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**Testbiotech comment on the IUCN report  
“Genetic frontiers for conservation, an assessment  
of synthetic biology and biodiversity conservation”**

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Updated version, August 2021

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**Imprint**

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## Summary

The IUCN report lacks an in-depth analysis of the potential impacts and risks of synthetic biology for nature conservation. In addition, it is written to a significant extent from an overly simplistic and ‘techno-fixated’ point of view. Large parts of the report seem to be promoting human intervention into the genomes of natural populations rather than protecting natural populations and their ecosystems for future generations.

If organisms derived from synthetic biology are introduced to persist and propagate withing natural populations it would mean the genetic engineering of the ‘germline of biodiversity’, with the risk of disrupting existing ecosystems and their future evolutionary dynamics.

The report, as it currently stands, creates the wrong impression, especially in the case studies and regarding the limits of knowledge, but also on the availability of methods of control requested by the precautionary principle. It appears to promote the introduction of genetically engineered organisms and even gene drive organisms, into wild populations without first considering the real spatio-temporal dimension.

If organisms derived from synthetic biology are introduced into natural populations as implied in the report, it would mean the genetic engineering of the ‘germline of biodiversity’, with the risk of disrupting existing ecosystems and their future evolutionary dynamics.

There is no plausibility in the hypothesis that human technical intelligence is ready to safely interfere with the fundamentals of life. Similarly to the spread of non-native diseases that are frequently vectored by non-native species or human activities, genetically engineered organisms introduced into natural populations may severely impact animal, plant and human health; and may also damage biodiversity and other values.

Whatever the case, the biological characteristics of the original GE organisms produced in the lab and grown under controlled conditions cannot be considered reliable for predicting potential hazards that may emerge in future generations, or for predicting hazards that may emerge after exposure to ongoing changes in the environment. Since the IUCN report fails to address these fundamental problems, it should not sbe adopted.

## Introduction

The International Union for Conservation of Nature (IUCN, 2019) report was drawn up to assess the following questions (as referred to in the report): *“examine the organisms, components and products resulting from synthetic biology techniques and the impacts of their production and use, which may be beneficial or detrimental to the conservation and sustainable use of biological diversity and associated social, economic, cultural and ethical considerations...”*

and to *“assess the implications of Gene Drives and related techniques and their potential impacts on the conservation and sustainable use of biological diversity as well as equitable sharing of benefits arising from genetic resources...”*

The questions raised concern recent developments in synthetic biology. The introduction of tools such as CRISPR/Cas enables a new depth of technical intervention at the level of genome. Overall, this is a rapidly developing field with an increasing number of applications. Compared to the first generation of genetically engineered organisms, many applications are not confined to domesticated plants or animals (or organisms contained in the laboratory). Instead, we are seeing an increasing number of projects looking at wild populations and a broad range of organisms, such as microorganisms, insects, rodents and trees, all of which are embedded in their own complex ecosystems. Therefore, the issues raised by IUCN are highly relevant for the future of nature conservation.

On the other hand, there is growing evidence of complex interactions between plants and animals as well as genomic mechanisms that allow for resilience, adaption and co-evolution of ecosystems, populations and species. The underlying mechanisms of these evolutionary dynamics are, by far, not fully understood. It has to be ensured that SynBio approaches do not negatively impact these natural dynamics within biodiversity by, for example, causing evolutionary mismatch effects between the Synbio organisms and their environment, or by causing destabilization and disturbance of the natural networks of co-evolution and resilience.

**There is growing evidence about unintended next generations effects in genetically engineered organisms that can persist and propagate in the environment. These effects include the biological characteristics of the offspring, complex interactions between the GE organisms and their environment as well as pleiotropic effects.**

In addition, there is growing evidence of unintended effects in following generations of genetically engineered organisms that can persist and propagate in the environment. These effects include the biological characteristics of the offspring, complex interactions between the GE organisms and their environment as well as pleiotropic effects.

However, it appears that the report failed to identify some of the most decisive issues alongside these developments. Furthermore, some chapters and findings seem to be biased towards the interests of those who intend to apply the respective technologies; some of whom were invited to be co-authors. Consequently, it does not give sufficient weight to protection goals such as the conservation of wild species within their natural ecosystems.

We exemplify our findings by discussing some of the case studies and then identifying some overarching issues. We conclude by pointing out the relevance of our findings for nature conservation.

## Huge potential, but ‘no free lunch’

Tools such as CRISPR/Cas make the genome more extensively available for changes than conventional breeding (see Kawall, 2019). They enable genetic changes which are otherwise unlikely to occur. Therefore, in many cases, the resulting intended and unintended changes as well as the risks are specific, and can be clearly distinguished from those arising from previous methods or natural mechanisms. Small changes, even without the insertion of additional genes, can have huge consequences.

