# CRISPR gene editing in conservation biology

Conservation biology requires a bigger toolbox to meet the biodiversity crisis. Is there a role for CRISPR and gene editing?

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**Genomic solutions to biodiversity problems**

Biodiversity loss is one of the major crises we face in the 21st century and we continue to fail in reversing this trend. Along with causing losses in natural ecosystems, the biodiversity crisis affects cultivated plants and their wild relatives. Climate change, habitat loss, invasive pathogens and pests have increased in recent years, resulting in substantial loss of diversity in the landraces and wild relatives of the crops we grow. These serve as pools of valuable alleles that can help crops withstand stresses related to climate change, such as drought, heat, and emerging diseases.

Conserving these important crop genetic resources requires big thinking. For example, this interesting review aticle goes beyond standard approaches to preserving genetic resources and biodiversity to discuss how the field of conservation biology requires a bigger toolbox to meet the challenges we have in this century. The author describes a number of genomic technologies that could provide solutions to conservation biology problems.

Table 1. Conservation Problems with Actual or Potential Genomic Solutions

| Conservation Problem | Actual and Potential Applications of Genomics |
|---|---|
| Identifying cryptic lineages to conserve | RADseq, ancient DNA |
| Delimiting conservation units | RADseq |
| Optimizing *ex situ* conservation | RADseq, RNAseq |
| Monitoring pathogens | RADseq |
| Identifying pathogen-resistant individuals | Associative transcriptomics |
| Selecting populations for reintroduction | RADseq, RNAseq |
| Assessing past and present connectivity | Various NGS techniques, ancient DNA |
| Assessing biodiversity | Metabarcoding, environmental DNA |
| Detecting invasive species | RADseq, metabarcoding, environmental DNA |
| Establishing baselines for restoration | Ancient DNA |
| Assessing adaptive potential | Genome-wide association studies, associative transcriptomics |
| Assessing acclimation potential | Transcriptional profiling |
| Controlling invasive species | CRISPR-based gene drives |
| Genetic rescue of inbred populations | RADseq, cellular and reproductive technologies |
| Reversing extinction | Synthetic biology |

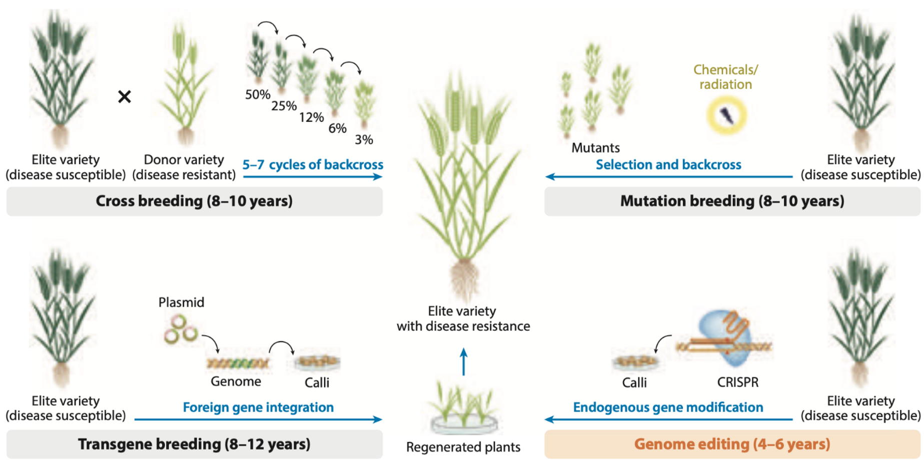
Genomic solutions to conservation biology problems. (Source: RT Corlett Trends in Biotechnology 2017)

## CRISPR—another tool in the conservation biology toolbox?

One potential tool in the conservation biology box is CRISPR-mediated gene editing (GE), a method which took biology by storm over the past 10 years. Before we address this topic, let me recap what the CRISPR technology is. CRISPR took off in August 2012 with this landmark publication, which described how a programmable guide RNA can be modified to target any sequence you wish and modify it (edit it!). This is the paper that led to the 2020 Nobel Prize in Chemistry for Emmanuelle Charpentier and Jennifer Doudna. The nomination refers to the technology as as "molecular scissors that can rewrite the code of life".


