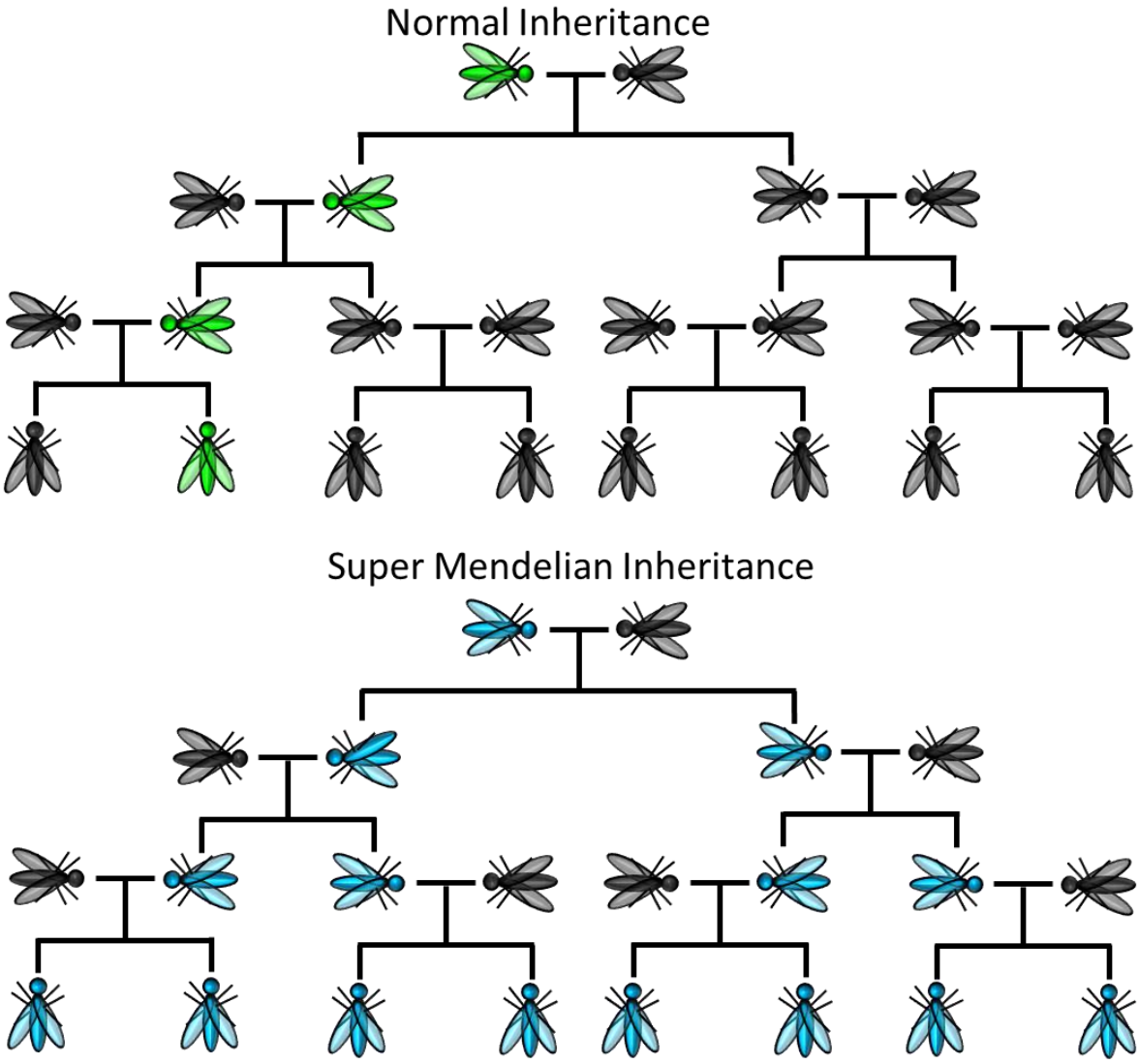
GDRA – Gene Drive Risk Assessment



Final Project Report:

August 2021

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Titelbild: Schemes of mendelian and supermendelian inheritance. Adapted from Esvelt et al. (2014)** by Johannes L. Frieß

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Abbreviation index

BAFU	(German) Bundesamt für Umwelt (Federal Office for Environment)
BfN	(German) Bundesamt für Naturschutz (Federal Agency for Nature Conservation)
BOKU	University of Natural Resources and Life Sciences, Vienna
Cas	CRISPR-associated (protein)
CBD	Convention on Biological Diversity
CRISPR	Clustered Regularly Interspaced Palindromic Repeats
CSM	Case-specific monitoring
EAA	Environment Agency Austria (German: Umweltbundesamt Wien: UBA)
EAS	Ecological Area Sampling (German: Ökologische Flächenstichprobe: ÖFS)
ERA	Ecological Risk Assessment / Environmental Risk Assessment
FFH	Fauna Flora Habitat
FOEN	Federal Office for the Environment (Switzerland)
GD	Gene Drive
GDO	Gene Drive Organism
GMO	Genetically Modified Organism
GS	General Surveillance
GTA	Gene Technology Act
HDR	Homology-Directed Repair
HEG	Homing Endonuclease Gene
HNV	High Nature Value
Medea	Maternal Effect Dominant Embryonic Arrest
MMEJ	Microhomology Mediated End Joining
MON 810	Genetically modified maize variety produced by Monsanto expressing Bt protein Cry1Ab
NHEJ	Non-Homologous End Joining
NNE	(German) Nationales Naturerbe (National Natural Heritage Sites)
ÖSM	(German) Ökosystem-Monitoring (ecosystem monitoring)
OTUS	Operational taxonomic units
PAM	Protospacer Adjacent Motif
PMEM	Post-market environmental monitoring
R	Resistance gene
RNA	Ribonucleid Acid
sgRNA	Single Guide RNA
TMD	(German) Tagfalter-Monitoring Deutschland
TO	Target organism.
UD	Underdominance

Executive Summary

Aim of the Study

The intended spread of genomic modifications into populations of wild organisms by synthetic gene drives represents a significant qualitative advancement in the GMO definition, as it expands the range of its functionalities to include potentially far-reaching spatial and temporal effects. It is therefore questionable whether the existing approaches for risk assessment can also be applied to gene drive-carrying organisms (GDOs) without neglecting essential risk-relevant properties. In order to be able to adapt and extend the risk assessment, sufficient knowledge must be available on the properties and possible applications of the different gene drive (GD) systems, their potential effects in exposed ecosystems, and methods for estimating their spread and subsequent effects. The present study aims to provide the scientific basis for adapting the risk assessment and monitoring of GDOs. Its analyses are therefore devoted to the following main topics:

a) a characterisation of existing GD approaches, their effectiveness, ways to control or limit them, and risk mitigation strategies,

b) an investigation of the possibilities for modelling GDs,

c) an investigation and evaluation of potential ecological and conservation impacts and the methods for estimating them,

d) an investigation of the requirements for monitoring released GDOs,
and as an initial scoping

e) a description of the legal framework for the release of GDOs.

Technical Characterisation of Gene Drives

A GD is a naturally occurring phenomenon known in population genetics in which a gene or group of genes is inherited with a probability that exceeds the 50% limit of the Mendelian inheritance rate. Therefore, a GD can spread a particular trait very rapidly within a population and may even cause its permanent presence in the population. GDOs have raised great expectations in public health, conservation, and agriculture, but also serious concerns. These are based primarily on the inherent ability of gene drives to spread and alter natural populations with great efficacy. This represents a paradigm shift for the release of genetically modified organisms (GMOs), as this new technology aims to spread into wild populations. Due to its inherently invasive nature, once released, a GD represents a significant irreversible intervention into ecosystems by actively altering the gene pool of natural populations and genetically modifying them itself.

The discovery of naturally occurring mechanisms that trigger super-mendelian inheritance of certain traits within a population was the starting point for the development and application of artificially created GDs. Many naturally occurring mechanisms exist that possess this remarkable property, such as transposable elements, meiotic segregation distorter genes, homing endonuclease genes, and Wolbachia bacteria. Some GDs secure their super-mendelian inheritance rather passively through a selection process, so that only offspring carrying the genetic information of the drive survive or are fertile. Others actively overcome the constraints of the mendelian inheritance pattern by affecting allelic segregation, i.e., fragmenting chromosomes, which can lead to altered sex ratios, for example. Active drives can also copy their genetic information between homologous chromosomes, resulting in homozygous offspring. Generally, a GD needs several generations to establish itself in a population. In the process, it can change over time through mutations. GDs not only affect the environment, but the environment also affects

the GDs. A laboratory-produced GD, once released, is confronted with evolutionary processes.

GDs can also be distinguished by their dispersal dynamics between self-limiting and self-maintaining techniques and between threshold-dependent and -independent (equivalent to local and global systems). Threshold refers to the proportion of GDO in a population above which their percentage increases over time.

Furthermore, we can distinguish between "modification drives," which aim to spread new traits, and "suppression drives," which aim to reduce or even regionally eradicate pest species or vectors of pathogens. Suppression drives are envisaged to strongly reduce the number of some prime vector mosquito species for infectious diseases like malaria and dengue. In addition, they are also being considered to decimate various invasive species that have become agricultural pests, such as the cherry fruit fly *Drosophila suzukii* in California or rodents like mice or rats in New Zealand which pose a severe threat to agriculture and the native environment. GDO are seen as a highly specific substitute for pesticides. This new technology is expected to provide far more targeted control of pests, invasive species or disease vectors than the use of chemicals. In addition to population suppression or eradication drives, 'modification drives' are also being developed to make, for example, mosquitoes resistant to the pathogens they transmit. In the case of the cherry vinegar fly, modification of the hard, serrated ovipositor would halt the agricultural damage caused by the egg-laying process.

A comparative technology characterisation of GDs revealed differences in the power and range, which correspondingly lead to different risk potential. For example, GDs may employ different mechanisms to ensure their mode of inheritance. From more or less sophisticated toxin-antidote systems such as Medea, Underdominance or Killer-Rescue to influenced segregation of sex chromosomes during meiosis (X-Shredder, Y-CHOPE). Extreme potential in terms of its power and range was found for homing endonuclease gene (HEG) based GDs using the CRISPR/Cas9 system. However, with this drive, as with some other GD techniques, the probability of failure is comparatively high. With increasing power and range of GDOs, uncertainty and lack of knowledge about their dynamics and potential impact also increase as the releases progress. Moreover, the inherent instability of genetic information becomes more relevant as the number of GD-modified organisms increases.

Studying naturally occurring drive systems can help understand the population genetics of synthetic GDs. Many naturally occurring GD systems, such as the natural Medea element, the t-haplotype, and many mechanisms that bias sex ratios in populations of mosquitoes and flies, are now known. It can be considered likely that a number of adaptations hinder the efficiency of drives in nature. Unfortunately, these are difficult to predict using modeling approaches, further increasing uncertainty about the fate of a synthetic GD in the wild.

Options for Control of Gene Drives

At the current stage of development, the dynamics and ecological consequences of a GD could hardly be retrieved post-release. Potential impacts of GD applications are complex and investigation into them is still in its infancy.

In recent years, a number of options to ensure control or even a kind of functional reversibility have been proposed for GDs. However, a proof of concept for their potential functionality, reliability and feasibility under the conditions of a release is still missing. A high exposure to GDOs presumably increases the possibility of unforeseen interactions in the environment significantly, and concomitantly increases the dimensions of ignorance about possible adverse effects. Thus, especially in anticipation of environmental release,

as a precautionary approach, it is advisable to primarily focus on strategies to limit or control the exposure potential of released GDOs. GDO spread could be controlled either by intrinsic molecular limitation mechanisms or by secondary release of specific organisms, GDOs, or chemicals.

Intrinsic containment refers to a concept wherein the GD-constructs or GDOs are dependent on synthetic substances or limited in spread due to their specific technical organization. The intrinsic containment of a GDO may either be linked to the reproductive incompatibility of the target species with wild type strains and related species or caused by the specific character of the GD. For instance, in case of HEG-drives the latter may arise due to a target sequence, only present in the genome of the target population. Accordingly, it is possible to differentiate between reproductive and molecular confinement as variants of intrinsic containment. All design variants of CRISPR drives with reduced risk potential are as yet insufficiently characterized with regard to their reliability under field conditions which inhibits reliable statements on their performance with regard to releases.

Secondary releases, such as overwriting drives, a guide RNA targeting the sequence of a released drive, or the release of sterile mating partners or wild type organisms (to reduce the proportion of GDOs within a population below the threshold of GDO) must be potent enough to cover all parts of a population and all populations affected by the primary released drive. Thus, it must be ensured that mutations or fitness losses do not interfere and reduce their efficiency. Evidence of the efficacy of secondary release options under more realistic conditions is still pending. Given the lack of reliable control options, the diversity of possible effects, and the high exposure potential of GDs, a precautionary approach that does not preclude screening of alternative techniques associated with potentially lower risks, uncertainties, and non-knowledge is indispensable.

The Prospective Assessment of Gene Drives Releases

Modelling can be useful in risk assessment to provide a basis for decision making. Depending on the design, models can help to represent and more concretely estimate the exposure and hazard potential of synthetic GDs. In order to reliably evaluate the efficacy and spatial and temporal spread of a given GD, application scenario modelling has become a helpful common method. For this purpose, in order to design a modeling approach that is as close as possible to the real-world conditions of a GD release, it is necessary to collect a set of data. These data can be divided into three main categories: 1) data specific to the GD system, 2) data specific to the target organism (TO) and 3) data specific to the environmental conditions of the corresponding ecosystems. In this study, a set of relevant criteria was identified for each categoryand it was investigated whether the respective data for the criteria are available. It was found that general statements about data availability are not possible because some data are available, but others are not available for the same criteria, but for a different technical design (GD type) or application context (e.g., different target organisms or ecosystems). In particular, GD-relevant ecological data are scarce, if available at all, due to the complexity of ecosystems.

Notwithstanding the incomplete data, the study reviewed 90 publications on models to examine the current state of development and applicability of models for GD risk assessment. Although some models are quite advanced in that they attempt to incorporate a high degree of realism, a comparison with environmental risk assessment (ERA) requirements shows that none of the identified models currently meet all ERA requirements. Nevertheless, four models were identified that have the potential to contribute to an ERA for released GDO in the future.

The model by North et al. (2019a) is a spatial, stochastic or deterministic agent-based simulation, which covers a large geographic area. It is directed at the life history of malaria

vectors *Anopheles gambiae* and *A. colluzzi*. The life history is implemented as well as abiotic factors such as the regional seasonality and perennial and non-perennial water bodies as breeding sites for the target organism. Biotic factors, such as larval competition which acts as the density dependent carrying capacity, migration, aestivation and long-distance migration are also considered. These models take into account to a comparatively high degree the biological characteristics of the target organisms and, to some extent, a spectrum of climatic and geographic conditions, albeit still quite limited.

Overall, the analysis of the current state of GMO modeling has shown that while some biotic traits associated with GMOs are considered, with the exception of interactions with pathogens, there are no models that consider interactions between GMOs and non-target organisms. In addition, in light of the requirements of the ERA, it became clear that there is a lack of comprehensive ecological data, particularly with respect to interactions with other species, habitats, and ecosystems.

A Modeling Concept for Gene Drives

The properties of GD constructs are highly diverse, depending on details of their molecular construction. Additionally, GDs can encounter and impact a wide range of conceivable ecological and demographic situations. Moreover, this makes it very challenging to convey their relative predicted properties to all but highly expert audiences. Furthermore, for proposed GD approaches to be critically evaluated in terms of their relative strengths and weakness, including of the modelling approaches employed or parameters selected, it is essential to broaden the pool of potential stakeholders that have an understanding. To facilitate this, we developed a unified mathematical paradigm for describing the properties of a wide variety of single construct GDs. This framework provides an intuitive and objective way to evaluate the properties and robustness of many GD approaches in terms of their expected end points. It is implemented within a user-friendly open source App called DrMxR – Drive Mixer, with expanding documentation including case studies. The framework provides the capacity to easily vary key drive parameters as a means to assess the sensitivity of parameter combinations and also as a means to identify assumptions that underlie published models (which are often not explicitly stated). Crucially, within this common framework, it is possible to recapitulate key published results derived using bespoke modelling frameworks. A user can choose the driving factor for the GD and its corresponding effect on the biology of the target organism. For the framework, we identified three factors responsible for the propagation of GD in the presence of an organismal fitness cost. These forces act during different stages of target organism's lifecycle and relate the gene driving mechanism with the organism's biology. Such a type of approach is arguably missing in earlier works on GD. The modelling approach also provides a classification of drives based on the biology of how the drive is designed (out of the three constituent forces) and avoids unnecessarily new and confusing terminology.

As case studies of our unified approach, the results of various GDs such as CRISPR homing endonuclease drive, Medea, Underdominance, Inverse Medea and Semele were recovered. Our result on the spatial model reveals that the inclusion of non-panmictic dynamics changes the invasion and fixation condition of the GD relative to the mixed population model. Flexibility to see the combined effect for various evolutionary factors influencing the spread of GD on the population dynamics is an essential feature of the DrMxR. In addition, a drive resistance allele was added to the model. With this extension, it is also possible to simulate the complexity of resistance evolution against GDs.

The framework is not intended to remove the need for continued bespoke modelling efforts or existing vocabularies, it can however provide a means to further expand the, explicit or intuitive, understanding of GD in the context of risk assessment, informing policies, and

enhancing public participation concerning potential application of proposed and future GD approaches.

Next, we extend our modeling framework to analyze the effects of three ecological factors on the population dynamics of gene drives. These are mate choice, mating systems, and mating networks. Apart from genetic resistance itself, these represent some of the complex mating conditions that the target population will face in the wild. We analyze and compare the results of two gene drive systems (distortion-based and viability-based gene drives) and quantify the negative effect of mate choice between the wild type and transgenics on the spread of the gene drive. Inefficient drive and fitness costs due to drive payload were found to exacerbate the situation, and the predicted threshold-dependent release is drastically different from the case where there is no mate choice bias. At higher levels of polygamy, the GD spread much faster, but the associated fitness costs reduced its rate of spread. Considering a finite population network model allowed us to understand the expected impacts of releasing the gene drive. Gene drive dispersal is faster and more effective when individuals have fewer connections in the mating network. The results highlight the need to consider various population-level ecological influences when modelling the spread of Gene drives. Such an analysis can better predict the threshold for release and the time frame for the spread of gene drives. Such analyses must be conducted before field trials can be considered.

Assessment of Ecological and Nature Conservation Effects

The main goal of this study part was to evaluate potential adverse effects the release of GDOs poses on the ecosystem and biodiversity. Therefore, current approaches to define and assess risk were reviewed and proposals were developed on how GDOs can be integrated into GMO risk assessment. The task was divided into three parts, (i) reviewing approaches to define protection goals, (ii) Evaluate ways to use and adapt Environmental Risk Assessment (ERA) for GDOs, including application to two case studies, and (iii) exploring the potential of ecological modelling as a tool used in ERA of GDOs. Finally, we assessed the extent to which the current ERA paradigm is applicable to the case of GDOs.

The definition of general protection goals is relatively straight forward and can be derived from legal documents of international, European, and national treaties. Based on the analysis of all the relevant agreements, there are two general goals: biodiversity and human well-being. More difficult is the identification of *measurable* specific protection goals, needed for ERA. Because the link between biodiversity and human wellbeing can be explained well by the ecosystem service concept, the recent tendency to define specific protection goals goes towards using concrete ecosystem services to derive measurement endpoints. We criticize this tendency because i) although through the ecosystem service concept it can be argued that maintenance of all biodiversity is providing all the ecosystem services, it does not necessarily work the other way round; ii) ecosystem redundancy could be used to argue that a concrete species could be removed from the system without losing a specific service; iii) unknown cascading effects of species removal are not taken into account; iv) a slight but regular adverse (non-significant) effect over a short period of time might still sum up to a negative impact over longer periods. The latter argument questions the definition of harm used in ERA in general and does apply to all specific protection goals, e.g. population size of any species. We provide a simulation for a hypothetical example.

In the framework of current ERA, the problem formulation phase is playing a crucial role, as it is this phase, when important information is gathered to assess potential adverse effects of the stressor on the environment. However, GDOs resemble in many ways invasive species as they are designed to spread and how they influence the ecosystems. For this reason, the analogies between invasive species and GDOs are suitable.

The intentional or unintentional spread of invasive species illustrates that local containment of GDOs is unrealistic in a globalized world. Furthermore, experience from failed containment of biological control agents (e.g. rabbit hemorrhagic disease was introduced to New Zealand by farmers) indicates that GDOs would be unintentionally but also likely intentionally introduced to other regions. Therefore, GDOs have aspects of different approaches to risk assessment related to their impact on populations and risk of spread. Similar to invasive species, GDOs can alter biological interactions within an ecosystem, leading to cascading effects within and outside the ecosystem into which they were originally introduced. For example, known effects of predator eradication include mesopredator release, herbivore release, disruption of predator social systems, and compensatory immigration. These different aspects of GDOs are difficult to translate into a conceptual framework. Therefore, we identified three distinct areas of risk:

1) **The effect of population declines on ecosystem and ecosystem services. This includes effect on species interacting with the target species, other cascading ecological effects, and not desired effects related to population size development of the target species.**
2) **The risk of escape of the GDOs into other geographical regions, i.e. overcoming geographical barriers. This is mainly relevant for applications were GD should be restricted to parts of a global range of species.**
3) **The risk of transfer of the GD to non-target populations or other species by hybridization independent from geography.**

A conceptual model for risk assessment of GDOs was developed, based on the analogies to invasive species and the fields of risk. As a GD application is as much a political and socio-economic as an ecological endeavor, we included also socio-economic and ethical aspects. With the model five basic-, however, interconnected pathways acting in feedback loops were identified: (1) the direct effect of the GDO in the target area on the wild type (intended effect), (2) the effect of the reduced population size on the ecosystem and on ecosystem services within the target area, (3) the effect on the population size and following ecological effects and effects on ecosystem services in the non-target area – here, a feedback between population size and establishment is expected, (4) the escape including all mechanisms to accidently overcome the restrictions of the drive, and finally (5) the effect of (1) and (2) but also (4) and (5) on socioeconomy and ethics including the resulting effect on the acceptance of the GD technique and the management target.

Further analysis of the conceptual model applied to two case studies showed that many of the data needed are lacking and that much of a potential risk assessment would have to be performed with high uncertainty. In addition, many of the processes are insufficiently understood. Ecological modelling could help to increase the understanding of processes but by no means can be a substitute for lacking data. The notion that modeling could be used instead of field studies must be dismissed, as well as the idea that ecological models could provide precise and unbiased predictions for measurement endpoints, i.e. specific protection goals.

Finally, the applicability of the current ERA paradigm to GDOs is discussed. We argue that GDOs do bring a new quality, because of the range and combination of ecological effects they can have: deliberate eradication of a species in the target area, unintended escape to non-target areas and or other species. We already outlined the impact of additive small effects and the inability to reliably take negligible effects into consideration. Given the ongoing biodiversity crisis, any ERA framework should account for ecological effects that may not be obvious but may cause harm in the long run, regardless of the applied technique. We do not think that this is the case in any of the current frameworks. However, when the removal of a species constitutes a potential hazard and the probability that the hazard causing ecological harm constitutes risk, the risk will increase with each application

of a suppression drive within the species, geographical area, areas into which transport occurs, or any escape scenario imaginable.

Gene Drive Monitoring

Before a release of a GD for testing purposes or even a large-scale release can be considered, an appropriate monitoring plan with study hypotheses and suitable indicators must be implemented in order to be able to observe and detect possible unintended impacts on the environment and human health in the first place.

The aim of the monitoring part of the study was to identify and compile all the characteristics and unique features of a GDO compared to a GMO, in order to identify and specify the specific requirements for GDO monitoring and the limits of monitoring and control of possible - in the worst case global - ecological impacts by a GDO. Based on these findings, recommendations are made for a future monitoring approach for GDOs.

Monitoring of GDOs should consider both approaches, case-specific monitoring and general surveillance. In addition, it should be able to identify (a) exposure and (b) adverse effects (hazards) on the environment. For the development of a monitoring system to determine the ecological impact of a GDO on the environment, a checklist of all relevant characteristics and parameters of a GDO that need to be taken into account is provided in order to present the requirements for a GDO monitoring system, as comprehensively as possible. Several characteristics of GDOs, such as their application in natural systems, their temporal and regional indefiniteness, and the broad effectiveness of GDOs, pose particular challenges for the design of a functional monitoring system. However, there is still a lack of sufficient fundamental knowledge to design appropriate monitoring plans. Therefore, it is not yet possible to design and implement adequate monitoring to observe the invasive behavior of GDOs. Furthermore, given the ability of GDOs to spread within and between populations through genetic exchange, monitoring of GDOs will be at the molecular level. Thus, there is a need for metagenomics aproaches. Existing national and international monitoring approaches and programs can currently only provide a starting point for GDO monitoring, such as a baseline study to detect impacts of GDOs on biodiversity, for example.

The monitoring procedures that are already mandatory for GMO monitoring must be incorporated into, or should form the basis of, the GDO monitoring program that is to be developed. Due to the potential global reach of impacts, it would be of utmost importance to establish future guidelines for the safest possible handling of GDOs and monitoring requirements using a globally uniform guideline in order to be able to ensure the comparability of global monitoring that is crucial in the case of GDOs. Existing guidelines for international regulations should be reviewed for their suitability. Comprehensive basic research on current developments in GD technologies and their ecological impact potential is needed for risk assessment and monitoring. Research on appropriate methods for monitoring GDO should be accelerated. If GDO releases were to actually occur, then sufficiently large budgets would need to be allocated to allow for long-term GDO monitoring including repeated monitoring runs, and to support the acquisition of basic knowledge to formulate risk hypotheses. However, monitoring only provides an observation system. Retrievability in the event of damage is not possible with monitoring alone.

Regulatory Framework for the Deliberate Release of Gene Drive Organisms

Various rules and standards at the national, European, and international levels are relevant to the deliberate release of GDOs. Most importantly, GDOs meet the definition of

GMOs under the European Biosafety Directive and the definition of living modified organisms (LMOs) under the Convention on Biological Diversity and its protocols mentioned below.

In addition, the German GMO Regulation implements the European Biosafety Framework at the Member State level. Therefore, the European GMO Regulation is of utmost importance for any deliberate release in the EU covering different biosafety aspects. The European Directive 2001/18/EC on the deliberate release of a GMO into the environment ensures that any deliberate release of a GMO requires an authorization through a governmental approval procedure based on an environmental risk assessment, emphasizing the importance of the precautionary principle. The Contained Use Directive regulates the biosafety of GDOs in the laboratory and establishes measures for contained use to ensure the protection of human health and the environment.

At the international level, there are rules and standards that are binding as international law, as well as the international treaties mentioned below. Because of its global recognition, the Convention on Biological Diversity is the most important international treaty that explicitly addresses the regulation of LMOs. It provides a binding international and near-universal general framework for the regulation of GDO that requires the assessment of risks and the establishment of appropriate risk management measures prior to a deliberate release.

In addition, the Cartagena Protocol, a binding international treaty and protocol to the Convention on Biological Diversity, contains specific provisions on how member states must proceed and conduct risk assessments for transboundary movements and deliberate releases of GDO, as well as on specific obligations related to risk management. Also of importance is the complementary Nagoya-Kuala Lumpur Protocol, the third binding international treaty in this area, which addresses the negative impacts on the conservation and sustainable use of biodiversity that could be caused by the transboundary movement of GDOs.

From a global trade law perspective, the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), a binding international treaty, provides a legal framework that states must follow when regulating the deliberate release of GMOs on their territory. Importantly, a zero-risk policy cannot be based on theoretical uncertainty regarding the risks of LMOs, an approach that differs slightly from that of the Cartagena Protocol.

From a general human rights perspective, binding universal human rights treaties (such as the International Covenant on Civil and Political Rights and the International Covenant on Economic, Social, and Cultural Rights) and regional human rights treaties (such as the Charter of Fundamental Rights of the European Union) are relevant because they set internationally legally binding standards for the regulation of biotechnology and include the right to scientific freedom, even if it is not explicitly mentioned.

From the perspective of customary international law, it is questionable whether the cross-border dissemination of GDO violates the obligation not to significantly affect the territory of another state. If this rule of international law is violated, the responsible state must make amends.

Finally, soft law and other guidelines such as the Codex Alimentarius are also relevant to the deliberate release of GDO. These have normative force even though they are not directly binding as law, but a violation of these rules does not make a state internationally responsible.

Conclusion

Synthetic gene drives represent a new quality of genetically modified organisms, as they can act independently to genetically modify wildlife and plants, or even eradicate individual species. Their spread and the wide range of potential ecological impacts, especially in the event of failure in the planned application process, can only be minimally assessed using current methods prior to potential releases. In addition, insufficient ecological data make it difficult to apply predictive approaches. For adequate environmental risk assessment of gene drive releases, greater consideration of less prominent and especially potentially additive effects is needed. How reliable strategies to control them spatially and temporally are cannot be adequately determined at this time. Existing monitoring concepts can only serve as a starting point or basic data reservoir for the development of optimized concepts. A reliable methodological basis for risk assessments and the monitoring of releases is thus by no means yet available. The consequences of gene drive releases cannot yet be predicted to the required extent and with sufficient reliability. The modeling software 'Drive Mixer' developed in this project can be used to improve the understanding of the properties of gene drives and to compare different GD approaches.

From a regulatory perspective, Gene Drives fall under existing international and national laws and treaties for genetically modified organisms, although there may be issues with recognition of damage in the event of transboundary spread and the respective impacts that a drive may cause in non-target regions.

However, if the technology path of gene drives is indeed pursued, precautionary risk management must find ways to adequately deal with a lack of knowledge to complete ignorance of potential negative impacts. In terms of the precautionary principle, the spread of Gene Drives, if it cannot be controlled, must be seen as a cause for great concern.

Zusammenfassung

Ziel der Studie

Die gezielte Verbreitung von genomischen Veränderungen in Populationen wildlebender Organismen durch synthetische Gene Drives stellt eine bedeutende qualitative Veränderung im Wesen von gentechnisch veränderten Organismen (GVO) dar, da sie das Spektrum der Funktionalitäten wesentlich erweitern und den Wirkungshorizont räumlich und zeitlich deutlich erweitern. Es ist daher fraglich, ob die bestehenden Ansätze zur Risikobewertung auch auf Gene Drive Organismen (GDOs) übertragen werden können, ohne wesentliche risikorelevante Eigenschaften zu vernachlässigen. Um die Risikobewertung anpassen und erweitern zu können, sollten ausreichende Kenntnisse über die Eigenschaften und Einsatzmöglichkeiten der verschiedenen Gene Drive (GD)-Systeme, ihre potenziellen Auswirkungen in exponierten Ökosystemen sowie Methoden zur Abschätzung ihrer Verbreitung und ihrer Folgewirkungen vorhanden sein. Die vorliegende Studie zielt darauf ab, die wissenschaftliche Grundlage als Basis für die Anpassung der Risikobewertung und Überwachung von GDO zu schaffen. Ihre Abschnitte sind daher den folgenden Hauptthemen gewidmet:

a) **einer Charakterisierung der bisherigen GD-Ansätze, ihrer Wirksamkeit, Möglichkeiten zur Kontrolle oder Begrenzung sowie Strategien zur Risikominderung,**
b) **eine Untersuchung der Möglichkeiten zur Modellierung von GD,**
c) **eine Untersuchung und Bewertung möglicher ökologischer Auswirkungen (inkl. ihrer Bedeutung für den Naturschutz) und der Methoden zu ihrer Abschätzung,**
d) **eine Untersuchung der Anforderungen an das Monitoring freigesetzter GDO,**
e) **und als erstes Scoping, eine Beschreibung des rechtlichen Rahmens für die Freisetzung von GDO.**

Technische Charakterisierung von Gene Drives

Ein GD ist ein in der Populationsgenetik bekanntes natürlich auftretendes Phänomen, bei dem ein Gen oder eine Gruppe von Genen mit einer Wahrscheinlichkeit vererbt wird, die die 50%-Grenze der Mendelschen Vererbungsrate überschreitet. Daher kann ein GD ein bestimmtes Merkmal sehr rasch innerhalb einer Population verbreiten und möglicherweise sogar seine dauerhafte Präsenz in der Population bewirken. GDO haben große Erwartungen im Bereich der öffentlichen Gesundheit, des Naturschutzes und der Landwirtschaft geweckt, ihre Anwendung im Rahmen von Freisetzungen ist jedoch auch mit großen Befürchtungen verbunden. Diese basieren vor allem auf der inhärenten Fähigkeit von GDs, sich auszubreiten und natürliche Populationen mit großer Wirksamkeit zu verändern. Der Fokus auf Wildpopulationen stellt einen Paradigmenwechsel für die Freisetzung gentechnisch veränderter Organismen dar. Aufgrund ihres inhärent invasiven Charakters kann die Freisetzung eines synthetischen GDs als erheblicher irreversibler Eingriff in Ökosysteme angesehen werden, da aktiv der Genpool von natürlichen Populationen verändert wird und die betroffenen Organismen in GVO (bzw. GDO) umgewandelt werden.

Die Entdeckung natürlich vorkommender Mechanismen, die eine supermendelsche Vererbung bestimmter Merkmale innerhalb einer Population auslösen, war der Ausgangspunkt für die Entwicklung und Anwendung künstlich geschaffener GDs. Es existieren viele natürliche Mechanismen, die über diese bemerkenswerte Eigenschaft verfügen, z. B. Transposons, meiotische Segregationsverzerrer-Gene, Homing-Endonuklease-Gene und *Wolbachia*-Bakterien. Einige GDs sichern ihre supermendelsche Vererbung eher passiv durch einen Selektionsprozess, so dass nur Nachkommen, die die genetische Information des Drives tragen, überleben oder fruchtbar

sind. Andere überwinden aktiv die Beschränkungen des Mendelschen Vererbungsmusters durch eine Beeinflussung der Allelsegregation, d. h. eine Fragmentierung der Chromosomen, was beispielsweise zu einem veränderten Geschlechterverhältnis führen kann. Aktive Drives können auch ihre genetische Information zwischen homologen Chromosomen kopieren, was zu homozygoten Nachkommen führt. Generell braucht ein GD mehrere Generationen, um sich in einer Population zu etablieren. Dabei kann er sich im Laufe der Zeit durch Mutationen verändern. GDs beeinflussen nicht nur die Umwelt, sondern die Umwelt beeinflusst auch die GDs. Ein im Labor hergestellter GD wird, sobald er freigesetzt wird, mit evolutionären Prozessen konfrontiert.

GDs können auch durch ihre Ausbreitungsdynamik zwischen selbstbegrenzenden und selbsterhaltenden Techniken und zwischen schwellenwertabhängigen und –unabhängigen (gleichbedeutend mit lokalen und globalen Systemen) unterschieden werden. Der Schwellenwert bezieht sich auf den Anteil von GDO in einer Population, ab dem ihr prozentualer Anteil sich im Laufe der Zeit erhöht.

Darüber hinaus kann zwischen "Modification Drives", die auf die Verbreitung neuer Merkmale abzielen, und "Suppression Drives", die den Fokus auf die Reduzierung oder sogar regionale Ausrottung von Schädlingsarten oder Vektoren von Krankheitserregern legen, unterschieden werden. Suppression Drives sollen die Populationsgrößen von Mückenarten reduzieren, die als Hauptüberträger für Infektionskrankheiten wie Malaria oder Dengue gelten. Darüber hinaus werden sie auch zur Dezimierung von verschiedenen invasiven Arten in Betracht gezogen, die zu landwirtschaftlichen Schädlingen geworden sind, wie etwa die Kirschessigfliege *Drosophila suzukii* in Kalifornien oder Nagetiere wie Mäuse oder Ratten in Neuseeland, die einerseits eine ernsthafte Bedrohung für die Landwirtschaft, andererseits sogar die einheimische Umwelt darstellen. GDO werden als Ersatz für Pestizide angesehen. Diese neue Technologie soll eine weitaus gezieltere Bekämpfung von Schädlingen, invasiven Arten und Krankheitsüberträgern ermöglichen, als sie mit chemischen Mitteln möglich ist. Neben den Drives zur Unterdrückung oder Ausrottung von Populationen werden auch ‚Modifikation Drives' entwickelt, um bspw. Stechmücken gegen die von ihnen übertragenen Erreger resistent zu machen. Für die Kirschessigfliege wurde vorgeschlagen, durch Modifikation ihres harten, gezahnten Legebohrers den durch den Eierablageprozess verursachten landwirtschaftlichen Schaden zu verringern.

Eine vergleichende Technikcharakterisierung von GDs ergab Unterschiede im Leistungsspektrum und in der Reichweite, die entsprechend zu einem unterschiedlichen Risikopotenzial führen. So können GDs beispielsweise unterschiedliche Mechanismen anwenden, um ihre Vererbungsweise zu gewährleisten. Von mehr oder weniger ausgeklügelten Toxin-Antidot-Systemen wie Medea, Underdominance oder Killer-Rescue bis hin zur beeinflussten Segregation der Geschlechtschromosomen während der Meiose (X-Shredder, Y-CHOPE). Ein extremes Potenzial in Bezug auf seine Wirkmächtigkeit und Reichweite konnte für Homing-Endonuklease-Gen (HEG) basierte GDs unter Verwendung des CRISPR/Cas9-Systems, festgestellt werden. Allerdings ist bei diesem Drive – wie auch bei einigen anderen GD-Techniken – die Versagenswahrscheinlichkeit vergleichsweise hoch. Mit der Leistung und der Reichweite von GDOs steigen die Unsicherheit und die Unkenntnis über ihre Dynamik und ihre potenziellen Auswirkungen im Verlauf der Freisetzungen. Darüber hinaus wird die inhärente Instabilität der genetischen Information mit zunehmender Anzahl GD-modifizierter Organismen immer relevanter.

Die Untersuchung natürlich vorkommender Drive-Systeme kann helfen, die Populationsgenetik synthetischer GDs zu verstehen. Viele natürliche GD-Systeme, wie das natürliche Medea-Element, der t-Haplotyp und viele Mechanismen, die die Geschlechterverhältnisse in Populationen von Mücken und Fliegen verzerren, sind heute

bekannt. Es kann als wahrscheinlich gelten, dass eine Reihe von Anpassungen die Effizienz von Drives in der Natur behindern. Leider lassen sich diese mit Modellierungsansätzen kaum vorhersagen, was die Ungewissheit über das Schicksal eines synthetischen GD in der freien Natur weiter erhöht.

Optionen für die Kontrolle von Gene Drives

Im gegenwärtigen Entwicklungsstadium könnten die Dynamik und die ökologischen Auswirkungen eines GD nach der Freisetzung kaum rückgängig gemacht werden. Die potenziellen Auswirkungen von GD-Anwendungen sind komplex und ihre Erforschung steht noch ganz am Anfang.

In den letzten Jahren wurde eine Reihe von Optionen vorgeschlagen, um die Kontrolle oder sogar eine Art funktionaler Reversibilität von GDs zu gewährleisten. Eine hohe Exposition gegenüber GDOs erhöht voraussichtlich die Möglichkeit unvorhergesehener Wechselwirkungen in der Umwelt deutlich und vergrößert damit auch das Ausmaß des Nichtwissens über mögliche schädliche Wirkungen. Daher ist es ratsam, sich vor allem im Vorfeld von GDO-Freisetzungen in die Umwelt vorsorglich auf Strategien zur Begrenzung oder die Kontrolle des Expositionspotenzials freigesetzter GDOs zu konzentrieren. Die Ausbreitung von GDOs könnte entweder durch intrinsische molekulare Begrenzungsmechanismen durch die sekundäre Freisetzung bestimmter wildtyp-Organismen, GDOs oder Chemikalien kontrolliert werden.

Die intrinsische Eindämmung (Containment) bezieht sich auf ein Konzept, bei dem die GD-Konstrukte oder GDOs von synthetischen Stoffen abhängig sind oder aufgrund ihrer spezifischen technischen Gestaltung in ihrer Ausbreitung beschränkt werden. Die intrinsische Eindämmung eines GDO kann entweder in der reproduktiven Inkompatibilität der Zielspezies mit Wildtyp-Stämmen und verwandten Spezies bestehen oder durch den spezifischen Charakter des GDO verursacht werden. Im Falle von HEG-Drives kann letzteres durch eine Zielsequenz verursacht werden, die nur im Genom der Zielpopulation vorhanden ist. Dementsprechend ist es möglich, zwischen reproduktiver und molekularer Eingrenzung (Confinement) als Varianten der intrinsischen Eindämmung zu unterscheiden. Alle Designvarianten von CRISPR-Drives mit reduziertem Risikopotenzial sind hinsichtlich ihrer Zuverlässigkeit unter Freilandbedingungen bisher noch ungenügend charakterisiert, was verlässliche Aussagen über ihre Leistungsfähigkeit in Bezug auf Freisetzungen verhindert.

Sekundäre Freisetzungen, wie das Überschreiben von Gene Drives, eine auf die Sequenz eines freigesetzten Drives abzielende guide-RNA, oder die Freisetzung von sterilen Paarungspartnern oder Wildtyp-Organismen (um den Anteil von GDOs innerhalb einer Population unter den für eine Ausbreitung des GD nötigen Schwellenwert zu senken) müssen potent genug sein, um alle Teile einer Population und alle Populationen, die von dem primär freigesetzten Drive betroffen sind, abzudecken. Es muss also auch sichergestellt werden, dass sie nicht durch Mutationen oder Fitnesseinbußen in ihrer Effizienz beeinträchtigt werden. Ein Nachweis der Wirksamkeit von Optionen für sekundäre Freisetzungen unter realistischeren Bedingungen steht noch aus. In Anbetracht des Mangels an zuverlässigen Kontrollmöglichkeiten, der Vielfalt möglicher Auswirkungen und des hohen Expositionspotenzials von GDs, ist ein vorsorgeorientierter Ansatz unabdingbar, der ein Screening alternativer Techniken nicht ausschließt, die mit potenziell geringeren Risiken, Unsicherheiten und Wissenslücken verbunden sind.

Die prospektive Bewertung von Gene Drive Freisetzungen

Modellierungen können bei der Risikobewertung hilfreich sein, um eine Grundlage für die Entscheidungsfindung zu gewinnen. Sie können dazu beitragen, die Exposition und das Gefährdungspotenzial synthetischer GDs darzustellen und konkreter abzuschätzen. Um die Wirksamkeit sowie die räumliche und zeitliche Ausbreitung eines bestimmten GD zuverlässig zu bewerten, haben sich Modellierungen von Freisetzungsszenarien als hilfreiche Methode etabliert. Zu diesem Zweck ist es zunächst notwendig, eine Reihe von Daten zu sammeln, um einen Modellierungsansatz zu entwickeln, der den realen Bedingungen einer GD-Freisetzung so nahe wie möglich kommt. Diese Daten können in drei Hauptkategorien unterteilt werden: 1) Daten, die spezifisch für das GD-System sind, 2) Daten, die spezifisch für den Zielorganismus (target organism, TO) sind und 3) Daten, die spezifisch für die Umweltbedingungen der entsprechenden Ökosysteme sind. In dieser Studie wurde für jede Kategorie eine Reihe relevanter Kriterien ermittelt und untersucht, ob die entsprechenden Daten für die Kriterien verfügbar sind. Es zeigte sich, dass allgemeine Aussagen über die Datenverfügbarkeit nicht möglich sind, da einige Daten verfügbar sind, andere jedoch nicht für dieselben Kriterien vorliegen, sondern für ein anderes technisches Design (GD-Typ) oder einen anderen Anwendungskontext (z. B. andere Zielorganismen oder Ökosysteme). Insbesondere GD-relevante ökologische Daten sind aufgrund der Komplexität von Ökosystemen nur mangelhaft, wenn überhaupt, vorhanden.

Es wurden in einem weiteren Schritt 90 Veröffentlichungen zu Modellen von GD ausgewertet, um den aktuellen Entwicklungsstand und die Anwendbarkeit von Modellen für die GD-Risikobewertung zu untersuchen. Obwohl einige Modelle insofern recht fortschrittlich sind, als sie versuchen, einen hohen Grad an Realismus einzubeziehen, zeigt ein Vergleich mit den Anforderungen der Umweltrisikobewertung, dass derzeit keines der identifizierten Modelle alle Anforderungen der Umweltrisikobewertung erfüllt. Dennoch wurden vier Modelle identifiziert, die das Potenzial haben, in Zukunft zu einer Umweltrisikobewertung für freigesetzte GDO beizutragen. Diese Modelle berücksichtigen in vergleichsweise hohem Umfang die biologischen Eigenschaften der Zielorganismen und zum Teil auch ein – wenn auch noch recht beschränktes – Spektrum von klimatischen und geografischen Bedingungen.

Insgesamt hat die Analyse des derzeitigen Stands der GDO-Modellierung gezeigt, dass zwar mit Ausnahme der Wechselwirkungen mit Pathogenen einige biotische Merkmale im Zusammenhang mit GDO berücksichtigt werden, es aber keine Modelle gibt, die Wechselwirkungen zwischen GDO und Nichtzielorganismen berücksichtigen. Darüber hinaus wurde angesichts der Anforderungen einer Umweltrisikobewertung deutlich, dass es an umfassenden ökologischen Daten mangelt, insbesondere im Hinblick auf die Wechselwirkungen mit anderen Arten, Lebensräumen und Ökosystemen.

Ein Modellierungskonzept für Gene Drives

Die Eigenschaften von GD-Konstrukten sind sehr vielfältig und hängen von den Details ihrer molekularen Konstruktion ab. Darüber hinaus können GDs in einer Vielzahl von denkbaren ökologischen und demografischen Situationen Effekte zeigen. Es erweist sich darüber hinaus als sehr schwierig, ihre aktuell bekannten Eigenschaften und Wirkweisen Laien zu vermitteln. Damit vorgeschlagene GD-Ansätze im Hinblick auf ihre relativen Stärken und Schwächen, einschließlich der verwendeten Modellierungsansätze oder der gewählten Parameter, von breiten gesellschaftlichen Kreisen kritisch bewertet werden können, ist es jedoch unerlässlich, ihnen die Möglichkeit zu geben, ein ausreichendes Verständnis der Eigenschaften und des Verhaltens von GDs zu erlangen. Um dies zu erleichtern, haben wir einen einheitlichen mathematischen Ansatz zur Beschreibung der Eigenschaften einer Vielzahl von GDs entwickelt. Diese Methodik bietet eine intuitive und

objektive Möglichkeit, die Eigenschaften und die Robustheit vieler GD-Ansätze im Hinblick auf ihre Einsatzziele zu bewerten. Der Algorithmus ist in einer benutzerfreundlichen Open-Source-App namens DrMxR - Drive Mixer implementiert und mit einer ausführlichen Dokumentation inklusive Fallstudien ausgestattet. Das Modell bietet die Möglichkeit, die wichtigsten Drive-Parameter auf einfache Weise zu variieren, um die Empfindlichkeit von Parameterkombinationen zu bewerten und die Annahmen zu ermitteln, die den veröffentlichten Modellen zugrunde liegen (und oft nicht explizit angegeben sind). Entscheidend ist, dass es innerhalb dieser einheitlichen Methodik möglich ist, bereits veröffentlichte Studien zu GDs nachzuvollziehen, die mit maßgeschneiderten Modellierungsalgorithmen erarbeitet wurden. Der Benutzer kann den maßgeblichen Faktor für die GD-Systeme und die entsprechenden Auswirkungen auf die Biologie des Zielorganismus auswählen. Für DrMxR haben wir drei Faktoren identifiziert, die für die Ausbreitung von fitnessmindernden GDs für den Organismus verantwortlich sind. Diese Faktoren wirken in verschiedenen Phasen des Lebenszyklus des Zielorganismus und setzen den Mechanismus des GDs mit der Biologie des Organismus in Beziehung. Ein derartiger Ansatz fehlt in früheren Arbeiten über GD. Der Modellierungsansatz bietet auch eine Klassifizierung der Drives auf der Grundlage der Biologie des jeweiligen Drives (aus den drei konstituierenden Faktoren) und vermeidet unnötig neue und verwirrende Terminologie.

