological promise and ethical complexity. Proponents tout gene drives as a way to prevent insect-borne diseases, wipe out rats and other invasive creatures, and even help prevent extinction of endangered species. But one set of organisms had stood apart from the excitement: plants.

Now, geneticists report that synthetic gene drives can work in flora, too. Circumventing a long-standing hurdle, two teams have independently engineered *Arabidopsis thaliana*, a small mustard popular for lab work, to carry a genetic payload that is inherited by up to 99% of offspring. Modeling suggests a similar gene could permeate a natural plant population in 10 to 30 generations. "What they've achieved is pretty amazing," says Paul Neve, a weed scientist at the University of Copenhagen. "It is clever and innovative."

In a pair of papers in *Nature Plants* last week, the teams described mimicking a natural gene drive involving "selfish" genetic sequences known as toxin-antidote elements. Their success opens the possibility of knocking back weeds that have evolved to resist many herbicides. Or gene drive could transform species to be less troublesome, so they can continue to provide food and habitat for pollinators and other wildlife.

A plant gene drive system could be "really valuable for sustainable weed management," says Mithila Jugulam, weed physiologist and molecular biologist at Kansas State University. But Todd Gaines, a weed biologist at Colorado State University, cautions, "I could see a lot of headwinds," including selling farmers on the technology and gaining regulatory approval.

It's still a long and expensive process to get a genetically modified (GM) crop past U.S. agencies, including the Department of Agriculture, which has a mandate to minimize the risk of new weeds and pests. So, winning approval to release GM weeds? "It's a horror story in the making," says University of Illinois Urbana-Champaign weed scientist Patrick Tranel.

Natural gene drives, instances where the rules of inheritance are broken, are rare. Normally, each copy of a gene, called an allele, has a coin-toss chance of being inherited. Some so-called selfish stretches of DNA, however, have evolved ways to cheat their way past other alleles, becoming ever more common in the population even if they don't enhance the success of the organism.

Many of the latest artificial gene drives rely on CRISPR, which can dramatically improve a DNA sequence's odds of being inherited. They consist of DNA for the genome editor, plus any attached gene, engineered into one of the two copies of a chromosome that an animal inherits from its parents. During reproduction, the sequence is duplicated with the rest of its chromosome, like any natural gene. But the genome editor then inserts the sequence into the other chromosome as well. Its inheritance by the next generation, no longer a coin toss, becomes a near certainty.

Plants can thwart standard CRISPR-directed genetic takeovers thanks to one of their DNA repair mechanisms. But some plants and animals do have the toxin-antidote elements. These sequences, which pair two genes, propel themselves through a population by taking advantage of reproduction's chromosome shuffling. In one manifestation of the system, gametes such as pollen or sperm that inherit only the toxin gene are doomed, while those with the entire toxin-antidote DNA survive.

Inspired by the toxin-antidote idea, geneticist Bruce Hay at the California Institute of Technology and colleagues in 2019 started developing a gene drive for insects they called a Cleave and Rescue system. It used CRISPR's DNA-cleaving enzyme Cas9 to cut and disable an essential gene in the gametes, dooming some. But those carrying the gene drive survived because they received a backup version of that gene, slightly modified to evade cleaving, along with a second gene carried along into surviving gametes. In this scenario, CRISPR created the toxin (the defunct essential gene), and the CRISPR-resistant rescue copy is the antidote that lets sperm or eggs live.

The team realized this approach should also work in plants. In its new paper, the group describes a system that would cut and disable a target gene, *YKT61*, which is required for plant cells to properly handle proteins and lipids. The gene drive also incorporated a rescue gene, a version of *YKT61* from an *Arabidopsis* relative that differs enough for CRISPR not to see it as a target. With a promoter attached to the CRISPR genes, they engineered plants that turn on this editor in gametes.

Pollen and ovules that carry the gene editor survive because of the rescue gene. Those without die when CRISPR destroys their *YKT61*. To show this could rapidly spread a gene, the team attached a marker gene that turns surviving seeds red. The gene drive was effective, as 97% to 99% of *Arabidopsis* produced red seeds. And it was stable, lasting for five generations.

In China, a second team took the same approach and achieved similar results. Led by Wenfeng Qian, a synthetic biologist from the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences and Peking University, it chose a different gene for CRISPR to cut, one required for pollen germination. The group's red marker showed up in 88% to 99% of seeds for two generations. The moment lab members opened the seed pods was "thrilling," Qian recalls.

The successes suggest new ways to control weeds that have evolved resistance to multiple herbicides. Take pigweed (*Amaranthus palmeri*), a plant that can choke off crops such as soybeans and cause allergies in people. Both teams calculate that in 10 to 30 generations their gene drive could saturate any plant population with a gene causing complete sterility. Or it could spread a cargo gene that would make the weed more benign without eliminating it—maybe one making the plant nonallergenic.

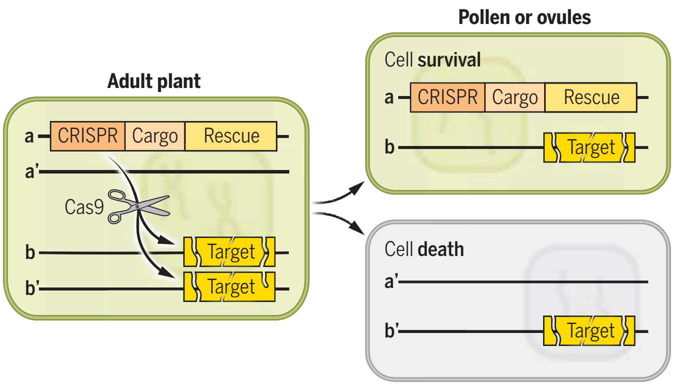
Hay says a gene drive aimed at eradicating a weed could be designed so that genetic recombination—the DNA shuffling that happens in reproductive cells—ultimately separates its genetic components and shuts it down, reducing the risk that the fatal gene drive spreads to weeds beyond the farm field. "It's very important that the technology can be targeted, but not completely destructive," says Kan Wang, a plant geneticist at Iowa State University. "That's a part I really appreciate."

However safe, a synthetic gene drive might have limited appeal for farm applications, Neve says. Waiting a decade to eradicate weeds could be a nonstarter for farmers. The model also assumes farmers would boost the existing weed population by adding 10% more weeds bearing gene drive—requiring a lot of planting and greater plant consumption of water and nutrients. Hay sees gene drive as an add-on to other measures, imagining that farmers would plant a fringe of gene drive–bearing weeds around their fields each year, bit by bit pushing the weed population to zero.

Tranel says there's also a basic biological constraint. Gene drives only work in plants that spread by pollinating neighbors, and a lot of troublesome weeds don't do that. Smooth pigweed and red root pigweed are major headaches in which each plant pollinates itself, so the gene drive would be stuck in park. Plants might also evolve resistance to toxin-antidote gene drives, Neve adds, like they have to herbicides. "This isn't going to be the cure-all, end-all for weed management," Tranel says. ■

## Search and rescue

A new gene drive for plants includes three elements. The DNA-cutting enzyme Cas9, part of the genome editor CRISPR, finds and disables both copies of a gene (target) essential for cell survival. A rescue gene, inserted along with CRISPR, has the same function but isn't disabled by Cas9. When chromosomes are shuffled as the plant generates pollen or ovules, some of these gametes end up without the rescue gene and die (gray). The others survive, and both the gene drive and a cargo gene attached to it become much more common in the next generation.

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