Chapter 2

Potential applications and risks

Ricarda Steinbrecher, Mark Wells, Ruthi Brandt, Elisabeth Bücking, Doug Gurian-Sherman

1 General introduction

Gene drives are genetic elements that are able to override the rules of inheritance. When genetically engineered and linked to a biological function, they are intended to be used to modify, and in some circumstances, eradicate, a whole population or even an entire species. This idea has largely remained just a theory until quite recently. With the arrival of the genome editing tool CRISPR/Cas9, the field of gene drives and the ability to eliminate undesired species and wild populations got a big boost. CRISPR/Cas-based gene drives were first proposed in 2014 (Esvelt et al. 2014) and the proofs of principle came swiftly, published in 2015 and 2016 for fruit flies (Drosophila), yeast and two species of mosquitoes (Anopheles stephensi & Anopheles gambiae) and in 2018 for mice. Gantz and Bier called it a “mutagenic chain reaction” when they delivered the first laboratory proof of principle (2015), showing that the genome editing tool CRISPR/Cas could in theory be turned into a self-spreading gene drive that might be capable of altering or eliminating wild populations or potentially whole species (Gantz and Bier 2015). Yet laboratory experiments picked up a flaw in this method – the emergence and build-up of ‘resistance’ to CRISPR/Cas, capable of stopping the functioning and spread of the gene drive, as discussed in Chapter 1. Crisanti’s team applied a different strategy, which by targeting highly preserved (conserved) genes, proved capable for the first time of completely crashing caged populations of the mosquito Anopheles gambiae in laboratory settings (Kyrou et al. 2018a). This strategy, however, comes with heightened new risks of the gene drive mechanism spreading beyond the target species, as such genes are often highly conserved across a whole species group. Hybridisation (cross-breeding) within these groups would move the gene drive and its action into closely related species, a distinct possibility for the Anopheles gambiae complex. Whilst this strategy might have overcome the resistance problem in this particular instance, it has to be noted that caged experiments do not address the complexities of how species will actually respond in the real world.

Other strategies and designs of CRISPR/Cas-based homing gene drive strategies are being developed under DARPA (US Defense Advanced Research Projects Agency) funding, and different non-homing gene drive systems and applications are also under development, utilising for example MEDEA (e.g. for fruit flies), t-haplotype systems (mice) or X-shredder (e.g. mosquito) (see Chapter 1 for technical details).

Gene drive organisms and gene drives are clearly not just research interests and research projects on their own – they include the clear intention of application. This is revealed by significant factors: the large sums of funding being provided; the categorisation into fields of applications found in many reviews and reports, e.g. the report by the National Academy of Sciences, Engineering and Medicine (NAS 2016); the rationale presented in scientific papers published by research groups; the substantial budgets for public and policy engagement in DARPA-funded gene drive projects; and the general benefit-focused portrayal in the media.

With such a strong application and benefit mindset and focus on “deployment”, there seems to be little room for crucial critical reflections, which should include: looking dispassionately at the real risks of this powerful technology; being clear about the fact that the claimed benefits are largely only
hypothetical and at best potential benefits, as none of them have undergone a scientific, and socio-economic, robust and transparent benefit analysis and assessment; taking note that there is no methodology in place for such needed assessments; taking time to investigate the spectrum of other solutions and approaches that might be cheaper or safer; trying to isolate and then address the underlying causes to the problems gene drives are supposed to address, which might actually require very different solutions; and finally, attempting to determine where the best answers to all these questions might lie - which may not always be with science and technology.

It would be problematic and inappropriate to view the use of engineered gene drives as some kind of a self-replicating, self-spreading, target-specific “pesticide”, to be readily ‘applied’ or released to attack any pest, disease vector, invasive species, unwanted or disliked species or nuisance populations; and yet that tendency is already present in media coverage and in promotional claims. Such a view of usage demonstrates an unwarranted sense of familiarity with, as well as a misconception of, what engineered gene drives are. We are talking about living organisms, living and highly interactive systems. We are talking about completely new and unknown numbers and levels of risks and impacts.

Gene drives - in particular CRISPR/Cas-based homing drives - are a technology which gives humans the potential to intentionally (or unintentionally) re-engineer whole ecosystems, perhaps altering or wiping out wild populations of various species across vast regions. If they are deployed, that is, released into the environment, there is no doubt there will be impacts. These include impacts on biodiversity, which is already in unprecedented and rapid decline (Ceballos, Ehrlich, and Dirzo 2017; Sanchez-Bayo and Wyckhuys 2019; FAO. 2019); impacts on ecosystem functions and services, including agricultural systems, at a time when many are already at the point of collapse; there will be co-evolutionary responses of pests and associated pathogens and parasites; and the disruption, unbalancing and shifting of ecosystems in unpredictable ways, which may not only be triggered by the suppression or elimination of a population or species or by the alteration of their traits, biological functions and behaviour, but also from the engineered gene drive moving or crossing to other species. For example, the mosquito species Anopheles gambiae is known to cross with other closely related species, such as A. quadriannulatus or A. arabiensis, both in the laboratory and in the wild, resulting in hybrid offspring with fertile females and at times also fertile males (Coluzzi, Sabatini, Petrarca, and Dideco 1979).

There will also be consequences from the technology not working as expected, or from it working differently than expected. This has been discussed in Chapter 1 and will also be considered in Section 3 on risks and risk assessment at the end of this chapter.

Suggestions for proposed gene drive applications cover a wide range and extend across plants, animals and fungi. To date, they include mice, rats, fish, birds, insects (e.g. various mosquitoes, flies, beetles, hornets, etc.); also spiders, feral cats, snails, nematodes, plants such as pigweed (Amaranth) and horseweed; and finally the phylum of fungi.¹

In order to understand the risks and identify the hazards of of gene drive organisms (GDOs), a categorisation into areas of application or envisaged potential benefits is of little help. Gene drive applications are frequently categorised into three areas: Public Health; Ecosystem Conservation (including combating alien invasive species); and Agriculture, mostly regarding pests, weeds, and diseases. Another category however, is military use; the potential weaponising of gene drives is commonly covered under the topic of “dual use”, as the knowledge creation and technological capacities achieved in civil research and for civil application can equally be used or misused for intentionally harmful purposes, including military ones. When looking at potential dual use scenarios, the US National Academy of Sciences (NSA) argues in their 2016 report on gene drives: “Yet, with a better understanding of the basis of mosquito—pathogen interactions, it is not in-

¹ See tables and special studies for references.
conceivable that rather than developing a resistant mosquito, one could develop a more susceptible mosquito capable of transmitting a specific pathogen more efficiently than wild-type mosquitoes. It might even be possible to develop mosquitoes that could transmit a pathogen that is not normally vector-borne, or that could even be able to deliver a toxin.” (NASEM 2016, 161) There are in fact many scenarios one could conceive of, especially for insects, given the recent research advances in that field. Whilst spreading toxins and diseases to humans, livestock or plants is a serious prospect, it would be of equal concern to intentionally weaken or eliminate beneficial insects. The US National Academy of Sciences hence states in its conclusions: “Governance mechanisms need to be in place to address questions about the biosecurity implications of gene drive research and consider developing mitigation strategies that are not dependent on the underlying technology.” (NASEM 2016, 171).

Such categorisations into fields of application does not only move the idea of benefit into the foreground, but also inadvertently raises sets of concerns and questions other than those necessary to identify hazards and understand risks. The former will look at questions related to doability, efficiency, reliability and simplification, that is, getting the gene drive to work despite or irrespective of environmental and ecosystem complexities; or to ideally creating a gene drive system that is easily transferrable to multiple species, e.g. to different insects that have become agricultural pests that could be new gene drive targets, (see for example criteria in (Marshall and Akbari 2016)).

Therefore, three points arise: Which categories are helpful to open up and reflect issues of hazards and risks? What questions need to be asked, and what kind of knowledge is required to answer them? Finally, is the experience we have with GMOs sufficient to deal with GDOs, or are there substantial differences?

Looking at the current experience with releases of approved GMOs (see Table 1), the vast majority are highly domesticated and uniform crop plants, as well as some trees and ornamental flowers. They are being released into simple, highly controlled (agricultural) and managed environments and are not intended to spread. Nonetheless, outcrossing and gene flow are regarded as serious problems and contamination incidents are frequently reported, such as for oilseed rape (Friesen, Nelson, and Van Acker 2003; Hecht et al. 2014), Maize in Mexico and South Africa (Pineyro-Nelson et al. 2009; Iversen et al. 2014) or for rice, alfalfa and creeping bentgrass in North America (Sharratt and Chopra 2019). With regard to GM animals, all are intended for rearing in enclosed systems, with strict containment requirements. The only open release of GM animals has been a few sterile GM insects, again not intended to spread, but instead incapacitated from doing so.

In contrast, GDOs will not be domesticated and uniform but genetically and behaviourally diverse. They will be released into open, wild, uncontrolled and highly complex environments. Unlike plants, most of them – especially insects - are highly mobile and are intended to spread and cross-breed, thus resulting in intentional contamination of wild populations and ecosystems.

Table 1: GM crops and GM animals that have gained approval in at least one country (source for plants: ISAAA 2019)

<table>
<thead>
<tr>
<th>GM crops</th>
<th>GM trees</th>
<th>GM flowers &amp; grasses</th>
<th>GM animals</th>
<th>GM insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predominantly: Cotton, Maíz, Olsed rape (canola), Soya bean</td>
<td>Apple, Papaya, Plum, Eucalyptus, Poplar</td>
<td>Creeping Bentgrass, Carnation, Petunia, Rose</td>
<td>In containment: Pigs, Goats, Salmon, Mice (for research)</td>
<td>For release: Mosquitos (sterile), Pink Bollworm (sterilised)</td>
</tr>
<tr>
<td>Also approved: Alfalfa, Bean, Eggplant, Flax, Melon, Potato, Rice, Safflower, Sugar, beet, Sugar cane, Sweet pepper, Tobacco, Tomato, Wheat</td>
<td></td>
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</tr>
</tbody>
</table>
Suggestions about using regular GM risk assessment and risk management methodology with this new technology displays a misconception of what engineered gene drives and GDOs are. The most advanced engineered gene drives to date are CRISPR/Cas9-based gene drives (including homing drives). Being equipped with an active CRISPR/Cas9 machinery that genetically modifies each individual anew in vivo in the wild allows for multiple changes to occur that cannot be predicted or assessed in the laboratory. The release of such GDOs into complex interactive systems is equivalent to introducing one complex system into another, even more complex system, the environment (Bar-Yam 2002). In systems theory, the result would by definition be unpredictable, or nearly impossible to compute; and assessing gene drive risks thus ascends to an unprecedented and potentially intractable level of complexity.\(^2\) This is one reason that we are suggesting a different categorisation, namely one guided by taxa and ecological ‘ranking’, focussing both on the individual species, with all knowledge required (including its ecological role, linkages and interconnections), as well as on each of the complex ecosystem(s) that are the potential recipients of the GDOs or that are directly or indirectly impacted.

This chapter will look at the different envisaged applications, the status quo of research and development, as well as at the risks and associated negative impacts with a focus on biodiversity and ecosystems.

## 2 GDOs - applications under development

### 2.1 Introduction

This section consists of three main components. First, we provide a detailed overview of the various gene drive applications under development, indicating the stage of development, i.e. the readiness for application. This is illustrated in the form of detailed tables which cover insects (Table 2a), small mammals (Table 2b) and fish, birds, mollusks, nematodes, flatworms and fungi (Table 2c).

These tables are followed by three main case studies, namely mosquitoes, mice and Palmer amaranth (pigweed).

We will then look at two fields of potential application. The first explores the issue of agricultural insect pests, why they have become such a problem in modern agriculture, what the arguments are for targeting them with engineered gene drives, and what other possible actions, agricultural practices and solutions are also available to counter the increasing emergence of such pests. The second application field briefly covers the issue of dual use, i.e. of civil as well as military use of gene drive technology. This will be done in the context of specific research being funded under DARPA.

### 2.2 Overview of Gene Drive applications under development

To give an overview of which species are being targeted and why, Tables 2a, b and c describe the gene drive concepts that are being developed for each target and the stated intentions for their development. In many cases there is an intention to eventually employ the drive against the target species in the wild; however we have also included important proof of concept experiments in laboratory model organisms, for example the fruit fly (Drosophila melanogaster) or baker’s yeast (Saccharomyces cerevisiae).

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\(^2\) A system that can be analyzed into many components having relatively many relations among them, so that the behavior of each component depends on the behavior of others. [Herbert Simon] [*https://www.informatics.indiana.edu/rocha/publications/complex/csm.html*]

\(^3\) "A central feature of a complex system is that attempting to understand it by breaking it down into smaller parts and studying those parts in isolation is likely to fail. Although any scientific endeavor must simplify a system in order to study it, the complex system itself cannot be simplified—it takes on its characteristics from the whole." (Vandermeer and Perfecto 2017, 698)
Scope of tables

We include:
- Representative published work on each drive concept from each research group. In this we combine into one group variations on a similar concept, for example the two insect X-shredder drives described by (Galizi et al. 2016) and (Galizi et al. 2014).
- Research proposals that have been funded, either for drive development or preliminary work.
- Representative examples of drives that have been proposed in the literature, but laboratory research has not yet begun.

We have not attempted to provide a comprehensive list of proposed gene drive concepts or applications as these are very numerous, especially those consisting mainly of the target idea without further reflections.

We have not included work aimed at:


2. Improving existing drive concepts by
   - multiplexing gRNAs in homing CRISPR/Cas9 drives (Champer et al. 2018; Oberhofer, Ivy, and Hay 2018);
   - optimising expression of CRISPR/Cas9 (Champer et al. 2018; Hammond et al. 2018);
   - improving biosafety by splitting drive components or targeting artificial sites (Champer et al. 2019).

3. Exploring feasibility of gene drives in the model organism Drosophila melanogaster using non-programmable Homing Endonuclease Genes (HEGs), which now appears to be superseded by CRISPR/Cas9 drives - (Chan et al. 2011; Chan et al. 2013).

2.2.1 Insects

Insects are increasingly becoming the main target for gene drive developments. On one hand there are the vectors of diseases such as the mosquitoes Anopheles gambiae, Anopheles stephensi, Aedes aegyptii and Culex quinquefasciatus (listed in Table 2a and in the Mosquito case study); on the other hand there is a long list of agricultural pests that are shifting more into focus as potential targets. Whilst research with Drosophila melanogaster has no direct application, it nevertheless has been the main laboratory model insect organism for almost 100 years. Research carried out on D. melanogaster has therefore to be viewed as R&D research for developing the “right” gene drive system and understanding innate technical problems (see six entries in Table 2a). Additional to the 8 agricultural pests listed in the table, there are many others being suggested and proposed, such as: gene drives against the brown plant hopper (Nilaparvata lugens), the silverleaf whitefly (Bemisia tabaci), the diamond-back moth (Plutella xylostella), and the New World screwworm fly (Cochliomyia hominivorax) (Scott et al. 2018). The question around agricultural pests is also explored in detail further on in ‘Agricultural pests as gene drive targets’.

2.2.2 Small mammals

Whilst small mammals are definitely potential targets for gene drive applications, the work has not progressed as rapidly as for insects, due to technical obstacles that are not yet fully understood (see Chapter 1). At present, most of this development work is being carried out in mice, as mice are the laboratory model animal for mammals. Nevertheless, mice are also targets themselves for elimination gene drives (homing drive, X-shredder and t-haplotype), as illustrated both in the case study and in Table 2b. It is understood that once the system works in mice that many more small mammals will become targets for elimination gene drives, such as various rodent ‘pests’, feral cats, brushtail possums and stoats, which have been especially named already in scientific reports; work on feral cats and brushtail possums has received preliminary funding.
2.2.3 Fish, birds, mollusks, nematodes, flatworms & fungi

As can be seen in Table 2c, fish, birds, mollusks, flatworms and nematodes, are all on the radar as potential gene drive targets, whilst fungi and possibly nematodes are being employed as model organisms for developing gene drive technology. The table includes drives to target invasive species (lionfish and starlings) and organisms involved in disease, including the soil transmitted helminths commonly called hookworm (*Trichuris trichiura*), whipworm (*Necator americanus* and *Ancylostoma duodenale*) and threadworm* (Strongyloides stercoralis), as well as the parasites that cause Schistosomiasis and their intermediate snail host. These examples illustrates that if gene drives become established as a tool for controlling pests, invasive species or agents of human disease, they will likely be employed against other taxa beyond insects and mammals. It is also worth noting, that in contrast to the strategy of targeting disease vectors, which is the motivation behind many of the proposed gene drives in insects, the planned suppression drives against nematode (roundworm) and flatworm parasites directly target the parasite themselves. This becomes an option when the parasite reproduces sexually.

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### Table 2a: Insects

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range¹</th>
<th>Problem it is aiming to address</th>
<th>Intended application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mosquitoes</strong></td>
<td></td>
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<tr>
<td><em>Anopheles gambiae</em></td>
<td></td>
<td>Morbidity and mortality from malaria in sub-Saharan Africa</td>
<td>Population suppression</td>
</tr>
<tr>
<td>African malaria mosquito</td>
<td>predicted distribution; red=present; blue=absent (MAP 2019, Wiebe et al. 2017)</td>
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</tr>
<tr>
<td><em>Anopheles gambiae</em></td>
<td></td>
<td>Morbidity and mortality from malaria in sub-Saharan Africa</td>
<td>Population suppression ‘collapsing vector population to levels that cannot support malaria transmission’</td>
</tr>
<tr>
<td><em>Anopheles stephensi</em></td>
<td></td>
<td>Morbidity and mortality from malaria in India and surrounding regions</td>
<td>Population modification to ‘interrupt parasite transmission’</td>
</tr>
<tr>
<td>Asian malaria mosquito</td>
<td>predicted distribution; red=present; blue=absent (MAP 2019)</td>
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<td></td>
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</tbody>
</table>

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¹ Please see separate bibliography for sources for maps and geographic range.

² Principal investigator

[Note: This is a different parasite to the one commonly known as threadworm in the U.K.]

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*π* Note this is a different parasite to the one commonly known as threadworm in the U.K.
Gene drives in agricultural weeds and other plants are speculative at this point in time, and we have not found examples that have been proven to work, even in the laboratory. Plants present several challenges for gene drive function. Their generally low rates of homologous recombination compared to other organisms makes it less likely that they will be suitable target organisms because of resulting mutations at target sites during NHEJ, or low rates of incorporation of genes used in drives. Seed buried in soil that may remain viable for many years could escape gene drive exposure, while many plant species are self-fertile or commonly reproduce vegetatively, also avoiding gene drive exposure.

<table>
<thead>
<tr>
<th>Type of gene drive</th>
<th>Global or ‘local’</th>
<th>Phenotype</th>
<th>Readiness of technology</th>
<th>PI, institution and funder Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autosomal ‘X-shredder’</td>
<td>In theory local (may spread)</td>
<td>Male only (drive results in &gt;95% male offspring)</td>
<td><img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /></td>
<td>A. Crisanti Imperial College Gates/DARPA/BBSRC (Galizi et al. 2016, Galizi et al. 2014, Facchinelli et al. 2019)</td>
</tr>
<tr>
<td>Homing CRISPR-Cas9</td>
<td>Probably global (no localisation strategy clear)</td>
<td>Intersex and sterile females (Kyrou et al)</td>
<td><img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /> <img src="#" alt="Red" /></td>
<td>A. Crisanti Imperial College Gates/DARPA BBSRC (Kyrou et al. 2018, Hammond et al. 2015)</td>
</tr>
</tbody>
</table>
| Homing CRISPR-Cas9 | Probably global (no localisation strategy clear) | Resistance to malaria parasite, \*P. falciparum* | ![Red](#) ![Red](#) ![Red](#) ![Red](#) ![Red](#) ![Red](#) ![Red](#) ![Red](#) | V. Gantz, E. Bier and A. James UCSD NIH/TATA/DARPA (Gantz et al. 2015)]

The table also shows alongside *Drosophila melanogaster* and *Mus musculus*, the baker’s and brewer’s yeast *Saccharomyces cerevisiae* is increasingly becoming a model organism for developing the technology (and that the soil nematode *Caenorhabditis brenneri* will also perhaps become established in this capacity). *Saccharomyces cerevisiae* is a widespread organism, and this is likely why two of the studies include molecular safeguards to reduce the risk of the drive and the GDO spreading outside the laboratory. These studies and others (e.g. Champer et al. 2019) raise the additional and important question: should molecular safeguards be mandatory in the design of more gene drive experiments, in addition to high level containment?
<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range¹</th>
<th>Problem it is aiming to address</th>
<th>Intended application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquitoes</td>
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<tr>
<td><em>Aedes aegypti</em></td>
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<tr>
<td>Yellow fever mosquito</td>
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<td></td>
<td></td>
<td>‘…predicted to occur primarily in the tropics and sub-tropics, with concentrations in northern Brazil and southeast Asia including all of India…’ (Kraemer et al. 2015)</td>
<td>Transmission of Zika and Dengue in California</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td></td>
<td></td>
<td>Population suppression</td>
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<tr>
<td><em>Culex quinquefasciatus</em></td>
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<tr>
<td>Southern house mosquito</td>
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<td>Population suppression</td>
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<td></td>
<td></td>
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<tr>
<td>Southern house mosquito</td>
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<tr>
<td><em>Drosophila melanogaster</em></td>
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<tr>
<td><em>Drosophila melanogaster</em></td>
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<td></td>
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<tr>
<td>Common fruit fly</td>
<td></td>
<td>cosmopolitan, present on all continents except Antractica (Miller 2000)</td>
<td>Providing proof of concept for homing CRISPR-Cas9 gene drive</td>
</tr>
<tr>
<td><em>Drosophila melanogaster</em></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Drosophila melanogaster</em></td>
<td></td>
<td></td>
<td>To provide proof of concept for specific gene drive system</td>
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<tr>
<td><em>Drosophila melanogaster</em></td>
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<tr>
<td><em>Drosophila melanogaster</em></td>
<td></td>
<td></td>
<td>To provide proof of concept for specific gene drive system(UD MEL)</td>
</tr>
<tr>
<td><em>Drosophila melanogaster</em></td>
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<td><em>Drosophila melanogaster</em></td>
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<tr>
<td><em>Drosophila melanogaster</em></td>
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</tbody>
</table>

³ The particular underdominance system is not specified in the article, but is likely to be similar to Floyd Reeds RPM drive developed in *Drosophila*

⁴ Not clear if this work is funded or not.

⁵ Not clear how much it would spread in the case of accidental release.