Als Fallstudien für unseren einheitlichen Ansatz wurden die Ergebnisse verschiedener GDs wie CRISPR HEG-Gen Drives, Medea, Underdominance, Inverse Medea und Semele nachgebildet. Unsere Ergebnisse für das räumliche Modell zeigen, dass die Einbeziehung von nicht-panmiktischen Dynamiken die Invasions- und Fixierungsbedingungen der GD im Vergleich zum gemischten Populationsmodell verändert. Die Flexibilität, den kombinierten Effekt verschiedener evolutionärer Faktoren, die die Ausbreitung von GD beeinflussen, auf die Populationsdynamik sichtbar zu machen, ist ein wesentliches Merkmal von DrMxR. Darüber hinaus wurde das Modell um ein Drive-Resistenz-Allel erweitert. Mit dieser Erweiterung ist es möglich, auch die Komplexität der Resistenzentwicklung gegen GDs zu simulieren.

DrMxR als universelles Modell ist nicht dazu gedacht, weitere maßgeschneiderte Modellezu ersetzen, es kann jedoch ein Mittel darstellen, um das Verständnis von GD im Zusammenhang mit der Risikobewertung zu erweitern, die Politik zu informieren und die informierte öffentliche Beteiligung zu potenziellen GD-Freisetzungen zu verbessern.

Im Rahmen der vorliegenden Studie wurde in einem zweiten Schritt der Modellierungsrahmen von DrMxR erweitert, um die Auswirkungen dreier ökologischer Faktoren auf die Populationsdynamik von GDs zu analysieren. Bei diesen Faktoren handelt es sich um die Partnerwahl, Paarungssysteme und Paarungsnetzwerke. Auf dieser Basis können komplexe Paarungsbedingungen, mit denen die Zielpopulation im Freiland konfrontiert sein wird, dargestellt werden. Mithilfe dieses erweiterten Ansatzes wurden die Ergebnisse zweier Gene-Drive-Systeme (verzerrungs- und viabilitätsbasierte GDs) verglichen und die negative Auswirkung der Partnerwahl zwischen dem Wildtyp und den transgenen Organismen auf die Ausbreitung des Gene Drives quantifiziert. Es stellte sich heraus, dass ein ineffizienter Drive und Fitnesskosten aufgrund der vom Drive verbreiteten Gene die Ausbreitung des Drives stark beeinträchtigen. Zudem unterscheidet sich der vorhergesagte, für die Ausbreitung notwendige Schwellenwert bei der Freisetzung deutlich vom Schwellenwert, der in einem Ansatz ohne Verzerrung der Partnerwahl ermittelt wurde. Bei einem höheren Grad der Polygamie breitete sich der GD viel schneller aus, die damit verbundenen Fitnesskosten verringerten allerdings seine Ausbreitungsgeschwindigkeit. Die Betrachtung eines endlichen Populationsnetzwerkmodells ermöglichte ein Verständnis der zu erwartenden Auswirkungen der Freisetzung des GDs. Auf diese Weise wurde deutlich, dass die Verbreitung des GDs schneller und effektiver ist, wenn die Individuen weniger

Verbindungen im Paarungsnetzwerk aufweisen. Diese Ergebnisse unterstreichen die Notwendigkeit, verschiedene ökologische Einflüsse auf der Populationsebene bei der Modellierung der Ausbreitung von GDs zu berücksichtigen. Mit einer solchen Analyse lassen sich der Schwellenwert für die Freisetzung und der Zeitrahmen für die Ausbreitung von GDs besser vorhersagen. Derartige Analysen sollten unbedingt vor etwaigen Feldversuchen durchgeführt werden.

Bewertung der ökologischen und naturschutzfachlichen Auswirkungen

Das Hauptziel dieses Studienteils bestand darin, die potenziellen negativen Auswirkungen der Freisetzung von GDOs auf das Ökosystem und die biologische Vielfalt zu bewerten. Daher wurden die derzeitigen Ansätze zur Definition und Bewertung von Risiken überprüft und Vorschläge entwickelt, wie GDOs in die Risikobewertung von GVO integriert werden können. Die Aufgabe war in drei Teile gegliedert: (i) Überprüfung von Ansätzen zur Definition von Schutzzielen, (ii) Evaluierung von Möglichkeiten, die Umweltrisikobewertung (ERA) für GDOs zu nutzen und anzupassen, einschließlich der Anwendung auf zwei Fallstudien, und (iii) Untersuchung des Potenzials der ökologischen Modellierung als Instrument für die Umweltrisikobewertung von GDOs. Schließlich wurde geprüft, inwieweit das derzeitige Paradigma der Umweltrisikobewertung auf den Fall von GDOs anwendbar ist.

Die Definition der allgemeinen Schutzziele ist relativ einfach und kann aus den Rechtsdokumenten internationaler, europäischer und nationaler Verträge abgeleitet werden. Auf der Grundlage der Analyse aller relevanten Abkommen können zwei allgemeine Ziele identifiziert werden: die biologische Vielfalt und das menschliche Wohlergehen. Schwieriger ist die Ermittlung messbarer spezifischer Schutzziele, die für die Umweltrisikobewertung erforderlich sind. Da der Zusammenhang zwischen biologischer Vielfalt und menschlichem Wohlergehen durch das Konzept der Ökosystemleistungen gut erklärt werden kann, tendiert die Definition spezifischer Schutzziele in aktuell dahin, konkrete Ökosystemleistungen zur Ableitung von Messendpunkten zu verwenden. Wir kritisieren diese Tendenz, weil i) mit dem Konzept der Ökosystemleistungen zwar argumentiert werden kann, dass die Erhaltung der gesamten biologischen Vielfalt alle Ökosystemleistungen berücksichtigt, dies aber nicht notwendigerweise auch umgekehrt funktioniert; ii) die Redundanz des Ökosystems genutzt werden könnte, um zu argumentieren, dass eine konkrete Art aus dem System entfernt werden könnte, ohne dass eine bestimmte Leistung verloren geht; iii) unbekannte Kaskadeneffekte der Entfernung von Arten nicht berücksichtigt werden; iv) eine geringfügige, aber regelmäßige negative (nicht signifikante) Auswirkung über einen kurzen Zeitraum sich dennoch zu einer negativen Auswirkung über längere Zeiträume summieren kann. Das letztgenannte Argument stellt die in der Umweltrisikobewertung verwendete Definition von Schaden im Allgemeinen in Frage und gilt für alle spezifischen Schutzziele, z. B. für die Populationsgröße einer Art. Wir führen eine Simulation für ein hypothetisches Beispiel durch.

Im derzeitigen Rahmen der Umweltrisikobewertung spielt die Phase der Problemformulierung eine entscheidende Rolle, da in dieser Phase wichtige Informationen gesammelt werden, um potenzielle schädliche Auswirkungen des Stressors auf die Umwelt zu bewerten. GDOs ähneln jedoch in vielerlei Hinsicht invasiven Arten, da sie darauf ausgelegt sind, sich zu verbreiten und die Ökosysteme zu beeinflussen. Aus diesem Grund eignet sich die Analogie zwischen invasiven Arten und GDOs.

Die absichtliche oder unabsichtliche Ausbreitung invasiver Arten verdeutlicht, dass eine lokale Eindämmung von GDOs in einer globalisierten Welt unrealistisch ist. Darüber hinaus zeigen die Erfahrungen aus der fehlgeschlagenen Eindämmung biologischer Schädlingsbekämpfung (z. B. wurde die Hämorrhagische Kaninchenkrankheit von

Landwirten nach Neuseeland eingeschleppt), dass GDOs unbeabsichtigt oder durchaus auch beabsichtigt in andere Regionen eingeschleppt werden könnten. Daher weisen GDOs Bezüge zu verschiedenen Konzepten der Risikobewertung auf, die sich auf ihre Auswirkungen auf die Populationen und das Risiko der Ausbreitung beziehen. Ähnlich wie invasive Arten können GDOs die biologischen Interaktionen innerhalb eines Ökosystems verändern, was zu Kaskadeneffekten innerhalb und außerhalb des Ökosystems führt, in das sie ursprünglich ausgesetzt wurden. Zu den bekannten Auswirkungen der Ausrottung von Raubtieren gehören beispielsweise die Freisetzung von Mesoprädatoren, die Freisetzung von Pflanzenfressern, die Störung der Sozialsysteme von Raubtieren und die kompensatorische Einwanderung. Diese verschiedenen Aspekte der GDO lassen sich nur schwer in einem konzeptionellen Rahmen umsetzen. Daher haben wir drei verschiedene Risikofelder identifiziert:

1) **Die Auswirkungen des Populationsrückgangs auf das Ökosystem und die Ökosystemleistungen. Dazu gehören die Auswirkungen auf Arten, die mit der Zielart interagieren, andere ökologische Kaskadeneffekte und nicht erwünschte Auswirkungen im Zusammenhang mit der Entwicklung der Populationsgröße der Zielart.**
2) **Das Risiko des Entkommens des GDO in andere geografische Regionen, d.h. die Überwindung geografischer Barrieren. Dies ist vor allem für Anwendungen relevant, bei denen ein GD auf Teile eines globalen Artenspektrums beschränkt werden soll.**
3) **Das Risiko der Übertragung des GDs auf Nichtzielpopulationen oder andere Arten durch Hybridisierung unabhängig von der geografischen Lage.**

Auf der Grundlage der Analogien zu invasiven Arten und deren Risikofelder wurde ein konzeptionelles Modell für die Risikobewertung von GDO entwickelt. Da eine GD-Anwendung ebenso ein politisches und sozioökonomisches wie ein ökologisches Unterfangen ist, wurden auch sozioökonomische und ethische Aspekte einbezogen. Mit dem Modell wurden fünf grundsätzliche, aber miteinander verbundene Wege identifiziert, die in Rückkopplungsschleifen wirken: (1) die direkte Wirkung des GDO im Zielgebiet auf den Wildtyp (beabsichtigte Wirkung), (2) die Wirkung der reduzierten Populationsgröße auf das Ökosystem und die Ökosystemleistungen im Zielgebiet, (3) die Auswirkung auf die Populationsgröße und die damit verbundenen ökologischen Effekte und Auswirkungen auf die Ökosystemleistungen im Nicht-Zielgebiet - hier wird eine Rückkopplung zwischen Populationsgröße und Etablierung erwartet, (4) das Entkommen, einschließlich aller Mechanismen zur zufälligen Überwindung der Drive-Beschränkungen, und schließlich (5) die Wirkung von (1) und (2), aber auch (4) und (5) auf Sozioökonomie und Ethik einschließlich der daraus resultierenden Wirkung auf die Akzeptanz der GD-Technik und des Managementziels.

Eine weiterführende Analyse des konzeptionellen Modells mit Bezug auf zwei Fallstudien, zeigte, dass viele der benötigten Daten fehlen und dass ein Großteil einer potenziellen Risikobewertung mit großer Unsicherheit durchgeführt werden müsste. Darüber hinaus sind viele der Prozesse unzureichend verstanden. Ökologische Modellierung könnte dazu beitragen, das Verständnis der Prozesse zu verbessern, kann aber keinesfalls ein Ersatz für fehlende Daten sein. Die Vorstellung, dass die Modellierung anstelle von Feldstudien eingesetzt werden könnte, muss ebenso zurückgewiesen werden, wie die Vorstellung, dass ökologische Modelle präzise und unvoreingenommene Vorhersagen für Messendpunkte, d. h. für bestimmte Schutzziele, liefern könnten.

Schließlich wird die Anwendbarkeit des aktuellen ERA-Paradigmas auf GDO diskutiert. Wir argumentieren, dass GDO aufgrund der Reichweite und Kombination von ökologischen Effekten, die sie haben können, eine neue Qualität mit sich bringen: absichtliche Ausrottung einer Art im Zielgebiet, unbeabsichtigtes Entweichen in Nicht-Zielgebiete und/oder andere Arten. Auf die Auswirkungen additiver kleiner Effekte und die

Unfähigkeit, vernachlässigbare Effekte zuverlässig zu berücksichtigen, wurde bereits hingewiesen. In Anbetracht der anhaltenden Krise der biologischen Vielfalt sollte jeder Rahmen für das ERA auch ökologische Auswirkungen berücksichtigen, die vielleicht nicht offensichtlich sind, aber langfristig Schaden anrichten können, unabhängig von der angewandten Technik. Wir sind der Meinung, dass dies in keinem der derzeitigen Rahmenwerke der Fall ist. Wenn jedoch die Beseitigung einer Art eine potenzielle Gefahr darstellt und die Wahrscheinlichkeit, dass die Gefahr einen ökologischen Schaden verursacht, ein Risiko darstellt, dann wird das Risiko mit jeder Anwendung eines GD zur Populationsunterdrückung innerhalb der Art, des geografischen Gebiets, der Gebiete, in die der Transport erfolgt, oder jedes denkbare Entweichungsszenario steigen.

Gene Drive Monitoring

Bevor eine Freisetzung eines GDs zu Testzwecken oder gar eine Freisetzung in großem Maßstab in Betracht gezogen werden kann, sollte ein geeigneter Monitoringsplan mit Untersuchungshypothesen und geeigneten Indikatoren implementiert sein, um überhaupt mögliche unbeabsichtigte Auswirkungen auf die Umwelt und die menschliche Gesundheit beobachten und erkennen zu können.

Ziel des Studienteils zum Monitoring war es, in vergleichender Analyse die im Vergleich zu einem GVO relevanten Merkmale eines GDO zu identifizieren, um die spezifischen Anforderungen an ein GDO-Monitoring und die Grenzen der Überwachung und Kontrolle möglicher - im schlimmsten Fall globaler - ökologischer Auswirkungen durch einen GDO zu ermitteln. Auf der Grundlage dieser Ergebnisse werden Empfehlungen für einen zukünftigen Überwachungsansatz für GDOs gegeben.

Das Monitoring von GDO sollte beide Ansätze, die fallspezifische Überwachung (case-specific monitoring) und die allgemeine Überwachung (general surveillance), berücksichtigen. Darüber hinaus sollte es in der Lage sein, a) die Exposition und b) die schädlichen Auswirkungen (Gefährdung) auf die Umwelt zu ermitteln. Für den Aufbau und die Entwicklung eines Überwachungssystems zur Ermittlung der ökologischen Auswirkungen eines GDO auf die Umwelt wurde eine Checkliste mit allen relevanten Eigenschaften und Parametern eines GDO erstellt, um die Anforderungen an ein GDO-Monitoring möglichst umfassend darzustellen. Mehrere Merkmale von GDO wie ihre Anwendung in natürlichen Systemen, ihre zeitliche und regionale Unbegrenztheit und die breite Wirksamkeit von GDO stellen besondere Herausforderungen für die Gestaltung eines funktionstüchtigen Monitoringsystems dar. Es fehlt allerdings noch an ausreichendem Grundlagenwissen, um geeignete Monitoringspläne entwerfen zu können. Daher ist es noch nicht möglich, ein angemessenes Monitoring zu konzipieren und umzusetzen, um das invasive Verhalten von GDO zu beobachten. Angesichts der Fähigkeit der GDO, sich innerhalb und zwischen Populationen durch genetischen Austausch auszubreiten, sollte die Überwachung von GDO zudem auf molekularer Ebene erfolgen, was metagenomische Ansätze notwendig macht. Bestehende nationale und internationale Monitoringkonzepte und -programme können derzeit nur einen Ausgangspunkt für das GDO-Monitoring bieten, wie etwa eine Grundlagenuntersuchung, um beispielsweise Effekte von GDO auf die Biodiversität erkennen zu können.

Die für das GVO-Monitoring bereits obligatorischen Monitoringverfahren müssen in das zu entwickelnde GDO-Monitoringprogramm einfließen bzw. sollten die Grundlage dafür bilden. Aufgrund der potenziellen globalen Reichweite der Auswirkungen wäre es von größter Bedeutung, künftige Richtlinien für den möglichst sicheren Umgang mit GDOs und die Anforderungen an das Monitoring mit Hilfe einer weltweit einheitlichen Richtlinie festzulegen, um die im Falle von GDO entscheidende Vergleichbarkeit eines globalen Monitorings gewährleisten zu können. Bestehende Leitlinien für internationale Regelungen sollten auf ihre Angemessenheit hin überprüft werden. Für die

Risikobewertung und das Monitoring ist eine umfassende Grundlagenforschung zu den aktuellen Entwicklungen der GD-Technologien und ihrem ökologischen Wirkungspotential erforderlich. Die Forschung nach geeigneten Methoden zum Monitoring von GDO sollte forciert werden. Falls es wirklich zur Freisetzung von GDO käme, müssten ausreichend große Budgets bereitgestellt werden, um ein langjähriges GDO-Monitoring inklusive wiederholter Monitoringläufe zu gewährleisten und den Erwerb von Grundlagenwissen zur Formulierung von Risikohypothesen zu fördern. Grundsätzlich sollte darauf hingewiesen werden, dass Monitoringkonzepte lediglich ein Beobachtungssystem darstellen. Eine Rückholbarkeit im Schadensfall ist allein durch Monitoring nicht möglich.

Rechtlicher Rahmen für die absichtliche Freisetzung von Gene Drive Organismen

Für die absichtliche Freisetzung von GDO sind verschiedene Regeln und Normen auf nationaler, europäischer und internationaler Ebene von Bedeutung. Vor allem erfüllen GDOs die Definition von GVO gemäß den Europäischen Richtlinien für die biologische Sicherheit und die Definition von lebenden veränderten Organismen (LMOs) gemäß der Biodiversitätskonvention und ihren Protokollen (siehe unten).

Außerdem setzt die deutsche GVO-Verordnung den Europäischen Rahmen für die biologische Sicherheit auf Ebene der Mitgliedstaaten um. Daher ist die europäische GVO-Verordnung für jede absichtliche Freisetzung in der EU, die verschiedene Aspekte der biologischen Sicherheit abdeckt, von größter Bedeutung. Die Richtlinie RL2001/18/EG über die absichtliche Freisetzung eines GVO in die Umwelt stellt sicher, dass jede absichtliche Freisetzung eines GVO einer Genehmigung durch ein staatliches Genehmigungsverfahren auf der Grundlage einer Umweltrisikobewertung bedarf, wobei die Bedeutung des Vorsorgeprinzips betont wird. Die Richtlinie über die Anwendung in geschlossenen Systemen regelt die biologische Sicherheit von GDO im Labor und legt Maßnahmen für die Anwendung in geschlossenen Systemen fest, um den Schutz der menschlichen Gesundheit und der Umwelt zu gewährleisten.

Auf internationaler Ebene gibt es Regeln und Normen, die als internationales Recht verbindlich sind, wie auch die unten genannten internationalen Verträge. Aufgrund seiner weltweiten Anerkennung ist das Übereinkommen über die biologische Vielfalt der wichtigste internationale Vertrag, der sich ausdrücklich mit der Regulierung von LMO befasst. Es bietet einen verbindlichen internationalen und nahezu universellen allgemeinen Rahmen für die Regulierung von GDO, der die Bewertung von Risiken und die Festlegung geeigneter Risikomanagementmaßnahmen vor einer absichtlichen Freisetzung vorschreibt.

Darüber hinaus enthält das Cartagena-Protokoll als verbindlicher internationaler Vertrag und Protokoll zum Übereinkommen über die biologische Vielfalt, spezifische Bestimmungen darüber, wie die Mitgliedstaaten bei der grenzüberschreitenden Verbringung und der absichtlichen Freisetzung von GDO vorgehen und Risikobewertungen durchführen müssen, sowie bei den spezifischen Verpflichtungen in Bezug auf das Risikomanagement. Von Bedeutung ist auch das ergänzende Nagoya-Kuala Lumpur-Protokoll, der dritte verbindliche internationale Vertrag in diesem Bereich, der die negativen Auswirkungen auf die Erhaltung und nachhaltige Nutzung der biologischen Vielfalt regelt, die durch die grenzüberschreitende Verbringung von GDOs verursacht werden könnten.

Aus Sicht des Welthandelsrechts bietet das WTO-Übereinkommen über die Anwendung gesundheitspolizeilicher und pflanzenschutzrechtlicher Maßnahmen (SPS-Übereinkommen) als verbindlicher internationaler Vertrag einen rechtlichen Rahmen, den die Staaten bei der Regelung der absichtlichen Freisetzung von GVO auf ihrem

Hoheitsgebiet beachten müssen. Wichtig ist, dass eine Null-Risiko-Politik nicht auf einer theoretischen Ungewissheit in Bezug auf die Risiken von LMOs beruhen darf, ein Ansatz, der sich leicht von dem des Cartagena-Protokolls unterscheidet.

Aus einer allgemeinen Menschenrechtsperspektive sind verbindliche universelle Menschenrechtsverträge (wie der Internationale Pakt über bürgerliche und politische Rechte und der Internationale Pakt über wirtschaftliche, soziale und kulturelle Rechte) und regionale Menschenrechtsverträge (wie die Charta der Grundrechte der Europäischen Union) relevant, da sie international rechtsverbindliche Standards für die Regulierung der Biotechnologie setzen und das Recht auf Wissenschaftsfreiheit beinhalten, auch wenn es nicht ausdrücklich erwähnt wird.

Aus der Sicht des Völkergewohnheitsrechts ist es fraglich, ob die grenzüberschreitende Verbreitung von GDO gegen die Verpflichtung verstößt, das Hoheitsgebiet eines anderen Staates nicht erheblich zu beeinträchtigen. Wenn diese Regel des Völkerrechts verletzt wird, muss der verantwortliche Staat Wiedergutmachung leisten.

Schließlich sind für die absichtliche Freisetzung von GDO auch das so genannte Soft Law und andere Richtlinien wie der *Codex Alimentarius* von Bedeutung. Diese haben normative Kraft, auch wenn sie nicht direkt als Gesetz bindend sind, aber ein Verstoß gegen diese Regeln führt nicht zur internationalen Verantwortung eines Staates.

Schlussfolgerung

Synthetische Gene Drives stellen eine neue Qualität gentechnisch veränderter Organismen dar, da sie eigenständig agieren können, um Wildtiere und Pflanzen gentechnisch zu verändern oder sogar einzelne Arten auszulöschen. Ihre Ausbreitung und die vielfältigen möglichen ökologischen Auswirkungen, insbesondere im Falle eines Scheiterns des geplanten Anwendungsablaufs, lassen sich mit heutigen Methoden vor möglichen Freisetzungen nur in minimalem Umfang abschätzen. Zudem erschwert die unzureichende ökologische Datenlage die Anwendung prädiktiver Ansätze. Für eine adäquate Umweltrisikobewertung von GD Freisetzungen ist eine stärkere Berücksichtigung von weniger deutlichen und vor allem potentiell additiven Effekten erforderlich. Wie zuverlässig Strategien zu ihrer räumlichen und zeitlichen Kontrolle sind, lässt sich derzeit nicht hinreichend bestimmen. Bestehende Monitoringkonzepte können nur als Ausgangspunkt oder Basisdatenreservoir für die Entwicklung optimierter Konzepte dienen. Eine verlässliche methodische Grundlage für Risikobewertungen und die Überwachung von Freisetzungen ist damit noch keinesfalls gegeben. Die Folgen von Freisetzungen von GDs sind bisher nicht im erforderlichen Umfang und mit ausreichender Zuverlässigkeit vorhersehbar. Die in diesem Projekt entwickelte Modellierungssoftware 'Drive Mixer' kann genutzt werden, um das Verständnis für die Eigenschaften von GDs zu verbessern und verschiedene GD-Ansätze zu vergleichen.

Aus regulatorischer Sicht fallen GDs unter die bestehenden internationalen und nationalen Gesetze und Verträge für gentechnisch veränderte Organismen, auch wenn es Probleme bei der Anerkennung von Schäden im Falle einer grenzüberschreitenden Ausbreitung und den jeweiligen Auswirkungen geben kann, die ein Drive in Nicht-Zielregionen verursachen kann.

Sollte der Technologiepfad der Gene Drives jedoch tatsächlich weiterverfolgt werden, muss ein vorsorgendes Risikomanagement Wege finden, um mit fehlendem Wissen bis hin zu völliger Unkenntnis über mögliche negative Auswirkungen adäquat umzugehen. Im Sinne des Vorsorgeprinzips muss die Verbreitung von Gene Drives, die im Zweifelsfall nicht kontrolliert werden können, als Grund zu großer Besorgnis angesehen werden.

1 Aim of the Study

The targeted rapid dissemination of artificially assembled genetic information in populations of wild organisms by gene drives represents a significant extension to the definition of a GMO because it expands the spectrum of its functionalities to include potentially far-reaching spatial and temporal effects. It is therefore doubted that the existing approaches and specifications for risk assessment are also applicable to gene drive-bearing organisms (GDOs) without neglecting essential risk-relevant properties (cf. Simon et al. 2018). However, in order to be able to adapt and extend the risk assessment, sufficient knowledge must be available on the properties and possible applications of the different gene drive systems, their potential effects in exposed ecosystems, and methods for estimating their spread and subsequent effects. The present study aims to provide the scientific basis for adapting the risk assessment and monitoring of GDO. Its analyses are therefore devoted to the following main topics:

a) **a characterization of existing gene drive approaches, their effectiveness, ways to control or limit them, and risk mitigation strategies,**
b) **an investigation of the possibilities for modeling gene drives,**
c) **an investigation and evaluation of potential ecological and conservation impacts and the methods for estimating them,**
d) **an investigation of the requirements for monitoring released GDOs, and**
e) **a description of the legal framework for the release of GDO in the context of an initial scoping exercise.**

2 Part A.0 - Technical Characterization of Gene Drives1

Johannes L. Frieß, Bernd Giese

As a basis for the risk-related consideration of Gene Drives, an overall view of technical Gene Drive systems was developed in this block. These include the gene drive approaches Medea ("Maternal Effect Dominant Embryonic Arrest"), single and two-locus Underdominance, killer rescue, autosomal and Y-linked X-Shredder and homing endonuclease-based systems (CRISPR incl. Daisy Chain Drives). For this purpose, prospective technology characterization is applied. It is designed to estimate the exposure and hazard potential based on early identifiable technical qualities and already known quantitative information on the use of technologies. From this analysis of risk-determining factors, indications of concern and relief criteria can be derived.

2.1. Gene Drives

A gene drive (GD) is a phenomenon in population genetics where a gene or set of genes is inherited with a probability higher than 50%, dictated by the Mendelian laws of inheritance. Hence, a gene drive may drive a certain trait quickly into a population and reach fixation. There is a number of natural mechanisms which possess this notable property. In 2006, Sinkins and Gould (2006) mentioned transposable elements, meiotic drive genes, homing endonuclease genes and *Wolbachia* as naturally occurring gene drives. Already in 1960, as a theoretical concept for gene drives as a method to drive a desired trait into a population, Craig et al. (1960) proposed: “Mass release of male-producing males might be used in control operations.”. Also the spread of chromosomal translocations has been already proposed as a means of population control in those years (Curtis, 1968; Serebrovskii, 1940). Hastings suggested to use so called “selfish genes” for that purpose (Hastings, 1994) and a practical implementation was explored with the use of the P-element for germline transformation of *Drosophila melanogaster* (Carareto et al., 1997). In 2003, Austin Burt suggested to use homing endonucleases for the design of self-replicating drives (Burt, 2003). Gene drives propagate even if they confer a fitness penalty, or in other words “Mathematically, drives are initially favoured by selection […] if the inheritance bias of the drive exceeds its fitness penalty.” (Noble et al., 2018, p. 201). Some secure their Super-Mendelian inheritance passively, so that only offspring carrying genetic information of the drive will survive or be fertile. Akbari et al. called this type of mechanism “selective embryonic lethality” (Akbari et al., 2015). Others actively overcome the limitations of the Mendelian inheritance pattern by a distortion of allelic segregation i.e. fragmentation of chromosomes, for example resulting in an altered sex ratio. Active drives may also copy their genetic information between homologous chromosomes resulting in homozygous offspring. Such approaches were termed „active genetics“ by Gantz and Bier (Gantz and Bier, 2015). Due to its inherently invasive character, a once-released gene drive represents a significant intervention into ecosystems. In principle, a gene drive needs several generations to establish itself in a population. It is thus a technology capable to reproduce itself and undergo mutational changes over time. Not only do gene drives affect the environment, the environment affects the gene drives as well. A gene drive engineered in the laboratory, once released will be confronted with evolutionary processes.

GDs can be divided into two groups according to how they secure their super Mendelian inheritance into active and passive drives. Further distinctions are made in the literature by their intention into modification or suppression drive, by their propagation dynamics between self-limiting and self-sustaining techniques and between threshold-dependent or

1 This chapter represents an update and extension of the work on technology characterization of gene drives in Frieß et al. (2020) and Frieß et al. (2019).

-independent, synonymous with local and global systems. The threshold value refers to a percentage within the population of the released GDOs or the gene drive will not spread. In Fig. 1, the estimated Threshold dependencies of different population control techniques are compared. Concomitantly, the techniques are ranked in their estimated persistence.

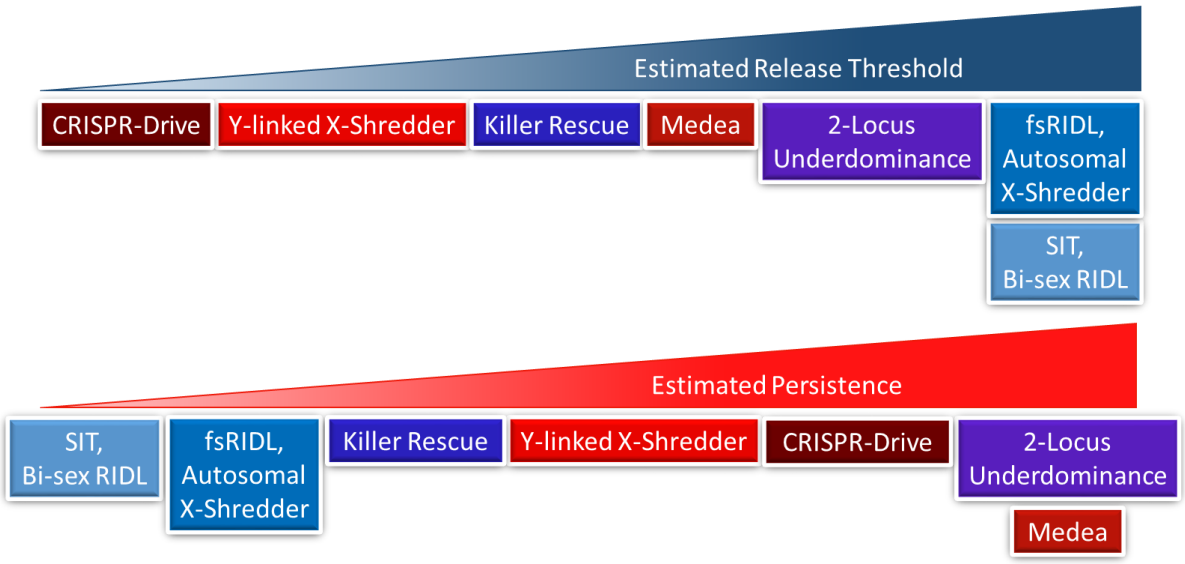

Fig. 1: Different population control techniques ranked for their estimated release thresholds and persistence.

2.2 Theory of Technology Characterization

In early stages of innovation processes and technology developments, application contexts and affected systems are usually still unknown, but the outlines and essential characteristics of the new technical application are already known. In such cases, prospective assessment relies on the use of early indicators of performance and impact of the intended application(s) of a technology. Technology characterization makes use of this approach and is therefore an appropriate method for the analysis of gene drives (GDs), since no experience from GD releases is yet available. The goal of technology characterization is to assess the hazard and exposure potential (reasons for concern) at an early stage and to evaluate various forms of missing knowledge to avoid path dependencies and costly mitigation measures at later stages of innovation (Fig. 2) (Frieß et al., 2019; von Gleich, 2013, pp. 51–73). In this way, technology characterization is an important approach to operationalize precautionary requirements.

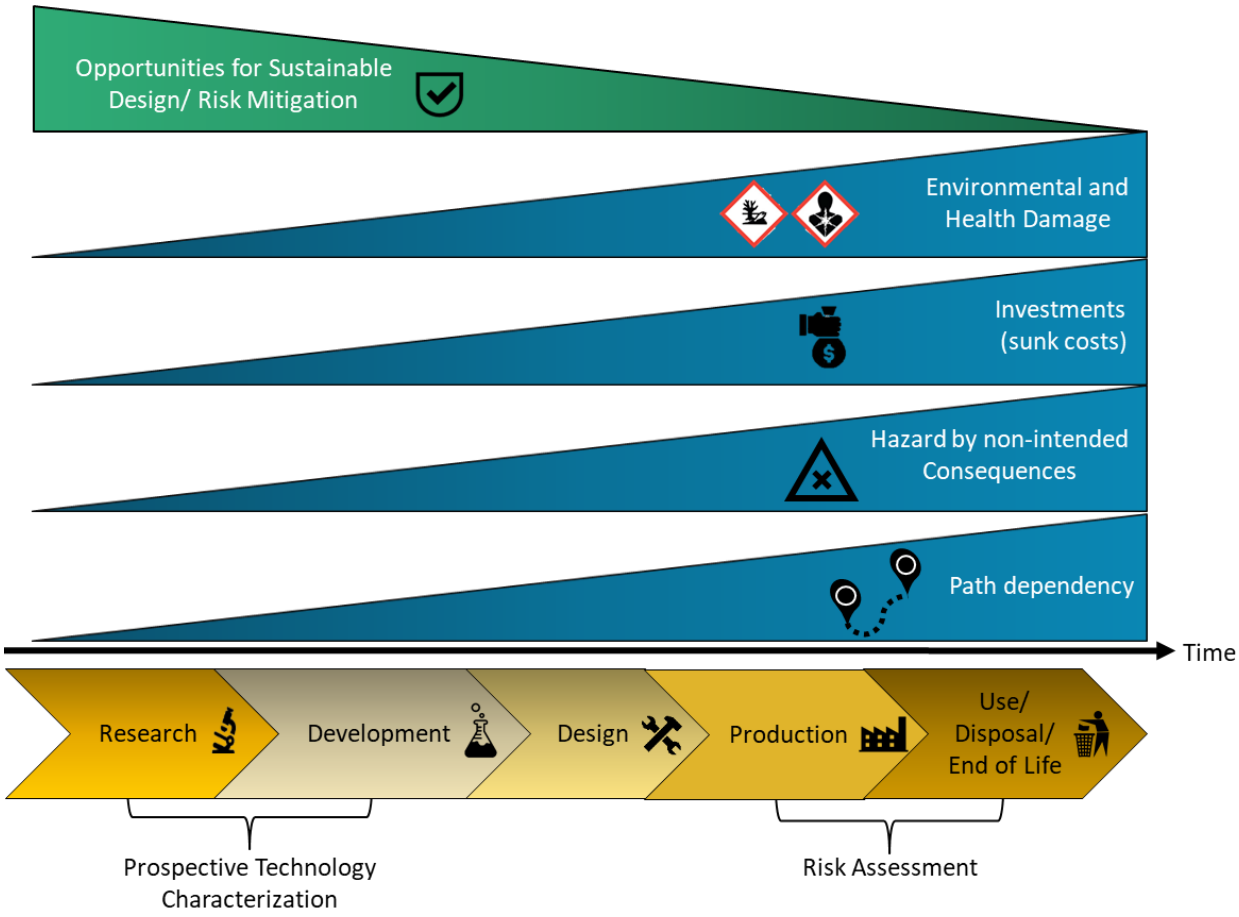

Fig. 2: Prospective technology characterisation is applied in early stages of innovations.
While early during research and development, application purposes may yet be unclear, path dependency is still low and allows for adaptation and direction to alternative development paths in case of emerging reasons for concern. On the opposite, the risk assessment of a newly introduced technology is conducted at the latest possible stage, when path dependency is high and adaptation is difficult.

Non-knowledge ranges from uncertainties to absolute ignorance. Thereby allowing to include complete surprises, meaning possible events for which currently no scientific approved 'model of effect' exists2. Such an approach for the assessment of different dimensions and forms of lacking knowledge regarding hazards and exposure already exists (Ahrens et al., 2005; Giese and von Gleich, 2015; Linkov et al., 2018; Owen et al., 2009; Steinfeldt et al., 2007). The underlying hypothesis of technology characterisation is, that the range and the forms of non-knowledge are not 'just there', but are to a large extend produced by the character of the technology. By scrutinizing their technological origin, the first criteria to investigate the range and forms of lacking knowledge are depth of technological intervention and also the intensity of intervention. The depth of intervention is a source of technological power and hence of potential effects, benefits or hazards on one side. On the other side, the depth of intervention presents sources of a high operating range of the created entities and thus the potential for exposure. High power and high range of exposure lead to a high extend of non-knowledge concerning possible effects. In order to provide additional information on the frequency and the corrigibility of the

2 As it was the case with DDT minimizing the thickness of bird eggs, ozone depletion triggered by CFC, the 'mad cow disease' and industrial chemicals functioning as endocrine disrupters (European Environment Agency, 2001) and is actually the case with the reduction of insect populations in Middle Europe.

expected effects, the quantitative aspects of the use of the technology (intensity of intervention i.e. quantity, frequency of its use), its reliability in practice, the probability of failure, and, finally, for cases of failure possible ways of limiting or mitigating harm have to be analysed. (von Gleich, 2013, pp. 51–73)

It is not the aim of prospective technology characterization to identify any possible adverse effect of technologies. Instead, it should provide a decision-making basis in the context of the precautionary principle (Commission of the European Communities, 2000; European Environment Agency, 2002; *The Rio declaration on environment and development*, 1992; United Nations, 2000). “The precautionary principle enables decision-makers to adopt precautionary measures when scientific evidence about an environmental or human health hazard is uncertain and the stakes are high” (European Parliament Think Tank, 2015) In cases when it is unwarrantable to wait until a risk is clear and proven, the precautionary principle legitimates precautionary action, because a probably occurring disaster would not be controllable then. Prerequisites to warrant precautionary action are therefore:

a) **lack of knowledge (from uncertainty to ignorance),**
b) **comprehensible reasons for concern (affecting extremely powerful and/or far reaching consequences),**
c) **a rudimentary cost-benefit analysis (in which e.g. medical applications with little less risky options are rated higher than applications in the food chain with plenty alternatives),**
d) **adequate measures (reaching from containment over substitution by less problematic alternatives to moratorium) (Fischer et al., 2006).**

The focus of technology characterization lies on the prevention of far reaching, by-trend-irreversible and global effects, of incidences with consequences that cannot be managed adequately, that cannot be retrieved, corrected or mitigated in case of their occurrence.

Based on the framework for technology characterization a comparison of various GD technologies will be performed. Thereby the following criteria are considered (Fig. 3).

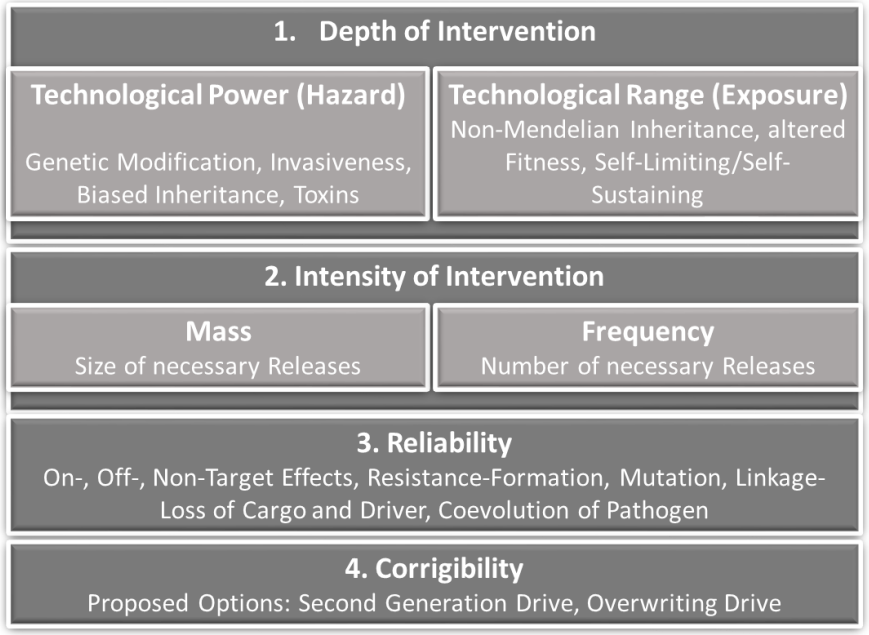

Fig. 3: Criteria of prospective technology characterization with gene drive-specific effects and options (taken from Frieß et al., 2019).

2.2.1 **Depth of Intervention (Technological Power, Range)**

Depth of intervention results from technological power and range. For GD technologies in general, the depth of intervention is much greater than for population control approaches not based on genetic modifications. One source of their technological power is that they are based on the manipulation of the very basis of organisms, their genetic characteristics. The other source of power depends on the functionalities of the applied genes and respective traits. The technological range describes potential spatio-temporal consequences of a gene drive, taking into account its lasting persistence in a population as well as the range with which it could spread across populations. Thus, the mono-generational suppression of a single population would be considered as a comparably low range, while the permanent replacement of a population with genetically altered specimens is considered a high range. At the same time range considers the possibility of either intended or unintended spread of a gene drive across multiple populations.

2.2.2 **Intensity of Intervention (Mass/Frequency)**

The intensity of intervention as mass or frequency of released organisms describes the necessary quantity of interventions to drive a desired trait into a targeted population. An approach requiring the released organisms to outnumber the wild type organisms would score as high intensity and if an initially low percentage of the population is sufficient it would correspond to a low intensity. The quality of released organisms, e. g. their capability of self-reproduction, which determines their range in a much higher proportion is determined by the criterion of depth of intervention.

2.2.3 **Reliability of the Technology**

This criterion describes the probability of failure of the technology with regard to its intended use. Important reliability issues are e. g. linkage-loss of the cargo gene and its driver system, the generation of resistances in the target population, coevolution of the pathogen and system decay (Alphey, 2014).

2.2.4 **Options of Risk Mitigation**

Can the damage of a failed gene drive be reversed by any means and if so, how laborious are they compared to the initially released construct? These questions address important aspects of risk management. For some GD technologies it is claimed that they can be somewhat remedied by a release of wild type organisms. But such an endeavour would not really reverse the damage done as populations do not exist in isolation but instead affect other populations and species.

Even more difficult to estimate are corrective actions such as a reversal drive which on one hand relies on the release of a second-generation gene drive to remedy the failures of the first. And on the other hand, the gene pool of the target population in any case retains transgenic elements.

In any case, in order for the mitigation strategy to be successful the organisms of the second release should outnumber the GDOs and the release area must at least cover the area that the GDOs have covered since their release. Since it is rather unlikely that such a mitigation strategy would be employed within the first generation post release, the whole idea of not just wild type – but all secondary releases seems unfeasible.

2.3 **Characterisation of Various Gene Drive Techniques**

In the following chapter, various GD techniques are characterized concerning their exposure and hazard potential, their reliability, options for risk mitigation and planned application (if any). Thereby, the focus is set on synthetic techniques, some of which have never been really established in a laboratory but are instead only theoretically explored in the literature. But all techniques are considered viable strategies for an anthropogenic gene drive. In this way, naturally occurring drive systems, such as the t-haplotype (Silver, 1993), the p-element in Drosophila melanogaster (Carareto et al., 1997), the naturally occurring driving Y in Aedes and Culex mosquitoes (Craig et al., 1960; Newton et al., 1976), nor the natural medea drive discovered in *Tribolium castaneum* (Beeman et al., 1992). Also, not part of this technical review are synthetic techniques which in theory could be applied as a gene drive but lack efficiency in comparison to the techniques introduced below. Therefore, transposable elements, Zincfinger Nucleases (ZFNs) and Transcription Activator-like Effector Nucleases (TALENs) (Xie et al., 2016) are not included in this chapter.

Furthermore, the GD techniques are categorized into two groups, active and passive, according to how they secure their super Mendelian inheritance. Further distinctions made between GD techniques are due to their persistence into self-limiting or self-sustaining, by their propagation dynamics, into threshold-dependent or –independent, synonymous with local and global systems, and by their intention into modification or suppression drives. The threshold value refers to a percentage within the population of the released GDOs or the Gene Drive will not spread. All the techniques more closely explored in the sections below are categorized in this way in Tab. 1.

Tab. 1: Categorization of gene drive techniques

Gene Drive Technique	Mode of Action	Threshold	Persistence	Intention
CRISPR-Drive	Active	Independent	Self-sustaining	Modification
Autosomal X-Shredder	Active	Independent	Self-limiting	Suppression
Y-linked X-Shredder	Active	Independent	Self-sustaining	Suppression
Cleave and Rescue	Active/Passive	Independent	Self-sustaining	Modification
Medea	Passive	Dependent	Self-sustaining	Modification
Inverse Medea	Passive	Dependent	Self-sustaining	Suppression
Medusa	Passive	Dependent	Self-sustaining	Suppression
Semele	Passive	Dependent	Self-sustaining	Modification
Underdominance	Passive	Dependent	Self-sustaining	Modification
Killer-Rescue	Passive	Dependent	Self-limiting	Modification

2.3.1 **HEG-Drive**

a. Exposure and Hazard Potential

The CRISPR drive is an active, self-sustaining, threshold independent, global modification technique. Its functional principle is based on the CRISPR/Cas system within the GD construct. This effectively turns a heterozygote into a homozygous GDO (Fig. 4, see Esvelt et al. (2014a)). This transformation is unique among GD techniques and thus this technique has the highest exposure potential of all GD techniques.

