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Genetic engineering endangers the protection of species

Why the spread of genetically engineered organisms into natural populations has to be prevented

Christoph Then

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Summary

This report is primarily concerned with the consequences of genetic engineering interventions into evolutionary processes. Leading scientists working in the field of 'new' genetic engineering are already talking about the 'end of the beginning' (George Church, 2012): in future, new life forms will no longer evolve from natural processes of self-reproduction and self-organisation, they will, instead, be designed by scientists. New genetic engineering technologies, such as the CRISPR/Cas gene scissors, are set to play an important role in this development.

Genetic engineering can indeed enable scientists to bypass natural mechanisms of genetic regulation and inheritance. We now have the technical ability to create cells and organisms that are very different to the 'first cell' (Popper, 1995). The advent of these new technologies means we can make interventions into the 'germline of biodiversity' and use designs from the 'gene lab' to influence the biosphere.

Biotechnological mutagens such gene scissors used for the interventions in the genome can create specific patterns of change, generating specific re-combinations of genetic information, even if no additional genes are inserted. These associated new biological traits go along with new risks, in particular, if the organisms are released into the environment and spread throughout natural populations.

'Monarch flies' – an example

'Monarch flies' were developed using CRISPR/Cas gene-scissor technology and are a useful current example with which to illustrate associated risks. Just three small changes in individual base pairs in their genome have effectively made the fruit flies resistant to toxins in specific plants. They now can ingest the toxin and thereby might become poisonous to predators (Karageorgi et al., 2019). This report warns about the possible consequences of releasing masses of new genetic combinations into the environment. While the 'Monarch flies' are not planned for release, there are plans to release many other genetically engineered insects, trees, rodents, corals and microbes. Some of these releases are proposed for the protection of species. This report contains examples of genetically engineered trees, corals and bees.

Based on fundamental evolutionary principles as well as observations regarding species extinction and experience with invasive species, our report comes to an important conclusion: releases of genetically engineered organisms able to propagate and spread throughout natural populations can rapidly damage the stability of ecosystems. These novel organisms can disturb or disrupt the networks of biological diversity in various ways, ultimately accelerating species extinction.

The threat of 'biological dementia'

In this context, it should be pointed out that DNA is the basis of inheritance and also functions as the memory of our shared evolution to present life forms. In fact, it represents around four billion years of shared evolution. The way in which information is stored and continuously changed is subject to multiple biological mechanisms that have evolved to protect existing biodiversity, and whose purpose is to enable coherent further development. The information stored in the genome is not only a mirror to the past, it also points to the present and the future of biodiversity. This 'shared memory' enables the ability of existing living organisms to adapt and interact in ecosystems, within species and between species.

If species are lost, then this 'shared memory' might also be lost. It is not only the extinction of species that can lead to 'biological dementia': interventions into the genome can change the collective information in its structure, consistency and function as well as trigger disruptive processes. Based on the principles of the modern theory of evolution, one could speak of "appearance of great masses of disharmonious gene patterns" which may endanger *"the integrity of the historically evolved arrays of genotypes which are the existing species"* (Dobzhanski, 1951).

New genetic engineering technology poses an enormous challenge

This report shows that new genetic engineering technology poses an enormous challenge in risk assessment:

- In order to assess risks, it is not sufficient to simply look at individual genetic changes in isolation. Instead, new patterns of genetic changes and new combinations of genetic information must be considered in context with the genome. The relevant questions of biology and risk that need to be asked are often much more complex than has so far been the case in genetic engineering.
- To make risk assessment meaningful, it must include the tools used in the process, all the steps in the process and all intended and unintended changes. This is because all the desired and undesired effects and their associated risks will be influenced by the respective technique used in the process.
- With the advent of the new genetic engineering technologies, there are plans to engineer the biological traits of natural populations. Issues arising in regard to interactions with the environment, epigenetics and lack of spatio-temporal control, make it highly questionable whether risk assessment can come to any meaningful and robust results at all. Extensive and long-term release experiments would be needed to research the actual effects: the consequences, however, would, in many cases, already be irreversible resp. uncontrollable.

The regulation of genetically engineered organisms

The following regulatory requirements are crucial in the approach to 'old' and 'new' genetic engineering technologies:

- Starting with the respective method, all organisms with genomes that have been changed using genetic engineering techniques, must be subject to regulation and mandatory authorisation even if no additional genes are inserted.
- The spatio-temporal controllability of genetically engineered organisms must be ensured. This means that each and every release must rigorously adhere to effective control and to the possibility of retrieval.

In addition, from the perspective of nature protection, it is necessary to explore to what extent natural species and biodiversity have a 'right' to the preservation of their own natural integrity and further development. Essentially, biodiversity should no longer be seen as a free resource for genetic engineering experiments.

1. Introduction

New genetic engineering techniques, such as CRISPR/Cas, enable radical interventions into the genomes of plants and animals. The tools used for these applications, known as nucleases (gene scissors), can be considered to be biotechnological mutagens that, unlike physico-chemical mutagens (chemical compounds or radiation), are able to interact in a targeted way with the biological mechanisms in the cell on the level of the genome and/or epigenome.

Very often, the question is raised, to which extent 'genome editing' can be compared to 'random mutagenesis' (physico-chemical mutagens) which has been used in plant breeding for several decades. To differentiate between methods of 'genome editing' on the one hand, and methods used in 'conventional' breeding on the other hand, the following criteria can be applied:

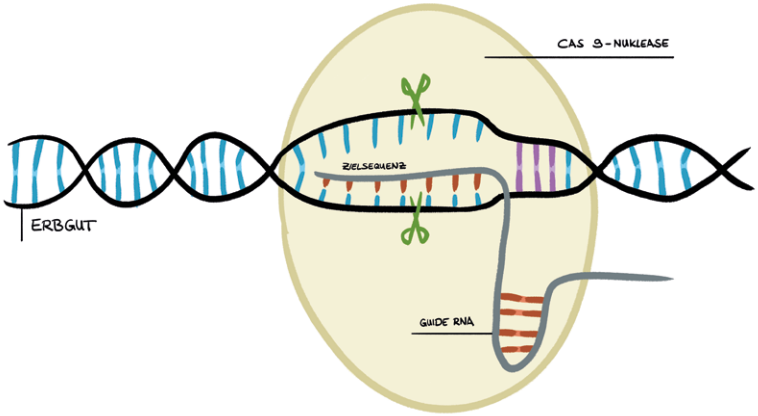
a. In the case of conventional breeding, the first step requires a high degree of genetic diversity that subsequently provides the basis for further crossings and selection. To increase genetic diversity in plant, but not in animal breeding, non-targeted mutagenesis can be applied by using chemical or physical mutagenesis. In this case, the resulting genomic changes are intended as they increase genetic diversity.
b. The situation regarding genome editing and other methods of genetic engineering is very different: (1) Genetic engineering uses biotechnological mutagens (molecules) which are intended to interfere with biological mechanisms on the level of the genome or gene regulation (epigenetic) in a targeted way (for overview see: Testbiotech, 2020). (2) These applications are typically not meant to increase genetic diversity in a non-targeted way. Therefore, unintended changes in the genome have to be seen as undesirable effects; (3) Tools such as CRISPR/Cas make a much larger part of the genome available for genetic changes compared to conventional breeding, and allow the generation of biological characteristics that were previously not achievable.