‘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 *ATPα* gene, which is important in many biological processes, was ‘edited’ to mimic the corresponding gene in Monarch butterflies. This was accomplished with a so-called SDN-2 application without inserting additional genes into the genome. The application involved reconstructing three specific locations in the genome to create new biological traits.

The resulting biological effect is complex: even without the insertion of any additional genes, the changes mediated by gene-editing confer higher fitness and an evolutionary advantage in the fruit flies. Similarly to Monarch butterflies (and some other insects), they are now tolerant to cardiac glycosides produced by various plants (such as milkweed), 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 in the DNA were needed (in total less than 10 base pairs). However, a certain combination of changes must be present to achieve the desired resistance to the toxin: it was found that some of the gene combinations led to weakness in the flies after a stress test. This is because this specific gene is involved in several biological processes, i.e. it has so-called pleiotropic effects. It was only after the combination of genetic changes was ‘optimized’ that the flies showed normal vitality and resistance to the toxins in the plants, and also are likely to be inedible for predators.

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 be necessary that single individuals could create large, stable populations. Even if the new gene combinations did become established, it would require long periods of time during which ecosystems could adapt.

Ecosystems can, in addition, 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 real effects could only be determined (probably irreversible) after a release.

The actual interactions of released genetically engineered organisms with the environment could likewise 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 may rapidly overwhelm the adaptability of ecosystems. Genetic engineering applications can – in addition to man-made effects such as climate change – contribute to a destabilization of ecosystems or intensify specific unfavorable effects.

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Huge potential, but ‘no free lunch’

While the ‘Monarch Fly’ is not meant to be released into the environment, a relatively large number of plants and animals with the potential to spread in natural populations have already been developed. In the same way as the fruit flies, the organisms have new biological and complex effects even without additionally inserted genes and despite very few genetic changes. 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.

Genome editing applications predominantly using CRISPR/Cas gene scissors in plants can also increase the possibilities and speed with which the genome can be changed. Again, it does not matter whether additional genes are integrated into the genome. A recent study (Kawall 2021) uses camelina (*Camelina sativa*) to explain possible unintended effects that the release of a genome-edited crop can have on ecosystems. Camelina is rich in polyunsaturated fatty acids. CRISPR/Cas was used to increase the amount of oleic acid in the camelina seeds and to reduce the amount of easily oxidizable fatty acids. This was intended to extend the shelf life of the oil extracted from the camelina. Gene scissors were used to simultaneously knock out 18 gene copies in the genome of the camelina and generate plants with a higher oleic acid content. Such interventions have until now hardly, or not at all, been possible with conventional breeding methods, and can give rise to completely new biological properties. In the USA, these plants have already been deregulated without having to undergo thorough risk assessment.

If the composition of the fatty acids is changed, unintended effects on various processes can occur in addition to the desired properties, e.g. effects on the formation of certain messenger substances with which plants communicate and with which they, for example, ‘warn’ of a pest infestation. A change in the composition of fatty acids can affect and influence existing food webs. In addition, there is also the possibility that genome-edited plants will hybridize with wild species leading to unintended effects in subsequent generations. At the same time, the genome-edited camelina has the potential to persist in the environment and spread uncontrollably.

**There is a risk that releases of genetically engineered organisms and their spread in natural populations may rapidly overwhelm the adaptability of ecosystems. Genetic engineering applications can – in addition to man-made effects such as climate change – contribute to a destabilization of ecosystems.**

## Completeness check - three case studies presented by IUCN

Several parts of the report, such as the explanations of genetic and evolutionary mechanisms as well as the risks and uncertainties in regard to nature conservation, are not sufficiently backed by due diligence. This can be exemplified by examining the three case studies given below. For our examination, we deliberately did not choose the examples concerning gene drive organisms, which are already part of a broader controversial debate. Instead, we chose to address applications in need of a much broader scientific and public debate. Nevertheless, our general findings and conclusions, as explained in Chapter 2, are also highly relevant for applications of gene drive organisms.

### 1. Chestnut trees

The report (IUCN 2019) highlights the example of genetically engineered blight-resistant chestnut trees. As explained in the report (page 87, 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 planting they are meant to breed with 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 were declared safe in regard to their 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 so-called chestnut blight was caused by accidental import from Asia; it releases a toxin that kills the trees and has led to a significant loss of the large chestnut trees. In 2018, surviving chestnut trees existed mainly in shrubby growth forms resulting from the formation of shoots from the root collar (NAS 2019).

The transgenic chestnut trees under discussion were developed some years ago but not released into the wider environment. The most recent version of these trees was generated by using promoters that strongly enhance gene expression of the inserted gene (derived from wheat). This recent generation of trees, that was first described only a few years ago (Zhang et al., 2013), is cloned from only one founder tree (see Popkin, 2018). Currently, there are discussions in the US on whether the genetically engineered trees should be deregulated. If this happens, the cloned and genetically engineered trees and their offspring could be planted in forests and cause gene flow to the remaining wild populations. At the same time, a conventional breeding program is underway to cross the American chestnut with the Asian chestnut, which is resistant to chestnut blight (Steiner et al., 2017). This program appears promising and could successfully result in blight-resistant trees; it is, however, time consuming.