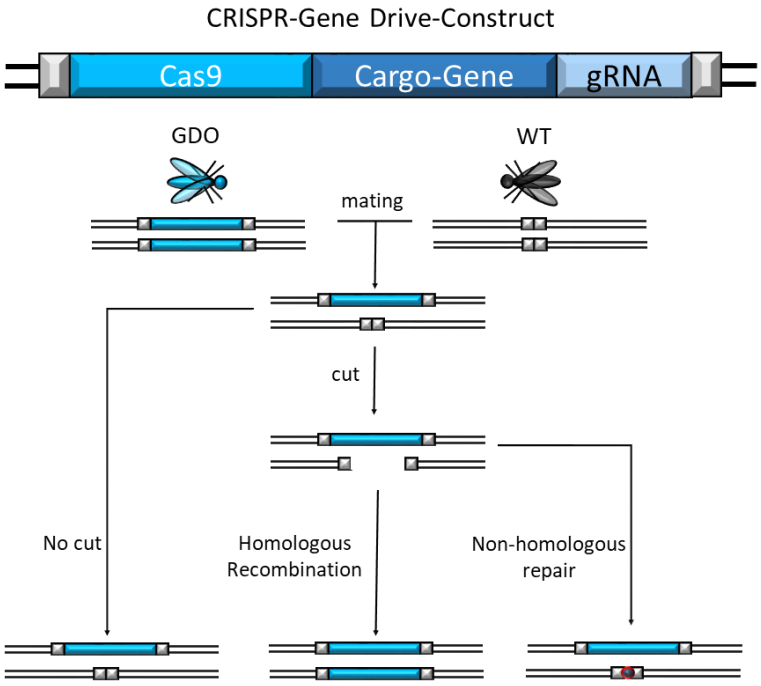

Fig. 4: Construction of a CRISPR gene drive allele and mechanism of action in the germline of the offspring. CRISPR/Cas cleaves the recognition sequence provided by the gRNA in the wild-type allele. As a result, homologous recombination occurs instead of doubling the Gene Drive allele, the incision is repaired by non-homologous repair, creating a resistance allele. (adapted from Esvelt et al., 2014).

b. Reliability

However, in most phases of the cell cycle, homologous recombination is not chosen as the repair pathway, but instead the ends created by the cut are rejoined by non-homologous end-joining (NHEJ), usually resulting in smaller insertions or deletions, so that the sequence is slightly altered and cannot be detected by CRISPR/Cas any more. This will ultimately produce resistant alleles that will quickly go to fixation in a population exposed to a GD (Marshall et al., 2017; Unckless et al., 2016). The same applies to sequence polymorphisms that already occur in the target population (Drury et al., 2017). Modelling suggests that the spread of a CRISPR-GD is followed by a spread of resistant organisms, if the fitness cost of the resistance is lower than that of the drive (Unckless et al., 2016). HDR vs. NHEJ efficiency could be as low as~10% (Lin & Potter, 2016). To reduce these events, CRISPR/Cas9 could be used to enhance HDR gene expression and repress NHEJ-genes. This could be achieved by the inclusion of HDR-genes and NHEJ-repressor genes. Furthermore, the generation of nucleases creating sticky-end overhangs as opposed to blunt ends may optimize the repair in the target organism. The rate of HDR depends on the species, cell type, developmental stage, and cell cycle phase. For example, faithful copying was achieved with up to 97% efficiency in mosquitoes but only 2% in fruit flies (Esvelt et al., 2014). Other studies yielded average homing rates of 56% (KaramiNejadRanjibar et al., 2018) and 97% (Gantz and Bier, 2015) in Drosophila and 98.8% in *Anopheles stephensi* (Gantz et al., 2015) and even 99% in wild yeast (DiCarlo et al., 2015a). Also a so-called maternal effect has been observed, where maternal Cas9 deposition in the oocyte during fertilization may cause cuts in the paternal target sequence that due to a lack of homologous templates in the vicinity are repaired non-homologously (Lin and Potter, 2016). To suppress this resistance formation to a large extent, a vital locus could be targeted, whose alteration would be fatal by non-homologous repair (Marshall et al., 2017). Additionally, pre-existing sequence variations can be covered with additional

gRNAs as already demonstrated in the laboratory (Yan and Finnigan, 2018). In a different strategy, it is feasible to exploit the functional constraints of highly conserved sequences to lower the selection of resistant sequence variants This particular strategy where the *doublesex* gene was targeted, however could only be used as a suppression drive (Kyrou et al., 2018).

c. Options for Risk Mitigation

Because of the potentially high invasiveness of a CRISPR-GD, various techniques for restriction and safety have been proposed, including the Reversal (Overwriting) Drive, Immunizing Drive (Esvelt et al., 2014), Split Drive (DiCarlo et al., 2015b), Daisy Chain Drive (Noble et al., 2019), Daisy Field Drive, Daisy Quorum Drive (Min et al., 2017a), Precision Drive (Min et al., 2018). All of these will be further explored in A.1 of this project.

d. Planned Applications

Up to now, the HEG-Drive has been implemented in yeast *Saccharomyces cerevisae* (DiCarlo et al., 2015a, 2015b; Yan and Finnigan, 2018), *Drosophila melanogaster* (Gantz and Bier, 2015) but not intended for release. Currently only in a model, a suppression drive against the agricultural pest, the medfly *Ceratitis capitata*. In lab experiments, this drive was so far only implemented into *Drosophila melanogaster* ,(KaramiNejadRanjibar et al., 2018), in mice *Mus musculus* (Grunwald, 2016) as a proof of concept, Target Malaria plans to use HEG-drives to control *Anopheles gambiae* and *A. coluzzi* and *A. arabiensis* in Africa (Gantz et al., 2015; Hammond et al., 2016; Kyrou et al., 2018). The cargo gene is said to cause female infertility, which should greatly reduce the populations3. Consideration of the necessary geographic spread of these mosquito species and taking into account the long time span involved, illustrates the extent of such GD intervention. If we imagine now the spread of the GD into other *Anopheles* species due to incomplete mating barriers, rare mating events and increasing prevalence of southern species in the North due to global warming, a global dissemination of the GD may be a consequence. For instance, the three Anopheles species *A. gambiae*, *A. coluzzi* and *A. arabiensis* are able to produce viable, fertile and not uncommon hybrid offspring (Pombi et al. 2017). Furthermore, all species within the *Anopheles gambiae s. l.* complex are able to hybridise4 On the other hand, *Aedes albopictus* and *A. aegypti* mosquitoes are predicted to spread further North up to Shanghai and Chicago, respectively within the next five to 15 years. By 2050, 49% of the world population may be exposed to the vectors of yellow fever, dengue, chikungunya and Zika due to climate change (Kraemer et al., 2019).

2.3.2 X-Shredder

a. Exposure and Hazard Potential

As with many metazoan species the sex determination of Anopheles mosquitoes is based on X and Y chromosomes. Females have identical sex chromosomes (XX) and males dimorphic (XY) (Aslamkhan, 1973). X-Shredder secures its super-Mendelian inheritance by cutting the X chromosome at conserved repeated sequences during spermatogenesis. This leads to a strong distortion of the sex ratio in the next generation. If the construct is

3 According to the Target Malaria Homepage; last accessed April 2, 2020
4 Target Malaria Fact Sheet 4 Ecology of *Anopheles gambiae*

located on the Y chromosome aka Driving-Y, it is an active, potentially self-sustaining, threshold-independent, and generally global suppression drive (Fig. 5). However, it is a suppression drive and these inherently limit themselves in their spread since a population without females eventually collapses. In the laboratory, a ratio of 95% male offspring was achieved (Galizi et al., 2014). Nevertheless, a purely male population would probably be likely to migrate in search of females. As male Anopheles mosquitoes form swarms flying about until they encounter females which leave the swarm in copula (Takken et al., 2006). North et al., (2019) modelled the spatial dispersal and suppression of Anopheles populations in an area including Burkina Faso with 42,360 settlements. They found releasing 10 males/year in only 1% of randomly chosen settlements would achieve 91.5-95.5% suppression after only 4 consecutive years. Whereas 1 Sterile Insect Technique (SIT) release of 50.000 males per year in every site caused between 0%-94%. Furthermore, they found that both releasing more than 10 Y-driving males in a given settlement or in more than 1% of settlements would only marginally increase suppression. Additionally, if cleavage rates are in an intermediate range, suppression is highest, due to the longer persistence of the drive in a population before it becomes eradicated, allowing for more migrational population exchange. (North et al., 2019).

An autosomal X-shredder would classify as an active, self-limiting, threshold-dependent suppression drive.

A comparable technique causing a Y-chromosome deletion using orthogonal programmable endonucleases (Y-CHOPE) was presented by Prowse et al. (2019). Acting in the germline this drive supposedly transforms XY-males into fertile X0 females with an efficiency around 90%.

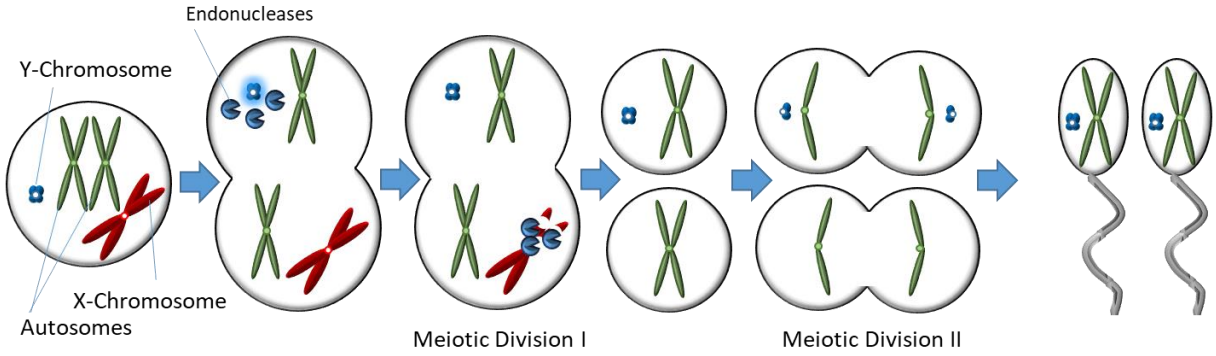

Fig. 5: Mode of action of a Y-linked X-Shredder drive.
During spermatogenesis, the drive cuts the X chromosome into many pieces using homing endonucleases. As a consequence, only the cells containing a Y chromosome can produce fertile offspring, which are of course male.

b. Reliability

Little is known about the vulnerability of X-Shredder. Despite the large fitness penalty attached to the drive, resistance formation seems unlikely, as most of the conserved repeated attack loci would have to mutate on the X chromosome at the same time. In contrast, a mutation in the endonuclease gene/s seems more likely.

c. Options for Risk Mitigation

Currently, there is no way to undo the damage caused by an X-Shredder release or to restrict its spread.

d. Planned Applications

Similar to the HEG-Drive, this technique is considered by Target Malaria for use against malaria mosquitoes (Facchinelli et al., 2019; Galizi et al., 2016). It would however in theory be possible to be designed for other species that have an XY-gonosome system.

2.3.3 Toxin-antidote-based gene drive techniques

a. Exposure and Hazard Potential

The super-Mendelian inheritance of these techniques is based on the combinations of toxin and antidote contained in the GD construct. Only offspring carrying the construct and thus the antidote are viable. This group includes Medea (Akbari et al., 2014), Underdominance (Akbari et al., 2013; Reeves et al., 2014), and Killer-Rescue Drives (Gould et al., 2008) as the most prominent examples. Regardless of the particular technique, at least the gene for the antidote is bound to an effector gene. These techniques are characterized as passive, mostly self-sustaining (exception killer-rescue), threshold-dependent, rather local modification drives. Their exposure potential is in principle lower than that of the previously presented techniques, but the exact estimation depends on the respective technique.

b. Reliability

The greatest vulnerability is the selection pressure of naturally evolved resistances, pre-existing resistances due to sequence variations or the inactivation of the toxin.

c. Options for Risk Mitigation

Since these techniques are threshold dependent, it is proposed to release wild types to limit the spread of GDs. But also, secondary drives have been proposed which in addition to a new toxin-antidote combination contain the antidote gene of the first drive. As with all secondary releases, they would have to cover the whole area the original drive release has spread to, which will become less feasible over time (as pointed out by Nick Barton at a workshop on the controllability of gene drives, (Giese et al., 2019).

2.3.4 Maternal Effect Dominant Embryonic Arrest (Medea)

a. Exposure and Hazard Potential

Maternal Effect Dominant Embryonic Arrest (Medea) is a threshold-dependent self-sustaining modification drive. It is named after the sorceress from Greek mythology who after finding out that Jason, her husband, cheated on her, decided to kill the children she had with him. Its exposure is probably the highest of the currently known toxin-antidote-based GD techniques. This Medea is a synthetic version of the naturally occurring Medea elements discovered in the flour beetle, *Tribolium castaneum* (Beeman et al., 1992) but has also been reported in mice (Peters and Barker, 1993; Weichenhan et al., 1996).

The synthetic gene drive construct consists of a tightly-linked toxin-antidote combination. The maternal miRNA toxins against a gene essential in embryogenesis are expressed during oogenesis. The antidote consists of a recoded version of the targeted gene, immune to the miRNAs. The targeted genes may be *myd88*, *o-fut1* or *dah* in Drosophila. The mother poisons the embryos and only if the embryo also carries the construct it possesses the necessary antidote and thus is able to survive. The molecular mechanism of Medea is depicted in Fig. 6. In Fig. 7, all genotype combinations in a Medea drive are depicted.

Medea has been established in *Drosophila melanogaster* and *Drosophila suzukii.* In Lab Trials 25% homozygous D. melanogaster were able to drive Medea to fixation within 10-12 generations, fitness costs were estimated to be between 27.3% to 17.4% for different constructs. In mathematical *D. suzukii*-models for the myd88-construct, fitness costs for hetero- and homozygotes of 28% and 65%, respectively are assumed. (Akbari et al., 2014; Buchman et al., 2018a).

There are certain variations of this system harboring some differences in inheritance, invasiveness and effect.

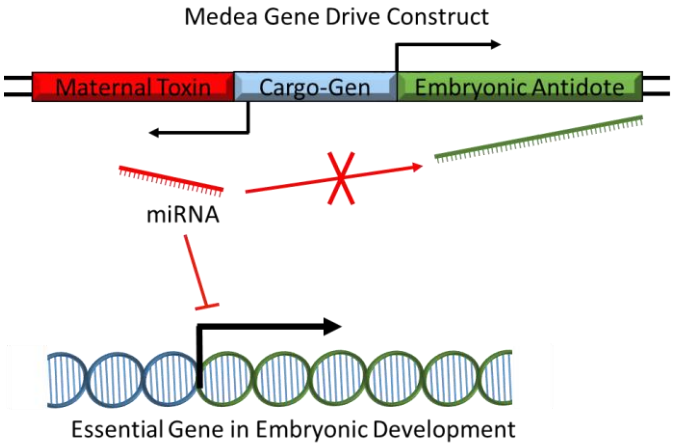

Fig. 6: The molecular mechanisms of the Medea technique is based on a toxin-antidote combination.
A maternally expressed miRNA toxin enriched in cytogenesis binds the mRNA of a gene essential in embryogenesis and thereby suppresses its expression, which would be lethal to the embryo. Only embryos which carry the construct also express a recoded version of the essential gene during embryogenesis. This version is immune to the toxin and allows the embryo to survive.

Medea			Female					
Male			+/+		M/+		M/M	
			+	+	M	+	M	M
	+/+	+	+/+	+/+	M/+	~~+/+~~	M/+	M/+
		+	+/+	+/+	M/+	~~+/+~~	M/+	M/+
	M/+	M	M/+	M/+	M/M	M/+	M/M	M/M
		+	+/+	+/+	M/+	~~+/+~~	M/+	M/+
	M/M	M	M/+	M/+	M/M	M/+	M/M	M/M
		M	M/+	M/+	M/M	M/+	M/M	M/M

Fig. 7: Possible genotype combinations in a Medea drive.
Only the genotypic wild type offspring of heterozygous Medea mothers are non-viable. +/+ = Wild type; M/+ = heterozygous GD-carrier; M/M = homozygous GD-carrier.

b. Reliability

Typically for toxin-antidote drives, the greatest vulnerability is the selection of by selective pressure naturally evolved resistances, pre-existing resistances due to sequence variations or the inactivation of the toxin. Buchman et al. (2018a) found pre-existing sequence variants of the targeted gene in five out of nine strains.

c. Options for risk mitigation

For Medea a secondary drive was proposed. This drive would consist of a novel toxin-antidote combination, as well as the old antidote (Akbari et al., 2014). This would make the secondary GDOs immune to the primary GDOs but not vice versa. This would mitigate the first drive but only by driving the second to fixation. Thus, the wild type population would be lost. Another option as with all threshold-dependent drives would be the release of enough wild types to push the GDO-ratio below the threshold.

d. Planned Applications

Medea was established in *Drosophila melanogaster* (Akbari et al., 2014; Chen et al., 2007) but is planned to be used against the cherry fruit fly *Drosophila suzukii* (Regalado, 2017). This invasive species is damaging the yield of cherry orchards in the US, as the sharp ovipositor allows females to lay their eggs in hard-skinned fruit. The aim is either to reduce the fertility of the flies, or possibly to induce a sensitivity to otherwise non-lethal chemicals in the population (Buchman et al., 2018a).

2.3.5 Inverse Medea

a. Exposure and Hazard Potential

While Medea causes the wild type offspring of Medea-mothers to die, Inverse Medea causes heterozygous offspring of wild type mothers to die. Instead of a maternal toxin and an embryonic antidote, Inverse Medea employs an embryonic toxin and maternal antidote. This is easily constructed, by switching the promotors in a Medea-construct. This drive

would classify as a passive threshold-dependent self-sustaining modification drive. Dependent on the assumed fitness cost of homozygous carriers the threshold varied in the model calculations. For instance, a homozygous fitness cost (s) of zero corresponds to a threshold of 50%, while s = 0.05 corresponds to a threshold of approx. 55%. Fig. 8 shows all possible genotype combinations in an Inverse Medea drive. On top of the fitness dependence, this drive system would require a higher threshold, since the only non-viable offspring are Medea-carriers. The authors see this as a benefit, ensuring confineability. (Marshall and Akbari, 2015; Marshall and Hay, 2011)

Inverse Medea			Female					
Male			+/+		M/+		M/M	
			+	+	M	+	M	M
	+/+	+	+/+	+/+	M/+	+/+	M/+	M/+
		+	+/+	+/+	M/+	+/+	M/+	M/+
	M/+	M	~~M/+~~	~~M/+~~	M/M	M/+	M/M	M/M
		+	+/+	+/+	M/+	+/+	M/+	M/+
	M/M	M	~~M/+~~	~~M/+~~	M/M	M/+	M/M	M/M
		M	~~M/+~~	~~M/+~~	M/M	M/+	M/M	M/M

Fig. 8: Possible genotype combination in an Inverse Medea drive.
Only offspring heterozygous for Inverse Medea of wild type mothers are non-viable. This reduces the spread of the drive as only drive-carrying mothers propagate viable carrier-offspring. +/+ = Wild type; M/+ = heterozygous GD-carrier; M/M = homozygous GD-carrier.

b. Reliability

Since this technique is only theoretically explored, no true vulnerabilities are known. It is however likely that Inverse Medea will suffer from the same vulnerabilities common to other toxin-antidote systems as well.

c. Options for Risk Mitigation

It is feasible that the same mitigation strategies as for Medea could be applied in an Inverse Medea drive.

2.3.6 Semele

a. Exposure and Hazard Potential

This theoretically explored single-locus system is based on a toxin expressed in the semen of the Semele-males. This toxin is supposed to either kill the females or render them infertile unless they carry the antidote. This technique, just as Medea, also refers to Greek mythology. Semele was a mortal woman with whom Zeus became infatuated, she died upon witnessing his divinity. The construct consists of a semen-based toxin targeting a gene essential for female survival or fertility. The toxin would be expressed either in the accessory glands or the male germ line, while the antidote would be expressed in female somatic tissues or in the female germline for deposition in the egg. Only-male releases

would cause population non-gene drive suppression. The threshold was calculated to be at 36%, assuming no fitness penalty associated with the drive. A Semele-construct is depicted in Fig. 9, while the possible genotype combinations are shown in Fig. 10. (Marshall et al., 2011; Marshall and Akbari, 2015).

Releases of both sexes would constitute a passive, threshold-dependent, self-sustaining modification drive.

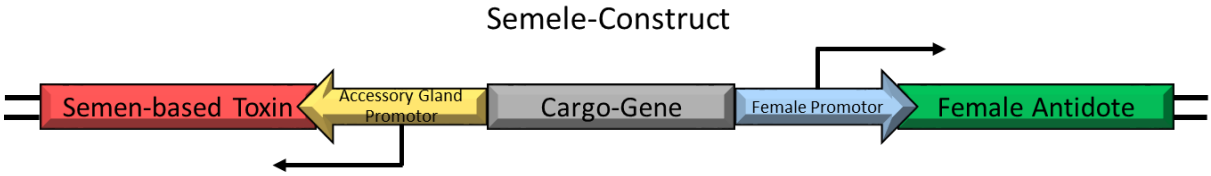

Fig. 9: Outline of a Semele-construct.
A semen-based toxin gene is under the control of an accessory gland promotor while the female antidote is under the control of a female promotor. Thus, all females mating with Semele-males die, unless they are Semele-females.

Semele		Female					
		+/+		S/+		S/S	
		+	+	S	+	S	S
Male	+/+ +	+/+	+/+	S/+	+/+	S/+	S/+
	+/+ +	+/+	+/+	S/+	+/+	S/+	S/+
	S/+ S	~~S/+~~	~~S/+~~	S/S	S/+	S/S	S/S
	S/+ +	~~+/+~~	~~+/+~~	S/+	+/+	S/+	S/+
	S/S S	~~S/+~~	~~S/+~~	S/S	S/+	S/S	S/S
	S/S S	~~S/+~~	~~S/+~~	S/S	S/+	S/S	S/S

Fig. 10: Possible genotype combinations in a Semele drive.
No offspring can arise between Semele-carrying males and wild type females. +/+ = Wild type; S/+ = heterozygous GD-carrier; S/S = homozygous GD-carrier.

b. Reliability

Since this technique is only theoretically explored, no true vulnerabilities are known. The authors propose Semele would be an excellent option for confined population replacement as it initially suppresses before replacement (Marshall and Akbari, 2015). It is however likely that Semele will suffer from the same vulnerabilities common to other toxin-antidote systems as well.

c. Options for Risk mitigation

It is feasible that the same mitigation strategies as for Medea could be applied in a Semele drive.

2.3.7 **Medusa**

a. Exposure and Hazard Potential

Medusa is an acronym for sex chromosome associated Medea. It consists of two Medea constructs where antidotes are switched (similar to two-locus Underdominance UD^{mel}). This theoretical construct is depicted in Fig. 11 and constitutes a passive threshold-dependent self-sustaining suppression drive. Both constructs are necessary to be viable all offspring with only one of the two constructs will die. Since one construct is located on the Y-chromosome, all female offspring will die (Marshall and Hay, 2014). Fig. 12 shows the possible combination of genotypes in a locally confinable Medusa suppression drive.

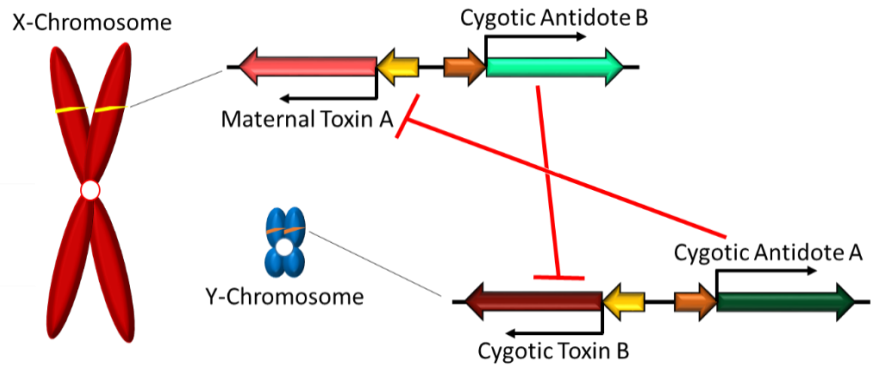

Fig. 11: Molecular mechanism of a Medusa drive.
Located on the X-chromosome, there is a construct with a maternal toxin and a zygotic antidote against a zygotic toxin located on the Y-chromosome together with the antidote to the maternal toxin. Necessity of both constructs to be viable when mothers express the maternal toxin selects for males carrying both constructs as well as females that carry the X-chromosomal construct.

Medusa			Female			
Male			X^aX^a		X^AX^a	
			X^a	X^a	X^A	X^a
	X^aY^b	X^a	X^aX^a	X^aX^a	~~X^AX^a~~	~~X^aX^a~~
		Y^b	X^aY^b	X^aY^b	~~X^AY^b~~	~~X^aY^b~~
	X^aY^B	X^a	X^aX^a	X^aX^a	~~X^AX^a~~	~~X^aX^a~~
		Y^B	X^aY^B	X^aY^B	X^AY^B	X^aY^B
	X^AY^B	X^A	X^AX^a	X^AX^a	~~X^AX^A~~	~~X^AX^a~~
		Y^B	X^aY^B	X^aY^B	X^AY^B	X^aY^B

Fig. 12: Possible genotype combinations in a Medusa drive.
The peculiar combination of a maternal effect male biased sex distorter causes female offspring of carrier mothers to be non-viable. Female carriers must have wild type mothers. X^a = wild type X-chromosome; X^A = Medusa X-Chromosome; Y^b = wild type Y-chromosome; Y^B = Medusa Y-chromosome.

b. Reliability

Since this technique is only theoretically explored, no true vulnerabilities are known. The authors propose Medusa an excellent option for confined population suppression in preparation for an invasive X-shredder drive (Marshall and Akbari, 2015; Marshall and

Hay, 2014). It is likely that Medusa suffers the same vulnerabilities as other toxin-antidote systems.

c. Options for Risk Mitigation

It is feasible that the same mitigation strategies as for Medea could be applied in a Medusa drive.

2.3.8 Underdominance

a. Exposure and Hazard Potential

The genetic phenomenon, also known as heterozygote inferiority, Underdominance (UD) describes alleles that when heterozygous confer a fitness penalty or a more severe fitness penalty than when homozygous. This phenomenon can be utilised in engineered gene drive techniques. There are different approaches, one UD^{mel} (Akbari et al., 2013) and *Rpl14* (Reeves et al., 2014) to mention the probably best known examples. Both approaches have been engineered in *Drosophila melanogaster*. One approach is operated by two gene constructs. Each construct consists of a maternal toxin gene and an embryonic antidote. However, the antidote to each toxin is located on the other construct. Thus, an embryo needs both constructs in order to have both antidotes to the maternally administered toxins (Fig. 13). Therefore, UD heterozygotes have a lower fitness than homozygotes (Reeves et al., 2014). The constructs can be located in the same locus on homologous chromosomes or on different chromosomes (two-locus Underdominance). Fig. 14 illustrates the molecular mechanism of the UD^{mel} Underdominance by Akbari et al (2013).

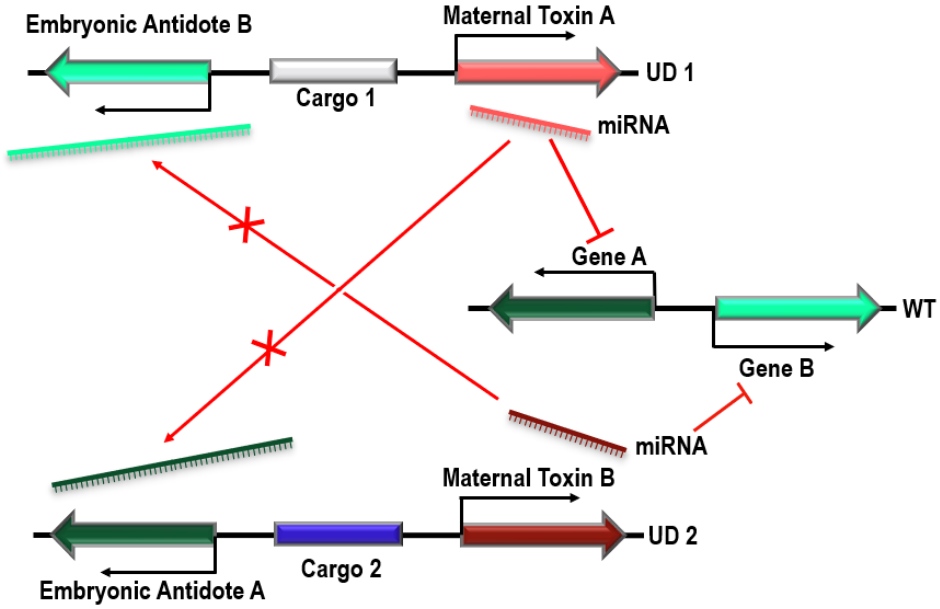

Fig. 13: The UD^{MEL} system is composed of two constructs.
UD^{MEL}-1 consists of maternal toxin A (light red) and embryo antidote B (light green), and UD^{MEL}-2 consists of maternal toxin B (dark red) and embryo antidote A (dark green).UD^{Mel} can be used in a single locus approach where each construct is located on one part of a homologous chromosome pair or a two locus approach where the constructs are located on non-homologous chromosomes.(adapted from Akbari et al., 2013).

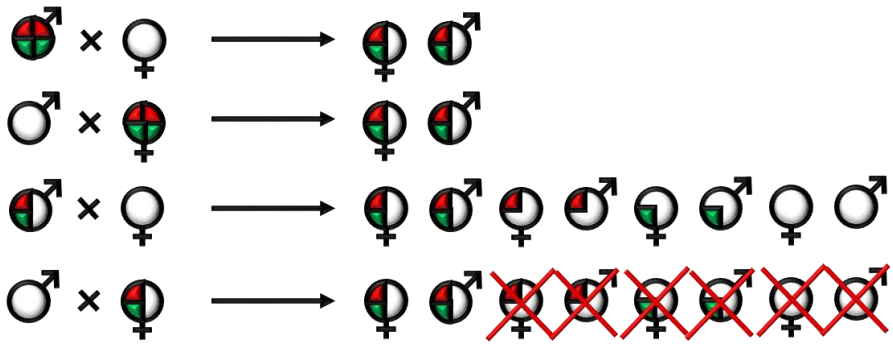

Fig. 14: Inheritance of Two-Locus Underdominance.
The lethal toxin is administered from GD-carrying mothers to their embryos. The necessity to carry both constructs in order to be viable results in Underdominance.

The toxins of the UD^{mel} Two-Locus Underdominance constructs are the same as utilised in the Medea technology: *myd88*, *dah* and *o-fut-1* (Akbari et al., 2013). Since these toxins are administered maternally, a release of wild type males into a replaced Underdominance population would lead to a population crash, as all offspring would inherit the wrong antidote (Akbari et al., 2013). A UD gene drive requires a high threshold release (National Academies of Sciences, 2016).

The single locus approach uses RpL14, a cytoplasmic ribosomal protein which is haploinsufficient as a target gene. It also relies on a miRNA toxin and a recoded version of the targeted gene as antidote. In this UD approach the underdominant genotypes do not die but have reduced fitness. The threshold is estimated to be as high as 61% of the total population (Reeves et al., 2014).

For both approaches, an intentional underdominant population transformation is inherently reversible where it is realistically possible to release sufficient wild type individuals to traverse the unstable equilibrium in the lower frequency direction (Gokhale et al., 2014).

b. Reliability

As with all toxin-antidote-based techniques, the greatest vulnerability is the selection of by selective pressure naturally evolved resistances or the inactivation of the toxin.

c. Options for Risk Mitigation

The release of wild type specimen represents the most obvious option to potentially mitigate adverse effects of the drive.

2.3.9 Translocation Drive

a. Exposure and Hazard Potential

First put forward by (Serebrovskii, 1940) and again later by Curtis (1968a), this technique may be called the first anthropogenic gene drive. As the name implies, this technique relies on the mutual exchange of chromosomal segments between two non-homologous chromosomes. At the time, it was considered to drive especially disease-refractory cargo genes which would be linked to a chromosomal break point. Translocation-strains were reared using radiation. This however led to a low fitness of specimens and the cargo genes

failed to spread (Lorimer et al., 1972). Progress in sequencing, synthetic biology (Egli et al., 2004; Golic and Golic, 1996) and not at last the discovery of programmable homing endonucleases, the induction of translocations is no longer reliant on radiation. Since a heterozygous translocation during meiosis leaves a gamete with a duplicated segment and a lack of another, these aneuploidy gametes are usually sterile, while homozygotes produce viable euploid gametes. Thus, a form of underdominance arises. The mode of action of translocations is a mode of its own, thus this technique has to be categorized neither as active nor passive. Despite this, Translocation Drives qualify as a high-threshold, self-sustaining modification drive technique. Although it stands to argue whether engineered translocations would be able to drive a trait to fixation in a wild population, *in vivo* and *in silico* observations warrant further exploration, as stated by Buchman et al. (2018b).

2.3.10 **Killer-Rescue**

a. Exposure and Hazard Potential

First proposed by Gould et al. in 2008, the Killer-Rescue System consists of two unlinked loci; one encoding a toxin (killer allele), the other encodes an antidote (rescue allele) (Gould et al., 2008). Apart from other toxin-antidote-based techniques, here the two genes are unconnected, positioned in different loci. Furthermore, a cargo gene can be fused to the antidote gene, depicted in Fig. 15. Homozygous carriers of both genes would be mass-released into wild populations, offspring which inherits the killer allele but not the rescue allele would be non-viable. Since both alleles are not linked in their inheritance the killer allele will be quickly selected from the population, while the rescue allele confers a clear fitness gain and will increase in its prevalence. As soon as the killer allele completely disappeared from the population, so will the rescue allele's fitness gain. Consequently, the rescue allele will again decline in its prevalence unless the cargo gene confers a gain in fitness. This system is designed to be a self-limiting modification drive in which, if the cargo gene bears a fitness penalty, its prevalence in the population would decrease after a number of generations. The inheritance of the Killer-Rescue GD is shown in Fig. 16. There is a possible variant where multiple copies of the killer allele are incorporated into the GDOs' genome, enhancing the selective benefit of the rescue allele.

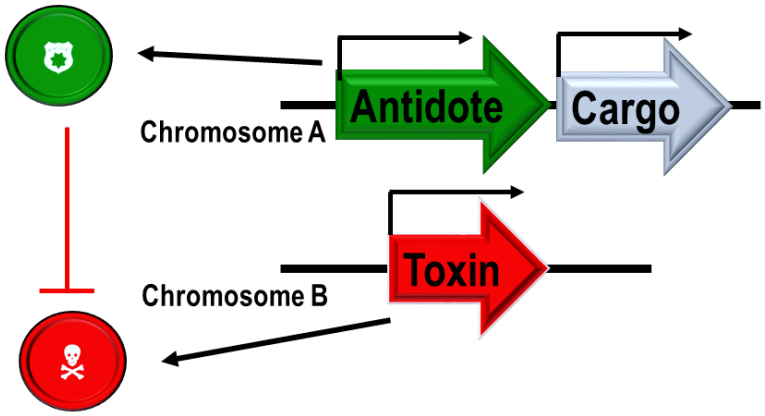

Fig. 15: Function of the Killer-Rescue construct.
Toxin and antidote gene are located on different loci. The cargo gene is fused to the antidote gene.

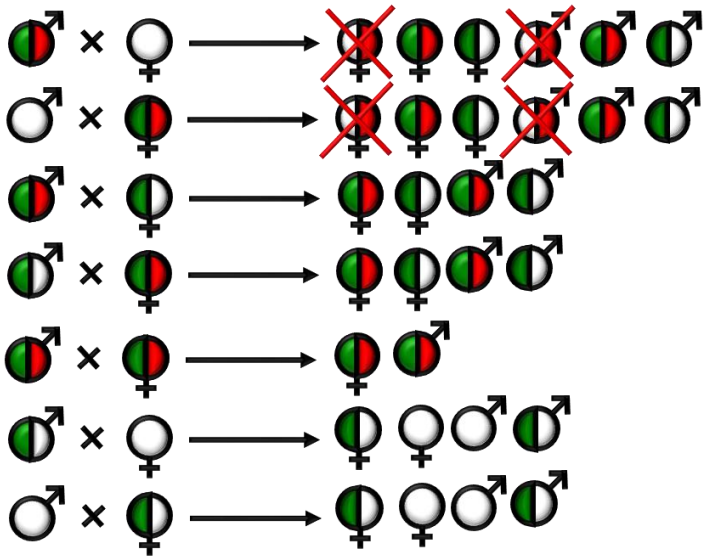

Fig. 16: Inheritance of Killer-Rescue gene drive.
Both constructs are independently inherited, carriers of the toxin gene (red) without the antidote gene (green) are none viable.

b. Reliability

Although the character of the toxin and antidote are not specified, it would be recommendable to use miRNA as a killer allele in order not to confer a potential toxic quality on the GDOs.

c. Options for Risk Mitigation

Since it is expected that the Killer-Rescue system has a high invasion threshold (although lower than that of two-locus Underdominance). The most feasible option to limit the spread of this gene drive would be the release of wild types.

2.3.11 Cleave and Rescue

a. Exposure and Hazard Potential

Engineered by (Oberhofer et al., 2019), this toxin-antidote system relies on CRISPR/Cas. Consisting again of a tightly linked combination of in this case CRSIPR and gRNA (cleave) toxin and an antidote, again a recoded version of a targeted essential gene (rescue). This technique apart from Medea and UD^{mel} does not rely on maternal effect killing, instead both parents cause death of non-carrier offspring, ensuring super-Mendelian inheritance. Although female carriers are more effective due to maternal toxin carryover. Also, the toxin does not only suppress gene expression by RNAi but completely deletes the targeted gene. This makes Cleave and rescue hard to shelf in the categories of active and passive drives. It has to be classified as both. Furthermore, it can be characterized as a self-sustaining threshold dependent modification drive. Its qualities make it comparably invasive as a HEG-drive while it seems less prone to resistance formation since it is not reliant on repair pathways.

It is even constructed in a way that both HDR and NHEJ in the targeted Gene cause a loss of function mutation. The authors imply the technique may be easily adapted to any target species without intricate knowledge of embryonic gene regulation as only the sequence of an essential gene must be known. Cleave and Rescues mode of action depicted in Fig. 17. (Oberhofer et al., 2019)

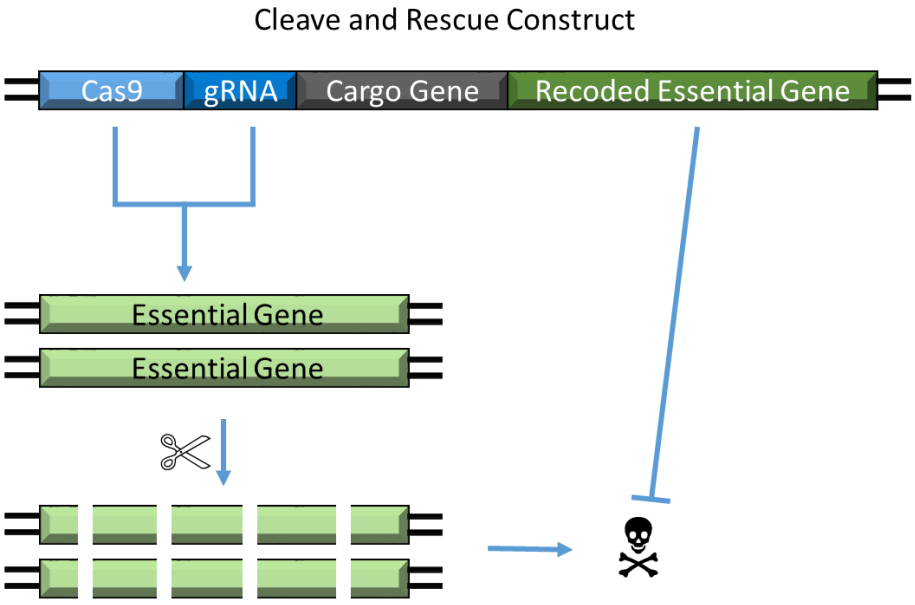

Fig. 17: Mode of action of a Cleave and Rescue drive.
The construct consists of Cas9 and gRNAs targeting an essential gene on a different chromosome pair, as well as a recoded version of that essential gene and potential cargo genes. The essential gene is cleaved by CRISPR/Cas only the recoded antidote gene rescues from death.

b. Reliability

Oberhofer et al. (2019) established this GD technique in *Drosophila melanogaster* and confirmed its efficiency in different strains from five continents. Arguing for a low probability of resistance formation and pre-existing resistances respectively. Although null-mutations in the Cas9 gene should stop the drive. It was shown that even if most Cleave alleles were deactivated in a population, the rescue-gene still confers a fitness gain keeping the drive active. (Oberhofer et al., 2019). The underlying assumption for this is that multiplexing gRNAs can prevent cleavage-resistant but functional alleles.

c. Options for Risk Mitigation

Since this technique is threshold dependent, although the threshold was calculated to be comparably low at 31.5%, it may be feasible to shift the ratio of carriers in a population by releasing wild types. But again, as Nick Barton pointed out at a workshop on control options for gene drives, wild type releases become increasingly unfeasible with an increasing number of GDOs over an increasing area. Hence, such wild type releases would have to be conducted shortly after the release of the original drive. Furthermore, the comparably low threshold of Cleave and Rescue would require very large quantities of wild types to be released. Therefore, secondary drives similar to those that target other HEG-drives may be more feasible.

d. Modelling Cleave and Rescue

To simulate the invasiveness of the Cleave and Rescue drive a generic, iterative deterministic model approach is chosen. The model is identical to the model presented by Frieß et al. (2019). It assumes panmixis in a large population with non-overlapping generations. The model solely focusses on the invasive capabilities of the drive due to its genetics and the the effect of maternal carry-over effect also assumed in the original publication by Oberhofer et al. (2019). Where, an egg of a heterozygous carrier already contains the CRISPR/Cas riboprotein complex during fertilization, even if the GD construct is not present in the genome. This is depicted in Fig. 18.

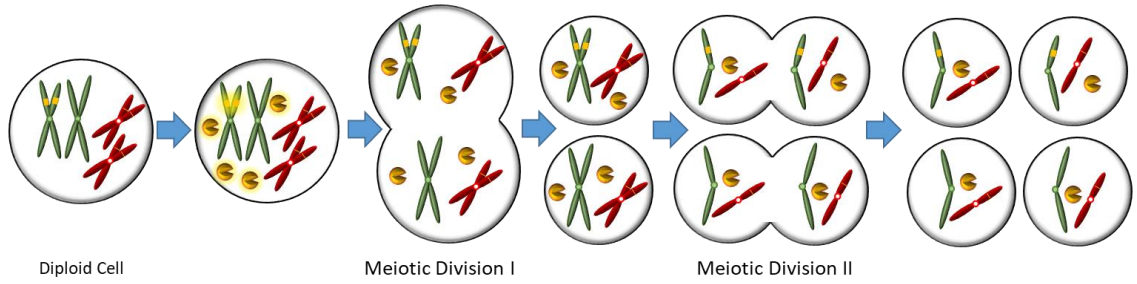

Fig. 18: Maternal carry-over effect with Cleave and Rescue.
During meiosis, previously formed CRISPR/Cas riboprotein complexes can be transferred into the newly formed gametes, irrespective of the presence of genes belonging to the gene product. This Carry-Over effect can thus also lethally cut the target gene in the absence of the Rescue gene.

As in the paper, the model assumes lethal hemicygosity with regard to the essential target gene. Furthermore, it is assumed that both the gene drive construct and the target gene are located on autosomes. Based on these assumptions, an inheritance scheme as shown in Tab. 2 is derived.

Tab. 2: Inheritance scheme of Cleave and Rescue with probabilities.
Green: female parent; Orange: male parent; Grey: offspring; Red: non-viable; +: wild type allele; C: Cleave and Rescue construct; *: cleaved target gene; ☠: dead

+/+♀	+/+	C/+*	C/C	□
+/+♂	1	0	0	0
C/+*♂	0	0.5	0	0.5
C/C♂	0	1	0	0
C/+♀	+/+	C/+*	C/C	□
+/+♂	0	0.5	0	0.5
C/+♂	0	0.5	0.25	0.25
C/C♂	0	0.5	0.5	0
C/C♀	+/+	C/+*	C/C	□
+/+♂	0	1	0	0
C/+♂	0	0.5	0.5	0
C/C♂	0	0	1	0

In the model, the probability of individuals mating and producing offspring is only dependent on their percentile occurrence in the population and the genotypic fitness. The fitness is also included as a value between 0 and 1 where the wild type always has a maximum fitness of one. The population ratio of gene drive organisms (GDOs) after release can be set by the user as well as the fitness of the transgenic genotypes. For the sake of automation, the fitness penalty for heterozygous GDOs was assumed to be half that of homozygous carriers. In a recurrence calculation the population percentages are determined for each generation allowing to follow the population dynamics and investigate the invasiveness of the Cleave and Rescue drive.

In a test run, we assume a fitness penalty of 20% for homozygotes, resulting in a 10% penalty for heterozygotes, respectively. If enough homozygous GDOs to make up 8% of the total population are released, then a population replacement will occur (Fig. 19). Note that, although after 50 generations 80% of the population make up homozygous GDOs, the other 20% will retain heterozygous for many generations. This is due to the non-viability of genotypic wild type offspring and the lowered fitness of the GDOs.

For the following test run, the initial release ratio is decreased by 1% to 7%. As Fig. 20 shows, this release ratio does not exceed the necessary threshold and thus the GDOs get selected from the population over many (50 to 60) generations.

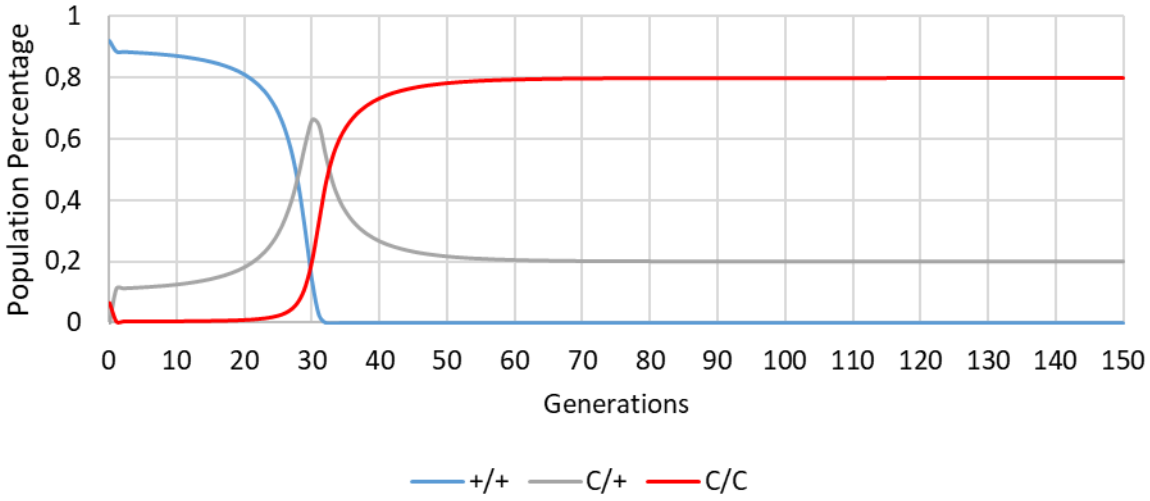

Fig. 19: Population dynamics with a 10% fitness penalty per GD allele and a homozygote release ratio of 8%.

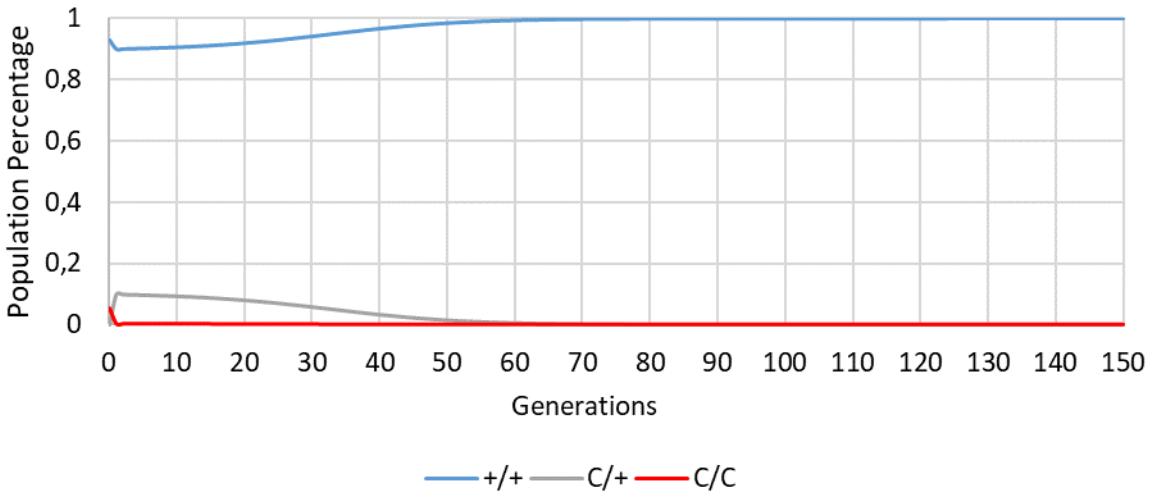

Fig. 20: Population dynamics with a 10% fitness penalty per GD allele and a homozygote release ratio of 7%.

In the model approach, whether a gene drive successfully replaces the wild type population or is lost over the generations is majorly dependent on the two variable factors fitness and initial population percentage. These may vary with respect to the applied gene drive technique, cargo gene, target organism, target region and other subtle effects and are hence case-specific. Therefore, the approach seems suitable to prospectively quantify the invasiveness of gene drive techniques. Wherein, a gene drive technique that would achieve a complete population replacement within a certain amount of generations with a lower release threshold than another system would be deemed more invasive.