⁶ Not clear if issues with resistance are present and need to be resolved.
<table>
<thead>
<tr>
<th>Type of gene drive</th>
<th>Global or ‘local’</th>
<th>Phenotype</th>
<th>Readiness of technology</th>
<th>PI(s), institution and funder Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>probably Homing CRISPR-Cas9</td>
<td>Intention appears to be ‘local’</td>
<td>Resistance to Zika Virus</td>
<td>4</td>
<td>O. Akbari UCSD NIH/DARPA (DARPA 2017)</td>
</tr>
<tr>
<td>CRISPR-Cas9 Daisy chain drive</td>
<td>In theory ‘local’ (but no proof of concept for this method)</td>
<td>Sterile females</td>
<td>3</td>
<td>Esvelt and Alphey MIT/Pirbright DARPA (DARPA 2017, BBSRC 2018)</td>
</tr>
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<td>3</td>
<td>K. Esvelt and L. Alphey MIT/Pirbright DARPA (BBSRC 2018, DARPA 2017)</td>
</tr>
<tr>
<td>Under-dominance</td>
<td>In theory ‘local’</td>
<td>Resistance to avian malaria parasite</td>
<td>4</td>
<td>F. Reed University of Hawaii (Goldman 2016)</td>
</tr>
</tbody>
</table>

3 The particular underdominance system is not specified in the article, but is likely to be similar to Floyd Reeds RPM drive developed in Drosophila

4 Not clear if this work is funded or not

5 Not clear how much it would spread in the case of accidental release

6 Not clear if issues with resistance are present and need to be resolved

<table>
<thead>
<tr>
<th>Homing CRISPR-Cas9</th>
<th>Not intended for release.</th>
<th>Yellow colour (due to lack of pigment)</th>
<th></th>
<th>V. Gantz and E. Bier UCSD NIH (Gantz and Bier 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underdominance: Maternal effect lethal under-dominance (UD MEL)</td>
<td>Not intended for release: in theory local</td>
<td>No detected phenotype (individuals not carrying drive do not hatch)</td>
<td></td>
<td>B. Hay California Institute of Technology NIH and others (Akbari et al. 2013)</td>
</tr>
<tr>
<td>MEDEA (Maternal Effect Dominant Embryonic Arrest)</td>
<td>Not intended for release: in theory local</td>
<td>No detected phenotype (individuals not carrying drive do not hatch)</td>
<td></td>
<td>B. Hay California Institute of Technology NIH and others (Chen et al. 2007, Akbari et al. 2014)</td>
</tr>
</tbody>
</table>
### Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range¹</th>
<th>Problem it is aiming to address</th>
<th>Intended application</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Drosophila melanogaster</em></td>
<td></td>
<td>NA (Laboratory model) To provide proof of concept for specific gene drive variation (ClvR) using CRISPR/Cas9 as ‘toxin’</td>
<td>Aim is demonstrating under-dominance system</td>
</tr>
<tr>
<td><em>Drosophila melanogaster</em></td>
<td></td>
<td>NA (Laboratory model)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Drosophila suzukii</em></td>
<td></td>
<td>Economic impacts of damage to soft fruit crops (e.g. cherries)</td>
<td>Here providing proof of concept for MEDEA in <em>D. suzukii</em> for population suppression or replacement</td>
</tr>
<tr>
<td>Spotted wing drosophila</td>
<td>Brazil, United States, Canada, Europe and Japan (Polo et al. 2016)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ceratitis capitata</em></td>
<td>Africa, Mediterranean area Australasia, North and South America (FAO/IAEA 2017)</td>
<td>Economic impacts caused by damage to fruit crops</td>
<td>Population suppression</td>
</tr>
<tr>
<td>Mediterranean fruit fly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Diaphorina citri</em></td>
<td>Central and South America, India, South East Asia and Saudi Arabia (Grafton-Cardwell et al. 2005)</td>
<td>Economic impacts of Citrus greening disease (caused by a bacterium which is transmitted by the psyllid)</td>
<td>Population modification or replacement</td>
</tr>
<tr>
<td>Asian citrus psyllid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhodnius prolixus</em></td>
<td>Venezuela, Columbia and parts of Central America (Sosa-Estani and Leonor Segura 2015)</td>
<td>Impacts of Chagas disease: <em>R. prolixus</em> is a vector for the causative parasite <em>Trypanosoma cruzi</em></td>
<td>Population suppression</td>
</tr>
<tr>
<td>Kissing bug</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lucilia cuprina</em></td>
<td>Warmer regions worldwide, including areas of Australasia, North America and Sub-Saharan Africa.</td>
<td>Blowfly infection of sheep causes lesions which can cause death and/or welfare issues</td>
<td>Population suppression, (eradication in New Zealand)</td>
</tr>
<tr>
<td>Australian sheep blowfly</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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1. Not clear how much it would spread in the case of accidental release
2. Not clear if issues with resistance are present and need to be resolved
3. Reported to be stable for 200 generations
4. The funders acknowledged here are the German Academic Exchange Service, and the Excellence Foundation for the Promotion of the Max Planck Society
5. This project apparently encountered difficulties with developing molecular genetics tools in the target species. It was funded until 2017, but it is not clear if work is still ongoing.
6. Not clear if this work is funded or not.
7. This investigator was recommended for funding but is a specialist in the parasite *T. cruzi*, so the gene drive itself would likely be constructed by a group specialising in insect molecular genetics.
<table>
<thead>
<tr>
<th>Type of gene drive</th>
<th>Global or ‘local’</th>
<th>Phenotype</th>
<th>Readiness of technology</th>
<th>PI(^2), institution and funder Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin-Antidote drive; Here via “Cleave and Rescue” (ClvR)</td>
<td>Not intended for release(^2)</td>
<td>Red and green fluorescence markers</td>
<td>B. Hay California Institute of Technology USDA (Oberhofer, Ivy, and Hay 2019)</td>
<td></td>
</tr>
<tr>
<td>Under-dominance RPM-drive (Ribosomal Protein Minute Drive)</td>
<td>Not intended for release: in theory local</td>
<td>No detected phenotype except red fluorescent marker. Heterozygotes develop slower and have less viable offspring</td>
<td>F. Reed University of Hawaii NSF and others (Reed et al. 2018, Reeves et al. 2014)</td>
<td></td>
</tr>
<tr>
<td>MEDEA</td>
<td>In theory local but capable of spread</td>
<td>Here for testing: red fluorescence throughout body, weak green in eyes</td>
<td>O. Akbari UCSD California Cherry Board (Buchman, Marshall, et al. 2018)</td>
<td></td>
</tr>
<tr>
<td>Homing CRISPR-Cas9</td>
<td>Potentially global with some discussion of theoretical potential for localisation</td>
<td>Either infertility or sex ratio distortion</td>
<td>E. Wimmer University of Göttingen(^10) (KaramiNejadRanjbar et al. 2018)</td>
<td></td>
</tr>
<tr>
<td>Possibly ‘cleavage drive’ (low threshold) or ‘reciprocal chromosome translocations’ (high threshold)</td>
<td>Both global and local are being considered</td>
<td>Various mechanisms under investigation to block transmission of the bacterium</td>
<td>B. Hay and others California Institute of Technology CitrusRDF (Turpen 2017)</td>
<td></td>
</tr>
<tr>
<td>Probably homing CRISPR-Cas9</td>
<td>Need for localisation noted but no strategy as yet</td>
<td>Not clear: probably female sterility or sex ratio distortion</td>
<td>N. El-Sayed(^13) University of Maryland PAF recommended for funding (Darrow et al. 2016)</td>
<td></td>
</tr>
<tr>
<td>Probably homing CRISPR-Cas9</td>
<td>Intention is probably ‘local’ to New Zealand</td>
<td>All male offspring</td>
<td>NA (Dearden et al. 2018)</td>
<td></td>
</tr>
</tbody>
</table>
### Chapter 2: Potential applications and risks

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range¹</th>
<th>Problem it is aiming to address</th>
<th>Intended application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tribolium castaneum Red flour beetle</td>
<td>present on all continents except Antarctica (IRAC 2019)</td>
<td>Economic impacts of consumption of stored grains</td>
<td>Population suppression</td>
</tr>
<tr>
<td>Vespula vulgaris Common wasp</td>
<td>North America, Asia, Europe (Holartic species) and Australia and New Zealand</td>
<td>‘Wasps attack native birds and insects and deplete critical food resources’</td>
<td>Population suppression, (Eradication in New Zealand)</td>
</tr>
<tr>
<td>Vespula germanica German wasp</td>
<td>predicted distribution; red/yellow = suitable; green = marginal (de Villiers, Kriticos, and Veldtman 2017)</td>
<td>‘Wasps attack native birds and insects and deplete critical food resources’</td>
<td>Population suppression, (Eradication in New Zealand)</td>
</tr>
<tr>
<td>Listronotus bonariensis Argentine stem weevil</td>
<td>South America (Argentina, Brazil, Chile, Bolivia, Uruguay), Australia and New Zealand</td>
<td>Economic impacts of damage to pasture grass</td>
<td>Population suppression, (Eradication in New Zealand)</td>
</tr>
</tbody>
</table>

14 Not clear how localisation would be achieved

**Table 2b: Small Mammals**

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range¹</th>
<th>Problem it is aiming to address</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mus musculus</td>
<td></td>
<td>Generating new lab mouse strains carrying multiple modifications is laborious</td>
<td>Proof of concept of CRISPR-Cas9 gene drive in mice as a mouse genetics tool</td>
</tr>
<tr>
<td>Mus musculus</td>
<td></td>
<td>Impacts of invasive populations on islands – and potentially wider applications: see case study</td>
<td>Population suppression to eliminate invasive populations</td>
</tr>
<tr>
<td>Mus musculus</td>
<td></td>
<td>Impacts of invasive populations on island biodiversity</td>
<td>Population suppression to eliminate invasive populations</td>
</tr>
<tr>
<td>Mus musculus</td>
<td></td>
<td>Economic costs of rodent ‘pest’ populations in UK and elsewhere</td>
<td>Population suppression ‘humane’ and cost-effective control of rodent populations</td>
</tr>
</tbody>
</table>

¹ Please see separate bibliography for sources for maps and geographic range.
<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range</th>
<th>Problem it is aiming to address</th>
<th>Application</th>
<th>Type of gene drive</th>
<th>Phenotype</th>
<th>Readiness of technology</th>
<th>PI, institution and funder Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vespula vulgaris</em></td>
<td>Common wasp North America, Asia, Europe (Holartic species) and Australia and New Zealand</td>
<td>‘Wasps attack native birds and insects and deplete critical food resources’</td>
<td>Population suppression, (Eradication in New Zealand)</td>
<td>Probably homing CRISPR-Cas9</td>
<td>Infertility or sex ratio distortion</td>
<td>NA (Dearden et al. 2018)</td>
<td></td>
</tr>
<tr>
<td><em>Vespula germanica</em></td>
<td>German wasp predicted distribution; red/yellow = suitable; green= marginal (de Villiers, Kriticos, and Veldtman 2017)</td>
<td>‘Wasps attack native birds and insects and deplete critical food resources’</td>
<td>Population suppression, (Eradication in New Zealand)</td>
<td>Probably homing CRISPR-Cas9</td>
<td>Infertility or sex ratio distortion</td>
<td>NA (Dearden et al. 2018)</td>
<td></td>
</tr>
<tr>
<td><em>Listronotus bonariensis</em></td>
<td>Argentine stem weevil South America (Argentina, Brazil, Chile, Bolivia, Uruguay), Australia and New Zealand</td>
<td>Economic impacts of damage to pasture grass</td>
<td>Population suppression, (Eradication in New Zealand)</td>
<td>Probably homing CRISPR-Cas9</td>
<td>Infertility or sex ratio distortion</td>
<td>NA (Dearden et al. 2018)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of gene drive</th>
<th>Global or ‘local’</th>
<th>Phenotype</th>
<th>Readiness of technology</th>
<th>PI, institution and funder Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homing CRISPR-Cas9</td>
<td>Theoretically global but current efficiency likely too low to effectively spread in wild</td>
<td>all white coats</td>
<td></td>
<td>K. Cooper (with V. Gantz and E. Bier) UCSD NIH and others (Grunwald et al. 2019)</td>
</tr>
<tr>
<td>T-haplotype</td>
<td>Not clear (but no localisation strategy given)</td>
<td>daughterless</td>
<td></td>
<td>D. Threadgill Texas A&amp;M DARPA (Leitschuh et al. 2018)</td>
</tr>
<tr>
<td>Homing CRISPR-Cas9</td>
<td>Not clear (but no localisation strategy given)</td>
<td>daughterless</td>
<td></td>
<td>P. Thomas University of Adelaide DARPA (GeneDriveFiles 2017)</td>
</tr>
<tr>
<td>Homing CRISPR-Cas9 or CRISPR-Cas9 X-shredder</td>
<td>Not clear</td>
<td>sterile females</td>
<td></td>
<td>B. Whitelaw Roslin Institute, UK BBSRC (McFarlane, Whitelaw, and Lillico 2018)</td>
</tr>
<tr>
<td>Species</td>
<td>Geographic range</td>
<td>Problem it is aiming to address</td>
<td>Application</td>
<td></td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------------</td>
<td>-------------------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><em>Peromyscus leucopus</em></td>
<td></td>
<td>Increasing incidence of Lyme’s disease in humans</td>
<td>Population modification (to reduce tick borne transmission of Lyme disease to humans)</td>
<td></td>
</tr>
<tr>
<td>White footed mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeting rats in UK,</td>
<td></td>
<td>Economic costs of rodent ‘pest’ populations in UK and elsewhere</td>
<td>Population suppression</td>
<td></td>
</tr>
<tr>
<td>probably <em>Rattus norvegicus</em> Brown rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Felis silvestris</em></td>
<td>Wild cat range (excluding feral populations)</td>
<td>Feral cat populations in Australia preying on native wildlife</td>
<td>Population suppression (eradication of Australian feral cat population)</td>
<td></td>
</tr>
<tr>
<td>House cat, wild cat &amp; feral cat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichosurus vulpecula</em></td>
<td>Native range</td>
<td>‘Predator on native birds and invertebrates, eats native plants, carrier for bovine TB’</td>
<td>Population suppression (eradication in New Zealand)</td>
<td></td>
</tr>
<tr>
<td>Brushtail possum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rattus rattus</em></td>
<td></td>
<td>‘Predator on native birds and invertebrates, eats native plants, carrier for diseases’</td>
<td>Population suppression (eradication in New Zealand)</td>
<td></td>
</tr>
<tr>
<td>Common rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mustela erminea</em></td>
<td></td>
<td>‘Predator on native birds and invertebrates, eats native plants, carrier for diseases’</td>
<td>Population suppression (eradication in New Zealand)</td>
<td></td>
</tr>
<tr>
<td>Stoats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Geographic range</td>
<td>Problem it is aiming to address</td>
<td>Application</td>
<td>Type of gene drive</td>
</tr>
<tr>
<td>---------</td>
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<td>---------------------------------</td>
<td>-------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Peromyscus leucopus</td>
<td>White footed mouse</td>
<td>Increasing incidence of Lyme’s disease in humans</td>
<td>Population modification (to reduce tick borne transmission of Lyme disease to humans)</td>
<td>Homing CRISPR-Cas9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Homing CRISPR-Cas9 or CRISPR-Cas9 X-shredder</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Probably homing CRISPR-Cas9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Probably homing CRISPR-Cas9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Probably homing CRISPR-Cas9</td>
</tr>
<tr>
<td>Rattus rattus</td>
<td>Common rat</td>
<td>Predator on native birds and invertebrates, eats native plants, carrier for diseases</td>
<td>Population suppression (eradication in New Zealand)</td>
<td>Homing CRISPR-Cas9</td>
</tr>
<tr>
<td>Mustela erminea</td>
<td>Stoats</td>
<td>Predator on native birds and invertebrates, eats native plants, carrier for diseases</td>
<td>Population suppression (eradication in New Zealand)</td>
<td>Probably homing CRISPR-Cas9</td>
</tr>
</tbody>
</table>

**Note:** The “Readiness of technology” column uses a scale from 1 to 8, where 1 indicates the least ready and 8 indicates the most ready.
<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range</th>
<th>Problem it is aiming to address</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pterois volitans</em></td>
<td>Indian Ocean, Red Sea, Invasive in Gulf of Mexico, Caribbean and Western Atlantic (FFWCC 2019)</td>
<td>“This invasive species has the potential to harm reef ecosystems... ...a top predator that competes with overfished native stocks”</td>
<td>Population suppression (eradication in Gulf of Mexico, Caribbean and Western Atlantic)</td>
</tr>
<tr>
<td><strong>Birds</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sturnus vulgaris</em></td>
<td>Dark colours = native; Light colours = invasive</td>
<td>Not stated but probably impacts of invasive starlings on agriculture and competition with native species</td>
<td>Population suppression (eradication in Australia)</td>
</tr>
<tr>
<td><strong>Mollusks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Biomphalaria glabrata</em></td>
<td>Parts of Brazil, and Venezuela, the Lesser Antilles (Mavarez et al. 2002)</td>
<td>Human health impacts of infection with schistosome parasites for which the snail is an intermediate host</td>
<td>Populations modification</td>
</tr>
<tr>
<td><strong>Nematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Caenorhabditis brenneri</em></td>
<td>Probably circum-tropical (Sudhaus and Kiontke 2007)</td>
<td>NA</td>
<td>Aim is development and testing of daisy chain drive and related concepts</td>
</tr>
<tr>
<td><em>Necator americanus</em></td>
<td>Circum-tropical and some temperate regions (Palmer, Reeder, and Dunn 2000)</td>
<td>Human health impacts of soil transmitted helminth infection</td>
<td>Population suppression</td>
</tr>
<tr>
<td><strong>Nematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ancylostoma duodenale</em></td>
<td>Mainly South East Asia, and Mediterranean (Palmer, Reeder, and Dunn 2000)</td>
<td>Same project</td>
<td>Same project</td>
</tr>
<tr>
<td><em>Trichuris trichuria</em></td>
<td>Circumtropical, Southern Europe and some other temperate regions (Palmer, Reeder, and Dunn 2000)</td>
<td>Same project</td>
<td>Same project</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>Endemic in Central and South America, sub-Saharan Africa, India and South East Asia (Varatharajalu and Kakuturu 2016)</td>
<td>Same project</td>
<td>Same project</td>
</tr>
</tbody>
</table>

1 It is not clear if this work has been funded or not
<table>
<thead>
<tr>
<th>Type of gene drive</th>
<th>Local or global</th>
<th>Phenotype to be propagated</th>
<th>Readiness of technology</th>
<th>PI, institution and funder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homing CRISPR-Cas9</td>
<td>Not clear if or how it would be localised</td>
<td>Not yet selected</td>
<td>1</td>
<td>P. Venturelli Ball State University Funder unknown (Vacura et al. 2018)</td>
</tr>
<tr>
<td>Probably Homing CRISPR-Cas9</td>
<td>Not clear if or how it would be localised</td>
<td>Not yet selected</td>
<td>NA</td>
<td>NA (Moro et al. 2018) (GISD 2019)</td>
</tr>
<tr>
<td>Probably Homing CRISPR-Cas9</td>
<td>Proposal to localise with daisy drive technology</td>
<td>Resistance to infection with schistosome parasites</td>
<td></td>
<td>J. Teem ILSI Foundation Funder not clear (Teem 2016)</td>
</tr>
<tr>
<td>CRISPR-Cas9 or CRISPR-Cpf1 Daisy chain drive (and variants)</td>
<td>Local (not intended for release)</td>
<td>Either a) change in fluorescence; or b) right hand coiled; or c) short (dumpy)</td>
<td></td>
<td>K. Esvelt MIT DARPA (Esvelt 2017)</td>
</tr>
<tr>
<td>Probably homing CRISPR-Cas9</td>
<td>Not clear</td>
<td>Possibly biasing sex ratios</td>
<td></td>
<td>M. Berriman, Sanger Institute J. Lok Uni. of Pennsylvania Recommended for funding (Darrow et al. 2016)</td>
</tr>
<tr>
<td>Species</td>
<td>Geographic range</td>
<td>Problem it is aiming to address</td>
<td>Application</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Flatworms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>Africa, the Middle East, South America and Caribbean (Weerakoon et al. 2015)</td>
<td>Human health impacts of schistosomiasis (bilharzia) caused by infection with this parasite</td>
<td>Population suppression</td>
<td></td>
</tr>
<tr>
<td><em>Schistosoma haematobium</em></td>
<td>Africa and the Middle East (Weerakoon et al. 2015)</td>
<td>Same project as above</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> Brewer’s yeast</td>
<td>Found globally in domesticated, human and wild environments. (Can hybridize with closest relative S. paradoxus) (Peter et al. 2018)</td>
<td>NA</td>
<td>Aim is to study gene drives over ‘hundreds of generations’, to understand emergence of resistance</td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> Brewer’s yeast</td>
<td>Found globally in domesticated, human and wild environments (Peter et al. 2018)</td>
<td>NA</td>
<td>Validation of CRISPR-Cas 9 gene drive in S. Cerevisiae</td>
<td></td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> Brewer’s yeast</td>
<td>Found globally in domesticated, human and wild environments (Peter et al. 2018)</td>
<td>NA</td>
<td>Testing of various methods to modulate gene drive activity (e.g. Cas9 expression level) (Roggenkamp et al. 2018) and multiplexed gRNAs (Yan and Finnigan 2018)</td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>A commensal organism in humans and animals (including mammals, and probably birds, reptiles, and fish)</td>
<td>NA</td>
<td>Aim is to easily create homozygous deletion mutants in diploid strains</td>
<td></td>
</tr>
</tbody>
</table>

2 The sex of schistosomes is determined by Z and W rather than X and Y. Females are ZW and males ZZ’. The proposed drive is conceptually similar to an X-Shredder design, the W-shredder would be encoded on the Z chromosome.

