Science & Society

OPEN ACCESS

EMBO reports

The viral era

New biotechnologies give humans an unprecedented control over Nature and require appropriate safeguards

Bernd Giese*

We are entering a new phase in our relationship with nature: after mechanization, automation and digitalization, a new era of autonomous technical objects is dawning. The most advanced of these technologies are characterized by viral behaviour. The COVID-19 pandemic has again aptly demonstrated the power of viral systems: not only because of the SARS-CoV-2 virus' ability to jump into and rapidly spread among the human population while wreaking havoc with human societies, but also because some of the vaccines developed against the virus are themselves based on viruses. Both developments give us some ideas of the possible impact of new biotechnologies that aim to create artefacts with viral behaviour in order to shape and control our natural environment. In this essay, the focus is on the use of genetically engineered organisms and the genetic manipulation of wild species. This change has a more direct relationship to our natural environment than autonomous software artefacts such as computer apps or digital viruses that "live" in their artificial "ecosystems" of information-processing devices. The development of artificial biological systems will therefore require new methods for monitoring and intervention given their potential to autonomously spread within natural ecosystems.

First steps

This change is not happening overnight nor has it just begun. Biological control has been used with great success, especially since the late 19th century using naturally occurring species to control or eradicate pests, weeds or pathogens (van den Bosch *et al*, 1982). However, even without genetic manipulations, classical biological control has risks: an exotic control agent introduced for pest control can become a pest species in itself if it is no longer under the control of its original environment as happened with the introduction of cats (to fight rodents) or the cane toad to fight cane beetles in Australia. Since, the field has gone through a learning process and established strategies to avoid adverse ecological effects. It also raised awareness among scientists and the general public about the critical implications of using living organisms as autonomous objects.

"Biological control has been used with great success, especially since the late 19th century using naturally occurring species to control or eradicate pests, weeds or pathogens…"

The development of the sterile insect technique (SIT) increased the power of biological control and the depth of intervention with natural species. SIT aims to control insect pests by releasing large numbers of preferably male individuals that are sterilized with radiation or chemicals. "Sterilized" in the context of SIT means that the treated males still produce sperm, but the majority of their eggs will not hatch. If a sufficiently large number of sterilized males are released over several years, the natural population eventually collapses as females find it increasingly difficult to mate with propagable males. SIT was first applied in the 1950s against the New World screwworm and has been successfully used to eradicate this pest in the United States, Mexico and Libya. A similar joint effort by Guatemala, Mexico and the United States under the Moscamed programme has also prevented the Mediterranean fruit fly, a major invasive pest of fruit and vegetable crops, from moving north of Guatemala. SIT is also being considered to eradicate disease-transmitting mosquitoes in Europe and North America.

However, the technique does have disadvantages. Radiation or chemical treatment to sterilize the males also reduces their fitness and thereby chance of mating. It therefore requires releasing an enormous number of sterile insects over several years to control or eradicate the target population. Moreover, in terms of induced genetic and phenotypic changes, SIT is a rather imprecise method that randomly damages the insects' genome and germline.

Sterile insect technique still represents an era where chemistry and physics dominated the technological approaches. The subsequent transgenic approach of "Release of Insects carrying a Dominant Lethal" (RIDL; Thomas *et al*, 2000) is more representative of the new era of biotechnologies and the life sciences. RIDL adopts the SIT strategy but uses genetic engineering instead of radiation or mutagenic chemicals to avoid random effects on the genome. Since the first releases in 2009–2010 in the Cayman Islands to eradicate *Aedes aegypti*, the mosquito vector of infectious diseases such as dengue or Zika fever, further applications of RIDL are being tested. Currently, a variant with female-specific lethality against *A. aegypti* (fsRIDL; Fu *et al*, 2010) is being released as

Institute of Safety and Risk Sciences, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
*Corresponding author. E-mail: bernd.giese@boku.ac.at
DOI 10.15252/embr.202153229 | EMBO Reports (2021) 22: e53229 | Published online 4 August 2021

part of a pilot study in the Florida Keys. fsRIDL is specifically designed as a self-limiting system the genetic "footprint" of which should be lost over time with subsequent generations. Other applications target insect pests such as the Diamondback moth or the Mediterranean fruit fly.

Another version of SIT currently being explored does no longer require genetic modification but uses RNAi to inhibit the expression of essential genes in the target species. Recently, Leonard *et al* (2020) showed that it is even possible to produce RNAi through a symbiotic gut bacterium and thereby kill *Varroa* mites, a devastating parasite of honeybees. RNAi technology is also used for another biological pest control strategy called maternal effect dominant embryonic arrest (MEDEA). MEDEA is based on selfish genes that naturally occur in flour beetles; in pest control applications, it can be used as a gene drive to spread traits that make populations susceptible to suppression.