There appear to be several shortcomings in the IUCN report e.g. the authors mostly ignore the life span of the trees as well as possible changes in the environment and resulting uncertainties. The trees could live up to 200 years, undergoing several stages of biological development such as flowering, producing fruits and aging. During their life span, they will be exposed to many changes in their environment, such as climate change and interactions with a diversity of stress factors (see, for example, Smolker & Petermann, 2019). It is therefore not unlikely that as the trees grow and propagate and are exposed to environmental changes, they will show characteristics not originally observed. This can be concluded from existing evidence (see below), but is not mentioned in the IUCN report.



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Further, the authors do not discuss the effects of further crossings of these trees: if the genetically engineered tree clones cross with other chestnut trees, the biological characteristics of the hybrid offspring might be quite different from those produced in the lab. This hypothesis can be concluded from existing evidence on next generation effects of already existing genetically engineered plants (see below), but is not mentioned in the report. We conclude it is impossible to argue safety, as implied in the report, just from testing genetically engineered and cloned trees for a few years, or by examining their interaction with some selected species that fail to represent the complexity and diversity of the ecosystem. For example, there are other known pathogens that harm chestnut trees such as *Phytophthora cinnamomi*. It seems largely unknown how the genetically engineered trees will react to this plant pest and other biological stressors (NAS, 2019). On the other hand, the trees derived from the conventional breeding program appear to show resistance to both pathogens (Steiner et al., 2017). If pollen is distributed by wind or seed and transported by human or animal activity, the transgenic trees and their offspring might spread in forests without any control. Given a sufficiently long period of time and continuing pressure from chestnut blight, the natural populations might be largely or completely replaced by the transgenic trees. If something goes wrong, it may be impossible to retrieve the trees from the environment.

A British-based NGO, GeneWatch UK, has also exposed these problems in risk assessment. One particular reason for concern: the trees might tolerate blight infections, and thus enable the pathogens to propagate on the transgenic trees; this might then become a reservoir for the further spread of the blight infection and add to increasing pressure on native populations (GeneWatch UK, 2020).

**The trees might tolerate blight infections, and thus enable the pathogens to propagate on the transgenic trees; this might then become a reservoir for the further spread of the blight infection and add to increasing pressure on native populations.**

Consequently, planting these trees where they can spread without control and allowing gene flow of the transgenes means ignoring the precautionary principle. The IUCN report fails to adequately discuss this problem.

## 2. Genetically engineered corals

The authors further discuss altering genes in corals or its associated symbionts using CRISPR/Cas9 mediated genetic engineering to enhance their thermal tolerance (Levin et al., 2017). Corals are complex organisms that depend on symbiotic interaction with algae and other microorganisms, such as bacteria and archaea also known as holobionts (Rosenberg & Rosenberg-Ziller 2016). Bleaching under ongoing climate change is commonly considered to be caused by disruption of the symbiosis between the coral host and its endosymbiotic microalgae (*Symbiodiniaceae spp*).

As admitted in the report, “genetic engineering methods are poorly developed for corals and their microbial symbionts”. However, what it should also have mentioned is that there are still many unknowns regarding the complex interactions between the host and its microbiome, which produces the compounds necessary for the coral system to live and survive.

Rosenberg & Rosenberg-Ziller (2016) describe how the microbial symbionts contribute to the overall genetic variation of the coral system and its adaptive, evolutionary processes. They refer to investigations showing that changes in the composition of symbionts do occur under changed environmental conditions, which can increase thermal tolerance of the corals by 1 to 2°C. These findings indicate there are mechanisms for natural adaptation of coral systems to climate change that are not yet fully known or understood.

There is further research showing that corals have a surprisingly high capacity to adapt to conditions caused by climate change (Kersting & Linares, 2019; Kenkel & Matz, 2016). It was shown that coral survival rates can also be aided by using simple methods: symbiotic microorganisms typically hosted by corals were first of all exposed to higher temperatures in contained systems. Subsequently, they were used to colonize corals – this had overall positive effects on the corals response to higher temperatures (Buerger et al., 2020).

There is research showing that corals have a surprisingly high capacity to adapt to conditions caused by climate change. It is unknown how these naturally occurring interactions of corals and their symbionts would be affected if their biological characteristics were changed by genetic engineering.

It is unknown how these naturally occurring interactions of corals and their symbionts would be affected if their biological characteristics were changed by genetic engineering. Therefore, this high degree of uncertainty should have been emphasised particularly from the perspective of nature conservation.

