NORRIE RUSSELL, UNIV. EDINBURGH

Pigs reared at the University of Edinburgh's Roslin Institute have had individual letters of their genetic code modified to protect them against African swine fever.

AGRICULTURE

A new breed of edits

Genome editing allows much smaller changes to be made to DNA compared with conventional genetic engineering. In terms of agriculture, this might win over public and regulator opinion.

BY CLAIRE AINSWORTH

In spring 2015, the first genome-edited crop, a herbicide-resistant oilseed rape, was planted in fields dotted across the United States. Although the plant's DNA has been directly altered by molecular biologists, the company that created it, Cibus, based in San Diego, California, explicitly markets the crop as non-genetically modified (non-GM). The company's argument is that only a few nucleotides of the plant's existing genes have been changed. No gene has been inserted from a different kind of organism, nor even from another plant.

A lot hangs on how governments around the world decide to regulate agricultural products that have had their genomes edited. The decisions will influence the types of edited crops and animal products that are developed. To US regulators, Cibus's oilseed rape is an example of mutagenesis, not of genetic modification. This is a relief to the company because preparing for regulatory approval of a GM organism in the United States can take more than five years and cost tens of millions of dollars. Europe is even stricter, and the European Commission has yet to publish its legal interpretation of how genome-edited crops, such as the Cibus oilseed rape, should be regulated. Several political groups are lobbying for a hard line, which would frustrate many researchers. "If Europe regulates genome-edited organisms in the same way it does GM organisms, it will kill the technology here for all except the biotech companies working with profitable traits in the major crops," says Huw Jones, senior research scientist at Rothamsted Research in Harpenden, UK, who is currently working on genome editing in wheat.

Yet the potential applications of genome editing for global agriculture — and disease vectors (see 'Hack the mosquito') — are huge. But so are the challenges that the world will face. According to projections by the United Nations, the world's population is set to soar from the current 7.3 billion to 9.7 billion by 2050. Agricultural output will have to increase to feed more mouths, even though the amount of fresh water available for irrigation is decreasing, and most of Earth's arable land is already under cultivation. Add in the effects of climate change — crop-damaging higher temperatures, drought and flooding, not to mention a rise in agricultural pests and diseases — and it is no surprise that food security is top of the international political agenda.

DIFFERENT FURROWS

Genetic modification and conventional breeding have long been available to assist in meeting these food-security challenges, but genome editing is different, argues Pamela Ronald, a plant pathologist at the University of California, Davis. Genetic engineering is typically ham-fisted: it often involves inserting a large section of DNA from an entirely different kind of organism — often in another kingdom — with little control over where in the genome it lands. Meanwhile, conventional breeders are limited not only by the time it takes to cross in new traits, but also by the need to ensure that in doing so, they do not breed out the plant's other desirable characteristics.

Compared with these alternatives, genome editing offers both subtlety and speed, wherever in the genome a researcher wants to target. "You can change even a single base pair, or you

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GENE DRIVES

Hack the mosquito

The mosquito has long held the title of the world's deadliest animal. The *Anopheles* genus causes hundreds of thousands of human deaths annually by transmitting malaria parasites. Editing *Anopheles* genomes — as well as those of *Aedes* mosquitoes, which spread viral infections such as yellow and dengue fevers — brings with it the possibility of new research and control methods.

Eric Marois of France's National Centre for Scientific Research in Strasbourg, is part of a team working with transcription activator-like effector nucleases (TALENs) to disrupt the gene *TEP1*, which is known to help *Anopheles gambiae* to resist infection by malaria parasites. Without the protection conferred by this gene, Marois's team found that the mosquito from sub-Saharan Africa became hypersusceptible to parasites[1]. That may not sound like an advance, but the research is helping scientists to understand the genetics that make this particular species such a good vector, and may lead to better malaria control, with or without gene editing.

Research with *Aedes*, which is easier to work with in the lab, is more advanced. A few groups have applied zinc-finger nucleases (ZFNs) and TALENs to the genus, but Ben Matthews, a mosquito specialist at Rockefeller University, New York, is trying out CRISPR–Cas9 because it is the cheapest and most user-friendly of the tools. Using the relatively simple technique also means his recent proof-of-concept paper is more likely to be picked up by other infectious-disease researchers. In the paper[2], Matthews and his colleagues demonstrated the use of CRISPR–Cas9 to delete parts of a target gene, which created mutations that were passed on in the *Aedes* germ line, and to insert a whole gene at a specific location.