In this computational approach, only two major factors of such a gene drive system can be altered, namely fitness and initial population percentage. Although possible, it would be tedious to now iterate the variables by hand to determine the thresholds for a population replacement or a suppression of the wild type, respectively. Instead, this is automated in a program written for this purpose in the following step. The program iterates each parameter in 1%-increments for a given generation post-release. This means the computation yields cross sections of 10,000 data points (100 × 100), each data point is a combination of fitness and release population percentage. The program judges upon

variable thresholds in which out of three categories each of the data points is to be put. The three categories are: wild type suppression/replacement, intermediate state and loss of gene drive construct. The thresholds were chosen as 5% and 95%. This means if the wild type population percentage at a given post-release generation reached values below 5% the data point is considered in the suppression category. If the wild type population percentage reached values above 95% the gene drive construct is considered lost for that data point. Any population percentages between 5% and 95% are considered as intermediate states. This is illustrated for Cleave Rescue 10 generations post release in Fig. 21. These cross sections can then be generated for any post release generation. For replacement drives, it is common that over the generations, the blue area grows from the lower left corner, while the red area grows from the upper right corner towards the centre until both areas collide. An overlay of cross sections in five generational steps from generation 5 to 35 is presented in Fig. 22.

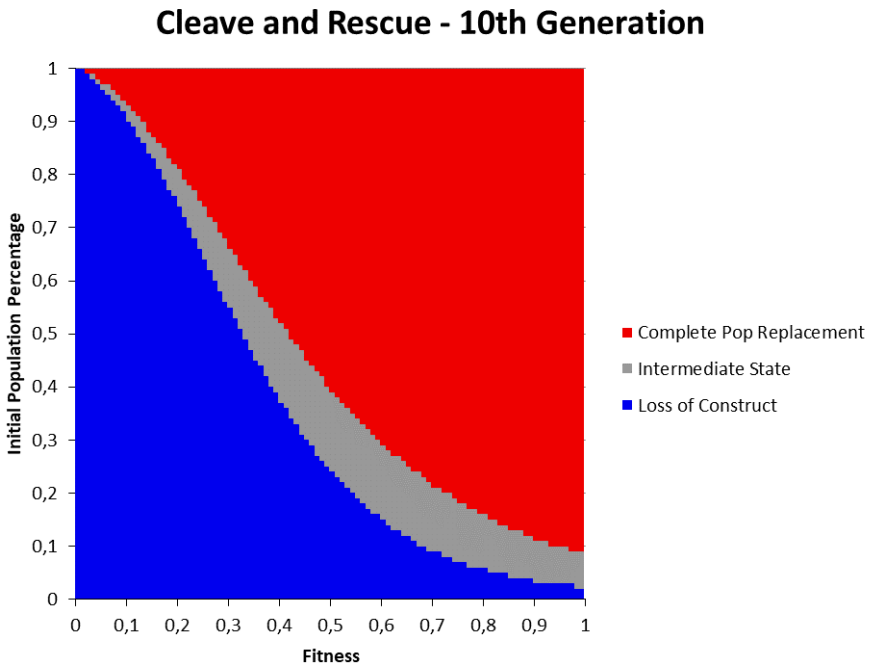

Fig. 21: Cross section of Cleave and Rescue in the 10th generation post homozygote release.
Red: Wild type population percentage below 5%; Grey: wild type population percentage between 5 and 95%; Blue: wild type population percentage above 95%.

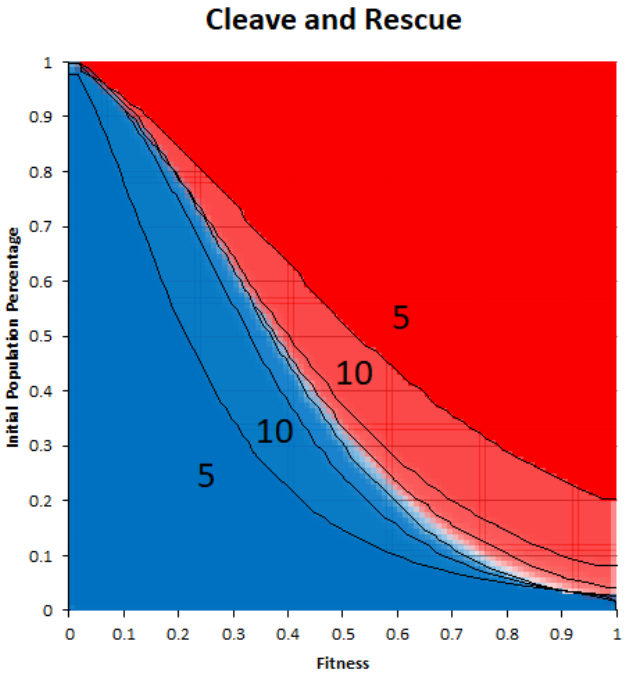

Fig. 22: Overlay of cross sections from generation 5-35 in 5 generational steps.
Red: Wild type population percentage below 5%; Grey: wild type population percentage between 5 and 95%; Blue: wild type population percentage above 95%. Black lines inserted by hand for clarity.

To then quantify the invasiveness of the drive system for each of those examined generations post release, the number of red data points is divided by the number of blue data points. When these ratios are plotted against the generations, the result is a curve that closes in on a fixed value. This value could be used as a means to represent the invasiveness of a gene drive system, dependent on the systems inheritance alone.

In

Fig. **23**, the curves for different gene drive techniques including Cleave and Rescue are shown. Note, that Cleave and Rescue scores as the second highest technique after CRISPR homing-drives, considering the point of approximation. Furthermore, including resistance formation into the CRISPR-homing drives elevates Cleave and Rescue to the most invasive technique yet.

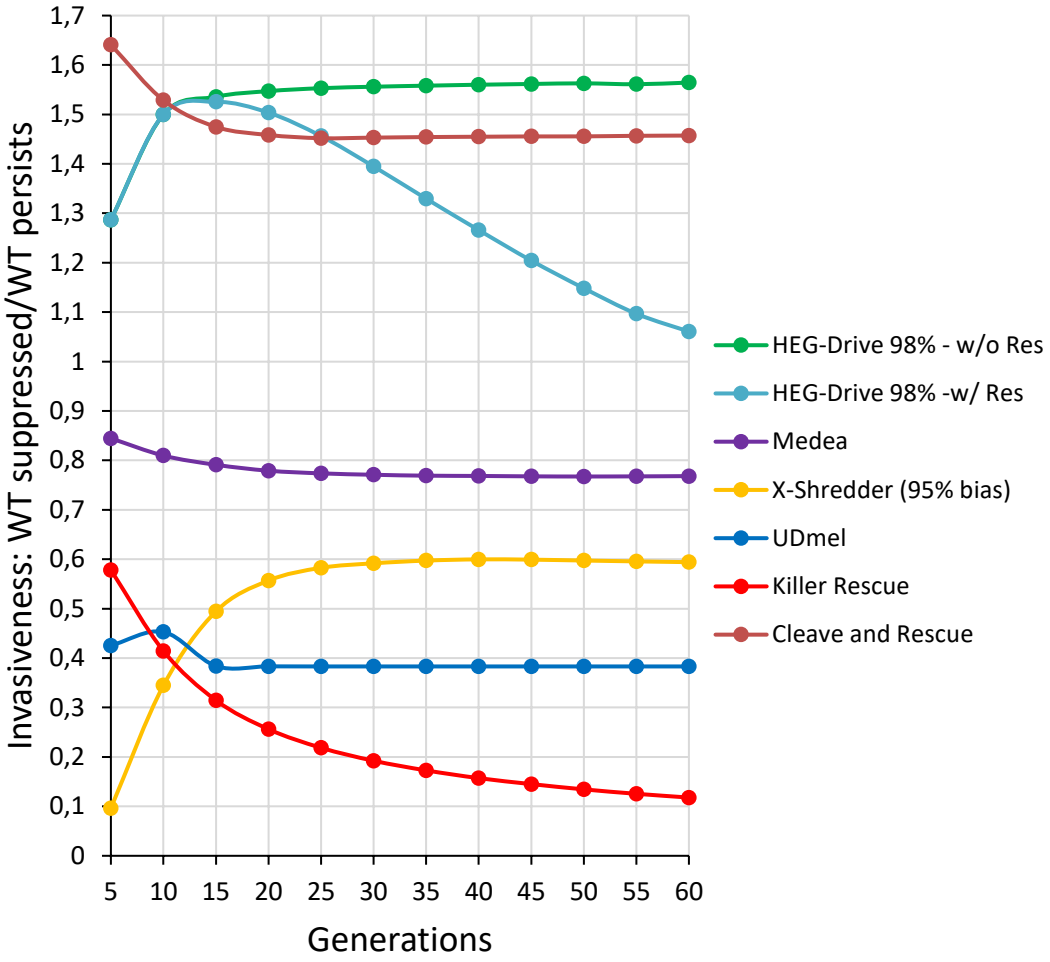

Fig. 23: Invasiveness as ratio of complete population replacement vs. loss of gene drive construct per generation.
Each gene drive technique shows an asymptotic behaviour with the exception of the CRISPR-GD, due to the formation of resistance alleles (labelled as w/ Res). A comparable CRISPR/Cas-mediated gene drive without resistance allele formation (labelled as w/o Res) shows asymptotic behaviour as well. Cleave and Rescue is highly invasive, even more invasive than a HEG-drive, considering resistance formation. (taken from Frieß et al. 2019 with the addition of a curve for cleave and rescue).

2.4 Technology Characterisation – Summary

The comparative technology characterisation revealed differences in the spectrum of power and range which inevitably lead to a range of potential hazards and exposure. For instance, GDs may employ different mechanisms to ensure their mode of inheritance. From more or less intricate toxin-antidote systems as Medea, Underdominance, Killer Rescue to the biased segregation of sex chromosomes during meiosis (X-Shredder, Y-CHOPE). An extreme potential with regard to power and especially range could be identified for endonuclease-based gene drives using the CRISPR/Cas9-system. Moreover, as for some other GD techniques, its probability of failure is comparably high. The outstanding potential of HEG-drives was also illustrated by the assessment of the range based on invasiveness of different gene drive-techniques according to their inheritance schemes in a publication by Frieß et al. (2019). Along with power and range uncertainties and ignorance rise with

a) **the extent of known unknowns regarding potential effects of known dependencies and relationships of the target species and possibly affected non-target species and**
b) **not yet determinable effects (unknown unknowns) due to unknown relationships or the inherent instability of genetic information which becomes more relevant with increasing numbers of gene drive-modified organisms.**

In the light of the absence of proven options to a) correct potential damage or b) just to limit the inherently self-propagating mechanism of GDs, these properties reveal important 'reasons for concern' with regard to the requirements of the precautionary principle.

3 Part A.1 - Confinement Strategies[5]

Bernd Giese, Johannes L. Frieß

Gene drives (GDs) have raised great expectations in terms of public health and nature conservation, but also serious concerns because of their inherent functionality to spread and invade natural populations. Besides posing a paradigm shift for the release of genetically engineered organisms (GMOs), as this novel technology is intended to spread within wild populations. Thus, a released GD would represent an unprecedented intervention into natural populations and their molecular foundation, actively interfering in their gene pool, transforming them into GMOs themselves. At the current stage of development, a GD could hardly be retrieved post-release. Potential impacts of GD-applications are complex and investigation into them are still at the very beginning. Up to now, consequences are not foreseeable in the case of malfunction. Thus, it is paramount, should this technology path really be pursued, precautionary risk management finds ways to adequately deal with lacking knowledge up to complete ignorance about potential adverse effects. In risk management, there are three main options for risk reduction. First, containment strategies to reduce exposure. Second, substitutional alternative technologies which fulfil comparable benefits but at lower hazard- and exposure potentials. Third, choosing low risk development paths during early innovation phases. This report focuses on and evaluates different strategies of confinement, namely containment and limitation strategies, proposed for GDs.

3.1 Introduction

The potential release of organisms carrying a GD bears a fundamental change in the release practice of GMOs. GMOs as gene drive organisms (GDO) would arise from wild populations in wild habitats, instead of in the laboratory or breeding facility and in controlled numbers. GDs are an ideal tool for the efficient manipulation of wild populations of sexually reproducing species, due to their inherent ability to overcome the limits of Mendelian inheritance – even for traits with detrimental effects on their fitness. The inspiration to use GDs came with the discovery of naturally occurring mechanisms like transposable elements or meiotic drives that trigger a super-Mendelian inheritance of certain traits within a population. The use of selfish genetic elements was proposed in 1994 by Hastings (Hastings, 1994) after early proposals to harness chromosomal translocations for population control (cp. Curtis, 1968). Already in 2003 by Austin Burt (Burt, 2003), the idea of using homing endonucleases to build self-replicating drives was put forward which are now realized with the help of the versatile molecular scissor CRISPR-Cas9. While so called 'modification drives' aim at the spread of new traits, 'suppression drives' are created to confer a reduction or even a regional extinction of pest species or vectors of pathogens. Suppression drives are envisaged to strongly reduce the number of some prime vector mosquito species for infectious diseases like malaria and dengue (Macias et al., 2017). GDs are considered to be applied against a number of invasive species that have become agricultural pests like the cherry fruit fly *Drosophila suzukii* in California (Buchman et al., 2018a; Regalado, 2017) or rodents like mice or rats in New Zealand which pose a serious threat to agriculture and the native environment (Dearden et al., 2018). In this regard, GDs are anticipated to be a highly specific replacement for pesticides, Even weeds have been proposed as targets for suppression drives (Neve, 2018). Currently, modification drives are developed to give disease refractoriness to mosquitoes or potentially to inhibit the agricultural damage caused by the cherry fruit fly due to its serrated ovipositor (Regalado, 2017).

5 This chapter represents an update and extension of the work on options for risk reduction of GDs in Giese et al. (2020).

In the course of the discussion about GD development and their potential applications, a number of reasons for concern have been raised (Esvelt and Gemmell, 2017; Ledford, 2016; National Academies of Sciences, 2016; Oye et al., 2014). GD represents a powerful genetic tool with an as of yet unprecedented range in time and space. While up to now, releases of GMOs have been limited to a certain number of engineered organisms (mostly plants) within a limited timeframe and confined to a limited area with more or less established separation from wild relatives to prohibit gene flow to wild relatives. In contrast, the very aim of a GD-application is focused on the fast, vertical gene transfer. In recent years, a number of options to ensure control or even a kind of functional reversibility have been proposed. However, a proof of concept for their potential functionality, reliability, feasibility and stage of development is still missing. Thus, additionally to variants of GD-techniques designed for higher controllability, by increasing reliability or decreasing hazard- and exposure potential as sources of possible risks, alternatives to GDs are as well included in the assessment.

But it would be a misconception to believe the technology itself is the only factor in the generation of risks. Additional exposure- and hazard potentials depend on the qualities and the vulnerability of the specific ecosystems into which the GD is introduced and on the specific aims and contexts of the application (e.g., agriculture, vector control or nature conservation). Beyond these known adverse properties nearly any biochemical quality, e.g., an enzymatic feature, may turn out hazardous in a particular context. Thus, a characterisation of the hazard potential is complicated by corresponding non-knowledge on the final behaviour of the GD in very early innovation phases when experimental test results are not yet available or more likely unobtainable. Thus, especially in anticipation of environmental release, as a precautionary approach, it is advisable to primarily focus on the exposure potential. High exposure strongly increases the possibility of unforeseen interactions in the environment, and concomitantly increases the dimensions of ignorance about possible adverse effects. Which is precisely the lesson that had to be learned from the release of persistent synthetic chemicals into the environment (e.g. CFC and POPs).6 Focusing on exposure-relevant qualities yields options on how to limit or even decrease the exposure potential emanating from GDs. The potential for unforeseen and unmanageable interactions of GDs in the environment may thereby be reduced. Thus, reduction of the exposure potential is a promising approach of risk reduction for GD.

The exposure potential of GDs is determined by qualities of the GD or the GDO that are related to a) the spatial and b) the temporal spread. These could be for example

- **stability of the GD against inactivation by mutations,**
- **impact of the GD on the fitness of the target species,**
- **frequency of inheritance.**

With regard to the target species the following qualities may have an influence:

- **mobility,**
- **life expectancy,**
- **inheritance,**
- **number of offspring,**
- **probability of crossbreeding,**
- **frequency of releases and initial number of released individuals carrying the GD,**
- **regional distribution of the target population,**
- **interconnections between subpopulations.**

6 CFC production was phased out under the Montreal protocol (1989). POPs are subject to regulation und directive (EC) 2019/1021).

For GDs exclusively applied in laboratories, exposure to the environment is mainly determined by the containment of the experimental settings. This may be called *extrinsic containment* which relies on physical barriers. For an overview of extrinsic containment strategies read (Akbari et al., 2015; Benedict et al., 2008). Ecological containment can be seen as a special form of extrinsic containment, where spatial separation serves as a form of barrier to provide safety. Where in the geographic region of the GDO release or laboratory experiment wild type populations of the target species or wild relatives are lacking. Additionally, the settlement of these wild species should not be hindered by the prevalent environmental conditions. However, ecological containment is an option of limited reliability because GDOs might be transported intentionally or unintentionally as a stow-away on cargo ships, trucks or planes and some may as well survive adverse climate (Min et al., 2017a).

Furthermore, as Wright et al. (2013, p. 1223) put it: “Biology can achieve a lot in a contained environment; however, physical containment alone offers no guarantees. For example, no matter how ingenious a protective device or material may be for a GMO field application, an inventive way will eventually be found by an operator to compromise it. Failure in this case is a matter of when, not if. Although some form of physical containment is obviously prudent, inbuilt biological mechanisms remain crucial to biosafety.” This quote refers to the concept of *intrinsic containment*. Wherein the GD-constructs or GDOs are dependent on synthetic substances or limited in spread due to their specific technical organization. Since extrinsic containment practically is only an option for laboratory GDs, focus will be set on approaches for intrinsic containment with relevance to environmental releases. In the following passages the developmental stage and the reliability of the different options for intrinsic containment of GDOs will be investigated to find out whether they could represent an alternative for physical containment as Wright recommends. Afterwards, alternative approaches to synthetic gene drives will be analysed.

3.2 Intrinsic Containment

The intrinsic containment of a GDO may either be linked to the reproductive incompatibility of the target species with wild type strains and related species or caused by the specific character of the GD. For instance, in case of homing endonuclease-based GDs (HEG-drives) the latter may arise due to a unique target sequence. Accordingly, (Min et al., 2017a) differentiate between reproductive and molecular confinement as variants of intrinsic containment (Min et al., 2017a, p. 55).

For GDOs used *in the laboratory,* a number of options for intrinsic containment have already been applied as safety measures for GMO experiments. For applications in laboratory facilities use of organisms not viable outside laboratory conditions is advisable. Containment strategies can make use of the following options:

1. **dependency on a synthetic substance unavailable outside the laboratory,**
2. **a kill switch activated when a certain food compound is lacking,**
3. **reproductive containment using laboratory strains incompatible with wild conspecifics, e.g., the use of Drosophila with compound autosomes, where the left arms of two chromosomes are joint together in one chromosome and the right arms in another, making these specimen infertile with wild types (Akbari et al., 2015).**

Safety strategies become more challenging for GD releases into the environment. Meanwhile a number of approaches to limit the spread of GDOs in time and space have been proposed (Esvelt et al., 2014; Noble et al., 2019). These options will be explored in the following sections.

3.3 Safety Options for GDO-Releases

Safety strategies for GD applications can be grouped into techniques that represent either modifications to gene drive and other transgenic constructs respectively or rather alternative approaches which are based on naturally occurring mutations and parasitic infections that enable population control in a comparable way. Options for both types of approaches, either relying on genetic engineering or harnessing naturally occurring anomalies are presented after the following sections.

3.3.1 Molecular Modifications of Gene Drives as Safety Strategy

a. Split Drive

The idea of a split drive of a GD is based upon the separation of the genetic components of CRISPR-drives to limit its spread. In a split drive the endonuclease gene and the genetic information of the single guide RNA (sgRNA) are located in different loci. Only one of the genes is inherited as a GD. For instance, if the sgRNA sequence resembles its own insertion site, only the inheritance of the sgRNA will be super-Mendelian. Inheritance of the endonuclease gene is by contrast determined by Mendelian dynamics and should therefore fade from the population as long as Cas9 does not provide a fitness gain (cf. DiCarlo et al., 2015b). A split drive strategy can help to keep the GD frequency in neighbouring populations low (Li et al., 2020). Terradas et al. (2021) and Kandul et al. (2021) successfully demonstrated the loss of separately encoded Cas9 transgenes in experiments with *Drosophila melanogaster* in cages over several generations. Initial evidence of self-limiting behavior due to loss of the Cas9 gene in *Drosophila* has also been shown for a split version of a "Cleave and Rescue" GD (Oberhofer et al., 2021a). Simplified examples of different Split Drive strategies are illustrated in Fig. 24.

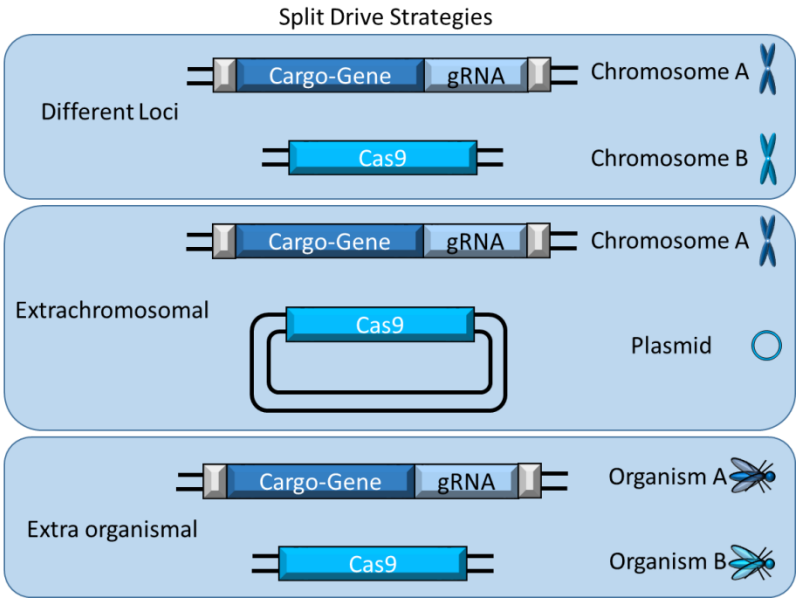

Fig. 24: Three Split Drive strategies.
Split Drives represent a safety option for CRISPR-Drives They are based on the separation of the Cas9 gene from the rest of the drive's components. These may be separated on different chromosomes, on a plasmid or even different organisms.

Molecular recombination events may cause malfunction of a split drive. If the Cas9-gene is translocated adjacent to the sgRNA sequence. If reading frames are intact the result would be

a complete GD consisting of the information for the endonuclease as well as a sgRNA and therefore potentially autonomous. Homology directed repair of subsequent sgRNA guided cleavage of a target site would then result in copying of sgRNA and Endonuclease genes. The unintended integration of sgRNA-sequences has already been observed (Li et al., 2016). However, the probability for such an event is low and it can be further reduced by a low homology between the locations of both elements of the split drive within the genome (Akbari et al., 2015). Additionally, GD developers recommend the combination of this strategy with a second form of containment (Akbari et al., 2015).

Besides a separation within the genome, other variants of split drives are imaginable. At least for some eukaryotic species, the genetic information of the endonuclease can be located episomally, outside the genome on an extrachromosomal plasmid. DiCarlo et al. experimentally verified the function of a split gene drive with episomal Cas9 gene in yeast (DiCarlo et al., 2015b). But additionally, to a verification of the gene drive-biased inheritance, an assessment of the limiting effect of this kind of split drive system is still lacking. The bias of inheritance decreases with each generation as plasmids get lost. And even if the endonuclease gene is integrated into the nuclear genome by recombination which is a rare but not impossible event, this gene will most likely be inherited by Mendelian dynamics and therefore the “drive” of the sgRNA fades over the next generations.

An even further expanded version of a split drive would be a constellation with different strains carrying parts of the genetic information of a gene drive. Here, the Cas9 gene can be part of the genome of a strain that mates with an sgRNA-bearing strain. This would require continuous releases of the Cas9-strain to keep the drive active, as if the sgRNA targets its own insertion sequence, the Cas9 gene will only passed on to offspring with a 50% chance of inheritance (Akbari et al., 2015).

However, for other reasons, there may be concepts for SplitDrive systems in which both the endonuclease gene and the gRNA sequence exhibit super-Mendelian inheritance. This is the case in the system presented by López del Amo et al. (2019) with two different gRNAs targeting the Cas9 integration site and the gRNA locus, respectively. Here, the most important safety feature lies in the fact that the alleles containing Cas9 and gRNA are initially stored as distinct lineages (female and male, respectively) until they are complemented into one genome by pairing.

b. Daisy Chain Drives

A daisy drive-system consists of a series of gene drives dependent on each other in a linear (or circular) manner, where each of the drives’ sgRNAs targets the sequences flanking next drive in the daisy chain. Therefore, no element of the chain drives itself. The single drives of a chain can even be located on different chromosomes. In a linear chain (of at least two elements), the first element would not be driven and would be lost first by the means of natural selection. Accordingly, the other elements of the chain would successively fade from the population over time. In the theoretical concept of a daisy drive proposed by Noble et al. 2019, the last element of the chain carries the “payload” (the cargo gene). If finally the last sgRNA of the chain would be lost, the top element would theoretically fade away as well, if it does not deliver any fitness gain (Noble et al., 2019). Edgington et al. proposed a daisy drive system for a killer-rescue GD (Edgington et al., 2020). Just as for split drives, recombination events may create an independent drive which then may overcome the limiting effect of the daisy chain. The concept of the daisy chain drive is depicted in Fig. 25.

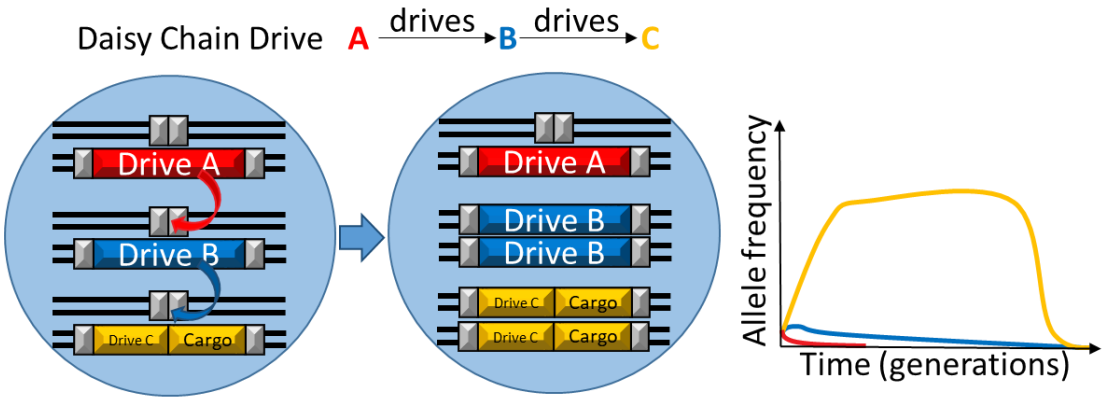

Fig. 25: Concept of a linear Daisy Chain Drive with the chain elements (recreated after Noble et al., 2019). In a Daisy Chain each element drives the subsequent. Only the final element of the chain carries the cargo gene. Each element is dependent on the previous element. In a linear chain the first element is not driven. Thus the first element (Drive A) will disappear from the population first, followed by Drive B (blue) and then Drive C (orange) will quickly fade as well.

c. Daisy Field Drive

In 2017, the Daisy Field Drive-system was proposed by (Min et al., 2018). The construct is depicted in Fig. 26. Here multiple sgRNAs are encoded separately from the locus harbouring the Cas9 gene and a potential payload gene. All sgRNAs share the same target sequence. Compared with a daisy chain drive, the daisy field system works with just a single cut-and-copy event and thus should be more reliable and less prone to non-intended recombination events that may create a global drive. According to Min et al. the fitness cost should be small because all elements of a daisy field drive except for the cargo genes and Cas9 consist of sgRNAs. Only for a number of initial generations, the genetic information for the nuclease and the payload is inherited to all offspring because with every generation the sgRNA daisy elements (N_{sgRNA}) are inherited with a mendelian probability. Because with every generation the number of sgRNAs per organism on average is cut in half, the nuclease and cargo-genes will be inherited by the drive for roughly (N_{sgRNA}+1) generations (Min et al., 2017a). According to this theory, the spread of the drive could be tuned by the initial number of sgRNAs. Daisy field drives can be combined with a daisy chain drive for instance as the first element of the chain.

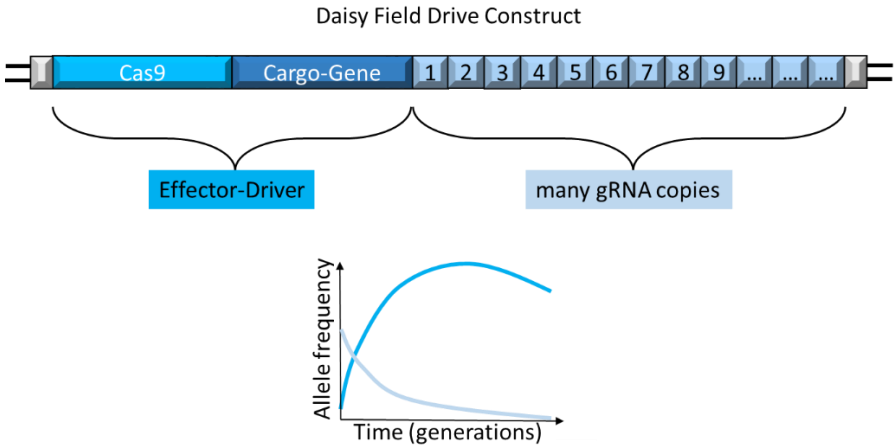

Fig. 26: Daisy Field Drive Construct (recreated from Min et al., 2017b).
The Construct constitutes a CRISPR-drive with many copies of the sequence-identical gRNAs. The Cas9-Gene and the cargo gene constitute the Effector-Driver combination, whose allele frequency will increase while that of the gRNA copies decreases. Once too few gRNAs are remaining the allele frequency of the Effector-Driver will decrease as well.

To prevent the occurrence of accidental generation of a global drive by recombination events that would translocate gRNA adjacent to the nuclease, sequences of gRNA and nuclease (including the Cargo genes) should not have sequence homology. Min et al. suggest to avoid more than 12 base pairs of homology. Additionally, they recommend to place the nuclease more than 100 kb apart from gRNA repeat sequences (Min et al., 2018). Whether this recommendation to avoid recombination would be prudent however, is highly dependent on the genetic context the specific loci and their recombination frequency.

d. Daisy Quorum Drive

In another prepublication, Min et al. propose the concept of “Daisy quorum” drives as an extension of a Daisy Drive-application by the subsequent release of wild-type organisms or a suppression drive targeting the previously altered population. This combination should lead to a low frequency of the engineered genes which then theoretically get lost over time by natural selection, if it does not provide a fitness gain for the organism expressing them (Min et al., 2017a). Since this approach is not yet experimentally validated, it is unclear if the daisy quorum will confer a fitness loss. Although fitness is dependent on the genetic background, target organism and environmental factors. This technique combines a CRISPR-Drive with Underdominance, wherein two haploinsufficient ribosomal genes on two chromosomes switch their loci in one of the sister chromatids.

e. Integral Drive

Nash et al. (2019), in search for a possibility to test the refractoriness conferring cargo gene in a test trial without the release of a fully functional GD invented the integral drive. The concept of the integral drive is depicted in Fig. 27. In the drive system native wild type genes are ‘hijacked’ to express the transgene under the control of natural promotors and concomitantly keep the fitness penalty relatively low. This approach may allow the collection of data on the population genetics of the cargo gene in field trials. If refractoriness proves stable under natural conditions, GDOs that only carry the Cas9 components could be released into the population offspring of the refractory transgenic organism and the GDO would then produce gametes that

carry both constructs. Should the refractoriness gene prove to confer a fitness gain, there may be no need to employ a gene drive.

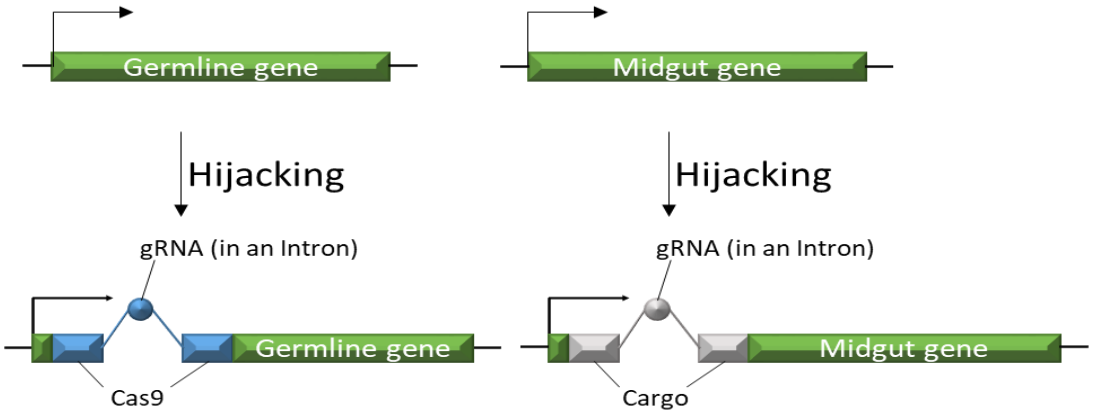

Fig. 27: Concept of Integral Drive (adapted from Nash et al., 2019).
The Cas9- and cargo gene are stored in different genomic regions, each upstream of a wild type gene. Each of the two transgenes has its respective gRNA encoded within an intron.

Hoermann et al. (2021) have shown the feasibility of an integral drive system in the African malaria mosquito *Anopheles gambiae*.

3.3.2 **Mitigation Strategies**

Early in the current accelerated GD developmental phase, secondary releases of sexually compatible members of the target species have been mentioned as a method to limit the spread of GDs and even reverse the functionality of the drive in the already affected individuals. The proposed approaches range from releases of sterile wild type individuals that should breed with the genetically altered organisms, thereby slowing down the spread of the drive (Montell cited in McFarling, 2017), to the release of GDOs equipped with overwriting drives that target the initial drive sequence. Particularly tricky approaches for the removal of CRISPR/Cas9-drives should even function without a complete GD-functionality: They only rely on gRNAs whose target sequences flank the previously released GD's sequence or are located within the coding sequence of Cas9. Due to the cellular presence of Cas9 from the released drive, which is now guided by the gRNA of the removal construct, excision or disruption of the GD and replacement with the coding cassette of the removal construct is possible (cp. Zentner and Wade, 2017). In a first laboratory experiment, Wu et al. (2016) demonstrate the functionality of an approach in which the gRNA targets a site within the DNA sequence of Cas9 in GD-bearing *Drosophila*. Xu et al. (2020) successfully showed that their two constructs, one for inactivation and one for replacement of the primary homing GD, suppressed its frequency in *Drosophila* cage population experiments.

Most probably, all these approaches for secondary releases are rather limited in their reliability as a means to restrict or reverse the impact of released GDs because their spread must at least cover if not exceed the spatial distribution as well as the number of carriers of the initial drive. A successful application of such a secondary drive, generations after the initial GD's release seems a rather challenging task. In particular, with regard to overwriting drives, a second (overwriting) drive has to reach every individual that was altered by the initial drive to exclude the possibility of recurrence – which cannot be excluded at least for very invasive drives with a low threshold such as a CRISPR-drive. It was also shown in simulations that overwriting drives irrespective of their fitness may not "catch-up" with the initial drive unless its fitness penalty is above 50% (Calvez et al., 2018). In any case, a secondary gene drive to

mitigate the first would be a poor decision. For one, it seems unwise to rely on the same technology to mitigate failure due to the vulnerabilities it itself suffers from. Secondly, the result may be similar to what was found in spatially explicit suppression drives, where long lasting cycles of invasion, extinction, recolonization and reinvasion would take place (as pointed out by Messer in Giese et al., 2019; North et al., 2019 fig 3 c and f). Furthermore, unless the GD is threshold-dependent, a release of sterile wild types is probably only able to slow down the spread of the drive. However, overwriting drives have been discussed in the community of scientists engaged in GD-development and Kevin Esvelt, on his webpage, demands that an overwriting drive should be built in parallel to any new gene drive.7 According to Esvelt, an overwriting – or "immunizing reversal" drive as he calls it – should not only target the individuals that are already altered by the initial drive: Besides overwriting the GD-code in the latter, it should render the wild type-population immune to further spread of the initial drive. He admits that "reversal" only refers to the phenotype, not the genotype of the altered organisms, because the second drive will not be able to restore the original genetic code. Traces of the genetic information of Cas9 and the sgRNA will remain in their genome.

3.3.3 **Limitation by Dependence**

Besides a specific genetic structure that may serve as a means to limit the spread of GDs, their continued super-Mendelian inheritance could be restricted by different types of dependence. External factors, such as environmental conditions may have an impact on the dynamics of GD dispersal. Other variants rely on a specific (synthetic) target sequence or a (synthetic) inductor molecule.

In the latter case, the inductor is necessary to induce the expression of the endonuclease or the sgRNA (in a CRISPR-drive). López Del Amo et al. (2020b) demonstrated the feasibility of an approach in *Drosophila* in which a synthetic, orally administered molecule leads to the stabilization of Cas9 and thus to a functional GD. If in toxin-antidote drives, the toxin is constitutively repressed, an inductor would be necessary to release the toxin and thereby activate the drive. But this method may turn out as difficult to realize for multicellular eukaryotic organisms, because the inductor has to be present in the germline and therefore cross several barriers of the organism's body. As an opposite strategy to an inductor, a toxin might be used which only impacts GDOs due to a sensitivity mediated by the genomic manipulation or the cargo of the gene drive.

A homing drive is engineered to target a specific sequence. If that sequence is unique to a certain number of individuals, the spread of the drive could be limited to these subpopulations of a species or previously released GMOs (for a deterministic model see Sudweeks et al. 2019). Esvelt and colleagues called the limitation to subpopulations a "precision drive" (cp. (Esvelt and Gemmell, 2017; Min et al., 2017a, p. 49). But according to Esvelt et al. it could be difficult to realize this drive type. First, to assure that the drive targets at least the subpopulation, it has to withstand the occurrence of resistant alleles. For that purpose, a multiplexed drive with at least three target sites is suggested. Additionally, these sites should be located within the sequence of essential genes. Moreover, for the application to be feasible, these "natural" sequences would have to contain a protospacer adjacent motif (PAM) to enable binding of the endonuclease to the target site.

To overcome these obstacles, the GD targets could only consist of synthetic sequences in genetically engineered organisms. As "synthetic site targeting" this safety approach was tested in yeast in an initial experiment (DiCarlo et al., 2015b). A major advantage of this approach is that depending on the sequence similarity with natural sequences the sgRNA of a CRISPR-drive must undergo several mutations before it may serve to place a drive in a natural sequence. For the application in isolated populations e.g., on islands, Min et al. suggested to

7 Link to the Esvelt "Sculpting Evolution" work group.

use target sequences for homing drives that are recoded by an initial drive to provide an appropriately prepared population (Min et al., 2018, p. 49). This sensitizing drive however, would probably suffer smaller fitness penalties because it would not carry a costly cargo gene. It might thus spread faster and more thoroughly than the the actual suppression drive itself. This could potentially pose even greater risks of transboundary movements.

Artificial sequences can also be introduced into populations using an underdominance approach to create reproductively isolated species, as demonstrated by Buchman et al. (2021) using a CRISPR/Cas system (without endonuclease activity) in *Drosophila melanogaster*.Craig Montell suggested a dependency on an environmental factor, to engineer GDOs with a self-destruct mechanism activated when an environmental parameter, e.g. temperature, reaches a threshold (Montell cited in McFarling, 2017). In order to reach every GDO with this technique, it has to be included in the cargo of the drive. Besides the necessary increase in size for the additional cargo information, the major drawback of this approach most probably lies in its vulnerability to mutations in the self-destruction mechanism. Instead of destroying the GD, Oberhofer et al. (2021b) developed a cleave-and-rescue drive in which the population suppression function is temperature-dependent and demonstrated its efficacy in laboratory experiments with *Drosophila melanogaster*.

3.3.4 **Limitation by Genetic Instability**

Experimental tests of CRISPR/Cas-homing drives revealed a significant restraining impact of resistance alleles in target populations. Selection of resistance to a CRISPR-drive was first documented by Hammond et al. in 2017. After an initial increase of GDO in caged mosquito populations over less than 10 generations, a gradual increase of the ratio of resistant alleles within the experimental time frame of 25 generations was observed (Hammond et al., 2017). Resistance allele formation may occur due to faulty repair by either Non-Homologous End Joining (NHEJ), Microhomology-Mediated End Joining (MMEJ), or incomplete Homology Directed Repair (HDR). But resistance may also be pre-existing due to sequence variations within the population (Champer et al., 2017). Within the sequence of essential genes of a species the probability is high that mutations compromise the viability of the organism. Thus, target sites are likely to confer more stability with regard to the spread of the GD if they are located within essential genes that are highly conserved among the members of a species. This strategy was shown to be successful in suppressing the selection of resistant alleles in *Anopheles gambiae* (Kyrou et al., 2018).

On the contrary, an approach for GD-limitation could be realized by a high probability for mutations due to only a single target site within a non-essential gene and only a single sgRNA locus. This approach however would greatly reduce the efficiency and predictability of the gene drive's spread and thereby might not be suitable to reach the desired goal.

3.4 **Overview of Potential Safety Mechanisms**

The different strategies presented here may help to overcome the potential risks of GD. The techniques vary remarkably in that they on the one hand rely on genetic engineering – partially even consist in GD-variants. Nonetheless, these approaches differ strongly in their qualities with regard to the aim of reducing exposure to GD and minimizing potential hazards associated with their release. And besides the fact that the effectivity of most of these options is not yet experimentally verified, they are connected to different vulnerabilities that may preclude particular applications. An overview on the approaches presented in this chapter, the basic strategy, their aim with regard to hazard and exposure of GD, major vulnerabilities as well as a rough characterization of their developmental stage are given in Tab. 3 and 4. Note that yet

no experimental proof exists for most design options for CRISPR-drives to increase controllability, although the concepts exist already for some years. Also, many approaches are reliant on the assumption that a genetic construct confers a fitness penalty and will fade from the population by evolutionary mechanisms. If this assumption is true may however vary depending on the genetic background, the target organism and its environment.

Tab. 3: Overview of design options for HEG-drives

Technique	Main strategy	Aim (Hazard / Exposure)	Vulnerability	Remarks/developmental stage
Split Drive	separation of genes for sgRNA and endonuclease	limitation of exposure to GDO (temporal and spatial)	co-localization of genes for Cas9 and sgRNA by recombination resulting in a global drive	first successful cage experiments with *Drosophila* in lab scale
Daisy Drives	chain of interdependent drives / multiple separately encoded sgRNAs for endonuclease (and cargo) target sequence	limitation of exposure to GDO (temporal and spatial)	co-localization of genes for Cas9 and sgRNA targeting its own insertion site by recombination resulting in a global drive	no experimental proof for the limiting potential so far
Integral Drive	Different wild type genes used to express the transgenes under the control of natural promotors	limitation of exposure to GDO (temporal and spatial)	co-localization of genes for Cas9 and sgRNA by recombination resulting in a global drive	first cage experiments show inheritance dynamics
(synthetic) Inductor molecule	dependency on the supply of a substance	limitation of exposure to GDO by GD deactivation	germline in multicellular organisms might be difficult to target with an inductor	first successful lab experiments with synthetic inductor in *Drosophila*
Specific (synthetic) target sequence	targeting of a unique target sequence	exposure limitation to GDO by targeting a genetic subpopulation	similarity to sequences in the general population	“synthetic site targeting” and engineered genetic incompatibility by artificial sequences tested in laboratory scale
Environmental conditions	self-destruction depending on environmental conditions	limitation of exposure to GDO	mutations deactivating the self-destruction system	no experimental proof for the deactivating potential so far (but temperature-dependent population suppression of a GD shown in laboratory scale)
Genetic instability	accumulation of GD-resistant target sequences due to mutation and sequence variations	limitation of exposure to GDO, slowdown of GD spread	incomplete reduction of the GD frequency	first experimental observations in laboratory scale

Tab. 4: Overview of secondary releases to limit or remove a GD.

Technique	Main strategy	Aim (Hazard / Exposure)	Vulnerability	Remarks/developmental stage
Overwriting drive	release of secondary GD targeting the sequence of the first drive	reducing exposure to GDO by deactivation/ limitation of the initial drive and immunization of the target population	dependence on perfect coverage of the first drive's distribution, sensitive to mutations	Successful cage experiments with *Drosophila* in lab scale
gRNA targeting a drive	release of organisms carrying gRNA against the sequence of the released GD	reducing exposure to GDO by deactivation/ limitation of the initial drive and immunization of the target population	dependence on perfect coverage of the first drive's distribution, sensitive to mutations	first experimental proof-of-principle in laboratory scale in *Drosophila*
Limitation by sterility	release of sexually compatible but sterile organisms	slowdown up to limitation of GD spread (in case of high threshold-drives)	dependence on perfect coverage of the first drive's distribution / spread of GD is only retarded	no experimental proof for the limiting potential so far

3.5 Safety and Containment Strategies – Summary

The list in Tab. 3 exemplifies the focus of design options for CRISPR-drives on the reduction of environmental exposure. The hazard potential of GDs will most probably be very case-specific because it is largely dependent on the genomic localization of the drive and the function of potential cargo genes. This focus on exposure minimization is hence justified. First successful applications in insects in the laboratory-scale have been reported for some design variants of CRISPR-Drives with reduced risk potential. However, tests under field-like conditions are still lacking, which prevents reliable conclusions about their performance with regard to releases. With an experimental release, the risk of uncontrolled spread in the case of malfunction is high. Secondary releases, such as overwriting drives, gRNA targeting the sequence of a released drive or the release of sterile mating partners must be potent enough to cover all parts of a population that have been affected by the primarily released drive. It is thus necessary to assure that mutations or fitness penalties do not interfere and reduce their efficiency. A first proof-of-principle in the laboratory scale was already published in 2016 in the form of a Cas9-triggered chain ablation (CATCHA) (Wu et al., 2016). However, a demonstration of the effectiveness of options for secondary releases under more realistic conditions is still pending. With regard to control by dependence mechanisms, the first experimental evidence is now available for the application of synthetic inducer molecules or the temperature dependence of population suppression at laboratory scale. Concepts for control of GD spread by genetic instability suffer from limited reliability

Given the current lack of safety and containment strategies for GDs that have been successfully applied under field-like conditions, the high exposure potential of GDs, and the variety of effects their use could cause, a precautionary approach is highly advisable.

4 Part A.2 - Base Data for the Prospective Assessment of Gene Drives Releases

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4.1 Data Categories

To reliably assess and evaluate the effectiveness, as well as the spread in space and time of a given gene drive system, a collection of base data is necessary. These data can be divided into three main categories:

1. **data specific to the gene drive (GD) system;**
2. **data specific to the target organism (TO) and**
3. **data specific to the environmental conditions of the corresponding ecosystems.**

For the assessment of the population dynamic behaviour of GDs, in a first step, the different types of necessary data to each category are identified. This constitutes a data wish list to be able to assess possible outcomes of a gene drive application as precisely as possible. For simplicity, the requests for the desired data are phrased in the form of a checklist.