There is also the problem that, were such genetically engineered holobionts to be released in coral systems, it would become impossible to retrieve them if something goes wrong. It can be acknowledged that

the IUCN report mentions this problem, but only in very general sense: “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” (page 92 of the printed version).

However, it would have been necessary to expand these considerations in more detail in order to fully integrate them into the case studies. Without this broader perspective, most of the case studies raise the dangerous impression that interventions in highly complex systems might be feasible and controllable in very near future.

### 3. Genetically engineered honey bees

The IUCN report introduces the idea of using synthetic biology to enhance the resilience of honey bee colonies to environmental stress factors by altering their microbial gut composition. Interestingly, the author comes to the conclusion that the proposed intervention might weaken the immune system of honey bees. Therefore, this example does not seem to recommend the use of synthetic biology.

However, it should not be overlooked that the nuclease CRISPR/Cas and genome editing have already been used to produce genetically engineered pesticide-resistant honey bees. The first insecticide-resistant honey bee was reportedly the goal of experimental work in South Korea (Lee, 2019). Other publications also suggest the use of CRISPR/Cas for this purpose (McAfee et al., 2019).

Researchers at the University of Austin (Texas) engineered the genome of bacteria found in the gut of honey bees and bumble bees to make them produce additional biologically active molecules (non-coding ribonucleic acid, ncRNA). The molecules are meant to be taken up from the gut and thus spread to other parts of the honey bee, including their central nervous system (Leonard et al., 2020).

It would be particularly problematic if honey bees with such microbes were to be released since there is no way of preventing the bacteria from infecting the gut of other honey bee colonies or wild relatives, such as bumble bees. Moreover, their synthetic genes can also be transferred to other species of bacteria. This problem triggers incalculable environmental risks: once released, the spread of the organisms and their synthetic genes could not be efficiently controlled.

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Some overarching issues that need to be considered

The most relevant gut bacteria in this context are *Snodgrassella alvi*. These microbes were only discovered a few years ago and are found in honey bees as well as in bumble bees. The bacteria play an important role in the vitality and the immune system of the bees. Changes in the populations of *Snodgrassella alvi* are known to substantially weaken the health of bee populations.

Given the ongoing research with genome editing tools such as CRISPR/Cas on many different levels, the IUCN report should critically assess these attempts promoted as new strategy for honey bee conservation.

**If honey bees with the SynBio microbes were to be released there is no way of preventing the bacteria from infecting the gut of other honey bee colonies or wild relatives, such as bumble bees. Moreover, their synthetic genes can also be transferred to other species of bacteria.**

Furthermore, given the extremely complex biology of honey bee colonies and their multitudinous interactions with the environment, such interventions on the level of their genome seem to be in contradiction to the aims of nature conservation and the protection of biodiversity. The IUCN report fails to address this problem adequately.

## **Some overarching issues that need to be considered**

It is known that the robustness and reliability of environmental risk assessment of genetically engineered (GE) organisms is largely influenced by the question of whether the GE organisms can spread in the environment. Very generally stated, if GE organisms (or organisms derived from synthetic biology) can persist in the environment, and if gene flow to wild relatives can be established leading to viable offspring, the uncertainties will increase and risk assessment will face more complex questions.

These issues are especially relevant for the assessment of potential applications of gene drive organisms as proposed in the IUCN report, and should be taken into account throughout the discussion of all applications concerning genetic engineering of wild populations.

In this context, it is important to be aware that existing experience with GE organisms cannot simply be extrapolated to assess these new applications: if GE organisms are released into natural populations, there are fundamental differences compared to, for example, risk assessment of GE crop plants grown for just one season. Under such conditions, the company / breeder might be able to check some seed characteristics each year before the plants are grown in the fields. However, volunteer hybrids and / or GE offspring spreading in wild populations do not undergo any additional quality or safety checks before they appear and spread in the environment. An overview of the comparison of existing experience to new challenges is provided in Table 1.

Some overarching issues that need to be considered

Table 1: New challenges in the risk assessment of genetically engineered organisms being released into natural populations in comparison to genetically engineered crop plants

<b>Some assumptions in the risk assessment of GE crop plants</b>	<b>New challenges in the risk assessment of genetically engineered organisms released in natural populations</b>
The majority of crop plants are cultivated for a single growing period. These plants are not meant to reproduce spontaneously.	Next generations will emerge spontaneously, without control in place to ensure, e.g. gene stability and gene expression rates.
Due to previous breeding processes, plant varieties used for genetic engineering are stable and have defined characteristics, as well as reduced genetic diversity. Seed quality can be controlled by breeders (or farmers) before and during cultivation.	Wild populations very often contain a broad spectrum of genetic backgrounds. As a result, genetically engineered organisms introduce their new genetic information into heterogeneous genetic backgrounds, without additional controls in place or checking for unintended gene interactions.
Crop plants are often grown in a managed agricultural environment with reduced biodiversity.	Wild populations very often interact with complex ecosystems. However, unintended impacts on other species might remain unnoticed due to lack of adequate methods for monitoring.
Crop plants of the same species are often cultivated under similar environmental conditions.	Wild populations, e.g. insects are often exposed to a wider range of environmental conditions due to their mobility. Further impact factors include, e.g. seasonal changes.

