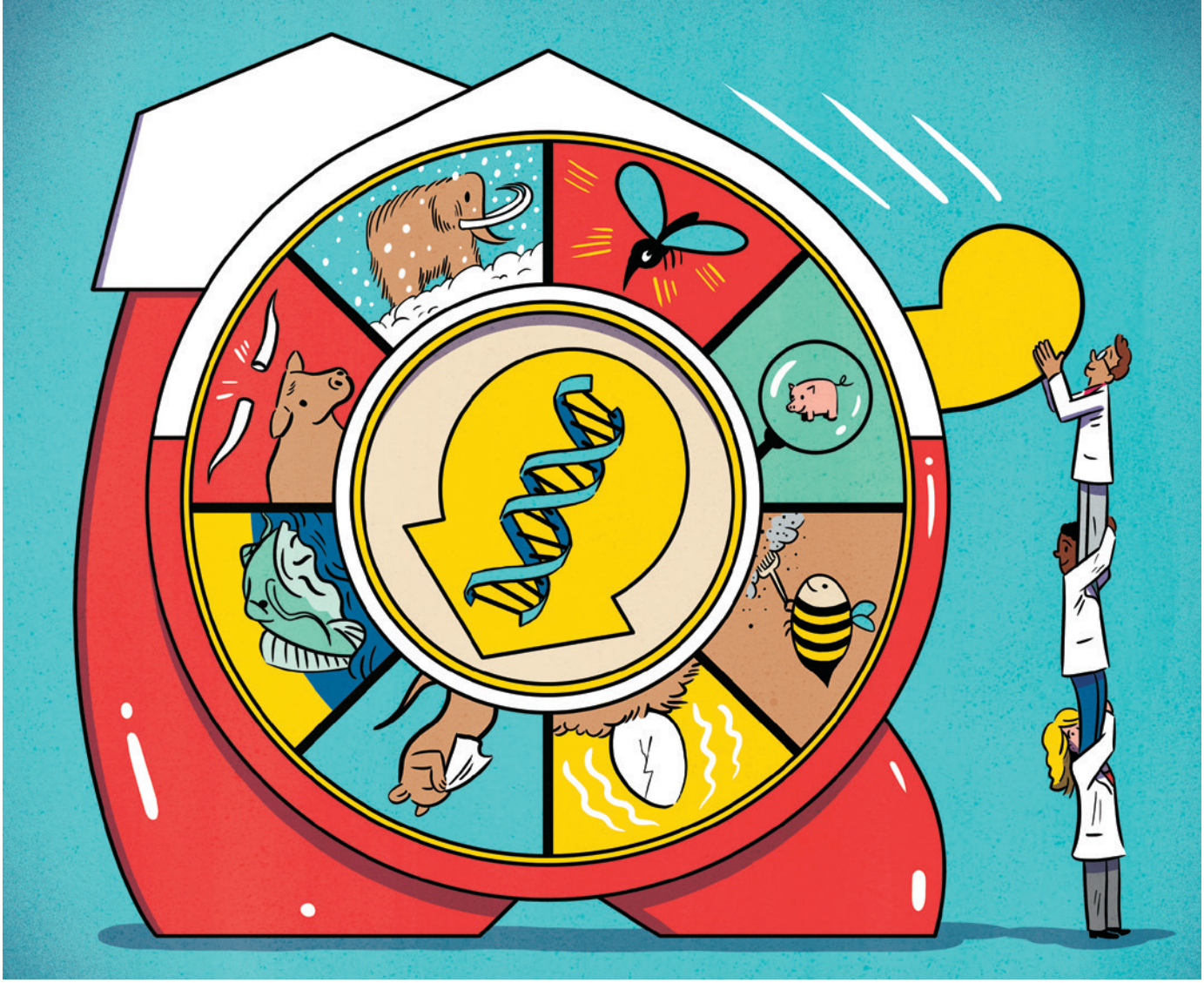


ILLUSTRATIONS BY RYAN SNOOK

THE CRISPR ZOO

Birds and bees are just the beginning for a burgeoning technology.

BY SARA REARDON

Timothy Doran's 11-year-old daughter is allergic to eggs. And like about 2% of children worldwide who share the condition, she is unable to receive many routine vaccinations because they are produced using chicken eggs.