4.1.1 Category 1: Data Related to the Gene Drive

The first data category is important as each gene drive system is different in multiple facets from any other system. When talking about GD it is paramount to understand that there is not one GD but many different kinds. General statements on GD, may be true for some but wrong for other systems. Furthermore, even if the same drive system is used, different cargo genes may lead to completely different outcomes. This further underscores the importance of a case-by-case scrutiny. Therefore, this first data category is important to frame the general implications to a GD application. Furthermore, as opposed to the other categories this one is mostly defined by genetics and molecular interaction. Comparisons might be drawn to the molecular characterisation conducted in the risk assessment of the European Food Safety Authority (EFSA).

First, the basic information on the gene drive system are required. It will be impossible to predict any dynamics of the drive without these basic data. The first five points on the checklist simply deal with the purpose or class of the GD, its persistence and mode of inheritance as well as the calculated threshold and number of estimated generations the drive is to persist. These points revolve around the basic design of the application. Thereby, the possibility of combined drive systems was also included. Say a CRISPR-homing drive that distorts the sex ratio (Kyrou et al., 2018) or a combination of Underdominance and Medea (Gokhale et al., 2014) or combining a meiotic drive with Underdominance (Huang et al., 2007b).

The following ten points (6 - 16) then concentrate on the genetic constitution of the system, what constructs it comprises, how they act and interact. Furthermore, this explores how the system affects the carrier individual in its genetics and gene expression up to complex yet basic physiological characteristics such as viability and fertility, not barring the occurrence of off-target effects and fitness penalties.

The final points in this category (17-19) aim at the obligatory mitigation strategy in case of failure and demands all the same data required for the original gene drive for the mitigation strategy should it entail a gene drive itself.

Basic Information on the GD system

1. **Purpose of the GD**

☐ Population suppression ☐ Population modification

2. **Propagation dynamics of GD**

☐ Self-limiting ☐ Self-sustaining

3. **Mode of super-Mendelian inheritance *(multiple answers possible)***

☐ Toxin-antidote-combination ☐ Sex ratio distortion ☐ Homing endonuclease (HEG)

☐ Homology-directed Repair (HDR)

4. **Calculated threshold for the GD to be successful**
5. **Number of generations the GD is planned/estimated to persist**
6. **Number of genetic constructs**
7. **Loci in which the constructs are integrated**
8. **Genes are included in the GD construct(s) and purpose or classification (marker gene, gRNA, miRNA-toxin, cargo gene, recoded antidote gene …)**
9. **Effect of the construct on the TO's gene-expression**
10. **Interaction of affected genes' expression with other genes and consequences**
11. **Construct(s) interaction**
12. **Observed off-target and on-target effects, especially resistance formation with a percentage of occurrence**
13. **GD effects on the TO's viability**
14. **GD effects on the TO's fertility**
15. **GD effects on the TO's mating behaviour/success**
16. **Estimated fitness for hetero-/homozygous GD-carriers**

Mitigation Strategy

17. **Proposed strategy to reduce and mitigate inflicted damage (e.g. ecosystem functions/services)**
18. **Estimated duration for mitigation strategy to be effective**
19. **All requested data above for the mitigation strategy if it is a GD itself**

4.1.2 Category 2: Data Related to the Target Organism

The second category of desirable data focuses on the target organism/s. The outcome of a gene drive is (of course) very dependent on its genetic blueprint. However, the specific characteristics of the target organism affect the course of population dynamics even more. A drive in mice would exhibit critically different population dynamics compared to a similar drive in mosquitoes. Thus, after specifying the TO and the reason why it is targeted for gene drive application (1 and 2) this category addresses data requests based largely on the TO's life history, its population dynamics, mating systems and partners (3 - 13), including, population structure assortative mating, inbreeding, and standing genetic variation and dormancies (17 – 19). The subsequent three issues focus on the migration of wild type and dispersal of GDO TOs (20 – 22), considering exchange between TO populations. Furthermore, the potential of the GD to cross species barriers and its prevention should be assessed. This is attempted by the demand for data on rare mating events and hybridization partners up to the second degree

(23 – 27). The final four points (28 – 31) focus on the specifics of releases, such as release size, -interval, -ratio, -number as well as the time of release during the annual cycle.

Introductory Data

1. **Taxonomic name of TO species**
2. **TO species' qualification for GD application**

Life History

3. **TO's generation time**
4. **TO's maturation time**
5. **TO's Life stages**
6. **TO's (meta-) population structure**
7. **Plant's reproductive morphology**

☐ perfect ☐ monoecious ☐ dioecous

8. **TO's mating system**

☐ iteroparous ☐ semelparous

9. **TO's mating practice**

☐ monogamous ☐ polygnous ☐ polyandrous

10. **Duration of TO's fertility in its life**
11. **TO's average population size**
12. **Density dependent factors on TO populations during life cycle**
13. **Average number of offspring per generation**
14. **TO's average litter size**
15. **Percentage of offspring that reach adulthood?**
16. **TO species' number of matings and mating partners in a lifetime.**
17. **Assortative mating, mate choice, possibly avoidance of GDO mates**
18. **TO's inbreeding or parthenogenesis rates, and effects on the GD**
19. **Duration of dormancies (like hibernation or aestivation) and a/biotic factors that influence it**

Migration and dispersal

20. **Dispersal distance of TO species**
21. **Dispersal distances of GDO**
22. **Exchange between populations**

Potential to cross species barriers

23. **Hybridization partners and percentage of fertile offspring**
24. **Hybridization partners of those hybridization partners and percentage of fertile offspring**
25. **State of conservation of GD targeted sequences, regarding standing genetic variation (inherent resistance) in TO and hybridisation partners**
26. **Overlapping habitats of hybridisation partners and their hybridisation partners**
27. **Inhibition of cross species GD spread**

Release data

28. **Estimated release ratios (wild type vs. GDO)**
29. **Time of first release during annual cycle**

30. **Number and interval of releases**
31. **Average release size**

To illustrate the complexity of interrelation connected to the different aspects explored in the target organism-specific data, Fig. 28 shows a graphic depiction of some factors that influence a population of target organisms in a system dynamics model environment. This kind of model is often used for classical population biology.

Note that this illustration is a rough simplification and does neither reflect the whole complexity nor does it include all factors that may be important. Even more so, it does not entail a gene drive which would be expected to extract further inter-relational influences. It however illustrates the dominant degrees of freedom inherent to wild ecosystems.

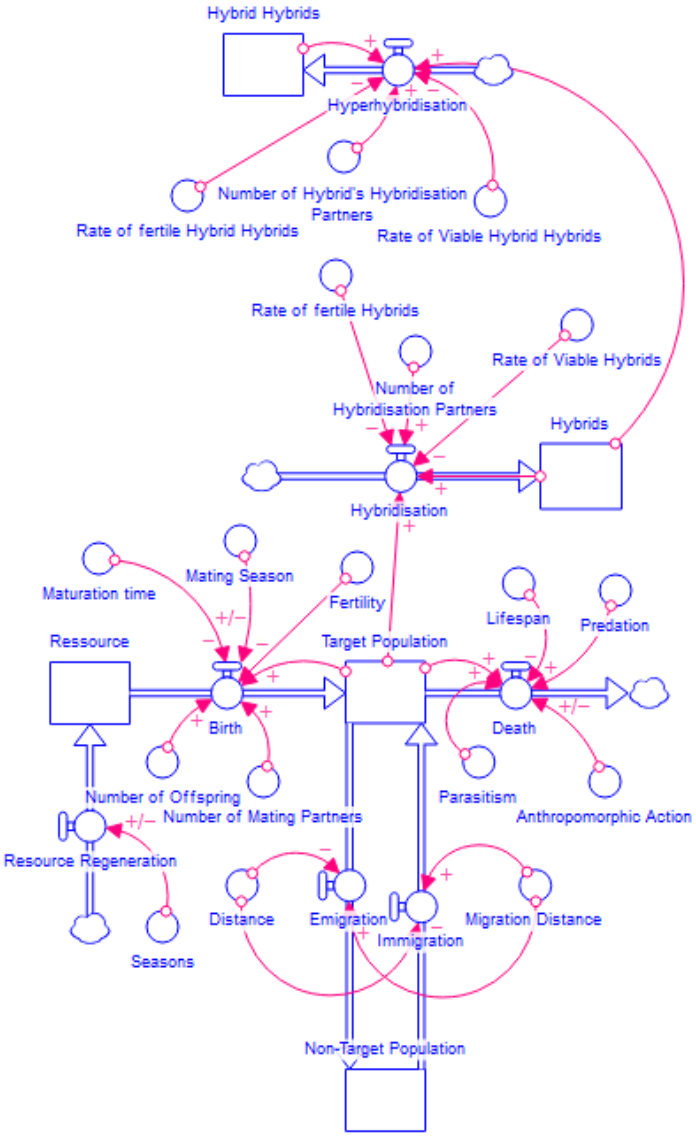

Fig. 28: System dynamics model depiction of some TO-specific factors.

This excerpt of important factors includes a target population, a non-target population and hybridization partners of the first and second degree and some of their influences (pink arrows). Note, that important influences such as resources, birth and death were omitted for clarity in all but the target population. This depiction does not claim to be exhaustive but serves to give

a rudimentary impression of the complexity of population dynamics even without the afflictions of a gene drive.

4.1.3 **Category 3: Data Related to the Receiving Environment**

The final category of information deals with the types of data focusing on the receiving environment. The environment is expected to have the greatest influence on the outcome of any GD application, since it is the vastest of the three categories. Unfortunately, it is also the category which is expected to be the least foreseeable, also harbouring the most problematic consequences. Thus, the acquisition of adequate data for this category is crucial for the accurate assessment of any GD application, however difficult it may be to obtain dependable data.

This category first addresses the area in which the GD is to be released and monitored, including other species, climatic and geographic characteristics and its duration (1 – 7). Then the presence, consent, and potential influence of humans and human intervention are assessed (8 – 11). Afterwards questions of confinement and mitigation are addressed (12 – 15).

Eventually, the next five issues (16 – 20) will certainly require the most effort to obtain relevant data because they deal with ecological interrelations up to the second degree. Without a doubt these are the most important issues as well as the most elaborative, where incomplete listings or falsely estimated values may lead to tremendous effects. For these questions the classification into ecosystem services or ecosystem functions may be employed.

The last point then closes with the consideration and enforcement of confinement on some of those inter-relational species within the monitoring area. At least for potential mating partners this should be a prudent step.

Monitoring

1. **Area of GD release**
2. **Size of monitored area**
3. **Other species in the monitored area**
4. **Distribution of habitats**
5. **Climatic and geographic/topographic characteristics (natural barriers) of the monitored area**
6. **Regularly occurring weather effects on TO directly (Dispersal by windstorm) and indirectly (e.g. on food sources or predators)**
7. **Duration of monitoring after GD release**

Human influence

8. **Presence of humans inside the monitored area**
9. **Residents informed consent to the releases**
10. **Consideration of (unintended) anthropogenic actions (such as pesticide spraying) regarding the effectiveness of the gene drive**
11. **Importance of action or omission of action (such as pesticide spraying)**

Confinement and mitigation

12. **Confinement of TO populations and GDO to monitored area**
13. **Effectivity of confinement strategies during monitoring**
14. **Counter measures should the confinement strategies prove to be ineffective**
15. **Necessary time period for counter measures to mitigate escapees**

Ecological interrelations

16. **(Multicellular) species that interact with the TO species and their relationship in their natural habitats (first degree interactions)**
17. **(Multicellular) species that interact with the species that interact with the TO species in their natural habitats (second degree actions)**
18. **First or second-degree interacting species reliant on TO**
19. **Occurrence of interacting species within monitoring area and expected population effects**
20. **Confinement of interacting species populations to monitoring area.**

4.1.4 Summary

In this study, a set of relevant criteria was identified for each category. It was also investigated whether the respective data for the criteria are available. It was found that general statements about data availability are not possible, as some data are available but others are not for the same criteria but a different technical design (GD-type) or application context (e.g., different target organism, or ecosystem). In particular, ecological data are the most deficient of the categories listed above. Models for studying the behavior and effects of released GMOs are useful for making predictions about specific risk-relevant properties of GMOs. In addition, an analysis of modeling approaches published so far also provides an overview of the availability of relevant data for this type of prospective methodology. Therefore, after an analysis of the informational value of natural gene drives a review of the current state of GD modeling will be presented in the following chapters.

4.2 What can be learned from Natural Gene Drives?

Many natural GD systems exist today, such as the natural Medea element, the t-haplotype and many sex ratio distorters in mosquitoes and flies. But it can only be guessed how many gene drive systems have existed throughout history. No data exists on species that may have gone extinct due to selfish elements. “Most eukaryote genomes carry a substantial burden from defunct transposons, and devote substantial genetic resources to combating selfish elements; those elements may well be an important cause of extinction” as Nick Barton put it in a report publication on the expert workshop during this project (Giese et al., 2019). But the existing natural drive systems show that organisms find a way to keep the drive in check, particularly interesting in this regard are so called ancient gene drive systems.

One such ancient drive is described by (Price et al., 2019). The sex ratio distorter (SR) is located on the X-chromosome in many populations of *Drosophila pseudoobscura* in North and Central America, skewing the sex ratio toward a female bias. This is accomplished by interference with Y-chromatid segregation at meiosis II eventually killing all Y-bearing sperm (Novitski et al., 1965). The inheritance scheme of the SR-drive is depicted in Tab. 5.The ancient drive is estimated to have persisted for perhaps hundreds of thousands of years. But during all this time the drive has neither spread to fixation and thereby driving *D. pseudoobscura* to extinction, nor could any trace of evolved resistance to the SR drive be found. It is yet unclear how this situation could be accomplished. Price et al. gather available information examining six factors that could play a role in the peculiar situation: the shortage of males and male fertility costs, female choice, polyandry and sperm competition, the cost to females, population structure and meta-population dynamics. The article concludes that the evidence to support shortage of males as a factor is weak (Price et al., 2008 a and b), while data on a reduced male fertility is unfortunately mixed (Policansky and Ellison, 1970; Price et al., 2012 a and b, 2008). There is strong evidence against mate choice being responsible for the persistence of the drive at intermediate frequencies (Price et al., 2012 a and b) but polyandry and resulting sperm competition seem to show strongly supportive evidence

(Holman et al., 2015; Price et al., 2014, 2010), while moderate evidence also points to high fitness cost to (at least homozygous) females. Lastly, there is unfortunately insufficient data concerning population structure as a factor to explain the drive's frequency. (Price et al., 2019)

Although this is only a solitary case study its significance is tremendously increased in consideration of the time span this drive has existed within the species. The SR X-chromosome constitutes a strong suppression drive with a sex distortion rate of up to 100% and yet it does not lead to population suppression as would be expected from SR's molecular characteristics likely due to ecological factors and behaviourisms within the population. This demonstrates that more emphasis should be laid on the study of the target organism and its ecology than the genetics-oriented focus of most studies and models in the current gene drive literature.

Tab. 5: Inheritance scheme of the ancient SR sex distorter drive in *Drosophila pseudoobscura.* (red symbolizes the SR X-chromosome)

SR			Females					
	Gonosomes		XX		XX		XX	
		Gametes	X	X	X	X	X	X
Males	XY	X	XX	XX	XX	XX	XX	XX
		Y	XY	XY	XY	XY	XY	XY
	XY	X	XX	XX	XX	XX	XX	XX
		Y	XY	XY	XY	XY	XY	XY

A study by Hammer and Silver (1993) could identify the t-haplotype to be of ancient origin as well. The t-haplotype encompasses 40 Mb at the proximal end of chromosome 17 in *Mus musculus* (Austin et al., 2009). Super-Mendelian inheritance is secured paternally, as t-carrying males inherit it with more than 90% while t-mothers only pass it on with 50% probability (Herrmann et al., 1987). The reason why the t-haplotype remains at relatively low frequencies of 10-25% in populations (Ardlie, 1998) is thought to be the detrimental effects on male homozygous carriers' fertility and viability. Manser et al., (2017) could show that sperm competition of t-haplotype males is reduced with respect to non-carrier conspecifics, leading to reduced gene drive prevalence in polyandric laboratory populations as opposed monandric populations. On the other hand, the reason why t-haplotype did not disappear over the course of over three million years was examined in a sequence and expression pattern analysis. This study led to the conclusion that although non-synonymous mutations suggested that no recent recombination event took place, occasional gene flow between the t- and the standard chromosome took place that may have regenerated accumulated mutations (Kelemen and Vicoso, 2018). It is however doubtful, such rejuvenating recombination events may be possible with synthetic drives.

The population dynamics of the natural Medea element in the flour beetle *Tribolium castaneum* have been examined in a study by Wade and Beeman (1994). The article concludes that in the absence of a fecundity cost, any degree of the maternal effect lethality permits Medea to spread. For Medea to spread the fecundity effects to mothers must be recessive, as Medea will not spread if it severely affects the fecundity of heterozygous females. Apart from the previous natural drives, the Medea element is so wide spread in the flour beetle it is used as a phylogenetic marker to trace gene flow (Beeman, 2003). This is of course only possible because Medea is not a suppression drive.

The study of naturally occurring drive systems is an important supporting tool to understand the population genetics of gene drives. It is currently the only way to study the behaviour of those constructs in natural environments and wild populations. The shown cases exemplify that especially with suppression drives that are attached to an inherently high fitness penalty may be impeded in their spread by simple features of the target organism's mating system. It can be considered likely that many other behaviouristic adaptations may hinder the efficiency

of drives in nature. Unfortunately, these can hardly be predicted by modelling approaches, further increasing the uncertainty of a synthetic gene drive's fate in wild habitats. The shortcomings of modelling approaches for gene drives will be further examined in the next chapter.

4.3 What can be learned from models in the literature?

The literature on gene drives has brought forth many papers in which the authors present various different model simulations of gene drives. In the following, the commonalities, differences and most importantly the significance of these papers and especially the importance of models in general to evaluate gene drive technology will be discussed in detail. We established a library of 90 publications concerning modelling of super-mendelian inheritance systems. This library was derived from a SCOPUS search query8 and an already existing topical database. The publications were published between 2001 and 2020. This list of publications may not be exhaustive but may in the least claim to have a reasonable representative quality for the different model simulations available on the subject of gene drives. The studies listed in Tab. 6 have been considered and examined:

Tab. 6: Considered publications that include model simulations of super-Mendelian inheritance systems.

1	(Davis et al., 2001)	46	(Champer et al., 2018)
2	(Boëte and Koella, 2002)	47	(Buchman et al., 2018a)
3	(Hall, 2004)	48	(Noble et al., 2018)
4	(Struchiner et al., 2005)	49	(Dhole et al., 2018)
5	(Rasgon and Gould, 2005)	50	(Wilkins et al., 2018)
6	(Magori and Gould, 2006)	51	(Khamis et al., 2018)
7	(Huang et al., 2007a)	52	(Lambert et al., 2018)
8	(Huang et al., 2007b)	53	(Edgington and Alphey, 2018)
9	(Deredec et al., 2008)	54	(Kyrou et al., 2018)
10	(Gould et al., 2008)	55	(Walker et al., 2019)
11	(Lambrechts et al., 2008)	56	(Haller and Messer, 2019)
12	(Marshall, 2009)	57	(Oberhofer et al., 2019)
13	(Magori et al., 2009)	58	(Wong and Holman, 2019)
14	(Xu et al., 2010)	59	(Edgington and Alphey, 2019)
15	(Windbichler et al., 2011)	60	(Heffel and Finnigan, 2019)
16	(Marshall and Hay, 2011)	61	(Backus and Delborne, 2019)
17	(Marshall et al., 2011)	62	(Noble et al., 2019)
18	(Huang et al., 2011)	63	(Frieß et al., 2019)
19	(Legros et al., 2011)	64	(North et al., 2019)
20	(Deredec et al., 2011)	65	(Sánchez C. et al., 2019)

8 Link to Scopus search query

21	(Akbari et al., 2013)	66	(Nash et al., 2019)
22	(Legros et al., 2013)	67	(Sudweeks et al., 2019)
23	(North et al., 2013)	68	(Manser et al., 2019)
24	(Robert et al., 2013)	69	(Prowse et al., 2019)
25	(Gokhale et al., 2014)	70	(Backus and Delborne, 2019)
26	(Marshall and Hay, 2014)	71	(Beaghton et al., 2019)
27	(Akbari et al., 2014)	72	(Dhole et al., 2019)
28	(Okamoto et al., 2014)	73	(Bull et al., 2019b)
29	(Unckless et al., 2015)	74	(Bull et al., 2019a)
30	(Beaghton et al., 2016)	75	(Edgington et al., 2020)
31	(Backus and Gross, 2016)	76	(J. Li et al., 2020)
32	(Hammond et al., 2016)	77	(S. E. Champer et al., 2020)
33	(de Jong, 2017)	78	(M. Li et al., 2020)
34	(Noble et al., 2017)	79	(López Del Amo et al., 2020a)
35	(Unckless et al., 2016)	80	(Sánchez C. et al., 2020)
36	(Gonen et al., 2017)	81	(Simoni et al., 2020)
37	(Vella et al., 2017)	82	(Cash et al., 2020)
38	(Eckhoff et al., 2017)	83	(Oberhofer et al., 2020)
39	(Marshall et al., 2017)	84	(Lester et al., 2020)
40	(Tanaka et al., 2017)	85	(North et al., 2020)
41	(Edgington and Alphey, 2017)	86	(Xu et al., 2020)
42	(Drury et al., 2017)	87	(Rode et al., 2020)
43	(Prowse et al., 2018)	88	(Champer et al., 2020a)
44	(KaramiNejadRanjibar et al., 2018)	89	(Champer et al., 2020b)
45	(Oberhofer et al., 2018)	90	(Champer et al., 2020c)

Fig. 29 depicts the distribution of the selected studies by publication year. It is evident that a strong increase has taken place since 2017. It may be estimated since the field of gene drive research and the broad discussion thereof is expanding that these numbers may even increase further in the upcoming years. Interestingly, out of the eleven considered papers from 2017, nine focus on CRISPR-homing drives with only one of them also concentrating on Driving-Y (Eckhoff et al., 2017)[38], one focussing on Underdominance (Edgington and Alphey, 2017)[41] and the last modelling a generic drive (Gonen et al., 2017)[36].

The earliest publications on simulations of super-Mendelian inheritance date back to the years 2001-2005, focusing on underdominance (Davis et al. 2001)[1], meiotic sex distorter X chromosome (Hall, 2004)[3] and the idea to manipulate inheritance by the use of transposable elements (Boëte and Koella, 2002; Rasgon and Gould, 2005; Struchiner et al., 2005)[2,4,5]. Only the latter mechanism so far has not been exploited to develop a synthetic gene drive system.

The next considered publication by Huang et al., (2007a)[7] explores the combined applications of gene drives systems in *Aedes aegypti* and discusses underdominance, meiotic drive and *Wolbachia*. Fig. 29 depicts the rising number of published papers per year that include models on super-Mendelian inheritance. This exemplifies that although the field of super-Mendelian

inheritance research is not new, the discovery of HEGs caused a substantial boost in interest in this field.

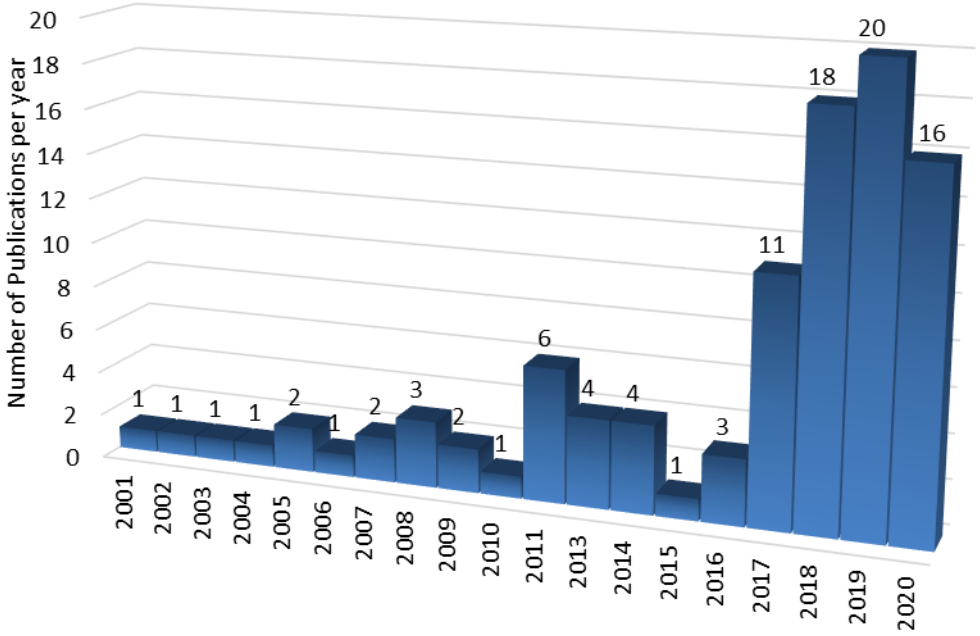

Fig. 29: Bar diagram on the considered 90 publications on gene drive models sorted by publication year. There was a visible increase in publications since 2017 with a trend that may suggest more annual model publications in the coming years.

4.3.1 **Study Focus**

These models aim at many different aspects depending on the focus of the study. Fig. 30 depicts an overview on the most common foci of the studies. In descending order, the most represented focus points are replacement, suppression, reversal, and invasion qualities of gene drives. The catch-all category 'other' consists of non-genetic, transgenerational fitness costs discovered in segregation distorters (Wong and Holman, 2019)[58], allele effects on gene drive spread, the spread of various drive systems (Marshall, 2009; Walker et al., 2019)[12,55], population dynamics (Legros et al., 2011; Magori et al., 2009; Xu et al., 2010)[13,14,19], model description (Haller and Messer, 2019)[56], synthetic resistance, reversal drives and immunizing reversal drives (Vella et al., 2017)[37], gRNA multiplexing (Champer et al., 2018)[48], the benefits of meiotic- vs. embryonic conversion in HEG-drives (de Jong, 2017)[33] and the post drive spread of parasites (Champer et al., 2020c)[74]. Some studies fall into several categories simultaneously and are therefore represented several times in the diagram.

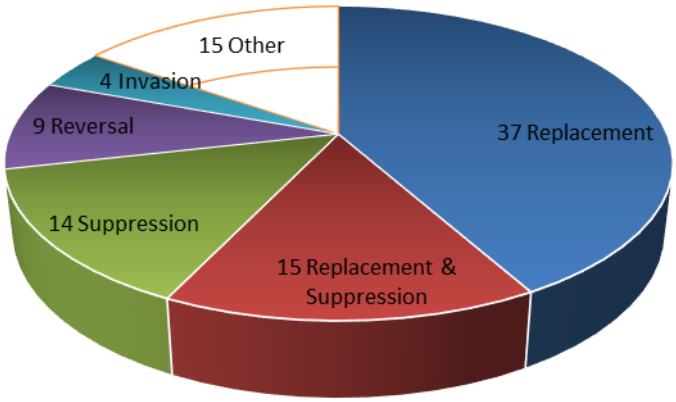

Fig. 30: Focus points of the considered studies shown in a pie chart.
In descending order, the most represented focus points are the gene drive qualities of replacement, suppression, reversal and invasion. Studies with multiple foci may be represented multiple times.

4.3.2 **Model Target Organism**

The target organism, in a way also defines the purpose of the gene drive application. For instance, gene drives on mosquitoes are of course focused on the application in public health, while flies as target organisms, namely *Drosophila suzukii*, *Cerratitis capitata* and *Tribolium castaneum* implies agricultural gene drive applications. *Drosophila melanogaster* as a laboratory model organism, on the other hand implies basic research applications. Finally, rodent target organisms often point toward gene drive applications in nature conservation or agriculture. However, in many cases the distinction of studies with regard to their addressed use is not that simple. Therefore, a more detailed review approach was conducted to specify to which of the three application uses, namely public health, agriculture and nature conservation, the respective study may be dedicated to. In 32 cases, a clear dedication to either one or any use could not be established. As depicted in Fig. 31, the majority of 46 studies subscribe to public health. Also, the studies Davis et al. (2001)[1] and Magori and Gould (2006)[6] were identified as leaning toward public health and scored accordingly. Seven studies addressed nature conservation[31,43,50,67,68,69,84] and five studies agriculture[27,36,44,47,76] as a potential use for gene drives.

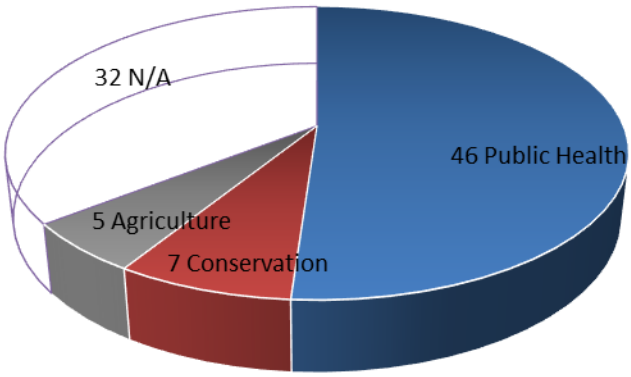

Fig. 31: Pie chart of the areas of application specifically addressed in the majority of examined studies.

Most of the considered publications, albeit focusing on specific target organisms, present generic models that do not consider the target organism's specific characteristics, like life history or population structure. Twelve out of 56 publications featuring generic models are directed at mosquitoes [2,5,7,12,16,24,30,40,65,66,73,81], seven generic models are directed at flies [27,45,46,57,58,65,83,], and one generic model on the red flour beetle (Drury et al., 2017)[42]. However, roughly one third of the publications orient their models towards specific organisms, incorporating the organism's life history (Fig. 32) including the red flour beetle (Cash et al., 2020)[82]. These subsets are further broken down in Fig. 33 and Tab. 7 to show the number of the publications that deal with specific species.

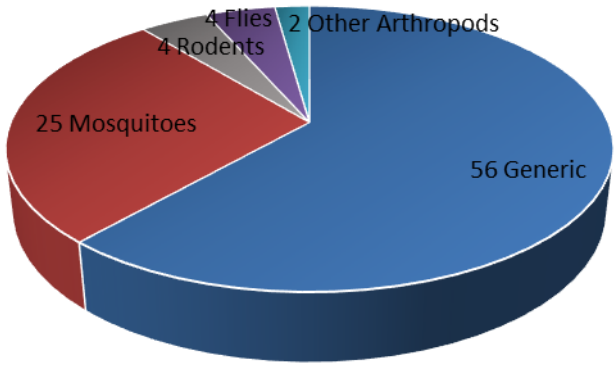

Fig. 32: The pie chart depicts the number of publications in which the presented models are either focused on specific target organisms or are kept generic. Roughly two thirds of the publications feature generic models. Roughly one quarter of the publications' models are specific to mosquitoes. Other models focus on flies, rodents or the red flour beetle.

The majority of publications with specific models focus on mosquitoes [8,13,14,15,18,19,20,22,26,28,38,39,41,51,52,53,54,55,64,65,78,79,80,85]. The publications featuring models specifically implementing the life history of mosquito species can be subdivided into twelve on *Anopheles gambiae* [15,20,23,26,39,52,51,54,55,64,65,85], one on *Anopheles arabiensis* (Eckhoff et al. 2017)[38], one on *Anopheles coluzzi* (North et al., 2019)[64] and twelve on *Aedes aegypti* [8,13,14,18,19,22,28, 53,65,78,79,80,].

An. Gambiae, An. arabiensis and An. coluzzi are mosquitoes in the *Anopheles gambiae* complex responsible as vectors, for the transmission of malaria in sub-Saharan Africa and cause the death of hundreds of thousands of people annually (WHO, 2017). *Ae. aegypti* is the vector responsible for transmitting several viruses including dengue, Zika and yellow fever throughout the world. For decades, different mosquito population control measures were set in place, with a significant decrease in cases and deaths. However, it continues to be a major problem and thus the research on controlling the populations of these species using gene drives is targeted at solving these issues in the public health sector.

Four publications illustrate models that are specifically directed at fly species [21,44,47,65]. One model on *Drosophila melanogaster* (KaramiNejadRanjibar et al., 2018)[44], which also accommodates the life history of *Cerratitis capita*ta, but also represented in one other model [44,65]. Only one publication features a model on *Drosophila suzukii* [44]. *Drophila melanogaster* serves as a model for research and has little applications in agriculture. However, *Cerratitis capitata* and *Drosophila suzukii* are important pests that cause the damage of fruits and consequently the loss of yield in the fruit growing industry. They are one of the most destructive pests that also became invasive species. The damage occurs when the females oviposit in fruits and other parts of the plants (in case of *C. capitata*).

Four publications specifically model gene drives in rodents [31,67,43,69]. The model by Backus and Gross (2016)[31] focuses on mice and the inheritance of a *t-Sry* gene drive construct. Sudweeks et al. (2019)[67] focuses on a CRISPR homing drive in mice, the model by Prowse et al. (2017)[43] covers mice, black rats and European rabbits with different variants of a CRISPR-based suppression drive. Finally, in Prowse et al. (2019)[69] focus on a homing meiotic drive called Y-ChOPE in mice. Mice, rats and rodents in general are targeted for eradication in Australia and New Zealand for the protection of native birds. Due to the introduction of these new predators, birds especially are being threatened with extinction because evolutionary they have not developed behaviors to protect their eggs or hatchlings.

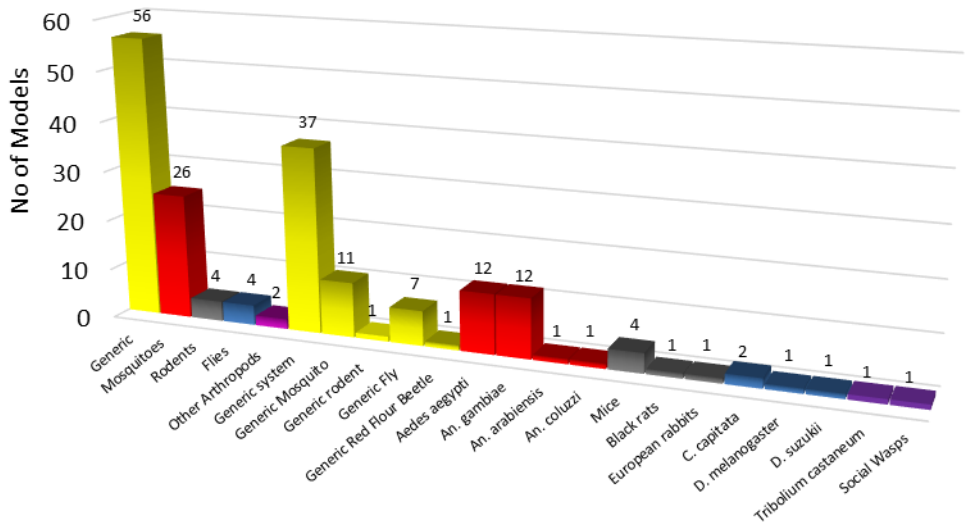

Fig. 33: The bar diagram further subdivides the considered publications' models by the considered target organism. Out of the 56 publications featuring generic models, 20 are generic despite the studies being directed at specific target organisms.

Tab. 7: Number of models dedicated to generic or specific target organisms.

Generic	***56***	***Mosquitoes***	***26***	***Rodents***	***4***	***Flies***	***4***	***Other Arthropods***	***2***
Generic systems	37	*Aedes aegypti*	12	Mice	4	*Ceratitis capitate*	2	*Tribolium castaneum*	1
Generic Mosquito	11	*Anopheles gambiae*	12	Rats	1	*Drosophila melanogaster*	1	Social Wasps	1
Generic Rodents	1	*Anopheles arabiensis*	1	Rabbits	1	*Drosophila suzukii*	1		
Generic Fly	7	*Anopheles coluzzi*	1						
Generic Flour Beetle	1								

Different gene drive techniques are explored in the various models. Wherein most notably HEG drives such as the CRISPR-homing drive is the most often represented technique, as this technique features in almost half of the models. Meiotic drives in various iterations are featured in only roughly one fifth of the considered models and thus represents the second most featured GD technique. In descending order, underdominance, Medea and CRISPR-TA systems are next, while BRAKE and Reversal drives, Killer-Rescue, transposable elements, RIDL, and no gene drives at all (category “none”) are all on the same level with four publications each. In the category “none”, three publications describe a model for population dynamics which is applied to gene drives in another study and one explores post-gene drive parasite spread. Lastly, publications on *Wolbachia*, generic drives and translocations are the least numerous. This is depicted in a bar diagram in Fig. 34.

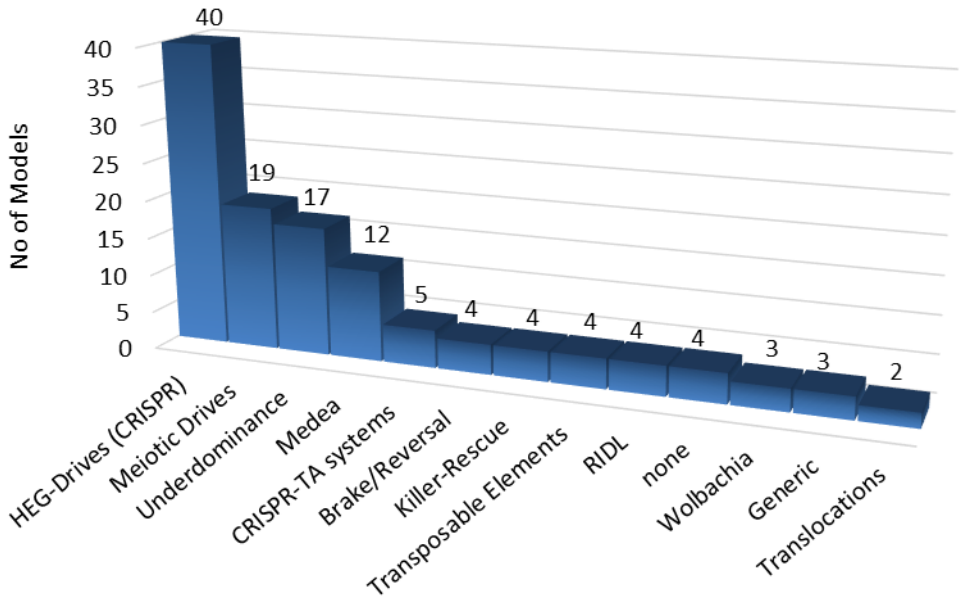

Fig. 34: Gene drive techniques considered in the models shown in a bar diagram.
The HEG-Drives are the most often modelled gene drive systems. The second most considered drive principle is Underdominance in various versions.

4.3.3 **Deterministic vs. Stochastic**

Usually models may be either deterministic or stochastic. However, within some of the considered studies models were introduced based on both approaches. Deterministic models utilize fixed values in their computations and thus also yield fixed results. However, stochastic models compute probability ranges for certain events and thus yield ranges of possible results. For the latter kind of model sensitivity analyses are very important to assess the impact dimensions of variations in the applied variables.

4.3.4 **Spatiality**

Furthermore, models may be either spatial or non-spatial. For this analysis spatial conditions were already granted when multiple non-randomly mating demes were assumed in the model. Tab. 8 shows the distribution of the models along these criteria. The majority of considered studies featured deterministic, non-spatial models [1,2,4,6,7,8,9,10,11,20,24,29,31,33,34,35,36,37,41,42,44,45,50,51,55,57,58,60,63,68,71,76,77,83,84,89,] followed by deterministic, spatial models [3,18,25,30,40,49,53,61,6267,70,72,73,74,] then stochastic, spatial [13,14,19,21,22,23,26,28,38,48,52,64,82,85,87,90] and stochastic, non-spatial models [5,12,17,27,39,43,46,47,69,75,86]. Among the eleven studies that feature deterministic as well as stochastic models, six models are spatial [16,56,65,78,79,80] and five are non-spatial [15,32,54,81,88].

Tab. 8: Distinction of models from considered studies into deterministic vs. stochastic and spatial vs. non-spatial

	Deterministic	*Stochastic*	*Det./Stoch.*
Spatial	14	16	6
Non-Spatial	38	11	5

4.3.5 **Mating System**

Since gene drives or other modes of super-Mendelian inheritance are dependent on sexual reproduction, any model must implement a way of reproduction. Most importantly in this regard is mate choice. True mate choices can only be implemented under stochastic conditions and dependent on the chosen target organism the models implemented reproductive strategies that are either semelparous (single mating) like that of mosquitoes or iteroparous (multiple matings) like that of some flies or mammals either polyandric or polygamous. However as shown before, the majority of models are deterministic and therefore feature a form of perfect panmixis (random mating). This means the offspring of a whole generation is produced according to the genotype percentages in the population. This is true for most mathematical models. Apart from this, almost all models assume panmixis. This assumption might be valid for cage experiments or very large populations, but likely not be valid for release experiments and especially considering shrinking populations in suppression drives. This problem is further exacerbated in deterministic models since in diminishing populations stochastic variations may have much stronger effects. Despite this, some studies that do model population suppression also implemented perfect panmixis (Edgington and Alphey, 2018, Fig. 4 and 5; KaramiNejadRanjibar et al., 2018, Fig. 4; Kyrou et al., 2018, Fig. 5b) [53,44,54] or are simply deterministic (Backus and Gross, 2016)[31]. All models considered spatial, at least limit panmixis to organisms within the same deme, patch, household or within the dispersal radius. Fig. 35 shows how often which kind of reproductive strategy is implemented. Obviously, about two thirds of all models rely on the above mentioned perfect or percentile panmixis. Albeit, perfect panmixis and itero-or semelparity are not mutually exclusive features, if a model exhibits

perfect panmixis, it is thus only counted towards that category. Interestingly, even some stochasticity-capable models were chosen to exhibit this less realistic procreation scheme [12,15,16,17,21,32,46,47,54,75,81,86,87]. And finally, one model was counted as relying on perfect panmixis due to its very unique way of utilizing seed-offspring for the subsequent generation in their cage experiment model (Edgington et al., 2020)[75]. The three models denoted with limited panmixis are deterministic, spatial models where the population is subdivided into patches of panmixis [18,70,73,74]. Eight models implemented iteroparity [23,31,43,48,50,69,82,84], three of which are directed at rodents [31,43,69], one at the red flour beetle [82], one at *Anopheles gambiae* [23] and the other three models are generic systems [48,50,68]. Twenty studies implemented semelparity in their models [5,13,14,19,20,22,26,28,39,52,56, 64,65,90,77,78,79,80,85,88,], of which fifteen are directed at mosquitoes [13,14,19,20,22,26,39,64,65,78, 79,80,28,52,85], while the remaining four models are generic systems that utilize the SLiM-model system [5,77,88,90]. The SLiM-model system is also the one model which allows to vary the reproductive strategy (Haller and Messer, 2019)[56].

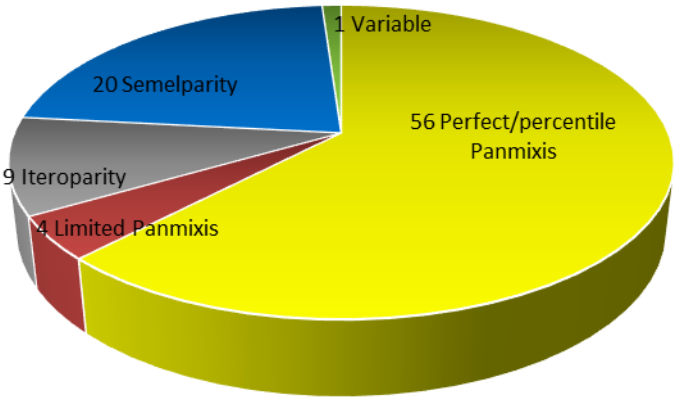

Fig. 35: Pie chart of the implemented reproductive strategies. 56 models implemented a kind of perfect panmixis, two deterministic, spatial models use forms of limited panmixis, seven models employ iteroparity, 20 semelparity and one can be set to various reproductive strategies.

4.3.6 **Implemented Features**

Fig. 36 depicts other features and how often they are implemented into the considered models. The most frequently implemented feature is **fitness cost**. Out of the 90 studies, only models from 15 studies did not implement fitness costs into the models [1,13,15,19,30,31,36,43,44,50,52,64,69,79,83]. In population genetics, fitness is mostly defined as a genotype's propagation success rate. For super-Mendelian inheritance modelling, fitness costs are applied synonymously with genetic load. Often (relative) fitness cost is implemented as a multiplier between 0 and 1 to the amount of offspring generated, in a few models this is done sex-specifically.

The second most frequently appearing factor is **gene drive thresholds**. Some gene drive techniques, mostly toxin-antidote based ones, require a certain ratio of gene drive carriers in the population for the gene drive to spread. Usually this is not an implemented feature but something that emerges due to other implemented features, most importantly the rules of the gene drive's inheritance and if implemented the fitness costs.

The third most often implemented feature is the **breakdown of drive**. This category includes modelling of acquired resistances such as sequence mutations due to non-homologous end-joining, microhomology-mediated repair or incomplete homology-directed repair, single nucleotide polymorphisms as expected from standing pre-existing resistances. As would be expected, the majority of studies concerned with the breakdown of the drive due to resistances focus on HEG-Drives like CRISPR homing drives or the novel CRISPR-TA systems [45,57,88]. But

also other drive systems like the synthetic as well as natural Medea drive are concerned with resistance-to-drive (Buchman et al., 2018a; Cash et al., 2020) [47,82]. Notably, the MGDrivE model also distinguishes between in-frame and out-of-frame resistance alleles (M. Li et al., 2020; López Del Amo et al., 2020a; Sánchez C. et al., 2020) [78,79,80]. And furthermore, self-limiting properties like that of killer-rescue or the reversal by wild type releases as for Underdominance (Akbari et al., 2013; Edgington and Alphey, 2019) [21,59] are also reflected in this category.

30.0% of models included **dispersal** as a metric. Logically, a model must be spatial in order to reflect the movement of the individuals either within or across habitats. Therefore, it is not surprising that all but seven out of the 36 considered spatial models comprise dispersal [14,19,52,67,72,73,87].

4.3.7 **Species Specificity**

The implementation of **life history** is the decisive factor whether or not a model was counted as dedicated to a specific target organism or a generic system. Life history may potentially encompass many parameters. In the narrow population biological sense, life history is the longevity, beginning of and frequency of reproduction and number of offspring. The mostly used parameters are merely life stages, mortality, reproductive strategy, number of offspring and dispersal in some models. Interestingly, many stochastic studies do not implement a definitive longevity for the final life stages but instead a mortality rate, which potentially allows for infinitely old individuals. Even less frequently implemented parameters are fertility and fecundity, population and assortative mating and mate choice preferences. Understandably, each one of these parameters may decisively influence the outcome of a model simulation. Therefore, the utilized values and functioning of the parameters should be met with diligent scrutiny. Most studies therefore rely on values derived from *in vivo* studies, naturally it is questionable in how far results from studies on laboratory strains or even caged wild specimen can be transposed onto the real-life conditions in the wild. While field data may often vary greatly from study to study, exemplifying great heterogeneity in different habitats and population dynamics of r-strategist species.

Fertility and fecundity respectively are implemented in 30.0% of models. Fertility is the ability to produce viable offspring and fecundity is the quantity of offspring. In most cases, those parameters are implemented in the amalgamated form of a multiplier to the amount of offspring with a value of 1 or below, just like fitness. In others, fecundity is implemented as a variable number of offspring generated from a mating. In some studies, a reduction in fecundity is the chosen way to implement fitness costs (MGDrivE) [65,78,79,80]. In yet others, fertility/fecundity is influenced by the drive's cargo gene (Eckhoff et al., 2017; Kyrou et al., 2018; Simoni et al., 2020) [36,54,81].