3 Not clear if issues with resistance are present and need to be resolved

### Readiness of Technology. Categories are:

1. Gene drive proposed
2. Gene drive proposed with published preliminary research (but potentially not done with intention of creating gene drive)
3. Funded preliminary research (genome sequences, molecular genetics tools, etc)
4. Funded research on gene drive construction (with no results published yet)
5. Limited proof of concept for gene drive (i.e. there are outstanding technical issues such as resistance, low efficiency, too high fitness costs)
6. Laboratory proof of concept
7. Proof of concept in contained simulated natural environments
8. Releases in natural environment

Grey bars denotes gene drives that are not intended for release.
<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic range</th>
<th>Problem it is aiming to address</th>
<th>Application</th>
<th>Type of gene drive</th>
<th>Readiness of technology</th>
<th>PI, institution and funder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flatworms</td>
<td>Africa, the Middle East, South America and Caribbean (Weerakoon et al. 2015)</td>
<td>Human health impacts of schistosomiasis (bilharzia) caused by infection with this parasite</td>
<td>Population suppression</td>
<td>CRISPR-Cas9-based ‘W-shredder’²</td>
<td>Not intended for public release</td>
<td>P. Brindley George Washington University (with K. Esvelt) Thomas Mather (Philanthropist) (Brindley and Esvelt 2019)</td>
</tr>
<tr>
<td>Schistosoma haematobium</td>
<td>Africa and the Middle East (Weerakoon et al. 2015)</td>
<td>Same project as above</td>
<td></td>
<td></td>
<td></td>
<td>S. Kryazhimskiy and J. Meyer (collaborating with O. Akbari, V. Gantz &amp; E. Bier) UCSD DARPA (Aguilera 2017 )</td>
</tr>
<tr>
<td>Fungi</td>
<td>Saccharomyces cerevisiae Brewer's yeast</td>
<td>NA</td>
<td>Aim is to study gene drives over 'hundreds of generations', to understand emergence of resistance</td>
<td>Probably Homing CRISPR-Cas9 (as Gantz and Bier use this technology)</td>
<td>Not intended for release</td>
<td>G. Church Harvard Medical School DOE, NSF and others (DiCarlo et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>Found globally in domesticated, human and wild environments. (Can hybridize with closest relative S. paradoxus) (Peter et al. 2018)</td>
<td></td>
<td>Validation of CRISPR-Cas9 gene drive in S. Cerevisiae</td>
<td>Homing CRISPR-Cas9</td>
<td>Not intended for release (Contained: Cas9 expressed on episome separate from drive)</td>
<td>G. Finnigan Kansas State University NIH and USDA (Roggenkamp et al. 2018, Yan and Finnigan 2018)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Testing of various methods to modulate gene drive activity (e.g. Cas9 expression level) (Roggenkamp et al. 2018) and multiplexed gRNAs (Yan and Finnigan 2018)</td>
<td>Homing CRISPR-Cas9</td>
<td>Not intended for release (Contained: Cas9 expressed on episome separate from drive and target sequence not in wild type)</td>
<td>G. Church Harvard Medical School DOE, NSF and others (DiCarlo et al. 2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CRISPR-Cas9</td>
<td>Not intended for release (containment strategy not described)</td>
<td>Collins MIT Various including NIH (Shapiro et al. 2018)</td>
</tr>
</tbody>
</table>

**Abbreviations for funders and other organisations:**
- **BBSRC** – UK Biotechnology and Biological Sciences Research Council
- **Gates** – The Bill and Melinda Gates Foundation
- **DARPA** – US Defense Advanced Research Projects Agency
- **NIH** – US National Institutes of Health
- **TATA** – TATA trusts
- **USDA** – US Department of Agriculture
- **PAF** – Philanthropy Advisory Fellowship
2.3 Knowledge required to understand the risks of using a species as a GDO

In the following case studies, we tried to address some main points that we regard as essential for gaining an understanding of the complexities, uncertainties and possible hazards that are involved in gene drive organisms. For this we used in part the brief check list below, which is not intended to be exhaustive, but rather to illustrate the breadth of elements and knowledge required. Some of these points will be picked up again in the final section on risk assessment.

A check list with important elements and questions for hazard identification

a) Ecological importance or “ranking” of gene drive target organism
   • Role within ecosystem; e.g. pollinators, place in food chain.
   • Knowledge of behaviour and interactions of GDO species
   • Listing of all predators, including their spectrum of prey and possible reliance on the GDO species.
   • Ability to spread; including speed of spread, distance of movement of individuals, ability to be carried by other organisms or by wind or water; dependence on particular ecological niche or ability to adapt easily to altered conditions.
   • Is it a “keystone species”?
   • Is it important for the survival of threatened or endangered species?

b) Global spread, ubiquity (only local, or everywhere)

c) Population genetics
   • Diversity of genetic background (within species)
   • Closely related species
   • Data on introgression (from hybridisation or in-crossing)

d) Are GDOs intended as a “solution approach”, and if so, at which level?
   • The perceived problem
   • What underlies this perceived problem, including the causes, the root causes and what enhances or what reduces the problem. Is the perceived or addressed problem more a symptom of underlying causes and problems.
   • Which answers/solutions are already present, have been tried, suggested, or may be possible? e.g. push-pull systems, plants with semiochemicals, biological controls (release of predators or parasitoids), drying up water, crop diversity and enhanced pest enemy habitat, protection from pesticides, breeding crops for resistance.

e) The gene drive approach
   • Description of suggested gene drive approach: what is it? Who is suggesting it, developing it, funding it? (e.g. national weapons researchers, infectious disease agency, agribusiness, biotech venture companies, conservation researchers).
   • Who is involved? How far has it gotten?
   • Is it the right approach (treatment of cause vs. symptom)?
   • Will gene drives actually be effective to solve the problem?
   • What negative implications, off-target effects and risks in general may this approach entail? (A central reference would be the CBD’s AHTEG Guidance on LM mosquitoes)\(^5\)
   • Does the gene drive approach provide important environmental co-benefits, and how does this compare to other approaches to solving the problem?
   • Has a similar approach to the problem been taken in the past (with other techniques)? What were the consequences? (e.g. SIT - sterile insect technique)
   • What would be a more sustainable approach or alternative solution?
   • Problems/solutions and other approaches (e.g. sterile insect technology SIT and GM RIDL mosquitoes; Wolbachia treatment for mosquitoes)

\(^5\) [https://www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf](https://www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf)
• Is the GDO acquiring an intended or accidental advantage as compared to the wild, non-modified population? (For example, endangered species might be given a resistance or advantage gene). What might those consequences be?

f) Social Implications
• Beyond the possible resolution of the immediate problem, who benefits from the gene drive approach compared to other possible solutions?
• Does the gene drive attract funding over other solutions because it benefits favoured segments of society?
• Does the gene drive have co-benefits for society broadly compared to other strategies?

All of the above considerations point to the complexity and challenges associated with the risk assessment of gene drives. Because of the intended or unintended spread of gene drives, their effects on the environment and society may be particularly complex compared to older technologies that are more contained and less likely to spread. Risk assessments for technologies like very widely used pesticides and GMOs in recent years have revealed many risks and harms several steps removed from direct impacts on target organisms. The scale of these technologies can be revealing for broad ecosystem of social effects. These effects have often been sub-lethal, on behaviour, fertility, the immune system, or other population level effects that have been difficult and time consuming for regulatory agencies to address. These have often not been effectively anticipated, and resulting harm only later detected. Similarly, social effects can be complex and favour some groups in society over others, prosing equity challenges. As the power of technologies like gene drives increases, their potential impacts can be much more complex.

2.4 Studies and specific applications

2.4.1 Case study 1: Mosquitoes

Introduction

Gene drives are actively being developed in at least four different mosquito species. Whilst technical issues remain, drives with the potential to suppress mosquito populations by biasing sex ratios or causing female infertility, or to modify populations with disease resistance genes, have been demonstrated in laboratories. In the light of these proof of concept studies, and the active pursuit by its developers for environmental release, focusing on the possible consequences of employing and releasing such technology has become paramount. Here we argue that, given the interwoven nature of ecosystems and the serious limitations in scientific understanding of these systems, and especially when combined with the unpredictability of the behaviour and actions of the engineered gene drives and GDOs, the attempted extinction or suppression of mosquito species would bring consequences that are difficult or impossible to fully or accurately predict, and which could be profoundly and irreversibly harmful.

Mosquitoes have existed at least since the beginning of the Jurassic, 200 million years ago (Reidenbach et al. 2009), co-evolving within a web of relationships to other species over this vast period of geological time (Tang et al. 2018). These relationships are not well explored; field studies usually only reveal single threads, or at best small parts of this web. Yet enough has been discovered to see these connections are likely to be significant. To consider some examples, one field study shows that for nesting house martens mosquitoes appear to be an important food source as they raise their young broods (Poulin, Lefebvre, and Paz 2010). Other studies reveal that for blunt-leaf orchids in the forests of North America, Scandinavia and Siberia, they are a major pollinator (Thien and Utech 1970; Gorham 1976). And in the tiny aquatic ecosystem inside the common pitcher plant, research shows their larvae are even a keystone predator, shaping...
the diversity of the microscopic community within (Peterson et al. 2008).

Taking a broader view, the mosquito family has adapted to virtually all land habitats around the globe, from the arctic tundra to the tropical forests, resulting in a huge variety of species in many ecological niches. More than two centuries of dedicated work by entomologists has described over 3500 species (Harbach 2013), yet this number continues to grow each year; some tropical regions probably contain numerous species which are still unknown to science (Foley, Rueda, and Wilkerson 2007).

At least 160⁶ species play some role in transmitting human pathogens and thus there is ongoing debate about the desirability of eliminating some or even all forms of the mosquito (Fang 2010), which has intensified with the arrival of CRISPR/Cas-based gene drives and suggestions that this technology could achieve such goals. To better understand the complexities, the potential hazards and the possible negative impacts of deploying such drives, we examine the various gene drive proposals, and briefly review the biology and ecological role of the mosquito, before moving on to consider the many uncertainties surrounding outcomes.

**Gene drive proposals**

**Population suppression or eradication**

At the time of writing, the most advanced gene drive technology targeting mosquitoes has been developed by a team at Imperial College London, UK, led by Andrea Crisanti, as part of the Gates Foundation’s Target Malaria project. This group is conducting advanced trials (in simulated natural environments, according to news reports)⁷ of at least two gene drive technologies theoretically capable of suppressing or eradicating populations of the African malaria mosquito, *Anopheles gambiae*, which they plan to use against wild populations (Molteni 2018). One technology under development is the X-shredder, here making the mosquito produce endonucleases⁸ to specifically target and sever sites on the X-chromosome during sperm production, resulting in the near absence of intact X-chromosomes in sperm and so producing almost entirely male offspring (Galizi et al. 2014; Galizi et al. 2016). If the gene for the endonuclease is engineered into an ordinary (autosomal) chromosome, the drive is not believed to spread rapidly, as it will be inherited in a Mendelian fashion. However, if the gene is engineered into the Y-chromosome (a sex-chromosome), the drive will be passed on to every male, making this drive theoretically highly invasive (Marshall and Akbari 2018). Whilst the first version has been tested in a laboratory (Galizi et al. 2016) the second version described by Marshall and Akbari 2018 has not yet been constructed. Following initial trials with conventional GM mosquitoes in Burkina Faso, the Imperial College group are proposing to release a theoretically self-limiting form of the X-shredder as a step towards gaining regulatory approval for more invasive and persistent drives (Molteni 2018).

Their second drive project is a CRISPR/Cas based homing drive (see Chapter 1). Resistance is a crucial issue in gene drive design (see Chapter 1): in the case of CRISPR/Cas9-based drives in particular, mutations frequently arise at the DNA target site as a result of erroneous repair after cutting, rendering individuals that inherit them resistant to the drive. Whilst it is unclear to what extent such resistance emerges to the X-shredder; the team has recently published details of a CRISPR/Cas-homing drive design which experimentally overcame this defence mechanism. They chose to disrupt a gene named doublesex, which results in sterility in females carrying the drive (Kyrou et al. 2018b). Because of its vital role, this gene has very little scope for mutation and therefore the minor mutations which normally allow resistance to evolve do not appear, allowing this drive to completely eradicate laboratory populations.

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⁶ See Table 3
⁷ Results have very recently been published on the I-PpoI X-shredder in simulated natural environments see Facchinelli et al, 2019.
⁸ Two versions of this approach have been described one using CRISPR/Cas9 (Galizi et al. 2016) and another employing an altered homing endonuclease gene I-PpoI from the slime mold Physarum polycephalum (Galizi et al. 2014)
Population modification

In contrast to the goal of population suppression, a consortium of researchers in California including Valentino Gantz, Ethan Bier, Anthony James and Omar Akbari aim to use gene drives to modify populations to confer resistance to pathogens, an approach which they believe will reduce the pressure for resistance to the gene drive to evolve and to spread. A principal target is Anopheles stephensi, a major malaria vector in India, which has been modified in a proof of concept experiment with a homing CRISPR/Cas9 drive to spread genes conferring a level of immunity to the malaria pathogen in mosquitoes in laboratory populations (Gantz et al. 2015b). As observed with similar CRISPR/Cas9 designs however, mutations giving resistance to the drive appear rapidly, which the group are exploring methods to overcome. The consortium is also being funded to develop gene drives in Aedes aegypti (DARPA 2017), which may include drives to propagate genes that inhibit the capacity of this mosquito to transmit Zika virus (Buchman et al. 2019)9.

‘Self-limiting’ gene drives

Because they wish to gain acceptance for the technology, the emerging view among gene drive researchers is that drives are required that will be self-limiting in their geographic reach or persistence, or indeed both. Theoretically, several designs of drive could achieve this goal (Marshall and Akbari 2018), including the autosomal X-shredder described above. Another gene drive technology, known as underdominance (see Chapter 1), has been successfully demonstrated in the laboratory in the fruit fly Drosophila melanogaster, a distant relative of the mosquito (Reeves et al. 2014), by a team led by Floyd Reed at the University of Hawaii. The same research team proposes to use this method to either modify or suppress populations of Culex quinquefasciatus to control avian malaria in Hawaii (Goldman 2016). A variant of the CRISPR/Cas9 homing drive proposed by Kevin Esvelt, known as the ‘daisy drive’, has also gained much attention owing to its theoretical potential to provide a self-limiting drive. In other words, as it ‘drives’ through the species population, it slows down; and, depending on frequency, may stop altogether - although the genetic modifications would likely remain present in the population, especially the payload gene. Whilst the method remains a theory and has not been demonstrated in the laboratory, a consortium based at MIT in the US and the Pirbright Institute in the UK has been funded by DARPA to develop daisy drives in Culex quinquefasciatus and Aedes aegypti. Given these projects and the efforts by others, it seems likely that variants of the mosquito gene drive concept will continue to proliferate over the coming years.

Resources

Much of the massive investment in gene drive research has been directed at research in mosquitoes. More than $200 million from institutions, including the Gates foundation, the US Defense Advanced Research Projects Agency (DARPA) and the TATA trust, has been invested in gene drive research as a whole, and these driving resources are beginning to produce results, raising questions about who will decide which species are targeted and where. Although numerous technical difficulties remain, field trials of gene drives in Anopheles gambiae are planned by the research consortium Target Malaria, potentially in as little as 5 years, according to news reports (Molteni 2018). It is very possible that trials of systems targeting other mosquito species could follow in their wake.

Ecological importance

Scope

To help understand the risks and hazards of employing gene drives to suppress or modify mosquitoes in the wild, we here give an overview of ecological roles within the mosquito family as a whole, with reference to specific cases from the literature10. To consider the implications of any particular gene

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9 This paper describes the modification of mosquitoes with genes encoding miRNAs that target Zika virus genes, which are reported to reduce the capacity of these mosquitoes to transmit Zika to mice.
10 A full literature review is beyond the scope of this report.
drive proposal, broad and detailed knowledge of the ecological roles of each potentially affected species would of course be required. This data is however lacking at the level of individual species, so taking a broader view is informative in identifying areas of concern. This bigger picture is also important because the numbers and identities of mosquito species that may eventually be suppressed or eliminated via gene drives, and the potential reductions in total mosquito biomass that would result, are both highly uncertain. As we later explore, gene drives may eventually be used against a wide range of mosquito species, and have potential to impact non-target mosquito species for example through hybridisation. It is also unclear what fraction of the total mosquito biomass, both locally and globally, would be represented by targeted species. It is beyond the scope to investigate this question in detail, but it is of ecological relevance that major vectors, which are likely to be among the initial targets, may be the more abundant species in at least some contexts.  

**Mosquitoes are an important food source**

Across the global range of their habitats, the different phases of the mosquito life cycle support a wide variety of species. The aquatic larvae for example are predated upon by species of water bugs (aquatic Hemiptera), beetles (Coleoptera), flies (Diptera), spiders (Arachnida), flatworms (Planaria), tadpoles (Amphibia), fish (Osteichthyes) and crustaceans (reviewed by (Collins et al. 2019)). For the African malaria mosquito *Anopheles gambiae*, it is estimated that around 95% of larvae are con-

**Figure 1: The life cycle of the mosquito** The complex life cycle of the mosquito allows it to perform a wide variety of ecosystem roles. Its life cycle has four distinct stages: egg, larva, pupa and adult, the first three of which need standing water (for more detail see (Rozendaal 1997)). The eggs are generally laid in water, or in some cases just above the water line or in wet mud. Hatching requires water, and the larvae feed and develop in this aquatic environment. Eventually a larva forms a pupa, a non-feeding though mobile stage which undergoes metamorphosis before shedding its case to emerge as an adult, winged mosquito. Contrary to popular belief, the airborne adults feed mainly on nectar and other sugary plant juices (Foster 1995), and it is only the females (of most but not all species) that require a blood meal to produce eggs.

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11 For example a study in a rice growing area of Kenya indicated that *Anopheles arabiensis*, *Culex quinquefasciatus*, both important disease vectors, together make up nearly 90% of the total mosquito population (Muturi et al. 2006).
sumed before reaching adulthood (Collins et al. 2019), implying that this stage makes the largest contribution to the food chain.

As adults, mosquitoes are consumed by a different spectrum of predators, including species of dragonflies and damselflies (Odonata), spiders (Arachnida), bats (Chiroptera) and birds (Aves) (Collins et al. 2019). Insights into the possible effects of removing mosquitoes and larvae from ecosystems can be gained from studying the impacts of the use of $Bti$ toxin\textsuperscript{12}, a selective biological control agent which is used to suppress or kill larvae. $Bti$ is toxic to mosquitoes (Goldberg and Margalit 1977) and close relatives such as midges, but at the lower doses used in mosquito control is claimed to generally be non-toxic to other insects\textsuperscript{13} (Lacey and Merritt 2004). Long term studies of the effects of $Bti$ spraying in the Camargue wetlands, a nature reserve in Southern France, have shown harmful indirect effects. For nesting house martens, the average size of their clutches\textsuperscript{14} and survival rate of fledglings were decreased (Poulin, Lefebvre, and Paz 2010);\textsuperscript{15} and for dragonflies and damselflies (Odonata) both species diversity and total numbers were roughly halved (Jakob and Poulin 2016). Since $Bti$ affects both midges and mosquitoes, these impacts cannot be exclusively attributed to the loss of mosquitoes, however they do illustrate that reducing populations of even a small group of species can have significant and unintended effects.

Whilst many predators of larvae and adult mosquitoes consume a variety of other prey, there are species that specialise in hunting mosquitoes, such as $Evarcha culicivora$, an East African jumping spider (Salticidae) (Wesolowska and Jackson 2003). Whether many more such highly specialised predators exist remains an open question.

Some mosquitoes have over time spread into other regions and areas distant to their ‘native’ area, where they have then become established and often integrated into those ecosystems. Populations of $Aedes aegypti$, for example, have become established in many areas globally, and are also possible gene drive targets. This raises questions about the possible impacts of their suppression. The extent to which $Aedes aegypti$ has become integrated into these new ecosystems has not been well investigated, but it would be expected that the species would be a food source for native predators in these new contexts, and there is some evidence that this is the case (Samannmali et al. 2018; Albeny et al. 2011).

**Larvae are an important predator in aquatic ecosystems**

Aquatic ecosystems contain a wealth of micro-organisms, including photosynthetic primary producers, phytoplankton (algae), alongside various bacteria\textsuperscript{16} and larger protozoa. At this microscopic scale, the protozoans are the predators feeding off the smaller microbes. Much of the diet of larval mosquitoes comes from this microbial community, including the protozoans, so the presence of larvae would be expected to have knock-on effects on this community. In the small pools of water inside common pitcher plants, the presence of larvae has indeed been shown to affect bacterial diversity, which is significantly higher when larvae are present (Peterson et al. 2008), presumably because they reduce numbers of protozoans.

In larger aquatic ecosystems, the effects of applying $Bti$ toxin suggest that eliminating mosquito larvae could create complex changes within the aquatic microbial community, but again because both midges and mosquitoes are affected it is not possible to completely isolate the effects of mos-
quito suppression given current data. In temporary wetlands in Sweden, a high dose of this toxin reduced the density of larvae by 97-100%, resulting in increases in the diversity and density of protozoans, as would be predicted when a predator is removed (Östman, Lundström, and Vinnersten 2008). The effects of removing larvae on other microbes appear complex. When samples of freshwater ecosystems are exposed to high doses of Bti toxin in a laboratory, thus reducing numbers of larvae, indirect effects are observed on microorganisms: phytoplankton densities are reduced, even though Bti is not toxic to these species; but bacterial diversity on the whole is increased (Duguma et al. 2015; Mulla and Su 1999). There is not always an immediate logic to such trophic chains and effects, which means that more detailed studies are required to more fully understand the processes involved. Long term studies of Bti mosquito control in the Camargue, for example, do not observe effects on phytoplankton (Fayolle et al. 2016), although there the doses of Bti were lower, reducing larval densities by around 80% rather than virtually eliminating them as in the Swedish experiment. Using these and similar studies to form a general understanding of the consequences of eliminating larvae is very difficult; the studies have observed different environments with different mosquito species, used different doses of toxin, and did not all observe the same groups of microbes. Thus, with current knowledge, the impacts on aquatic ecosystems of attempting to eliminate or suppress mosquitoes cannot be predicted with any confidence, and harmful knock-on effects, such as suppression of phytoplankton, cannot be excluded.

Mosquito larvae contribute to nutrient recycling

Because decaying organic detritus also forms part of the diet of larvae (Daugherty, Alto, and Juliano 2000; Daugherty and Juliano 2003), they contribute to recycling the nutrients in dead animal and plant matter into the food chain. As larvae represent a significant amount of biomass in some aquatic ecosystems (Yurchenko and Belevich 2016), their role in processing detritus in these contexts may be significant. Laboratory studies on the effects of using Bti toxin to suppress larvae have shown that both nitrogen and phosphorous in the water column are reduced when high doses of the toxin are used, although the mechanism for this effect is unclear (Duguma et al. 2015). Again, knowledge on this area is limited, but is sufficient to suggest that nutrient recycling could be disrupted by removing larvae.

Mosquitoes are important pollinators for certain plants

With the exception of females of certain species feeding on blood to support reproduction, adult mosquitoes generally feed on nectar. This would imply possible roles in pollination, and whilst this question has not been well investigated, the mosquito’s role as pollinator has been confirmed for some species. For example, six species of the genus Aedes have been shown to pollinate the orchid Platycrater obtusata, which has a large range covering the northerly regions of Europe, Asia and North America (Gorham 1976). Whilst it can be inferred statistically that the vast majority of the ca. 350,000 known flowering plant species rely on animal pollination the actual pollination of most wild flowering plants has not been studied (Ollerton, Winfree, and Tarrant 2011), so with current information it is not possible to know how significant the role of mosquitoes in pollination is. It is thus possible that mosquitoes are important pollinators for other flowering plants, giving potential for elimination programmes to impact these plant species and their related communities, particularly given that wild insect pollinators are already in decline in many areas (IPBES 2016).