But that is all in the laboratory. Getting insects with edited genomes to thrive in the wild — so that the edited genes spread throughout a population — presents an entirely different challenge. Researchers have to pick their gene edits carefully, because experiments show that seemingly advantageous genetic manipulation can reduce a mosquito's ability to survive and reproduce compared with its wild counterparts. Another problem is that if an edit succeeds in making an insect immune to infection, it also creates a strong selective pressure for the pathogen to evolve a means of getting around the modification, potentially encouraging new and greater challenges to disease control.

To circumvent some of these problems, scientists have proposed tricks, collectively known as gene drives, that artificially force the dissemination of gene modifications through the generations. During normal inheritance, there is a 50% chance that offspring will inherit a modified gene carried on one chromosome. The gene-drive system, however, cuts the partner to this chromosome and, during the repair process, the mutation is copied to the partner chromosome so that an edited organism will transmit the altered gene to almost all of its offspring. In 2011, a team led by scientists at Imperial College London showed that genetic elements known as homing endonucleases could work as gene drives in *Anopheles*[3]. And earlier this year, researchers at the University of California, San Diego, used CRISPR–Cas9 to generate a 'mutagenic chain reaction' whereby a mutation that is present in just one of a pair of chromosomes copies itself to the other chromosome of the pair[4].

Yet many researchers worry about the potential ecological affects of unleashing gene drives in the wild. As much as these modifications have the potential to eliminate the proliferation of insects that transmit disease to humans, they could also accidentally destroy a key segment of a food web, facilitating the invasion of another species. How to test gene drives properly without losing control of them is a catch-22 situation. C.A.

can delete a gene very precisely," says Ronald. The speed comes from the technologies' ability to remake an existing gene in the image of a more useful one, which might be present in the breeding population at very low frequency. Useful traits that are found only in wild populations or related species — perhaps a species that encounters similar pathogens — can be quickly brought in. "Genome editing basically provides the variation you want, where you want it," says Bruce Whitelaw, an animal biotechnologist at Scotland's Roslin Institute, near Edinburgh.

In a barn at the Roslin Institute, pigs snuffle around, unaware that they illustrate Whitelaw's point perfectly. As fertilized eggs, they had one of their immune-system genes edited. The gene in question, *RELA*, is thought to trigger the overblown immune reaction that kills pigs infected with the haemorrhagic virus that causes African swine fever. Whitelaw's team was inspired by the fact that warthogs (which belong to the same family as domestic pigs) tolerate the infection well, even though their version of *RELA* differs from that of domestic pigs by only 3 amino acids out of more than 500. Whitelaw's team began the research using editing tools called zinc-finger nucleases and then transcription activator-like effector nuclease (TALEN) technology, and has since moved on to CRISPR–Cas9, with the aim of editing the pig gene to achieve the exact warthog *RELA* sequence. The edited pigs will soon be exposed to the pathogen, for which there is no vaccine or cure. If the pigs make it through unharmed, the team will have found a way to protect farmers from devastating losses, particularly those in regions where the disease is hard to eradicate, such as sub-Saharan Africa and Eastern Europe.

Whitelaw's pig project will largely benefit poor farmers — a rarity for editing research. The prospect of tough regulation and consequently an expensive market-approval process has meant that a much more common goal among livestock-focused genome editing has been to generate higher-profit cattle, pigs and sheep with increased muscle mass — often by disabling the *MSTN* gene, which restricts muscle growth.

Similarly, it is of little surprise that the first genome-edited crop to emerge — Cibus's oilseed rape — has a business rationale. Instead of focusing on an edit that could, for example, boost the vitamin content of the plant's oil to combat malnutrition, the edits allow a farmer to spray weedkiller more liberally over his or her fields. "I don't think it's too extreme to say that the way that the technology will be used for plant breeding in the future will hinge on how is regulated," says Jones.

The question of how to regulate genome-edited crops in Europe has been on the table for years; the European Commission started to look at the issue back in 2007. The commission generally considers an organism to be GM if its genes are altered in ways that cannot occur naturally, suggesting that edited crops should be classified as GM. But it also has a record for making exceptions for crops in which mutations have been induced using chemicals or radiation. Jones sorely hopes that genome editing falls into the latter category. Placing it alongside older genetic engineering would, in his eyes, be unfair. "It's almost like comparing chalk and cheese," he says. ■

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