In conclusion, genetically engineered organisms that can persist and propagate in the environment and / or enable gene flow to wild populations, pose new challenges for risk assessment. In many cases, significant uncertainties remain and some unknowns might prevail that make the risk assessment inconclusive: the multiplex interrelations with the closer and wider environment pose a real challenge for the risk assessor. While genetic stability over several generations might be demonstrated in domesticated varieties under normal field conditions or green house cultivation, genome x environmental interactions and introgression into heterogeneous genetic backgrounds can still trigger unpredictable next generation effects (see Bauer-Panskus et al., 2020).

**The biological characteristics of the original GE organisms produced in the lab and tested under controlled conditions, cannot be regarded as sufficient to predict all relevant effects that can emerge in the next generations, and in interaction with the receiving environments.**

Gene drive organisms generated by the nuclease CRISPR/Cas add a further layer of complexity to these findings. These organisms replicate the process of genetic engineering in a self-organised way: in every generation the offspring receive one chromosome carrying the genetic material encoding the CRISPR/Cas components (e.g. the nuclease Cas9 and a gRNA) and potentially associated cargo-genes. Thus, all offspring in the next generations will receive the gene drive construct. As a result, the newly introduced gene drive cassette can spread throughout a population exponentially, and much more rapidly than could be expected under the Mendelian pattern of inheritance. This process was rightfully named ‘mutagenic chain reaction’ (Gantz & Bier, 2015). Many technical and biological uncertainties surround these applications which can be exacerbated by interactions with the environment or by next generation effects (see Then et al., 2020).

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Some overarching issues that need to be considered

Whatever the case, the biological characteristics of the original GE organisms produced in the lab and tested under controlled conditions, cannot be regarded as sufficient to predict all relevant effects that can emerge in the next generations, and in interaction with the receiving environments.

In this regard, some of the existing evidence from GE plants are summarized in Table 2.

Table 2: Summary of the literature review investigating GE plants and next generation effects with specific relevance for risk assessment of GE organisms that can spread in natural populations

Issue	Findings
New biological characteristics	Next generations of GE organisms can show effects that were not observed or intended in the original event (Kawata et al., 2009; Cao et al., 2009; Yang et al., 2017).
Effects emerging from genetic background	Unintended effects can emerge from interaction of the newly inserted genes with the genetic backgrounds (Bollinedi et al., 2017; Lu & Yang, 2009; Vacher et al., 2004; Adamczyk & Meredith, 2004; Adamczyk et al., 2009).
Interaction with the environment on the level of the genome (genome x environment interactions)	Unintended genomic effects can be triggered by changing environmental conditions or biotic and abiotic stressors (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).
Pleiotropic effects	The additional EPSPS enzymes were shown to impact plant growth, the number of seeds and seed dormancy (Beres, 2019; Beres et al., 2018; Wang et al., 2014, Fang et al., 2018; Yang et al., 2017; Jiang et al., 2021). These characteristics are known to enhance fitness and may result in invasiveness.
Complex interactions	Complex interactions with ants, pest insects and viruses were shown in GE cotton and soybean (Almeida et al., 2021; Vázquez-Barrios et al., 2021; Xiao et al., 2021). These effects are likely to increase pest damage in fields with GE crops. In the case of Bt cotton, they may render invasive characteristics that can spread through cotton biodiversity hotspots (Vázquez-Barrios et al., 2021).

Based on the evidence provided above, we conclude that there is a more fundamental problem with GE organisms that can persist and spontaneously propagate in wild populations without control: if the spatio-temporal dimension cannot be controlled, the risk assessment of genetically engineered organisms has to consider evolutionary dimensions. In these circumstances, evolutionary dynamics combine large numbers of individuals on the population level and singularities on the molecular scale. Thus, evolutionary processes make it possible to turn events with a low probability of ever happening into events that may feasibly happen (Breckling, 2013). Under these conditions, for example, the fitness of new genomic constituents cannot be calculated in absolute terms; it will depend on the environment and future changes.

Very generally, it has to be concluded that at some point, the uncertainties and unknowns in risk assessment will become predominant in comparison to the knowledge available, affecting the ability to conclude on the safety of GE organisms (Bauer-Panskus et al., 2020; Then et al., 2020). Table 3 gives an overview of questions that need to be answered to address the problem of spatio-temporal controllability before risk assessment can be finalised.