Whatever the case, there are fundamental differences between methods of genetic engineering and 'conventional' methods of breeding.

There is an increasingly broad range of applications emerging from the new techniques of genetic engineering. Compared to earlier genetic engineering techniques, the new genetic engineering techniques are not only different in their technical features but also in their aims. Target organisms are no longer just confined to domesticated plants and animals or those kept in the laboratory. Instead, we are seeing a growing number of projects targeting wild populations such as insects, rodents and trees that are all embedded in their own complex ecosystems.

For example, in the USA, sweet chestnut trees, apparently genetically engineered to be resistant to fungal infections (Popkin et al., 2018), are being proposed for release. At the same time, there is continuing debate about whether to modify insects and rodents with gene drives, so that whole populations could either be genetically altered or wiped out (Critical Scientists Switzerland, 2019). To help combat the transmission of malaria, mosquitoes could be infected with a transgene fungus that produces an insecticide (Lovett et al., 2019). A further ongoing debate is about whether to use insects to spread genetically engineered viruses in the environment (Reeves et al., 2018). Some of these applications are listed in the current overview issued by the International Union for Conservation of Nature (IUCN, 2019).

Selected examples described by the IUCN are included in this report together with a critical analysis. The report was drawn up for the German League for Nature and Environment (DNR).



What is 'new' genetic engineering?

New genetic engineering is collectively known as 'genome editing' and includes a whole range of different methods: the site-directed nucleases (SDNs) and oligonucleotide-directed mutagenesis (ODM). The site-directed nucleases are widely known as 'gene scissors', the most well-known of which is CRISPR/Cas9, i.e. Clustered Regularly Interspaced Palindromic Repeats/CRISPR associated). CRISPR/Cas9 is a relatively young technology that was originally found in bacteria as a natural self-defense mechanism against invading viruses. Scientists first researched this natural mechanism in bacteria and then adapted it in the laboratory to edit the genomes of plants, animals and human cells.

A requirement for the application of CRISPR/Cas9 is that the genome of the target organism is already decoded by the use of genome sequencing methods. The genomes of many model organisms were already sequenced. The genomes of the remaining species are supposed to be decoded within the next ten years in a large sequencing project (Lewin et al., 2018). Scientists will then easily be able to find gene sequences and use these reference genomes for targeted genome editing.

CRISPR/Cas consists of two key elements: a 'cutting' component and a 'guide' component. The guide component, the 'guide RNA' (gRNA), guides the 'cutting' component, i.e. the 'gene scissors', to a target sequence in the genome of the organism where it 'cuts' the DNA structure.

The cell recognises that the DNA is cut (i.e. damaged) and activates its own repair mechanism to quickly fix the damage. These repair processes are error-prone and can be activated either leading to an erroneous repair or restoring of its original condition. This is collectively known as SDN-1 (Site-directed Nuclease-1). SDN-1 applications typically lead to small deletions, insertions or substitutions which will impair the function of the respective gene(s). Alternatively, other repair mechanisms can be triggered that use a DNA template to introduce a DNA template meant to cause specific change at the DNA target site. This could be either individual DNA letters, i.e. so-called SDN-2 applications, or the insertion of whole genes, i.e. SDN-3 applications.

SDN applications can be used to switch genes on or off in the target genome, as well as to remove or insert other genes. Moreover, genes may be 'read' differently, or their functionality might be changed.

2. Genetic engineering – better than evolution?

With powerful tools such as CRISPR/Cas9, scientists can now add new genetic information, change genomes or silence gene functions to a larger extent than ever before. They have found a way of by-passing the natural mechanisms of inheritance and gene regulation. According to the views of George Church and Jennifer Doudna, both of whom were lead scientists involved in the development of CRISPR/Cas9, the new methods even allow far-reaching intervention into evolution itself. They believe that this is the 'end of the beginning' (Church & Regis, 2012, page 225).

Jennifer Doudna wrote in her book 'A Crack in Creation' (2017):

> *"Gone are the days when life was shaped exclusively by the plodding forces of evolution. We are standing on the cusp of a new area, one in which we will have primary authority over life's makeup and all its vibrant and varied outputs. Indeed, we are already supplanting the deaf, dumb, and blind system that has shaped genetic material on our planet for eons and replacing it with a conscious, intentional system of human-directed evolution."* (Page 243/244)

And George Church wrote (2012):

> *"Synthetic genomics has the potential to recapitulate the course of natural genomic evolution, with the difference that the course of synthetic genomics will be under our own conscious deliberation and control instead of being directed by the blind and opportunistic processes of natural selection."* (Page 13)

The technical possibilities continue to increase in range; they now reach from the synthesis of whole genomes (Gibson et al., 2010; Fredens, 2019) and changes in complex biological traits (Zsogon et al., 2018) to gene drives with which whole natural populations can be genetically engineered (see, for example, Noble et al., 2018; Gantz & Bier, 2015). Proposals are being made to use genetic engineering to bring back extinct species and even resurrect Neanderthals (Church & Regis, 2012).

Is genetic engineering really suitable for shaping life on our planet? Can we really improve evolution through interventions into nature? Doudna and Church are, in fact, right about one thing: evolution follows different biological mechanisms compared to genetic engineering. The natural biodiversity of living organisms has not emerged from conscious design.

If humans intervene in evolution, then this is indeed the 'end of the beginning': until now, all life forms emerge from natural processes and ultimately from a natural origin. Life in its present forms is a continuum from its origins that lie billions of years in the past. Or as the philosopher Karl Popper wrote (Popper, 1987):

> *"The first cell is still living after billions of years, and now even in many trillions of copies. Wherever we look, it is there. It has made a garden of our earth and transformed out atmosphere with green plants. And it created our eyes and opened them to the blue sky and the stars. It is doing well."*

Now, for the first time we have technical tools to create cells that are substantially different to descends of the 'first cell'. We can create organisms that interfere with the further development of current life forms in their self-regulation and self-organisation, and lead to changes, disruption or even the destruction of their ecosystems. There are many reasons to believe that we could have, in fact, ushered in the 'end of the beginning' for natural life. Scientists can intervene in the 'germline' of biodiversity and the future of the biosphere could be designed in a 'gene lab'.

3. Evolution: more complex than genetic engineering wants to acknowledge

Until now, all species owe their development and preservation to processes and mechanisms of evolution. But is, as Doudna has written, this evolutionary system after many millions of years now actually just *deaf, dumb, and blind*?