Doran, a molecular biologist at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Geelong, Australia, thinks that he could solve this problem using the powerful gene-editing tool CRISPR–Cas9. Most egg allergies are caused by one of just four proteins in the white, and when Doran's colleagues altered the gene that encodes one of these in bacteria, the resulting protein no longer triggered a reaction in blood serum from people who were known to be allergic to it[1]. Doran thinks that using CRISPR to edit the gene in chickens could result in hypoallergenic eggs.

The group expects to hatch its first generation of chicks with gene modifications later this year as a proof of concept. Doran realizes that it could be some time before regulators would approve gene-edited eggs, and he hopes that his daughter will have grown out of her allergy by then. "If not, I've got someone ready and waiting to try the first egg," he says.

Chickens are just one of a menagerie of animals that could soon have their genomes reimagined. Until now, researchers had the tools to genetically manipulate only a small selection of animals, and the process was

often inefficient and laborious. With the arrival of CRISPR, they can alter the genes of a wide range of organisms with relative precision and ease. In the past two years alone, the prospect of gene-edited monkeys, mammoths, mosquitoes and more have made headlines as scientists attempt to put CRISPR to use for applications as varied as agriculture, drug production and bringing back lost species. CRISPR-modified animals are even being marketed for sale as pets. "It's allowed us to consider a whole raft of projects we couldn't before," says Bruce Whitelaw, an animal biotechnologist at the Roslin Institute in Edinburgh, UK. "The whole community has wholeheartedly moved towards genome editing."

But regulators are still working out how to deal with such creatures, particularly those intended for food or for release into the wild. Concerns abound about safety and ecological impacts. Even the US director of national intelligence has weighed in, saying that the easy access, low cost and speedy development of genome editing could increase the risk that someone will engineer harmful biological agents.

Eleonore Pauwels, who studies biotechnology regulation at the Wilson Center in Washington DC, says that the burgeoning use of CRISPR in animals offers an opportunity for researchers and policymakers to engage the public in debate. She hopes that such discussions will help in determining which uses of CRISPR will be most helpful to humans, to other species and to science — and will highlight the limits of the technology. "I think there is a lot of value in humility about how much control we have," she says.

DISEASE CONTROL

Disease resistance is one of the most popular applications for CRISPR in agriculture, and scientists are tinkering across a wide spectrum of animals. Biotechnology entrepreneur Brian Gillis in San Francisco is hoping that the tool can help to stem the dramatic loss of honeybees around the world, which is being caused by factors such as disease and parasites.

Gillis has been studying the genomes of 'hygienic' bees, which obsessively clean their hives and remove sick and infested bee larvae. Their colonies are less likely to succumb to mites, fungi and other pathogens than are those of other strains, and Gillis thinks that if he can identify genes associated with the behaviour, he might be able to edit them in other breeds to bolster hive health.

But the trait could be difficult to engineer. No hygiene-associated genes have been definitively identified, and the roots of the behaviour may prove complex, says BartJan Fernhout, chairman of Arista Bee Research in Boxmeer, the Netherlands, which studies mite resistance. Moreover, if genes are identified, he says, conventional breeding may be sufficient to confer resistance to new populations, and that might be preferable given the widespread opposition to genetic engineering.

Such concerns don't seem to have slowed down others studying disease resistance. Whitelaw's group at the Roslin Institute is one of several using CRISPR and other gene-editing systems to create pigs that are resistant to viral diseases that cost the agricultural industry hundreds of millions of dollars each year.

Whitelaw's team is using another gene-editing technique to alter immune genes in domestic pigs to match more closely those of warthogs that are naturally resistant to African swine fever, a major agricultural pest[2]. And Randall Prather at the University of Missouri in Columbia has created pigs with a mutated protein on the surface of their cells, which should make them impervious to a deadly respiratory virus[3]. Other researchers are making cattle that are resistant to the trypanosome parasites that are responsible for sleeping sickness.

Whitelaw hopes that regulators — and sceptical consumers — will be more enthusiastic about animals that have had their genes edited to improve disease resistance than they have been for traits such as growth promotion because of the potential to reduce suffering. And some governments are considering whether CRISPR-modified animals should be regulated in the same way as other genetically modified organisms, because they do not contain DNA from other species.