**The requirement to demonstrate ‘spatio-temporal controllability’ could be introduced into environmental risk assessment as cut-off criteria: If spatio-temporal controllability is not demonstrated, the organisms cannot be released into the environment.**

Some overarching issues that need to be considered

**Table 3: Overview of relevant questions for the risk assessment of genetically engineered plants that can persist, spontaneously propagate and cause gene flow in the environment (cut-off criteria for risk assessment).**

<b>Question</b>	<b>Relevance</b>
(1) Can genetic stability be controlled in following generations?	Self-replication and environmental as well as epigenetic effects can lead to emergence of next generation unintended effects not observed in the first generation.
(2) How can genetic diversity in wild populations of the same species be taken into account?	In most cases a high degree of genetic diversity exists in natural populations. These heterogeneous genetic backgrounds can trigger unexpected effects not observed in domesticated populations.
(3) Will there be any gene flow to other species?	If gene flow is possible and hybrid offspring are viable, the resulting organisms have to be seen as new events that have to be assessed separately from the original GE organisms.
(4) How can population dynamics and life cycle aspects of the target species be integrated?	For example, bottlenecks in the population dynamics can have a significant impact on tipping points within the populations.
(5) Can the receiving environment be defined in regard to relevant interactions and confined in regard to potential spread?	Adverse effects can emerge from interaction with closer (associated microbiomes) or wider environments (such as food webs, predators, beneficial organisms). Complex interrelations (such as signaling pathways) have to be taken into account.

The requirement to demonstrate ‘spatio-temporal controllability’ could be introduced at the beginning of environmental risk assessment in the form of cut-off criteria as part of the data check: if spatio-temporal controllability is not demonstrated, the application should be rejected and the organisms cannot be released into the environment (Bauer-Panskus et al., 2020; Then et al., 2020).

## Synthetic biology on micro-organisms

Applications of synthetic biology on micro-organisms (SynBio MO) including, for example, bacteria, viruses, fungi and yeast, may become a threat to the global biosphere to greater extent compared to many other applications of genetic engineering in more complex species. Micro-organisms (MO) are the basis of life on earth and the common network of all existing species. They are essential for mobilizing and exchanging abiotic and biotic resources. Further, they transmit biologically active molecules and information across boundaries between species. The emergence of diseases further adds to the complex interactions between MO and other species. MO reproduce fast compared to more highly developed species, and are thus adept at spreading rapidly in their environments.

There are an increasing number of projects which may include releases of SynBio MO into the environment. Some examples:

- Potential uses of SynBio MO imply the engineering of ecosystems, microbial communities for purposes such as changing biodegradation, waste treatment and bioremediation (Wang et al., 2013; Mee et al., 2014; Qian et al., 2020).
- Several projects aim to change gut microbiota in animals and humans (Mimee et al., 2015; Kim et al., 2018; Ronda et al., 2019).
- SynBio or GE applications to change gut microbiota are also being discussed for insects such as flies (De Vooght et al., 2014; Gilbert et al., 2016) mosquitoes (Ren et al., 2008; Fang et al., 2011; Bilgo et al., 2017; Lovett et al., 2019) and bees (Rangberg et al., 2012; Leonard et al., 2018; Leonard et al., 2020). Some of these approaches are called ‘paratransgenesis’, which means the biological characteristics of a target host are changed by genetically engineering its symbiotic bacteria, for example, to eliminate a pathogen from insects via the expression of effector molecules (Wilke et al., 2015).
- Similar approaches are being discussed in regard to corals (Levin et al., 2017).
- In agriculture, there are ongoing discussions in regard to applications that change the microbiomes of plants, e.g. mycorrhiza or endophytes (Vorholt et al., 2017; Checcucci et al., 2018; Hettiarachchige et al., 2019; Arif et al., 2020).
- In agriculture, SynBio applications for soil microorganisms are also being discussed (Temme et al., 2012; Shelake et al., 2019; Shulse et al., 2019).
- Further potential uses include the usage of SynBio MO as pesticides (Leclère et al., 2005; Tseng et al., 2005; Wang et al., 2011; Fang et al., 2014; Scheepmaker et al., 2016; Azizoglu et al., 2020).
- Other applications include viral systems for engineering plants and animals in the environment (Nuismer & Bull, 2020; Killiny N., 2020) and even the dissemination of genetically engineered viruses via insects (‘insect allies’) for potential military purposes (Reeves et al., 2018).