The CRISPR gene editing revolution.

CRISPR can be used to knock out genes, make large and small deletions, knock in or replace genes, and edit specific bases. For those of you who want to find out more, I encourage you to read this review article by Caixia Gao and colleagues in Beijing, who is one of the pioneers of applying CRISPR technology to crop plants. Caixia and colleagues describe CRISPR as enabling precision plant breeding. Indeed, changing one or a few letters in a plant genome is the ultimate in genetic alteration.




Gene editing compared to other plant breeding approaches. (Source: Chen et al., Annual Reviews of Plant Biology 2019)

## How can CRISPR be used in conservation biology

Now that we covered how CRISPR enables precise genetic modification of plants, let's explore how the method can be used in conservation biology. Conservation genomics of crops can be divided in these 3 activities: genome sequencing to unravel genetic variation, facilitated adaptation and de-extinction.

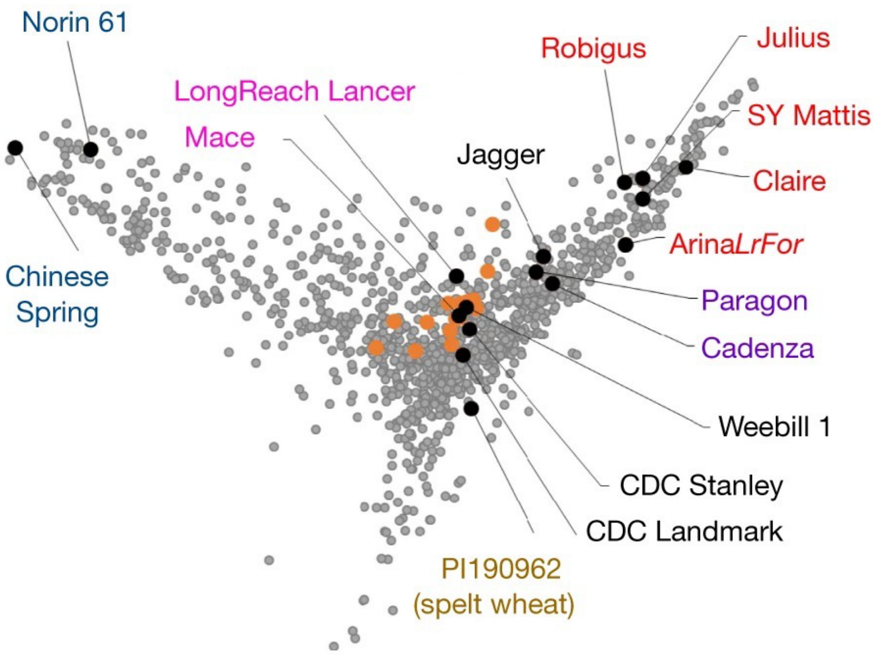
- Conservation genomics—before we conserve, we need to know what's out there
- Facilitated adaptation—introduce adaptive traits for conservation of endangered crops
- Crop de-extinction?—CRISPR can deliver it

Conservation genomics of crops in 3 steps — understand the genetic variation, use gene editing for facilitated adaptation of endangered crops, bring extinct crop varieties s back to life.

**Conserving diversity by understanding genetic variation**

One of the key prerequisites for conserving genetic resources, and for effective gene editing, is understanding the variation in genes and the effects this variation has on phenotype. CRISPR can be used to study the phenotype of different gene variants—a topic covered in the review by Chen *et al.* and an ever-increasing number of articles. But to achieve this, we need to know the standing natural genetic variation in the first place. We need to know plant germplasm better, starting with a reference genome and continuing with re-sequencing of different varieties, landraces, and wild relatives to produce a pan-genome. Although a few studies, such as the 3000 rice genomes project, have made inroads in understanding the variation in crop genomes, such studies have only scratched the surface of the variation present in crop genomes.

