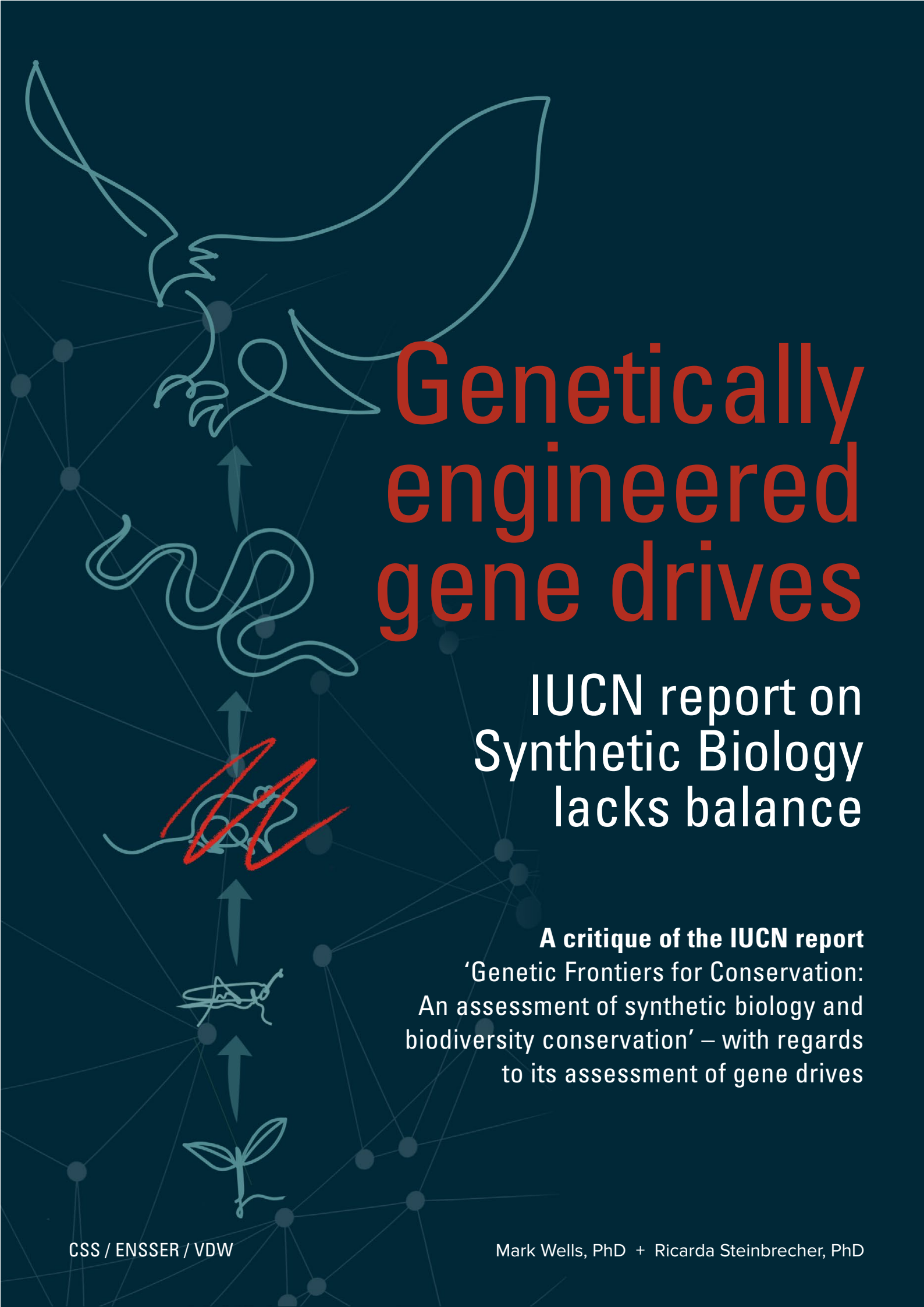

Genetically engineered gene drives

IUCN report on Synthetic Biology lacks balance

A critique of the IUCN report 'Genetic Frontiers for Conservation: An assessment of synthetic biology and biodiversity conservation' – with regards to its assessment of gene drives

CSS / ENSSER / VDW

Mark Wells, PhD + Ricarda Steinbrecher, PhD

Genetically engineered gene drives: IUCN report on Synthetic Biology lacks balance

A critique of the IUCN report 'Genetic Frontiers for Conservation: An assessment of synthetic biology and biodiversity conservation' – with regards to its assessment of gene drives

May 2021

Authors: Mark Wells, PhD & Ricarda Steinbrecher, PhD

Editor: Holly Dressel

Published by: Critical Scientists Switzerland (CSS)
European Network of Scientists for Social and Environmental Responsibility (ENSSER)
Vereinigung Deutscher Wissenschaftler (VDW/FGS)

weblink: https://genedrives.ch/new-publications/

Translations: The present report is also available in French and Spanish (summaries in different languages). https://genedrives.ch/fr/nouvelles-publications/ & https://genedrives.ch/de/neue-publikationen/

Authors' biographies

Dr Ricarda Steinbrecher is a biologist and molecular geneticist based in Oxford, UK, educated at the University of London and the University of Kiel, Germany. First specialising in gene regulation and gene modification, she worked in the field of mutational analysis, gene identification and gene therapy. Since 1995 her focus has been on biosafety aspects of genetically modified organisms. More recently, she has been concentrating on synthetic biology, new genetic engineering techniques such as CRISPR-Cas9, and gene drive organisms. Dr Steinbrecher is co-director of EcoNexus, a public interest research organisation focusing on new technologies and their impacts on biodiversity, ecosystems, food security and agriculture.

Dr. Steinbrecher has been actively involved in UN-led processes since 1996, especially of the Convention on Biological Diversity (CBD) and its Cartagena Protocol on Biosafety. Since 2015 she has served on the Technical Expert Group on Synthetic Biology of the CBD, also covering gene drives. She is a member of the Federation of German Scientists (FGS/VDW) whom she represents at the international UN negotiations, and is a founding and board member of the European Network of Scientists for Social and Environmental Responsibility (ENSSER).

Recent publications as co-author include:

Chapters 1 and 2 in *'Gene Drives: A report on their science, applications, social aspects, ethics and regulation'* - published in 2019 by Critical Scientists Switzerland (CSS), ENSSER and VDW.

Eckerstofer MF, M Dolezel, A Heissenberger, M Miklau, W Reichenbecher, RA Steinbrecher and F. Wassmann. An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs)', *Frontiers in Bioengineering and Biotechnology (*2019) 7:31. doi: 10.3389/fbioe.2019.00031.

Dr Mark Wells is a biological chemist and a researcher for Econexus, where he analyses and assesses developments in gene drive technology, especially with regard to risks. Dr Wells has nine years of research experience in molecular biology and protein chemistry – including postdoctoral research at the Medical Research Council Centre for Protein Engineering in Cambridge, and a senior scientist position in a biotechnology start-up. He is co-author of the two chapters in '*Gene Drives – A report on their science, applications, social aspects, ethics and regulation*, mentioned above.

Contents

EXECUTIVE SUMMARY

The report from IUCN 'Genetic Frontiers for Conservation'[1] is a consideration of the biodiversity implications of a range of old and new genetic engineering technologies, as well as some hypothetical ones, all currently presented under the banner of synthetic biology. **In this critique, we focus solely on the IUCN report's discussion of engineered gene drives and gene drive organisms (GDOs). Our concern is that the IUCN discussion tends to downplay many risks and uncertainties surrounding this technology**. In particular, important risks and lack of predictability in terms of behaviour and outcomes are often framed in a way that detracts from their importance. The possible 'unintended consequences' of using engineered gene drives are not explored in any depth (even though historical experience with new technologies suggests such consequences are likely). A non-specialist reader could easily fail to see the importance and implications of particular points, or might miss them all together. It is also of concern that at a time when a real commitment to the precautionary principle is needed, this report actually side-lines this important international principle and even tries to re-define it in a manner that markedly weakens it. In combination, these concerns call into question the manner in which the report has been written, and especially its appropriateness as a basis for policy decision making.

What are gene drive organisms?

Gene drive organisms (GDOs) are genetically modified organisms (GMOs) with specially constructed genetic material that is capable of overriding the normal rules of inheritance. When these organisms reproduce, specific traits, as well as the gene drive mechanism itself, are passed on to the offspring at a much higher rate than would normally occur.

The term 'gene drive' (also known as engineered/synthetic gene drive) can have different meanings, including:

(1) a method used to increase the inheritance of specific genes or traits;

(2) the modified genetic material within a gene drive organism that causes such altered inheritance, and is itself passed on at an artificially high rate.[2]

Unlike previous GMOs, gene drive organisms (GDOs) are not meant to stay where they are released, but are designed to spread and actively 'drive' their modified genes far and wide.[3] They could be used to rapidly alter the genetic make-up of wild populations, with the aim of either changing certain characteristics, collapsing these populations, or even eradicating them altogether.

In contrast to previous GMOs, where the genetic modification was carried out in a laboratory, most gene drive organisms are designed to transport the engineering action into the wild, with genetic modifications repeatedly taking place from generation to generation.

1 International Union for Conservation of Nature (IUCN), 2019 report download: https://portals.iucn.org/library/node/48408

2 Some researchers also use the term 'gene drive' to describe certain natural phenomena, a practice we find problematic - see page 17 of this critique.

3 This is particularly true for the CRIPSR-Cas9-based homing gene drives, the currently most advanced system.

Clarification: Throughout this document we use the term 'gene drive' to refer to engineered/synthetic gene drives. This reflects the original usage of the term, which was conceived to describe artificial systems to drive genes or traits into a population. The terms 'engineered' or 'synthetic' gene drives are used synonymously in the critique as in the wider scientific literature.

Overly optimistic tone and outlook of the report

It must be highlighted that the tone of important sections of the report (for example the first and last chapters and much of the two chapters on applications) is one of enthusiasm about the *potential* of genetic technologies in conservation and other fields, as opposed to a cooler and more objective presentation. The first chapter portrays synthetic biology as part of a 'Fourth Industrial Revolution', and cites a claim that innovation in this field is 'exponential',[4] without, at the same time, providing any caveats to such enthusiasm. Such an introduction will tend to influence the way the report is perceived, especially regarding subsequent discussion of the many serious risks associated with these technologies. In this chapter and other sections, the report may be faulted for its lack of sufficient critical consideration given to *claims from developers* concerning timelines, intended functionality and benefits, and assumptions of safety, which are likely to all be overly optimistic.

Lack of expected discussion of root causes and alternatives

These new technologies are framed in the report as meeting a need for 'new tools' (e.g. p. 121) to address biodiversity loss, with very little consideration being given to the fact that this loss is complex and its drivers multi-systemic; it cannot therefore be readily addressed by any single technology or approach. This critique cannot analyse all the interwoven drivers of biodiversity loss, including such factors as: agricultural production systems, land rights, land-use change, climate crisis, over-exploitation, extractive industries, or indeed dominant value systems. However, the inclusion of such considerations would not only open the way to a discussion of a much broader range of possible solutions and approaches to biodiversity loss, but would particularly help to put an as yet untried technology like gene drives into proper context for assessment.

Besides gene drives, the current IUCN report covers other genetic technologies, such as gene-edited crops and first-generation genetic modification, for example the transgenic American chestnut. This critique is not at this time commenting on those sections of the IUCN report (which is not to say that there are not problems with them); rather we are concentrating only on gene drives.

This critique identifies eight major areas of concern[5]

These need to be properly addressed before the IUCN make policy decisions on the use or release of gene drive organisms.