The genome, the DNA, the chemical basis of inheritance, has stored all our shared memory throughout the evolution of all life. In fact, it represents around four billion years of shared memory and information, from the very beginning of life, from cells and organisms to actual species, all of which has been stored, evaluated and selected. Moreover, the genetic information stored in the genome is not just simply the past result of evolution; it is also its present and its future.

It is this shared 'knowledge' and stored memory that provides the basis for contemporary lifeforms to further adapt and interact in ecosystems, as well as within species and between species. Regardless of whether it is the interactions between bees and flowers, microbes and roots, or reproduction and relationships within food webs, DNA is the memory that directs behaviour and interrelationships.

At the same time, the information stored in DNA is by no means based on completely random events. The way in which information can be stored and changed is subject to multiple biological mechanisms that both protect existing lifeforms and enable a coherent development going forward. Evolution in its entirety is neither predictable nor completely random. These mechanisms have very little in common with the prevalent distorted view of evolution that generally believes it is simply a matter of random mutations and the 'survival of the fittest'.

The boundaries between the species are just one example illustrating the processes and mechanisms of evolution. It is these boundaries which actually enable diversity in all its steadily increasing complexity: indeed, if genetic information could be randomly exchanged, there would be no stable traits in species. Basically, the traits would, for the most part, be extensively and rapidly levelled out; all life would have similar genetic 'information' that would average out around a midpoint, with no direction or ability to find a way to develop further. Evolution would have no way of transcending the level of single cell organisms. It would certainly never be able to develop the complex interdependencies in stable ecosystems and chaos would abound.

Boundaries between species is certainly not the only mechanism that nature has developed to protect the DNA that stores the 'common memory' giving us the basis for all biodiversity. In particular, the mechanisms and processes that determine how chromosomes and cells multiply and divide, as well as make it possible to pass on the 'common memory' to daughter cells and following generations in 'ordered genomic distribution', are important for the preservation of the species (Vogel & Angermann, 1998).

We know a lot about these mechanisms, the processes based upon them and gene regulation. This includes the way in which DNA is stored in the chromosome: the chromatin packed around the DNA has an effect on the emergence of mutations. These occur in dependence on the structure of the DNA and are not simply random (Makova & Hardison, 2015). Further mechanisms and repair processes include those occuring after base-pair mismatching (Belfield et al., 2018) and multiple copies of genes as back-up (Sanchez-Leon et al., 2018; Kanan et al., 2018). In addition, there are other regions in the genome that mutate more often or have higher rates of recombination during meiosis, and change more frequently than other locations. These are so-called 'recombinatorial hotspots', where nature fosters new gene combinations (Choi et al., 2018; Si et al., 2015; Rogozin et al., 2003). One could say that after 4 billion years, evolution has achieved a high degree of 'sophistication' and is by no means *'blind, deaf and dumb'*. Indeed, evolutionary mechanisms prevent genetic chaos and make a balance between species preservation and their continual further development and adaptation possible.

Without doubt, this new scientific evidence substantiates what has been taught in modern theory of evolution for many years. Theodosius Dobzhansky (1900–1975) worked in the USA in the Rockefeller Institute at the University of Colombia. Even today his work remains one of the pillars upon which the modern synthetic

theory of evolution rests, it is an expansion on Darwin's classic theory of evolution (see, for example, Beurton in Jahn & Schmitt, 2001, pages 146 ff). Between 1937 and 1966 there were many editions of his published work "Genetics and the Origins of the Species". In his book, he explains just how important the boundaries between species are for the preservation of biodiversity. He describes how different mechanisms ensure that specific genetic information and sites on the genome change more than others. At the same time, he emphasises the importance of the entirety of the genome for the functions of the individual genes. Dobzhansky shows that evolution can indeed make use of random occurrences that arise, but that species are nevertheless not simply the product of random occurrences:

> *"It is frequently stated, particularly in popular scientific writings that mutations are haphazard, chance, accidental, random etc., changes of the genes. Such a characterisation is misleading when given without qualification. For the only respect in which mutations are haphazard is that they arise regardless of the needs of the organism at a given time, and hence are far more likely to be deleterious than useful. But the kinds of mutations that a gene is capable of producing as well as the frequencies with which it produces them are far from intermediate. They are controlled by the structure of the gene itself as well as the genetic constitution of the organism."*
> (3rd edition, 1951, page 58)

According to Dobzhansky, even seemingly random processes are by no means only trial and error:

> *"Such trial and error mechanism is provided primarily by mutation and sexual reproduction, which are able to generate a practically limitless variety of genotypes. But this does not mean that the modern theory of evolution is based on a belief in „chance", as it is often but groundlessly alleged. „Chance" enters only to the extent that any mutation has a finite probability of happening, and consequently mutations occur regardless of whether they will be immediately, over ever, useful. But the evolutionary changes in Mendelian populations are far from automatic results of lucky throws of the genetical dice, or even of the demands of the environments. The relation between the genetic system and the external milieu are so complex that the evolutionary process can be described as a creative one. Indeed, this process gives rise to previously nonexistent coherent entities, new organisms fit to perpetuate themselves in certain habitats."* (3rd edition, 1951, page 278)

And because his book is about 'the origin of the species', he emphasises just how important the boundaries between species are for the preservation of existing species and biodiversity arising from evolution:

> *"(...) reproductive isolation prevents the appearance of great masses of disharmonious gene patterns, and thus preserves the integrity of the historically evolved arrays of genotypes which are the existing species."*
> (3rd edition, 1951, page 297)

Dobzhansky not only describes the origin of the species, he also describes how the balance between stability and change is secured through the biological mechanisms of evolution. Doudna and Church, on the other hand, represent the perspective of 'scientific engineering'. Genetic engineering embodies an approach that believes life can be reduced to a series of 'building blocks' available for a diverse number of interventions. There is no room in this approach for recognition that the structure and function of the genome are orchestrated by highly complex and specific structures as well as by multiple biological processes and mechanisms.

Biodiversity is not deliberately 'designed', it is also not based on a lottery of genes or something that arises completely randomly. Conventional breeding does not mean that the genome is just 'whirled around', as it was described by Emmanuelle Charpentier, one of the inventors of the CRISPR/Cas gene scissors, to a German Newspaper, the *Süddeutsche Zeitung*.

What does this mean for the assessment of risks associated with genetically engineered organisms?

4. Extinction of species, stability of ecosystems and genetic engineering

Once a species is lost, its extinction means that other species can fill the gap. Some evolutionary developments repeat themselves, or come to similar outcomes through different means. The loss of existing species and the appearance of new species is in itself a natural process and an outcome of adaptation. However, once a certain range is exceeded, the loss of species can cause whole ecosystems to collapse. For example, Elizabeth Kolbert in her book "The Sixth Extinction: An Unnatural History" (2014), or Dave Goulson in his book "A Buzz in the Meadow" (2018) warn of an ongoing man-made mass extinction of species.