Nevertheless, scientists continue to make progress and even the most complex plant genomes are now accessible. For example, wheat was one of the most challenging plant genome to sequence given its complicated hexaploid hybrid nature and massive ~16 Gigabase size—about 120x the size of the Arabidopsis genome and 2.5x the size of the human genome. The first somewhat complete sequence of a wheat genome was only published in 2018. That's the first genome sequence where you have the different haplotypes separated, different genomes separated, and a close to complete sequence. Then just two years later, you have the very first comprehensive documentation of the genetic variation with genomes of multiple cultivars providing a snapshot of the diversity of the modern varieties (see the two papers here and here). That's a glimpse of the future to come.




Patterns of genome variation reveal the genetic relatedness of 1,200 wheat lines. Source: Walkowiak et al. 2020.

**Facilitated adaptation**

Preserving seeds in seed banks and preserving habitat to allow organisms to thrive are essential approaches for conservation biology. For crops that we want to grow each year, another potential approach is to alter plants to grow and thrive under current conditions. This is where gene editing technology can help make our landraces more resilient, and therefore help ensure their survival and improve their adaptation to current conditions. For example, many of the valuable landraces we have are no longer as well suited to the climate as they used to be. So, we could consider using gene editing to improve landraces to help them handle the changing climate. This is known as *facilitated adaptation*.

Although facilitated adaptation sounds simple, understanding how to modify plants to better survive climate change requires a deep understanding of the genome, its variation, and how that variation affects plant traits—the causal link between genes and phenotypes. Therefore, we need to better understand fundamental processes of plant biology to fully take advantage of gene editing. As my plant biologist colleague Jane Langdale put it in the roadmap of the UK plant science research strategy, "*research and innovation involving plants is crucial if we are to tackle the global challenges*".

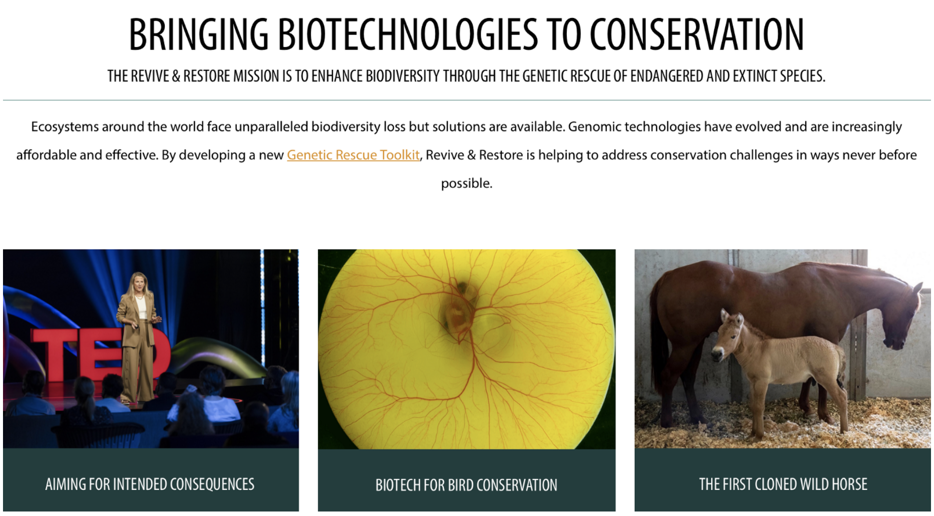

UK Plant Science Research Strategy — the urgent need for research and innovation in plant biology .