In 36.66% of the considered studies the models implemented **density dependence** into their models. The majority of the other models assume a population of a fixed size. Density dependence revolves around the implementation of a carrying capacity, however the consequences when closing in on that carrying capacity varies. Some models reduce growth down to zero (Prowse et al., 2018) [43]. Others reduce fecundity (Bull et al., 2019a; Champer et al., 2020a; Eckhoff et al., 2017; Haller and Messer, 2019) [38,56,73] or increase larval mortality (Beaghton et al., 2016; Deredec et al., 2011, 2008; Huang et al., 2011; Legros et al., 2013; M. Li et al., 2020; López Del Amo et al., 2020a; Magori et al., 2009; Marshall et al., 2017; Marshall and Hay, 2014; North et al., 2019; Sánchez et al., 2020, 2019) [9,13,18,20,22,26,30,39,64,65,78,79,80], some reduce births and increase death (Backus and Gross, 2016) [31], yet other models reduce fitness (Champer et al., 2020c; Edgington and Alphey, 2018) [53,90].

Twenty models implemented **competition** which in half the cases is implemented only in the larval stage. This larval competition is reminiscent to density dependence limited to a

premature life stage. Only the model by (Manser et al., 2019) [61] implemented sperm competition.

Ten models incorporated **geography/topography**[13,14,19,22,23,38,52,56,64,85], relying on either real life geography or urban households to plot the target organisms' habitats or at least continuous space for agents to move in. Relatedly, in eight publications **seasonality or weather data**[12,13,14,19,22,38,52,64,85] are implemented. Four of which represent the Skeeter Buster model (Legros et al., 2013, 2011; Magori et al., 2009; Xu et al., 2010)[13, 14, 19, 22]. Both features may be decisive factors to predict gene drive spread in real life applications.

Only seven publications include various mitigation techniques [37,59,60,61,86,87,80] such as the introduction of synthetic resistances[37,59 52], reversal drives[37,60], immunizing reversal drives[37] and wild types [59,80], brake [61] are discussed in these publications and show varying effectiveness.

Inbreeding was shown to be another important factor implemented in the models of four studies [42,48,56,74]. In two studies inbreeding is shown to be a decisive factor (Bull et al., 2019b; Drury et al., 2017) [42,74].

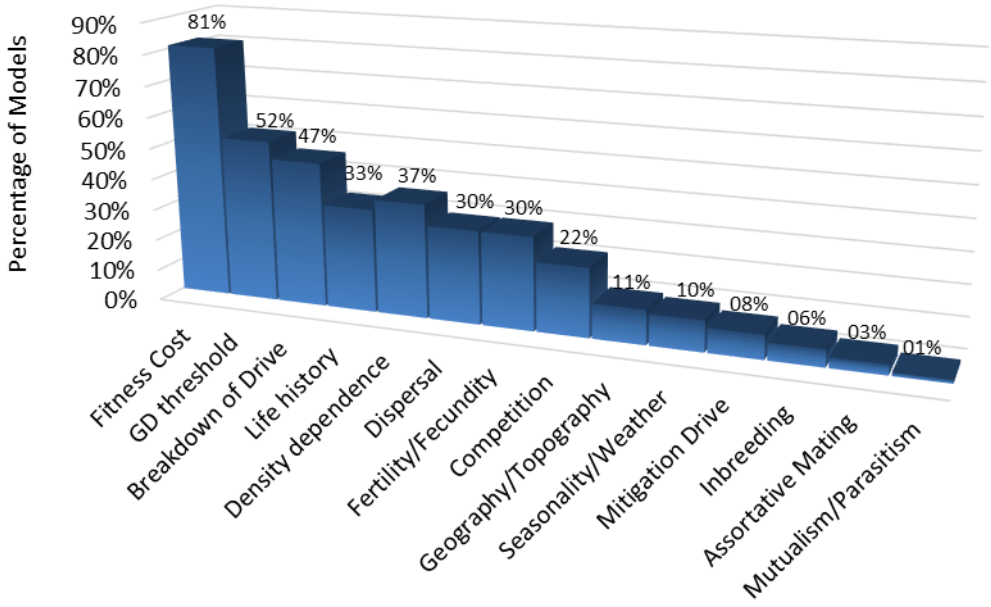

Fig. 36: Frequency of implemented features in the models shown in a bar diagram.
Fitness cost is the most frequently implemented feature followed by gene drive threshold, breakdown of drive, life history, density dependence and dispersal. All those features are implemented in upward of one third of the examined models.

Another issue concerning data on target organisms is that these in most cases were obtained from laboratory observations. For instance, the duration of life stages, longevity, fertility and mortality in *Drosophila suzukii* are to a great degree temperature-dependent. It would thus be the logical step to construct a model that implements temperature data translating it onto the population parameters. This together with the general availability of annual temperature data for almost every area on the globe would make such a model a versatile tool. The data on these factors however, were gained in laboratory studies which exposed the flies to constant temperatures over long time-spans (Ryan et al., 2016). Hence, the reliability of these data is limited considering natural conditions. The alternative would be to, if available, rely on field observations which are mostly scarce, less exact with high degrees of variability and often

contradictive. For instance, estimates on the population size of *D. suzukii* are based on trapping experiments, numbers of catches together with their sex ratio vary with the applied kind of trap. Yet even when data from observations in the natural habitat are available. These are mostly more general and not as specific as required from an input into a model. It is unlikely that any ecological study would yield a table of temperature dependent alterations in abstract parameters such as fecundity/fertility or fitness as they are employed in models. Maybe it is due to such unavailability of suitable data why Buchman et al. (2018a) focused solely on the genetics in their model on Medea spread in *D. suzukii* with standing genetic variation.

Maybe this however only owed to the early stage of development of this particular gene drive application. Evidently the more advanced development of an application in Anopheles mosquitoes has fathered models that focus more on the specific characteristics of the target organism. Looking into one of the more sophisticated spatial models on the release of gene drive in Anopheles mosquitoes and in the later publication including geographic data from Burkina Faso (North et al., 2013; North et al., 2019) the supplementary material on the model parameters can be very interesting.9 As the numbers for many parameters seem more like rough estimates most often lacking information of their variances.

4.3.8 **Requirements for Models in Risk Assessment**

Data sources for the performance of a GMO environmental risk assessment are predominantly laboratory experiments, semi-field or field experiments, primary literature and models. To assess the adverse effects of releasing GMOs, there is a need to study all levels of organization, from genetic, to species, population and landscape, including their interaction. Oftentimes, there is a need to enlarge the research scope, especially to evaluate the potential delayed effects that might arise, through the use of mathematical models.

The specific challenges of gene drives are critical for environmental risk assessment and substantially increase the importance of computational model simulations to understand and predict spread and consequences of a gene drive in the environment. Such extrapolations can only be interpreted as indicative, and cannot simulate the complexity of the environment. However, since information cannot be generated through field experiments in the case of GDs, these models represent the only available means to predict GD dynamics in the field, they will play an important role in risk assessment (EFSA GMO Panel et al., 2020, p. 55). While Modelling approaches for efficacy testing and for risk assessment pursue some overlapping goals, they differ fundamentally in the level of certainty they need to provide. From the developer's perspective, the risk of failure, i.e. too little efficiency needs to be excluded, while from a risk assessor's perspective e.g. the following scenarios of exposure need to be evaluated. Loss of the gene drive does not exclude the possibility that synthetic gene drives remain in the environment. Also, if synthetic gene drive systems are more effective than expected, this might trigger unintended enhanced spatial or temporal exposure.

According to chapter 3.7 in the guidance for the release of GM animals (EFSA GMO Panel, 2013), models used in ERA should provide information on its parameters, verification, validation, sensitivity analysis and evaluation of unquantified uncertainties. However, our focus is not on the design and mathematics of the models, but rather on how far the complex environment has been simulated and which ERA characteristics are being examined.

We analyzed the existing models in the focus of this study against the specific properties of a GM insect that need to be addressed in the European risk assessment (EFSA GMO Panel, 2013). There are seven major areas of interest when assessing the release of a GMO insect

9 Link to Table S1
Link to supplementary material of North et al. (2019), download additional File 6

into the environment: persistence and invasiveness of GM insects, vertical and horizontal gene transfer, pathogens, infections and diseases, interactions between the GMO with target and non-target organisms, impacts on human and animal health and impacts on techniques for the management of GM insects (EFSA GMO Panel, 2013, chap. 4.2). Included in these major areas of risk, there are specific endpoints that could potentially be predicted through modelling. These endpoints are presented in Tab. 9.

Tab. 9 Requirements for ERA of GM insects (EFSA GMO Panel, 2013) and consideration in GD models.

Specific areas of risk for the ERA of GM insects	*EFSA 2013 characteristic*	*Implemented in GD models?*
Abiotic factors	Temperature	✓
	Humidity	✓
	Temporality	✓
	Climatic /geographical barriers	✓
	Occurrence	✓
	Fitness	✓
	Reproductive biology (fertility and fecundity) before and after release	✓
	Survival	✓
	Dispersal	✓
	Population size, structure, sex ratio (before and after GM release)	✓
	Reduction in efficiency/resistance development against GM	✓
	Changes in interactions (behavioural, genetic) between GM-TO	✗
	Adverse effects due to ”low quality GM insects” or reduction in GM efficiency	✓
Biotic characteristic GMO related	Reproductive potential	✓
	Hybridization	✗
	Male mating competitiveness	✓
	Female mating success	✓
	Fecundity (GM and hybrids)	✗
	Fertility (GM and hybrids)	✗
	Heterosis (hybrid vigour)	✗
	Development	✓
	Dispersal (Potential to explore new niches)	✗
	Ability to survive (disease, predation, competition, food availability, abiotic factors)	✗
	Fertile offspring production	✓
	Horizontal gene transfer	✗
	GM genetic stability	✓
	Immigration/emigration	✓
Interactions with other species (non-target organisms)	Competition with other species	✗
	Hybridization	✗
	Pathogens (altered transmission range and frequency), increased vector competence	✓
	Adverse effects due to ”low quality GM insects” e.g. increased human biting rate or disease transmission	✗
	Prey	✗
	Predators/predation	✗
	Symbionts	✗
	Hosts (plants, animals)	✗
	Parasites, pathogens	✗
	Trophic level/food web effects	✗
	Competitors (abundance, species composition)	✗
	Ecosystem services	✗
	Toxins/allergens associated with GMO	✗

Simulating the complexity of the natural world is a task that can only be solved in approximations. However, as shown in Tab. 9, current models consider (at least partially) abiotic factors as well as biotic factors related to the target organism such as reproductive

biology, fitness, etc. Some GMO-related biotic traits are also considered, but with the exception of interactions with pathogens, there are no models that consider interactions between the GM insect and non-target organisms. The overall analysis of models in the light of ERA requirements showed that there is a lack of ecological data, especially concerning interactions with other species, habitats and ecosystems. In addition, the potentially very long-time frame of persistence can lead to inaccuracies in predictions. Whereas models on classic GMOs may rely on lab and field data of scaled releases with finite time frames that can serve as a trajectory to predict behaviour and properties in the field, simulations for GD systems seem far less trustworthy in this regard.

4.3.9 **Advanced Models for Gene Drive Risk Assessment**

For the following paragraphs the likely most powerful models were selected to closer examine them according to their suitability towards an environmental risk assessment. These models are that of North et al., SliM3, Skeeter Buster and MGDrivE.

The model by North et al. is a spatial, stochastic or deterministic agent-based simulation, which covers a large geographic area of one million square miles. It is directed at the life history of malaria vectors *Anopheles gambiae* and *A. colluzzi*. The life history is implemented as two stages, juvenile and adult. Adults are males and multiple female stages of mate-, host or breeding site-seeking. So far publications focused on a meiotic sex distorter Y-drive (North et al., 2019)[64] and a CRISPR/Cas-based homing drive with a female infertility cargo gene (North et al., 2020)[85] in simulated eight year time-frames. The model considers abiotic factors such as the regional seasonality and perennial and non-perennial water bodies as breeding sites for the target organism (North and Godfray, 2018). Each breeding site in the vicinity of human settlements is an agent. Biotic factors, such as larval competition which acts as the density dependent carrying capacity, migration, aestivation and long-distance migration are also considered by this model. Although especially these latter two parameters rely more on assumptions rather than field data.

The SliM3 model (Haller and Messer, 2019)[56] can be used in a deterministic or stochastic fashion and in a spatial and non-spatial manner. It is a based on a Wright Fisher model, but exceeds its predecessor by implementing age structured populations, mate choice, inherent offspring generation, overlapping generations, migration, hard selection, continuous space maps for different parameters and populations, interactions with interaction strengths and radii, genetics, different types of mutations and individual organisms. The so far published simulations examined HEG Drive (Champer et al., 2018; S. E. Champer et al., 2020) [46,77], CRISPR-TA (Champer et al., 2020b)[89], as well as four different Underdominance variants (Champer et al., 2020c)[90]. All these publications have been generic, although the model would allow customization towards the life history of a specific target organism. The simulated time frames varied between 40 to 100 generations. Although SliM3 would be a very versatile program to use in environmental risk assessment, this versatility comes at the lack of modularity. The continuous space can be defined with areas of parameter changes such as fitness, population density and so on, but it would prove difficult for an assessor to define real landscapes according to abstract terms of population dynamics. Likewise, the software can be designed to accommodate specific target organisms which would be a challenging endeavour to do.

The Skeeter Buster (Magori et al., 2009)[13] software is a stochastic, spatial model directed at *Aedes aegypti*. The model simulates individual water containers in 612 (in fourfold copy of 153) households in Iquitos, Peru (Legros et al., 2011)[19]. Each container is modeled with a water level, nutrition, and temperature. Those factors determine larval weight and thereby development and competition. Weight also determines the fecundity and mating capability of adult mosquitoes. The model distinguishes eggs, larvae, pupae and adult mosquitoes. This software is the most detailed considering the life history of its target organism. It features two

modes of dispersal: migration to neighbouring households or long distance (~200 m) by either adults or the displacement of entire containers. Furthermore, different release strategies and weather conditions such as temperature precipitation and humidity from Iquitos, Peru and Buenos Aires, Argentina have been considered (Legros et al., 2011)[19]. Medea and Killer-Rescue (Legros et al., 2013)[22], as well as RIDL and an anti-pathogen gene were until now simulated with this model (Okamoto et al., 2014)[28]. The modelling time frames were set between two to five years.

MGDrivE (Sánchez C. et al., 2019)[80] is a deterministic or stochastic, spatial modelling software, that has a modular build. Each model run consists of an inheritance cube module, a life history module and a landscape module. The inheritance cube module is designed according to the respective genetic system. So far, inheritance cubes exist for CRISPR-based homing drives for replacement or suppression, Medea, RIDL, Wolbachia, Underdominance, Translocations (Sánchez C. et al., 2020)[80], transcomplementing homing drive (López Del Amo et al., 2020a)[79], and a split HEG-Drive (M. Li et al., 2020)[78]. Inheritance may also be linked to a set sex-specifically variable parameters and emerging resistance alleles that can vary in fitness penalty and probability of occurrence. The life history module works with lumped age-class structures that may vary in a set of parameters and are predefined for *A. gambiae*, *Ae. aegypti* and *C. capitate*. The landscape module consists of a network of interconnected habitats. Migration probability between different habitats may be variable. Finally release schemes specifying habitat, size, number, frequency and beginning can be customized. While the inheritance cube modules are quite detailed and comprehensive, life history and landscapes seem to be covered rather superficially and more abstract. While other ecological factors are completely disregarded. But a pre-print on the follow-up version MGDrivE 2 (Wu et al., 2020) alleviates these issues, as now life history and migration parameters can be changed over time. Furthermore, additional inheritance cubes are included such as remediation systems (CHACR, ERACR) and CleaveR (Oberhofer et al., 2019)[57]. Most notably, an epidemiology module was included that harbors a lumped class human population that can progress through the states of susceptible, latently infected or infectious and in the case of arboviruses also 'recovered'. Likewise, except for recovered, the mosquitoes now possess the same denominators. Nevertheless, the genetic component of the model remains the most elaborated module as opposed to the rather crude and abstract other modules.

5 Part A.3 - Gaining Knowledge through Modelling

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Verma, Prateek; Reeves, R. Guy; Simon, Samson; Otto, Mathias; Gokhale, Chaitanya S.

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Gene drive techniques increase the frequency of a synthetic genetic element in populations in a manner only partially determined by its impact on organismal fitness (and stochastic events). As an example, the natural Segregation Distorter (SD) locus in Drosophila melanogaster imposes an enormous organismal fitness cost, in that it is homozygous lethal (and only viable as heterozygotes) (Crow, 1991; Sandler et al., 1959; Sandler and Golic, 1985). Consequently, in most circumstances, natural selection, at the organismal level would act to eliminate the SD allele. However, because of its capacity to bias the production of SD functional sperm in +/SD heterozygotes, the allele has rapidly increased to an equilibrium frequency of 1-5% in most natural populations around the globe (Brand et al., 2015; Hartl, 1975; Hiraizumi and Thomas, 1984). This natural drive element illustrates how drive elements can increase in frequency even where there is a substantial cost to (overall) organismal fitness. Since the development of molecular biological techniques, there has been an interest in developing synthetic drive elements used to push linked genes into wild populations in a self-perpetuating manner. This is generally termed replacement drive, to distinguish from suppression drive that aims to reduce or completely eradicate the size of target populations upon release.

As in the case of SD, it does not necessarily follow that any synthetic drive element will likely increase to a frequency to the extent that it displaces all wild type alleles at its chromosomal location that were initially present in the wild population. This fixation property is dependent on various drive parameters of the developed system. Other such properties of interest are the speed of action, reversibility and potential to be spatially confined to only target populations. The sensitivity of such fundamental properties of drive systems to drive parameters has been a topic of interest of numerous recent theoretical studies.

Developments in the theories and models of gene drive, to some extent, out-stripped the experimental approaches. However, the fast-pace of developments in the field of molecular biology allow us to design complicated drive systems which may be substantially better in the properties of interest than their natural counter-parts. The need for theoretical sandboxing of such technology with planetary consequences is therefore imperative before field deployment. It is also critically important to provide the stakeholders of such a technology, sufficient understanding to evaluate the basis of crucial projected outcomes. However, the number of publications on theoretical and experimental synthetic gene drive systems is overwhelming and ever-increasing. Generally, the properties of each of the sequentially proposed synthetic drive approaches are described using bespoke modelling frameworks (Davis et al., 2001; Unckless et al., 2016; Ward et al., 2011). Even with adequately described mathematical models, a recapitulation of crucial results is often beyond all but expert theoreticians. The capacity to quickly compare the relative sensitivity of fundamental properties of different drive scenarios to parameter changes would be of potential value to both experts and non-experts alike.

We constructed a representative literature database on synthetic gene drive system to be cognizant of the current trends in this rapidly growing field of research. The database consists of 50 publications from year 1995 to 2019. The literature is sorted on the basis of gene drive

type (replacement or suppression), the model system under study, theoretical methodology, consideration of breakdown of drive, the possibility of gene drive reversibility and public accessibility of the literature. From the analysis of the literature database, we found that studies on replacement drives (Gantz et al., 2015; Marshall and Akbari, 2015) are given no less importance compared to suppression drives (Beaghton et al., 2017b; Hammond et al., 2016; Kyrou et al., 2018). The complete database and the summary statistic can be found online on GitHub. The majority of research studies have considered resistance evolution in synthetic gene drive system (Noble et al., 2017; Unckless et al., 2016). Analytical methodologies mainly employed deterministic and stochastic models. The focus of research is now trending to consider spatial features in their models (Bull et al., 2019a; Calvez et al., 2018; Champer et al., 2021a; Dhole et al., 2018; Eckhoff et al., 2017; Huang et al., 2011a; Tanaka et al., 2017a). The model organism on gene drive studies have been chiefly mosquitoes (Gantz et al., 2015; Hammond et al., 2016; Windbichler et al., 2011), fruit files (Buchman et al., 2018a; Gantz and Bier, 2015; Larracuente and Presgraves, 2012) and rodent (Grunwald et al., 2019; Lindholm et al., 2013; Lyon, 2003) respectively. Several theoretical studies also use generic organisms that allow for generalized prediction on the spread of drive organism. Analysing the select literature, we have distilled the primary components of synthetic gene drive models in a succinct theoretical model and a handy, user-friendly tool DrMxR - Drive Mixer.

We develop from the principles of standard population genetics, incorporating the processes that subvert the generally dominant role that organismal fitness plays in how natural selection can impact the frequencies of alleles within natural populations.

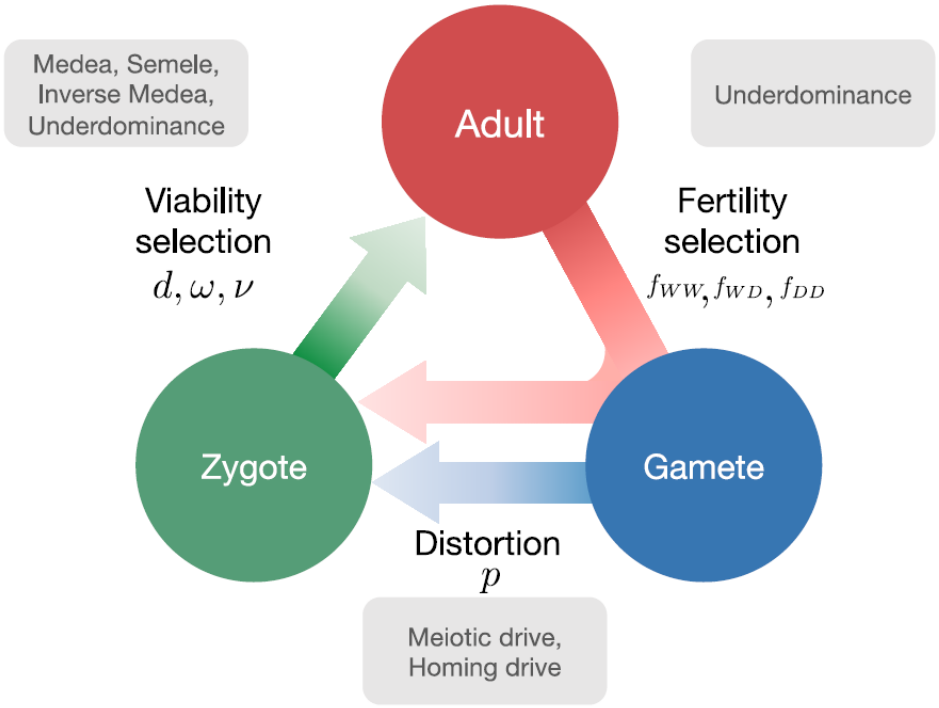


Fig. 37: Lifecycle of an individual organism for a generic gene drive model.
Assuming that individuals reproduce sexually and that the lifecycle has three stages, adult, gamete and zygote. Adults produce gametes which combine to form zygotes. Zygotes grow up to become adults. Three factors can act during the life stages of an organism: distortion, viability selection and fertility selection (represented as arrows). Each can influence the probability of inheritance of a gene in the population and can be potentially manipulated to engineer gene drive constructs. Parameters, described in the text, are associated with each of the three arrows. Examples of named drive systems that can be generated are provided associated with the respective arrow.

To develop the model, first, we consider the lifecycle of a generic diploid organism through the various stages of development, from an adult, forming gametes to zygote and then back to an adult. We discuss how the drive can act at any one or all of these stages. We then proceed to combine the knowledge into a single population dynamic model. We test our developed model by extending it in different ways, thus recovering the specific cases of gene drives discussed in previous theoretical and experimental studies. Next, we extend our analysis to the ecological dimension as well. We determine the risk level of losing a wild population, through accidental introduction or migration of drive capable individuals. Further, we test our results in spatially explicit conditions and determines the extended conditions required for the invasiveness of drive elements.

We thus show that a single theoretical approach when minimally extended provides specific cases of different drive systems. This exercise provides us with a universal vocabulary as opposed to the invention of new terms for every different drive system, which makes comparing them prohibitively time consuming. To this end, we begin by detailing the process of theory development in the following section.

5.1 **Results**

One of our main results is the generation of a user-friendly application called DrMxR (Drive Mixer) shown in Fig. 38 and available on GitHub. With an intuitive interface, both experts and non-experts alike can explore the properties of previously described drive systems across their entire parameter space. Besides, users can combine drive systems to represent the likely properties of largely unexplored combinations. For developing this application, we have assumed an obligate sexually reproducing organism, a likely necessity for successful gene drive where organismal fitness is negatively impacted. The life cycle of the organism is split into three tractable stages; the minimal abstraction required to recover the established results in the field of engineered gene drive systems. Further complications can indeed be added depending on the exact case study in focus.

Fig. 37 shows the life cycle of an individual in our model. We focus on two allelic types wild type (W) and the driven gene (D). Thus, we have adults of three genotypes, wild type homozygotes WW, heterozygotes WD and drive homozygotes DD. Adults are chosen from the population pool for reproduction. Adults produce gametes which combine to form zygotes. The zygotes grow up to become adults, and the cycle continues. We allow for overlapping generations, a realistic assumption for numerous target species such as mosquitoes, drosophila or rodents (Backus and Gross, 2016; Buchman et al., 2018a; Windbichler et al., 2011). We assume that the alleles during gamete formation are segregated independently according to Mendel's inheritance laws. Hence, the total number of alleles in the absence of any evolutionary processes remain conserved over successive generations. Frequencies of genotypes, therefore, reach Hardy-Weinberg equilibrium in the limit of infinite population, random mating and no selection (black parabola connecting WW and DD in the Fig. 38).

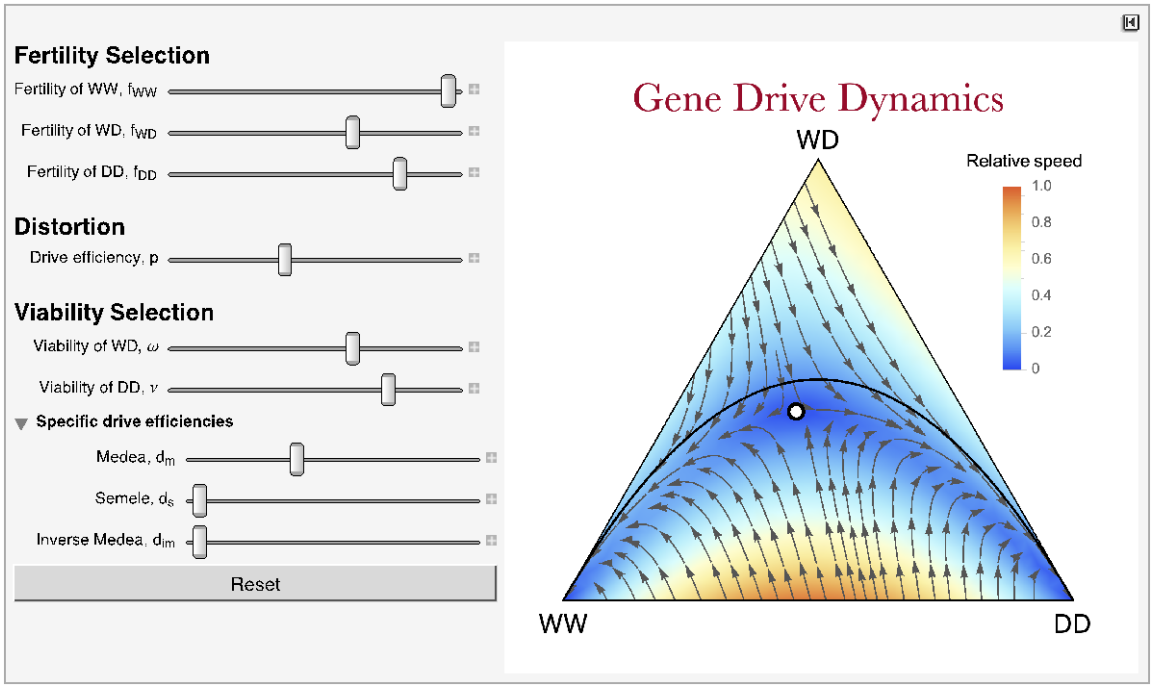


Fig. 38: DrMxR (Drive Mixer): a handy tool to explore the population level consequences of different drive systems.
Different types of drive mechanisms can be designed by biasing the three fundamental phases in the lifecycle of a diploid organism. In DrMxR the user can select the magnitude of the impact of the selected drive. Further-more the consequences of a combination of different types of drives can be visualised in the space of the three genotypes denoted by the wild type (W) and the Drive (D) homozygotes and the WD heterozygotes. Note that even when parameters embodying multiple types of drive are employed simultaneously the dynamics described by DrMxR is always for a single combination drive construct. The tool is available on GitHub.

The essential feature of a gene drive is biasing the chance of inheritance of the desired gene in the population (Champer et al., 2016). The expected outcome, however, is that the population composition is modifiable in a controlled fashion. Interventions along the lifecycles can accomplish the change via the process of distortion, viability selection and fertility selection. These processes act at different stages of an individual's life cycle. Distortion acts at the gamete level and biases the transmission of the drive allele in the heterozygote. Gametes combine to form zygotes, but some are non-viable and die. Fertility selection acts at the adult stage when individuals are chosen to reproduce with probability proportional to their fitness. Distortion, viability selection and fertility selection, thus, together or even independently can drive the population away from the Hardy-Weinberg equilibrium. Synthetic gene drive techniques allow us to engineer such selection pressures.

5.1.1 Individual Dynamics

a. Viability Selection

Viability selection acts during the zygote phase of an individual's lifecycle. The viability finesses represent the inherent variation in the fitness of the three genotype WW, WD and DD. The fitness can also capture the payload costs of the drive allele. Viability fitness is defined here as the probability of survival of the zygotes up-to-the adult stage. ω and ν denotes the genotypic viabilities of WD and DD respectively. The above parameters have been normalized with respect to the viability of WW that is kept to 1.

Well described synthetic drive systems that work principally by manipulating viability selection parameters include those using zygotic toxin-antidotes. In these systems, a proportion of zygotes of specific genotypes may become non-viable. Medea (Maternal effect dominant embryonic arrest) is an example of a naturally occurring toxin-antidote gene drive found in flour beetles (Beeman et al., 1992; Wade and Beeman, 1994). In Medea drive wild type homozygous offspring of heterozygous mothers are non-viable. Population dynamics of Medea drives have been studied in (Gokhale et al., 2014; Ward et al., 2011). A synthetically engineered Medea drive first demonstrated in Drosophila (Chen et al., 2007) has been extensively studied (Akbari et al., 2014; Buchman et al., 2018a). Similarly, a synthetic viability selection based underdominant population transformation system was developed for Drosophila melanogaster in (Reeves et al., 2014). Fig. 39A shows the population dynamics of Medea drive and deviation from Hardy-Weinberg equilibrium parabola.

Using DrMxR, Medea and other related synthetic drive systems can be seamlessly modelled including inverse Medea (Marshall and Hay, 2011), or Semele (Marshall et al., 2011). The drive efficiencies of Medea, Inverse Medea and Semele drive is represented by parameters d_m, d_{im} and d_s respectively. The framework used by DrMxR is general and applicable to other single construct gene drive system also entirely or partially based on viability selection.

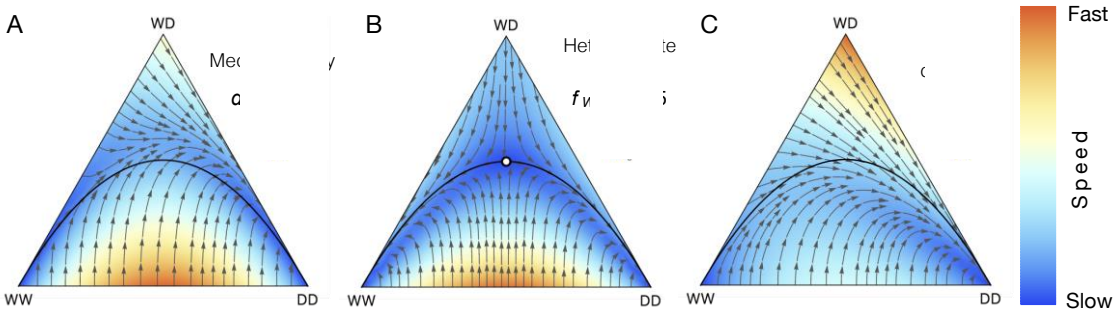


Fig. 39: Effects of fertility selection, distortion and viability selection on population dynamics of the three genotypes.
Population consist of single genotype at the vertices of a triangle in de Finetti diagram. A point in the interior corresponds to the population composition where all three of the genotypes potentially exist. Their relative abundance is proportional to the distance from the vertices. The black parabola curve represents Hardy-Weinberg equilibrium. The white open point represents the population composition of the fixed point. Colours exhibit speed of the dynamics inside de Finetti plots. The speed of the dynamics has been normalized for each plot and their absolute values are not directly comparable between diagrams through the flowlines are. **(A)** Viability selection for Medea gene drive with drive efficiency $d_m = 1$. **(B)** Fertility selection for the underdominance case where fertilities of the of the genotypes are $f_{ww} = 1$, $f_{WD} = 0.5$, $f_{DD} = 1$. An unstable point appears in the interior of de Finetti diagram and is denoted by a white circle at $x_{WW}, x_{WD}, x_{DD} = (0.25, 0.50, 0.25)$. A small release of WD or DD will invade the wild population exclusively consisting of WW. **(C)** Distortion when drive heterozygous individuals contribute drive allele with 100% efficiency i.e. $p = 1$.

b. Fertility Selection

Specific genotypes may experience fitness advantages because of preference for traits during mating and or because some genotypic pairings are more fertile than others. Both of these fitness components are modelled using the fertility selection parameters. The fact that both mating success and fecundity are considered jointly dictates that the fertility selection arrow on Fig. 37 traverses three life stages, rather than the two indicated for the other types of selection. The fertility fitness component arising from mating success is included in the parameter f_{WW}, f_{WD} and f_{DD} for the three genotypes. Fertility selection is an evolutionary phenomenon that drives the population away from the Hardy-Weinberg equilibrium. In our

model, we did not differentiate between sexes of the same genotype which is studied in (Hofbauer and Sigmund, 1998) where the fitness for all possible mating pair is different.

Work by (Feldman and Liberman, 1985; Nagylaki, 1987) shows the rich dynamics that ensue when fertility selection is considered. The population dynamics of two alleles system for different fertilities and sex-dependent viabilities have been extensively studied in (Hofbauer and Sigmund, 1998). The authors have also accounted for non-random mating between the mating pairs by introducing additional parameters (Hofbauer and Sigmund, 1998). We have accounted for variable fertility rates by introducing suitable parameters in the context of the gene drive system (as shown in Fig. 39B).

c. Distortion

Gametic distortion alters the transmission of drive alleles in heterozygotes, so they substantially exceed the Mendelian expectation of a half and is controlled by the single parameter p in our model. Biologically such distortion happens in natural meiotic drives where meiosis is subverted due to intra-genomic conflict (Lindholm et al., 2016; Palopoli and Wu, 1996; Sandler and Novitski, 1957a). Examples of naturally occurring gene drive elements based on distortion are segregation distorter and t-haplotype in heterozygous fruit fly and mice respectively (Larracuente and Presgraves, 2012; Lyon, 2003). These drive elements bias their transmission during spermatogenesis by killing sperm carrying non-driving alleles (W). Though the killing of non-carrier sperm also has the potential to reduce fertility (Lindholm et al., 2016; Price and Wedell, 2008), 'distortion' can be conceived as an independent evolutionary force responsible for biased transmission of drive allele. The synthetic homing drive also distorts the transmission of alleles in heterozygotes. To keep the model tractable, both analytically and in terms of user comprehension, DrMxR does not currently consider sex-ratio gene drives (Y-driving, X-Shredder) (Burt, 2003; Burt and Deredec, 2018). Fig. 39C shows the effect of distortion on the population dynamics of the three genotypes: WW, WD, DD.

All the above methods of biasing the inheritance pattern of a gene can be captured by the means of our generic model. We first derive the mathematical formulations of the processes independently and then combine them in a single dynamical model system. To demonstrate the generality of our approach we recover the results of Noble et al. (2017), Marshall and Hay (2011), Marshall et al. (2011) and Gokhale et al. (2014) as special cases of our model formulation in Appendix A. Ecologically, it is important to characterise the spread of a genetic construct. We do this in panmictic as well as spatially constrained populations (constrained in the sense that the probability of mating between two individuals in not uniform across the range of the population). We provide an analytical form for calculating the zone of refraction (the safe amount of drive heterozygotes and homozygotes), when released, the population recovers the wild type state. For spatially constrained systems we show the exact form in which the probability of invasion and fixation of a drive element depends on the connectivity of the network.

5.1.2 Combined Dynamics

The three evolutionary forces viz. distortion, viability and fertility selection have the potential to act during the three stages of an organism's lifecycle. The individual impact of the forces on the population dynamics is illustrated in Fig. 39 by varying parameters using our application DrMxR. The equilibrium dynamic changes in different ways relative to the Hardy-Weinberg equilibrium line in Fig. 39. Besides individual impact, our application allows intuitive exploration of scenarios when more than one of these three evolutionary forces acts in combination. Realistically, such scenarios arise when a drive element impacts simultaneously both distortion

and fertility selection (Lindholm et al., 2016; Price and Wedell, 2008). In the Drosophila segregation distorter, sperm carrying wild type allele in heterozygous males is selectively killed biasing the transmission of drive allele and also potentially reducing the fertility of the males. Homing Endonuclease gene drive based on CRISPR Cas9 has also been mathematically modelled to bias transmission and reduce the fertility of the genotype carrying payload gene (Noble et al., 2017).

Our approach recovers the result of (Noble et al., 2017) showing the combined effect of distortion and fertility selection on population dynamics. Fig. 38 recovers the result of (Gokhale et al., 2014) shows the combined effect of fertility selection (underdominance) and viability selection (Medea gene drive). Similarly, population dynamics of other drive combinations across their entire parameter range can be intuitively explored using the DrMxR, for example, Medea (viability selection) and homing endonuclease (distortion) can be studied.

5.1.3 **Ecological Factors**

In the context of field deployment, understanding only the population genetics of the system is not enough. The properties of gene drive constructs are diverse, depending on their molecular construction, and differential selection pressure they impose in the varied ecological situations. Conversely, the ecology of the target species itself can disrupt the intended dynamics of the driven gene. Taking the demographic parameters such as migration or population structure into account is, therefore, imperative when assessing the impact of gene drive deployment. Below we derive the invasion threshold of a drive system and evaluate the impact of spatial structure on the invasion (from rare) and fixation of the drive.

a. Invasion Threshold

The unintended spread of certain types of drive to non-target populations has been a significant concern ever since the conception of synthetic gene drives. This interest is particularly the case for replacement drives (not intended to alter the size of populations) since the negative selection costs (fertility and viability) imposed by replacement-drive constructs are generally much smaller than for suppression drives. In this context, the option of making the replacement gene drive localized to target populations has been a significant focus for some scientists developing gene drives (Backus and Delborne, 2019). A mechanism for localizing the driven construct is the imposition of a suitably high invasion threshold. The invasion threshold is the property of the drive system that quantifies the minimum frequency of drive organisms necessary to be released to replace the wild target population. If the invasion threshold is high, the drive is more spatially restricted because the invasion of the non-target populations will require a large number of introduced individuals. Similarly, as high threshold drives theoretically limit their spatial spread, they also may mitigate the spread of drives taxonomically into partially interfertile species (or subspecies) that they may encounter. Accidental release of a few drive organisms may completely transform wild populations for gene drives with low or no threshold (Noble et al., 2018). A recent review of different types of gene drives based on a quantitative analysis of their invasiveness can be found in (Frieß et al., 2019).

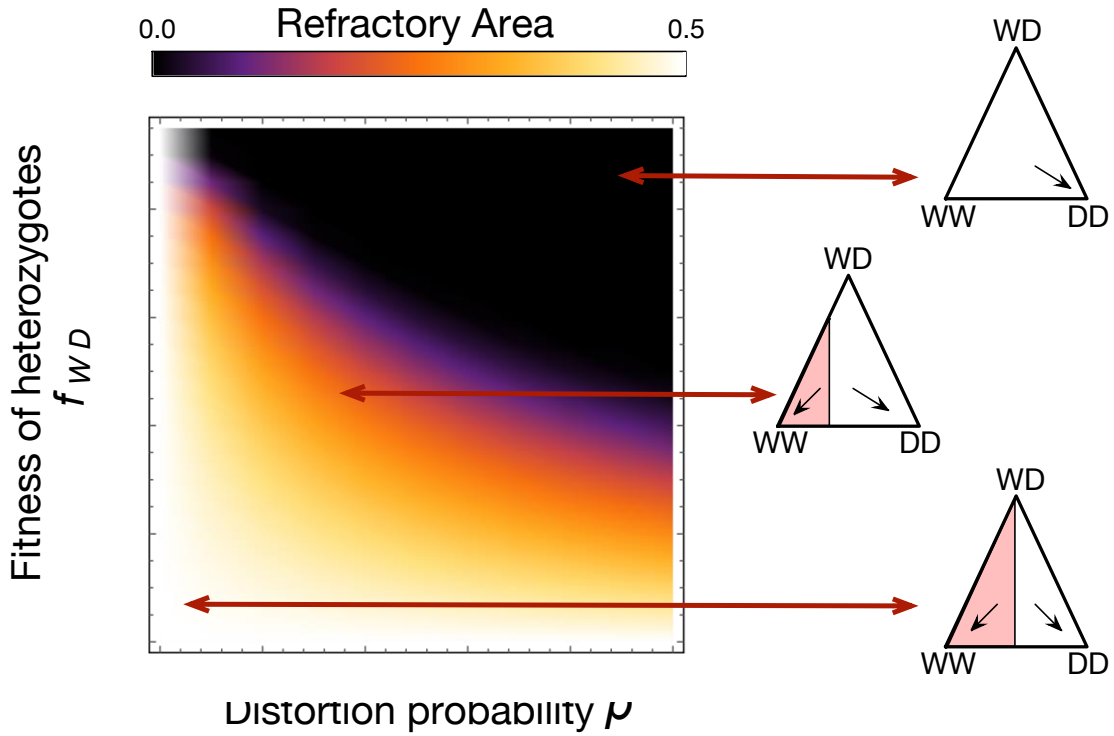


Fig. 40: Heat-map showing the refractory zone with variation in distortion probability p and fertility fitness of heterozygotes f_{WD}.
Illustration of refractory zone for specific values of p and f_{WD} of the heat-map. Trajectories of a de Finetti diagram when $2pf_{WD} > f_{WW}$, drive individuals invade the wild population. Refractory zone is zero and is shown by black colour in the heatmap. $p = 0.5$ corresponds to 'no distortion' case. The values of other parameters are fixed to $f_{WW} = 1, f_{DD} = 1$.

A relevant quantity of interest is the possible combination of heterozygotes and homozygotes release required for the successful invasion (if possible) of a wild population. In our model, the invasion threshold can be quantified based on the direction of the flow lines in the de Finetti diagram. We define the refractory zone as the area of the flow lines towards the fully wild population in the de Finetti diagrams. Thus, we quantify the amount of accidental release or migratory influx that a population may sustain and still revert to the wild type. Simply, we quantify the basin of attraction of the wild type vertex.

We calculated the refractory zone by analytically computing the equation of invariant manifold separating the flow lines through approximations. The refractory zone quantifies the minimal number of drive heterozygotes (WD) and homozygotes (DD), either released or migrating into the target population and capable of transforming the wild type population (WW).

Modelling predicts that variation in the drive efficiency and fitness of different genotypes affects the refractory zone of a gene drive system. Using the insight provided from the simplified Fig. 37, we consider the case of distortion-based gene drive along with fertility selection. Fig. 40 shows the heat-map of the refractory zone with variation in distortion probability p and fertility fitness of heterozygotes f_{WD}. When both the drive efficiency and fitness of heterozygous is high, the refractory zone for the distortion drive is zero. Hence an accidental release of only a small frequency of drive organism may lead to complete replacement of the wild population. In this scenario the gene drive system is, therefore predicted to be non-localized. Low distortion drive efficiency and fitness of heterozygotes predicts that the drive system is increasingly localized, so a significant release of drive organism would be required for a successful transformation of the wild population. For intermediate values of p and f_{WD}, the model predicts that the gene drive system is localized and does not require a massive release.

5.1.4 **Spatial Organisation within a Population**

Recent work has highlighted the need for realistic spatial modelling for more accurately predicting the outcome of gene drive release, especially for suppression drives (Calvez et al., 2018; Champer et al., 2021). Assuming random mating (were all fertile individuals in a population have an equal probability of mating) may in some circumstances give an incorrect prediction about the invasion condition of the gene drive. Here we derive the condition for a gene drive to invade a single wild population in a continuous landscape environment. We tune the spatial structuring between individuals within a population using the parameter k (where k tending to infinity corresponds to complete mixing, a simplifying assumption common to many models including DrMxR, see right side of Fig. 41). Consequently, we have developed a framework to explore the consequences of relaxing this assumption. In this derivation, we use the framework of evolutionary game theory and track the allele frequency instead of genotype. The link between games and gene drive have been previously explored for the meiotic drives (Haig, 2010; Traulsen and Reed, 2012). Under suitable assumptions, the payoff matrix for the meiotic drive, i.e. with distortion and selection is given by:

$$\begin{matrix} & W & D \\ W & f_{WW} & 2f_{WD}(1-p)) \\ D & 2f_{WDp} & f_{DD} \end{matrix}$$

The equation that governs the population dynamics at allele level is then given by the standard selection equation (Crow and Kimura, 1970; Hofbauer and Sigmund, 1998):

$$\dot{x_D} = x_D(f_{DD}x_D + 2f_{WD}p(1-x_D) - \phi) \tag{1}$$

Where $\phi = f_{DD}^2 x_D^2 + 2f_{WD}px_D(1-x_D) + f_{WW}^2(1-x_D)^2$ is the average fitness of W and D alleles. The drive allele can invade if $2f_{WD}p < f_{WW}$ (as derived in [Noble et al.2017]) and fix in the population if $p > 1 - \frac{f_{DD}}{2f_{WD}}$. Describing the dynamics using selection equation allows us to write the population dynamics of the gene drive on a regular graph specifically infinitely large Bethe lattices of degree k using the pair-approximation method. Incidentally, this equation is the replicator equation with transformed payoff matrix used in studying evolutionary games on networks (Ohtsuki and Nowak, 2006). The payoff matrix transformation is different for different update rules. We will use the birth-death update rule in our analysis. In the birth-death update rule, first, an individual is selected proportional to its fitness which then replaces one of its randomly chosen neighbours. When the payoff matrix of the game is A = $[a_{ij}]$. The payoff matrix for the birth-death update rule is transformed to A' = $[a_{ij}]$ + $[b_{ij}]$. (Ohtsuki and Nowak, 2006) where,

$$b_{ij} = \frac{a_{ii} + a_{ij} - a_{ji} - a_{jj}}{k-2} \tag{2}$$

Driven gene will invade (from rare) and fix in the population if $a_{21} + b_{21} > a_{11} + b_{11}$ and $a_{22} + b_{22} > a_{12} + b_{12}$. The conditions for invasion from rarity for the case of distortion and fertility selection is:

$$a_{21} + b_{21} > a_{11} + b_{11}$$
$$\Rightarrow p > \left(\frac{f_{WW}}{2f_{WD}}\right) + \frac{1}{k}\left(\frac{2f_{WD} - f_{DD} - f_{WW}}{2f_{WD}}\right) \quad (3)$$

If $2f_{WD} > f_{DD} + f_{WW}$, the critical p required for invasion increases relative to the mixed population scenario. Hence a lower network degree k results in higher critical p_c. If $2f_{WD} < f_{DD} + f_{WW}$, the critical p required for invasion decreases. The condition obtained for the mixed population regime is recovered in the limit of $k \to \infty$. The additional condition for the fixation of the gene drive is:

$$a_{22} + b_{22} > a_{12} + b_{12}$$
$$\Rightarrow p > \left(1 - \frac{f_{WD}}{2f_{WD}}\right) - \frac{1}{k}\left(\frac{2f_{WD} - f_{DD} - f_{WW}}{2f_{WD}}\right) \quad (4)$$

Here also the condition for fixation can be recovered for the mixed population regime in the limit of $k \to \infty$. It is also worth noting that the condition for invasion and fixation remains intact with variation in k if $2f_{WD} = f_{DD} + f_{WW}$. But a constraint is also put on the invasion and fixation conditions.