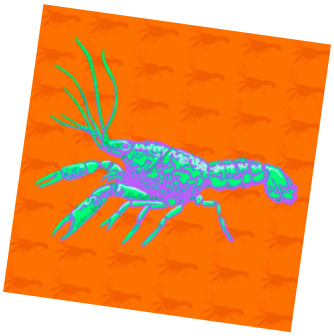
Each and every existing species can be seen as having a unique 'memory' going back millions or even billions of years through evolution. If the extinction of the species reaches a critical mass, then the whole network, the common thread that defines their ecosystem, can be severely disrupted or even destroyed. Or as Goulson says, 'the complex tapestry of life' will disappear. If we understand DNA as being the stored common memory and shared knowledge from millions or even billions of years of evolution, then in an analogy, we could talk of the risk of 'biological dementia': losing 'important' species means that the function of existing ecosystems is threatened, and with them their future development.

It is not only the extinction of species that can lead to 'biological dementia': interventions in the genome can threaten the internally stored information in its structure, consistency and function, as well as trigger disruptive processes. In keeping with our analogy of 'biological dementia', there is a risk that an uncontrolled spread of genetically engineered organisms into natural populations could lead to their 'shared memory' of evolution becoming 'confused'. Dobzhansky describes this as *"appearance of great masses of disharmonious gene patterns"* that are not in tune with those arising from evolutionary processes that represent *"the integrity of the historically evolved arrays of genotypes which are the existing species"* (Dobzhansky, see above).

When organisms that are changed in their genetic makeup, with novel biologically active molecules or changed genetic patterns, spread in natural populations, they can act as 'interfering transmitters' in ecosystems. They can change or disrupt both food webs and communication networks. There are a multitude of possible effects: the ecosystem could collapse and it could lead to an acceleration of species becoming extinct or a shift in ecological balance and evolutionary development pathways. At the same time, all of these factors can be mutually disruptive in their intensity and acceleration.

Invasive species are a good example with which to illustrate how ecosystems are affected by non-adapted organisms (neobiota), for which, in many cases, human intervention is responsible. At the same time, a clear distinction has to be made between genetically engineered organisms and the spread of neophytes or neozoa: these spread through environments to which they are not adapted. Genetically engineered organisms, on the other hand, are species which in their original form were adapted to their environment, but their gene pool has been changed by either adding genes or altering their genetic information by biological mutagens. The dynamic and consequences of the spread of new genetic information within an established species can be very different to the spread of new species within existing ecosystems.

4.1 Example 1 – Marbled crayfish: uncontrolled spread without genetic engineering



Marbled crayfish or Marmorkrebs (*procambarus virginalis*) are a good example with which to illustrate and discuss the commonalities and differences between the risks associated with neobiota and genetically engineered organisms. The 'original organism' was the slough crayfish (*procambarus fallax*) which is found in ecosystems in Florida. It took just one biological event, known as a 'macro-mutation' (Lyco, 2017), to change the slough crayfish into a new species, i.e. the marbled crayfish. This new species is substantially different in its outward appearance, behaviour and genetic makeup to the original species (Lyco, 2017). The most noticeable change in contrast to the slough crayfish is an increased invasiveness – they can spread much more rapidly to new habitats and displace native species.

Marbled crayfish probably developed randomly by coincidence and through human influence. Crayfish bred in an aquarium played a central role. It is not exactly clear where the marbled crayfish originated. They first appeared in 1995 in a German aquarium (Lyco, 2017) but were not recognised as a new species. From there, the crayfish spread into the environment via the pet trade as well as via sewage, fish bait and other human activities. The way in which marbled crayfish reproduce is one of their specific characteristics: all individuals are female and can reproduce without mating. This is known as parthenogenesis. It enables large populations to be established within a very short time, since the crayfish can lay large numbers of eggs. Therefore, any aquarium with just one crayfish is rapidly threatened by over-population. The crayfish are not particular about their nutrition and can eat both plants and animals, they quickly become a threat to the biocoenosis in the aquarium. To avoid killing the crayfish, they were either given away or disposed of in the environment. With dire consequences.

Within only twenty years, the marbled crayfish rapidly outgrew the confines of aquariums and are now even found in Madagascar, where they are a threat to native crabs and their ecosystems (Gutekunst et al., 2018). Other countries where they can be found include Germany, Hungary, Croatia and Ukraine (Lyko, 2017). In the meantime, the invasive spread of the marbled crayfish has reached such an extent the commercial value of the animals is being considered and discussed (Adriantsoa et al., 2019).

Marbled crayfish are most closely related to slough crayfish, but they differ genetically in that they carry three copies of each chromosome. Whether these multiple sets of chromosomes are a prerequisite for parthenogenesis, is still being debated (Martin, 2015). Potentially, it could be other effects of an aberrant cell division after mating that led to a change in biological traits and, in particular, gene regulation (Gatzmann et al., 2018; Vogt et al., 2008). Compared to the original species, the slough crayfish, many of the marbled crayfish genes have lost some or all of their epigenetic markers (methyl groups) with which gene activity can be regulated and restricted. At the same time, there is stronger variation in the expression of these genes (Gatzmann et al., 2018). All the offspring of the marbled crayfish originate from a single clone, and the animals themselves only show slight genetic variations. Consequently, there is ongoing debate about whether the epigenetic changes are the reason for their ability to adapt (Gatzmann et al., 2018).

Their very aggressive behaviour enabled marbled crayfish to become super-invasive and replace other crab species. At the same time, this distinctive behaviour appears to give them an advantage: in contrast to other crab species, if they come into contact with natural enemies, they do not show fight-or-flight behaviour, instead, they first of all just 'freeze' their movement (Linzmaier et al., 2018). This means that they do not display normal behavioural patterns and therefore may have an advantage by surprise. One other possible advantage for

the marbled crayfish is that they are either unaware of or partly ignore the warning signals which other native crabs secrete in their urine. Whatever the case may be, they do not react with typical behavioural patterns (Linzmaier et al., 2018). Compared to the non-invasive original species, i.e. slough crayfish, their shell and pincers are also much bigger and they produce a lot more offspring (Lyko, 2017).

As an example, marbled crayfish demonstrate interesting aspects in connection with the risks of an uncontrolled spread of genetically engineered organisms: (i) Changed traits in marbled crayfish are due to changes in their genome. Knowing about this macro-mutation does not mean that all the new traits were predictable. Their adaptability is not simply due to a change in genetic information; an increased variability in gene activity also plays a part; (ii) In order to accurately assess their invasive potential in the environment, it is important to assess their actual behaviour in the environment as well as their specific phenotypic traits. The traits relevant for the risk assessment of marbled crayfish cannot be seen solely in their changed (epi-) genetics, but must also include their interactions with the environment.

Marbled crayfish are an extremely rare example of the emergence of a new species which, reinforced by human activity, has rapidly spread in the environment. In this case, the spread of the marbled crayfish was much faster than the ecosystem was able to adapt. Normally, evolutionary processes are much slower. Single mutations that happen in a few individuals of a population are often lost again. Even if these traits do become established, it takes a very long time until the new traits have reached the whole population and have substantial effects on the ecosystems.