**De-extinction: from alleles to entire species**

Variation genomics goes hand in hand with conserving plant genetic resources. In addition to sequencing extant materials, we can also access extinct crops whenever we have herbarium material or other stored tissue, identify the earliest variants, and then introduce them back into modern materials. Indeed, this field of genomics— known as ancient DNA or aDNA—has implications for conservation biology. We can essentially resurrect genes, alleles and variants that have gone extinct by introducing them into modern varieties. This is known as de-extinction and it can only involve resurrecting a single extinct genetic change.

As gene editing becomes widely used, having complete information on sequence variation and knowing what the valuable alleles and mutants are will prove to be as important as having the seeds themselves because breeders can introduce those mutations into plant materials at will. Therefore, genomics and scaling up sequencing of crops, including diverse types of the same crop is important to identify and conserve valuable traits and alleles that could be incorporated into modern varieties now and in the future.

Genome editing could also revive species that are extinct or endangered. For example, George Church's Revive and Restore initiative targets endangered or even extinct organisms and produces a framework for how to enhance their capacity to survive or even revive them. Although this may sound like science fiction—mammoths and Przewalski's horses—this is something we're inching towards for charismatic megafauna, and hopefully important plants too.

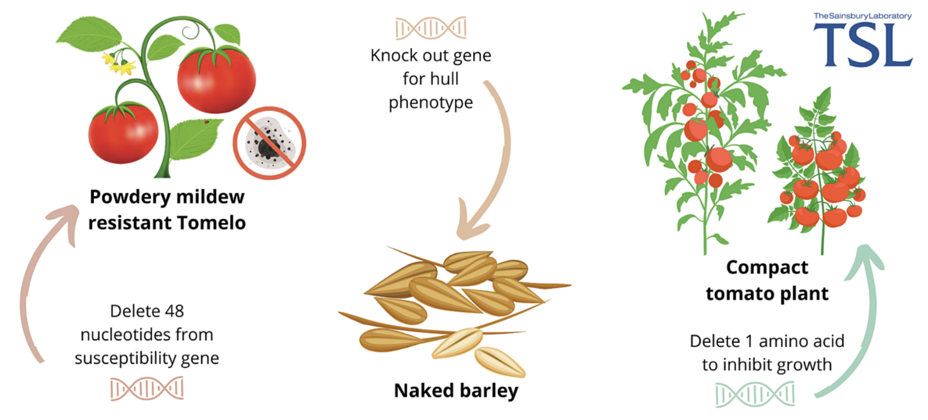


Revive & Restore — bringing biotech to conservation.

**Gene Editing (GE): GM or no?**

What about gene editing? Is it GM? Is it non-GM? We've been improving crops and modifying the code of life ever since humans domesticated plants. Let's think of the genome as a book. Some of the early types of improvements were based on hybridization, and that would be like bringing Volume One and Volume Two together and binding them into a single book. Using transgenes for traditional GM approaches is more like moving words and even sentences or a paragraph from a different book into a new one. We have also been modifying the genome with 'traditional methods' such as random mutagenesis, including insertion mutagenesis, essentially randomly changing text in the book.

Where does CRISPR fit into this continuum of genetic improvement of plants? The nice thing about CRISPR, if you use that metaphor of the book, is that it's the ultimate in terms of precision, because now with gene editing, we can change one letter, we can change a few letters, we can remove a few letters, and we can do this precisely in a specific location in the book that we wish to target. So essentially, it's like a word processor. We can change one single letter, but also delete words, paragraphs, and even chapters. This is different from what is loosely known as genetic modification (GM) or transgenics, the introduction of DNA from one organism to another. GE is about modifying the genome of the organism, sometimes to introduce genetic variation that already exists in nature, but in different varieties.