1. Global spread / eradication of entire species

The risks and potential consequences of engineered gene drives spreading uncontrollably within the target species - and potentially modifying or eradicating the entire species - are not adequately explored. It is of real concern that nowhere in the IUCN Report is it explicitly stated that there is a real possibility of these gene drive organisms causing alteration, suppression, or extinction

4 For a more detailed discussion of these issues, please see page 16 of this critique.

5 For details with references see main section.

of the target species, far beyond the intended geographic area. Current gene drive designs have the capacity to spread very rapidly, with potentially global reach (so-called 'global gene drives').[6] No consideration is given in this report to the consequences such an outcome could cause to ecosystems; for example, complex and potentially irreversible effects on food webs and biodiversity.

2. Lack of control

The report suggests that gene drives are controllable designer tools, whereas the current state of this technology makes it clear that any level of control is at present *purely speculative*. The introductory [first] chapter gives the impression that creating a localised gene drive system is easy and currently possible, which, simply put, is not true. It claims that '[some] types of drive systems are inherently localised' (p. 8), without acknowledging the very limited capabilities of localised gene drive systems to date, and the uncertainty about how they would perform under real life conditions. More sophisticated designs that might overcome these limitations *remain purely theoretical*. If gene drive organisms are ever released, the **geographic spread and the longevity of an engineered gene drive would be very difficult or in many cases impossible to predict**. This in turn should trigger the application of the precautionary principle.

3. Irreversibility

Little attention is given to the irreversible nature of the genetic changes that gene drives cause or could cause, and the risks and consequences arising from being unable to reverse gene drives that might have unwanted effects. The absence of feasible methods to reverse gene drives (and their modifications) is not discussed at all in the section on 'Potential adverse effects' (p.76) nor in the summary of risks in the concluding chapter (p. 122). Although critical to understanding potential adverse effects, the lack of capacity for reversal only receives a brief discussion in an earlier chapter on governance. Current proposals for so called 'reversible' gene drives are at a very early stage, and are not actually capable of reversing the set of genetic changes caused by the first gene drive mechanism (e.g. by CRISPR-Cas9. Their envisaged design is rather to stop the further active spread of a problematic gene drive or to 'overwrite' the changes such a drive has made with further genetic modifications. Such 'reversal' drives would come with their own risks and dangers.

4. Transmission to non-target species

The risk of gene drives 'jumping' species[7] and affecting species other than the intended target is downplayed. For example, concerning the most prominent current gene drive target, the mosquito *Anopheles gambiae*, researchers have noted that a gene drive could be transmitted to closely related species via cross-breeding (as the IUCN report's case study acknowledges, p. 102). The discussion on the risks of gene drives moving into non-target species (introgression) notes the concern (p. 76), but fails to explore the possible consequences at any level. What are offered instead are overstated and out of context arguments that seek to reassure the reader that this outcome of gene transfer is unlikely. This pattern of stating concerns and immediately countering them - instead of exploring their implications - is unfortunately the manner in which the report treats many important issues. This section further fails to address the hazards and risk of the well-known phenomenon of horizontal gene transfer (HGT), in which genetic material jumps species by processes other than sexual reproduction. Instead it gives the erroneous impression that horizontal gene transfer relies on sexual reproduction (which *by definition* it does not), and that therefore there is little need for concern. **The presence of such an error and misrepresentation in an important report that states it is taking an 'evidence-based approach' (p. vii) is worrying, particularly as the effect is to dismiss an important risk**.

6 Also referred to by some as 'standard gene drives', see for example Noble et al. 2019 and Esvelt et al., 2014

7 Engineered gene drives may cross species barriers either by cross-breeding (so-called sexual or vertical gene transfer) or by non-sexual means, so-called horizontal gene transfer (HGT).

5. Biodiversity effects of GDO use for agricultural pest control

There are clear intentions of using gene drive organisms for the suppression or eradication of agricultural 'pest' species, yet the report does not make this use explicit and instead only alludes to it. Gene drives targeting agricultural pest species, especially invasive alien species, are a significant focus of both current funding and research, and there is concern that the use of gene drives in agriculture could become widespread, with potentially severe negative consequences. The avoidance of clearly discussing this issue is one of the most significant omissions in the IUCN report, given the serious implications for biodiversity and conservation. One important concern would be the possibility of the gene drive organism getting back to the native range of the targeted species and unintentionally altering or eradicating the species in its original or common habitat.

Often, organisms targeted as 'pests' by certain forms of industrial agriculture may not be pests in their place of origin or in all habitats they occur in. They could also be integral parts of non-agricultural ecosystems, which provide important ecosystem services (e.g. micro-climate, water retention).

6. Assumption that any risk can be managed or predicted

Discussions of risk in the report move too quickly from exploring risks to asserting that they can be 'managed' (see for example the case study on gene drives targeting mice, p.70). **There appears to be an assumption that risks can always be predicted and managed, and that risks should therefore not stand in the way of acceptance and deployment of this technology.** However, there cannot be confidence that the hazards and risks arising from the deployment of gene drive technology have all currently been even identified, much less fully understood. It may be that some risks cannot be managed; for example, the uncontrolled spread of a highly invasive gene drive would be virtually impossible to stop.

7. Bias or conflict of interest of the authors

As the IUCN report itself acknowledges, **many of its authors are involved in developing gene drive technology and therefore cannot be considered neutral observers**. As demonstrated in this critique, there is a bias towards the acceptance and deployment of gene drives in prominent sections of the IUCN report, in particular the first and last chapters.[8] Bias is also evident in the failure to properly explore important risks and concerns.

8. The IUCN report's concluding 'key messages' are not balanced

The synthesis section in the report's key concluding chapter does not appear impartial. **Speculative and potential benefits are discussed at some length, whereas possible negative or unintended impacts, together with the current inability to understand or predict outcomes at this stage of the technology's development, receive scant attention**. Only a single point out of the ten key messages, number 6, even mentions risks, stating that 'Synthetic biology and engineered gene drive may be detrimental to conservation and sustainable use of biodiversity'. Whilst listing a few potential hazards in very broad terms, this single point does not provide details to help the reader grasp what might happen in the event of unwanted spread or demands for recall, and fails to provide clarity on the potential severity of the effects on ecosystems and biodiversity.

8 See points 2 and 8 of this critique

These eight major concerns are discussed in more detail and referenced to the literature in the following section. This critique also identifies a number of additional issues, detailed on pages 16 to 22 of this critique. Whist some sections of the IUCN report do offer sceptical perspectives (see pages 22 and 23 of this critique for examples), key sections often appear to have a biased perspective on gene drive organisms. **The inescapable conclusion is that the current IUCN report does not give an adequate basis for policy decisions regarding the development and use of engineered gene drives**. It would appear that the vast sums[9] being invested in gene drive research are influencing the views of parts of the academic community, and the IUCN should be far more aware of this when seeking advice on this topic.

For an overview of risks and uncertainties relating to gene drive technology, we recommend the recent report by 8 European Environment and Nature Conservation Agencies, 'Gene Drive Organisms: Implications for the Environment and Nature Conservation'.[10] For a more detailed exploration of risks and social, ethical and regulatory concerns, please also consult 'Gene drives: A report on their science, applications, social aspects, ethics and regulation'.[11] This report concludes that there currently exists a clear lack of knowledge concerning basic functions of gene drive technology; that many of the inherent hazards and risks of altering the evolution of entire species are neither fully understood nor manageable; and that a robust application of the precautionary principle is therefore required for the present.

9 By early 2019 Investments totalling more than $200 million have been made by the US Defence Advanced Research Projects Agency, the Bill and Melinda Gates Foundation, and TATA trusts, in addition to numerous other investments by national science funding agencies (Lebrecht et al. 2019 page 161-2)

10 Dolezel et al. 2020a https://www.umweltbundesamt.at/fileadmin/site/publikationen/rep0705.pdf

11 https://genedrives.ch/report/ -The two authors of this critique also contributed to the first and second chapters of this CSS/ENSSER/VDW report.

MAJOR CONCERNS

1. **Risks of gene drives affecting non-target populations of the same species are not adequately explored**

The IUCN report acknowledges that a critical concern with gene drives are adverse effects on 'non-target populations due to their spread beyond the target population', and that technical developments to make gene drives self-limiting are either theoretical or at an early stage (p. 76). However, it does not give a full picture of the problem itself, including the possibility of uncontrolled spread of the engineered gene drive and the serious consequences which could follow. Instead, the crucial discussion on potential adverse effects (p. 76) moves too quickly from actually exploring the possible consequences of an engineered gene drive spreading beyond the target populations, to proposing unproven ways of managing a risk that could in fact be catastrophic and irreversible. Modelling work predicting that most current gene drive designs 'are likely to be highly invasive in wild populations' (Noble et al. 2018), should, for example, have been highlighted in this section, but receives no mention.[12] Likewise, **the possible consequences of uncontrolled spread of a gene drive, which include irreversibly modifying, crashing, or even making extinct non-target populations of the same species, and the resulting unknown effects to ecosystems and their functions, are not explored, highlighted, or made explicit**.[13] Instead, the text quickly moves on to arguing that concerns about spread could *potentially* be addressed by selecting 'sites from which organism dispersal is naturally limited, and/or can be limited through management' or by targeting drives to so called 'private' or 'locally fixed' alleles[14] in the target population (Sudweeks et al. 2019). Firstly, limiting the dispersal of insects or even small mammals is likely to be extremely difficult. Secondly, it needs to be mentioned that the so called 'private' or 'locally fixed' alleles suggestion is an approach which is currently unproven, and most likely would result in rapid resistance to the engineered drive[15] (Champer et al. 2017, Hammond et al. 2017, Kyrou et al. 2018), rendering it useless. These enormous challenges to basic gene drive design and management are not discussed or even acknowledged.

12 This work is referred to in the first chapter of the IUCN report (p. 8), but the discussion immediately moves to considering 'localised drives', which therefore reveals an intention to mitigate the concern.

13 The single mention that gene drives could cause extinction of non-target populations is found in a brief discussion on their possible use against the crown of thorns starfish, within a case study dealing with a different topic – namely the use of genetic techniques to adapt corals to acidification and climate stress.

14 Alleles are alternative versions of a gene that have arisen through mutation. Such sequence variants of a gene are relatively common and will mostly have no or little impact on the phenotype, though some will do. The larger a population and the more frequent the exchange with other populations, the higher the frequency and number of sequence variants, i.e. alleles. A 'fixed allele' describes a situation where there is only one version of the gene (an allele) present in a population. The approach of using 'locally fixed alleles' is based on the assumption that, in genetically isolated populations, for example on islands, there will be genes with fixed alleles, whereas the same gene will have different variants in other populations. If a gene drive targeting the isolated population is directed to the fixed allele, it would in theory be more difficult for the drive to spread into the more genetically diverse population. But this theory remains to be proven in practice, and other hurdles remain.

15 To date the emergence of alleles resistant to CRISPR-Cas9 cleavage has been shown to be a major obstacle for the design of functional homing gene drives (Champer et al. 2017, Hammond et al. 2017). The only exception to this is when drives are targeted to highly conserved and essential genes, which would not be subject to the variation that gives rise to alleles (Kyrou et al. 2018).

Case study 1 (pp. 70-72) looks at the potential use of engineered gene drives to control invasive rodents on islands, but the possibility of effects on non-target populations of the same species does not receive adequate discussion. In particular, the possibility of a crash in mainland populations, if mice or rats carrying a suppression gene drive were to somehow migrate off islands, is not stated explicitly, nor its consequences explored. This serious risk could thus easily be missed by a reader without specialist knowledge. Mice and rats are well known to stow away on various forms of human transportation (Baker 1994), which is of course one of the key factors that has allowed them to invade such islands and become such widespread and invasive species in the first place. It is suggested that the chances of unintended spread can be managed by using isolated islands and bio-security protocols; yet if the gene drive were deployed on many islands (as is intended) the probability of escape or intentional transport by humans would increase. Experience with previous island mouse and rat eradications, using toxicants, also shows that islands are sometimes re-invaded;[16] which suggests that *the risk of unintended movements cannot be reduced to zero*. Given there are strong economic incentives to control rodents in other settings, for example food stores, the possibility of intentional release - authorised or unauthorised - on mainland areas also needs consideration.