4.2 Example 2 – 'Monarch Flies'

Genetic engineering enables the release of large numbers of organisms into the environment that are not adapted to ecosystems. In contrast to marbled crayfish, such releases are not rare accidental occurrences. In fact, such releases could happen with a diverse range of organisms in rapid succession and in huge numbers. Instead of a single occurrence, as is the case with marbled crayfish, there is a danger of multiple, repeated and sustained overload of natural ecosystems – with unpredictable consequences for the environment.

'Monarch flies' are an interesting example with which to illustrate such risks: CRISPR/Cas was used to make three changes in a single gene in fruit flies (*Drosophila melanogaster)* (Karageorgi et al., 2019). The so-called ATPalpha gene that is involved in many biological processes, was 'edited' to mimic the corresponding gene in Monarch butterflies. This was accomplished with a so-called SDN-2 application in which no additional genes are inserted into the genome, but where three specific sites in the genome are reconstructed to create new biological traits (see 'What is new genetic engineering?).

The biological effect is complex: even without any additional genes being inserted, the changes as mediated by gene-editing confer a higher fitness and an evolutionary advantage in the fruit flies. Like Monarch butterflies (and some other insects), they are now immune to cardiac glycosides produced by various plants, and therefore both the fruit flies and their larvae have a wider range of food they can ingest. Moreover, the fruit flies, resp. their larvae, might protect themselves against predators by ingestion and storage of the toxins.

Ultimately, only three small changes of the DNA were needed (in total less than 10 base pairs) that, however, have to be present in a certain combination to achieve the desired resistance to the toxin: it was shown that some of the gene variations led to weakness in the flies after a stress test. This is because the specific gene is

involved in several biological processes, it displays a so-called pleiotropic effect. It was only after the combination of genetic changes were 'optimised' that the flies showed a combination of normal vitality and resistance to the toxins in the plants, as well as inedibility for predators.

The Central Commission on Biological Safety (ZKBS, 2012) and the EU Joint Research Centre (Lusser et al., 2011), – at least with plants – claim that organisms with changed DNA sequences, but comprising less than 20 nucleotides in total, are not subject to genetic engineering regulation. As an example, 'Monarch flies' show that it is not solely about the number of genetic changes or their range, but rather a matter of specific patterns of genetic change and the resulting combination of genetic information.

Even if these respective combinations did actually appear spontaneously in fruit flies, it is by no means certain that these traits would spread through a population. To this end, it would have to be very clear that single individuals could create large, stable populations. Additionally, if the new gene combinations did become established, it would take long periods of time during which ecosystems could adapt.

Conversely, ecosystems can be overwhelmed if masses of fruit flies with changed genetic traits are released into the environment, as could be the case with releases of genetically engineered organisms. The actual effects could only be determined (probably irreversible) after a release. Likewise, the actual interactions of released genetically engineered organisms with the environment could not be reliably predicted.

Fruit flies and their larvae are predominantly beneficial to other insects or amphibians as food. There are other insects that have a trait conferring resistance to cardiac glycosides, but in these cases the ecosystems have had sufficient time to adapt. Apart from Monarch butterfly caterpillars, there are, for example, some other species resistant to toxic cardiac glycosides that are brightly coloured (*Oncopeltus fasciatus B; Aphis nerii; Myzus persicae*).

In general, there is a risk that releases of genetically engineered organisms and their spread in natural populations will rapidly overwhelm the adaptability of ecosystems. Genetic engineering applications can – in addition to man-made effects such as climate change – contribute to a destabilisation of ecosystems or intensify specific effects.

In the meantime, a relatively large number of plants and animals with the potential to spread in natural populations have been developed. There are plans to release several of these organisms and some have already been released. In the same way as the fruit flies, even without additionally inserted genes and despite very few genetic changes, the organisms have new biological and complex effects. These include, amongst others, changes in: (i) the composition of their components; (ii) metabolic processes and (iii) resistance to pests, from which diverse changed interactions with the environment can emerge.

Taking a look, for example, at approvals issued for genetically engineered plants in the USA, reveals (Testbiotech, 2019) that many crop plants have been approved with no controls whatsoever for release and cultivation; these plants have frequently been modified with 'old' and 'new' genetic engineering technology. Many of them have traits that would not have been achieved either with conventional breeding or through evolutionary processes. Some species, such as camelina, can persist, propagate and spread in the environment. Eventual long-term impacts on ecosystems were not investigated in any of the approval processes. Releases intended to extensively change natural populations of insects, trees and other wild plants and animals could follow in the near future.

In case of the marbled crayfish it is no longer possible to remove the new species from the environment. One can only hope that they disappear on their own. There is, however, no sign of this happening. On the other hand, it is not too late in cases of genetic engineering. We still have an opportunity to decide against genetically engineering of natural populations and being 'cleverer than evolution'. Whereas it is unclear just how much human intervention was involved in the emergence of marbled crayfish, there is no doubt at all who would be responsible for disastrous consequences resulting from genetic engineering applications.

4.3 Example 3 – Mammoths

One possible application of genetic engineering could be to bring back long extinct species (IUCN, 2019; Church & Regis, 2012). Church and his co-author are trying to resurrect both woolly mammoths and Neanderthals:

> *"A later technique under development in my Harvard lab will allow us to resurrect practically any extinct animal whose genome is known or can be reconstructed from fossil remains, up to and including the woolly mammoth, the passenger pigeon, and even Neanderthal man. (…) the genome sequence of both the wooly mammoth and Neanderthal man have been substantially reconstructed; the genetic information that defines those animals exist, is known, and is stored in computer databases. The problem is to convert that information – those abstract sequence of letters – into actual strings of nucleotides that constitute the genes and the genomes of the animals in question. This could be done by MAGE technology – multiplex automated genome engineering."* (Page 11)

Church & Regis (2012) cite an application known as multiplex automated genome engineering (MAGE, see, e.g. Carr et al., 2012). The purpose of this application is not to transfer large gene sequences, but rather the 're-programming' an existing genome step-by-step (gene editing). In this case, the genes of the woolly mammoth can be edited into the Asian elephant genome. The individual base pairs in the DNA are exchanged gradually until the genetic information is modified to such an extent that the original elephant genome resembles that of a mammoth. Each step appears to be straightforward and simple. However, in total, all these numerous steps amount to create a completely new organism (mammoth) that in comparison to the original species (elephant) has very different biological traits and behaviour, and whose interactions with the environment are equally and fundamentally different.

This example illustrates just how powerful these new genetic engineering tools are and how different the patterns of genetic modification can be, even if seemingly only small changes are gradually and repeatedly made: although it might not be possible to bring back extinct species, it is still possible to modify large sections of the genome.