Gene drives

Synthetic gene drives represent another qualitative revolution not only for population control but also in terms of genetically modified organisms (GMOs) because these are deliberately designed to spread in wild species (Simon *et al*, 2018). As MEDEA, they have archetypes in selfish genes; the specific molecular setup of a synthetic gene drive—partially copied from naturally occurring drives—enables a propagation of the drive itself along with "cargo" genes within the population even when these transgenes represent a high fitness burden for their carriers. Some elaborated types of synthetic gene drives use homing endonucleases like the CRISPR/Cas system to multiply themselves within the target genome and thereby convert heterozygous individuals to homozygous. Gantz and Bier presented the experimental proof for their CRISPR-homing drive under the telling label "mutagenic chain reaction" (Gantz & Bier, 2015). Gene drives pass themselves and their target genes on the offspring of sexually reproducing organisms with a higher frequency than the 50% normal Mendelian inheritance: it is a technology for "cheating evolution" (Champer *et al*, 2016).

Some of the applications currently under development aim to suppress or eliminate populations of disease vectors, pests and invasive species. Others aim to spread a new trait in wild species. Both strategies are currently being pursued in the fight against infectious diseases such as malaria. Another development targets the vinegar fly *Drosophila suzukii*, an invasive crop pest native to Southeast Asia that causes severe damage to stone fruit and berries. In Australia and New Zealand, gene drives have been proposed to control invasive species such as rats, stoats, possums, cane toads and other species that harm agriculture and nature. Gene drives have also been discussed as a means to protect endangered species from pathogens, such as birds from avian malaria.

Whereas the elimination of major plagues would potentially require unlimited propagation, control of invasive species could be achieved with locally acting drives. At present, however, it is not clear whether gene drives can be controlled in any way to prevent their spread and adverse effects outside the intended location. In addition, a spread to non-target species via horizontal gene transfer or rare cross-species mating events must also be considered, even if, depending on the species, this probability is very low.

Gene drives would clearly and profoundly change the nature of pest control, and laboratory experiments indicate a very high effectiveness in particular for gene drives based on the CRISPR tool. If we assume that such drives will also be successful in the wild, their application represents an enormous expansion of human intervention possibilities. Many scientists are well aware of this potential and call for careful selection and control of its applications to avoid detrimental effects beyond their target species and region (Esvelt & Gemmell, 2017).

"If we assume that such drives will also be successful in the wild, their application represents an enormous expansion of human intervention possibilities."

Virus-based approaches

A major limitation of gene drives is the fact that they depend on vertical transmission via sexual reproduction. Applications to target rapidly reproducing insect species will probably show effects after a few months or a year, but in mammals such as rodents, it may take several years to reach the majority of individuals in a population.

Research is now underway to shorten the time by exploiting virus-based technique to spread transgenes horizontally. Virus-based approaches have already been successfully used for genome editing in plants: Ma *et al* (2020) used a negative-strand RNA virus to introduce the large expression cassette of the endonuclease Cas9 and its guide RNA (gRNA) into plant tissue for genome editing even beyond the infection site. However, these approaches are still confined to the laboratory, as the technique uses *Agrobacterium tumefaciens* to infect the plant cells with these viruses. The use of natural vectors like aphids or cicada would theoretically enable the genetic modification of plant populations on a much larger scale within a few generations.

In 2018, Reeves *et al* (2018) discussed the dual-use character of a research programme that had received little attention so far: the development of genetically engineered viruses to alter crop plants outside the laboratory. They called this new type of mediated genetic engineering "horizontal environmental genetic alteration agents" (HEGAAs). Funded by the US Defense Advanced Research Projects Agency (DARPA), three research consortia were tasked to realize such systems with the aim of "addressing national security challenges in agriculture domestically and abroad" (DARPA, 2016). As mentioned above, the key feature is to transmit genetically engineered viruses via plant-eating insects: this could enable a rapid response to pathogens and pests, but also increase drought or flooding tolerance of plants within one growing season. Nevertheless, the self-propagating nature of the technology would also allow misuse as an offensive weapon to destroy important crop plants. One group of the consortium already demonstrated a heritable effect in plants via this path (Ellison *et al*, 2020). This approach, however, still relied on Cas9 endonuclease expression by the plant because it was not possible to include the Cas9 gene in the virus.