Case study 6 (p. 100 – 103), which considers the possible use of engineered gene drives to supress *Anopeheles* mosquitos in their native range in Africa, makes no mention of the difficulties involved in limiting the spread of such drives.

For a more in-depth discussion of the issues regarding the use of engineered gene drives against mice and mosquitoes, please see footnote.[17]

2. The report suggests that gene drives are controllable designer tools, when in fact any level of control outside the laboratory remains speculative

The introductory first chapter of the report states that '[some] types of drive systems are inherently localised due to some sort of frequency dependence…' (p. 8) and 'The geographic spread of local drive systems is limited by their dependence on the frequency of other genetic elements…' (p. 9). These statements give the misleading impression that employing a localised drive is currently possible, when in fact the capabilities of such technology remain highly speculative. **As acknowledged much later in the report, many proposed approaches to 'limit' or 'localise' drives have not yet been shown to work, even in the laboratory (p.76)**.

Whilst there are many types of engineered gene drives, the so called 'spatially controlled' or 'localised' drives that have been constructed thus far are predicted to have very limited capabilities. At present, the only forms of potentially 'localised' gene drives, that have been demonstrated to work within a laboratory experiment, employ a principle called 'threshold dependence' (Akbari et al. 2013, Reeves et al. 2014) . The 'threshold' for such drives is the minimum percentage of a population that must be made up of gene drive organisms for the drive to eventually spread right through the population. In many cases, the threshold is 50% or higher (Akbari et al. 2013, Reeves et al. 2014).[18] This means that the release of gene drive organisms would have to be done at a ratio of at least 1:1 to the wild type population. For threshold-dependent

16 The Database of Island Invasive Species Eradications (http://diise.islandconservation.org/) shows that rats and mice have re-invaded 139 islands where they have previously been eradicated. For comparison, around 560 islands where rats and mice have been eliminated have remained free of these rodents.

17 Please see case studies 1 and 2 in Steinbrecher et al. 2019; and Chapter 6 in NASEM 2016.

18 A paper entitled 'Small molecule control of super Mendelian inheritance in gene drives' - published in June 2020 - describes a CRISPR-Cas9-based drive in Drosophila, where the gene drive activity is inducible with a specific small molecule (López Del Amo et al. 2020). The authors suggest such a system might provide control of a gene drive in the field, which would require the administration of a particular small molecule in the feed to activate the drive. However, amongst other technical hurdles, the concentrations of the small molecule needed to activate the drive would be very difficult to achieve outside of a controlled environment.

systems, the lowest threshold reported in the literature is 24% (Champer et al. 2020). Some designs are even predicted to require multiple releases at a 1:1 ratio to become established (Li et al. 2020). The relatively high thresholds for the systems described thus far mean that such drives would require the release of a *very large number* of cage-reared gene drive organisms (GDOs) to treat even a small geographic area. It must also be noted that these theoretical localised gene drives are classed as 'replacement drives' - meaning their purpose is to replace natural populations with genetically modified ones; a goal which holds clear risks in itself.

In the case of insect pest control, the requirement for such large releases would make such systems comparable to sterile insect techniques (SIT, including transgenic SIT) in terms of the resources required. It is furthermore very difficult to predict how any proposed 'localised' drives would perform outside of a controlled laboratory environment; there can be no certainty that such systems would effectively restrict the spread of genetic modifications or deliver the intended outcomes. Proposals for more complex 'temporally controlled' gene drives, which could perhaps overcome the requirement for large releases – so called 'daisy chain' drives - lack any laboratory proof at present. Unfortunately, none of the above is made clear by the IUCN report, and most readers would be left unaware of these severe limitations on methods to control the technology once it is deployed.

The limitations of genetic 'control' systems that are intended to prevent the spread of genetic changes in wild populations through the release of genetically modified organisms, is illustrated in reports concerning the release of (non-gene drive) Oxitec transgenic mosquitoes in Brazil. These engineered mosquitoes were designed to categorically prevent the spread of genetic modifications into wild populations, by passing a 'killer gene' on to all their offspring, causing these offspring to die at an early stage in development (e.g. larval stage). In this case, genetically engineered material was *nevertheless transferred into wild populations*, despite assertions and theoretical predictions to the contrary (Evans et al. 2019). **This is an important early example demonstrating that engineered lethality can neither be completely effective nor relied upon as a safeguard**.

3. The expected irreversibility of gene drives and the serious hazards arising from it receive little attention

Most gene drive designs are expected to cause irreversible genetic changes in wild populations of their target species, so there is great concern that engineered gene drives could cause harmful effects that cannot be undone. For example, the gene drive organisms developed to modify *Anopheles stephensi* described by Gantz *et al.* (2015), carry a section of genetic material inserted into their genomes that includes a CRISPR-Cas9 'genome editor' (DNA-cutter), a gene that causes carriers to develop red eyes, plus two genes conferring resistance to the malaria parasite. If such altered organisms were ever released, both the genetic elements they carry and the intended and unintended genetic alterations created by the DNA-cutter could spread into the population, either through further action of the engineered gene drive, or, if some form of resistance to the drive emerges, through normal Mendelian inheritance. It is difficult to even imagine how these genetic alterations in the wild population could be undone. Should there be the need to remove the modified organisms for urgent safety, ethical, legal or socio-economic reasons, that goal *would be impossible to achieve*.

This vital issue is, however, avoided in the relevant sections of the report. It is not mentioned in the summary of risks in the key messages (p. 122), nor the section on potential adverse effects (pp. 76-77). This is another area in which the IUCN report does not give a full picture of risks. Discussion is in fact limited to a single paragraph (p. 41) in the chapter on governance, which itself is open to criticism.

Most importantly, the report's treatment of this issue does not attempt to clearly state or differentiate the different levels of irreversible effects that gene drives could cause in one or more species. These would likely include effects on:

- genome sequences (genotype)
- characteristics/traits of organisms (phenotype)
- populations and abundance of a species, including local or global eradication

The paragraph on page 41 of the report argues that 'in some cases, as in use of engineered gene drives to eradicate a species from a certain habitat, irreversibility could be seen as part of the intent'. This statement is problematic because it only addresses effects at the population level and does not acknowledge the serious concerns about irreversible effects on genotype and phenotype. Imagine, for example, if a gene drive organism designed to crash a population of insect pests were released, but resistance to the drive emerged in the target population due to a mutation arising from the cutting process. The result could be a new and unintended population made up largely or completely of genetically modified organisms (Friess, von Gleich, and Giese 2019). These new GM insects would not only be numerous and possibly exhibiting a wide range of GM variations, but they would also be untested and unassessed (Dolezel et al. 2020a).[19] **This illustrates a particular risk of CRISPR-based gene drives, which is that the action of genetic modification will no longer be confined to the laboratory, but rather is taken out to the wild. Thus genetic modification occurs again and again with each new generation of gene drive organisms (Simon, Otto, and Engelhard 2018), which means unpredictability increases**.

A related question concerns the difficulty or impossibility of stopping or reversing the action of an engineered gene drive once it has been initiated. **The discussion in the IUCN report considers how risks of 'indirect or unintended environmental impacts' might be managed by releasing a second gene drive to partially overwrite the genetic changes made by the first. Whilst it is rather uncertain if a first gene drive would work as predicted, it is also not known whether or not a second or third would be any more predictable**. Although in principle shown to work for yeast in laboratory settings (DiCarlo et al. 2015), so-called 'rescue', 'killer', or 'reversal' drives are largely theoretical.[20] Even if they were able to stop the further super-Mendelian spread of the engineered drive, they would still most likely leave a trail of genetically modified organisms behind.[21] Indeed, in the case of CRISPR-Cas9 gene drives, active CRISPR-Cas9 genes and molecules would remain in the population with the potential to initiate yet further unintended changes[22] over time, and to some extent would maintain a 'lab in the field' capacity. The fact that such approaches are very unlikely of being capable of restoring a population to its wild-type genetic state means that calling them 'reversal drives' is a misleading terminology, which also should be clarified in the report. As Champer et al. point out: '*Of note, despite their name, reversal gene drives do not restore the original modification to the wild type; rather, they induce further changes that may undo a phenotypic alteration caused by the initial gene drive* [our italics].' (Champer, Buchman, and Akbari 2016). None of these serious issues are discussed in the present report.

19 See also Dolezel et al. 2020b and pp. 99-100 (CSS/ENSSER/VDW 2019)

20 Working with the yeast Saccharomyces cerevisiae, DiCarlo et al. (2015) used a first CRIPSR/Cas-based 'homing gene drive' to disrupt a gene (ADE2) and then a second one to reinsert the (slightly altered) gene, thus repairing the loss of function. However, as they stated: 'In principle, such an overwriting drive can restore the original phenotype - in this case the loss of ADE2 - but the resulting organisms will still be transgenic due to the presence of a residual Cas9 gene and guide RNA.'

21 A reversal drive using a CRISPR-Cas9 homing drive would leave a genetically modified population behind – as already detailed in the previous footnote for both the homing drive (DiCarlo et al. 2015) and the 'Cleave and Rescue' replacement drive (Oberhofer et al. 2019 & 2020). There has been a complex theoretical proposal to use a combination of different engineered gene drives to first modify a population and then to restore the population to a wild-type state. This proposal relies on an entirely theoretical drive system named 'Daisy-Quorum drive' that is intended to be localised (see Min et al. 2017). If one wanted to re-establish wild-type population this would be reliant on reinvasion from surrounding areas or on large-scale releases of wild-types.

22 Dolezel et al. 2020a, see also p. 127 Steinbrecher et al. 2019.

4. The risks of gene drives jumping between species are erroneously defined and downplayed

Not enough consideration is given to the possibility that synthetic gene drives could directly affect non-target species. This is an important concern. For example, the gene drive target *Anopheles gambiae* can hybridise (breed) with other members of the *An. gambiae* species complex (Fontaine et al. 2015). This means there is potential for a gene drive to jump species; indeed, this possibility is identified as a risk in the case study in the IUCN report (pp. 102-103). The risk is highlighted by recent results showing introgression of an engineered sex ratio distortion system (resembling a gene drive), from *An. gambiae* into another member of the complex, *An. arabiensis*, in a laboratory environment (Bernardini et al. 2019). Were a gene drive targeting *An. gambiae* to spread into other members of the complex, it could potentially impact the species *An. quadriannulatus*, which is not considered a malaria vector. Another important gene drive target, the house mouse, is also known to hybridise with close relatives (Zechner et al. 1996, Payseur, Krenz, and Nachman 2004),[23] but this is also not mentioned in the IUCN report.

As with other important issues, the risk of engineered gene drives jumping to non-target species receives only a very brief discussion in the relevant section of the report (p. 76). Not only is it not sufficiently explored, arguments are made suggesting that it should not be a concern. For example, the report states that the 'potential for gene transfer via interbreeding is generally relatively low, since instances where interbreeding may occur can in most cases be identified from existing knowledge' (p. 76). The logic here is not completely clear, but the sentence seems to be suggesting that if this risk is identified as being present from 'existing knowledge', it is not a real risk and can somehow be 'managed'. But no detail is given as to how this potentially very difficult task could be accomplished. It must be added that 'existing knowledge' of hybridisation between species is far from complete. To counter concerns about gene transfer between species, the report also states that 'multiple genetic changes are generally needed for phenotypic change' (p. 76). This seems intended to be reassuring, but is misleading; it does not reflect how most gene drives are designed in practice. Whilst the statement may be factually correct, disruption of a single gene in many cases can and does cause phenotypic changes. The report's reassuring claims may thus seem plausible at first glance, but since many gene drive designs target genes whose modification will alter phenotype (e.g. SRY in mice, *doublesex* in mosquitoes), these claims do not stand up to more rigorous scrutiny.