Potential impacts of mosquito suppression

Whilst mosquitoes have been well studied compared to many insects, research has generally been driven by interest in identifying disease vectors and controlling numbers, and the ecosystem roles of the thousands of species within the family have only been investigated in individual and narrow circumstances. Nevertheless, the limited knowledge available is sufficient to show they are embedded in a wide network of relations to many other species. Where they are abundant they will be an important food source both as larvae and adults, will exert complex influences on the community of aquatic mi-
croorganisms, and will contribute to recycling nutrients into the eco-system. Knowledge of their roles in pollination remains limited, which means that the possibility of important pollinator relationships. Indeed other eco-system roles in certain circumstances cannot be excluded. Any proposals to eradicate mosquito species or groups of species, even in a localised setting, should therefore be viewed within this context of science’s limited understanding of their complex system of relationships.

To summarise, at the ecosystem level, effects could occur in five broad areas, although their nature and extent is extremely difficult to anticipate:

- Decline in numbers and/or diversity of predators
- Reductions of other species that have become the new prey of predators
- Complex effects on aquatic microbial communities
- Reductions in nutrient availability in aquatic ecosystems
- Potential reduction in pollination and ensuing consequences

Whilst it is likely that empty ecological niches would be filled or that populations could rebound due to some form of resistance, even a temporary reduction in mosquito populations could have significant impacts on predator species and aquatic ecosystems, particularly if they were already under stress from other factors.

Role in human and animal disease

The requirement for blood feeding brings most mosquitoes into relationship with other sets of species, including humans. Recent genetic evidence suggests that mosquitoes have evolved rapidly to adapt to blood feeding on humans (Neafsey et al. 2015) and to live in anthropogenic environments (White, Collins, and Besansky 2011), seizing the opportunity created by the large fraction of biomass represented by humans (Bar-On, Phillips, and Milo 2018) and the increasing areas of land devoted to human activities. Not surprisingly, the species composition of the mosquito community is affected by the presence of humans and human influences on habitat. A study from Thailand has shown that even over short distances, the diversity of species and the relative abundance of disease vectors varied across forest and different anthropogenic habitats, with vectors of disease lowest in intact forest (Thongsripong et al. 2013).

A number of pathogens have evolved to exploit mosquitoes feeding on humans, including the single-celled plasmodium parasites which cause malaria; several viruses, such as Dengue, Zika and yellow fever; and various parasitic nematodes, which cause filariasis. Of these, malaria parasites cause the most infections and the highest mortality, contributing to an estimated 430,000 deaths in 2015 (WHO 2016). The two most significant malaria parasites, *Plasmodium falciparum* and *Plasmodium vivax*, are responsible for the vast majority of infections globally, with *falciparum*, which is common in sub-Saharan Africa, by far the most deadly form. The health impacts of other mosquito-borne pathogens are also substantial; for example, Dengue is reported as being responsible for tens of thousands of deaths every year (ECDC 2019).

Co-evolution of humans, pathogen and vectors

Inevitably, vector-borne pathogens are involved in an evolutionary ‘arms race’ with their human hosts, and it should be considered how the use of gene drives to spread disease refractory genes in mosquitoes might affect this process.

The evolutionary relationship between humans and the two most significant malaria parasites is now at least partly understood. In fact, resistance to malaria is thought to be the strongest selection pressure in recent human evolution, driving some of the most rapid evolutionary changes known. For example, over the last 40,000 years a variant in a gene known as DARC has swept through African populations (McManus et al. 2017), apparently because it gives protection against *Plasmodium vivax*. Given that *P. vivax* is rarely lethal in modern humans, the
strength of the selection pressure driving DARC into the human population is difficult to explain from today’s situation, and it has been suggested that this form of malaria was more deadly in the past and that humans have evolved considerable resistance (Price 2017). The most deadly parasite, Plasmodium falciparum, originated in gorillas and is thought to have been transmitted to humans relatively recently in evolutionary terms, probably around 10,000 years ago (Loy et al. 2017). Since then, mutations providing resistance have been strongly selected for in human populations, including the variant in the haemoglobin-gene that gives rise to sickle cell anaemia. It is believed that in exposed populations, additional resistance variants are present that have yet to be identified (Hedrick 2011). In turn, however, the parasites also evolve to evade human resistance, with some strains of P. vivax now apparently gaining the capacity to infect humans who carry the protective DARC mutation (Mendes et al. 2011).

Like humans, mosquitoes can evolve a high level of immunity to the malaria parasite, but intriguingly this strong immune response is only present in some species: the species Anopheles quadriannulatus, widely considered a non-vector, shows a robust immune response to Plasmodium falciparum, whereas the important vector, Anopheles gambiae, shows little immunity (Habtewold et al. 2008). Similar results were found when these two species were infected with the Plasmodium berghei parasite, and it was also shown that other Anopheles vector species showed a weak immune response (Habtewold, Groom, and Christophides 2017). The most probable explanation for the absence of immunity in these cases is that the costs to the insect of deploying an immune response can outweigh or balance out the costs of infection (Hurd et al. 2005).

Humans, mosquitoes and malaria parasites are thus involved in a dynamic three-way process of co-evolution that is unlikely to end with the modification of certain vector mosquitoes through gene drives. If gene drives do succeed in spreading disease refractory genes in mosquitoes, and even if they only partially or temporarily succeed, this alteration would interact with powerful evolutionary forces. It seems appropriate to reflect on the uncertainties and potential consequences this might entail.

**How many species could be affected?**

Given the ambition of gene drive developers to deliver health outcomes by modifying or suppressing disease-vector mosquitoes, it is worthwhile to consider how many species this could reach. To our knowledge, at least four (and probably five) species, representing three major genera, are being targeted by gene drive development. However, given the number of species implicated as vectors, which we estimate at 160 to 190, there is clearly scope for many more (Table 3). Here we consider the total numbers of known disease vectors, the species targeted, and the scope for gene drives to spread beyond target species by hybridisation for each of the three affected genera (Anopheles, Aedes and Culex).

**Anopheles**

Whilst attention is often given to a handful of prominent vector species, there are in fact 40-70 species of Anopheles that are capable of transmitting the human malaria parasite. Among these are two of the principal targets for gene drive projects: Anopheles gambiae, the major vector in sub-Saharan Africa, and Anopheles stephensi, a major vector in the Indian sub-continent. Anopheles gambiae has seven closely related species that together form a ‘species complex’, which, whilst almost identical in appearance, exhibit different behaviours in their choice of host, have different capacities to transmit malaria, and have different, yet overlapping, preference of habitats. Anopheles gambiae, for example, prefers to feed on humans and is susceptible to infection with the malaria parasite, whereas quadriannulatus shows no preference for humans over livestock, is relatively resistant to infection, and is not considered a vector for human malaria (Pates et al. 2001; Habtewold et al. 2008). However, a synthetic

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17 Figures for the total number of Anopheles species which can transmit disease vary in the literature. The Malaria Atlas Project states that ‘Approximately 40 Anopheles species are able to transmit malaria well enough to cause significant human illness and death’ (MAP 2019). Neafsey et al give a figure of 60, while Manguin et al state that around 70 species are of ‘epidemiological significance’.
gene drive has the potential to spread into this species and to affect, suppress or potentially eliminate it, because *gambiae* and *quadriannulatus* are capable of hybridising and producing fertile offspring, as are most other species in the complex (Pates, Curtis, and Takken 2014; Fontaine et al. 2015). Genetic studies show evidence of extensive historic gene flow between members of the complex (Fontaine et al. 2015; Coluzzi, Sabatini, Petrarca, and Di Deco 1979), observations that illustrate the inherent difficulty in defining separate species by reproductive isolation. Genetic comparisons of *Anopheles gambiae* with *quadriannulatus* have also revealed that *gambiae* has adapted to feeding on humans, for example by evolving a capacity to detect human odours (McBride 2016; Rinker et al. 2013), another aspect of the triangular evolutionary relationship between mosquitoes, humans and the plasmodium parasite.

### Aedes

The *Aedes* are the largest mosquito genus, with over 900 species (Wilkerson et al. 2015), and are the dominant group globally, with a range extending from the tropics to the arctic (Harbach 2013). More than 80 of these are known vectors for human diseases, including viruses such as Dengue and the nematodes which cause filariasis (Wilkerson et al. 2015). Prominent among them is the gene drive target *Aedes aegypti*, a species that acts as a vector for viruses such as Dengue, yellow fever and Zika (Guerbois et al. 2016; Wilkerson et al. 2015). *Aegypti* originally evolved in Africa but has adapted to feed off humans (Ponlawat and Harrington 2005), allowing it to spread alongside its human host and to become established in tropical and warm temperate regions around the globe (Powell and Tabachnick 2013; Kraemer et al. 2015). According to one source, research on gene drives in another invasive species, *Aedes albopictus*, a vector for many of the same diseases, is also underway (Darrow et al. 2016). Like *Anopheles gambiae*, *Aedes aegypti* can produce fertile hybrids with closely related species (Motara and Rai 1977).

### Culex

After *Aedes*, *Culex* are the second largest genus, with nearly 800 species and a range reaching from the tropics up to cool temperate latitudes (Harbach 2013). At least 15 species have been shown to act as vectors for human pathogens, including West Nile virus, encephalitis viruses, and filarial nematodes.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Number of species</th>
<th>Number of known vectors of human disease</th>
<th>Examples of human pathogens carried</th>
<th>Gene drive targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles</td>
<td>475</td>
<td>40-70</td>
<td>Malaria, filariasis</td>
<td>Anopheles gambiae; Anopheles stephensi</td>
</tr>
<tr>
<td>Culex</td>
<td>769</td>
<td>≥15</td>
<td>West Nile virus, filariasis</td>
<td>Culex quinquefasciati</td>
</tr>
<tr>
<td>Aedes</td>
<td>931</td>
<td>≥84</td>
<td>Dengue, yellow fever, Zika</td>
<td>Aedes aegypti, Aedes albopictus</td>
</tr>
<tr>
<td>Psorophora</td>
<td>49</td>
<td>≥10</td>
<td>West Nile virus</td>
<td>Currently none</td>
</tr>
<tr>
<td>Haemagogus</td>
<td>28</td>
<td>≥4</td>
<td>Yellow fever</td>
<td>Currently none</td>
</tr>
<tr>
<td>Armigeres</td>
<td>58</td>
<td>≥2</td>
<td>filariasis</td>
<td>Currently none</td>
</tr>
<tr>
<td>Mansonia</td>
<td>25</td>
<td>≥8</td>
<td>filariasis</td>
<td>Currently none</td>
</tr>
<tr>
<td>Total</td>
<td>163–193</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(Harbach 2013) as well as acting as vectors for other diseases in mammals, birds and reptiles. The well-known species *Culex quinquefasciatus*, which is found in tropical and sub-tropical regions around the world (Samy et al. 2016), has become a focus for gene drive development, owing to its role in human disease and avian malaria. As with *Anopheles gambiae* and *Aedes aegypti, Culex quinquefasciatus* (Gomes et al. 2012) can produce fertile hybrids with closely related species.

**Technical issues**

Without downplaying the possibility that drives could have major and potentially very harmful impacts, it must be noted that a variety of technical hurdles create a significant likelihood that drives won’t behave as expected or deliver the promised outcomes.

**Resistance: technical and behavioural issues that may thwart planned outcomes**

At least three mechanisms could give rise to resistance to gene drives, preventing them from propagating in the target population and suppressing or modifying populations as planned (Sarkar 2018): natural genetic variations at the target site may block the drive; mutations can arise that generate evolved resistance; and selection pressures against the drive may result from non-random mating behaviours, (see Chapter 1). One example of ‘behavioural resistance’ would be the evolution of sibling mating behaviours, which modelling studies show could emerge in response to gene drives (Bull, Remien, and Krone 2019).

In the case of suppression drives, it is obvious that there is a huge evolutionary pressure for resistance to emerge. However, if modification drives were ever released in the wild, it is rather uncertain how rapidly they would spread. In this case, the payload gene is not intended as a burden to the mosquito, thus avoiding selection pressure. However, if either the gene drive or the payload gene confer a high fitness cost which is counter to the design criteria, resistance to the drive could spread faster and selection pressure could reduce the presence of the payload gene.

**Implementation in wild populations will be challenging**

There are also numerous difficulties in modifying populations in the wild, as opposed to a cage. For example, success would be dependent on a level of geographic mobility of mosquitoes to spread the drive, and could be undermined if gene drive mosquitoes are less successful at finding mates than their unmodified counterparts. In addition, there are significant practical difficulties, such as mass rearing of mosquitoes for release and in most scenarios ensuring that biting females are not released.

**Risks and uncertainties**

There are a number of different issues and risks with gene drive technology that need to be brought into the foreground (see also Section 3 on risk assessment); in particular, its unpredictable nature must be emphasised.

**A wide spectrum of unplanned outcomes is possible**

The likelihood of some form of resistance emerging to a mosquito gene drive makes the actual outcomes of a drive in the wild very difficult to predict. Figure 2 illustrates the range of possible outcomes in a highly simplified case of a single use of a suppression gene drive against one species. Even in this case, a wide range of outcomes are possible, depending on how widespread resistance is in the initial population or how rapidly it appears through evolutionary and behavioural processes. At the two extremes of the range of possibilities are: complete collapse of the drive; or complete eradication of the target species. Perhaps more likely than either is a partial suppression of the target, ranging from a limited drop in the population to near complete eradication, followed by the spread of resistance and the rebounding of the population to near its original level, with notable public health implications (see below). Depending on how the drive behaves at a molecular level and how it is impacted by mosquito behaviour, the new population could...
also contain a large number of genetically modified mosquitoes, bringing additional risks (see below). In reality, this scenario is overly simplistic, and factors such as geographic limitations on its spread and the locations and timings of probable multiple releases would all add additional layers of complexity to predicting outcomes.

For example, in the case of gene drives containing active CRISPR/Cas9 (either as a homing system or a simple endonuclease for cleaving DNA), the development of resistance will not stop this machinery from cutting alternative target sites. The presence of an active CRISPR/Cas9 system in the population has the potential for frequently setting new and unintended mutations, thus potentially constantly adding to the genetic alteration or modification of the population in a way that most likely cannot be predicted.

**Geographic range and species scope are difficult to control**

If drives do propagate as intended, there is considerable uncertainty about concerns such as the extent of the geographic areas they will eventually reach and the numbers of species that might be affected. Many gene drive technologies have the potential to become highly invasive, which could lead to impacts that are both global and irreversible (Noble et al. 2018). Even with proposals for theoretically self-limiting drives (many of which have not yet

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**Figure 3**

Spectrum of outcomes for a suppression gene drive
(assuming it remains in one species)

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Population outcome</th>
<th>Genetic outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular resistance appears rapidly</td>
<td>Very limited suppression</td>
<td>Wild type</td>
</tr>
<tr>
<td>Complete pre-existing behavioural resistance</td>
<td>Partial suppression</td>
<td>Mosaic of wild type and genetically modified</td>
</tr>
<tr>
<td>Molecular resistance evolves more slowly</td>
<td>Near complete suppression</td>
<td>Eradicated</td>
</tr>
<tr>
<td>Behavioural resistance evolves</td>
<td>Complete suppression</td>
<td></td>
</tr>
</tbody>
</table>

Range of suppression levels possible

Population rebounds
been demonstrated even in a laboratory), the scope of the effects is difficult to predict and could be far wider ranging than intended (Dhole et al. 2018).

Given that most of the mosquito species being targeted are known to hybridise with closely related species, the capacity to confine a drive within one species is also questionable. Significantly, the doublesex gene sequence (targeted by the suppression drive developed by Crisanti and co-workers) is completely conserved across the *Anopheles gambiae* species complex, which means the drive would function just as effectively in these sibling species. Members of this complex are known to hybridise, so if this drive were released in the wild, it could potentially affect the entire species complex alongside *gambiae* – and the ecosystems linked to them.

**Risks from generating GM mosquito populations**

One significant issue is that drives could generate vast populations of GM mosquitoes, potentially carrying genes encoding the genome editing agent CRISPR/Cas9. This would of course occur with intentional modifications designed to alter or suppress mosquitoes, and these alterations may have unexpected or unintended effects and consequences that will need to be investigated and risk assessed prior to release.

More difficult to risk assess are unintended alterations; as every time a CRISPR/Cas9 gene drive is active in the cell, it could theoretically cut the DNA off-target with a non-homologous end joining (NHEJ) mutation arising (see Chapter 1). With replacement drives, these modifications could well spread. In the case of suppression drives, if resistance arises, then unintended modifications may get fixed in the population. In all cases these GM insects would constitute a risk of their own, one that has not been assessed and in fact probably cannot be assessed beforehand. The question arises, how this could be adequately addressed in a risk assessment, especially when no data are available.

**Several mechanisms could confound any health benefits**

The consequences on human health of attempting to eradicate or of eradicating a mosquito population or species are likely to also be difficult to predict. Whilst we are not experts on public health, we believe it is important that certain scenarios are considered, in which gene drives do not give the intended results. We therefore wish to draw attention to important questions in this area and to highlight relevant concerns that have been raised by others.

**Could other disease vectors occupy empty ecological niches?**

The response of the ecosystem to the eradication of a mosquito species is one important unknown quantity in predicting health outcomes. One possibility would be that other related species of mosquitoes or other insects would simply fill the newly empty niche. It is known, for example, that *Aedes albopictus* competes with *Aedes aegypti* in many settings (Braks et al. 2004); so removal of *aegypti* would perhaps simply result in dominance of *albopictus*, which, as stated already, is a vector for many of the same diseases. If other mosquito or insect species expanded to fill emptied ecological niches, then it could be possible they could adapt to feed on humans in a similar way as *Anopheles gambiae* has done. Humans account for a large proportion of vertebrate biomass in many contexts, pointing to a plausible evolutionary pressure or advantage for such specialisation.

**Impacts of partial or temporary removal**

As already discussed, there is a possibility that drives could suppress vector mosquitoes temporarily or even for some substantial time, with populations later rebounding. What might the consequences for public health be for such a scenario? According to reports, with regular exposure to malaria adults develop a natural acquired immunity to the disease (NAI), in addition to the various levels of genetic immunity that already exist in exposed populations. The authors of one review argue that NAI “should be appreciated as being virtually 100%
effective against severe disease and death among heavily exposed adults” (Doolan, Dobaño, and Baird 2009). This review goes on to state:

“Interventions that reduce exposure below a level capable of maintaining NAI risk the possibility of catastrophic rebound, as occurred in the highlands of Madagascar in the 1980s, with epidemic malaria killing more than 40,000 people. (Romi et al. 2002).” (Doolan, Dobaño, and Baird 2009) p14, emphasis added.

In light of this, we would recommend that the risks associated with temporary population suppression of vectors are investigated and assessed by those with relevant expertise.

Evolution or replacement of pathogens

The capacity of pathogens to evolve in order to evade immune responses is well documented, and arboviruses such as Dengue have already evolved mechanisms to suppress mosquito host defences (Sim, Jupatanakul, and Dimopoulos 2014). Thus it could be asked: if efforts to modify populations to generate immunity to pathogens succeed, would pathogens not simply evolve in ways that avoid this immunity? Similarly, if existing vectors were wiped out, then couldn’t selection pressure push pathogens to evolve to spread via other vectors, which might be just as difficult to control. Mosquito species can in many cases transmit more than one pathogen, and as the Convention on Biodiversity (CBD) guidance on living modified (LM) mosquitoes (Andow 2012) states, a mosquito in which the ‘capacity of transmission of one of these pathogens has been modified, may enhance the transmission of other pathogens.’

Overview of existing and proposed alternatives

Mosquito-borne diseases have been impacting human health for tens of thousands of years, and human ingenuity has developed approaches, both ancient and modern, to counter them. This is a field where we cannot offer expertise, and so we are not seeking to give advice on public health strategies.

We are aware however that an overview of current practices, as well as current developments, would be helpful in giving a sense of the wider context in which mosquito gene drives are being developed.

Current malaria control methodology

A concerted global programme of malaria control saw deaths from malaria halve in the period from 2000 to 2015 (Gulland 2015), and even included complete eradication of the disease in countries such as Sri Lanka (in 2016) and Paraguay (as recently as 2018). This progress was based on widespread implementation of policies recommended by the WHO, principally the use of: long lasting insecticide-treated bed nets (LLINs); indoor residual spraying (IRS) of insecticides in homes at risk for malaria; preventative treatments for children and pregnant women; and access to diagnosis and treatment for malaria infections (WHO 2016). Progress since 2015 has stalled, however, with deaths remaining around 430,000 (WHO 2018). The reasons for this are not fully clear, although the WHO is highlighting reductions in funding for malaria control in many countries with a high disease burden (Kelland 2017; WHO 2018).

Vaccination

Vaccination has made substantial impacts on the incidence of yellow fever, and many view it as a promising approach for tackling other mosquito-borne diseases (Frierson 2010; WHO 2017; Draper et al. 2018). A relatively safe and effective vaccine was developed against yellow fever in the 1930s, and mass vaccination campaigns resulted in the eventual disappearance of the disease in many areas (Frierson 2010). Development of a vaccine against Dengue involves significant technical challenges, and whilst the currently licenced vaccine is only recommended in certain circumstances, the WHO states that “the current Dengue vaccine pipeline is advanced, diverse and overall promising” (WHO 2017). Malaria vaccine research has also encountered considerable difficulties, yet much pro-

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19 Treatment contributes to reducing malaria transmission: people who have been treated with anti-malarial medication are less likely to infect mosquitoes and thereby transmit the parasite to others (WHO 2015).
ggress is being made; one vaccine has now been approved which is said to offer effective protection for infants and young children (although the immunity is partial and not long-lasting), and over 20 other vaccine candidates are in clinical trials or advanced pre-clinical trials (Draper et al. 2018; WHO 2019).

**Emerging mosquito control methodologies**

Public health specialists are also drawing attention to a variety of new and existing techniques that could supplement the two current existing foundations of vector control, LLINs and IRS (Barreaux et al. 2017; Killeen et al. 2017). A recent review (Barreaux et al. 2017) highlights five complementary approaches, each with an evidence base, which could begin to be deployed immediately:

- **Attractive toxic sugar baits (ATSBs):** These take advantage of mosquito sugar feeding to administer an oral toxin and are capable of locally reducing malaria vector populations.

- **Swarm sprays:** Many vector mosquitoes form swarms when mating which can be sprayed with insecticide by local volunteers, giving reductions in vector density and mating success.