The example with the mammoths highlights a further aspect: even if the utopian scenario envisaged by George Church was actually to become possible on the DNA level, it would achieve very little: the modified genome would also need to be aligned in its epigenetics and gene regulation with that of a mammoth. Only then would it be possible to create animals capable of surviving, or that even looked and possibly behaved like the original mammoth species. However, their epigenetics were adapted to their environment at that time. The question of which epigenetics would be required in the present cannot be answered.

Errors in gene regulation affect, amongst others, embryonic development and animal health. The grave con sequences of flawed epigenetics can be regularly seen in experiments to clone cattle, sheep and pigs: most of these animals, which are not genetically engineered, die at birth or suffer from serious illnesses due to flawed gene regulation having a substantial effect on embryonic development (EGE, 2008). Therefore, it must be assumed that the same problems would occur with mammoths, since they would probably have to develop in the uteruses of elephants.

The importance of epigenetics is not only evident in the proposed reprogramming of existing genomes; it generally has an important role to play in the risk assessment of genetically engineered organisms. The procedure used to modify genes can also lead to changes in gene regulation, which, in different environmental conditions, may cause new traits to emerge that were not observed in the laboratory or field trials (see Table 1). Therefore, these genetically engineered organisms, which can persist and propagate in the environment, need to be observed and assessed over several generations under various environmental conditions before risk assessment can be concluded. However, the inherent problem in this scenario is that many of these organisms can simply no longer be retrieved.

New genetic engineering technology can create new genetic combinations

Until now, small changes in DNA have most commonly been achieved using SDN-1 applications. These single, small changes are frequently presented as safe and precise in a restricted way. In fact, CRISPR/Cas enables a wide range of possible changes in the genome far beyond just single changes, so-called point mutations. Ultimately, these changes can lead to the re-programming of an organism:

- Different genes can be changed simultaneously in an organism. The aim in doing this is to change complex metabolic processes in order to, for example, create plants that are better adapted to stress conditions. Alteration of multiple different DNA sequences in an organism simultaneously or successively, is known as multiplexing. For example, eight different genes were targeted in rice leading to an increase in yields as well as a change in fragrance and appearance (Shen et al., 2017).
- Plants can have complex genomes. Some plants carry several sets of chromosomes and/or have a large genome with repetitive DNA sequences. This means that they often have multiple copies of genes, and it is unlikely to knock-out and/or change all the copies of a gene using conventional methods. Genome editing, however, can be used to change all copies of a gene (alleles of a gene) at once. For example, in sugar cane the TALEN gene scissor was used to change 107 of 109 gene copies of the COMT gene that led to improved qualities for the production of biofuels (Kannan et al., 2018).
- CRISPR/Cas9 gene scissors can be used to separate linked genes which would naturally be inherited together in following generations. In barley and tomatoes large parts of their genomes are genetically linked and are inherited together. In tomatoes, the gene responsible for the development of the separation zone between ripe tomatoes and the stalk is, for example, inherited together with a gene that is responsible for the shape of the fruit. If the gene responsible for the separation zone is changed, then the tomato plants produce deformed tomatoes. Both of these traits can now be separated using CRISPR/Cas9 and are inherited separately (Roldan et al., 2017).
- Naturally occurring mutations happen spontaneously and are untargeted. They can happen during DNA replication or be caused by external factors, such as sunlight or chemical substances. The immediate result can be a mismatch of individual letters in the DNA that would trigger the DNA mismatch repair mechanism. If the repair mechanism is disabled, this will trigger a cluster of mutations at specific gene locations (Belfield et al., 2018). These locations can be changed using CRISPR/Cas9 because there are indications that CRISPR/Cas9 applications result in a change at the target sequence rather than its repair (Brinkman et al., 2018).

CRISPR/Cas9 can be used to create new genetic combinations that have so far largely not been possible using conventional methods (Kawall, 2019). Altogether, it is possible that this will result in organisms with specific genetic combinations that would most likely never have been generated with natural or induced mutations.

Unintended side effects of CRISPR/Cas9

CRISPR/Cas9 applications can have various side effects that need to be considered:

- The recognition component of the CRISPR/Cas9 gene scissors can sometimes target DNA sequences that are very similar to the actual target sequence, and thereby cause unintended modifications at those genomic regions. The occurrence of such off-target effects depends on many different factors, each of which must be evaluated individually. Whole genomic sequencing can be used to sequence the altered genomes and to search for any off-target effects without any bias. However, as yet, most publications use biased techniques to analyse off-target sites only at predefined genomic regions (Modrzejewski et al., 2019).
- Besides off-target effects, fragments of DNA can unintentionally become incorporated within or near the target sequence, which was shown to occur both in plants (Liang et al., 2017) and animals (Norris et al., 2019; Young et al., 2019).
- Additionally, unintentional modifications at the target site can lead to an altered reading frame of the respective gene. In consequence, a shortened protein might be expressed, and/ or the protein is altered in its functions. It has been shown that, in some cases, whole sections of a gene are not translated into the respective protein, an effect known as exon skipping (Sharpe et al., 2017; Tuladhar et al., 2019; Kapahnke et al., 2016; Mou et al., 2017).
- DNA can be extensively rearranged around the target region. This has already been shown in human and animal cells (Kosicki et al., 2018). According to Hahn and Nekrasov (2019), such effects can (theoretically) also occur in plants, but the methodologies to identify these effects are hardly ever used. Thus, such on-target effects might often be overlooked in plants.
- In most cases, new genetic engineering applications in plants also involve the application of older genetic engineering methods, such as agrobacterium-mediated transformation or a so-called gene gun. The risks associated with the use of these older methods include unintended changes of the genome (Forsbach et al., 2003; Jupe et al., 2019; Kim et al., 2003; Latham et al., 2006; Makarevitch et al., 2003; Rang et al., 2005; Windels et al., 2003).
- In contrast to older methods of genetic engineering, which are often used to insert genes conferring herbicide resistance or the production of insecticides, the new methods are designed to change, for example, metabolic pathways in the plants. In most cases, unintended effects of the intended changes of genome-edited plants are not investigated. Biomolecules, such as proteins, RNA and DNA, are strongly interconnected. If a gene involved in metabolism is silenced or changed, this can have consequences for many other pathways. Therefore, even the smallest changes in the genome carry the risk of unintended changes in plants.

5. The IUCN Report

The International Union for Conservation of Nature published a report in 2019, in which various 'old' and 'new' methods of genetic engineering technology for the conservation of biodiversity were presented and discussed (IUCN, 2019). These were collectively described as 'synthetic biology'. The report covered a broad range of topics, from applications of genetic technology in the laboratory to applications used in agriculture and natural populations, e.g. the genetic engineering of trees, insects, corals, amphibians and mammals.

The report supports genetic engineering technology in natural populations rather more than it calls it into question. This is possibly due to the fact that many of the scientists who compiled the report have an interest in developing and applying the technology.