MAKING DRUGS

Doran's quest to modify allergens in chicken eggs requires delicate control. The trick is to finely adjust a genetic sequence in a way that will stop the protein from triggering an immune reaction in people, but still allow it to perform its normal role in embryonic development. CRISPR has made such precise edits possible for the first time. "CRISPR has been the saviour for trying to tackle allergens," says Mark Tizard, a molecular biologist at CSIRO who works with Doran on chickens.

Using the technique in birds still presents problems. Mammals can be induced to produce extra eggs, which can then be removed, edited, fertilized and replaced. But in birds, the fertilized egg binds closely to the yolk and removing it would destroy the embryo. And because eggs are difficult to access while still inside the hen, CRISPR components cannot be directly injected into the egg itself. By the time the egg is laid, development has proceeded too far for gene editing to affect the chick's future generations.

To get around this, Tizard and Doran looked to primordial germ cells (PGCs) — immature cells that eventually turn into sperm or eggs. Unlike in many animals, chicken PGCs spend time in the bloodstream during development. Researchers can therefore remove PGCs, edit them in the lab and then return them to the developing bird. The CSIRO team has even developed a method to insert CRISPR components directly into the bloodstream so that they can edit PGCs there[4].

The researchers also plan to produce chickens with components required for CRISPR integrated directly into their genomes — what they call CRISPi chickens. This would make it even easier to edit chicken DNA, which could be a boon for 'farmaceuticals' — drugs created using domesticated animals.

Regulators have shown a willingness to consider such drugs. In 2006, the European Union approved a goat that produces an anticlotting protein in its milk. It was subsequently approved by the US Food and Drug Administration, in 2009. And in 2015, both agencies approved a transgenic chicken whose eggs contain a drug for cholesterol diseases.

DE-EXTINCTION

About 4,000 years ago, hunting by humans helped to drive woolly mammoths (*Mammuthus primigenius*) to extinction. CRISPR pioneer George Church at Harvard Medical School in Boston, Massachusetts, has attracted attention for his ambitious plan to undo the damage by using CRISPR to transform endangered Indian elephants into woolly mammoths — or at least cold-resistant elephants. The goal, he says, would be to release them into a reserve in Siberia, where they would have space to roam.

The plan sounds wild — but efforts to make mammals more mammoth-like have been going on for a while. Last year, geneticist Vincent Lynch at the University of Chicago in Illinois showed that cells with the mammoth version of a gene for heat-sensing and hair growth could grow in low temperatures[5], and mice with similar versions prefer the colder parts of a temperature-regulated cage[6]. Church says that he has edited about 14 such genes in elephant embryos.

But editing, birthing and then raising mammoth-like elephants is a huge undertaking. Church says that it would be unethical to implant gene-edited embryos into endangered elephants as part of an experiment. So his lab is looking into ways to build an artificial womb; so far, no such device has ever been shown to work.

There are some de-extinction projects that could prove less challenging. Ben Novak at the University of California, Santa Cruz, for example, wants to resurrect the passenger pigeon (*Ectopistes migratorius*), a once-ubiquitous bird that was driven to extinction in the late nineteenth century by overhunting. His group is currently comparing DNA from museum specimens to that of modern pigeons. Using PGC methods similar to Doran's, he plans to edit the modern-pigeon genomes so that the birds more closely resemble their extinct counterparts.

Novak says that the technology is not yet advanced enough to modify the hundreds of genes that differ between modern and historic pigeons. Still, he says that CRISPR has given him the best chance yet of realizing his lifelong dream of restoring an extinct species. "I think the project is 100% impossible without CRISPR," he says.

VECTOR CONTROL

For decades, researchers have explored the idea of genetically modifying mosquitos to prevent the spread of diseases such as dengue or malaria. CRISPR has given them a new way to try.

In November, molecular biologist Anthony James of the University of California, Irvine, revealed a line of mosquitoes with a synthetic system called a gene drive that passes a malaria-resistance gene on to the mosquitoes' offspring[7]. Gene drives ensure that almost all the insects' offspring inherit two copies of the edited gene, allowing it to spread rapidly through a population.

Another type of gene drive, published last December[8], propagates a gene that sterilizes all female mosquitoes, which could wipe out a population. The outbreak of mosquito-borne Zika virus in Central and South America has increased interest in the technology, and several research labs have begun building gene drives that could eliminate the Zika-carrying species, *Aedes aegypti*.