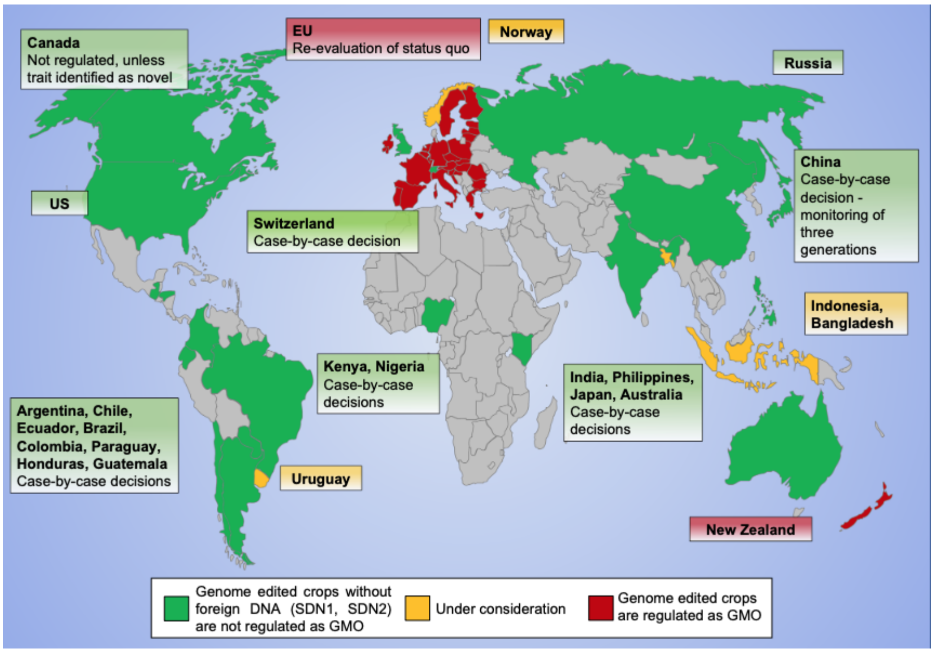

Examples of beneficial GE modifications of plants. Source: The Sainsbury Laboratory.

Back in the pre-CRISPR days (2012), my colleague Eric Ward and I wrote a commentary that was based on some of the first applications of a different method of gene editing, known as TALEN. At that time, gene editing technology was used to remove genes from crop genomes. We argued that this is the exact opposite of what we do with transgenic GMOs, because transgenics are all about bringing new genes into your crop from other species, not removing them. In the case of gene editing whether by TALEN or CRISPR, we're doing the exact opposite.

I want to emphasize that whether or not gene editing is GM is not a scientific question. CRISPR is just part of the continuum of genetic modification techniques we have implemented using mutagenesis. The focus on regulating crop plants based on the method that was used to produce them rather than the end product—the modified plant itself—is irrational and unscientific. You can, for instance, use CRISPR to introduce naturally occurring mutations in a different crop background. In terms of the product, in most cases, gene editing is not actually producing anything different from what you would obtain through natural processes of evolution or classical methods of plant breeding. In a way, the question of whether gene editing is GMO or not is more of a regulatory question rather than a scientific one.

**The globalization of GE technology**

The answer to the "GM or no" question varies depending on the country because different countries have taken different approaches to regulation. Some regulatory frameworks are process-based whereas others are product-based. There is a lot of diversity in how countries regulate gene editing. Importantly, some countries that have been traditionally more conservative like Japan, for example, have decided not to regulate targeted mutagenesis or gene editing in the same way that they regulate GM transgenic technology. To keep up with the ever-evolving regulatory landscape, follow the Global Gene Editing Regulation Tracker and Index.




June 2022 status of plant GE and GM regulation across the world. Source: Buchholzer and Frommer, 2022.