- **Housing improvements:** Modern housing and modifications to existing homes can provide protection against malaria transmission.

- **Treatment of livestock:** Many mosquitoes also target livestock, so treatment of livestock or the structures housing them with insecticides can reduce mosquito numbers.

- **Spatial repellents:** These are airborne chemicals that cause changes in insect behaviour and which show potential for reducing transmission.

**Other perspectives on malaria control**

A detailed review of the malaria control literature is beyond the scope of this report, but it should be highlighted that there are many potentially valuable perspectives that could be further explored. Laporta and colleagues, for example, are considering the protective role that healthier ecosystems might play. On the basis of modelling studies, they have suggested that in tropical forest areas where biodiversity of both mosquitoes and wild warm-blooded animals is high, humans are to some extent protected from malaria (Laporta et al. 2013). There is also the consideration that traditional medicines and healers are widely used in many communities affected by malaria (Suswardany et al. 2015), and that some researchers have proposed that giving them a greater role in public health programmes could improve outcomes (Graz, Kitua, and Malebo 2011).

**Conclusions**

Gene drives are an inherently risky mosquito control technology. The considerable enthusiasm of some actors is drawing attention away from both the risks of the technology failing to deliver the promised benefits, but also, more crucially, from its unpredictability. This obscures the potentially serious and irreparable harms that may be caused by the release of gene drive mosquitoes on ecosystems and biodiversity, and tends to ignore the possibility of negative human health impacts.

If drives fail to suppress or modify mosquito populations as planned, then clearly the health benefits will not be realised. Equally, even if drives do achieve these goals in the short term, shifts in the composition of the mosquito community, adaption of feeding preferences and evolution of pathogens could all potentially rapidly counteract any benefits and perhaps pose new risks.

Mosquitoes and their larvae are likely to be an important component of ecosystems in many circumstances. If major mosquito species are removed, the ecosystem will shift in complex patterns that are not fully predictable, but because other species rely on them in various ways, are likely to be harmful to biodiversity. Deployment of these drives could easily lead to knock-on effects that impact predators such as birds, bats or dragonflies, which may already be under stress because of other damage to ecosystems, resulting in further declines in their numbers. Similarly, impacts on the microbial community and nutrient recycling could, for exam-
ple, harm the photosynthetic phytoplankton at the base of food chain, leading to further chains of consequences. So whilst swatting an individual mosquito is no threat to biodiversity, taking a similar step at an ecosystem level would be dangerous leap into the unknown.

The question also arises of just how many mosquito species may eventually become gene drive targets? Will gene drives be deployed against a handful of major vectors, or all disease vectors, or even all mosquitoes? The consequences would be different in each of these scenarios, yet we must reflect on all of them because no one, including the developers, knows where this technology may ultimately lead us. As stated earlier, at least 160 mosquito species are known to transmit human pathogens, and given that many species have not been well investigated, there could be more, all in addition to species that may be poor vectors, or could potentially adapt to act as vectors. Given that even when they are not disease vectors, mosquitoes are considered a nuisance, it is not hard to imagine a situation where gene drives are used against a large proportion of the mosquito family, normalising an unprecedented level of intervention in the natural world and opening up the prospect of ‘designer ecosystems’ starting to replace natural ones.

There are a wide range of approaches for controlling mosquito-borne diseases, with some proven methods not receiving enough investment. In this context, and in line with the Precautionary Principle, it would be wisest to avoid any approach that risks failing to deliver health benefits and could also cause significant collateral damage to ecosystems.

2.4.2 Case study 2: Mice

Introduction

In one of Aesop’s fables, a sleeping lion is woken by a mouse and is so angered by the disturbance that he threatens to kill the mouse. The mouse replies that he would not be worthy prey, and so the lion agrees to spare his life. To the lion’s amusement, the mouse responds that he will one day return the favour, and they go their separate ways. Sometime later, the mouse finds the lion caught in a hunter’s net and on recognising him, chews through the threads to free him and save him from the hunters’ spears.

This story, which dates back to ancient Greece, illustrates not just the benefits of mercy and how beings can be interconnected in unexpected ways, but also the long-standing place mice have in the human imagination. This is hardly surprising, given that the house mouse (Mus musculus) has lived in close association with humans at least since the development of agriculture about 12,000 years ago (Auffray, Tchernov, and Nevo 1988), accessing human food supplies in houses, out-buildings, stores and cropland. Whilst the species originated in the Indian sub-continent, their commensal relationship with humans probably emerged in the Middle East (Weissbrod et al. 2017), and this association, combined with remarkable adaptability, has allowed them to spread widely: first travelling with bronze-age traders around the Mediterranean and into Europe (Cucchi, Vigne, and Auffray 2005), and more recently to the Americas and other landmasses along shipping routes (Boursot et al. 1993). Indeed, together with rats, they are now probably the most widely distributed vertebrate in the world, after humans (GISD 2019), inhabiting environments from the tropics to the Arctic and sub-Antarctic (Musser 2016).

Methods to control commensal rodent populations have been actively pursued at least since the domestication of the cat, and considerable investment is now being made to add eradication via gene drive to the existing range of tools. At least three research teams are pursuing gene drives intended to be capable of suppressing or eradicating wild mouse populations, either by biasing sex ratios or spreading infertility. As the most intensively studied mammalian laboratory organism, the development of effective gene drives in Mus musculus is also being pursued as an intentional step towards engineering drives in other mammals. Here we give a brief overview of the biology of the house mouse, and review the motivations behind mouse drives along with the current state of research, so as to
better comprehend the spectrum of potential hazards and multitude of risks in the application of this technology.

Overview of ecological role and relevant biology

House mice are very widespread globally and stow away easily

Whilst the house mouse has thrived in this close relationship with humans, the species has existed much longer than modern Homo sapiens\(^\text{20}\) and populations continue to flourish in wild and semi-wild environments. The house mouse is generally very successful in anthropogenic habitats, yet can also occupy grasslands and shrublands at a wide range of latitudes, as well as some coastal and wetland habitats (Musser 2016). The wide range of new territories colonised by Mus musculus reflects its remarkable capacity as a stowaway. A small study in the United States found mice in transported hay, straw, grain, dog food, and even a vehicle cab, leading the author to estimate that thousands of mice are unintentionally transported globally each year (Baker 1994).

Diet and influence on invertebrates

House mice are omnivores, and this dietary flexibility is important in allowing them to occupy such a range of habitats. Their diet comprises a variety of plant material, which can include grains, seeds, fruits, leaves, stems and roots, in addition to insects and other invertebrates (Tann, Singleton, and Coman 1991; Shiels et al. 2012b; Wilson et al. 2006). The range of invertebrates found in mouse stomachs is considerable, and includes true flies (Diptera), true bugs (Hemiptera), beetles (Coleoptera), caterpillars (Lepidoptera), spiders (Araneae) and earthworms (Annelida), suggesting that mouse predation may be a significant influence on some invertebrate populations.

The relative fraction of invertebrates and plant matter varies considerably depending on their habitat. In some contexts, for example croplands in Australia, cereal seeds have been shown to make up the majority of their diet (Tann, Singleton, and Coman 1991). In other environments, including the sub-Antarctic Marion Island (Gleeson and Van Rensburg 1982), the Hawaiian Islands (Shiels et al. 2012a), and in alpine and coastal New Zealand habitats (Wilson et al. 2006; Miller and Webb 2001), they have been shown to be predominantly insectivores.

An important food source for many species

A great variety of carnivores and omnivores eat house mice in all of their many habitats; they include domestic cats (Felis silvestris), foxes (Vulpes), weasels (Mustela), ferrets (Mustela), mongooses (Herpestidae), wolves (Canis lupus), large lizards (Squamata), snakes (Serpentes), hawks (Accipitridae), falcons (Falconidae) and owls (Strigiformes) among others (Ballenger 1999; Alberto et al. 1991) (see Figure 4). The predators of Mus musculus will of course be different in different environments, and knowledge of how important the species is in sustaining different predators is limited to certain narrow contexts that have been studied in detail. For some, like wolves, house mice are one food source among many (Alberto et al. 1991). For others, for example barn owls, long eared owls and kestrels, which were studied in urban environments, they form a large proportion of their diet (Charter et al. 2007; Laiu and Murariu 1998; Kečkéšová and Noga 2008). So whilst house mice can be considered pests in cities, they are also important in sustaining birds of prey in these habitats. In the light of the number of species that prey on them, what might be the effects of suddenly removing mice?

Closely related species, scope for hybridisation and spread of gene drives

House mice belong to the genus mus, which contains about 40 species, and are closely related to genera such as field mice (Apodemus) and rats

\(^{20}\) The subspecies of Mus musculus are estimated to have diverged from a common ancestor around 500,000 years ago (Geraldes et al. 2008)
Mus musculus itself has diverged into at least three sub-species, all of which show commensal behaviour: M. m. domesticus, which is present in Western Europe, the Americas, Africa and Australia; M. m. musculus, in Eastern Europe and much of Asia; and M. m. castaneus, in India and South East Asia. (Geraldes et al. 2008). Whilst these are sometimes considered separate species, their reproductive isolation is by no means absolute. Hybridisation between M.m. domesticus and M.m. domesticus is known to occur in the wild (Payseur, Krenz, and Nachman 2004), generally producing fertile offspring. M.m. domesticus can also produce fertile female hybrids with closely related species such as Mus spretus (Orth et al. 2002) and Mus spicilegus (Zechner et al. 1996). Indeed, some gene flow has been shown to have occurred between M.m. domesticus and spretus (Liu et al. 2015). The capacity for interbreeding between subspecies and closely related species, and the overlap in their distributions (Phifer-Rixey and Nachman 2015), therefore makes...
it uncertain whether any gene drive could be confined to a particular target species or subspecies. It is not clear exactly which subspecies are being targeted for gene drive development: it is probable that initial experiments would use standard inbred laboratory mice, which are hybrids largely derived from *M. m. domesticus* (Yang et al. 2011).

**Knowledge of ecological roles is limited**

Inevitably, given the limitations of what has been or can be systematically studied, knowledge of the ecological roles of *Mus musculus* is limited. So it is possible, and even probable, that the species is interacting with and sustaining other species besides those listed, and contributing in other manners to ecosystems, in ways that have not yet been observed. For example, it has been proposed that mice could play a role in formation of new soils, by transporting oribatid mites - an important component of soil - to locations where new soils are forming; and these mites have indeed been found on other mice in field studies (Teunkens 2016). It is also possible that house mice play a role in seed dispersal. Rodents of the murid family (which includes house mice) have been shown to pass intact seeds through their digestive systems (Corlett 2017), and at least in some cases these seeds are viable (Duron et al. 2016). Thus the impacts of any sudden reduction in house mouse populations may not be limited to the obvious effects on their predators and prey.

Might closely related species also be vulnerable to gene drives targeting *Mus musculus*? This possibility is discussed in more detail below, but if this risk was present then the ecological roles of related species would also need to be considered. For example, a related species, the western Mediterranean mouse (*Mus spretus*), buries acorns in scattered hoards, and has been shown to be important in dispersing acorns of the holm oak (*Quercus ilex*) (Muñoz and Bonal 2007) the dominant tree species in many western Mediterranean forests (Sheffer 2012).

**Drivers for mouse gene drive research**

Whilst house mice cause a number of undesirable effects for humans, including minor damage to building fabric and in rare cases the spread of pathogens, it is the consumption of crops, stored food and animal feed that is likely one of the foremost drivers of gene drive research to suppress this species. Unstated assumptions underlying the logic of suppressing mice and other pests should be carefully examined: namely, that humans societies are entitled to maximise harvests by eliminating any species that seek to use even a small fraction of those same resources. To consider the point of view of those wishing to maximise economic returns, the reported monetary costs that *Mus musculus* brings are certainly significant, creating powerful incentives to employ new ‘pest control’ measures. An explosion in numbers in Australia in 1993/4 is estimated to have caused damage to crops totalling U.S. $60 million (Brown and Singleton 2000). In farms and other anthropogenic environments, they often co-exist with black and brown rats (*Rattus rattus* and *Rattus norvegicus*), with the annual costs incurred by rodents to farmers estimated at around U.S.$30 billion in the United States (Pimental 2007) and U.S.$2 billion in South East Asia (Nghiem Le et al. 2013). It is clear in at least some cases that suppressing pest populations is the primary motivation of this research. The UK’s Roslin Institute states that they are exploring how disruption of fertility in mice and rats via gene drive could ‘curb pest rodent populations’ (Roslin Institute 2017).

On many islands *Mus musculus* can become a problematic invasive species; in one unusual but high profile case, Gough Island in the South Atlantic, by predating chicks of ground-nesting birds (Cuthbert et al. 2016). The eradication of mice on certain islands has therefore become a conservation goal. Elimination of rodents, especially rats, has been achieved on many islands through the use of toxicants (Campbell 2015), but this approach cannot presently be applied in all circumstances. Advocates of mouse gene drive research therefore argue that a drive capable of eradicating mouse populations should be developed as a conservation tool (Leitschuh et al. 2018), and a consortium
calling itself Genetic Biocontrol of Invasive Rodents (GBIRd) is now seeking to deliver gene drives which they state are for conservation purposes. However, it is questionable if any future mouse gene drive would remain exclusively as a conservation tool. Communications between researchers obtained through open record requests show that the research community is well aware of the potential to use this technology in agriculture and elsewhere. A memorandum of understanding between the partners in the GBIRd consortium (Gene Drive Files 2017a) from April 2017 states:

“The Participants seek to assess the potential of this technology for advances in agriculture, food security, and human health.”

Emails between GBIRd Steering Committee members sent later, in July 2017, reveal a discussion about whether communications should be “noting the potential future benefits in other areas” or “focus solely...on eradication of invasive rodents from islands...” (Gene Drive Files 2017b)

**Current state of gene drive research**

Mice have been chosen as the first candidate for gene drive development in mammals for several reasons; they are the foremost mammalian laboratory organism, and researchers possess well-developed molecular genetic tools, a complete genome sequence, and a high level of understanding of their physiology and development. Significant funding is being committed to achieve this goal, from institutions that include the US National Institutes of Health (NIH), the US Defence Advanced Research Projects Agency (DARPA) and the UK Biotechnology and Biological Science Research Council (BBSRC), and in January 2019 results were published from a team at the University of California San Diego describing a mouse gene drive (Grunwald et al. 2019). In this case, the technology is a homing CRISPR/Cas9 drive using a visual trait (white coats) to test the feasibility and performance of such an approach. However, so far the method has only limited efficiency: the drive increases the probability of an individual inheriting the desired allele from 50% to 73% on average, and only functions in the female germline. Whilst higher efficiency would be needed for a gene drive to function in the wild, the authors suggest that the technology could be useful for constructing new strains of laboratory mice for medical research.

At least three other groups are working on mouse gene drives, with the aim of suppressing or eradicating populations of mice in the wild. One proposal is to construct drives that cause mice to produce all male offspring. This could in theory be achieved by constructing drives to propagate a gene named Sry, that leads to the development of male characteristics. A team at the University of Adelaide is seeking to achieve this with a homing CRISPR/Cas9 drive (Gene Drive Files 2017b), whereas a group at Texas A&M University are coupling Sry to a naturally occurring selfish genetic element named the T-haplotype, which behaves much like a synthetic gene-drive (Leitschuh et al. 2018). A drive based on the X-shredder method could also bias sex ratios towards males, and this is one of the proposals being explored by a group at the Roslin Institute, UK (McFarlane, Whitelaw, and Lillico 2018). Alongside this, that group have proposed a second approach, which would use a homing CRISPR/Cas9 drive to disrupt female fertility genes (McFarlane, Whitelaw, and Lillico 2018). Given that the Grunwald study indicates there are additional technical barriers to constructing efficient gene drives in mammals as compared to insects, there is uncertainty if any of these methods can reach high enough levels of efficiency to eradicate wild populations. Equally, with the current levels of investment in this technology, a mouse gene drive for population suppression could soon be a technical possibility.
Risks and uncertainties of using a gene drive against mice

Risks from use exclusively on islands

Mouse gene drive developers are promoting a scenario where the drive would be used to eradicate mouse populations from certain islands, and would thus be contained by water and could not spread to other land masses. Such a scenario in itself already holds direct and indirect problems and risks. Should mice suddenly, within a few generations, be eliminated from islands, what would be the knock-on effects? While removal of (recently) invasive species often benefits a native ecosystem, unpredicted and negative effects can occur where interactions between different invasive species are present (Zavaleta, Hobbs, and Mooney 2001). For example, because rats and mice are competitors, one scenario would be a competitor release effect, where elimination of the mice would result in an increase in the population of rats. Such effects have been observed in the opposite scenario, where rats have been eliminated, thus causing an increase in mouse numbers (Caut et al. 2007). Further complexity arises from interactions with predators23, as described by Leitschuh et al.: “The presence of an invasive species, especially species that are food sources for predators, can attract other species in search of food, as seen on the Channel Islands and the Farallon Islands [Collins, Latta, and Roemer 2009, SouthEastFarallonIslandsEIS 2013]. If the invasive food source is removed too quickly, the predator may turn to consuming endemic species rather than leave the island [Courchamp, Woodroffe, and Roemer 2003, Collins, Latta, and Roemer 2009].” (Leitschuh et al. 2018, S125)

The potential to spread to continental landmasses

The most dangerous possible outcome of releasing gene drive mice on islands, or indeed anywhere else, is that they may escape, stow away, and migrate to other landmasses, resulting in uncontrolled spread of the drive and widespread elimination or suppression of the species. This is a very significant risk, and the difficulty in containing gene drive mice is well recognised. In a preliminary risk analysis on the use of gene drives in Australia, Australian government scientists concluded that “…biocontainment of house mice and black rats will be challenging to manage because of their propensity to stowaway and survive among cargo and vessels.” (Moro et al. 2018). There are other scenarios that could result in spread of gene drive mice on continental landmasses. Many actors have an economic interest in controlling mouse populations, and it is not hard to imagine an unauthorised release of gene drive mice, or of a state using them unilaterally. Accidental release from a laboratory is also a possibility.

Risks from a gene drive in continental mouse populations

What might the consequences be of the arrival of a gene drive in mainland populations? One scenario would be a crash in mouse numbers, which would likely have serious negative impacts on the predators that rely on them, and could well be disastrous. Often these predators are already under pressure from other factors, for example barn owl populations in the UK have declined due to road mortality, a loss of nesting and roosting spots, and intensive farming practices that have reduced food availability (Meek et al. 2003; Toms, Crick, and Shawyer 2001). Given the wide geographic range of the house mouse and the number of predator species it helps sustain, effects from suppression or eradication could be very widespread and harmful to a large number of species. Other ecological effects, for example complex changes in the invertebrate communities the house mouse feeds on, are also possible, and similarly could be very wide ranging.

23 There is an interesting case reported from Italy of how one prey species can protect another species. In this example, introduced crayfish were found to protect native amphibians from consumption by the invasive American bullfrog (Bissattini, Buono, and Vignoli 2018)
consequences of reductions in populations of these alternative prey species? And what species might increase in numbers to fill the gap left by mice?

**Risks from hybridisation**

The scope for hybridisation of *Mus musculus*, both with other subspecies and with the closely related species *Mus spretus* and *Mus spicilegus*, widens the potential range of impacts further. What might the consequences be of a gene drive spreading to such other species? Hybridisation between subspecies makes the possible geographic impact of a gene drive global, while suppression of related species that live in natural habitats would bring further ecosystem consequences. The dispersal of acorns by *Mus spretus* is a good example of the sort of relationship that might not be obvious, but which if disrupted could bring significant and harmful results.

**A drive could have complex effects on mouse populations and genetics**

The effects of any eventual engineered gene drive on both mouse populations and genetics would be highly uncertain and unpredictable. Over time, resistance to the drive could emerge through various mechanisms (see Chapter 1), which could create scenarios where the population first drops and then rebounds. What might the ecosystem consequences of such an outcome be? What about a situation where the gene drive becomes ineffective, yet the mice are all genetically modified, and - depending on which gene drive system was used - might actually have more and different alterations than at the point of release (as CRISPR/Cas9 for example, when acting as an endonuclease, has a capacity to cut off-target.)

There are many scenarios that need to be considered and that people - that means all of us - haven’t yet envisaged, as well as many possible consequences that no one has yet been able to perceive.

**What are the alternatives?**

The control of rodents is a significant area of study and it is not our intention to recommend particular approaches, nor do we have the expertise to do so. We do wish to highlight though that there are many existing technologies for controlling rodent populations, and some proposed new methods, which do not carry the risks that come with gene drives.

**Island ecosystems**

At the time of writing, more than 560 islands have been successfully cleared of invasive rodents, almost all through the use of toxicants, with success rates for eradication campaigns relatively high\(^{24}\) (DI-ISE 2018). The ambition of rodent removal projects is also increasing as expertise develops. In 2005 New Zealand’s Campbell Island, at 117 km\(^2\), became the largest island to be successfully cleared of rodents (Howald et al. 2007). Yet by 2018, South Georgia, with an area of more than 3500 km\(^2\), was also declared free of mice and rats, after a 10-year elimination project (Harvey 2018). Whilst there are legitimate animal welfare questions to be asked about toxicants, relative to gene drives they do at least avoid the risk of uncontrolled elimination of target species beyond the intended area.

**Rodent control in other settings**

Rodent control in agricultural environments, in food storage and transport and in domestic settings, is a well-developed science, drawing on long established traditional techniques as well as more modern ones. Methods include physical barriers, various forms of trapping and stalking, using cats or other predators, and removal of cover, alongside the more recent development of toxicant usage. Practices in rodent management continue to evolve. Since the 1990s, Ecologically Based Rodent Management (EBRM) has emerged as an approach to design more effective control strategies, by drawing on knowledge of animal biology and behaviour (Singleton et al. 1999); it has been shown to be ef-

\(^{24}\) For comparison, failures have been reported on 100 islands.
fective in various farming communities (Singleton, Brown, and Jacob 2004; Taylor et al. 2012). Alongside the push towards gene drives, other more predictable new technologies are also in development (Campbell 2015), for example self-resetting traps have shown promise in controlling rats and mice at landscape scale in New Zealand (Carter et al. 2016; Carter and Peters 2016).

### Beyond gene drive mice – what’s next?

Current efforts to construct a mouse gene drive are motivated in large part by the desire to apply gene drives to other mammals. The comments in an opinion piece accompanying Grunwald and co-workers’ mouse gene drive study in the journal Nature illustrate this: “Grunwald and colleagues’ work is an important proof-of-concept that will surely be followed by modifications that might lead to improvements in future mammalian gene drives. If gene drives become efficient in mammals, one possible way in which they might be used is to tackle pests or disease-causing agents.” (Conklin 2019).