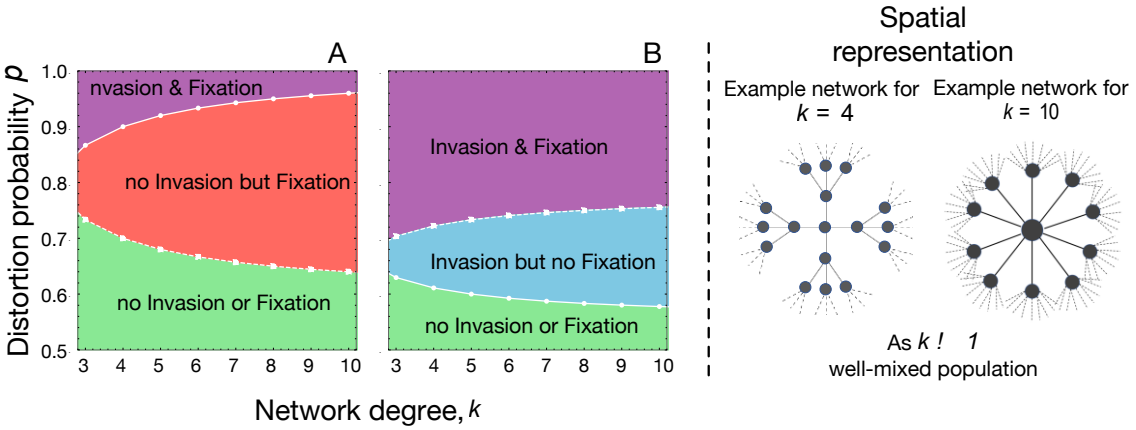


Fig. 41: Spatial structure affects the condition for the invasion from rare and fixation of the driven gene. **(A)** Variation in invasion (full line with circles) and fixation (dashed line with squares) conditions with respect to network degree (k) and distortion parameter (p) for $f_{WD} = 0.5$ and **(B)** $f_{WD} = 0.9$. The values of other parameters are fixed to $f_{WW} = 1$, $f_{DD} = 0.4$. Population dynamics changes when the population becomes more structured on the Bethe lattice parameterized by k. Lower k means more structured population and higher k represents less structure (closer to well-mixed case). The change in population dynamics properties can be seen by the change in invasion/fixation condition and combinations of them, such as no invasion from rare but fixation, if sufficient drive individuals are released/migrate.

Fig. 41 shows that the invasion and fixation outcomes within a single population vary depending on the degree of spatial mixing and the efficiency of distortion. Increasing network degree can move a population where the drive cannot invade or fix to a situation where the drive can fix but cannot invade from rare for lower to moderate values of p ($p = 0.65$ to 0.80). The fixation but no-invasion case corresponds to the introduction of the invasion threshold that can help in local confinement of the gene drive. Interestingly, one can move to this regime by regulating the degree of the network. For higher values of $p > 0.80$ when the drive can both invade and fix in the population, increasing the network degree again can introduce an invasion

threshold. A similar trend ensues in Fig. 41B but here increasing network degree may allow the drive to invade the wild population but does not allow it to get fixed in the population. This scenario corresponds to the over-dominance case, and mathematically, the dynamics correspond to a stable fixed point in the interior of the simplex. The condition for the fixation and the invasion is expected to tend towards a well-mixed population regime for higher k.

5.1.5 **Discussion**

We have developed a minimalist modelling framework and identified three forces/factors responsible for the propagation of gene drive in the presence of an organismal fitness cost. These forces act during different stages of target organism's lifecycle and relate the gene driving mechanism with the organism's biology. Such a type of approach is arguably missing in earlier works on gene drive. For example, (Noble et al., 2017) studied the population dynamics of CRISPR gene drive without explicitly stating that the fitness they incorporated belongs to fertility selection parameters. In other models, fitness costs have been introduced through viability fitness parameter (Gokhale et al., 2014; Marshall et al., 2011; Marshall and Hay, 2011). One can demonstrate using DrMxR that the evolutionary outcome for the two cases (drive acting through viability or fertility but leading to similar costs) differs substantially. Thus our work stresses the biology of the target organism and knowing the exact phases of the lifecycle where the synthetic construct will be expressed. The current modelling approach also provides a classification of drives based on the biology of how the drive is designed (out of the three constituent forces) and avoids unnecessarily new and confusing terminology.

As with different applications of translational evolutionary biology, the eventual aim of several synthetic gene drive constructs is their potential deployment in the field. Any drive technology thus needs to be compared with other available techniques, not by experts of the particular system but decision makers who need a broader perspective. Our work employs standard population genetics methods while keeping our model as generic and minimal as possible. The resulting model allows us to provide a birds-eye view of the dynamics over the space of different drive mechanisms. Educators and regulators would benefit from using our DrMxR for studying the population dynamics of the gene drive. A user can choose the driving factor for the drive and its corresponding effect on the biology of the target organism by tweaking the various parameters as explored in this manuscript. Deviations from the null Hardy-Weinberg equilibrium may be studied via the effect of the three driving factors, individually or combined. Conditions for invasion and fixation of the drive and its tolerance to fitness cost that is highly relevant for drive deployment can be investigated (relevant code provided on GitHub). As case studies of our unified approach, we have recovered the results of various drives such as CRISPR homing endonuclease drive, Medea, Underdominance, Inverse Medea and Semele.

Empirical studies have shown that the selfish genetic elements based on transmission distortion can reduce both fertility (offspring production) (Dyer and Hall, 2019; Larner et al., 2019a) and viability (egg to adult ratio) (Finnegan et al., 2019) of the target species. In order to estimate the evolutionary outcome, we have allowed to jointly vary the factors influencing the propagation of such gene drives. Flexibility to see the combined effect for various evolutionary factors influencing the spread of gene drive on the population dynamics is an essential feature of the DrMxR. We believe that analytical results for the evaluation of refractory zone would be useful for the regulators to frame their investigations of invasiveness of the studied drive. Methodologically, the calculation of the refractory zone is general enough to allow an interdisciplinary dialogue, e.g. with evolutionary games and population genetics (Altrock et al., 2010; Traulsen and Reed, 2012).

Our result on the spatial model reveals that the inclusion of non-panmictic dynamics changes the invasion and fixation condition of the gene drive relative to the mixed population model. We found that for lower values of network degree, the region of phase space in Fig. 41 for

invasion & fixation and no invasion or fixation increases. Hence, introducing spatial feature during interaction may make the drive either highly invasive or redundant.

In this study, we develop a mathematical model that encompasses a variety of synthetic (and natural) gene drive techniques. Currently, we limit our study to replacement drives – spreading the drive gene along with its trait to the entire population by leading it to fixation. Suppression drives – intended to eradicate or reduce the target population or 'reversal drives' – intended to reverse the genetic alteration brought by the first gene drive (DiCarlo et al., 2015b; Edgington and Alphey, 2019; Esvelt et al., 2014a; Vella et al., 2017) are not included. Self-exhausting drives that first rapidly spread in the population and then self-exhaust after limited generations are also excluded in this study (Noble et al., 2019). Our work focuses on a single locus and highlights the complexities that single locus drives can generate.

We have extended our analysis to spatial systems as per (Ohtsuki and Nowak, 2006). Studying density-dependent migrations between patches (Altrock et al., 2011) could be included to understand the spread of different drive systems. Inclusion of ecological parameters such as seasonality and environmental disturbances would be necessary (Eckhoff et al., 2017) utilising the theory to model a specific target species. For specific species, considering detailed life history and influences in the lifecycle of the organism would be a valid extension. For example, a mosquito's lifecycle consists of egg, larva, pupae and adult stage. Density-dependent effects due to larval competition become relevant and changing fitness components such as viability and fertility may have a small effect. Hence adding appropriate lifecycle history depending on the model organism is necessary for a potentially more reliable prediction of gene drive spread. The theoretical framework that inspires our study allows for such complications (Hofbauer and Sigmund, 1998) to be added. However, we emphasise the disparity between the theoretical developments in simple synthetic drive scenarios and urge towards a unified understanding at the primary level.

5.2 Recovering Results from Models in the Literature.

In this section, we will demonstrate the flexibility of our generic modelling approach by recovering the results of earlier work on different gene drive systems. Here we present population dynamics of the three genotypes WW, WD and DD for some special cases using our generic model. Please note that the results shown here are only a subset of the work done in the original studies.

5.2.1 Recovering Noble et al. (2017)

Noble et al. (2017) studied the population dynamics of CRISPR based homing endonuclease gene drive (Noble et al., 2017). These gene drive construct induces a double strand break at the target sequence (wild type allele). The drive is then copied at the break site using homologous recombination. If resistance evolution is ignored, the final consequence is that the heterozygous individuals only transmit drive allele during recombination. Looking from the perspective of our generic model, the drive acts in the gamete stage and uses distortion for propagating the drive allele in the population. The authors in their study also accounted for the variation in the fertility rates of genotypes due to the drive construct. Hence every individual undergoes both distortion and fertility selection during its life cycle. The authors derived the following condition which lead to the invasion of wild type population by the gene drive:

$$2pf_{WD} > f_{WW}$$

The above invasion condition of Noble et al. (2017) is demonstrated in Fig. 42. The original study also analysed the implication of resistance evolution and utility of multiple guide RNAs construct on the evolutionary dynamics. These features can also be included in our model and would entail addition of more genotypes and their corresponding dynamics.

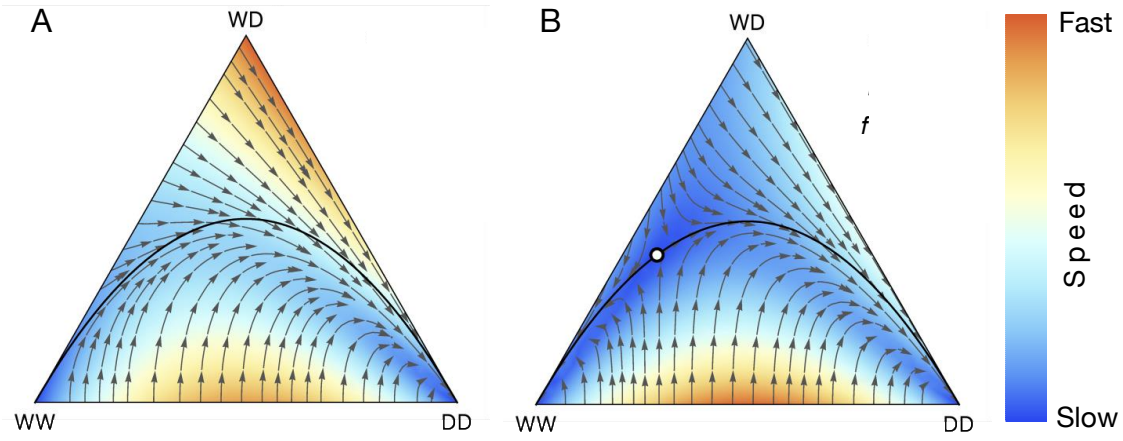


Fig. 42: Population dynamics of CRISPR based homing endonuclease gene drive.
(A) When the fertility rate of heterozygous adults is 0.7 and drive efficiency is 100%, we have $2pf_{WD} > f_{WW}$. A small release of WD/DD will invade the population consisting entirely of WW. (B) When the fertility rate of heterozygous adults is 0.3, we have $2pf_{WD} < f_{WW}$. Successful invasion by gene drive would require threshold release of WD/DD in the population. The position of the unstable fixed point is $(\mathrm{WW}, \mathrm{DD}) = (0.286, 0.354)$. Other parameters are fixed to $f_{WW} = 1, f_{DD} = 1$ for both A and B.

5.2.2 **Recovering Gokhale et al. (2014)**

Gokhale et al (2014) analysed the synergistic effect of combined Medea and underdominance in a single transgenic construct (Gokhale et al., 2014). Medea gene drive utilize viability selection which acts during zygote stage of an organism. In the Medea constructs, wild type homozygous offspring of heterozygous mother becomes non-viable. In underdominance, the heterozygotes are less fit than both wild and drive homozygotes. Fig. 43 recovers the results of Gokhale et al. (2014) for special parameter set.

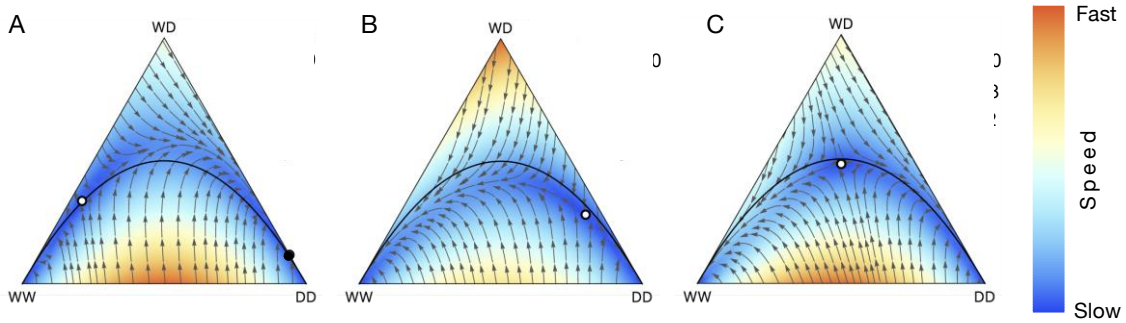


Fig. 43: de Finetti diagram showing the population dynamics of Medea, underdominace and their combined effect.
(A) Medea only **(B)**Underdominance only **(C)** Combined effect of Medea and Underdominance.

5.2.3 **Recovering Marshall and Hay (2011)**

In Inverse Medea (Marshall and Hay, 2011), homozygous offspring of a wild type mother are non-viable. Fig. 44 recovers the results of Marshall and Hay (2011) for special parameter set.

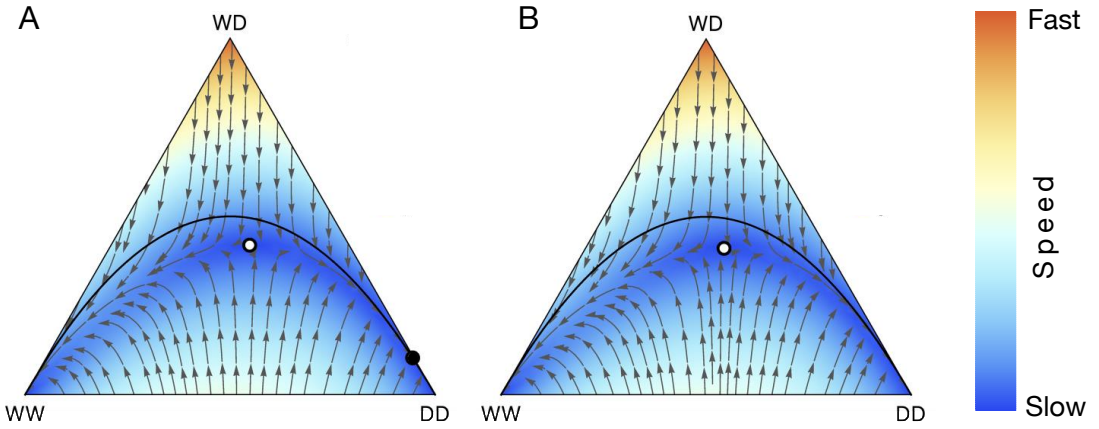


Fig. 44: Population dynamics of Inverse Medea.
(A) For $\omega = 0.975$ and $\nu = 0.95$ if transgenic individuals are released above a threshold, population converges to a stable point consisting of 99.7% of DD and WD. The stable and unstable fixed point is represented by black and white circle on the de finetti diagram. **(B)** For $\omega = 0.95$ and $\nu = 0.95$ above a threshold release, drive homozygous (DD) invades the whole population. $d_m = 1$.

5.2.4 **Recovering Marshall et al (2011)**

Semele drive was first proposed in Marshall et al. (2011) and is based on toxin-antidote system. Transgenic males carry toxin and transgenic females carry the corresponding antidote. Offspring of transgenic male carrying toxin and wild type female with no antidotes are non-viable. *Semele* drive like Medea and Inverse Medea utilise viability selection and acts during zygote stage (Fig. 45).

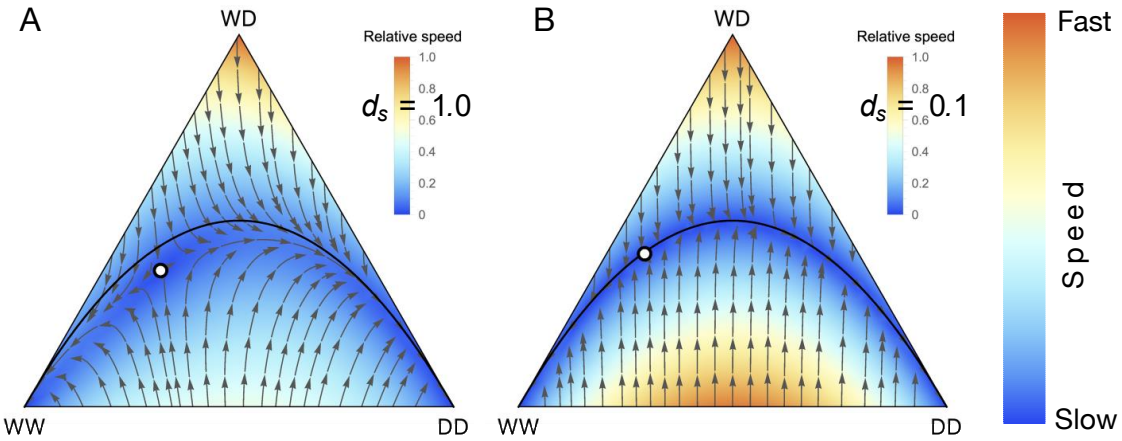


Fig. 45: Population dynamics of Semele drive when there is no fitness cost.
Drive efficiency is 100% **(B)** Drive efficiency is 10%.

5.3 Gaining Knowledge Through Modelling – Summary

Synthetic gene drive technologies aim to spread transgenic constructs into wild populations even when they impose organismal fitness disadvantages. The properties of gene drive constructs are highly diverse, depending on details of their molecular construction, additionally, gene drives can encounter a wide range of conceivable ecological and demographic situations. This makes it very challenging to convey their relative predicted properties to all but highly expert audiences. Furthermore, for proposed gene drive approaches to be critically evaluated in terms of their relative strengths and weakness, including of the modelling approaches employed or parameters selected, it is essential to broaden the pool of potential stakeholders that have an understanding. To facilitate this, we have for the first time developed a unified mathematical paradigm for describing the properties of a wide variety of single construct gene drives. This framework provides an intuitive and objective way to evaluate the properties and robustness of many gene drive approaches in terms of their expected end points. Implemented within a user-friendly open source App, with expanding documentation and case studies (Fig. 46). It provides the capacity easily vary key drive parameters as a means to assess the sensitivity of parameter combinations and also as a means to identify assumptions that underlie published models (which are often not explicitly stated). Crucially, within this common framework, it is possible to recapitulate key published results derived using bespoke modelling frameworks.

The described framework is not intended to remove the need for continued bespoke modelling efforts or existing vocabularies, it can however provide a means to further expand the, explicit or intuitive, understanding of gene drive in the context of risk assessment, informing policies, and enhancing public participation of proposed and future gene drive approaches.

We also discuss a method for analytically assessing the invasiveness of a drive construct and explore their resilience in a spatially explicit manner.

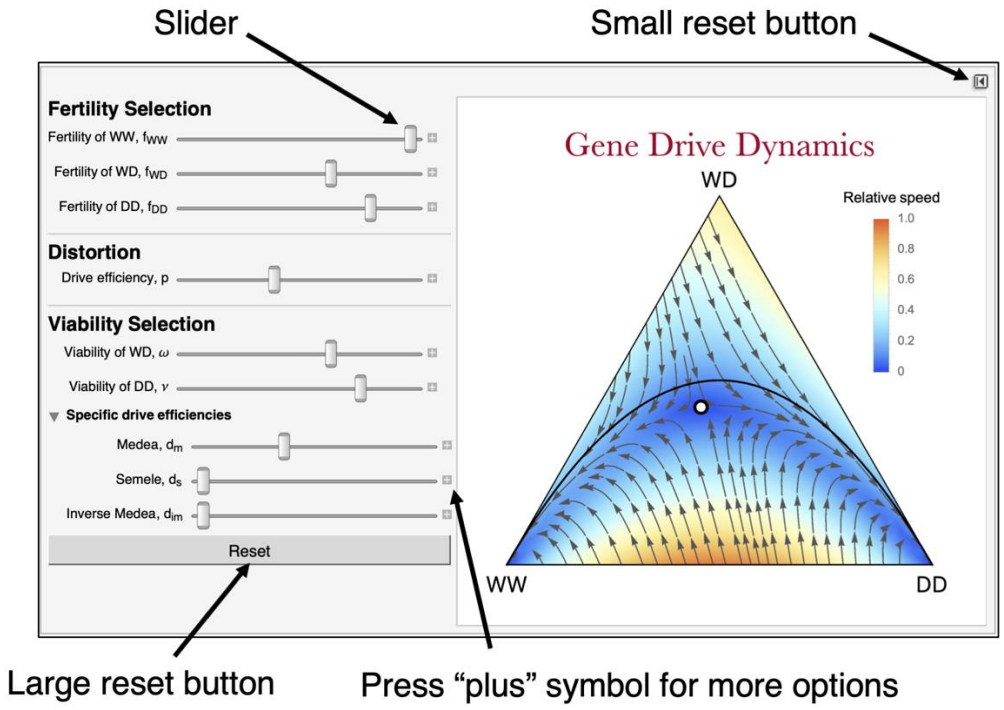


Fig. 46: Screenshot from the DrMxR gene drive model.

5.4 Multi Allele System

5.4.1 Resistance Evolution

Gene drives are prone to resistance evolution due to standing genetic variation or because of the inefficiency of the drive mechanism (Burt, 2003; Deredec et al., 2008; Esvelt et al., 2014). For example, in CRISPR based homing drives, resistance could arise because the cell repairs the double-stranded break by CRISPR through non-homologous end joining (NHEJ) instead of expected homologous recombination (HR) (Noble et al., 2017). Many studies have suggested that the drive resistance can severely impact the spread of the gene drive unless mitigating strategies are included (Burt, 2003; Champer et al., 2018; Deredec et al., 2008; Esvelt et al., 2014; Gomulkiewicz et al., 2021; Noble et al., 2017). Here, we extend our base model to include a drive resistance allele (R). Our mathematical framework is flexible to include the complexity of such resistance evolution in gene drives. It is important to note that these extensions demonstrate our modelling framework's flexibility to include more complexity. They have not been deployed in the current instance of our DrMxR app. Including an extra allele results in six possible genotype combinations for a single locus diploid population: WW, WD, DD, WR, DR, RR. The table 1 shows the proportion of different genotypes produced from 36 (6 _ 6) possible mating pairs. To keep things simpler, we do not show here any fitness variation due to viability or fertility selection and take the example of resistance evolution in CRISPR based homing gene drives. The rate of production of different genotype is given by:

$$F_{WW} = \left(x_{WW}^2 x_{WR} + \frac{1}{4}x_{WR}^2\right)$$

$$F_{WD} = \left(\frac{1+h}{2}x_{WW}^2 x_{WD} + x_{WW}x_{DD} + \frac{1}{2}x_{WW}x_{DR} + \frac{1+h}{2}x_{WW}x_{WD} + \frac{1+h}{4}x_{WD}x_{WR} + x_{DD}x_{WW} + \frac{1}{2}x_{DD}x_{WR} + \frac{1+h}{4}x_{WR}x_{WD} + \frac{1}{2}x_{WR}x_{DD} + \frac{1}{4}x_{WR}x_{DR} + \frac{1}{2}x_{DR}x_{WW} + \frac{1}{4}x_{DR}x_{WR}\right)$$

$$F_{DD} = \left(\frac{(1+h)^2}{4}x_{WD}^2 + \frac{1+h}{4}x_{WD}x_{WW} + \frac{1+h}{2}x_{DD}x_{WD} + x_{DD}x_{DD} + \frac{1}{2}x_{DD}x_{DR} + \frac{1+h}{4}x_{DR}x_{WD} + \frac{1}{4}x_{DR}x_{DR}\right)$$

$$F_{WR} = \left(\frac{1-h}{2}x_{WW}x_{WD} + \frac{1}{2}x_{WW}x_{WR} + \frac{1}{2}x_{WW}x_{DR} + x_{WW}x_{RR} + \frac{1-h}{2}x_{WD}x_{WW} + \frac{1-h}{4}x_{WD}x_{WR} + \frac{1}{2}x_{WR}x_{WW} + \frac{1-h}{4}x_{WR}x_{WD} + \frac{1}{2}x_{WR}^2 + \frac{1}{4}x_{WR}x_{DR} + \frac{1}{2}x_{WR}x_{RR} + \frac{1}{2}x_{DR}x_{WW} + \frac{1}{4}x_{DR}x_{WR} + x_{RR}x_{WW} + \frac{1}{2}x_{RR}x_{WR}\right)$$

$$F_{DR} = \left(\frac{1-h^2}{2}x_{WD}x_{WD} + \frac{1-h}{2}x_{WD}x_{DD} + \frac{1+h}{4}x_{WD}x_{WR} + \frac{1}{2}x_{WD}x_{DR} + \frac{1+h}{2}x_{WD}x_{RR} + \frac{1-h}{2}x_{DD}x_{WD} + \frac{1}{2}x_{DD}x_{WR} + \frac{1}{2}x_{DD}x_{DR} + x_{DD}x_{RR} + \frac{1+h}{4}x_{WR}x_{WD} + \frac{1}{2}x_{WR}x_{DD} + \frac{1}{4}x_{WR}x_{DR} + \frac{1}{2}x_{DR}x_{WD} + \frac{1}{2}x_{DR}x_{DD} + \frac{1}{4}x_{DR}x_{WR} + \frac{1}{2}x_{DR}^2 + \frac{1}{2}x_{DR}x_{RR}\frac{1+h}{2}x_{RR}x_{WD} + x_{RR}x_{DD} + \frac{1}{2}x_{RR}x_{DR}\right)$$

$$F_{RR} = \left(\frac{(1-h)^2}{4} x_{WD}^2 + \frac{1-h}{4} x_{WD}x_{WR} + \frac{1-h}{4} x_{WD}x_{DR} + \frac{1-h}{2} x_{WD}x_{RR} + \frac{1-h}{4} x_{WR}x_{WD}\frac{1}{4} x_{WD}^2 + \frac{1}{4} x_{WR}x_{DR} + \frac{1}{2} x_{DR}x_{RR} + x_{RR}^2\right) \quad (1)$$

where h is the homing efficiency of the CRISPR gene drive hence the probability with which drive heterozygotes parent WD produces gamete with haplotype D and R are 0.5 (1 + h) and 0.5 (1 - h) respectively. The rate of change in the frequencies of each genotype can be obtained by inserting F_i's in the following equation in (2):

$$\dot{x}_\alpha = F_\alpha - x_\alpha \bar{F} \quad (2)$$

where $\bar{F}$ is the sum of the production rate of the three genotypes:

$$F = \sum_\alpha F_\alpha \quad (3)$$

The resulting dynamical equations are equivalent to the equations obtained by Noble et al. 2017 when there is one resistant allele and all genotypes have equal fitness (Noble et al., 2017). The frequencies of the six genotypes has been normalized to one:

$$x_{WW} + x_{WD} + x_{DD} + x_{WR} + x_{DR} + x_{RR} = 1 \quad (4)$$

Given the six genotypes, the system's population dynamics proceeds in a fivedimensional space and cannot be represented in a de Finetti diagram. The specific dynamics could still be studied by numerically solving the equation for various initial conditions.

5.4.2 **Precision Drives**

One of the concerns of the regulators is that the released gene drive could spread to non-target population. This may be mitigated using a precision gene drive system where unique genes of the target species or even a subpopulation is chosen to cut via CRISPR machinery. Hence, if the chosen sequence is sufficiently distinct, the gene drive would not be able to propagate across non-target populations (Esvelt et al., 2014). Precision gene drive works by releasing two drives in quick succession in a population with no or limited gene flow. First, the target population is tagged with a unique sequence by releasing drive A, which does not affect the organism. Then, drive B is released, which only spreads through drive A and does not target wild type allele W. Assuming drive A does not escape and is completely replaced by drive B, then successive precision drives would be able to target population B without the risk of spreading to other populations. The proportion of offspring for the precision gene drive system is given in the Tab. 11.

5.4.3 **One Locus Two Toxin (1L2T) Gene Drive**

Interestingly, the dynamical equation obtained using Eq (1) demonstrates the addition of multiple alleles to our base model. In this case, the third allele (R) happens to be the resistant allele, but that is not a general case. Like the two-allele system, if we remove the distortion because of homing (h = 0) and add the effect of fertility or viability selection, the other three

allele gene drive systems could be captured through our model. One locus two toxins (1L2T) system is an example of a system where two different drive alleles exist at a single genomic locus like D, and R (Champer et al., 2020c; Davis et al., 2001; Dhole et al., 2018). The two drive alleles, D and R, both encode a different toxin and carry an RNAi (the "antidote") that neutralizes the other drive allele's toxin. Therefore, the genotypes containing toxin but no corresponding antidote (WD, RR, DD and WR) are non-viable. In contrast, the viable genotypes are heterozygotes with the two drive alleles (RD) and wild-type homozygotes (WW).

5.4.4 **Multi Locus Gene Drives – Summary**

Here we demonstrate that our basic model could be extended to include several multi locus gene drive system (Champer et al., 2020c; Davis et al., 2001; Dhole et al., 2018; Noble et al., 2019). Daisy chain gene drive is an example of such a drive system (Noble et al., 2019). It consists of a linear series of genetic elements on different loci where one element drives the next. The last genetic element in the chain is driven to a high frequency, while the element at the base cannot be driven and is lost over time due to natural selection. This process causes the next element to stop driving in the population, and so on. The process continues until the whole population returns to an all wild type state. Again, owing to plural terminology, the daisy chain system is also referred to as a self-exhausting gene drive (Noble et al., 2019). To model a multi locus gene drive system, we illustrate a two-locus diploid organism with loci 1 and 2. There are two alleles, the wild type (W) and the drive type (D). The allele at first locus can therefore be 1_W or 1_D. Similarly, the allele at the second locus is represented by 2_W or 2_D. The genotype corresponding to wild type homozygous individual at both the loci is $1_{WW}2_{WW}$. There are in total nine possible genotypes: $1_{WW}2_{WW}$, $1_{WW}2_{WD}$, $1_{WW}2_{DD}$, $1_{WD}2_{WW}$, $1_{WD}2_{WD}$, $1_{WD}2_{DD}$, $1_{DD}2_{WW}$, $1_{DD}2_{WD}$ and $1_{DD}2_{DD}$. A daisy chain drive uses CRISPR genome editing technology to engineer drive alleles. The drive allele (1_D) in the first locus induces the cutting of the 2_W allele. Considering the nature of distortion outlined in the original paper (Noble et al., 2019), the proportion of offspring from all possible 81 mating pairs can be computed to yield equivalent population dynamic equations (Noble et al., 2019). A natural extension would be to generalize the framework for any number of locus and allele. Other multi locus gene drive systems such as two-locus two toxin (2L2T), reciprocal chromosomal translocation (RCT) underdominance system and killer & rescue drive can also be modelled through our framework (if distortion due to homing is not considered). Specific genotype becomes non-viable because of the toxin carrying drive element (Champer et al., 2020c; Dhole et al., 2018). Besides the wild type allele, this system consists of two drive alleles at the two loci (say 1_D and 2_D). In reciprocal chromosomal translocation (RCT), the only viable genotypes are homozygotes for the wild-type alleles ($1_{WW}2_{WW}$), homozygotes for the translocated alleles ($1_{DD}2_{DD}$), heterozygotes for the translocated alleles ($1_{WD}2_{WD}$) (Champer et al., 2020c; Curtis, 1968). While in two locus two toxin (2L2T) system the viable genotypes are homozygotes for the wild-type alleles ($1_{WW}2_{WW}$) and those which carry at least one copy of each drive allele ($1_{WD}2_{WD}$, $1_{DD}2_{WD}$, $1_{WD}2_{DD}$, $1_{DD}2_{DD}$) (Champer et al., 2020c; Davis et al., 2001). Killer & rescue gene drive constructs consist of two alleles, namely killer (K) and rescue allele (R), and their corresponding wild type counterparts are 'k', and 'r' respectively (Gould et al., 2008). If the locus of insertion of allele K or R is independent of other loci, there are nine possible genotypes. Out of nine genotypes ($1_{KK}2_{RR}$, $1_{KK}2_{Rr}$, $1_{Kk}2_{RR}$, $1_{Kk}2_{Rr}$, $1_{kk}2_{RR}$, $1_{kk}2_{Rr}$, $1_{kk}2_{rr}$, $1_{Kk}2_{rr}$, and $1_{KK}2_{rr}$), the genotypes which carry only killer allele K and no rescue allele are non-viable ($1_{Kk}2_{rr}$, and $1_{KK}2_{rr}$). Underdominance tethered homing drive (UTH) consist of two components and three alleles with either a transgenic (D) or wild type (W) (Dhole et al., 2019). This gene drive system can have 27 different diploid genotypes and hence 729 mating possibilities. The details about the fitness of viable and non-viable genotype can be found in the supplementary material of the original study (Dhole et al., 2019).

The wild type genotype can be represented as $1_{ww}2_{ww}3_{ww}$. First component is a two-locus engineered underdominance drive which we have already described. The second component is an unlinked locus to be inserted into a haploinsufficient gene, that is, two copies of a functional gene are required at this locus for viable offspring. The homing component at the third locus is driven by the presence of the other two constructs. The guide RNA and Cas endonuclease target the wild-type (3W) alleles for multiple double-stranded breaks. Repairs through non-homologous end-joining (NHEJ) or homology-directed repair (HDR) that did not produce a functional copy of the haploinsufficient results in individuals that are incapable of producing viable offspring. This gene drive system thus helps to prevent the emergence of resistance due to NHEJ (Esvelt et al., 2014).

Tab. 10: Offspring proportions for CRISPR based homing gene drive with resistance.

Parents		*Offspring*					
♂	♀	WW	WD	DD	WR	DR	RR
WW	WW	1.0					
WW	WD		0.5(1+h)		0.5(1-h)		
WW	DD		1.0				
WW	WR	0.5			0.5		
WW	DR		0.5		0.5		
WW	RR				1.0		
WD	WW		0.5(1+h)		0.5(1-h)		
WD	WD		$0.25(1+h)^2$			$0.5(1-h)^2$	$0.25(1-h)^2$
WD	DD			0.5(1+h)		0.5(1-h)	
WD	WR		0.25(1+h)		0.25(1-h)	0.25(1+h)	0.25(1-h)
WD	RR					0.5(1+h)	0.5(1-h)
DD	WW		1.0				
DD	WD			0.5(1+h)		0.5(1-h)	
DD	DD			1.0			
DD	WR		0.5			0.5	
DD	DR			0.5		0.5	
DD	RR					1.0	
WR	WW	0.5			0.5		
WR	WD		0.25(1+h)		0.25(1-h)	0.25(1+h)	0.25(1-h)
WR	DD		0.5		0.5		
WR	WR	0.25			0.5		0.25
WR	DR		0.25		0.25	0.25	0.25
WR	RR				0.5		0.5
DR	WW		0.5		0.5		
DR	WD			0.25(1+h)		0.5	0.25(1-h)
DR	DD			0.5		0.5	
DR	WR		0.25		0.25	0.25	0.25
DR	DR			0.25		0.5	0.25
DR	RR					0.5	0.5
RR	WW				1.0		
RR	WD					0.5(1+h)	0.5(1-h)
RR	DD					1.0	
RR	WR				0.5		0.5
RR	DR					0.5	0.5
RR	RR						1.0

Tab. 11: Offspring proportions for CRISPR based precision gene drives.

Parents		*Offspring*					
♂	♀	WW	WA	AA	WB	AB	BB
WW	WW	1.0					
WW	WA		1.0				
WW	AA		1.0				
WW	WB	0.5			0.5		
WW	AB				1.0		
WW	BB				1.0		
WA	WW		1.0				
WA	WA			1.0			
WA	AA			1.0			
WA	WB		0.5			0.5	
WA	AB					1.0	
WA	BB					1.0	
AA	WW		1.0				
AA	WA			1.0			
AA	AA			1.0			
AA	WB		0.5			0.5	
AA	AB					1.0	
AA	BB					1.0	
WB	WW	0.5			0.5		
WB	WA		0.5		0.5		
WB	AA		0.5		0.5		
WB	WB	0.25			0.5		0.25
WB	AB				0.5		0.5
WB	BB				0.5		0.5
AB	WW				1.0		
AB	WA					1.0	
AB	AA					1.0	
AB	WB				0.5		0.5
AB	AB						1.0
AB	BB					1.0	
BB	WW				1.0		
BB	WA					1.0	
BB	AA					1.0	
BB	WB				0.5		0.5
BB	AB						1.0
BB	BB						1.0

5.5 On the effect of mating complexity on gene dynamics

Gene drive technology being designed to deliver on some of the critical challenges in human health, agriculture or biodiversity conservation (Brossard et al., 2019; Buchman et al., 2018a; Johnson et al., 2016; Prowse et al., 2017; Windbichler et al., 2011). Malaria, for example, is a poster example where driving genes in mosquitoes populations that make them resistant to the malaria parasite is a sought after application (Carballar-Lejarazú et al., 2020; Gantz et al., 2015). For biodiversity conservation, gene drives possibly can help control the spread of invasive species or make the endangered species resilient to disease or other threats (Godwin et al., 2019; Johnson et al., 2016; Prowse et al., 2017). In agriculture, gene drive could control pest populations like fruit flies (Buchman et al., 2018a) in cherry plantations or transform the pest population to make them more susceptible to pesticides (Barrett et al., 2019). Theoretical and some experimental studies indicate that the genetically modified organism may spread through the wild population in 10-20 generations. However, such results are valid only under ideal conditions such as random mating and the absence of ecological stressors (Burt, 2003; Deredec et al., 2008; Simoni et al., 2020; Windbichler et al., 2011) and therefore do not provide a realistic estimate of the drive's behaviour under field conditions. Several studies relating to the risk assessment of gene drives have highlighted the relevance of ecological and technological bottlenecks like resistance evolution, mate-choice, mating system, and spatial interaction in successfully deploying gene drive organisms (Collins, 2018; Dhole et al., 2020; Giese et al., 2019; Moro et al., 2018; National Academies of Sciences Engineering and Medicine, 2016; Oye et al., 2014). Thus, assessing model assumption's validity is an essential task that any gene drive technology needs to overcome to become an option for a field release. While numerous assumptions made in the laboratory may be violated in the wild, we choose to focus on aspects of mating complexity to stress our point. We show how the effect of mate-choice, mating systems and mating networks can change the course of eco-evolutionary trajectories of gene drive systems.

Gene drive leverages sexual reproduction by biasing the inheritance of a specific gene from one generation to the next. A gene construct can successfully spread in the population only when the released transgenic organism can spread faster than the wild type population, even with an organismal fitness cost. Hence, it becomes imperative to account for the target species' reproductive biology and mating pattern to predict the release threshold of GMOs (Moro et al., 2018; National Academies of Sciences Engineering and Medicine, 2016). While the theoretical explorations and laboratory techniques of gene drive techniques often assume random mating, factors such as inbreeding, mate-choice and mating systems are common in the wild that can cause non-random mating. This important aspect has been recognised in gene drive research (Deredec et al., 2008; Noble et al., 2017; Qureshi et al., 2019; Unckless et al., 2015). Inbreeding could diminish the frequency of heterozygotes in the population, reducing and slowing the spread of gene drive. For example, (Qureshi et al., 2019) found that mosquito populations exposed to higher levels of male competition evolved higher competitive mating success compared to populations that evolved in the absence of competition. In natural meiotic drive, females of some species can discriminate against males carrying drive when the region containing the drive gene is linked to the gene of mate-choice ornament (Price and Wedell, 2008; Wedell and Price, 2015). In some studies, naturally occurring selfish genetic element (*t*-complex) in *Mus domesticus* shows mate preference whereby both sexes appear to avoid heterozygous mate using olfactory cues (Lenington, 1983; 1991; Lindholm et al., 2013). A newly evolved natural distorter system may be inefficient due to reduced fertility of drive carrying organism and possible evolution of mating bias in response to reduced fertility (Charlesworth and Charlesworth, 2010; Wedell and Price, 2015). Though it is not clear that bias in the mate preference can quickly evolve for laboratory-engineered synthetic gene drives, concerns still hold, mostly when the gene drive might incur high fertility costs. A study by (Drury et al., 2017) showed that non-random mating caused by inbreeding (inbreeding) could render the CRISPR based gene drive inefficient against standard genetic variation resistance for Cas9 target sites in the flour beetle *Tribolium castaneum*. (Bull, 2017) suggested that a mild level of

inbreeding can lead to the evolution of selfing in hermaphrodites (plants) in response to the homing endonuclease gene drive. Suppression gene drive, aimed at the extinction of target species, can lead to the evolution of sib-mating, significantly hampering the spread of the driven gene (Bull et al., 2019b). Recently, a study on the efficacy of CRISPR-based gene drives targeting the gene doublesex of mosquitoes (*Anopheles gambiae*) was done in large indoor cages under more ecologically relevant settings to bridge the gap between laboratory and field (Hammond et al., 2021). We show that if the driven gene's pleiotropic effects impinge on the mating behaviour of the target species, the gene's spread is constrained.

The mating system of the target species will play an essential role in deciding the population dynamics of the spread of gene drive. For example, even in the absence of pre-copulatory mate-choice, *t*-haplotype gene drive in mice can be limited by the polyandrous mating system where females mate with multiple males in a breeding cycle (Lindholm et al., 2016; Manser et al., 2017). The *t*-haplotype carrying males have reduced fertility, so when a female mates with multiple males, the fertilization of non-drive carrying male due to sperm competition is more likely (Manser et al., 2020, 2017). A sex linked gene drive based on utilising *t*-haplotype is being proposed for the suppression of rodent population (Godwin et al., 2019; Leitschuh et al., 2018). The impact of polyandry on the population-level dynamics of one such gene drive (*t-Sry*) have been studied by (Manser et al., 2019). With a focus on an age-structured population, (Huang et al., 2009) showed that the mating system for Medea and engineered under-dominance gene drive can significantly change the predicted threshold number of released transgenic individuals for successful population transformation. They also found that low polyandry levels can hamper gene drive spread if only males are released. When the gene drive causes male scarcity (Y-shredder), in polygamous systems where males mate with multiple females, the efficacy of spread is hampered (Prowse et al., 2017).

Most wild populations do not exist in a single panmictic population but multiple heterogeneous communities across rugged, disconnected landscapes. In a spatially segregated population, individuals are more likely to interact with others in their vicinity than randomly with everyone in the population. To account for spatial interaction, some mathematical models of gene drive use reaction-diffusion models (Beaghton et al., 2017a; Girardin et al., 2019; Tanaka et al., 2017). In these systems, the time required for a gene to spread depends on the interaction zone where the wild type meets the transgenics. This zone is the leading edge of the wave in the reaction-diffusion models. The wave in the case of suppression-drives sweeps through the wild type population leaving behind empty space (Barton and Turelli, 2011; Bull et al., 2019a; North et al., 2013). Compared to the panmictic models the suppression drive can be less effective and slow in spatial models (Champer et al., 2021; Champer et al., 2020c; North et al., 2013). When considering long-range dispersal, the wild types could occupy the empty space created by the suppression drive resulting in local cycles of drive eradication and reoccupation by the wild type (Champer et al., 2021). Similar cyclical dynamics is possible for reversal drives released to convert the previously established homing drives (Girardin et al., 2019). A question primarily ignored in some of these spatial models concerns the effect of heterogeneous interaction between mating pairs. For example, the interactions in mathematical models using reaction-diffusion equations are assumed to be homogeneous. The spread of the gene drives relies on sexual reproduction, which is not uniform for all individuals in a population. A population structured on a network can help account for the natural heterogeneity in mating success. We use concepts from network theory and build a model to investigate how spatial mating networks could affect the gene drive's spread.

Risk assessors are facing fundamental challenges here. First, understanding modelling approaches and the underlying assumptions for complex applications like synthetic gene drives is far from trivial and second, evaluating the effects of ecological factors on gene drive efficacy is not intuitive. Hence, in general, risk assessment of gene drives will be complex as synthetic gene drive systems show some fundamental deviations from other GMOs developed for release into the environment (Simon et al., 2018). Modelling can be a valuable tool for risk

assessment of GMOs, acknowledging that modelling is complex even for presumably simple questions like the impact of Bt Toxins from transgenic maize (Dolezel et al., 2020). While modelling ecological effects is still in its infancy (Dhole et al., 2020), much research focuses on efficacy modelling when it comes to synthetic gene drives. The view of risk assessors here has to assess all possible outcomes, as different scenarios can open varying risk hypotheses, e.g. superefficient drives, inefficient drives or resurgence effects due to ineffective drives.

The population-dynamic consequences of the three stressors mate-choice, mating systems, and mating structure on gene drives are crucial while predicting the transgenic organisms' probability and time to fixation and release thresholds required to replace the target population completely. The effects of mate-choice and mating systems are studied using deterministic ordinary differential equations, while the mating structure uses a network model. Even though we use different modelling frameworks for individual stressors, the underlying gene drive model extends from a population genetic perspective. We have previously categorised various gene drive systems based on standard terminology (distortion, fertility selection and viability selection) (Verma et al., 2021). Here, we develop an approach by adding a generalizable understanding of the effect of some aspects of mating complexity on gene drive dynamics.

5.5.1 **Model and Results**

As is typical for a functioning gene drive, we assume a diploid organism whose life cycle consists of three stages: zygote, adults and gametes. An adult produces gametes that combine to form a zygote. The zygote grows up to become an adult, and the cycle continues. We also assume that the organisms are diploid with two alleles for the gene of interest, the wild type allele (W) and the modified allele aimed to be driven (D). Hence, an individual can be either of the three genotypes: WW, DD and WD. In our previous work, we have shown that the gene drive could emerge if certain drive carrying genotype undergoes distortion, viability or fertility selection which acts during the different life stages of an organism (Verma et al., 2021). Hence, a common framework was developed to categorize variety of different gene drive system based on pre-existing standard population-genetic terminology (distortion, fertility selection and viability selection) Manipulating the strength of these forces via the engineered construct influences the probability of inheritance, giving rise to gene drive (Verma et al., 2021).

Distortion acts at the gamete level and biases the transmission of the drive allele in the heterozygote. It can give rise to gene drive like meiotic drive (Lindholm et al., 2016; Sandler and Novitski, 1957) and CRISPR based homing endonuclease gene drive (Noble et al., 2018, 2017). Gametes combine to form zygotes, but certain genotypes may become non-viable. The engineered constructs that work principally by manipulating viability selection are those using zygotic toxin-antidote mechanisms as Medea (Beeman et al., 1992; Gokhale et al., 2014; Ward et al., 2011), Inverse Medea (Marshall and Hay, 2011) and Semele (Marshall et al., 2011). Fertility selection acts at the adult stage. Empirical studies have shown that selfish genetic elements can reduce the fertility of drive allele carrying organisms (offspring production) (Dyer and Hall, 2019; Larner et al., 2019). These evolutionary forces can become the source or the by-product of the gene drive mechanism. The population dynamics of these systems have been studied independently in (Verma et al., 2021). Here, we subject the target population to the three stressors: mate-choice, mating structure and mating systems to understand their effect on gene drive population dynamics (Fig. 47).