China has fully embraced biotechnology in general and CRISPR in particular. For example, the company Biogle, based in Changzhou, specializes in CRISPR gene editing technology, particularly in plants, as a service to the Chinese scientific community. I had the opportunity to visit Biogle in April 2018 and, at that time, Biogle scientists were busy doing gene editing in maize at an industrial scale. Nowadays, they work on many other organisms and have published a number of papers. One example of the resources they produced is a large-scale library of targeted mutants in rice. Any Chinese scientist who would like to obtain a mutant in a specific gene just needs to order it through their gene bank, and would simply get the mutant in the mail a few days later. More recently, they published a mutant library for maize. *This is the future of plant breeding, the large-scale production of genetic variation using GE methods.*

**Sophien Kamoun**
@KamounLab

The future of plant biology is here. In Changzhou.
biogle.cn


5:33 AM · Apr 8, 2018 · Twitter for iPhone

The future of plant biology is in large-scale GE production as witnessed at Biogle, Changzhou, China.

**I didn't come here to tell you how this is going to end…**

CRISPR and related GE technologies are in their infancy. Even though you hear about it all the time, it's remarkable that CRISPR was only invented in 2012, and first applied to plants only in 2013. So, we're talking about a very young technology that we haven't yet explored at its full potential. I'd like to end with this quote from Neo in *The Matrix*, which I find very appropriate for this topic: "I didn't come here to tell you how this is going to end, I came here to tell you how it's going to begin". I think that we're really at the beginning of the applications of gene editing to agriculture and conservation biology and only the future will tell how it ends.

**Sophien Kamoun**
@KamounLab

"I didn't come here to tell you how this is going to end. I came here to tell you how it's going to begin." Neo 'The Matrix'

CRISPR was only invented in 2012.

**Concluding remarks—a bigger toolbox for crop conservation**

Conservation biology requires a bigger toolbox to meet the biodiversity crisis. Genomics has a major role to play in conservation biology. We can use gene editing to produce genetic variation, whether by introducing natural variants into our landraces and crops or tode-extinct long gone variants from the sequence alone. Moi Exposito-Alonso and colleagues recently estimated that over 10% of genetic diversity has already been lost, and the trend is most probably accelerating. Perhaps, in the future, GE will help reverse this loss by generating genetic variation.

All of this first requires sequencing the genomes of the crops we grow and of their wild relatives before they go extinct. This should be massively scaled up and germplasm collections need to be sequenced before it's too late. In addition to physically storing the invaluable seeds of our crops, we need to also determine and preserve the genetic information in that germplasm. Given the possibilities of the modern GE technology, the genome sequences are almost as valuable as preserving the seeds themselves. It's this type of forward-looking thinking that can help us deal with the biodiversity crisis.

**Special thanks to…**

This article was adapted from the GROW webinar "CRISPR crops with Sophien Kamoun" that I gave to the Crop Trust in March 2022 as part of a monthly series on genebanks. I'm grateful to Janny van Beem, Global Genebank Partnership Coordinator at the Crop Trust, for tasking me to talk about CRISPR and gene editing in the context of conservation of biological resources. I really have to thank Janny for the invitation because I hadn't given this topic as much thought as it deserves. The invitation prompted me to do a lot of reading and catch up on the topic. The article is not meant to be written by an expert in conservation biology, but more as a way to kick off a discussion on the role of genomic technologies to fend off the dramatic loss of biodiversity we are facing.


GROW webinar: CRISPR crops with Sophien Kamoun

**Acknowledgements**

As often, I'm most grateful to Jennifer Mach at PlantEditors for turning the chaotic transcript of the talk into a readable and coherent draft. I thank many colleagues for their input and ideas. In particular, I thank Moi Exposito-Alonso for prompting me to wrap up the article following his presentation at WeigelFest on the genomics of extinction.

**Competing Interest Statement**

My lab is actively engaged in apply GE and other biotech approaches to crop improvements. I hold patents and I have close associations with the biotech industry. My full list of competing interests is available as part of my online CV.

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Did you know it's possible to delete 48 letters in the genetic code of a tomato? 🍅 🔬

This **#BritishScienceWeek**, we visit **#STEM** experts at **@TheSainsburyLab**.

They're using genome editing to make pathogen resistant plants 👇 **#BSW22**

Generating genetic variation, one mutant at a time.