Funds are already being directed for preliminary research towards gene drives to eradicate populations of other mammals. For example, the Australian Wildlife Conservancy and the Australian government agency CSIRO are funding a project to sequence the genome of feral cats (AWC 2018) - an invasive species in Australia. Gene drives have also been proposed for the eradication of invasive populations of rats, stoats and brushtail possums in New Zealand (Dearden et al. 2018), for the elimination of rats, mice, red foxes, and rabbits in Australia (Moro et al. 2018), and for control of indigenous rats in the UK (McFarlane, Whitelaw, and Lillico 2018). If mammalian gene drives can be made more efficient, a rapid proliferation of target species is very probable, similar to that which is already starting with insects (see Table 2a). It is also being suggested by some that mammalian gene drives might be used in the wild as a test case before insect ones; the opinion piece cited above goes on to propose that they could be a better test case because the movements of mammals might be “more easily restricted” - a statement which is of course debatable for mice and rats, which are the most likely targets. Nevertheless, the enthusiasm with which some funding agencies and researchers view mammalian gene drive technology is apparent. If the use of gene drives to eradicate mice proves technically possible and gains regulatory approval, the house mouse is likely to be just the first of many species targeted.

### Concluding remarks

A highly unpredictable technology

Much like proposed gene drives in mosquitoes, the behaviour of any drives deployed against mice or other mammals, and the ecological consequences they would bring, is very difficult to predict with any confidence. As discussed for mosquitoes in this chapter, various molecular mechanisms or behavioural tendencies could give rise to resistance, causing the drive to either fail completely or only partially suppress populations. However, there is the risk that drives could be highly invasive and spread rapidly, and given that species of *Mus musculus* are present over much of the globe, can hybridise with each other, and frequently stow away, the geographic reach of any drive could prove to be impossible to control. The removal, or even temporary suppression of mice, could also have powerful ecological effects, which are difficult to predict with any accuracy, but could be harmful to biodiversity, agriculture or human health. A spectrum of predators could decline or even disappear in some circumstances; invertebrate communities could increase or shift in composition due to reductions in predation; and other species could eventually expand to occupy the empty ecological niche, bringing further consequences.

Functional gene drives would tighten human control over the biosphere

 Whilst the consequences of experimental releases of gene drive organisms are highly unpredictable, it is necessary to consider the scenario humanity would reach if researchers succeed in their intention of building operational gene drives in mammals. Through agriculture, humans have gained control over the types of plants produced in the biosphere and the species that feed on them, to the extent that...
humans and their livestock now account for more than 95% of all land mammals by biomass (Bar-On, Phillips, and Milo 2018). Animals like *Mus musculus*, whilst from one perspective considered pests, divert biomass out of human control into the sphere of the remaining wild animals. To put it another way, the limitations of current ‘pest control’ methods are valuable in maintaining biodiversity. For example, a study of barn owls living on agricultural land in California found that 99.5% of their prey were ‘pest’ species (Kross, Bourbour, and Martinico 2016). If gene drives do fulfil their developer’s ambitions, they would instead offer a new level of pest control, potentially going as far as wide-scale eradication. Would it be possible to control the use of these tools against mice and other vertebrate ‘pests’ in the face of strong economic pressures to employ them? If some individuals or organisations did use them, could they be contained—and if not, what might be the consequences? It is possible that drives could crash not just mouse populations, but also greatly reduce the numbers of snakes, lizards, owls, hawks, falcons, foxes, stoats, weasels, and many others that feed on them. Thus gene drives could have the capacity to further shrink the already much diminished realm of the wild animals. We find the prospect of this further tightening of human control over the biosphere extremely disturbing.

### 2.4.3 Case study 3: Plants in agriculture – Palmer amaranth

**Introduction – Brief description of the biology of Palmer amaranth (*amaranthus palmeri*), and the broader agricultural context**

Palmer amaranth (PA) is a member of an important group of annual plants found in North America and other parts of the world. There are several aspects of the biology of the species in the PA group that makes them of high interest as crops and as food sources for animals. However, in instances which include certain industrial agriculture systems, this same biology is responsible for their classification as agricultural ‘weeds’, candidates for biological controls.

The current interest in using gene drives to control PA weeds could lead to several harmful results. Gene drives created in an attempt to eradicate the weed could spread into non-agricultural populations, potentially damaging their role in native ecosystems. This means that the potential of this species as a human food source, or potentially as a source of conventionally bred genes for related crop species, could thereby be threatened. The highly desirable traits possessed by Palmer amaranth described below, especially its nutritional value and ability to adapt to high temperatures and drought, both of which are exacerbated by climate change, illustrate some of the risks of eliminating this species, either intentionally or accidentally.

On the other hand, there are systems-based solutions for controlling pests such as PA that can be achieved by following the principles of agroecology, the widely recognised and sophisticated science that applies ecology to the design and management of agricultural systems (Altieri 1995, Gliessman 2014; UN Food and Agriculture Organization 2011; Abate et al. 2008). It emphasises the optimisation of biodiversity of crops and supportive organisms, as part of strategies to build long-term, healthy agro-ecosystems and secure livelihoods. Generations of indigenous and peasant farmers’ knowledge and skills pioneered this practice, and they continue to contribute to the growth and use of agroecology. Because local farming communities must be healthy and adaptive to local ecosystems for agroecology to be effective, it is a form of agriculture that recognises the importance of climate justice, food justice and food sovereignty to its functioning (Altieri 1999; Francis et al. 2003).

The agroecological approach avoids the risks and uncertainties of gene drives and also provides multiple co-benefits for the environment, including much cleaner water, increased biodiversity and substantially reduced greenhouse gas emissions (Dooley et al. 2018; Han et al. 2017; Isbell et al. 2017; Kremen and Merenlender 2018; Kremen and Miles 2012; Liebman and Schulte 2015; Ramankutty et al. 2018).
Indeed, one of the more general and usually unmentioned risks presented by gene drives is that if they succeed, they may further lock agriculture into a chemically dependent industrialised system that is causing multiple, global scale environmental challenges. The harm that results from industrial agriculture includes hundreds of coastal hypoxic zones ("dead zones") and toxic algal blooms, heavy reliance on pesticides, major contributions to the largest loss of biodiversity in millions of years, and a quarter to a third of greenhouse gas emissions (Breitburg et al. 2018; Dudley et al. 2017; Garnet 2011; Kremen and Miles 2012; Maxwell et al. 2016; Ramankutty et al. 2018; Scavia et al. 2014; Smith et al. 2014).

Gene drives aimed at reversing resistance to herbicides, glyphosate in particular, could simply further lock in an herbicide-dominated approach to weed control that has been shown to cause direct and indirect harm to the environment and to people.

Characteristics of Palmer Amaranth and its Value

In addition to the use of some species of amaranths as domesticated crops, wild species, including Palmer amaranth, have been used by indigenous peoples and native farmers globally as important food sources. Several native American tribes in the Southwest US used PA seeds and foliage as food (Moerman 1998). Species in Sub-Saharan Africa are similarly used. Not only are the seeds highly nutritious, but the foliage contains 25 percent protein (in the leaves of *A. cruentus*), as well as vitamins and minerals such as calcium (National Academies of Sciences 2006). These can be particularly important for subsistence farmers as supplements to their cultivated crops, providing important nutrients.

The family *Amaranthaceae* contains 79 genera globally, while the closely related *Chenopodiaceae* contains 104 genera (Hernandez-Ledesma et al. 2015). These two families are often considered to make up a single taxonomic clade (having a mutual ancestor). The genus *Amaranthus* contains about 75 species (Ward et al. 2012), including the important grain amaranth (*A. hypochondriacus*), a highly nutritious crop containing high quality protein. The *Chenopodiaceae* contains quinoa (*Chenopodium quinoa*), which was domesticated in South America about 3,000+ years ago and is highly nutritious, also containing high levels of high-quality protein. The amaranth family also contains several important weed species, including Palmer amaranth (also known as Palmer pigweed), and several other species (often known as pigweeds).

Palmer amaranth is dioecious, meaning that it typically produces male and female flowers on separate plants, while several other species of amaranths are monoecious, as are many other plant species, producing both male and female flowers on the same plant.

The dioecious characteristic results in high genetic adaptability through obligate outcrossing (Ward et al. 2012). This characteristic also makes it a good potential target for the use of gene drives, since it facilitates the spread of the drive. Many plants that are monoecious can be self-fertile, and this trait reduces the dispersal of the drive during reproduction.

Palmer amaranth is native to the arid areas of the Southwestern U.S. and northern Mexico, typically living in or adjacent to the washes (seasonal streams and rivers) (Saurer 1957). As a species adapted to desert conditions, it is capable of growing at high temperatures and tolerating drought.

It does not do this primarily by resisting desiccation, as do species like cacti (in the western hemisphere) or euphorbs (in the eastern hemisphere). Those species use several characteristics to retain water, such as thick cuticle covered by or permeated by wax, and broad stems that hold substantial amounts of water. Palmer amaranth also does not rely on a deep root system to tap deeply buried groundwater. Instead, it takes rapid advantage of limited seasonal precipitation by growing extremely quickly and developing huge numbers of seeds before soil moisture is lost. It also maintains high solute levels that help to retain water in its tissues to temporarily resist wilting when water is not plentiful in the environment (Ward et al. 2012).
This allows it to grow quickly by using substantial amounts while water is available, and to continue growing for some time beyond, when many plants, including crops that do not develop high solute levels, would fatally wilt. Since it produces seed before water is completely gone, it avoids the worst conditions of drought. In fact, PA can respond quickly to loss of water by accelerating seed production. This can be a highly desirable ability to adapt to climate change.

Another important characteristic of amaranths is that they use C4 photosynthesis (Wang et al. 1992). This mechanism greatly improves photosynthetic efficiency by largely avoiding a process called ‘photorespiration’, that occurs in C3, the other main type of photosynthesis. C4 plants typically grow faster than C3 plants, at least under historic atmospheric carbon dioxide levels and higher temperatures. They also tend to be more drought- and heat-tolerant. C4 metabolism evolved separately in monocots such as grasses, and dicots, such as PA.

Much more common among monocots, C4 metabolism is unusual among dicots. Important examples of C4 monocot crops are corn, sorghum, and millets (but this metabolism is not found in several other grains, such as rice, wheat, barley, oats or rye). Corn is known for its extremely high productivity, and sorghums and millets for their drought and heat tolerance.

The C4 property, as well as the others described above, makes PA and other amaranths of particular interest. There is work and consideration focused on potentially further developing several amaranths as food crops, due to such properties and high nutritional value (National Academies of Sciences 2006). At the same time, these physiological and genetic properties of PA and other amaranths have also made them invasive weeds under certain conditions (see below).

### Outcrossing between Palmer Amaranth and Related Species

Hybridisation between PA and other related species could provide a route for a gene drive to enter the population of these other species, with unknown, potentially harmful consequences for the species and their ecosystems. For this to occur, some of the hybrids must be fertile and have the ability to backcross with that species in order to cause the introgression of the gene drive into the population.

Plants have higher potential to outcross and produce viable hybrids and fertile offspring more commonly than do most animal species. It was believed as recently as several decades ago that crops would rarely outcross to wild relatives, but this has now been shown to commonly occur (Ellstrand 2003). Plants have been sometimes found to produce fertile hybrids, not only with other species in the same genus, but occasionally even with species from other genera in the same family.

The ability to hybridise with other species is important both for evolution and crop breeding, in providing important sources of genetic diversity and adaptability (Baack and Rieseberg 2007). It is also as a concern for the potential unwanted spread of gene drives from one species to another, or to a crop, and could entail serious potential health and ecological consequences.

Palmer amaranth has been shown to most readily hybridise and produce fertile progeny with the sympatric monocious species called spiny amaranth (A. spinosus) (Gaines et al. 2012). One report provided evidence of hybridisation and introgression of a herbicide resistance gene through backcrossing from PA into common waterhemp (A. rudis) (Wetzel et al. 1999). Hybridisation was also found with tall waterhemp (A. tuberculatus), and at very low rates with smooth pigweed (A. hybridus) (Gaines et al. 2012). However, I could find no research on its sexual compatibility with amaranths from other regions.

The ability of PA to form fertile hybrids with other species could extend the geographic range or ecological impacts of a gene drive. While PA was originally found in arid areas of the U.S. Southwest and Mexico, the natural range of several sexually
compatible species extends farther north and into more humid areas.

Ward and colleagues summarise several studies of interspecies hybridisation attempts, showing differing results in different experiments, with some producing fertile progeny and others, although using the same species, failing to do so (Ward et al. 2012). This may be attributed to the very low hybridisation frequencies found in these studies, as well as differences in methodology or the genetics of the specimens used in the studies. This could result in rare fertile hybridisation events, with various species being missed in such studies.

As Ward et al. recognise when referring to the potential for herbicide resistance to be transferred to other amaranths, even low levels of gene flow between these species could be cause for concern. This statement could also be applied to low levels of transfer of a gene drive to other species. If a gene drive is effective at spreading through the genome, even very low frequencies of gene flow through hybridisation and backcrossing to another species could result in the effective interspecies spread of the drive.

It should also be noted that the previous research on natural hybridisation and gene flow cited above does not address whether PA may be a source of genes for improving cultivated grain amaranths. Techniques such as protoplast fusion or embryo rescue have sometimes been used successfully to breed crops with wild relatives, techniques which may allow fertile hybridisation in cases where it may not occur naturally or is exceedingly rare without such assistance. These techniques were not used in studies of natural hybridisation.

To summarise the available research, hybridisation and gene flow between PA and other sympatric species has been demonstrated. But these data are limited, and it is unknown how many other species, whether in North America or elsewhere, might be sexually compatible with PA. Even when it comes to the potential spread through species already known to be sexually compatible with PA, there could be significant risk from a gene drive, since these species are common native members of North American ecosystems.

Ecology of Palmer Amaranth

As a fast-growing seed-producing plant in the US Southwestern deserts, PA has a role in providing food for numerous seed-eating species. Desert birds are reported to use this food source, with recovery from 11 bird species’ digestive tracks (Proctor 1968). It is also consumed by multiple duck and goose species, with seed remaining viable after digestive track recovery (Farmer et al. 2017). Survival in bird digestive tracks may facilitate long distance dispersal of this plant.

It is important to note that its adaptive strategy of emphasising fast growth, rather than high conservation of water, compared to the typically slower growth of many other desert species, may complement the latter’s ecological function. Its small seeds and large seed production also facilitates colonisation of new and disturbed sites, which may facilitate later succession to slower-growing species.

Perhaps because of its dual food and weed roles, much of the research on PA focuses on these aspects of its biology. The research on its wider roles in ecosystems is more limited. Therefore, the consequences of gene drives that may impact whole populations or species like PA is very difficult to predict. This limited ecological understanding makes adequate risk assessment difficult at best.

How Palmer Amaranth became a Serious Weed Problem in Agriculture - The Bigger Picture

Palmer amaranth is an example of a plant that has become a major weed only recently, largely due to technological changes in US and South American agriculture in the last few decades. In particular, the advent of genetically engineered glyphosate-resistant crops, in the mid-90s, led to the large majority of corn, soybean and cotton acreage in the US containing this trait, which resulted in the extreme over-use of this herbicide (Mortensen et al. 2012). Prior to that time, PA was not considered to be a highly important weed. Besides the US, engineered her-
bicide-resistant crops are most common in South America, where PA and other resistant weeds have also become a substantial problem.

Many other countries have been reluctant to grow these crops. However, they are nonetheless part of trends toward further industrialisation of agriculture and emphasis on reducing dependence on labour, being dependent on chemical fertilisers, large machinery and pesticide use, along with various other technologies. In this broader sense, this trend toward industrialisation and reduced biological diversity can also be found in many parts of the world, including Europe. This is due in part to international neoliberal trade regimes that emphasise productivity and price, at the expense of more multifunctional aspects of agriculture. Crop and habitat diversity support predators of pests and pollinators, and continuous plant cover of the soil enhances fertility and water quality and also helps to limit soil erosion. In other words, technologies that emphasise herbicide and other pesticide use are the antithesis of agricultural systems such as agroecology, that are based fundamentally on diversity—biological and genetic, as well as cultural.

As such, there is continuing pressure globally to further adopt policies and technologies, such as engineered crops, that have coincided with falling crop and landscape diversity in the United States (Lark et al. 2015; Plourde et al. 2013; Stern 2012). Gene drives, by enhancing herbicide overreliance or avoiding systems based on ecological diversity, could well increase this trend.

The widespread planting of herbicide-resistant crops and overuse of glyphosate-based herbicides led to the development and rapid selection for PA and several other weeds resistant to this herbicide (Webster and Nichols 2012). Their spread through many states has been facilitated, especially in PA’s case, by its production of large numbers of small, easily dispersed seeds.

Therefore, although PA is widely established now, making it hard to control, measures not highly dependent on glyphosate or other herbicides that were previously used, as well as newer measures, can be useful to regain control of this weed.

In fact, as discussed below, integrated weed management using the principles of agroecology could not only resolve the PA problem, but also provide other important co-benefits for the environment and rural communities. Gene drives aimed at ridding the agroecosystem of PA or making it susceptible to glyphosate again might instead, if successful, allow industrial agriculture to avoid needed changes. These needed changes include achieving broad environmental goals such as cleaner water, reduced water use, greater biodiversity and lower greenhouse gas emissions.

This points to a general problem that afflicts narrowly targeted strategies like gene drives: They do not address the often complex and broader issues that led to the problem in the first place. This in and of itself does not mean that technologies could never have a positive role in solving agricultural problems; but it does suggest that they may obscure more systemic problems and foreclose better systemic solutions that have multiple benefits.

**Specific Origins of the Glyphosate-Resistant Weed Problem**

Palmer amaranth was reported to begin moving from its regions of origin in the early 20th century (Ward et al. 2012). However, except for a few southeastern states, it was not recognised as a major agricultural weed until the adoption of conservation tillage (reduced tillage with crop residues left in the field) and no-till farming became more common following incentives in the 1985 U.S. Farm Bill, and also after glyphosate resistant crops became widespread after the mid-1990s. With the reduction of tillage, which can substantially contribute to effective control of PA, weed control became even more dependent on herbicides, especially glyphosate used in conjunction with the major crops corn, soybeans, and cotton.

This led to the massive overuse of the single herbicide, glyphosate, and to PA’s subsequent resistance to it and its geographic spread (Mortensen et
al. 2012). For example, while not listed among the important US weeds of corn in 1994, it was ranked 7th by 2009. It was ranked 23rd in soybeans in 1995, and rose to 2nd in 2010 (Webster and Nichols 2012). This means the main reason for the increased importance of PA as a weed was the development during this period of glyphosate resistance, with reduced tillage also playing a role.

In the several decades prior to HR crops and incentives to reduce ploughing and tillage, along with several herbicides, were commonly used to control weeds by the industrial farms that dominate commodity crop production in the US and Western Europe. This includes crops like corn, soybeans and cotton that are grown on huge areas of land. Palmer amaranth is susceptible to tillage in part because it is an annual and so does not grow back from root fragments that may occur after ploughing, as many perennial weeds can. Moreover, its seeds are very small; when buried by ploughing, they cannot germinate and sprout. However, because, unlike many other weeds, new PA seedlings emerge throughout most of the growing season, control measures like tillage or herbicide applications often need to be repeated.

**Gene Drives for Palmer Amaranth**

Because of its importance as a weed and its biological characteristics, PA is considered one of the primary targets for CRISPR/Cas9 based gene drives in plants, as noted by the U.S. National Academies of Sciences (National Academies of Sciences 2016). Because it is dioecious, it is an obligately outcrossing species. However, there are reports of low level agamospermy (seed produced from ovules without fertilisation) that could short-circuit a gene drive system (Ward et al. 2012). The large number of seeds produced by PA plants, on the other hand, could facilitate faster spread of the drive.

There are several recognised challenges that would affect the performance of gene drives in weeds or other plants. First, it could take several years for a gene drive to adequately penetrate a population, and persistent seed banks can add to that time period—seed banks are the seed left in the soil (Neve 2018). Depending on the plant species and environmental conditions, these may last from a few years to decades. Seed banks are an important consideration in weed control, because once they have built up to significant levels, weeds will continue to emerge in a field, even if no further immigration of seeds from elsewhere occurs. PA does not have a very persistent seed bank. In one experiment, after four years of burial in soil, only about 0.01 – 0.03 percent of seed remained viable. On the other hand, a single plant can produce 600,000 seeds, which still means many viable seeds after several years even with low persistence rates (Jha et al. 2014). Resistance, as reported above, is also a possibility for several gene drives and could readily occur in the field (Unckless et al. 2017 – and see Chapter 1).

More fundamentally, homologous recombination does not seem to function as well or as readily as a DNA repair mechanism in plants as it does in many other organisms (Neve 2018). The predominant repair mechanism for DNA double strand breakages in plants is the NHEJ mechanism (non-homologous end joining), which, instead of facilitating the insertion of the gene drive construct, results in small random mutations at the DNA breakage point. A homologous repair pathway is required to ensure a functioning gene drive system, whether the goal is to disrupt a target gene with insertion of CRISPR/Cas9 as a “genetic chain reaction”, or to spread an effector gene through the population. Low rates of homologous recombination repair could greatly slow the spread of the drive, which is already a challenge with weeds because of relatively low rates of reproduction and/or lack of reliable outcrossing. If low enough, these rates could prevent the drive from working (e.g., if lower than any possible reduction in fitness resulting from the drive). Research to date has not been found that demonstrates any proof of concept for gene drives in plants.

**Risks and Other Issues**

Two general types of approaches for gene drives in PA have been suggested, 1.) drives altering the sex ratio (e.g. reducing or eliminating female plants), which would aim for eliminating weed populations,
2.) sensitising drives, to make subsequently treated plants susceptible to a treatment, likely a proprietary chemical, that would then kill them (Neve 2018). The latter would also include re-sensitising weeds that have become resistant to a herbicide, such as glyphosate. These approaches would be based on CRISPR/Cas9 methods.

The most commonly discussed sensitising drive for PA is to restore its original sensitivity to glyphosate-based herbicides. Theoretically this could be possible, since the genetic mechanism for resistance to the herbicide has been discovered. It has been found that gene duplication has created numerous copies of the native EPSPS gene, part of the aromatic amino acid biosynthesis pathway. The EPSPS enzyme is the target of glyphosate herbicidal activity. The effect of multiple EPSPS gene duplications is to dilute the herbicide relative to its target to the point where it no longer can kill the plant at normal or even very high application rates (Gaines et al. 2010).

However, a substantial limitation with this approach, in addition to those already discussed above, is that the herbicide could not be used for a number of years while the drive was spreading through the weed population, and while viable seed remained in the soil seed bank. Otherwise, the plants with the drive would be killed prior to all plants acquiring the drive. If some plants escaped the drive, they would ultimately be strongly selected for by the use of the herbicide.