As well as transfer between species via hybridisation (sexual reproduction), there is also the potential for gene drives to jump to unrelated or more distantly related species via horizontal gene transfer events (Rode et al. 2019). *Horizontal* gene transfer (HGT) is generally defined as the acquisition of genetic material by one organism from another that is not its parent (in contrast to *vertical* gene transfer, where genetic material is transferred from a parent organism to offspring). HGT of 'selfish' genetic elements such as 'transposable elements' (also problematically termed 'natural gene drives' by some) has been shown to have occurred frequently in the evolution of insects (Peccoud et al. 2017) as well as vertebrates (Zhang et al. 2020). It has been suggested that such horizontal transfer is mediated, for example, by viruses (Gilbert and Cordaux 2017), intracellular symbiotic bacteria such as Wolbachia and spiroplasms (Dunning Hotopp et al. 2007), parasitoids such as wasps (Yoshiyama et al. 2001), or parasites like mites (Houck et al. 1991; for a review of HGT in *Drosophila melanogaster* see Loreto, Carareto, and Capy 2008).

23 Hybridisation between the mouse *M.m. musculus* and *M.m. domesticus*, which are sometimes considered subspecies, is known to occur in the wild, generally giving fertile offspring (Payseur et al. 2004). *M.m. domesticus* can also produce fertile female hybrids with closely related species such as *Mus spicilegus* (Zechner et al. 1996).

The risk of horizontal gene transfer (HGT) of engineered gene drives[24] therefore needs very careful evaluation. However, this core issue receives no analysis at all in the IUCN report. The discussion of this important issue on page 76 is unfortunately completely muddled by the entirely incorrect assertion that HGT relies on *sexual reproduction*. The paragraph then proceeds to discuss the risk of gene drives crossing species barriers via hybridisation (vertical gene transfer), without any consideration of the actual subject: *Horizontal* Gene Transfer (HGT), which, for clarity, is also known as *non-sexual or lateral gene transfer.* Whatever the reason for this rather worrying error, the result is that another important risk is brushed aside, in this case on the unsound basis that HGT is the opposite of what it actually is.

Similarly, despite the evidence that over evolutionary timescales successful HGT is relatively common between insects (Peccoud et al. 2017), that risk is not even discussed in the case study concerning the use of gene drives in mosquitoes. In the case study on invasive rodents (p. 70), the risk of HGT *is* raised[25] but then immediately countered with the statement that 'animals are largely unaffected by HGT'. Recent genome analysis shows HGT does occur in vertebrates, including in mammals (Zhang et al. 2020), so this is not a reason to dismiss this risk. Whilst the likelihood may be regarded as small, the consequences, should it occur, could be very significant. A second argument is then made by the report, stating that 'multiple genes are generally needed for phenotypic change' (p. 70); which we would question on the same grounds as outlined earlier (see discussion on phenotypic changes, three paragraphs above).

5. The possible conservation and biodiversity impacts of gene drive use in agricultural pest control are ignored

Gene drive developers are well aware of the intention to apply this technology to agricultural pest control. For example, a report from the Australian Academy of Sciences states that 'Australian agriculture is a promising area for gene drive applications.' Significant funding is being directed to developing drives to target agricultural pest species,[26] and at least one project, for the fruit fly *Drosophila suzukii*, has reached proof of concept stage (Buchman et al. 2018) . Preliminary research to underpin gene drive development is being carried out in a number of other agricultural pest species, including the diamondback moth (*Plutella xylostella*), the olive fruit fly (*Bactrocera oleae*), the oriental fruit fly (*Bactrocera dorsalis*) and fruit flies in the *Anastrepha* and *Ceratitis* genera (Koidou et al. 2020, Harvey-Samuel et al. 2019, Zhao et al. 2019, Sim et al. 2019). A range of other insect pests have also been proposed as possible targets, including the brown plant hopper (*Nilaparvata lugens*), the silverleaf whitefly (*Bemisia tabaci*), and the Asian citrus psyllid (*Diaphorina citri*) (Scott et al. 2018). Whilst some agricultural pests are invasive alien species, all agricultural pests will have a native range or natural habitat where they are an integral part of ecosystems and contribute to biodiversity. What if a GDO finds its way back to the native range of the target organism? What if we eliminate a species unwanted by certain actors, and it turns out to have been an important component in maintaining ecological balances or resilience? Whether fruit flies or moths or caterpillars, they are commonly part of extensive foodwebs that need to be maintained for biodiversity to survive and

24 Here constituting any 'synthetic selfish genetic elements'

25 The risk of HGT is also noted in a single sentence in a case study that considers the use of gene drive to control crown-of-thorns starfish, yet this case study does not also note the risk of the drive transferring between species via hybridisation (vertical gene transfer) (p. 91)

26 E.g. by the California Cherry Board (Buchman et al. 2018) and the US Department of Agriculture (https://nifa.usda.gov/announcement/nifa-invests-research-implications-gene-editing-technologies)

flourish. **Incomprehensibly, given the funding and literature on the subject, the potential to target agricultural pests with gene drives is never directly mentioned or made explicit anywhere in the IUCN report.**[27] **The enormous harm to biodiversity and ecosystems that such use could cause is not explored at all**.

Whether intentionally or not, the report has avoided any proper consideration of one of the most significant concerns regarding the practical use of this technology. For a more in-depth consideration of the issues related to employing gene drives for agricultural pest control please see Medina (2017), Preu *et al.* (2020) and 'Agricultural insect pests as gene drive targets' in Steinbrecher *et al.*, 2019 (pp. 120-124).

6. The assumption that any risk is 'manageable' is problematic and unfounded

Underlying much of the discussion in the IUCN report is a tacit assumption that any and all risks identified with this technology can be 'managed', and so should not stand in the way of its acceptance and deployment. This statement on the scope of Chapter 5 (p. 67) is an example: '[the chapter] explores the potential positive conservation outcomes from such applications [of synthetic biology including gene drives] and details important considerations, while also recognising that many situations, *if not managed appropriately*, could potentially also have negative impacts on conservation [our italics] '. This implies that the tools for proper management exist and simply have to be deployed, whereas so far, they do not; as explained in points 1 to 4 the technology brings risks that would in fact be very difficult to 'manage'. A second example is that more than half of the section that is supposedly considering 'Potential adverse effects and limitations' of engineered gene drives for conservation (pages 76-77) instead is given to discussing the potential for risk management through various methods, in particular the choice of release sites (as a means of risk management) or the so far hypothetical utilisation of private and fixed alleles to reduce the spread to non-target populations. In other words, once again the report does not describe the actual risks but emphasizes supposed or theoretical ways of dealing with them.

It must also be emphasised that by presenting the known hazards with gene drives as controllable through 'risk management', the IUCN report is implying that gene drive technology will proceed on the basis of case-by-case risk assessment that will formulate risk management strategies. Such an assumption is vastly premature, given that **there cannot yet be any confidence that the hazards and risks arising from any utilisation of a gene drive have all been identified or fully understood** (Breckling and von Gleich 2020, Then 2020, Friess, von Gleich, and Giese 2019), nor that current risk assessment frameworks and methodologies are adequate (Dolezel et al. 2020b).

For example, a spectrum of poorly understood risks emerges from permanently inserting 'genome editors' or 'DNA-cutters' like CRISPR-Cas into eukaryotic genomes. As Dolezel et al. (2020a) state, 'In the long term, off-target effects and unintended effects at the molecular level may occur with unknown ramifications for the resulting phenotype as well as for genome-environment interactions.'

Instead of assuming that all the risks and consequences of engineered gene drives are already understood and can be managed or mitigated, a more responsible approach would be to recognise that much deeper and wider analysis and knowledge are required to assess such a new and powerful technology, with regard to its possible applications and

27 The text of the IUCN report appears to avoid making this point completely explicit. Only with careful reading one can see it is alluded to in just three places. Section 6.3 states 'Various applications have been proposed using synthetic biology to combat different types of pest responsible for damage to both agricultural and human health' and 'The development of engineered gene drive strategies for malaria vector control and other synthetic biology pest control applications is an emerging field…' (p. 99). Section 6.2 mentions gene drives could be used in agriculture but does not say for what (p. 98).

to making policy. This would have to include the trajectory of what changes using GDOs might make to an ecosystem, and all the consequences and problems that might arise. **Without better knowledge, which will take time and research to acquire, and without fully understanding the complexity of the underlying problems, all the issues this technology seeks to address — including loss of biodiversity — may well not be alleviated, but very significantly increased**.

7. Too many of the IUCN report's authors are actively involved in developing gene drive technology

The report itself (p. 57) says that many of its authors are developers of synthetic biology or gene technologies. One would therefore expect them to be advocates for the technology. For balance, the assessment group should also have included voices that were more critical of these technologies, and should have given space to such voices in each of the chapters, including their assessments regarding potential adverse impacts. The tone of much of the report suggests that few such individuals were involved or such voices included.

The case studies concerning GDOs are a particular area of concern. As Chapter 4 tells us, the authors involved in drafting them in most cases 'also have a strong interest in investigating the feasibility of the application, *so they are not entirely neutral observers*' (p. 62, italics ours). It is however emphasised that principles of 'objectivity and robustness' have been applied in order to ensure that the case studies were reviewed by other members of the technical subgroup. Unfortunately, we cannot be sure of these other members' neutrality either, or if there were sufficient breadth of knowledge amongst them to be able to thoroughly consider all risks. It is not necessarily the case that the reviewers (or perhaps authors) are fully informed of all the issues, risks and concerns, and some of the enthusiasm of the authors is - in our opinion - unduly reflected in the final texts.[28]

The choice of authors also raises the concern that the report may have a tendency to advocate the technology, which we now observe to be the case. This is evident from the seven other points discussed in this part of the text, and the additional issues raised in the following section.

8. The IUCN report's concluding 'key messages' are not balanced – they are heavily weighted towards speculative potential benefits and give very little attention to the numerous possible negative impacts and current lack of basic predictive knowledge

The synthesis section in the report's concluding chapter finishes with a list of 'Key Messages' (pp 121-123) that predominantly draw attention to speculative benefits, as opposed to risks, and thus do not appear impartial. Point 2 states that 'Some synthetic biology and engineered gene drive applications, if appropriately designed and targeted, could enhance biodiversity conservation…'. Point 4 enthuses that 'Engineered gene drive systems can be a transformative tool for direct conservation as well as in other sectors like public health….', while point 5 again states only the unproven positive side of this as yet unperfected technology, namely that 'Synthetic biology and engineered gene drive may be beneficial to conservation and sustainable use of

28 See points 1 and 4 for concerns regarding case study 1 on the use of gene drives to target invasive mouse populations

biodiversity'. Only a single point out of the ten key messages, number 6, considers risks, stating that 'Synthetic biology and engineered gene drive may be detrimental to conservation and sustainable use of biodiversity'. Whilst listing a few potential hazards in very broad terms,[29] this single point does not provide details to help readers grasp the possible dangers; it also provides no discussion of merit on the potential severity of the effects on ecosystems and biodiversity.

This section of the report does note that all of the points described above are speculative, but does not go any further to acknowledge the continuing scientific uncertainty surrounding the effects of engineered gene drives, including the speculative beneficial outcomes so often mentioned. **There is no recognition of two very key practical points: first, the inherent difficulty of predicting the results of gene drive deployment; and second, the current lack of knowledge on which to base predictions and foresee risks**.

29 Point 6 then lists: 'Detrimental effects may stem from the movement of genes, or escape of engineered gene-drive-carrying organisms, impacting non-target populations or species {5.2–5.3, 6.2-6.4} (speculative), changes to ecological roles played by target organisms {5.2, 6.3} (speculative), broader ecosystem effects {6.2} (speculative), product replacement that exacerbates a conservation problem {5.2.2} (competing explanations), socioeconomic effects of product replacement on livelihoods and on production and consumption patterns {6.4} (competing explanations), distracting funding from other conservation approaches {5.1, 5.4} (speculative), and moral hazard reducing the urgency and importance of biodiversity conservation {2.3, 5.1} (speculative).'

