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

Content

Summary

The IUCN report lacks in-depth analysis on 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 the protection of natural populations and their ecosystems for future generations.

The report, as it currently stands, creates the wrong impression, in particular in the case studies and on the limits of knowledge as well as availability of methods for 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 considering the real spatio-temporal dimension.

If organisms derived from synthetic biology are introduced into natural populations as implied, it would mean the genetic engineering of the ‘germ line’ of biodiversity, with the risk of disrupting the functioning of 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, 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 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 not be considered reliable for predicting potential hazards that may emerge in future generations; and after exposure to ongoing changes in the environment. Since the IUCN report fails to address these fundamental problems, it should not be adopted.

Introduction

The report of the International Union for Conservation of Nature (IUCN, 2019) was produced 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 on the level of genome. Overall, developments in this field are rapid 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 regarding wild populations with a broad range of organisms such as insects, rodents and trees, all of which are embedded in their complex ecosystems. Therefore, the issues raised by IUCN are highly relevant for the future of nature conservation.

However, from the report it seems that some of the most decisive issues alongside these developments were not identified. 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, sufficient weight is not given to protection goals such as conservation of the 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.

Completeness check of three case studies

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 (see CSS, 2019). Instead, we chose to address applications in need of 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 that result 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 promotors 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., 2016). 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 as 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.

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., 2016).

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. Consequently, planting these trees where they can spread without control and cause gene flow 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 symbiontic interaction with algae and other microorganisms, such as bacteria and archea 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 have also 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 adaption of coral systems to climate change that are not yet fully known or understood.

It is still 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 honeybees. 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 for this purpose (McAfee et al., 2019).

Given the ongoing research with genome editing tools such as CRISPR on many different levels, the IUCN report should also take into account those attempts promoted as new strategy for honey bee conservation.

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.

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

Some assumptions in the risk assessment of GE crop plants	New challenges in the risk assessment of genetically engineered organisms released in natural populations
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.

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 (see, for example, CSS, 2019) which can be exacerbated by interactions with the environment or by next generation effects.

Whatever the case, the biological characteristics of the original GE organisms produced in the lab and grown under controlled conditions, cannot not 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 summarised in Table 2.

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

Issue	Findings
Next generation effects	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).

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. 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.

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.

Question	Relevance
(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 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.

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.

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.

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 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 (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.

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