Many scientists are worried about unintended and unknown ecological consequences of releasing such a mosquito. For this reason, Church and his colleagues have developed 'reverse gene drives' — systems that would propagate through the population to cancel out the original mutations[9,10].

But Jason Rasgon, who works on genetically modified insects at Pennsylvania State University in University Park, says that although ecology should always be a consideration, the extent and deadliness of some human diseases such as malaria may outweigh some costs.

Mosquitoes are some of the easiest insects to work with, he says, but researchers are looking at numerous other ways to use gene drives, including making ticks that are unable to transmit the bacteria that cause Lyme disease. Last year, researchers identified a set of genes that could be modified to prevent aquatic snails (*Biomphalaria glabrata*) from transmitting the parasitic disease schistosomiasis[11].

BETTER FOOD PRODUCTION

Last November, after a lengthy review, the US Food and Drug Administration approved the first transgenic animals for human consumption: fast-growing salmon made by AquaBounty Technologies of Maynard, Massachusetts. Some still fear that if the salmon escape, they could breed with wild fish and upset the ecological balance.

To address such concerns, fish geneticist Rex Dunham of Auburn University in Alabama has been using CRISPR to inactivate genes for three reproductive hormones — in this case, in catfish, the most intensively farmed fish in the United States. The changes should leave the fish sterile, so any fish that might escape from a farm, whether genetically modified or not, would stand little chance of polluting natural stocks. "If we're able to achieve 100% sterility, there is no way that they can make a genetic impact," Dunham says. Administering hormones would allow the fish to reproduce for breeding purposes. And Dunham says that similar methods could be used in other fish species.

CRISPR could also reduce the need for farmers to cull animals, an expensive and arguably inhumane practice. Biotechnologist Alison van Eenennaam at the University of California, Davis, is using the technique to ensure that beef cattle produce only male or male-like offspring, because females produce less meat and are often culled. She copies a Y-chromosome gene that is important for male sexual development onto the X chromosome in sperm. Offspring produced with the sperm would be either normal, XY males, or XX females with male traits such as more muscle.

In the egg industry, male chicks from elite egg-laying chicken breeds have no use, and farmers generally cull them within a day of hatching. Tizard and his colleagues are adding a gene for green fluorescent protein to the chickens' sex chromosomes so that male embryos will glow under ultraviolet light. Egg producers could remove the male eggs before they hatch and potentially use them for vaccine production.

There are other ways that CRISPR could make agriculture more humane. Packing cattle into trailers or other small spaces often causes injuries, especially when the animals have long horns. So cattle farmers generally burn, cut or remove them with chemicals — a process

that can be painful for the animal and dangerous for the handler. There are cattle varieties that do not have horns — a condition called 'polled' — but crossing these breeds with 'elite' meat or dairy breeds reduces the quality of the offspring.

Molecular geneticist Scott Fahrenkrug, founder of Recombinetics in Saint Paul, Minnesota, is using gene-editing techniques to transfer the gene that eliminates horns into elite breeds[12]. The company has produced only two polled calves so far — both male — which are being raised at the University of California, Davis, until they are old enough to breed.

IMPROVING PETS

Last September, the genomics firm BGI wowed a conference in Shenzhen, China, with micropigs — animals that grow to only around 15 kilograms, about the size of a standard dachshund. BGI had originally intended to make the pigs for research, but has since decided to capitalize on creation of the animals by selling them as pets for US$1,600. The plan is to eventually allow buyers to request customized coat patterns.

BGI is also using CRISPR to alter the size, colour and patterns of koi carp. Koi breeding is an ancient tradition in China, and Jian Wang, director of gene-editing platforms at BGI, says that even good breeders will usually produce only a few of the most beautifully coloured and proportioned, 'champion quality' fish out of millions of eggs. CRISPR, she says, will let them precisely control the fish's patterns, and could also be used to make the fish more suitable for home aquariums rather than the large pools where they are usually kept. Wang says that the company will begin selling koi in 2017 or 2018 and plans to eventually add other types of pet fish to its repertoire.

Claire Wade, a geneticist at the University of Sydney in Australia, says that CRISPR could be used to enhance dogs. Her group has been cataloguing genetic differences between breeds and hopes to identify areas involved in behaviour and traits such as agility that could potentially be edited[13]. Sooam Biotech in Seoul, best-known for a service that will clone a deceased pet for $100,000, is also interested in using CRISPR. Sooam researcher David Kim says that the company wants to enhance the capabilities of working dogs — guide dogs or herding dogs, for example.