ADDITIONAL AREAS OF CONCERN

The tone of key sections often veers into 'techno-hype'. This gives the report's arguments the sense of a sales pitch and undermines the impartiality expected of such a document.

The term 'techno-hype' refers to rosy descriptions of new technologies against a claimed background of 'ever-accelerating change'.[30] In several places, particularly in the introductory and concluding chapters, there is an unquestioning restatement of proponents' claims about the pace of developments in synthetic biology, along with an emphasis on the power of these technologies; which seems to both express and elicit a naïve enthusiasm for the field. Similarly, the graphics in the first chapter seek to communicate a positive picture of a rapidly growing field with many potential applications. The tone in several cases becomes almost breathless: 'Much of synthetic biology innovation, especially in enabling technologies, is considered to be exponential, and is considered to be a domain of the Fourth Industrial Revolution, blurring the lines between the physical, digital and biological spheres.... ...and is characterised by its "velocity, scope and systems impact"...' (p. 2). **Inflated claims for the potential of new approaches and technologies are unfortunately common in popular as well as in some academic literature relating to biotechnology, and often reflect the desire to secure visibility and funding**. This creates a difficulty in discriminating between actual experimental results and speculation about what may be achieved in the future. The IUCN's report should have exercised much greater care in trying to avoid this popular, yet problematic, stance.

Readers of the IUCN's analysis may take claims and speculations regarding synthetic biology as fact, since they are being presented in what they understand to be a scientific report. Such statements also may leave readers with the false impression the technology is completely functional and ready to be deployed. However, as a whole, synthetic biology is proving to be slow to deliver many real-world applications. For example, despite initial optimism and substantial investments over the last two decades, synthetic biology based biofuel technologies have not delivered the promised outcomes.[31] Some of the claims about synthetic biology may perhaps in time become feasible, but many such new technological possibilities take much longer to develop than anticipated, as for example the history of gene therapy teaches us;[32] or they may never materialise at all.

30 See 'Ever Accelerating Hype' (https://www.prospectmagazine.co.uk/magazine/everacceleratinghype) (Edgerton 1997)

31 'Why the promise of cheap fuels from super bugs fell short' https://www.technologyreview.com/2014/02/05/14111/why-the-promise-of-cheap-fuel-from-super-bugs-fell-short/ (LaMonica 2014); also see Ferry (2015) and Winters (2020)

32 Research on correcting genetic defects in mammalian cells began in the 1960s (Goswami et al. 2019). In the mid 1980s, with the widened tools of genetic engineering, it was believed that at most it would take another 10 years for human gene therapy to be delivered safely and effectively for human [inherited] genetic diseases (such as haemophilia, cystic fibrosis or sickle cell anaemia). Yet despite tremendous efforts, it is only in the last five years that approved treatments have reached double digit figures – and only a few of these are being clinically used (Shahryari et al. 2019).

Arguments in favour of deploying gene drives or other genetic technologies are too often given precedence over calls for caution

Whilst the report nominally considers different perspectives on genetic technology, their weight and presentations are not well balanced. For example, arguments in favour of deploying gene drives or other synthetic biology 'solutions' are often presented *after* arguments that question the wisdom of such 'solutions'. This form of presentation has the effect of brushing the concerns aside and giving the 'last word' to the proponents of these technologies, implying that any arguments calling for caution have been addressed. Examples include the discussion on attitudes within the conservation community regarding synthetic biology in paragraphs 2 and 4 of page 3; a moratorium on the release of synthetic organisms on page 21; or the paragraph on the precautionary principle on page 123. The report's discussion and analysis of risks follows a similar pattern; see this document's main points 1 and 4.

Lack of rigour in distinguishing between engineered gene drives and naturally occurring selfish genetic elements

The report adopts the problematic practice of describing naturally occurring 'selfish' genetic elements as a form of 'gene drive'. For whatever reason, this practice has more recently become widespread, yet because evolution has allowed species to adapt to these natural phenomena, it is important to clearly distinguish natural, selfish genetic elements from human-made, engineered gene drives. Because the synthetic forms are often simply called 'gene drives', using the same term for both hinders clear and meaningful discussion of the issues. The report itself helps create this confusion, stating that 'gene drives' are natural phenomena, but then, in certain places, uses the terms 'gene drive' and 'engineered gene drive' interchangeably when referring only to the engineered systems. We are concerned that this blurring of the language contributes to making engineered gene drives look 'natural', suggesting safety and familiarity, whilst the opposite is true.

The term 'gene drive' has most commonly been used to describe a *human technology* consisting of engineered genetic elements that display non-Mendelian or 'super-Mendelian' inheritance. It may also be used to refer to the use of such elements to *intentionally* propagate genetic changes at the population level.[33] Many in the research community however, have recently started employing the term 'gene drive' to describe *natural* genetic phenomena that result in an inheritance bias (so-called 'selfish genetic elements').[34] This

33 We note that in the English language the word/verb 'drive' is almost always associated with human agency, and always with intent.

34 For an explanation of 'selfish genetic elements' please see the review by Agren and Clark (2018).

problematic and ambiguous use of 'gene drive' is used several times in the IUCN report. However, the two papers cited by the IUCN report as showing that most sequenced genomes contain 'gene drives' (Feschotte and Pritham 2007, de Koning et al. 2011), actually refer to DNA transposons and transposable elements. Neither of these papers use the term 'gene drive'. A number of genetic mechanisms or genetic elements occurring in nature have thus been *retrospectively* termed 'gene drives'.

Making a clear distinction between natural phenomena and engineered ones is important, because the former is embedded in evolution. Over time, evolutionary adaptation to naturally occurring 'selfish genetic elements' counteracts the fitness costs or disadvantages of such inheritance. Indeed, such elements -for example transposons- are increasingly seen to play an important role in the evolution of genomes, as well as in speciation (Zhang et al. 2020). On the other hand, *engineered* gene drives are a man-made mechanism intended to forcibly modify ecosystems and entire species, and to do so over relatively short timescales. **Since synthetic gene drives and gene drive mechanisms would be newly introduced, it is unknown how evolutionary forces will react to them or on them**. Accordingly, and especially given the intended speed of the alterations and spread, it is highly unpredictable how the evolution of species as well as ecosystem evolution will respond over time.

The IUCN report thus fails to employ a basic element crucial in any scientific discussion: to define terms clearly and to use them in a consistent manner. Clarity on such a key distinction is crucial in such a report, which is intended to inform and underpin policy and decision making. A lack of clear term definitions not only hinders discussion of the issues, but also increases the likelihood that the technology will not receive proper scrutiny from policy makers.

The framing of genetic modification in the introductory chapter obscures key concerns about the technology

The introductory chapter unduly portrays genetic modification (referred to by the IUCN report as synthetic biology) as benign developments of selective breeding (p. 5), without making important distinctions clear. In particular, it omits the crucial point that most modern biotechnology involves the transfer of genetic material from very different organisms (such as transferring CRISPR-Cas9 DNA-cutters, Bt-toxin or antibiotic resistance genes from bacteria into animals or plants), which is not what happens in breeding. Moreover, none of the known risk issues arising from the genetic engineering processes are mentioned. These would include unintended process-induced genome-wide mutations, such as accidental sequence deletions and disruptions of functional genes; rearrangement and scrambling of genomic sequences near the insertion site; unintended addition of bacterial DNA; or scattered genome-wide sequence alterations (Wilson, Latham, and Steinbrecher 2006). In the case of genome editing techniques, additional unanticipated off-target and on-target effects of this or similar nature have been observed and are to be expected (Eckerstorfer et al. 2019). A particular risk issue here is the unintentional production of new or truncated proteins and of novel mRNAs, as well as altered RNA regulation and altered ribosomal entry, due to CRISPR-Cas9-induced frameshift mutation, as for example observed in 50% of 'genome-edited' mammalian (here human) cell lines (Tuladhar et al. 2019). The fact that altered and new proteins and messenger RNAs were found is clearly worrying, as this can: give rise to altered behaviour and phenotype; weaken or strengthen organisms; change their interactions; make them toxic; or make them easy prey. Whilst at times there will be no effects on an organism, at other times there will be, resulting in unknown and unpredictable outcomes and thus constituting serious risk.

Current levels of understanding of genomes, and, by implication, the developers' ability to *foresee* the consequences of genetic modification, are over-stated. Early on, the reader is told that 'Digital sequence information… …enables researchers to view and *understand* the blueprints of an organism… [our italics]' (p. 6), yet elsewhere in the report it is rightly noted that scientific understanding of genomes (p. 94) and epigenetics (p. 9 and 10) is still very incomplete. The continuing difficulties in researchers' understanding of even the simplest bacterial genomes are illustrated by attempts to generate 'artificial' cells with a 'minimal' genome, in which all non-essential genes are cut out of the genome. The resulting cell contains around 470 genes, but for about 100 of these genes *it is not understood why they are essential*. Even the simplest genome in one of the simplest organisms is still not 'understood' (Powell 2018) in the way that the report's discussions imply.

The representation of the precautionary principle does not reflect its intent, nor how it is used in practice.

The IUCN report fails to capture the essence and intent of the precautionary principle: to safeguard against serious negative impact of new technologies or substances that may cause significant harm to the environment[35] or to human and animal health. Examples of the failure to employ the precautionary principle are the significant harm and suffering caused by asbestos, thalidomide, chlorofluorocarbons (CFCs), or polychlorinated biphenyls (PCBs). As detailed and discussed in the European Environment Agency 2001 report 'Late lessons from early warnings' (EEA 2001), the requirement to apply the precautionary principle is meant to halt the deployment of a new and potentially harmful technology/substance prior to it causing irreversible damage, not to help open doors to them. This stance is based on a history of serious damage to the environment as well as to human health, where there had been early indications and warnings of damage. These were ignored until very serious damage had occurred on a wide scale and restitution or repair became difficult or impossible. However, section 7.2 of **the report proposes a 180-degree flip in terms of how to interpret the precautionary principle, to be specifically applied to the technologies and substances of synthetic biology. Presenting such counter-interpretations of this established, single principle as 'dual interpretations' (p. 123) with equal value, can here be seen as a step towards eroding or perverting the precautionary principle as it stands and is intended to be applied**. Similarly, the notion of replacing the precautionary principle with cost-benefit analysis is sympathetically discussed on page 29, without any other analysis or consideration of the dangers this would entail. These stances seem to be trying assiduously to erode one of the few safeguards available to society for the conservation and sustainable use of biodiversity, as well as human health. **Coming from a world-renowned conservation body, these suggestions to change a key protective definition are therefore very disturbing**.

Calls for a moratorium on release of SynBio organisms (including gene drive organisms) are misrepresented

The report fails to clarify what a moratorium is, along with what it is not, and invites the reader to draw erroneous conclusions. It would have been important to state that a moratorium is not a ban, but rather a clear agreement on a set of conditions that must be met before specific activities can take place, be they commercialisation and use of new pharmaceuticals, or release of synthetic organisms, including gene drive organisms, into the environment.

35 e.g. to conservation and sustainable use of biodiversity

In the 'Synthesis' section, the report seems to be trying to exaggerate the consequences of a possible gene drive moratorium, and indeed to undermine it as an option, by making the statement that, if society decides 'that some research should not proceed', there 'will be no new evidence'. This fails to recognize that a vast range of research and knowledge from different disciplines and perspectives is relevant to risk assessment and decisions on releases of engineered gene drives into the environment. Most of this research could continue and would be ongoing even if there were a moratorium on such releases.[36] Indeed, much research would need to have been newly initiated and undertaken prior to any releases.