The IUCN should address these issue, emphasizing that, in dealing with SynBio MO, the precautionary principle deserves to be given the highest priority. The European Food Safety Authority states (EFSA, 2020) that: *“Even with the complete genetic information of a synthetic micro-organism, it is beyond the capacity of any existent bioinformatic analysis to fully predict the capability of a synthetic organism to survive, colonise and interact with other organisms under natural conditions, given the uncountable diversity of potential microhabitats and their temporal variability.”*

In the discussions on these applications, the IUCN should take into account that MOs are part of the microbiome of plants, insects, mammals and humans. Under such circumstances, the biological effects, and potential

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adverse effects of SynBio MO will emerge from these symbiotic interactions in a non-linear pattern. Therefore, these biological systems cannot be assessed simply by looking at single parts and pieces in isolation, they all have to be considered as larger units known as holobionts or hologenoms, taking into account that all species in the same habitat interact and influence each other (see, for example, Richardson, 2017; Sanchez-Canizares, 2017; Arif et al., 2020). It is not only the SynBio MOs which may act upon target and non-target organisms, but also the host and the hologenome may impact the

characteristics of the SynBio MO. Furthermore, risk assessment of genetically engineered hosts, which may be combined with a SynBio microorganism by accident or on purpose, also need to be considered.

## Climate change considerations

As explained in the IUCN report, climate change due to human activity has caused greenhouse emissions that are putting increasing pressure on ecosystems and biodiversity. Invasive species, pests and pathogens are endangering biodiversity and food security. More extreme weather conditions, including drought, flooding and cold, are already endangering large regions. As a result, biodiversity, ecosystems and agriculture will undergo rapid changes and be severely damaged.

In many cases, the environment is already being adversely impacted by other human activities and climate change is causing additional stress, leading to potential tipping points in the ongoing extinction of many

Are we cleverer than four billion years of evolution? Can we protect endangered species and safeguard food security by designing organisms with an optimized genome? And should we erase less advantageous species with gene drives? Why not?

species. Currently, species are becoming extinct at a much faster rate than the average for the last 10 million years.<sup>1</sup>

Which role might genetically engineered (SynBio) organisms play in this context? Could we, for example, use genome editing to create organisms which are tolerant to various environmental stressors just at the right time? Some researchers appear to be raising these kinds of expectations. For example, Jennifer Doudna, inventor of CRISPR/Cas technology and noble price laureate, 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.”<sup>2</sup>

So are we cleverer than four billion years of evolution? Can we protect endangered species and safeguard food security by designing organisms with an optimized genome? And should we erase less advantageous species with gene drives? Why not?

Evolution has given many species deep links to earlier stages of evolution with similar environmental conditions (see, for example, Shubin, 2020). More generally, evolution builds on genetic and biological diversity

<sup>1</sup> <https://ipbes.net/news/Media-Release-Global-Assessment>, see also Kegel, 2021.

<sup>2</sup> Page 243/244



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which, as a system, can continue to evolve, very often using already existing solutions to problems. It is not simply about the single ‘fittest’ organism to survive, but about populations and ecosystems which are diverse and flexible enough to respond to new environmental conditions.

In general, species and their populations evolve several strategies for responding to changes in local environmental conditions (see, for example, Waldvogel et al., 2019): (i) shifting their range of distribution; (ii) persisting in their local habitat through epigenetic responses; (iii) persisting in their local habitat through genetic adaptation to new conditions; (iv) persisting in their ecological niche but experiencing demographic decline or even extinction; (v) a further strategy may be based on adaptation within symbiotic networks (as, for example, shown in corals).

Recent research has found that, in many cases, it is not a new mutation or new genetic conditions that are needed for survival, but solutions to problems – and these may already be available in the genome of the species or in the networks of ecosystems. For example, recent research in amphibians and reptiles has shown that they can adapt much faster to changes in environmental conditions than expected (Wiens et al., 2019). The authors conclude: *“These species clearly did not adapt evolutionarily to these conditions instantaneously, but instead must have been able to tolerate them before their introduction”*

Genetic diversity within species and ecological networks is key to providing a sufficiently broad range of possible solutions. Against this backdrop, there is an abundance of scientific evidence in support of strategies aiming to increase diversity in agroecology systems<sup>3</sup>. The same is true for forests (see for example Morin et al., 2018) and grasslands (see for example Isbell et al., 2015).

Furthermore, it has also been shown that, for example, bees and pollinated plants can evolve together and survive conditions arising from climate change in a kind of orchestrated process of development (Bartomeus et al., 2011). One reason for this could be interactions between gene regulation in honey bees and biologically active molecules (e.g. miRNAs) produced by pollinated plants, which it has been suggested interfere with gene regulation in several metabolic pathways in the honey bees (Gharehdaghi et al., 2021). The authors conclude that *“the results provide evidence of cross-species regulation function of miRNA between honey bee and flowering host plants, extending our understanding of the molecular interactions between plants and animals.”* Further studies in honey bees show that the miRNA fed by honey bees to their larvae regulates their cast development (Ashby et al., 2016; Zhu et al., 2017).