Beyond horizontal gene transfer, the number of applications for genetically modified viruses has grown in recent years. In addition to virus-based human vaccines against SARS-CoV-2, most developments concern vaccines for livestock and pest control and, potentially, wild species.

Recombinant transmissible vaccines, genetically engineered chimeras of two viral genomes—a harmless "vector" virus containing genes from a pathogenic virus—have already been developed for wildlife and are now being considered for use in humans. Compared to attenuated live viruses, they have the advantage that they cannot revert to wild-type virulence. However, controlling their autonomous spread to suitable recipients and the risk of mutations that could alter their antigenic potential remains a challenge (Bull *et al*, 2018).

> *"Recombinant transmissible vaccines, genetically engineered chimeras of two viral genomes [. . .] have already been developed for wildlife and are now being considered for use in humans."*

Implications of a viral age

If we consider SIT as a technology from the pre-biotechnological era when physics and chemistry shaped the character of technologies, RIDL already represents the age of biotechnology and begins to unlock the potential of the information age. Gene drives and HEGAA now further make use of biotechnology and information technology to advance our possibilities to control and manipulate nature. The evolution from SIT to RIDL to gene drives and virus-based technologies also means that less and less human actions and labour are required to apply it.

Meanwhile, the increasing viral character in the sense of autonomous action shows similarities to information technology's self-replicating software and computer viruses as well as communication via social networks that enables messages to go "viral". The difference to software autonomous agents is that engineered viruses and gene drives leave the artificial environment of technical infrastructures and act in the natural environment.

The hallmarks of the viral age are the increased autonomy of means and a focus on manipulating the genotype instead of the phenotype. Indeed, we just begin to realize the true relevance and power of information as represented by the genotype; it enables dematerialization, a distinctive feature of the information era, and an increased autonomy and invasiveness of its technologies. Instead of producing, shipping and spraying pesticides to control an insect pest at great cost, we can now directly control a pest and/or its host organism by releasing autonomous agents that manipulate its genome. This gives us a much more precise and far-reaching influence on our natural living environment, but these technologies have also limited reversibility compared to information technology, where deletion or reformatting remains as a last resort.

Information is power

Information has another key advantage: it does not wear off. It is flexible and can be easily and quickly adapted. Manipulating the genotype allows infinite possibilities for shaping the natural environment and, at the same time, gives humans an unprecedented power over nature. Whatever organism—virus, prokaryote, animal or plant—it can in principle be manipulated by accessing its software in the same way as any other data-processing artefact. Biology and biotechnology now allow to remotely rewrite the code that determines the shape and function of the living world. And the living world in turn becomes a gigantic system of interacting Turing machines that invites us to control them. In this way, gene drives and HEGAA help to fulfil a central claim of synthetic biology: the ability to shape nature according to our specific needs.

> *"Whatever organism – virus, prokaryote, animal or plant – it can in principle be manipulated by accessing its software in the same way as any other data-processing artefact."*

In the course of this, genetic modifications would leave the laboratory and become self-perpetuating processes in the wild (Simon *et al*, 2018). But modifications in the wild require a systemic ecological understanding of the target population and its relationships with other elements of the ecosystem, to be effective and avoid negative impacts. Moreover, the specific character of genetic information presents some difficulties: The genetic information sent into the wild is not consumed like a substance but may become unusable owing to mutations. Furthermore, it might reach only a limited part of the target population due to naturally occurring genetic variations in the target sequence. Additional problems can arise from unintended directions of horizontal spread in the case of virus-based approaches that are not intended to be inherited or vertically through hybridization with closely related non-target species. Steffen *et al* (2015), in their much-noticed publication on planetary boundaries, introduced novel entities as another control variable for which a safe operating space should be defined, but their focus is mostly on pollution by synthetic chemical substances. Under their term "modified life forms", one could include genetically modified organisms, which do require much more attention since genetic artefacts are both mobile and able to reproduce. Even if the problems caused by chemicals are still in the foreground—due to the large quantities released as well as their persistence and diversity—it is to be expected that genetic artefacts of the viral age will gain in importance.

Command and control

Autonomous genetic artefacts allow control of living things and remote domestication of wild species. But with the increased power, efforts to control these technologies have to increase as well. Systems of unprecedented reach and power require correspondingly potent means of control and containment. Unless genetic artefacts contain a built-in expiration date, control can hardly be achieved without harnessing the potential of living entities to be replicative and mobile. Inherent containment by expiration dates and release of secondary control agents are the main control strategies.