In the section on the precautionary principle, however, the report implies that a moratorium means opting to reject possibly viable methods of addressing a problem due to a 'desire for caution' regarding the risk of (any) intervention in general (p. 21). This line of argument edges the reader towards an interpretation of the precautionary principle that calls for measures taken to be 'cost effective' and allow some 'well-regulated risks'.

Whilst the text does not illuminate what a 'well-regulated risk' in these cases would actually be, this section nurtures the assumption that a moratorium would be ill-considered, and might constitute a problem, rather than performing its intended role as a protective safety measure. In practice, a moratorium is intended as a pause to allow for further deliberation and further study, leading to a better understanding of the possible consequences of the technology and its utilisation. **As generally understood internationally, a well-implemented moratorium offers the space — and especially the time — to create data, high quality analyses, and wider, more participatory technology assessments**. This is needed to develop guidance and regulations in order to ensure social and ecosystem safety. In short, moratoria allow society the time and space to arrive at real solutions that are environmentally sustainable, ecosystem-based, and resilient.

The IUCN report states that 'some civil society and scientific organisations' are arguing that the precautionary principle 'necessitates' a moratorium on release and commercial use[37] until government bodies, with full public participation, 'have conducted assessments and developed international oversight mechanisms'.[38] This, however, is an incomplete reflection of the 'The Principles for the Oversight of Synthetic Biology' to which the IUCN report refers,[39] which covers a much wider spectrum of requirements, namely:

36 Such research may for example include detailed ecological studies such as on food webs; long term studies of the behaviour of gene drive organisms in laboratory environments and with different genetic backgrounds; studies on the potential for introgression of the gene drive into other species. It might also include advancing and testing theoretical and modelling studies for ecological and environmental impacts in general, and testing predictabilities. Studies to provide better understanding of the long-term effects of CRISPR-Cas9 on genome stability would be important and would be a valuable addition to the body of research in this field.

37 'of synthetic organisms, cells or genomes'

38 Unfortunately, the listing of sources for this in the IUCN report is problematic (p. 21). The link given to the reference 'Friends of Earth (FOE), 2012' does not lead to the relevant publication 'The Principles of Oversight of Synthetic Biology' - (see following footnote for correct link). The listing does however provide a functional link to an open letter by gene drive developers and proponents, dated 2018, making a case for gene drives from their particular perspective, which we find is ill-placed here. This reference is then followed by a badly copied and thus non-functional link to a critical open letter termed *A Call to Protect Food Systems from Genetic Extinction Technology: The Global Food and Agriculture Movement Says NO to Release of Gene Drives* (https://www.etcgroup.org/sites/www.etcgroup.org/files/files/etc_ftfsignonletter113018engweb_1.pdf). Interestingly, an important open letter 'A Call for Conservation with a Conscience: No Place for Gene Drives in Conservation' from 2016, is missing altogether (http://www.etcgroup.org/files/files/final_gene_drive_letter.pdf).

39 *The Principles for the Oversight of Synthetic Biology (2012)*. Published by Friends of the Earth U.S., the International Centre for Technology Assessment, and ETC group and undersigned by 116 civil society and scientific organisations. http://www.synbiowatch.org/wp-content/uploads/2013/05/Principles-for-the-oversight-of-synthetic-biology-web-2.pdf or https://www.etcgroup.org/sites/www.etcgroup.org/files/The%20Principles%20for%20the%20Oversight%20of%20Synthetic%20Biology%20FINAL.pdf

'until government bodies, with the full participation of the public, have:

- Developed a research agenda guided by the public interest.
- Ensured that alternative approaches to synthetic biology applications have fully been considered.
- Conducted full and inclusive assessments of the implications of this technology, including but not limited to devising a comprehensive means of assessing the human health, environmental, and socio-economic impacts of synthetic biology and preventing harms where they are present.
- Developed national and international oversight and security mechanisms equipped to keep pace with the risks as synthetic biology technologies develop'

Civil society organisations and scientific organisations have repeatedly argued that primary attention should be directed to addressing underlying problems, causes and drivers within each issue, rather than focussing on symptoms and any technological fixes that might be quickly applied to them. **To offer an experimental technology as a solution for many of these problems is a very over-simplified and short-term approach to complex issues**. There have also been calls within the UN system for the establishment of horizon scanning and technology assessment processes for new and emerging technologies, which would include the full and effective participation of civil society, indigenous peoples and local communities. Such processes would ultimately lead to a more considered and *mature* relationship with new technologies, in contrast with the tendency to rush towards acceptance of a technological symptom-fix, without sufficient reflection.

Discussions on 'moral hazard' imply that speculative technical fixes are viable options

Chapter 2 also briefly considers 'moral hazard', by which in this context they mean 'that new technologies may correct the symptoms of, and provide an excuse not to address, more fundamental socio-political failures which caused the symptoms in the first place.' (p. 46). **Whilst it is important to be aware of such 'moral hazards', the discussion on p.46 unfortunately proceeds to vastly inflate the potential effectiveness of the technological fix it had supposedly set out to warn against, and so - perhaps unwittingly - falls once again into techno-hype**.

The authors claim, 'With regard to synthetic biology, examples of "moral hazard" could include new technologies such as drought resistant crops, creating excuses for decision makers not to implement mitigation policies to prevent droughts' (p. 46). However, by using the media-effective but scientifically incorrect term 'drought resistance' (no crop is 'resistant' to drought; all crops need water to grow) and by failing to explain that *'drought-tolerance'*- like any type of stress tolerance - is a highly complex response system involving multiple genes and interconnected traits, the authors create an unrealistic sense of technological possibilities, whilst ostensibly making a point on ethics. Drought-*tolerance* can be found in many farmers varieties[40] of domesticated crops (including in maize) and has been successfully bred using specific breeding procedures. However, the appropriate answer to drought and other stressors would be the internationally supported creation and development of resilient farming systems, using ecosystem approaches such as agro-ecology, agro-forestry etc., in particular in conjunction with heterogenous seed. Unlike uniform seed, these complex approaches would function both as adaptation and mitigation measures to provide protection

40 Farmers' varieties are biodiverse and heterogeneous seeds, sometimes ancient varieties.

against many aspects of climate change, including unpredictable weather patterns (IAASTD 2009, HLPE 2019, FAO 2018, IPES-Food 2016, UN-HRC 2010).

Concerns regarding 'dual use' of gene drives are barely mentioned

There is a strong concern in the wider scientific community that gene drives, and indeed other synthetic biology applications, could be employed with the intention of causing harm, for example, being weaponised for military use.[41] The fact that the U.S. Defense Advanced Research Projects Agency (DARPA) is one of the largest funders of gene drive research adds weight to this concern. Such potential for deliberately harmful applications generally goes by the rather mild term 'dual use.' Whilst this issue receives extensive consideration in the high profile 2016 report from the US National Academies 'Gene Drives on the Horizon' (NASEM 2016),[42] it is only briefly touched on in the IUCN report. The potential to use synthetic biology for military ends is mentioned explicitly only twice in the latter (p. 25 and 57), while the possibility of weaponising gene drives is only alluded to by quoting NASEM, who in this case use the term "misuse" (p. 38 of IUCN report). Many malicious applications of gene drive organisms could be contrived; examples would be the deliberate suppression of important insect pollinators, or modification of insects to intentionally make them new or more potent vectors for plant, animal, or human diseases. Whilst the intention behind such uses would likely be to impact human populations, the implications for ecosystems, conservation and sustainable use of biodiversity would likely be equally damaging. **Taking the time to explore how a powerful new technology could actually be used in the real world seems an important aspect of any assessment that attempts to inform policies to control it; but that is missing here**.

Points that should have been expanded or explored in more detail

Bias in engagement

Questions are raised about 'bias in engagement… where it is undertaken by the proponent of the technology' (p. 47), referring to the concern that public consultations about employing gene drives will be led or unduly influenced by those actively involved in developing that technology. Similarly, this concern should also apply to the materials that are created to inform such discussions. These are important issues for IUCN members, and deserve an in-depth discussion. Concerns relating to a 'limited identification of who is entitled to give consent and how consent is sought' (p. 47) are also raised in this section, and should have been explored in much greater detail. Likewise, the report ought to have considered in more depth the question of who ultimately decides who will be allowed to deploy these gene drive organisms and how to ensure that no release takes place unless there is explicit free prior and informed consent of indigenous peoples and local communities living on the territories where such organisms are released, or the territories to which they may spread. A clear distinction between 'consultation' and free, prior and informed consent (FPIC) would have brought the discussion into its proper context.

41 See section 2.4.5 on Dual use in Steinbrecher et al. 2019

42 See for example page 9 (in the Summary), pages 69-70 (in Chapter 4 on values), pages 159-161 (in Chapter 8 on governance) of the NASEM report (2016).

Who does synthetic biology benefit?

Chapter 2 of the IUCN report covers governance, and mentions that concerns have been raised by the ETC Group 'that synthetic biology will benefit private over public interests, continue enclosures of genetic commons through aggressive intellectual property practices, concentrate power in the hands of elites, and undermine more holistic and traditional approaches to sustainability'. (p. 46). The report's chapter 3, which considers how evidence should be used in decision making, notes the concern that the use of gene drives in conservation may smooth the way for more 'questionable synthetic biology applications, such as those involving military-related ends or the corporate control over agriculture' (p. 57). This chapter also highlights how factors such as economic profit and political power contribute to shaping the questions asked in research (p. 54). These important and related points should have been given a much higher profile in the report. Besides getting only cursory treatment in chapter 3, these points are completely absent both from the introductory first chapter and from the concluding final chapter.

'Unknown unknowns' and uncertainty

The discussion about 'unknown unknowns' in chapter 3 (p. 54) is very relevant and also deserves a considerably higher profile than has been allotted. **Engineered gene drives alter interlinked, highly complex systems - cells, organisms, ecosystems and evolutionary dynamics – which means that the results and risks of their introduction are unlikely to be sufficiently predictable for safe use**. The discussion on 'Engaging with uncertainty' with regard to the need for evidence-based decision making on synthetic biology, rightly acknowledges that 'Uncertainty concerning the impacts of synthetic biology applications – intended and unintended – may be caused by a variety of factors, such as limitations of modelling or low levels of empirical evidence' (p. 53). One conclusion which should have been stated is that any evidence used to inform decision making on such applications must include robust 'uncertainty assessments'.

The technology may not deliver what is promised

The report does go some way towards reflecting the uncertainties regarding what gene drives and other synthetic biology technology will actually deliver. The conclusion of chapter 5, which covers the potential use of synthetic biology in conservation, acknowledges that there is a chance that these technologies won't deliver the promised outcomes: 'Application and efficacy of proposed synthetic biology approaches (including gene drive) in the field are likely to encounter multiple hurdles which will require further development to overcome, or may even prove to be intractable barriers to useful application' (p. 94). The text also notes that genetic manipulation is not fully predictable because 'much remains to be learned about how the information encoded in the genome is transcribed into function'. The concluding chapter also affirms that there 'remains a great deal of hype for synthetic biology' (p. 125). These points should have been highlighted in the introductory first chapter of the IUCN report and at appropriate places throughout the text.

The report as a whole draws far too little attention to uncertainty regarding the extent to which this technology will actually function or deliver promised 'benefits'. As presented, it also does not do enough to moderate the highly ambitious claims of those developing the technology.

CONCLUSIONS

The IUCN's report "Genetic Frontiers for Conservation" is meant to provide a solid scientific and contextual basis to help the IUCN — its members as well as its commissions — make informed policy decisions concerning Synthetic Biology, including gene-drive organisms.

Genetically engineered gene drives are a new, untried, laboratory-based technology intended to be released into wild species and open ecosystems. A cascade of knock-on effects can be expected as they begin to interact with complex systems, all interlinked by a multitude of processes and feedback loops. This makes the outcomes of engineered gene drive releases very difficult to predict - and consequences may be expected at genetic, species, ecosystem, and evolutionary process levels, along with human economic, social and cultural effects.