Jeantine Lunshof, a bioethicist who works in Church's lab at Harvard, says that engineering animals just to change their appearance, "just to satisfy our idiosyncratic desires", borders on frivolous and could harm animal well-being.

But she concedes that the practice is not much different from the inbreeding that humans have been performing for centuries to enhance traits in domestic animals and pets. And CRISPR might even help to eliminate some undesirable characteristics: many dog breeds are prone to hip problems, for example. "If you could use genome editing to reverse the very bad effects we have achieved by this selective inbreeding over decades, then that would be good."

DISEASE MODELS

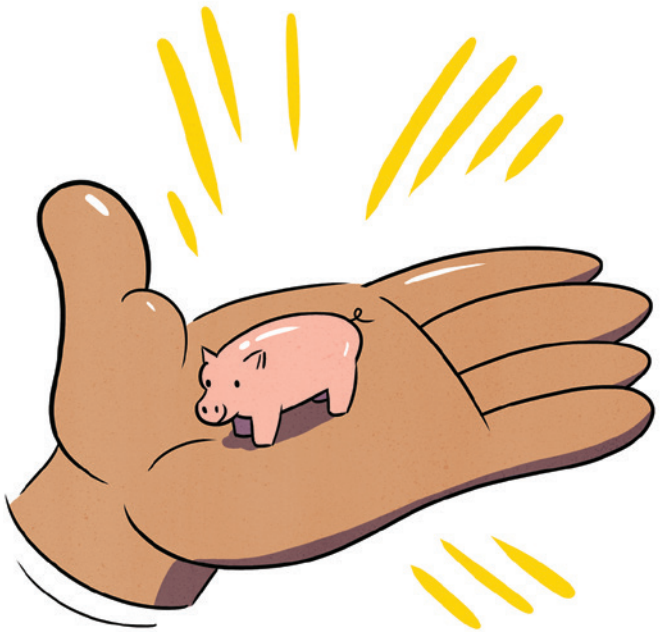
Ferrets have long been a useful model for influenza research because the virus replicates in their respiratory tracts and they sometimes sneeze when infected, allowing studies of virus transmission. But until the arrival of CRISPR, virologists lacked the tools to easily alter ferret genes. Xiaoqun Wang and his colleagues at the Chinese Academy of Sciences in Beijing have used CRISPR to tweak genes involved in ferret brain development[14], and they are now using it to modify the animals' susceptibility to the flu virus. He says that he will make the model available to infectious-disease researchers.

Behavioural researchers are particularly excited about the prospect of genetically manipulating marmosets and monkeys, which are more closely related to humans than are standard rodent models. The work is moving most quickly in China and Japan. In January, for instance, neuroscientist Zilong Qiu and his colleagues at the Chinese Academy of Sciences in Shanghai published a paper[15] describing macaques with a CRISPR-induced mutation in *MECP2*, the gene associated with the neurodevelopmental disorder Rett syndrome. The animals showed symptoms of autism spectrum disorder, including repetitive behaviours and avoiding social contact.

But Anthony Chan, a geneticist at Emory University in Atlanta, Georgia, cautions that researchers must think carefully about the ethics of creating such models and whether more-standard laboratory animals such as mice would suffice. "Not every disease needs a primate model," he says.

Basic neuroscience could also benefit from the availability of new animal models. Neurobiologist Ed Boyden at the Massachusetts Institute of Technology is raising a colony of the world's tiniest mammal — the Etruscan tree shrew (*Suncus etruscus*). The shrews' brains are so small that the entire organ can be viewed under a microscope at once. Gene edits that cause neurons to flash when they fire, for instance, could allow researchers to study the animal's entire brain in real time.

The CRISPR zoo is expanding fast — the question now is how to navigate the way forward. Pauwels says that the field could face the same kind of public backlash that bedevilled the previous generation of genetically modified plants and animals, and to avoid it, scientists need to communicate the advantages of their work. "If it's here and can have some benefit," she says, "let's think of it as something we can digest and we can own." ■



Sara Reardon *writes for* Nature *from Washington DC.*

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