Genetically engineered organisms may promote evolutionary mismatch-effects within such complex interactions, which may, in turn, interrupt the finely-tuned interactions between the species and the dynamics of co-evolution. For example, it was shown that interactions between genetically engineered cotton and associated ant species are disturbed. This is likely to foster the spread of Bt cotton in biodiversity hotspots (Vázquez-Barrios et al., 2021).

**Genetically engineered organisms may promote evolutionary mismatch-effects within such complex interactions, which may, in turn, interrupt the finely-tuned interactions between the species and the dynamics of co-evolution.**

Genetically engineered microorganisms, such as honey bees, corals, amphibians, trees or crops, might look promising as short-term solutions. However, in the long-term, once these genotypes are introduced into complex natural networks and interactions, they may disturb and destabilize existing mechanisms of resilience and climate adaption. We should remain pessimistic. Climate change caused by human activity goes far beyond anything that the natural world has ever been exposed to over millions of years. Therefore, Mother Nature’s resilience mecha-

3 <http://www.fao.org/3/I9037EN/i9037en.pdf>

nisms are likely to be too slow to save biodiversity as we know it and would like to protect. On the other hand, research carried out on corals, honey bees, amphibians and reptiles shows that many species have an amazing potential to adapt and co-evolve in changing environmental conditions. However, these effects are hard to predict. For example, even if diverse plant societies can survive drought conditions for several years, the adverse impacts might be much stronger if the drought continues for a longer period of time (Komatsu et al., 2019). As this research shows, the biological effects cannot be predicted, and can turn out to be very different even though conditions are similar to start with.

We need to stop climate change because our technologies might cause additional damage or even trigger a new man-made crisis. At the same time, we cannot rely and trust in the resilience of Mother Nature if we want to protect existing species and ecosystems.

In the light of these findings, potential releases of genetically engineered organisms to combat or mitigate climate change appear to be driven by particular interests, and are not suited to providing adequate solutions. We should not put our faith in false hopes: if we do not stop climate change, no technology will ever be able to prevent the extinction of thousands of species. And no! We are still not cleverer than evolution.

## **Conclusion: Why the IUCN report needs major revision**

The above findings are highly relevant for the discussion on potential usage of synthetic biology for conservation purposes. However, these issues are not, or only randomly, mentioned in the IUCN report. Therefore, instead of it being adopted, the report should undergo major revision. As it currently stands, the report and especially the case studies, give the wrong impression about the limits of knowledge and the availability of methods for spatio-temporal control.

**There is no plausibility in the hypothesis that human technical intelligence is ready to safely interfere with the fundamentals of evolutionary processes.**

The precautionary principle referred to in the report is based on the idea that new risks might be taken, but only as long as effective measures are available and can be implemented if something ‘goes wrong’. Such measures depend on being able to control the release of GE organisms in their spatio-temporal dimension.

If, however, genetically engineered organisms are introduced into natural populations without effective control, it would mean the genetic engineering of the ‘germ line’ of biodiversity, with the risk of disrupting functioning existing ecosystems and their future evolutionary dynamics.

The biosphere in which we all are embedded is based on a multitudinous network of mutual interactions. Genetics are not only decisive for the organisation of the organisms but also, e.g. for signaling pathways, complex behaviours and instinctive reactions, interactions within symbiotic communities, and also for the establishment of specific structures within populations, such as bee colonies or other eusocial insects. Life has evolved to a degree of complexity that has allowed mankind to develop a high level of technical intelligence. However, this does not mean that living organisms in nature should be designed in accordance with human wisdom and perception. There is no plausibility in the hypothesis that human technical intelligence is ready to safely interfere with the fundamentals of life and evolutionary processes.

Furthermore, a parallel can be drawn to the risks posed by invasive species or non-native diseases mentioned in the IUCN report: similarly to the spread of non-native diseases, frequently vectored by non-native species or

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human activities, genetically engineered organisms introduced into natural populations may severely impact animal, plant and human health; also damaging biodiversity and other values.

Therefore, nature conservation should aim to protect existing life forms, ecosystems and their future dynamics that are the result of evolutionary processes over several billion years. From the perspective of nature conservation, it would be irresponsible to allow organisms derived from synthetic biology to interfere with the future of life and the trajectories of evolution. The protection goals of nature conservation should not only encompass endangered species, but also the diversity and dynamics of future biodiversity.

We should respect the rights of future generations to experience and live in a world which has emerged from its own intrinsic dynamics and not one that was made in the laboratory. Currently we are experiencing an increased awareness that nature and living beings should not only be treated with respect but considered as rights holders against misuse and destruction (see for example Chapron et al., 2019). Whatever the case, nature conservation should protect natural populations that have emerged from the evolution over billions of years and not promote human intervention into their genomes.

Similarly to the spread of non-native diseases, frequently vectored by non-native species or human activities, genetically engineered organisms introduced into natural populations may severely impact animal, plant and human health; also damaging biodiversity and other values.

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