Whatever the case may be, the details show that, in many cases, not enough importance has been placed upon species protection and the precautionary principle. In the following section, we have included three examples from the report. In choosing these examples, we have deliberately chosen not to include gene drives, since these are already being widely discussed (for overview see CSS, 2019; Giese et al., 2019; v. Gleich & Schroeder, 2020; Dolezel et al., 2020). Instead, we have chosen examples which have so far been given very little public attention. Our conclusions are nevertheless relevant for the discussion around gene drives.

5.1 Sweet chestnuts in the USA

The IUCN report (2019) focusses attention, in particular, on sweet chestnut trees apparently modified to be resistant to specific fungal diseases. As explained in the report (page 87 of the printed version):

> *"Researchers at the College of Environmental Science and Forestry in Syracuse, New York, have produced American chestnut trees that show promise to tolerate blight infections (Zhang et al., 2013). This was achieved by inserting a single gene from wheat into a new line of American chestnut trees."*
> *(Zhang et al., 2013)*

After release, the trees are meant to spread through natural populations:

> *"Outcrossing lab-produced transgenic trees with surviving wild American chestnuts has the potential to incorporate the necessary genetic diversity and regional adaptations in future generations of American chestnuts, while also protecting them from chestnut blight (…)."*

The trees are described as being safe for the environment:

> *"Transgenic chestnuts have been tested for safety to many other organisms, including ectomycorrhizal fungi (symbiotic fungi associated with roots that aid in water and nutrient uptake), tadpoles which consume leaf litter, and native seeds, and tests to date have shown no adverse effects compared to traditional breeding (…)."*

The fungal disease (chestnut blight, *Cryphonectria parasitica*) was originally unintentionally imported from Asia. The pathogenic fungus produces a toxin that has led to drastic losses in the natural populations of the trees. In 2018, in the affected regions, all that was left were mostly smaller trees and shoots growing out of the roots of the dead trees (NAS, 2019).

The transgenic trees were developed some years ago but never released into the environment. In the latest version of the transgenic trees, the effect of the gene, which originally came from wheat, has considerably been enhanced (Zhang et al., 2013). These most recent variants of the tree are all clones of a single predecessor (see, Popkin, 2018).

Currently, in the USA, there are continuing discussions about whether these genetically engineered trees can be released for planting without any further restrictions (Smolker & Petermann, 2019). At the same time, there is an ongoing project using conventional breeding methods to cross the American tree species with Asian sweet chestnut trees, which have a natural resistance to the pathogenic fungus (Steiner et al., 2016). This project appears to be quite promising, but very lengthy.

There are significant weaknesses in the IUCN report in regard to the risks associated with genetically engineered trees: the trees can live for over 200 years and therefore live through different stages of growth, bloom, seed formation and aging. During this time, they will be exposed to multiple changes in their environment, such as climate change (see, e.g. Steiner et al., 2016).

There are, in addition, further pathogenic fungi, e.g. *Phytophthora cinnamomi*, which are also known to infest the trees. It is mostly unknown how the genetically engineered trees will react to these pathogens and other stress factors (NAS, 2019). In contrast, the conventionally bred trees appear to be resistant to both these fungal diseases (Steiner et al., 2016).

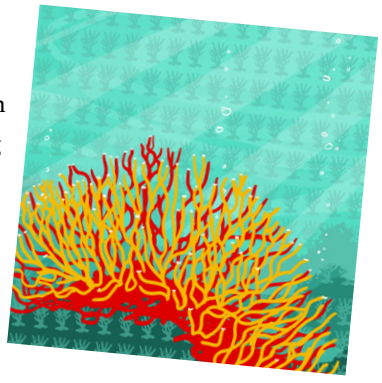
It is not improbable that the trees, or the offspring thereof, will develop traits in response to stress factors which were not observed in the original first generation of the modified trees. Although not mentioned in the IUCN report, this would cause considerable uncertainty in risk assessment.

The IUCN report also fails to address the possible effects that may emerge from crossings with other trees: when the genetically engineered clone trees cross with trees in natural populations, the biological traits of the hybrid offspring can be significantly different to those observed in trees grown in the laboratory, greenhouse or field trials. One reason could be the genetic variations in the genome of the wild trees, which are considerably more heterogenous than in the genome of the genetic clones.

From this it can be concluded, that it is not possible to reliably assess the risks of genetically engineered trees purely on the basis of observational research carried out over a number of years (see also Bauer-Panskus et al., 2020). The investigations of interactions with the environment mentioned in the IUCN report are based on a very few chosen species, which do not represent the overall complexity and diversity of the ecosystems.

When pollen from the trees is distributed by wind, or seeds are spread due to human or animal activity, the trees and their offspring can spread uncontrolled in the woods and forests. Their modified genes can then spread into any remaining wild populations. If any damage was to be subsequently observed in ecosystems, it could be much too late to remove the trees from the environment. Planting the trees knowing that they have the potential to spread in the environment, is a violation of the precautionary principle. Clearly, the IUCN report should have addressed this issue.

5.2 Genetic engineered corals



The authors of the IUCN report included corals, as well as a discussion on whether their associated symbiotic microorganisms might be modified using CRISPR/Cas9 to strengthen their ability to adapt to climate change and higher temperatures. Corals are complex organisms, and also holobionts, that are dependent on a symbiotic relationship with algae and other microorganisms, e.g. bacteria and archaebacteria, that produce substances essential for their life and survival (Rosenberg & Rosenberg-Ziller, 2016). It is believed that in coral

bleaching due to climate change, disruption of the symbiosis between the multicellular body of the corals (the polyps) and their endosymbiotic microalgae (*Symbiodiniaceae spp*) plays an important role. Initial studies are already developing ideas on how new genetic engineering methods can be applied to protect microalgae (*Symbiodiniaceae spp*) from the effects of heat-induced damage (Levin et al., 2017).

Rosenberg & Rosenber-Ziller (2016) describe how the symbioses contribute to genetic diversity of corals as well as their evolutionary adaptation. They refer to research showing that the composition of the associated microorganisms can change spontaneously in altered environmental conditions, and therefore enable the adaptation of the corals in water that is warmer by one or two degrees (see, for example, Oliver & Palumbi, 2010). Other research has reported a surprising natural adaptability of corals (Kersting & Linares, 2019; Kenkel & Matz, 2016).

The various mechanisms with which specific coral systems can adapt to climate change are still not clearly understood. For example, it is not predictable how interactions between the corals and their symbionts could be influenced through genetic engineering. There should therefore have been much more emphasis in the report on the potential effects of the interactions between the corals and their microbiome, including a high degree of uncertainty in risk assessment.

In addition, there is the problem that genetically engineered holobionts cannot be removed from the coral colonies after release. The IUCN report only makes a very general mention of this problem:

> *"Where synthetic biology is used to alter the fundamental niche of a species (the entire set of conditions under which it can survive and reproduce itself), that it could potentially alter the ecological and evolutionary trajectories for that species (with potentially deleterious long-term consequences; e.g. a climate change adaptation is engineered, and climate change is eventually reversed) should also be considered."* (see page 92 of the printed version)

Indeed, it would be essential to expand these issues taking these concerns systematically into account in the respective case studies. Otherwise, there is a danger of creating the impression that such interventions into complex systems could happen in the near future, and could also be controlled.