But more fundamentally, this approach would maintain the heavy use of this herbicide, with all its attendant harms to the environment, biodiversity and human health. The heavy use of glyphosate is likely the primary cause of the decimation of monarch butterflies in the US by nearly eliminating the milkweeds necessary for larval growth (Pleasants et al. 2017), and herbicide drift generally harms important uncultivated habitat near treated crop fields. Glyphosate has also been determined to be a probable human carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer 2015; Levin and Greenfield 2018). As such, a gene drive restoring sensitivity of PA to glyphosate would also further forestall the implementation of truly sustainable weed control methods like agroecology, and could have other negative consequences to the environment and human health.

The second approach, population suppression, neglects the fact that PA is only a weed in agricultural systems. Its other properties, discussed above, make it a potentially valuable plant as a human food source or as a source of valuable genes for breeding in related species. In particular, its adaptation and fast growth in hot and water-limited environments, and its highly nutritious seed and foliage, means that it may have enhanced value as a potential crop in the future.

Given how easily dispersed the seed of this plant is, both by wind or animals but also farm machinery, it seems highly possible that a gene drive could invade native populations of PA in the US southwest and Mexico. Palmer amaranth is now found widely in the lower 48 mainland US states, so there are no major geographic barriers such as oceans to prevent its invasion of the native range of the species, which could threaten the species’ existence.

PA also has ecological significance that is not well understood. The ability of PA to hybridise with several other species of amaranths means that the drive may eventually spread to related species. Even if PA and related species are not driven to extinction, reduced populations in wild habitats could have negative ecological consequences. As for the potential of these species to provide genes useful for crop breeding, population reduction short of extinction could reduce their genetic diversity, possibly reducing the number of valuable genes or alleles. The collective impact could cause significant harm to the environment.

Agroecology as a Way to Control PA Sustainably and as an Alternative to Gene Drives

Agroecology relies on biodiversity and a diversity of farming practices and management methods like long crop rotations, cover crops and provision of habitat for pest predators, in order to control
pests and weeds. It relies on knowledge developed by farmers over millennia to sustainably grow crops, along with application of modern ecological sciences (Altieri 1999). Modern ecological research can help to optimise systems and breed crop varieties adapted to those systems and to the needs of farmers. For example, the ecological sciences recognise that ecosystems vary locally and regionally, and therefore optimisation of cropping systems for productivity and co-benefits can best be achieved by designing such systems to best take advantage of local conditions.

In essence, all organisms, including pests, are adapted to particular environmental conditions and plants they can feed on or crops they can infest as weeds. By varying those conditions over time and space through practices like crop rotation and the use of cover crops, pests are usually prevented from building up to harmful levels. Using these strategies have been shown to be effective in controlling weeds (Liebman et al. 2004). Similarly, crops can be bred to better compete with or suppress weeds (Worthington and Reberg-Horton 2013). In addition, the increased biodiversity created by these crops and nearby uncultivated areas, together with limited use of pesticides, encourages the proliferation of organisms that reduce pest and weed numbers (Altieri 1995; Liebman et al. 2004).

For example, long-term experiments in Iowa have shown that herbicide use can be reduced by about 90 percent or more to obtain weed control, and crop yields for corn and soybeans as high or higher than for typical herbicide-dependent industrial agriculture (Davis et al. 2012). Others have demonstrated similar results in Europe and elsewhere, especially under drought stress (Gaudin et al. 2015; Lechenet et al. 2014). While more labour is often required, the cost of chemical treatment is reduced, so the net profit to the farmer is as high or higher than for chemical-and GMO dependent industrial agriculture. PA was not found in the area of these experiments, but a related amaranth, waterhemp, is a major weed in Iowa. Even though limited tillage was used in the system of Davis et al. and others, water quality is substantially higher than for industrial agriculture, due to the incorporation of the perennial alfalfa (lucerne) and cover crops in the winter (Davis et al. 2012; Isbell et al. 2017).

More specifically, separate experiments have shown that winter rye cover crops, combined with modest tillage, can effectively reduce PA, providing the biomass of the cover crop is high enough (Aulakh et al. 2012; Aulakh et al. 2013; Price et al 2016). Combined with the positive weed control effects of greater crop diversity in agroecological systems, these practices have promise to provide long-term control of PA, as well as providing the multiple benefits noted above (Liebman et al. 2004). At present however, there appears to be no research on the use of diverse agroecology specifically to control PA.

Although agroecological systems provide substantial co-benefits in reduced pesticide and fertiliser use, increased soil fertility, higher biodiversity, and cleaner water, also in reduction of greenhouse gas emissions, farmers are often locked into current industrial practices for several reasons. These include: inexperience with knowledge-intensive agroecology; peer pressure; farm policy that discourages them (e.g. insurance or loan unavailability); higher labour requirements; and debt service due to the purchase of expensive specialised equipment (Roesch-McNally et al. 2017; Vanloqueren and Barret 2009). These could be remedied over time with proper policies and incentives, along with more research to optimise agroecological systems (Delonge et al. 2016). These measures would be highly justified, given the large public benefits of this kind of farming.

**Summary and Conclusions**

There is considerable interest in developing gene drives to address the substantial challenges of agricultural weeds. Palmer amaranth in particular has been discussed as a desirable target. This is due both to its great importance as a weed in the US and South America, and also because of its biology of obligate outcrossing. On the other hand, there are so far no proof-of-concept examples of gene drives functioning in plants, and there are several considerable biological barriers, which are greater than
for some other organisms (such as mosquitoes), for such an approach to work.

Despite the current barriers, the potential attractiveness of commercial gene drives that control weeds like PA or other crop pests is likely to drive further research projects seeking to overcome these challenges. It is therefore important to consider the many potential harmful consequences of agricultural gene drives such as might be used for PA.

In doing so, this chapter finds many ways that such gene drives could cause harm, as well as too little information about how such gene drives could negatively affect either the environment or human health. Some of this harm could result from reduction or elimination of populations or PA in the natural environment, or through damage to populations of related species via gene flow. This is an area for which there is far too little information.

Substantial harms and dangers could also occur through the re-sensitisation of PA to glyphosate herbicide, which is something that will probably find financial backing, as such a gene drive would hold considerable interest to the companies that sell this chemical.

This re-sensitisation pathway is often considered to be less risky than population elimination. However, the analysis here finds that there are considerable indirect risks that are not often well considered by regulatory agencies. These include the established harms of supporting forms of agriculture overly dependent on herbicides, along with other ecologically and socially harmful technologies or practices.

The risks of using gene drives in these contexts also include the potential opportunity costs of forestalling movement to more agroecologically-based systems, which are needed to address the multiple harms caused by industrial agriculture, currently propped up in part by overuse of herbicides. These kinds of concerns, although they have huge social and environmental implications, are rarely considered by risk assessment agencies.

This also points to a general concern about gene drives as an example of a piecemeal techno-fix, rather than a systems-based, holistic approach to solving complex environmental and social problems. Because specific problems in agriculture are inevitably part of complex interconnected systems, they are often symptoms of much larger problems. Addressing these as individual issues alone can lead to other unanticipated harms.

Our starting point should therefore be a broad analysis of the biology and ecology of the organisms that may be considered to be targets of gene drives. We also must evaluate the social systems with which these organisms interact, and understand exactly what kind of systems in which these drives would be used, or could facilitate. We must also evaluate systems-based alternatives before gene drives are considered for use in the environment.

2.4.4 Agricultural insect pests as Gene Drive targets

In considering pest insects as potential gene drive targets and in order to adequately determine other potential options for pest control, it is important to first understand how these insects became significant pests in the first place. Instead, the starting point for considering whether gene drives might be appropriate is typically the current severity of the pest and whether typical conventional options such as pesticides are sufficient to control it. In particular, the industrial agriculture production system itself may have played a large role in terms of whether an insect became a pest in the first place, as well as how severe its effects might be. Therefore, consideration should be given to whether altering the production system might also be able to provide opportunities for control that would avoid the risks and uncertainties associated with gene drives.

A second important consideration in evaluating the use of gene drives for agricultural insect control is the state of knowledge about the ecological role of the pest in the wider environment, including the different geographies into which the gene drive may spread. These considerations will be evaluated below.
Because in practice a very large number of species might eventually be considered by proponents of this technology as gene drive targets, in order to briefly illustrate these issues, the focus here is on only a few examples. However, it needs to be emphasised that to date, only some proof of concept research has been published, which is a long way from showing that gene drives can work in the complex context of the open environment or that they can be safe.

Several of the species that have been mentioned as potential targets are listed in Table 2a and references cited therein, while several others are considered by Scott and colleagues (Scott et al. 2018). These include: spotted wing drosophila (SWD, Drosophila suzukii); the wasp species Vespula vulgaris and V. germanica; Argentine stem weevil (Listronotus bonariensis); Australian sheep blowfly (Lucilia cuprina); red flour beetle (Tribolium castaneum); and the Asian citrus psyllid (Diaphorina citri); the New World screwworm fly (Cochliomyia hominivorax); diamondback moth (Plutella xylostella); Western corn rootworm (Diabrotica virgifera virgifera); and silverleaf whitefly (Bemisia tabaci).

The Role of the Agriculture System in the Development of Insect Pests

Many important insect pests cause substantial damage because current intensive agriculture production systems are particularly vulnerable to pests due to their limited biological and genetic diversity, which can be exacerbated by heavy dependence on chemical pesticides (Bennett et al. 2012; Douglas and Tooker 2016). Research showing that biologically diverse organic farming systems tend to have less pest damage also demonstrate productivity in these organic systems at near industrial farm levels (Ponisio et al. 2015). In contrast, low-diversity organic systems that are similar to industrial systems in all respects other than pesticide use, had yields approximately 19% less than comparable intensive industrial plots. Overall, more diverse organic systems yielded only about 8-9% less than industrial systems (Ponisio et al. 2015).

As these authors point out, this is despite the fact that only a few percent of US research dollars currently being spent is supporting the improvement of organic and other agroecological systems (De-longe et al. 2015). Ponisio and colleagues suggest that with adequate research support, even the small yield gap between the two systems might be eliminated. At least one other study at the global scale suggests that organic can be close to, or sometimes more productive than, conventional agriculture, especially in developing countries (Badgley et al. 2007). These agroecological systems can apply advances in ecological science to augment more traditional systems, but currently receive little research funding compared to industrial methods, despite their notable successes.

Although factors other than reduced insect pest damage could likely have contributed to these results, it is unlikely that high yields could have been produced alongside substantial insect damage. Similarly, diversified farms in Europe maintained yields as high as industrial systems, despite dramatic pesticide reduction in these more diversified farming systems (Lechenet et al. 2014). Long-term experiments at farm scale have shown that agroecological systems in the US Midwest can be as, or more, productive than industrial systems, while dramatically reducing pesticide use and fertilisers, thus conferring large benefits to biodiversity and water quality (Davis et al. 2012; Liebman and Schulte 2015).

A substantial factor in higher yields in more diverse systems is biological insect pest control by pest predators and parasitoids, which are also known as pest natural enemies (Grab et al. 2018; Rusch et al. 2016). Current simplified industrial systems generally have lower abundance and diversity of pest enemies than more diverse farming systems (Letourneau et al. 2011).

A proposed gene drive target, Western corn rootworm (WCR) (Scott et al. 2018) provides a well-studied example of how current simplified cropping systems lead to higher crop damage. Western corn rootworm is considered the worst corn insect pest in the US, but in most areas crop rotations eliminate the need either for insecticides, or gene drives, to
control it (Gray et al. 2009). These rotational systems are as, or more profitable than, monoculture corn (Davis et al. 2012). In some areas WCR has developed resistance to the common corn-soybean rotation. This rotation, however, consisting of only two crops, is considered too simple to fully qualify as agroecology, and is part of typical industrial crop systems heavily reliant on chemical pesticides (Davis et al. 2012).

**Invasive Species as Agricultural Insect Pests**

Many of the most challenging insect pests, and most of those noted in Table 2a and elsewhere as gene drive targets, are actually invasive species where they occur as agricultural pests (Scott et al. 2018). A substantial reason for the damage they cause may be the lack of adequate local biocontrol (in terms of natural predators) in their new habitats (Tscharntke et al. 2016). It is therefore important to note that the lack of diverse habitats may reduce the efficacy of introduced biocontrol agents. For example, strawberry farms embedded in less diverse habitats had more damage and lower yields because of the tarnished plant bug (*Lygus lineolaris*), due to reduced populations of an introduced wasp biocontrol parasitoid in the less diverse landscape (Grab et al. 2018).

Similarly, insecticides used in simplified industrial systems are known to harm biocontrol agents (Douglas and Tooker 2016; Tscharntke et al. 2016). This has been demonstrated for an increasingly important introduced wasp parasitoid of the invasive Asian soybean aphid, the most important insect pest of soybeans in the US (Frewin et al. 2012; Frewin et al. 2014).

These examples may have important implications for accepted biocontrol methods that could be more viable and desirable approaches than the creation of targeted gene drives. For example, several parasitoid wasps, both domestic and from the region of origin of the spotted wing drosophila (SWD) (another target of gene drives), could be more or less effective depending on both farm landscape diversity and insecticide use (Staccconi et al. 2017; Wang et al. 2018).

In particular, the efficacy of an introduced biocontrol agent could be hampered in the simplified industrial farm landscapes common in the U.S. and parts of Europe. This could mask the potential of some of these agents, and thereby encourage the use of gene drives, if care is not taken to determine the suitability of the farm landscape, and to encourage a favourable environment for the biocontrol agents. Unlike gene drives, which target only one particular pest at a time, agroecologically diverse farming systems also provide multiple environmental and social benefits that include better water quality, climate adaptation, and biodiversity (see Case Study 3 on Palmer amaranth), as well as their basic pest control function.

Those who oppose biocontrol methods may point to how long it takes to develop them, but gene drives may take fully as long and could be far more dangerous. Pest natural enemies also may adapt over time to reduce harm to crops or livestock from an invasive pest. It is important, therefore, to consider how long an invasive pest has existed in its new environment, and how long efforts other than gene drives have been under development. For example, invasive SWD first was identified in Europe and the US just over 10 years ago, and potentially effective biocontrol agents have only been identified in the past 3 or 4 years and require more testing (Wang et al. 2018). By comparison, the Asian soybean aphid was first found in the U.S. in 2000, and the unintentionally introduced biocontrol parasitoid wasp, *Aphelinus certus*, has been increasing in abundance and efficacy in recent years (Kaser and Heimpel 2018). There is also evidence that generalist pest predators may adapt over time for greater control of some invasive insect pests (Symondson 2018).

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25 It is not known whether WCR would have developed resistance to longer crop rotations, but it seems likely that such rotations would impose higher fitness costs to rotation-resistant variants, reducing this possibility.

26 A parasitoid is an insect, especially wasps, that lay their eggs in other insects, including pest insects. The larvae of the wasps grow in the insect host and kill it.

27 The soybean aphid is not a target for gene drives because it frequently reproduces asexually. It is used here as an example of an important invasive insect species, which provides important relevant similarities for gene drive targets.
et al. 2002). Other helpful approaches to invasive pests also take time to develop, such as breeding for crop resistance (McCarville et al. 2014), or cultural methods such as soil micronutrient additions, which show promise for remedying harm from the Huanglongbing (citrus greening) pathogen transmitted by the invasive Asian citrus psyllid, another potential gene drive target (Table 2a) (Corchrane and Shade 2019). In fact, gene drives themselves may take several years to develop, and more years to adequately test and assure their safety (if their safety, or adequate testing, is even possible). This means that they have no obvious advantage in terms of how quickly they may be safely deployed.

**Risk Assessment of Gene Drives for Agricultural Insect Pests**

An important component of risk from gene drives is the harm they may cause to the ecosystems from which the agricultural pest originated. In most cases we know little about these risks, because we know little about the role of many of these pests in non-farming ecosystems. In their review of potential agriculture insect targets of gene drives, Scott et al. (2018) note that eradication of the New World screwworm (NWSW) from Texas by using sterile insect technology may have contributed to increases in whitetail deer populations—and deer overpopulation can result in harm to tree understories and tree reproduction, as well as to the spread of Lyme disease. They also note that little is known about the ecology of New World screwworm, probably because research emphasis has been on its control, not its ecosystem purpose. These authors also note that there is similar need for more ecological research for other insect pest targets of gene drives: “Further, the screwworm experience highlights the need for more basic ecological studies for other pest insects before and after a population suppression program.”

In research for this report, it was similarly noted that the preponderance of the research on *Drosophila* has been on *D. melanogaster*, a model organism for genetics research since the early 20th century, and now again for gene drives (see Table 2a). Proof of concept was demonstrated in *D. melanogaster* for a “mutagenic chain reaction” using CRISPR/Cas9 (Gantz and Bier 2015). More recently, Buchman et al. (2018) used a MEDEA-based maternal gene drive to achieve near 100% population suppression in lab in Spotted Wing Drosophila, including several wild-type populations from different regions, although this is still far from demonstrating function in the environment.

But knowledge of *D. melanogaster* cannot substitute for understanding the actual ecological functions of the pest species SWD, including behavior, effects on plant species composition, role as a food source and other factors. Research for this report was unable to identify substantial research on the role of SWD in its native habitats; there seems to be very little. Some aspects of the physiology or anatomy of SWD that make it a particular pest problem, such as its serrated ovipositor (egg laying organ), which facilitates egg laying in ripening fruit, unlike many other species of better known *Drosophila*, also may have implications for its ecological roles that differ from better known species. The same processes that would spread SWD could be reversed, to bring gene drive individuals into contact with non-target populations (i.e. in region of origin) (Webber et al. 2015). Further, recent modelling suggests that efficient gene drives could be highly effective and spread through all populations, even if introduced at low frequencies (Nobel et al. 2018). Several species of drosophila have been shown to be able to hybridise, resulting in gene flow (Kaneshio 1990).

This all means that, in addition to possible extinction of the target pest SWD, it may be that other species, especially closely related species sympatric to the region of origin of SWD, might be driven to extinction as well, without researchers having, or

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26 Parasitoids often have only a few species that they attack, which makes them attractive as imported biocontrol agents. They are therefore specialised biocontrol agents. Generalist biocontrol agents, by contrast, prey on many species, including pests and others.

29 It is too early to determine how effective this approach may be, but the main point is that these approaches take some time to develop and test.

30 Scott et al. 2018, S104
even attempting to have, a clear understanding of their role in their ecosystems.

Ecological assessments of environmental risks by or for regulatory authorities and agencies for other pest control technologies have been shown in recent years to be inadequate. For example, resistance to glyphosate herbicide and the weed control crisis (see Case Study 3 on Palmer amaranth) resulting from the commercialisation of genetically engineered herbicide resistance crops, was not predicted or prevented by authorities in the US.31

More recently, widespread harm to invertebrates, such as pollinators, from the use of systemic neonicotinoid insecticides, was not foreseen by regulators in any country. This is due at least in part to the inadequacy and difficulty in determining harmful sub-lethal and trophic level effects of these insecticides in the broader environment, for example, identifying harms to behavior, fecundity, or immune function of invertebrates (Pisa et al. 2015). It has taken years of research by dozens of scientists to begin to determine these effects. While the precise types of ecosystem effects caused by gene drives would not be identical to the effects of a pesticide, they are likely to also be at least as complex and take considerable time and effort to understand. The history of the regulation of pest control technologies does not provide comfort that adequate complex ecological assessments will in fact be undertaken before application if they are developed. And while pesticide use can be discontinued, gene drives intended to spread through the environment, or those that spread beyond their intended range, so far cannot be recalled or reversed.

A general problem with piecemeal approaches like gene drives is that even if nominally successful in controlling some particular pests, they could leave intact harmful industrial agriculture and perhaps forestall systemic ecological changes in these activities that are needed in order to reduce water pollution and climate change emissions, conserve biodiversity, and improve resilience to climate change (i.e. temperature and moisture extremes).

Agroecology has been shown to benefit all of these societal needs. Although it is not necessarily inherent in the development of gene drives that its use would replace efforts to pursue agroecological systems, new technologies need to be considered in a current social context, one which already favours industrial agriculture in terms of research and funding, and which could be propped up and further entrenched with gene drive technologies (Roesch-McNally et al. 2018; Vanloqueren and Baret 2008).

2.4.5 Dual use - military (& civilian) research & potential use

As already outlined in the introduction, this is a powerful technology that has high potential for misuse and destruction, and is as such recognised as a ‘dual-use technology’, that is, a technology that can be utilised for peaceful civilian purposes as well as for military ones. The National Academy of Sciences report on gene drives makes an important point under “biosecurity considerations”, explaining that gene drives add a new dimension, a new opportunity for weaponising insects, because of their self-sustaining nature: “The actual and potential use of insects as weapons has been discussed; for example, by releasing insects infected with human pathogens or releasing agricultural pests (Lockwood 2012). However, the availability of a gene drive provides a new opportunity for malicious use because its self-sustaining nature poses a perhaps more significant threat.” (NASEM 2016, 161). Kenneth Oye has also repeatedly warned about the potential ease of misuse of this technology, e.g. (Oye and Esvelt 2014).

When looking at potential dual use scenarios, the US National Academy of Sciences, Engineering, and Medicine (NASEM) argues in their 2016 report on gene drives: “Yet, with a better understanding of the basis of mosquito—pathogen interactions, it is not inconceivable that rather than developing a resistant mosquito, one could develop a more susceptible mosquito capable of transmitting a specific pathogen more efficiently than wild-type mosqui-

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31 Weed resistance was predicted by environmentalists, but even they did not anticipate the scale of harm from this process.
toes. It might even be possible to develop mosquitoes that could transmit a pathogen that is not normally vector-borne, or that could even be able to deliver a toxin.” (NASEM 2016, 161) There are in fact many scenarios one could conceive of, especially for insects, given the recent research advances in that field. Whilst spreading toxins and diseases to humans, livestock or plants is a serious prospect, it would be of equal concern to intentionally weaken or eliminate beneficial insects. NASEM hence states in its conclusions: “Governance mechanisms need to be in place to address questions about the biosecurity implications of gene drive research and consider developing mitigation strategies that are not dependent on the underlying technology.” (NASEM 2016, 171)

The DARPA Safe Genes programme and possible military applications

The US Defense Advanced Research Projects Agency (DARPA) is investing at least $65 million over four years into research on gene drives and genome editing technology through a programme named ‘Safe Genes’ (DARPA 2017). The programme was announced in July 2017, along with outlines of seven major research projects it would support, five of which are wholly or partly focused on gene drive research. The goals are broad and include: overcoming the remaining technical barriers to creating gene drive systems capable of modifying wild populations; development of control systems to allow limitation of their geographic range; and finding methods for reversing drives, including counter-acting drives released by other actors. DARPA emphasises that no gene drives will be released in the wild as part of this programme, though many of the projects include public consultation elements or engagement with policy makers which are likely to be aimed at achieving consent for an eventual release.