The report's analysis of the potential impacts of this new technology on these complex systems is not adequate, because it limits the areas and levels addressed and relies on the naïve belief that *intended* effects will be more common and significant than any *unintentional* (and damaging) consequences. To do a proper analysis, a wide range of interdisciplinary and transdisciplinary expertise is required, involving, for example, new modelling tools from complexity science as well as empirical data from observations, experiments and simulations. Governance of technologies with impacts on complex systems requires information that can only be provided by interdisciplinary and cross-sectoral cooperation; a similar approach is needed for the analysis of systemic risks and the search for systemic solutions with multiple benefits. The IUCN report does not attempt such an approach, and fails to identify very crucial knowledge gaps. Instead it focusses on technological fixes to narrowly perceived problems in isolation from wider systems.

This outcome is largely due to the composition of the group of experts responsible for the report, many of whom are connected to gene drive development or other synthetic biology programmes. Many prominent organisations have established 'Conflict of Interest Policies and Strategies' (such as the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) and the Convention on Biological Diversity (CBD)). They did so to safeguard the credibility of their expert advice. Such practices must first identify the asymmetries of power and finance between the constituencies needed for a systemic assessment, and second address them, to ensure that there is a balance between the different inputs. If such methods are not observed, recommendations and decision making are compromised, and developing countries, indigenous peoples and local communities will suffer most. Conflicts of interest are only noted in the middle of the report, when they should have been made clear at the outset, and there is no evidence that capacity for input from all necessary constituencies was considered.

This lack of balance and the bias towards technological solutions has led to serious failings and omissions in the IUCN report:

- Risks, knowledge gaps and the potential for unforeseen outcomes are repeatedly downplayed or ignored, in particular with regards to ecological impacts and effects over space and time.

43 See point 2, page 8 of this critique.

- The tone of important sections is one of enthusiasm about the potential of genetic technologies, when an objective presentation and analysis is required. For example, the report gives the impression that in the near future engineered gene drives could be used to eradicate invasive rodents, when in fact the available evidence shows current gene drive technology is ineffective in mammals (Grunwald *et al.* 2019, Pfitzner et al. 2020).

- Research into the use of engineered gene drives in agriculture is being funded and is ongoing, but is barely mentioned in the report. Such agricultural use could become widespread and would be almost impossible to contain or control, with great potential for harm. Moreover, release of gene drive organisms is inherently linked to questions of unintended but uncontrollable transboundary movements, which would potentially violate the sovereignty of neighbouring countries.

- The potential of this new technology for hostile and 'dual use', such as developing biological weapons against crops, livestock and people, is barely mentioned. This is worrisome in view of the present institutional weaknesses of the Convention on Biological and Chemical Weapons.

Important intergovernmental processes and agreements, like the Rio-Process and its Declaration on Environment and Development, have established principles that apply to the governance of new technologies. In the context of the environment and human health, the Precautionary Principle plays a guiding role, in particular with new technologies. It specifically mentions 'indications of serious or irreversible harm'. Risk-benefit analysis on the other hand suffers from a time-lag. Benefits show up quickly, especially in the form of careers and finance for some players, such as shareholders and scientists. However, the demonstration of the many possible categories of harm to nature and society takes much more time and requires multi-facetted scientific research as well as other relevant inputs. It is nonetheless crucial for informed governance decisions.

A moratorium on gene drive release would not impede responsible research to properly understand GD technology, its consequences and alternative options. On the contrary it could be the starting point for serious, multi-disciplinary work based on a more systemic conceptual framework. The present IUCN report fails to acknowledge the need for such patient and robust analysis, nor does it provide an adequate basis for informed deliberations on policy. Delivering the diverse Sustainable Development Goals in such a way as to bring people and nature into harmony is a serious challenge. Efforts to meet this challenge would benefit from examples of best practice, following an interdisciplinary, inclusive and systemic approach. As the IUCN moves towards forming policy on gene drives and synthetic biology, the opportunity is still open to exemplify such an approach.

References

Ågren, J. A., and A. G. Clark. 2018. "Selfish genetic elements." *PLoS Genet* 14 (11):e1007700. doi: 10.1371/journal.pgen.1007700.

Akbari, O. S., K. D. Matzen, J. M. Marshall, H. Huang, C. M. Ward, and B. A. Hay. 2013. "A synthetic gene drive system for local, reversible modification and suppression of insect populations." *Curr Biol* 23 (8):671-7. doi: 10.1016/j.cub.2013.02.059.

Baker, A. E. M. 1994. "Stowaway transport rates of house mice (Mus domesticus) and deermice (Peromyscus maniculatus)*." Proceedings of the 16th vertebrate pest conference., Davis, CA: University of California, February 1994.

Bernardini, F., A. Kriezis, R. Galizi, T. Nolan, and A. Crisanti. 2019. "Introgression of a synthetic sex ratio distortion system from Anopheles gambiae into Anopheles arabiensis." *Sci Rep* 9 (1):5158. doi: 10.1038/s41598-019-41646-8.

Breckling, B. , and A. von Gleich. 2020. "Chapter 2: *Gene Drives Touching Tipping Points."* In Gene Drives at Tipping Points: Precautionary Technology Assessment and Governance of New Approaches to Genetically Modify Animal and Plant Populations, edited by A. von Gleich and W. Schröder, 29-56. Switzerland: Springer Open.

Buchman, A., J. M. Marshall, D. Ostrovski, T. Yang, and O. S. Akbari. 2018. "Synthetically engineered Medea gene drive system in the worldwide crop pest Drosophila suzukii." *Proc Natl Acad Sci U S A* 115 (18):4725-4730. doi: 10.1073/pnas.1713139115.

Champer, J., A. Buchman, and O. S. Akbari. 2016. "Cheating evolution: engineering gene drives to manipulate the fate of wild populations." *Nat Rev Genet* 17 (3):146-159. doi: 10.1038/nrg.2015.34.

Champer, J., R. Reeves, S. Y. Oh, C. Liu, J. Liu, A. G. Clark, and P. W. Messer. 2017. "Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations." *PLoS Genet* 13 (7):e1006796. doi: 10.1371/journal.pgen.1006796.

Champer, J., E. Lee, E. Yang, C. Liu, A. G. Clark, and P. W. Messer. 2020. "A toxin-antidote CRISPR gene drive system for regional population modification." *Nat Comm* 11 (1). doi: 10.1038/s41467-020-14960-3.

CSS/ENSSER/VDW. 2019. Gene drives - a report on their science, applications, social aspects, ethics and regulations.

de Koning, A. P. J., W. Gu, T. A. Castoe, M. A. Batzer, and D. D. Pollock. 2011. "Repetitive elements may comprise over two-thirds of the human genome." *PLoS Genet* 7 (12):e1002384-e1002384. doi: 10.1371/journal.pgen.1002384.

DiCarlo, J. E., A. Chavez, S. L. Dietz, K. M. Esvelt, and G. M. Church. 2015. "Safeguarding CRISPR-Cas9 gene drives in yeast." *Nat Biotechnol* 33 (12):1250-1255. doi: 10.1038/nbt.3412.

Dolezel, M., S. Samson, M. Otto, M. Engelhard, and W. Zughart. 2020a. Gene Drive Organisms: Implications for the Environment and Nature Conservation. Vienna: Umweltbundesamt (Environment Agency Austria).

Dolezel, M., C. Lüthi, and H. Gaugitsch. 2020.b "Beyond limits – the pitfalls of global gene drives for environmental risk assessment in the European Union." *BioRisk* 15. doi: 10.3897/biorisk.15.49297.

Dunning Hotopp, J. C., M. E. Clark, D. C. Oliveira, J. M. Foster, P. Fischer, M. C. Muñoz Torres, J. D. Giebel, N. Kumar, N. Ishmael, S. Wang, J. Ingram, R. V. Nene, J. Shepard, J. Tomkins, S. Richards, D. J. Spiro, E. Ghedin, B. E. Slatko, H. Tettelin, and J. H. Werren. 2007. "Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes." *Science* 317 (5845):1753-6. doi: 10.1126/science.1142490.

Eckerstorfer, M. F., M. Dolezel, A. Heissenberger, M. Miklau, W. Reichenbecher, R. A. Steinbrecher, and F. Wassmann. 2019. "An EU Perspective on Biosafety Considerations for Plants Developed by Genome Editing and Other New Genetic Modification Techniques (nGMs)." *Front Bioeng Biotechnol* 7:31. doi: 10.3389/fbioe.2019.00031.

Edgerton, D. 1997. "Ever accelerating hype." *Prospect*, April 20, 1997.

EEA. 2001 (2002 online publication). Late lessons from early warnings:the precautionary principle 1896–2000. Copenhagen: European Environment Agency.

Esvelt, K. M., A. L. Smidler, F. Catteruccia, G. M. Church, Emerging Technology: Concerning RNA-guided gene drives for the alteration of wild populations. Elife 2014 doi: 10.7554/eLife.03401

Evans, B. R., P. Kotsakiozi, A. L. Costa-da-Silva, R. S. Ioshino, L. Garziera, M. C. Pedrosa, A. Malavasi, J. F. Virginio, M. L. Capurro, and J. R. Powell. 2019. "Transgenic Aedes aegypti Mosquitoes Transfer Genes into a Natural Population." *Sci Rep* 9 (1):13047. doi: 10.1038/s41598-019-49660-6.

FAO. 2018. Food and Agriculture Organisation of the United Nations. Scaling up Agroecology Initiative - transforming food and agricultural systems in support of the SDGs.

Ferry, D. 2015. "The promises and perils of synthetic biology." Newsweek, March 11, 2015

Feschotte, Cédric, and Ellen J. Pritham. 2007. "DNA Transposons and the Evolution of Eukaryotic Genomes." *Annual Review of Genetics* 41 (1):331-368. doi: 10.1146/annurev.genet.40.110405.090448.

Fontaine, M. C., J. B. Pease, A. Steele, R. M. Waterhouse, D. E. Neafsey, I. V. Sharakhov, X. Jiang, A. B. Hall, F. Catteruccia, E. Kakani, S. N. Mitchell, Y. C. Wu, H. A. Smith, R. R. Love, M. K. Lawniczak, M. A. Slotman, S. J. Emrich, M. W. Hahn, and N. J. Besansky. 2015. "Extensive introgression in a malaria vector species complex revealed by phylogenomics." *Science* 347 (6217):1258524. doi: 10.1126/science.1258524.

Friess, J. L., A. von Gleich, and B. Giese. 2019. “Gene drives as a new quality in GMO releases-a comparative technology characterization.” *PeerJ 7*:e6793. doi: 10.7717/peerj.6793.

Gantz, V. M., N. Jasinskiene, O. Tatarenkova, A. Fazekas, V. M. Macias, E. Bier, and A. A. James. 2015. “Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi.” *Proc Natl Acad Sci U S A* 112 (49):E6736-43. doi: 10.1073/pnas.1521077112.

Gilbert, C., and R. Cordaux. 2017. “Viruses as vectors of horizontal transfer of genetic material in eukaryotes.” Curr Opin Virol 25:16-22. doi: 10.1016/j.coviro.2017.06.005.

Goswami, R., G. Subramanian, L. Silayeva, I. Newkirk, D. Doctor, K. Chawla, S. Chattopadhyay, D. Chandra, N. Chilukuri, and V. Betapudi. 2019. “Gene Therapy Leaves a Vicious Cycle.” *Front Oncol* 9:297. doi: 10.3389/fonc.2019.00297.

Grunwald, H. A., V. M. Gantz, G. Poplawski, X. S. Xu, E. Bier, and K. L. Cooper. 2019. “Super-Mendelian inheritance mediated by CRISPR-Cas9 in the female mouse germline.” *Nature* 566 (7742):105-109. doi: 10.1038/s41586-019-0875-2.