5.3 Genetically engineered honeybees

The IUCN report includes a discussion on whether new genetic engineering techniques should be used to increase the resilience of honeybees to environmental influences. This would mean using genetic engineering to change the composition of their gut bacteria. Interestingly, the report comes to the conclusion that the suggested intervention could weaken the immune system of the bees, and advises against this approach.

However, it should not be overlooked that CRISPR/Cas9 applications and new genetic engineering techniques are already being used to genetically change honeybees themselves. The aim here is to create pesticide-resistant bee colonies. Experiments carried out in South Korea (Lee, 2019), aimed to make honeybees resistant to insecticides. Other publications propose similar projects (McAfee et al., 2019).

Given the extremely complex biology of honeybee colonies and their multilayered interactions with the environment, these approaches are clearly contrary to the aims of species protection and preservation of biodiversity. This problem was not sufficiently elucidated in the report.

6. Problems in the approach to new genetic engineering technology

All genetic engineering methods, whether 'old' or 'new', are associated with specific risks (for more details see: Testbiotech, 2020). At the same time the processes often involve several steps. For example: (i) with plants, 'old' and 'new' methods are often combined; (ii) with livestock, often involves cloning; (iii) in many cases, several genetic changes are combined through crossing; (iv) the respective methods are often repeatedly applied (see, for example, Eckerstorfer et al., 2019).

Ultimately, it is not sufficient to examine the 'end product' resp. the resulting organism and its desired traits. In order to ask the relevant questions in risk assessment, it is necessary to include each step of the process in every individual case. Therefore, it is necessary that, according to EU GMO legislation (Directive 2001/18), all genetically modified organisms must undergo risk assessment. According to EU legislation, all methods involving the insertion of material prepared outside the cells and then inserted to genetically engineer traits, are subject to mandatory regulation.

This must include interactions with ecosystems and associated microorganisms as well as responses to stress factors, including possible effects that might occur in following generations. This applies, in particular, to genetically engineered organisms that can persist and even propagate in the environment. The aim of risk assessment must be to facilitate an assessment of genetically engineered organisms that is as realistic as possible under changing environmental conditions over several generations.

Based on existing knowledge (see Table 1), we can conclude that the traits of genetically engineered organisms (plants) investigated in the laboratory or under controlled conditions, are not sufficient to predict all relevant traits that might occur in offspring and in interaction with the environment (Bauer-Panskus et al., 2020).

Table 1: Existing knowledge of genetically engineered organisms with particular relevance for risk assessment of genetically engineered organisms that can persist and propagate in the environment

Issue	Findings
Genetic stability in following generations	The offspring of genetically engineered organisms can show traits that did not occur in the original generations (Kawata et al., 2009; Cao et al., 2009; Yang et al., 2017)
Interactions with the genetic background	Unexpected effects can emerge from interactions with the genetic background (Bollinedi et al., 2017; Lu and Yang, 2009; Vacher et al., 2004; Adamczyk & Meredith, 2004; Adamczyk et al., 2009).
Interactions with the environment that effect the genome	Unexpected effects can emerge from interactions with the environment (Zeller et al., 2010; Matthews et al., 2005; Meyer et al., 1992; Trtikova et al., 2015; Then & Lorch, 2008; Zhu et al., 2018; Fang et al., 2018).

For many of the examples included in the IUCN report, an investigation of their traits and effects would only be possible after release. This would, of course, cause considerable problems in the case of corals, insects, microorganisms and also trees, since releases could not be clearly defined in terms of duration and possible spread.

This highlights a fundamental problem: if the spatio-temporal dimension is not sufficiently defined, risk assessment would have to take evolutionary dimensions into account. However, evolutionary processes, due to their lengthy duration, can often turn events which only have the slightest probability of ever happening into events that actually happen (Breckling, 2013). This can make reliable, robust risk assessment impossible (see also Then et al., 2020). Without sufficient spatio-temporal control, every release of genetically engineered organisms is therefore akin to playing Russian roulette.

7. Conclusions and recommendations

The biosphere we all inhabit is based on a multidimensional network of interactions. Genetic information is not the only decisive element for the physiological characteristics of organisms, it is also decisive for signalling pathways, behaviour, instincts, their symbioses and the emergence of complex structures within populations, e.g. superorganisms building communities.

There is absolutely no plausibility in the assumption that human intelligence can, with the help of genetic engineering, intervene in the complex foundations of life in a safe and predictable way.

On the contrary: similarly to the way in which pathogens can threaten the existence of many species via dispersal and spread due to human activity, genetically engineered organisms that are released into natural populations can endanger the health of humans, animals and plants as well as biodiversity.

What is more, a further consideration that the traits of genetically engineered organisms as they appear in the laboratory or in controlled experimental conditions, can be considerably different in changed environmental conditions or after several generations.

Against this backdrop, it would be completely irresponsible to release genetically engineered organisms that can spread and propagate in the environment without any possibility of effective spatio-temporal control.

Irrespective of whether old or new genetic engineering methods are applied, or whether genetic information is added, changed or deleted, these organisms are not based on 'experience of the evolutionary process. Uncontrolled spread threatens – to cite Dobzhansky (see above) – to cause *"appearance of great masses of disharmonious gene patterns"* which may endanger *"the integrity of the historically evolved arrays of genotypes which are the existing species"*.

In general, it can be said that problems in protecting biodiversity cannot be solved by replacing endangered species with genetically engineered organisms. Faced with a high number of species that are endangered due to climate change and human influences, it is obvious that measures designed to curb environmental damage need to be given the very highest priority. We cannot restore natural biodiversity by recreating mammoths or making pesticide-resistant honeybees. On the contrary, we need effective limits to be set for genetic engineering so that it does not additionally contribute to and accelerate the destabilisation of ecosystems and loss of species.

In this context, two regulatory requests are crucial in dealing with 'old' and 'new' genetic engineering:

- Starting with the respective method, all organisms with genomes that have been changed using genetic engineering techniques, must be subject to regulation and mandatory authorisation even if no additional genes are inserted.
- Spatio-temporal controllability must be ensured, i.e. every release must be strictly dependent on the possibility of effective control and 'retrieval'.

Our actions must take the rights of coming generations into account, so that they may live in a world full of biodiversity that has emerged from its own dynamic and is not designed in a 'gene lab'.

In this context, we welcome the fact that there appears to be an increasing willingness to treat nature and living beings with respect, and to understand that they have their own intrinsic 'rights' – so that we can better protect them from misuse and destruction (Chapron et al., 2019; Boyd, 2018).

Against this backdrop, it should be examined to what extent a 'right' can be accorded to natural species and biodiversity, so that their natural genetic integrity and further development can be protected by law. In any case, biodiversity and its further development must no longer be seen as a free resource for genetic engineering experiments.

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