Who is being funded?

‘Safe Genes’ is directing funding to most of the leading figures in gene drive research, alongside high profile individuals in the CRISPR/Cas9 genome editing community. Whilst many of these projects have been described, an overview is helpful to illustrate the reach of the programme:

- Omar Akbari at UC San Diego is leading a $14.9 million project to engineer gene drives to modify or eradicate populations of the mosquito Aedes aegypti (Warren 2017), alongside the development of drives in Saccharomyces cerevisiae as a model system (Aguilera 2017). His collaborators include Ethan Bier, Valentino Gantz, Anthony James and others.

- A consortium led by John Godwin at NCSU is receiving $6.4 million to develop gene drives capable of eradicating mouse (Mus musculus) populations (NCState 2017). Collaborators include David Threadgill and Paul Thomas, who are spearheading the mouse genetics work.

- Kevin Esvelt at MIT is leading a collaboration to validate ‘daisy chain’ drives and related concepts in the nematode Caenorhabditis brenneri (Esvelt 2017), and along with Luke Alphey, to apply them in the mosquito species Culex quinquefasciatus and Aedes aegypti (BBSRC 2018).

- Andrea Crisanti’s team at Imperial College are continuing their development of gene drives in Anopheles gambiae mosquitoes (Neslen 2017), as part of an $11 million project led by CRISPR/Cas9 expert Keith Joung at Massachusetts General Hospital (MGH 2017).

- A project to develop controllable gene drives in Anapholes stephensi mosquitoes is going ahead, led by CRISPR/Cas9 specialist, Amit Choudhary at the Broad Institute (DARPA 2017). It has not been announced who is directing the mosquito work, though it is likely to be Valentino Gantz and Ethan Bier who work with A. stephensi and have stated they are involved in a second ‘Safe genes’ project (Aguilera 2017).

As well as focussing on gene drives, ‘Safe genes’ is funding efforts to develop new CRISPR/Cas9 technologies, including: engineering small molecule regulated forms; expanding methods to inhibit CRISPR; improvement of genome editing specificity; increas-
ing the range of sites on the genome that can be targeted; and the development of new applications for CRISPR beyond genome editing. These efforts involve Joung’s and Choudhary’s teams, alongside projects led by George Church of Harvard Medical School and Jennifer Doudna of UC Berkeley.

What are the motivations?

To speculate on the underlying logic of ‘Safe Genes’, consider first how a gene drive could be weaponised, for example to crash populations of insect pollinators for important crops, or to suppress fish populations in an important fishery. Even if the US was not interested in gaining this capacity, there would be motivation to find out how to counteract a gene drive released by a hostile actor. Given that the most plausible strategy to counteract a gene drive is another gene drive (as discussed in Chapter 1), this creates an imperative for US defence agencies to develop a functioning gene drive before any rival states or other actors do. Therefore, from a military perspective, there are powerful motives for the US and others to develop this technology, and to do so as rapidly as possible.

The Safe Genes programme should also be seen in the light of another recently announced DARPA programme named ‘Insect Allies’ (DARPA 2016). This $27 million programme seeks to develop methods to genetically modify crops using infectious viruses that would be delivered by insects (some of the methods being explored to do this would use CRISPR/Cas9) (Reeves et al. 2018). Whilst the stated motive for this programme is to be able to directly modify crops whilst they are already growing in the field to protect them from stressors such as drought, disease or insect attack, it has been observed that the proposed technology could be weaponised in various ways, for example to disrupt the formation of viable seeds from targeted crop varieties (Reeves et al. 2018). There may then be the view in the defence community that it would be desirable to gain the capacity to modify crops, insects and perhaps other organisms on a very large scale, whether through viruses or gene drives, and to be one step ahead of rivals in terms of being able to counteract these measures. This is a disturbing prospect, and concern about proliferation of such biological weapons has led to calls for the Insect Allies programme to be scrapped (Reeves et al. 2018).

3 Risks, potential negative impacts and risk assessment limitations

3.1 Risk assessment of GDOs

Current risk assessment (RA) of GMOs is mainly focused on crop plants. With increased research into gene drives we may expect a shift that has already started with the development of GM-sterile insect technology: environmental release of organisms carrying a gene drive will be in wild living animals.

Risk assessment of GM crops is mainly focused on toxicological effects of the expressed transgenic components. Environmental risk assessment (ERA) is complex even for sessile (‘classical’) GMOs like crop plants, which are cultivated in field sites. The release of gene drive differs from these ‘classically’ genetically engineered crops in the following ways, which add additional layers of complexity to any effective ERA, since it means that first release will most likely be:

1. With mobile animals (mosquitoes or rodents);
2. in natural or semi-natural environments (island ecosystems);

32 The term GDO was coined by van der Vlugt et al. 2018 and is also used in Simon et al. 2018
3. carrying a transgene designed to outcross and spread in natural populations;

4. with the intention to either wipe out or persist and permanently genetically modify populations.

The competent authority for ERA in the EU is the European Food Safety Authority, EFSA. In the past, it has published guidance for ERA of crop plants (EFSA 2010), as well as on GM animals (EFSA 2013), which briefly covers gene drives. It has to be noted, however, that this guidance has been developed in the pre-CRISPR era and so will not be fully applicable to recent developments, especially the ability to create global gene drives (global is used in the sense that they might cover the whole geographic range of the affected species).

Risk assessment and especially environmental risk assessment of gene drives will be concerned with multiple layers of effects, caused either directly by the genetic modification or by the direct effects of this modification. For low threshold (global) drives, like most CRISPR-based systems, an intrinsic problem with field testing will occur: namely, that a small release can easily escalate into a full release (Noble et al. 2018). CRISPR-based gene drives can act as “mutagenic chain reactions” (Gantz et al. 2015a), which spread exponentially by inheritance in the given population or species. To obtain field data to support the actual ability to do proper RA of gene drives will thus be difficult, and in some cases might even prove impossible. Modelling effects are seen as an attractive alternative to extensive field testing. However, most modelling approaches for gene drives have so far been performed in order to evaluate efficacy and spread of the desired genetic modification e.g. (Unckless et al. 2015), not in order to anticipate risks. Modelling of ecological effects caused by gene drives which would be useful for ERAs has yet to be developed. In comparison to the modelling of efficacy for purpose, approaches to simulate ecosystem effects are far more complex.

Complexity of ecological modelling will be determined by the questions asked in RA/ERA.

The potential full release resulting from an intended small (test) release has another striking consequence for RA/ERA: Limits of concern for possible risks have to be defined before first releases are authorised. Only then may intolerable adverse effects on a global (species)-wide level be prevented.

3.1.1 Molecular considerations

Currently, the most promising concept leading to the creation of an effective gene drive would be a global CRISPR gene drive. Synthetic CRISPR gene drives differ significantly in their concept and make-up from current GMOs released into the environment, in that they have a mutagenically active component integrated into their genome. Furthermore, in eukaryotic or complex organisms, the stable integration of CRISPR, a component of an anti-pathogenic system in bacteria, creates a high level of complexity, opening many questions on the molecular level. With regards to CRISPR/Cas as a genome editing tool, it is currently used in research as well as in the development of GM organisms for commercialisation. After application, the CRISPR/Cas system is intentionally removed from the organism to prevent unintended effects. Cas itself is an endonuclease, which are “restriction” enzymes that in their original context cleave foreign DNA in an organism, thus eliminating foreign DNA from outside. Stable insertion of an endonuclease into an organism might create toxic effects, a finding that is related to the question of why a defence system based on homing endonucleases is very successful in single-celled prokaryotes, but absent in multicellular eukaryotes. Data on the influence of a permanent, long term exposure of homing endonucleases (such as the CRISPR/Cas based gene drives) on eukaryotic genome stability is currently lacking.
3.1.2 Outcrossing and spreading

Spreading of the molecular construct is another important consideration in the ERA of organisms carrying a gene drive. The inheritance of the transgene is crucial for the functionality of any given synthetic gene drive, and therefore is a desired trait of an organism with a gene drive. Outcrossing of transgenes into closely related taxa is commonly assessed in RA of GMOs. Important for the evaluation of outcrossing is the likelihood of the event, but also the potential of the transgene to establish in feral or natural populations. Even with a high potential to cross into wild relatives, certain GM traits may not be advantageous or favoured by selection, and therefore are expected to disappear over time. For organisms carrying a global gene drive, the chance of transfer for a single transgene into a closely related wild relative is comparable to that of a GMO release, and the likelihood might even increase with the spreading of the gene drive into many organisms.

The important difference is that gene drives do not need to confer a selective advantage in order to spread. The likelihood of an unlimited spread of that given gene drive into the whole geographic range of that species is vastly increased. In the case of an intended population suppression gene drive, this could have fatal consequences for an entire species. Global gene drives, like CRISPR/Cas based systems, are sequence specific. For efficacy reasons, those gene drives will most likely be based on conserved gene sequences, thus increasing the risk of any outcrossing event becoming established. Therefore, any assessment of the outcrossing potential needs to take into consideration the DNA sequence space at the target site(s) of potential crossing partners of the desired species, in order to be capable of evaluating a given risk. This data is not even available for most target organisms currently discussed, and has to additionally be generated for all potential crossing partners of the potential crossing partners. Because of these facts, performing RA/ERA for gene drives is not possible, given currently available data.

3.1.3 Risk assessment of the intended effects

Population suppression using gene drive organisms will have ecological consequences for the entire ecosystem into which it is released. In a best-case scenario, e.g. rodents eradicated from islands, the gene drive will relieve the ecosystem of the target species (in this case invasive) and help to balance the existing ecosystem and strengthen ecological diversity, with little or no detrimental effects. In practice, however, effects on ecosystems have not proven to be so easily predictable, not even in rather simple examples such as the eradication of rabbits and cats from islands (Bergstrom et al. 2009).

Eradication of mosquitoes or weeds poses more complex scenarios, as such efforts will affect not only organisms when they are invasives, but organisms within their native environments. Data and knowledge about their roles in ecosystems is often lacking, but experts warn the likelihood of severe effects on ecosystems (Hochkirch et al. 2018). Evaluation of the impact of any given species’ demise relies on the interaction of the target species within the food web and its full ecological context. Besides being a pest or disease carrier, the target species may provide many positive ecosystem services such as: pollinator, food source (prey), predator, ecosystem builder.

As ecological effects are often long term, the proper problem formulation, data acquisition, modelling and/or practical testing will be both complex and demanding for any gene drive.

3.1.4 Risk assessment of the unintended effects through escape

What happens if the gene drive “escapes” its geographic area of application? Eradicating invasive alien species from their non-native habitat has proven to be a difficult task. Using gene drives to solve this problem is a tempting quick-fix. But one has to carefully consider the fact that in cases where an

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35 In this example, the influence of eradicating invasive cats on rabbit populations (which were under biological control as well) was underestimated and resulted in an explosion of the rabbit population, causing substantial damage by herbivory.
invasive species might potentially be removed from say an island ecosystem where they are considered a problem, might then, under similar circumstances of spread, unwitting carriers, etc., be able to return to their native or other habitats, now carrying a deadly gene drive.

This point leads to one of the most pressing questions posed by developers and risk assessors: Is it even possible to efficiently contain a gene drive? Containment of gene drives is not trivial. Even using island ecosystems for rodents as an example cannot be considered sufficient protection against GDO spread. Alternatively, concepts for containment can also be based on the molecular design (e.g. high threshold, or ‘local’ CRISPR drives). But those concepts (once fully developed in the laboratory) have yet to be proven safe, which might be impossible considering the myriad of unforeseen effects that can occur in nature.

As population suppression is the most widely proposed application of gene drives today, escape scenarios will be a primary focus of RA/ERA. Evaluations for assessing escape are coupled to space (geographic ranges of populations and species and migration boundaries, unintended movements, etc.); but time is also an important factor. The self-perpetuating nature of gene drives does not allow predictions about the timely spread of a given synthetic construct. In fact, spread of the gene drive depends on migration and reproduction parameters of the target species and population, which can result in very different dynamics (i.e., fast and exponential, vs. slow and steady, as well as all mixtures). Alongside the intention that gene drives persist infinitely in the wild, or until the goal of suppression is reached, time becomes an important factor for RA, especially because, even with low likelihood, the probability of an escape event will increase with time.

Factors to consider in the risk assessment of escape scenarios are:

1. molecular design of the gene drive (global vs. local gene drive, specificity for a given population)
2. life history of the population and species
3. space (geographic characteristics) and time (infinite persistence) dynamics

3.2 Monitoring

Monitoring of GDOs has to be able to identify and detect a given gene drive in the wild. Due to its molecular mechanisms, a simple detection might not be sufficiently able to determine whether a gene drive is active. CRISPR gene drives harbour a complex copy/paste mechanism, which is error prone. Fragments of inactive gene drives can nonetheless be inherited and thus be detected in monitoring approaches. An in-depth molecular characterisation, potentially by sequencing, might be needed to monitor active gene drives.

A second layer of monitoring could aim at detecting gene drives that have outcrossed in untargeted populations and species. For this task, sampling and molecular characterisation has to extend well beyond the target population and species.

Finally, monitoring has to be able to detect the effects on the environment that are caused intentionally and unintentionally by the gene drive and the GDOs. Those effects also have to be investigated, even if a gene drive has already vanished (due to failure or success). Hence monitoring will need to be complex and long-term.

Should a GDO be released (intentionally or unintentionally), early and efficient monitoring would be crucial, as risk management procedures are aggravated due to the intrinsic properties of gene drives, that is, the spread of a GM trait independent of time and space.

As there is a strong call for not releasing gene drives unless they can be reversed, recalled or overwritten, monitoring will also need to be able to assess the effectiveness of such counter-measures as well as to monitor for environmental impacts of these measures.
4 Conclusions

In this chapter we have addressed numerous points linked to the application of synthetic gene drives and their potential negative impacts. We have carefully explained why categorisation based on the different areas of intended application (agriculture, health etc.) and desired or claimed benefits, which so often is used as the starting point for introducing the topic of gene drives, is problematic and often misguided. We believe it must be stringently examined, and the possibility seriously considered that this technology may not be desirable, for either ecosystems or its stated purposes. Given the potentially severe and undeniable negative impacts that can arise from the release of gene drive organisms, we find it inappropriate to be guided by the excitement of technical advances or the lure of benefits only. We have noted that it is often the case that the underlying causes of the problems gene drives are intended to solve have actually been self-created by human practices and activities, or could be addressed by less problematic means; yet the necessary political or economic support has not been available. As we have illustrated in the case studies, modern agriculture is vulnerable to pests in large part because of the biological and genetic simplification involved in industrial agricultural practices, which also harm the natural predators of pests through pesticides and by limiting habitat. More diverse farming systems based on agroecology collectively present substantial defences against pests.

In our approach, we have thus placed the organism itself and the ecosystems linked to it centre stage. Understanding the full biology of an organism, including its genetic diversity, its mating behaviour, speed of dispersal, feeding patterns and sources, its place in the foodweb, its role in the ecosystem and its ecological value, are all essential for understanding the hazards, and for identifying the negative consequences that may arise from the release of a GDO. This also requires detailed knowledge of the respective ecosystems and their many complexities.

To illustrate and investigate this more closely, we have provided three case studies focusing on taxonomic categories, namely, mosquitoes, mice and Palmer amaranth, all of which highlight the lack of sufficient knowledge and understanding of the organism, its behaviour in the wild and its roles and functions in the ecosystems associated with it. Whilst the data are insufficient and the complexities too intricate to currently (if ever) allow for clear and reliable predictions of the outcomes and the impacts from a release of invasive gene drives, this is additionally confounded by the inability to identify and address various concerns. Such concerns are among others: how the wild populations will behave in response to the gene drive (e.g. altered mating behaviour, unintended behavioural effects due to the modifications); how the gene drive will behave at the molecular level within the wild populations and under real life conditions; whether the gene drive will fail to work, either at once or gradually, causing unpredictable population rebounds and changes; which genetic modifications will arise in response to the presence of active CRISPR/Cas9 elements in the genome; and how, in turn, to predict the consequences of these. We also discussed the possible spread of the engineered gene drives into closely related species, the consequences of which would additionally need to be addressed in any risk assessment.

Given the high level of unpredictabilities, the lack of knowledge and the potentially severe negative impacts on biodiversity and ecosystems, including agroecosystems, we recommend that there should be no intentional releases into the environment (including experimental releases) of GDOs; and that such releases should only be considered if and when it is demonstrated that there is full knowledge and understanding that allows for robust and reliable performance and risk assessments that can verify that no serious or irreversible negative impacts will arise as a consequence of the release of gene drives and also that there are no other, possibly safer options for dealing with the problem (and its underlying causes) that each drive is intended to solve.
This places the search for, development and support of other sustainable approaches high on the agenda. As shown for example in our agricultural case studies for Palmer amaranth (pigweed), systems-based, agroecological approaches have been shown to provide substantial control. Applying gene drive technology to Palmer amaranth, only recently considered a major pest due to industrial and especially herbicide practices which are largely linked to GMO crops, could put its useful and nutritious related species at risk. And in fact, Palmer amaranth itself has traits and food qualities that would be lost if it was driven to extinction. At present, CRISPR/Cas-based homing gene drives have not been shown to be viable for plants, due at least in part to the fact that the levels of homology directed repair in plants are too low for gene drives to spread. Therefore any potential use of gene drives in plants remains highly speculative at this point.

Similarly, insect pests are more of a threat in simplified industrial agroecosystems. Most of the current insect pests under consideration or mentioned as possible targets for gene drives are invasive. Invasive species may be less susceptible to control by natural pest enemies in the geographies where they have spread. But introduced biocontrol agents like parasitoid wasps to control such pests also depend on suitable habitat that may not be adequately provided in simplified industrial systems. This is yet another reason why more diverse agroecosystems are needed. Of course, it takes time to find and test new biocontrol agents and methods that often prove effective against established invasive insects over time. Therefore, these methods need time and investment to develop, rather than a precipitous resort to gene drives. In fact, gene drives themselves take time to develop and test for safety, and therefore have no obvious advantage in that respect. Furthermore, at this stage, gene drives in pest insects have not been shown to be reliable in the environment or safe for a variety of alarming possibilities, including spread throughout the species or even to other species.

It must also be emphasised that the history of risk assessment (as exemplified by herbicide resistant GMOs leading to extremely problematic resistant weeds like Palmer amaranth, harm to monarch butterflies, or neonicotinoid insecticide harm to non-target invertebrates), raises serious doubts about whether risk assessment authorities are equipped to adequately evaluate the risks of gene drives. In the case of neonicotinoids, several types of sub-lethal effects have caused widespread harm at the population level and at multiple trophic levels. These kinds of complex problems, even if different in specifics, will be difficult for risk assessment to evaluate in gene drives.

The case study on gene drive mice also revealed the unpredictability and limits of this technology. It highlights that even if gene drives were only ever used for eradication on islands, there would be serious risks from (stow-away) gene drive mice unintentionally ending up in and decimating mainland wild populations. It also showed that the deliberate use of a gene drive in mainland populations is likely, given that a major motivation for developing gene drive mice is the intention to eradicate pest populations that cause economic damage to crops, seed and feed. We pose the questions: Were it possible to make gene drives work reliably in small mammals, would they be seen as the next level of pest control, potentially going as far as wide-scale eradication? And, given the economic pressures involved, would it be possible to control the use of these tools against mice and other vertebrate ‘pests’?

In considering mosquitoes, we draw attention to the complex web of relationships with other species, and potential of severe knock on effects to ecosystems of suppressing mosquito species. Also important is the very real possibility that gene drives will not achieve their intended results in terms of human health: the behaviour of synthetic gene drives in the wild is difficult to predict but population rebounds are one very plausible outcome; similarly the interaction of drives with the many evolutionary forces at play is extremely difficult to foresee. However, if gene drives do achieve population suppression, even temporarily, the ecological implications could be profound. The high level of uncertainties and unpredictabilities is further confounded by the multitude of scenarios arising from the wide spectrum of possible performance and behaviour of gene
drive technologies and gene drive mosquitoes in real life – also raising concerns regarding negative implications for human health. We further raise the question of how many species may eventually be targeted, as there are at least 160 known for being vectors of human diseases, and 40-70 of these transmit Malaria. Setting aside their individual roles and functions in the ecosystems, how much of the total mosquito biomass would these species represent in particular ecosystems? An important concern is that the vectors targeted by gene drives may be among the more abundant species in some contexts. Given the large numbers of mosquito species involved in human disease transmission, mosquito gene drives could eventually be employed against numerous species representing a significant proportion of the total mosquito population, escalating and broadening the likely ecological consequences, including negative impacts on species that depend on mosquitoes for food.

We have also covered the aspect of dual use, the use of gene drive technology for military and harmful purposes. This in particular needs urgent attention.

The range of organisms intended as gene drive targets is broad and continuously growing. As discussed, the intention is to make the technology widely applicable for small mammals and for a wide range of insects, which we regard as alarming, both as an approach to dealing with problems, as well as with regards to the impacts of such practices.

In conclusion: in terms of the science and current knowledge, we cannot see how to make the release of gene drive organisms safe, or even how to perform an adequate and robust risk assessment that would cover all the points we have raised and that we regard as essential to safeguard biodiversity as well as human health. For the present, the strict application of the Precautionary Principle might be our best guide in terms of this new and potent technology.
for references for the tables and case studies see sections below


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**Table references**

**References Table 2a**


References Table 2b


References Table 2c


References for geographic range of target species


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Where no source is specified information and maps are from Wikipedia or Wikimedia Commons. Sources for the maps are as follows:

**Mus musculus**
Osado, (corresponds to IUCN Red List data though source not cited) https://commons.wikimedia.org/w/index.php?curid=9482429

**Peromyscus leucopus**
Izvora, corresponds to IUCN Red List data though source not cited https://commons.wikimedia.org/wiki/File:Peromyscus_leucopus_range_map.png

**Rattus norvegicus,**
Oknavezad, does not correspond well to IUCN data, source not cited https://upload.wikimedia.org/wikipedia/commons/archive/2/20/20190402152405%21Brown_rat_distribution.png

**Rattus rattus**
Rikiraru123, corresponds to IUCN Red List data though source not cited https://commons.wikimedia.org/wiki/File:Rattus_rattus.png

**Sturnus vulgaris**

**Trichosurus vulpecula,**
Chermundy using the IUCN Red List spatial data: IUCN Red List of Threatened Species, species assessors and the authors of the spatial data. https://commons.wikimedia.org/wiki/File:Common_Brushtail_Possum_area.png

**Mustela ermine,**
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