Hammond, A. M., K. Kyrou, M. Bruttini, A. North, R. Galizi, X. Karlsson, N. Kranjc, F. M. Carpi, R. D’Aurizio, A. Crisanti, and T. Nolan. 2017. “The creation and selection of mutations resistant to a gene drive over multiple generations in the malaria mosquito.” *PLoS Genet* 13 (10):e1007039. doi: 10.1371/journal.pgen.1007039.

Harvey-Samuel, T., V. C. Norman, R. Carter, E. Lovett, and L. Alphey. 2019. “Identification and characterization of a Masculinizer homologue in the diamondback moth, Plutella xylostella.” *Insect Mol Biol*. doi: 10.1111/imb.12628.

HLPE. 2019. Agroecological and other innovative approaches for sustainable agriculture and food systems that enhance food security and nutrition. A report by the High Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome.

Houck, M. A., J. B. Clark, K. R. Peterson, and M. G. Kidwell. 1991. “Possible horizontal transfer of Drosophila genes by the mite Proctolaelaps regalis.” *Science* 253 (5024):1125-8. doi: 10.1126/science.1653453.

IAASTD. 2009. Agriculture at a Crossroads: Synthesis Report – A report by the International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD).

IPES-Food. 2016. From Uniformity to Diversity: A paradigm shift from industrial agriculture to diversified agroecological systems. A report by the International Panel of Experts on Sustainable Food Systems.

Koidou, V., S. Denecke, P. Ioannidis, I. Vlatakis, I. Livadaras, and J. Vontas. 2020. “Efficient genome editing in the olive fruit fly, Bactrocera oleae.” *Insect Mol Biol*. doi: 10.1111/imb.12640.

Kyrou, K., A. M. Hammond, R. Galizi, N. Kranjc, A. Burt, A. K. Beaghton, T. Nolan, and A. Crisanti. 2018. “A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes.” *Nat Biotechnol* 36 (11):1062-1066. doi: 10.1038/nbt.4245.

LaMonica, M. 2014. “Why the promise of cheap fuels from super bugs fell short.” MIT Technology Review, February 5, 2014

Lebrecht, T., H. Wallace, and I. Castro. 2019. “Chapter 3: Social Issues.” In Gene Drives: A report on their science, applications, social aspects, ethics and regulations, 159-214. CSS, ENSSER & VDW.

Li, M., T. Yang, N. P. Kandul, M. Bui, S. Gamez, R. Raban, J. Bennett, H. M. C. Sanchez, G. C. Lanzaro, H. Schmidt, Y. L. Lee, J. M. Marshall, and O. S. Akbari. 2020. “Development of a confinable gene drive system in the human disease vector Aedes aegypti.” *Elife* 9. doi: 10.7554/eLife.51701.

López Del Amo, V., B. S. Leger, K. J. Cox, S. Gill, A. L. Bishop, G. D. Scanlon, J. A. Walker, V. M. Gantz, and A. Choudhary. 2020. “Small-Molecule Control of Super-Mendelian Inheritance in Gene Drives.” *Cell Rep* 31 (13):107841. doi: 10.1016/j.celrep.2020.107841.

Loreto, E. L., C. M. Carareto, and P. Capy. 2008. “Revisiting horizontal transfer of transposable elements in Drosophila.” *Heredity* (Edinb) 100 (6):545-54. doi: 10.1038/sj.hdy.6801094.

Medina, R. F. 2018. “Gene drives and the management of agricultural pests.” *J Responsible Innov* 5 (sup1):S255-S262. doi: 10.1080/23299460.2017.1407913.

Min, J., C. Noble, D. Najjar, and K. M. Esvelt. 2017. “Daisy quorum drives for the genetic restoration of wild populations.” *bioRxiv*:115618. doi: 10.1101/115618.

NASEM. 2016. Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values. Washington (DC): National Academies Press (US).

Noble, C., B. Adlam, G. M. Church, K. M. Esvelt, and M. A. Nowak. 2018. “Current CRISPR gene drive systems are likely to be highly invasive in wild populations.” *Elife* 7. doi: 10.7554/eLife.33423.

Noble, C., J. Min, J. Olejarz, J. Buchthal, A. Chavez, A. L. Smidler, E. A. DeBenedictis, G. M. Church, M. A. Nowak, and K. M. Esvelt. 2019. “Daisy-chain gene drives for the alteration of local populations.” *Proc Natl Acad Sci U S A* 116 (17):8275-8282. doi: 10.1073/pnas.1716358116.

Oberhofer, G., T. Ivy, and B. A. Hay. 2019. “Cleave and Rescue, a novel selfish genetic element and general strategy for gene drive.” *Proc Natl Acad Sci U S A*. doi: 10.1073/pnas.1816928116.

Oberhofer, G., T. Ivy, and B. A. Hay. 2020. “Gene drive and resilience through renewal with next generation Cleave and Rescue selfish genetic elements.” *Proc Natl Acad Sci USA* 117 (16):9013-9021. doi: 10.1073/pnas.1921698117.

Payseur, B. A., J. G. Krenz, and M. W. Nachman. 2004. “Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice.” *Evolution* 58 (9):2064-78. doi: 10.1111/j.0014-3820.2004.tb00490.x

Peccoud, J., V. Loiseau, R. Cordaux, and C. Gilbert. 2017. "Massive horizontal transfer of transposable elements in insects." *Proc Natl Acad Sci USA* 114 (18):4721. doi: 10.1073/pnas.1621178114.

Pfitzner, C., J. Hughes, M. White, M. Scherer, S. Piltz, P. Thomas. Development of zygotic and germline gene drives in mice BioRxiv 2020 DOI: 10.1101/2020.06.21.162594

Powell, K. 2018. "How biologists are creating life-like cells from scratch." *Nature* 563 (7730):172-175. doi: 10.1038/d41586-018-07289-x.

Preu, M. , J. L. Friess, B. Breckling, and W. Schröder. 2020. "Chapter 4 - Case Study 1: Olive Fruit Fly (Bactrocera oleae)." In Gene Drives at Tipping Points: Precautionary Technology Assessment and Governance of New Approaches to Genetically Modify Animal and Plant Populations, edited by A. von Gleich and W. Schröder, 187-217. Switzerland: Springer Open.

Reeves, R. G., J. Bryk, P. M. Altrock, J. A. Denton, and F. A. Reed. 2014. "First steps towards underdominant genetic transformation of insect populations." *PLoS One* 9 (5):e97557. doi: 10.1371/journal.pone.0097557.

Rode, N. O., A. Estoup, D. Bourguet, V. Courtier-Orgogozo, and F. Débarre. 2019. "Population management using gene drive: molecular design, models of spread dynamics and assessment of ecological risks." *Conserv Genet* 20 (4):671-690. doi: 10.1007/s10592-019-01165-5.

Scott, M. J., F. Gould, M. Lorenzen, N. Grubbs, O. R. Edwards, and D. O'Brochta. 2018. "Agricultural production: assessment of the potential use of Cas9-mediated gene drive systems for agricultural pest control." *J Responsible Innov* 5 (sup1):S98-S120. doi: 10.1080/23299460.2017.1410343.

Shahryari, A., M. Saghaeian Jazi, S. Mohammadi, H. Razavi Nikoo, Z. Nazari, E. S. Hosseini, I. Burtscher, S. J. Mowla, and H. Lickert. 2019. "Development and Clinical Translation of Approved Gene Therapy Products for Genetic Disorders." *Front Genet* 10:868. doi: 10.3389/fgene.2019.00868.

Sim, S. B., A. N. Kauwe, R. E. Y. Ruano, P. Rendon, and S. M. Geib. 2019. "The ABCs of CRISPR in Tephritidae: developing methods for inducing heritable mutations in the genera Anastrepha, Bactrocera and Ceratitis." *Insect Mol Biol* 28 (2):277-289. doi: 10.1111/imb.12550.

Simon, S., M. Otto, and M. Engelhard. 2018. "Synthetic gene drive: between continuity and novelty: Crucial differences between gene drive and genetically modified organisms require an adapted risk assessment for their use." *EMBO Rep* 19 (5). doi: 10.15252/embr.201845760.

Steinbrecher, R., and M. Wells. 2019. "Chapter 1: What are gene drives? The science, the biology, the techniques." In *Gene Drives:* A report on their science, applications, social aspects, ethics and regulations, 21-68. CSS, ENSSER & VDW.

Steinbrecher, R., M. Wells, R. Brandt, E. Bücking, and D. Gurian-Sherman. 2019. "Chapter 2: Potential applications and risks." In Gene Drives: A report on their science, applications, social aspects, ethics and regulations, 69-158. CSS, ENSSER & VDW.

Sudweeks, J., B. Hollingsworth, D. V. Blondel, K. J. Campbell, S. Dhole, J. D. Eisemann, O. Edwards, J. Godwin, G. R. Howald, K. P. Oh, A. J. Piaggio, T. A. A. Prowse, J. V. Ross, J. R. Saah, A. B. Shiels, P. Q. Thomas, D. W. Threadgill, M. R. Vella, F. Gould, and A. L. Lloyd. 2019. "Locally Fixed Alleles: A method to localize gene drive to island populations." *Sci Rep* 9 (1):15821. doi: 10.1038/s41598-019-51994-0.

Then, C. 2020. "Chapter 8: Limits of Knowledge and Tipping Points in the Risk Assessment of Gene Drive Organisms." In *Gene Drives at Tipping Points: Precautionary Technology Assessment and Governance of New Approaches to Genetically Modify Animal and Plant Populations*, edited by A. von Gleich and W. Schröder, 187-217. Switzerland: Springer Open.

Tuladhar, R., Y. Yeu, J. Tyler Piazza, Z. Tan, J. Rene Clemenceau, X. Wu, Q. Barrett, J. Herbert, D. H. Mathews, J. Kim, T. Hyun Hwang, and L. Lum. 2019. "CRISPR-Cas9-based mutagenesis frequently provokes on-target mRNA misregulation." *Nat Commun* 10 (1):4056. doi: 10.1038/s41467-019-12028-5.

UN-HRC. 2010. Agro-ecology and the right to food. Report by the UN Special Rapporteur on the Right to Food, Olivier De Schutter, at the 16th Session of the UN Human Rights Council (HRC).

Wilson, A. K., J. R. Latham, and R. A. Steinbrecher. 2006. "Transformation-induced mutations in transgenic plants: analysis and biosafety implications." *Biotechnol Genet Eng Rev* 23:209-37. doi: 10.1080/02648725.2006.10648085.

Winters, J. 2020. "The myth of algae biofuels." Harvard Political Review, January 26, 2020

Yoshiyama, M., Z. Tu, Y. Kainoh, H. Honda, T. Shono, and K. Kimura. 2001. "Possible horizontal transfer of a transposable element from host to parasitoid." *Mol Biol Evol* 18 (10):1952-8. doi: 10.1093/oxfordjournals.molbev.a003735.

Zechner, U., M. Reule, A. Orth, F. Bonhomme, B. Strack, Guenet, H. Hameister, and R. Fundele. 1996. "An X-chromosome linked locus contributes to abnormal placental development in mouse interspecific hybrid." *Nat Genet* 12 (4):398-403. doi: 10.1038/ng0496-398.

Zhang, H.-H., J. Peccoud, M.-R.-X. Xu, X.-G. Zhang, and C. Gilbert. 2020. "Horizontal transfer and evolution of transposable elements in vertebrates." *Nat Comm* 11 (1):1362. doi: 10.1038/s41467-020-15149-4.

Zhao, S., Z. Xing, Z. Liu, Y. Liu, X. Liu, Z. Chen, J. Li, and R. Yan. 2019. "Efficient somatic and germline genome engineering of Bactrocera dorsalis by the CRISPR/Cas9 system." *Pest Manag Sci* 75 (7):1921-1932. doi: 10.1002/ps.5305.