> *"Systems of unprecedented reach and power require correspondingly potent means of control and containment."*

To be effective, secondary control agents must reach a certain number of individuals to prevent the primary agent from spreading further. Relying on natural evolution of resistance to a gene drive would be largely unpredictable, although drive-immune organisms

may, over a few generations, replace individuals with the original first drive if they provide a fitness advantage. Another mean for inherently limiting its spread is designing gene drives with several elements that are dependent on each other but are successively lost over subsequent generations, such as the "Daisy Drive" (Noble *et al*, 2019). Such so-called split-drive systems separate the transgenes necessary for the gene drive in two parts, whereby only one part is inherited as a gene drive and the other is correspondingly lost. This split approach could also prevent uncontrolled spread for virus-based approaches to genome editing. However, the question remains whether accidental recombination of the separated parts can be excluded to an acceptable degree. Furthermore, there is the possibility of recombination events between genetically modified and naturally occurring viruses.

"The key question is whether our understanding of nature is too simple to allow a comprehensive management of ecological and socio-ecological systems."

In synthetic biology, orthogonality—in the sense of lack of interference—between different systems or system components is sought for new creations. Current approaches to controlling gene drives also aim for orthogonality, but at a higher level of organisms, populations and species. To keep information under control, orthogonality and reversibility have to be guaranteed before any releases. As with chemical substances, where persistence and bioaccumulation are reasons for concern, the same should apply to genetic information released into nature.

Complementing the increasingly powerful artefacts of the viral era requires creating and constantly updating safeguards against misuse and failure, such as we have created for computer networks. However, any control is necessarily bound by the limits of our understanding of natural systems. The key question is whether our understanding of nature is too simple to allow a comprehensive management of ecological and socio-ecological systems. The greater the depth of intervention, the greater the need for knowledge to be able to predict and control the consequences of our actions since we do not have the freedom for trial and error like evolution has. Test runs as with pesticides are not possible given the self-propagating nature of the new autonomous techniques and the mobility of organisms and seeds. The release of autonomous genetic agents will therefore largely become a major experiment in our environment.

References

van den Bosch R, Messenger PS, Gutierrez AP (eds.) (1982) The history and development of biological control. In *An Introduction to Biological Control*, pp 21–36. New York, NY: Springer US

Bull JJ, Smithson MW, Nuismer SL (2018) Transmissible viral vaccines. *Trends Microbiol* 26: 6–15

Champer J, Buchman A, Akbari OS (2016) Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nat Rev Genet* 17: 146–159

DARPA (2016) Insect allies. Broad Agency Announcement HR001117S0002. Defense Advanced Research Projects Agency (DARPA), Biological Technologies Office, Arlington, VA. https://sam.gov/opp/c1b79c54a1aa8f5990f7b4a3cc7f6576/view

Ellison EE, Nagalakshmi U, Gamo ME, Huang P, Dinesh-Kumar S, Voytas DF (2020) Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. *Nat Plants* 6: 620–624

Esvelt KM, Gemmell NJ (2017) Conservation demands safe gene drive. *PLoS Biol* 15: e2003850

Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P, Berendonk TU, White-Cooper H, Scaife S, Kim Phuc H *et al* (2010) Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci USA* 107: 4550

Gantz VM, Bier E (2015) Genome editing. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. *Science* 348: 442–444

Leonard SP, Powell JE, Perutka J, Geng P, Heckmann LC, Horak RD, Davies BW, Ellington AD, Barrick JE, Moran NA (2020) Engineered symbionts activate honey bee immunity and limit pathogens. *Science* 367: 573

Ma X, Zhang X, Liu H, Li Z (2020) Highly efficient DNA-free plant genome editing using virally delivered CRISPR–Cas9. *Nature Plants* 6: 773–779

Noble C, Min J, Olejarz J, Buchthal J, Chavez A, Smidler AL, DeBenedictis EA, Church GM, Nowak MA, Esvelt KM (2019) Daisy-chain gene drives for the alteration of local populations. *Proc Natl Acad Sci USA* 116: 8275–8282

Reeves RG, Voeneky S, Caetano-Anollés D, Beck F, Boëte C (2018) Agricultural research, or a new bioweapon system? *Science* 362: 35–37

Simon S, Otto M, Engelhard M (2018) Synthetic gene drive: between continuity and novelty. *EMBO Rep* 19: e45760

Steffen W, Richardson K, Rockstrom J, Cornell SE, Fetzer I, Bennett EM, Biggs R, Carpenter SR, de Vries W, de Wit CA *et al* (2015) Planetary boundaries: guiding human development on a changing planet. *Science* 347: 1259855

Thomas DD, Donnelly CA, Wood RJ, Alphey LS (2000) Insect population control using a dominant, repressible, lethal genetic system. *Science* 287: 2474–2476