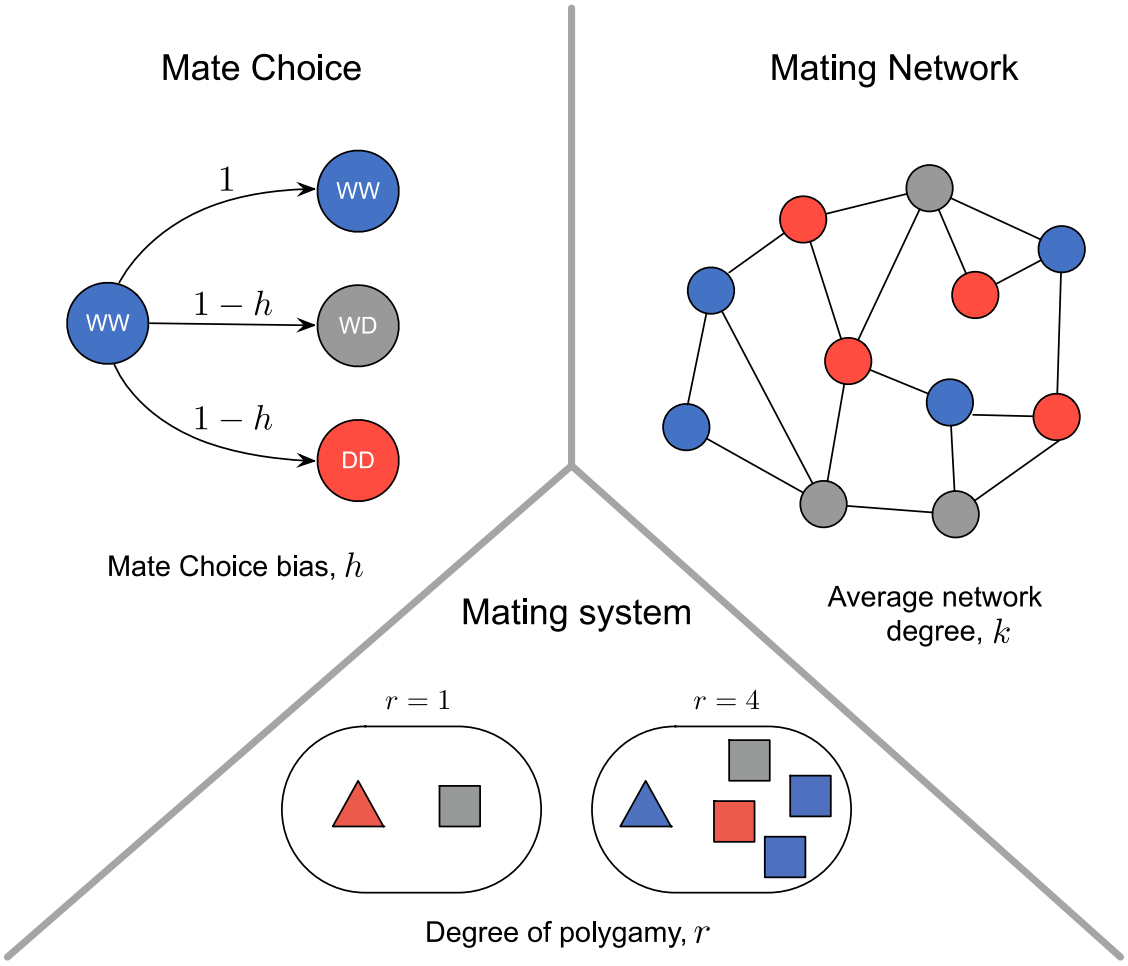


Fig. 47: Pictorial representation of the three mating complexities: mate-choice, mating network and mating system that can affect the population dynamics of gene drive.
Blue, grey and red colours represent individuals with genotype WW, WD and DD, respectively. When there is no distinction between the two sexes, individuals are represented by circles, while triangles and squares denote individuals belonging to different sexes. Under mate-choice bias, the wild type genotype (WW) are less likely to mate with drive carrying genotype (DD and WD). Mate-choice bias is denoted by h in our model where $(1 - h)$ is the mating rate between the wildtypes (WW) and the transgenics (WD or DD). In structured mating, individuals mate and reproduce with other individuals in their vicinity, and their likely interactions are modelled on a mating network of average degree k. The consequence of mating with one (monogamy $r = 1$) or multiple mating partners (polygamy, $r > 1$) on the gene drive dynamics is studied under the mating systems.

5.5.2 **Mate-choice**

We will first consider the null case where there is no gene drive and understand how mate-choice bias of wildtype against transgenic alone will affect the population dynamics. Mate choice bias in our model is captured by the parameter h (Fig. 47). If $h = 0$, the wildtype (WW) is equally likely to mate with the drive carrying genotype (WD and DD). While if $h = 1$, the wildtype (WW) and drive type (WD and DD) do not mate at all. The mating rate among the wildtypes is set to one. Similarly, the mating rate among the drive types is also one. During the exploration of parameter space (h), we work under the assumption that the wildtype genotypes are less likely to mate with individuals carrying the drive allele (WD and DD); therefore, $0 \leq h \leq 1$. The above assumption can be justified with observation that for natural gene drives and even in sterile insect technique (SIT) when female choice of mates is "active" i.e. females choose among males, wild females preferred wild males over drive carrying males or mass reared sterile males (Price and Wedell, 2008; Robinson and Hendrichs, 2005; Wedell and

Price, 2015). In our model, both sexes (male and female) of WW have an equal bias against mating with WD or DD. Assuming an infinitely large population and random segregation of alleles during meiosis, the rate of the production for the three genotypes is given by,

$$\begin{aligned} F_{WW} &= x_{WW}^2 + (1-h)x_{WW}x_{WD} + \frac{x_{WD}^2}{4} \\ F_{WD} &= (1-h)x_{WW}x_{WD} + x_{WD}x_{DD} + 2(1-h)x_{WW}x_{DD} + \frac{x_{WD}^2}{2} \\ F_{DD} &= x_{DD}^2 + x_{WD}x_{DD} + \frac{x_{WD}^2}{4} \end{aligned} \quad (1)$$

)

Where x_α and F_α are the frequency and rate of genotype production respectively, and $\alpha \in (WW, WD, DD)$. The following set of differential equations governs the population dynamics of the genotypes in continuous time:

$$\dot{x}_\alpha = F_\alpha - x_\alpha \overline{F}. \quad (2)$$

where $\overline{F}$ is the average fitness of the three genotypes,

$$\overline{F} = \sum_\alpha F_\alpha . \quad (3)$$

The total population remains constant hence the frequencies of all genotypes sum to unity.

$$x_{WW} + x_{WD} + x_{DD} = 1 \quad (4)$$

The above constraints on frequencies allows us to represent the dynamics of equation $\dot{x}_\alpha = F_\alpha - x_\alpha \overline{F}.$ (2) on a de Finetti diagram. The frequency of the three genotype (WW, WD and DD) without mate-choice ($h = 0$) converge to Hardy Weinberg equilibrium (Gokhale et al., 2014; Verma et al., 2021). When we introduce mate-choice parameter into the rate equations*)*, the dynamics deviates from Hardy Weinberg equilibrium and is governed by the fixed points which appears in the interior of de Finetti diagram. In our context, a fixed point is a specific composition of the population ($x^*_{WW}, x^*_{WD}, x^*_{DD}$) where the proportion of all the genotype does not change. Specifically, where $\dot{x}_\alpha = 0 \; \forall \; \alpha \in (WW, WD, DD)$. Primarily, there are two types of fixed point: stable and unstable. If the population is at the stable fixed point, small change in the population composition would bring the population to the stable fixed point. While in unstable fixed point, population composition would diverge and move away from unstable fixed-point composition. The position of these fixed points governs the overall population dynamics of a specific case. For example, population dynamics for a special case of $h = 0.9$ is shown in the inset of Fig. 48A where the position of an unstable interior fixed point decides the evolutionary fate of the population.

In Fig. 48, we plot the positions and trajectories of these interior fixed points for different values of mate-choice (h) under scenarios such as null case, viability selection, distortion, fertility selection. The null case is when only the effect of mate-choice is considered without any gene drive arising from viability selection, distortion, fertility selection (Fig. 48A). Even under slight mate-choice bias ($h = 0.01$), the dynamics quickly deviates from the Hardy Weinberg

equilibrium. An unstable fixed point (saddle point) appears in the interior of the de Finetti diagram. The threshold frequency of transgenic genotype (DD or WD) required for population transformation is closely related to the position of these unstable fixed points. The area to the left of the unstable fixed point is the basin of attraction of wildtype genotype. The trajectories of the initial conditions in this area lead to the extinction of the modified allele. In contrast, the area on the right is the basin of attraction of drive homozygotes (DD), leading to population transformation. Increasing the mate-choice bias (or as h increases from 0.01 to approximately 1), the position of the interior fixed point moves towards the middle of WW and DD line (Fig. 48A). It implies that when the mate-choice bias increases, the threshold amount of transgenics (DD and WD) required to transform the wildtype population also increases even without the gene drive.

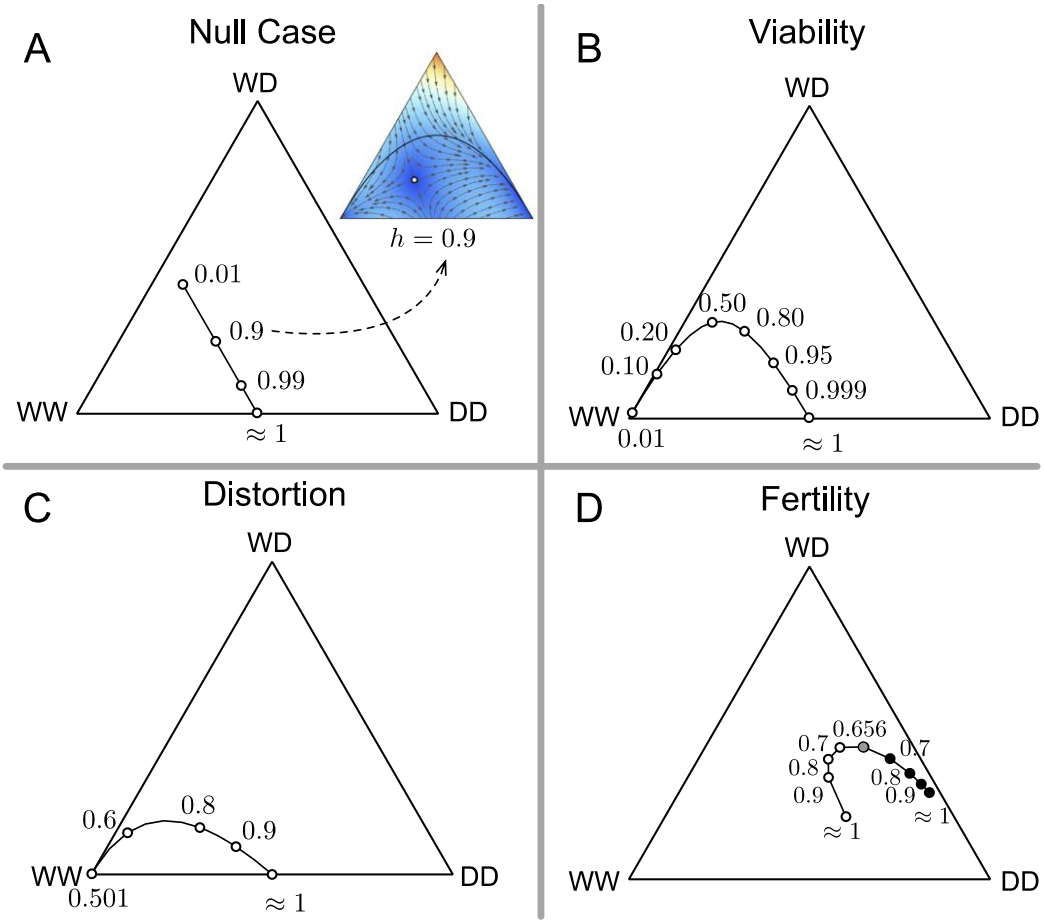


Fig. 48: Effect of mate-choice bias on the internal fixed point of the population dynamics without (null case) and with gene drives system based on viability selection, distortion and fertility selection.
Fixed points appear in the interior of the de Finetti diagrams when the fitnesses of all the genotypes are the same. Open circles denote unstable fixed points of the dynamics, while closed black circles denote stable fixed points. Grey circle denotes the bifurcation point where both unstable and stable points emerge. The position of these fixed points changes with mate-choice bias (h) and hence the overall population dynamics, including the release threshold. Solid black lines show in the trajectory of these fixed points for varying mate-choice parameter h. **(A)** Null case (without drive) considers the effect of mate-choice alone on the population dynamics. The population dynamics for a specific case of $h = 0.9$ is shown in the inset of figure 2A. The position of the fixed point is pointed out through a dashed line. **(B)** Medea drive efficiency is set to 100%, $d = 1.0$ **(C)** Distortion based drive is assumed to be fully efficient (probability $p = 1.0$) **(D)** Fertility fitness cost, $c = 0.2$. When other parameters are not changed their values are: $d = 0, p = 0.5, f_{WW} = 1, f_{WD} = 1, f_{DD} = 1$.

a. Mate-choice with Viability Selection (Medea)

Viability selection is observed in many toxin-antidote gene drive constructs such as Medea, Inverse Medea, Semele and engineered under-dominance drive (Beeman et al., 1992; Marshall et al., 2011; Marshall and Hay, 2011). In such systems, specific offsprings become non-viable during the zygote stage of the life cycle. We have focused on Medea gene drive system in our analysis where d measures the drive efficiency. In Medea drive, wildtype homozygous offspring of heterozygous mothers becomes non-viable (Akbari et al., 2014; Buchman et al., 2018a; Gokhale et al., 2014; Ward et al., 2011). The rate of zygote production in the next generation for Medea gene drive with the incorporation of mate-choice bias can be written as:

$$\begin{aligned} F_{WW} &= x_{WW}^2 + (1-h)(1-0.5d)x_{WW}x_{WD} + (1-d)\frac{x_{WD}^2}{4} \\ F_{WD} &= (1-h)\frac{x_{WW}x_{WD}}{2} + x_{WD}x_{DD} + 2(1-h)x_{WW}x_{DD} + \frac{x_{WD}^2}{2} \\ F_{DD} &= x_{DD}^2 + x_{WD}x_{DD} + \frac{x_{WD}^2}{4} \end{aligned} \quad (5)$$

Fig. 48B shows the position and trajectory of the unstable fixed point for viability selection-based Medea gene drive with 100% efficiency i.e., $d = 1$. The population dynamics equation can be derived using equation $\dot{x}_\alpha = F_\alpha - x_\alpha \overline{F}$. (2) and (5). When the rate of mating between transgenic and wildtype decreases via h, the unstable fixed point moves towards DD vertex in the de Finetti diagram following a projectile like trajectory (Fig. 48B). Hence here, mate-choice has a negative impact on the frequency of threshold release of transgenics. For $h \approx 1$, the number of transgenics released needs to be almost half the target population size for achieving total population replacement. These results are also consistent with the invasion condition of equation (A 3) derived in the appendix A for Medea gene drive.

b. Mate-choice with Distortion

Let us now consider the case of distorted allele transmission in addition to mate-choice bias introduced by h. There are several distortion-based gene drives, but here we will focus on a meiotic drive where the distortion efficiency is p. More specifically, p is the probability of transmission of drive allele from heterozygous parent to offspring. If $p = 1$, the gene drive system mimics CRISPR/Cas-9 based homing endonuclease drive with 100% efficiency (Noble et al., 2017). If a drive allele is transmitted from heterozygous parents with probability p, the rate of genotype production then changes to,

$$\begin{aligned} F_{WW} &= x_{WW}^2 + 2(1-h)(1-p)x_{WW}x_{WD} + (1-p)^2 x_{WD}^2 \\ F_{WD} &= 2(1-h)px_{WW}x_{WD} + 2(1-p)x_{WD}x_{DD} + 2(1-h)x_{WW}x_{DD} + 2p(1-p)x_{WD}^2 \\ F_{DD} &= x_{DD}^2 + 2px_{WD}x_{DD} + p^2 x_{WD}^2 \end{aligned} \quad (6)$$

Again, the population dynamics for the distorted case is given by equation $\dot{x}_\alpha = F_\alpha - x_\alpha \overline{F}$. (2), but the effective genotype production rate changes according to equation (6). In Fig. 48C we focus on the scenario when the distortion-based gene drive such as meiotic drive or CRISPR drive with 100% efficiency (refer equation (6) for $p = 1$). We observe that the interior unstable fixed point appears only after the mate-choice bias becomes greater than 50% or $h > 0.5$ unlike viability-based gene drive Medea (Fig. 48B & C). For $h < 0.5$, a small

transgenic release is enough for population transformation to drive homozygotes (DD). Hence, the distortion-based gene drives appear to be more robust against the mate-choice stressors than viability-based gene drive Medea. These results are also consistent with the condition of invasion derived in appendix A for the distortion-based gene drive
(see equation $2(1\text{-h})\mathrm{pf}_{\mathrm{WD}} > \mathrm{f}WW$ (A 6).

c. Mate-choice with Fertility Selection

The relative number of offspring produced may differ because of the variation in the adult mating pairs' fertilities. The fitness component due to differential fertilities is included in the parameters f_α where $\alpha \in$ (WW, WD, DD). The rate of the offspring production for the three genotypes because of fertility selection changes to,

$$\begin{aligned} F_{WW} &= f_{WW}^2 x_{WW}^2 + (1-h) f_{WW} f_{WD} x_{WW} x_{WD} + f_{WD}^2 \frac{x_{WD}^2}{4} \\ F_{WD} &= (1-h) f_{WW} f_{WD} x_{WW} x_{WD} + f_{WD} f_{DD} x_{WD} x_{DD} + 2(1-h) f_{WW} f_{DD} x_{WW} x_{DD} + f_{WD}^2 \frac{x_{WD}^2}{2} \quad (7) \\ F_{DD} &= f_{DD}^2 x_{DD}^2 + f_{WD} f_{DD} x_{WD} x_{DD} + f_{WD}^2 \frac{x_{WD}^2}{4}. \end{aligned}$$

To observe the effect of fitness cost on fertility, we consider a scenario where $f_{WW} = 1$, $f_{WD} = (1-c)$, $f_{DD} = (1-c)^2$ for the dynamical equations derived using equation (7). Here, we assume multiplicative fitness cost and c denotes the fertility-fitness cost of the drive allele. The two internal fixed point appears only after substantial mate-choice bias $h \approx 0.656$ (Fig. 48D). One of the fixed points is unstable, and the other is stable. Therefore, with multiplicative fitness cost on the fertility of the transgenic organism, due to drive-allele payload, mate-choice can result in the coexistence of all the three genotypes. When $h < 0.656$, the global stable fixed point lies at the vertex of wildtype population (WW); hence no amount of drive release can replace the wild population.

Besides understanding the impact of mate-choice on the population dynamics, we also indirectly probe the threshold fraction of transgenic organisms needed to be released for complete population replacement relative to the target population size. In Fig. 49, we numerically calculate the threshold frequency of drive homozygotes (DD) necessary for the invasion of a population consisting of wild types (WW). We evaluate the impact of mate-choice bias (h), gene drive efficiency and fertility-fitness cost for two gene drive systems, namely meiotic drive and Medea. Fig. 49A shows that the mate-choice bias negatively impacts the invasion threshold frequency of DD required for complete population replacement for Medea drive. The threshold frequency of DD also slightly increases with decreasing drive efficiency. The change in threshold frequency due to drive-efficiency reduces for a higher bias in mate-choice. For lower mate-choice bias, the release threshold is close to zero, represented by the heatmap's light colour. The position of fixed point for the case of 100% drive efficiency ($p = 1$ and $d = 1$) for both Fig. 49A & B corresponds to the scenario studied in Fig. 48B & C respectively. For the distortion-based drive, lower mate-choice and sufficiently high distortion probability do not change the threshold frequency. The region in the heatmap where a minimal transgenic release can transform the population is significantly high for the distortion-based drive than Medea drive. When the mate-choice bias is high enough $(h > 0.5)$, an increase in distortion probability only slightly decreases the invasion threshold of DD. In this regime $(h > 0.5)$, a substantial frequency of DD is required for the population of wiltype to be invaded even for very high distortion probability.

In Fig. 49C & D corresponds to the case when there is a cost on the fertility fitness of the drive carrying organism ($c = 0.1$ hence $f_{WD} = 0.9$ and $f_{DD} = 0.81$). Fitness cost leads to an increase in the invasion threshold frequency for both the gene drive systems overall. Moreover, for

inefficient drives under low mate-choice bias, any DD release is insufficient to invade the wild type population. The dark colour represents this region in the heatmap. Interestingly, increasing the mate-choice bias can facilitate the invasion by DD even for less efficient drives. The distortion-based gene drive appears to be more robust against the ecological stress of mate-choice bias even when considering the fitness costs.

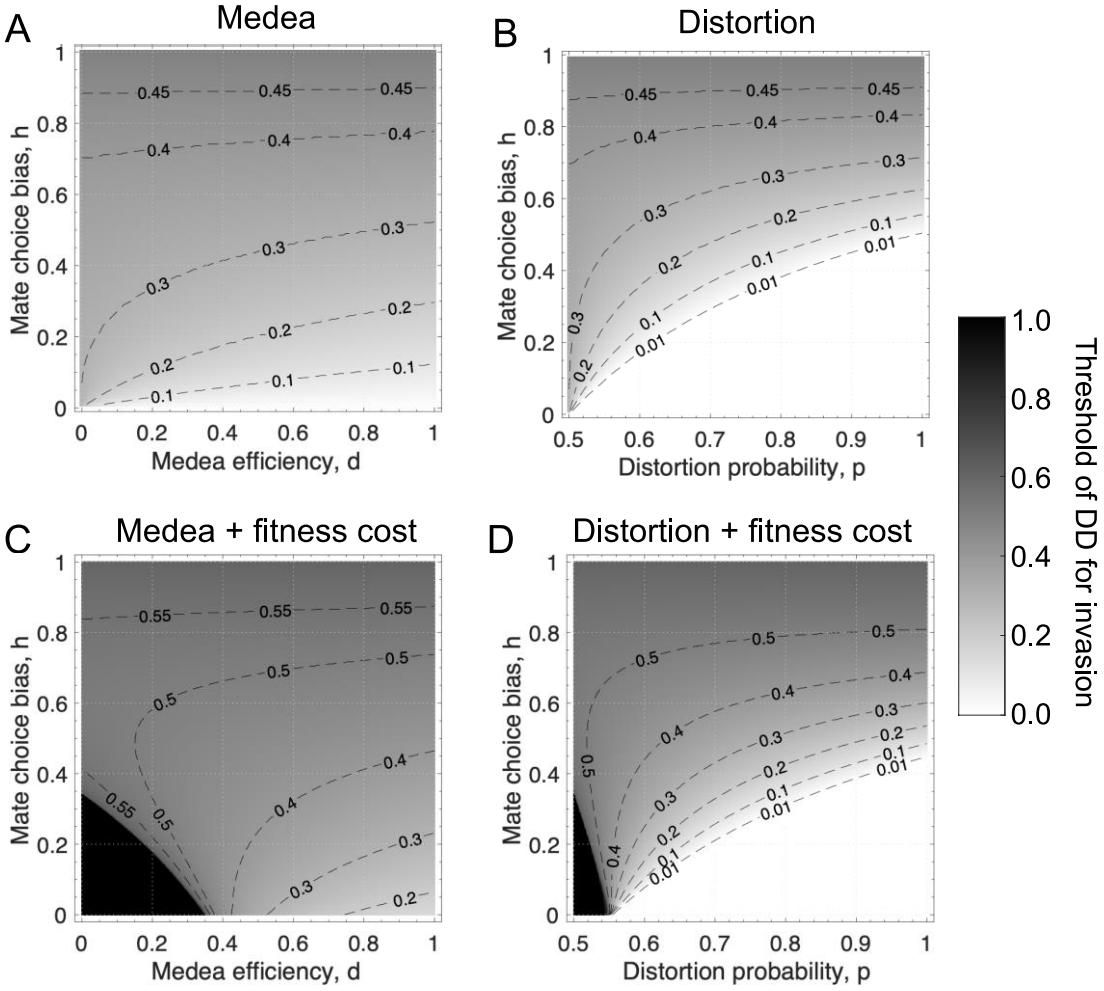


Fig. 49: Heatmap shows the threshold frequency of drive homozygotes (DD) required to invade a population of wild type homozygotes (WW) with respect to variation in mate-choice bias (h) for the following gene drive systems: Medea and distortion-based drive.
Black dashed lines correspond to the contour lines showing the threshold frequency of drive homozygotes (DD). (**A**) Medea gene drive with no fitness cost i.e. $c = 0$. (**B**) Distortion based gene drive with no fitness cost to drive i.e. $c = 0$. (**C**) Medea gene drive where the fitness cost due to drive alelle is $c = 0.1$ hence $f_{WD} = 0.9$ and $f_{DD} = 0.81$. (**D**) Distortion based gene drive where the fitness cost due to drive alelle is $c = 0.1$ hence $f_{WD} = 0.9$ and $f_{DD} = 0.81$.

Mating systems

Gene drive technology relies on sexual reproduction between the mating pairs for its transmission in the population. Most of the target species of interest have a polygamous mating system instead of the commonly assumed monogamous mating system (Moro et al., 2018; Rode et al., 2019). As introduced in the previous section of mate-choice, the model is modified here to incorporate the mating system. In this model, we will consider two separate populations of the two sexes. We assume that the offspring of both sexes are produced in equal proportion.

The frequency of male and female's genotypes are denoted using x_i and y_j. There are three possible genotypes: wild type (WW), drive heterozygotes (WD) and drive homozygotes (DD) respectively. Let us consider the mating system when one male mates with r females. Hence $r = 1$ represents monogamous mating system while $r > 1$ corresponds to polygamous mating system. The following set of equations gives the frequencies of the genotypes produced with the polygamous mating system for both males and female:

$$F_k(r) \quad = \quad \sum_{\alpha,\beta_1,\beta_2,\ldots\beta_r} \sum_{j=1}^{r} M_k\left(\alpha,\beta_j\right) f_\alpha x_\alpha \prod_{i=1}^{r} f_{\beta_i} y_{\beta_i} \tag{8}$$

Here, $M_k(\alpha,\beta_j)$ is the proportion of genotype k produced from the mating between male of genotype α and female of genotype β_j. α and β_j are dummy index for any of the three genotype WW, WD or DD. The elements of the matrix $M_k(\alpha,\beta_j)$ will depends upon the type of the gene drive as well. Matrix M_k for Medea (equation (A 7)-(A 9)) and distortion-based gene drive system (equation (A 10)-(A 12)) is given in appendix A. The summation over α and β_j is carried out over set of all genotypes (WW, WD, DD). We have also assumed a polygamous mating system of mating ratio r, i.e. one male mates with r female or vice-versa. Equation (8) may be interrupted in parts as selecting a male of genotype α and selecting r females of genotype $\beta_1, \beta_2, \ldots, \beta_r$. Finally, the contribution of all possible matings in producing genotype k is summed up.

Simplifying equation (8) by expansion formula for multinomial expression yields,

$$F_k(r) = rF_k(1)(f_{WW}y_{WW} + f_{WD}y_{WD} + f_{DD}y_{DD})^{r-1} \tag{9}$$

The following set of differential equations governs the population dynamics of the genotypes in continuous time:

$$\begin{aligned} x_\alpha &= \tfrac{1}{2}F_\alpha(r) - x_\alpha\overline{F}(r) \\ \dot{y}_\alpha &= \tfrac{1}{2}F_\alpha(r) - y_\alpha\overline{F}(r) \end{aligned} \tag{10}$$

where $\overline{F}$ is the sum of rates of genotype production,

$$\overline{F}(r) = \sum_\alpha F_\alpha\,(r) \tag{11}$$

The total population of both males and females remains constant and sum up to unity.

$$x_{WW} + x_{WD} + x_{DD} = 1 \tag{12}$$

$$y_{WW} + y_{WD} + y_{DD} = 1 \tag{13}$$

In equation (9), $F_k(r = 1)$ and $F_k(r > 1)$ is the production rate of genotype k for monogamous $(r = 1)$ and polygamous $(r > 1)$ mating system respectively. It implies that the equilibrium population dynamics for both monogamous $(r = 1)$ and polygamous $(r > 1)$ mating system even with gene drives are equivalent. In other words, the final population composition with respect to genotypes abundance remains same for both polygamous and monogamous mating system. Previous studies without any gene drive also supports that the equilibrium dynamics for both monogamy and polygamy remain same (Karlin, 1978; O'Donald, 1980). However, the difference lies in the relative time to reach population equilibrium. It can be shown that after simplifying the equation (10) obtained for $r > 1$, the rate of increase of different genotypes is equivalent to the case of monogamy $(r = 1)$ with rescaled time. The expectation is that the

gene drive will spread faster in polygamous mating species compared to monogamy (Moro et al., 2018). Hence, the time required for the drive allele to spread through the population should increase for monogamous mating system. Our result also supports the expected outcome. Here we quantify the same.

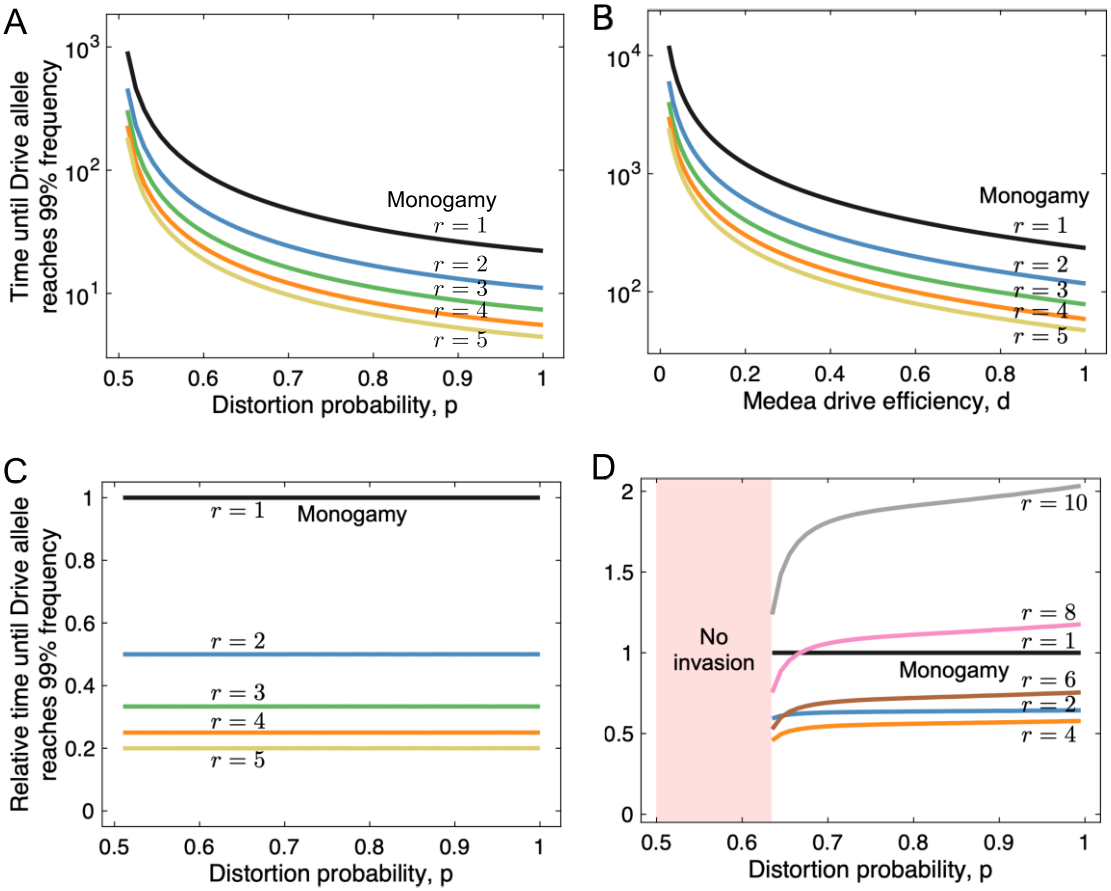


Fig. 50: Effect of mating system and drive efficiency on the time for the drive allele to reach 99% frequency. We start from a population consisting of the 99% wild types (WW) and 1% the drive heterozygotes (DD) with 100% drive efficiency and varying fitness cost. The population is evolved until frequency of drive allele reaches 99%. **(A)** Absolute time is plotted for distortion-based gene drive with no fitness cost, $c = 0$ and $p = 1$. **(B)** Absolute time is plotted for Medea gene drive with no fitness cost, $c = 0$ and $d = 1$. **(C)** Relative time with respect to monogamy ($r = 1$) case is plotted for distortion-based gene drive without fitness cost, $c = 0$ and $p = 1$. **(D)** Relative time with respect to monogamy ($r = 1$) case is plotted for distortion-based gene drive with fitness cost, $c = 0.2$ and $p = 1$. The red shaded area is the region where the drive heterozygotes are not able to invade the wild type population.

Let us first look at the case where there is no fitness cost of the gene drive and only the efficiency of the two gene drive system based on distortion and viability selection are varied. Fig. 50A, B & C shows that gene drive will spread faster for species with high degree of polygamy I. It can also be seen by comparing Fig. 50A & B that the distortion-based gene drive will spreads faster compared to viability-based Medea drive. Infact, the time for the gene drive to reach 99% frequency is an order of magnitude higher for Medea drive compared to CRISPR homing drive or meiotic drive. Higher degree of polygamy (r) reduces the time required to reach critical drive frequency (99%) for both the gene drive system. This reduction in the value of absolute time becomes more pronounced when the gene drive is less efficient (Fig. 50A & B).

In Fig. 50C, it is clearly evident that the relative time required for the drive allele to reach 99% frequency is rescaled exactly by a factor of $1/r$ for the polygamy relative to monogamous mating system. This is in line with the relation obtained in equation (9). When $f_{WW} = f_{WD} = f_{DD}$, the production rate of offsprings for polygamy is r times that for the monogamous mating system. But, when we have a fitness cost c for carrying a drive allele, the relation between the time to reach 99% frequency and degree of polygamy becomes more complex (Fig. 50D). Increase in the degree of polygamy first decreases the relative time to reach drive allele's critical frequency ($r = 2$ and $r = 4$) but further increase in the degree of polygamy ($r = 6,8,10$) elevates it. In Fig. 50, it can also be noted that when the distortion probability is low ($p < 0.625$), drive allele is not able to invade the wildtype population. This is congruence with the condition of invasion derived for monogamous case in equation $2(1-h)pf_{WD}$>fWW(A 6) in the appendix.

The above result can be understood from the equation (9) where the fitness cost makes the factor $(f_{WW}y_{WW} + f_{WD}y_{WD} + f_{DD}y_{DD})^{r-1}$ less than one. The factor $(f_{WW}y_{WW} + f_{WD}y_{WD} + f_{DD}y_{DD})^{r-1}$ decreases exponentially with increasing level of polygamy r. Since the time is rescaled by the factor of $\frac{1}{r(f_{WW}y_{WW}+f_{WD}y_{WD}+f_{DD}y_{DD})^{r-1}}$, it first decreases when dominated by $1/r$ with increase in r but later on decreases when dominated by $1/(f_{WW}y_{WW} + f_{WD}y_{WD} + f_{DD}y_{DD})^{r-1}$. When the fitness cost is $c = 0.2$, the relative time until drive allele reaches 99% frequency with respect to monogamy decreases for $r = 2$ and $r = 4$ but then it starts to increase for $r = 6$. For $r = 8$ and $r = 10$ spread of gene drive becomes slower compared to monogamy. Another way to understand the results is that the rate of production genotype DD first increases up to a point for increasing level of polygamy r but later decreases for moderate fitness cost (Fig. B 1). Hence the time in spreading gene drive is lowest for intermediate levels of polygamy. Further increase in the degree of polygamy reduces the production of DD and therefore increases the time to spread the drive allele.

5.5.3 **Spatial network interaction**

The population dynamics of CRISPR based homing endonuclease gene drive have been extensively studied for well-mixed infinitely large (Noble et al., 2017) and finite population (Noble et al., 2018). But most species occur in a partially heterogeneous landscape where they interact and mate with other individuals in their vicinity. Hence, a network-based population is an appropriate framework to model dynamics in such structured populations.

We considered a structured population of n individuals. The individuals live on a random network with an average degree of k; thus, each individual has k connections on average. Here k controls the number of mating opportunities and the level of competition for an individual. The population is updated via a death-birth process (Fig. 51) described as follows: First, an individual is chosen randomly for death. Then two parents are selected, who are neighbours of the dead individual with probability proportional to their fertility fitness. According to their genetic archetype, the selected parents contribute their gametes, where other genetic effects like distortion can come into play. The combination of these contributed gametes forms the offspring that replaces the dead individual in the network. The population is updated until it fixes to all WW or all DD state.

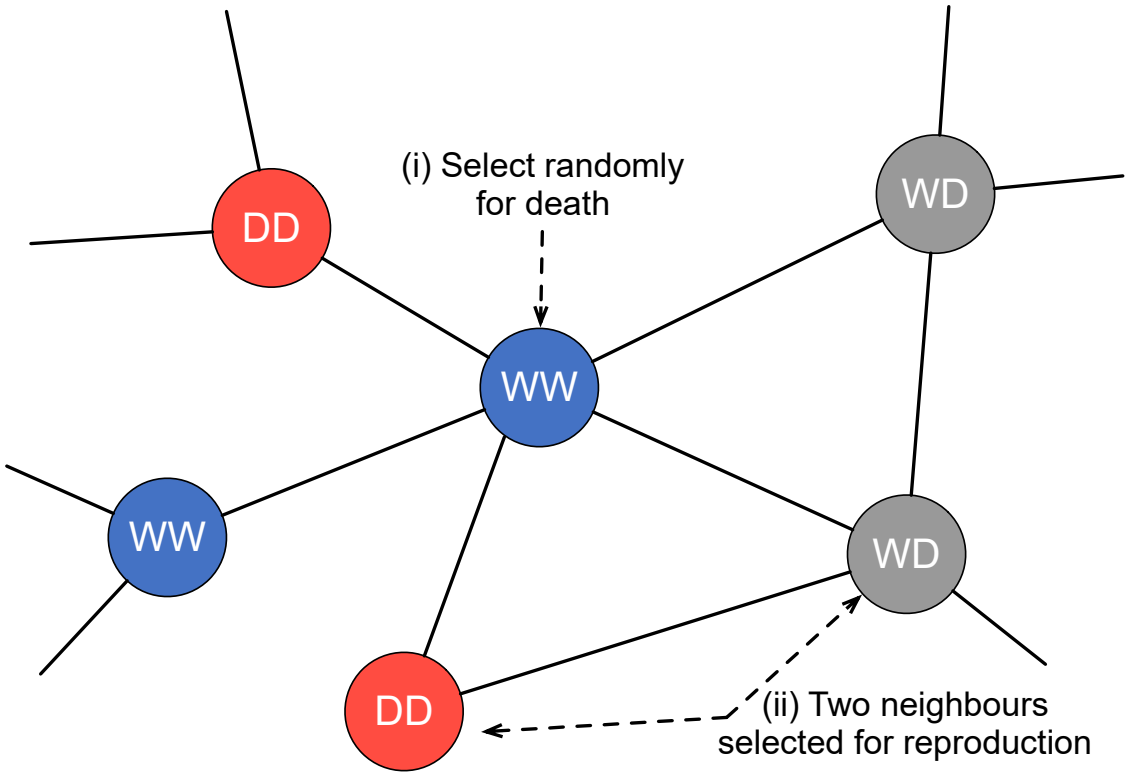


Fig. 51: Spatial model explaining the population update mechanism.
The blue, grey and red colours represent individuals of WW, WD and DD genotype, respectively. Population update happens in 2 steps: firstly, a random individual is selected for death. This step creates space at that particular network position. Secondly, two random neighbours of the dead individuals are chosen as parents to produce offspring. The genotype of the offspring is determined from the parents, and it replaces the dead individual.

In Fig. 51, we exhibit the stochastic network model by running the simulation several times and plotting fixation probability and conditional fixation time with the dependence on the average number of interacting individuals per site (represented by k). We also studied the impact of increasing the number of released transgenic (WD and DD) and different genotype (WD and DD). Here, k controls the number of mating opportunities and competition during the birth process. When k increases, the fixation probability of DD decreases mainly due to higher competition during the birth update per site (Fig. 52A). As expected, distortion probability has a positive impact on the fixation probability of DD. The impact is more pronounced for lower values of an average degree since the heterogeneity in the number of connected individuals is also high for this case. Fixation probability also increases as the number of released DD increases (Fig. 52A). Unexpectedly, DD transgenic release has a lower chance of getting fixed than a WD release (Fig. 52B). This observation is mainly because the fitness cost of DD is quite high compared to WD ($f_{WD} = 0.50, f_{DD} = 0.25$). If the fitness cost is small and drive efficiency is low, release of DD genotype is expected to fix the gene drive with higher probability. The effect on fixation probability by the release of WD compared to DD becomes more pronounced with the increase in average degree k (Fig. 52B). It increases first with an increase in the release number of transgenic, attains a maximum and decreases latter. We also plotted conditional fixation time with variation in the number of releases and the network degree (Fig. 52C). The conditional fixation time is lower for a high number of releases and lower values of average degree k. The difference in the value of conditional fixation time is high when the release number hence the loss of drive due to stochasticity is high. In all of our simulations for the release nodes of transgenic are chosen at random.

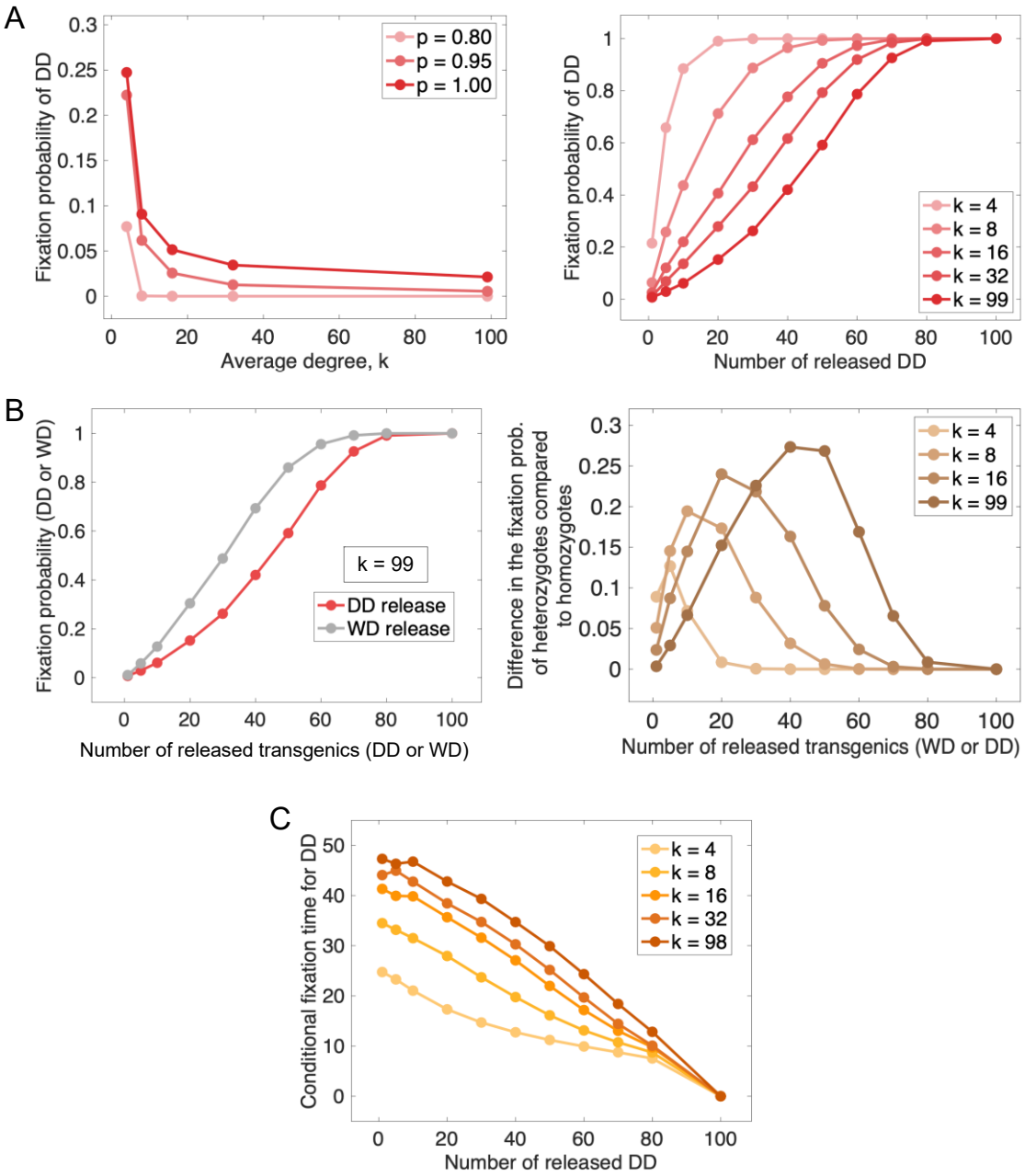


Fig. 52: Fixation probability and conditional fixation time of DD with variation in average degree k, distorsion probability p and initial number of released transgenic individuals WD or DD.
(A) Plots show the fixation probability of drive homozygotes against average degree k (left panel) and the number of released DD (right panel) for different values of p and k respectively. Left: one DD individual is initially released in the population consisting only of WW. **(B)** Left: Fixation probability is plotted against the number of released DD and WD for a complete graph ($k = 99$). Right: the difference between the fixation probability of WD and DD release is plotted against the number of released transgenic for varying average degree k. **(C)** Shows the average number of generations when the drive individuals get fixed in the population against an initial number of released DD with varying average degree k. A generation consists of n death-birth step. Hence in a generation, the whole population is updated on an average. All simulations were performed for a population size of $n = 100$ and 10,000 trials to estimate fixation probability and conditional fixation time. If not mentioned distortion probability and fitness cost are fixed to $p = 0.95$ and $c = 0.5$, respectively.

5.5.4 **Discussion**

Gene drive is one of the tools of synthetic biology that has the potential to transform whole populations. The transformation uses and modulates one of the foundational tenets of evolution – the inheritance of traits through sexual reproduction. Thus, variation in the reproductive biology and the mating behaviour of the target species can affect the eventual spread of the gene drive. Previous studies have emphasized the understanding of the impact of genetic resistance on gene drives (Champer et al., 2018; Noble et al., 2017; Unckless et al., 2017). Herein, we have examined a few of the mating complexities that the target population will face in the field beside the genetic resistance itself. In particular, we focus on how mating complexities, namely mate-choice, mating system, and mating network, affect the spread of gene drive. We found that incorporating the above factors is crucial to correctly predict the outcome of releasing a specified number of transgenic gene drive individuals into a population for invasion of the target population. It is also required to estimate better the fixation time of the drive gene to plan any field release.

We found that if the drive gene has linkage with the ornaments of mate-choice, a mate-choice bias can develop with a significant effect on the release threshold of a gene drive, as shown in Fig. 49. Inefficient drive and fitness costs due to drive-payload aggravate the situation, and the predicted threshold release is drastically different compared to the case when there is no mate-choice bias. We also found that distortion-based gene drive fares much better than viability-based gene drive under ecological stress of mate-choice. Hence for regulatory checks, gene drive constructs should be evaluated for their robustness against various ecological stressors. Though it is not clear if the target species could evolve such mate-choice preferences, their evolution will be fast since the target species for gene drives are mostly targeted towards fast reproducing species. Moreover, experience from sterile insect techniques has taught that different rearing conditions in the lab and wild can also give rise to different behavioural and genetic traits leading to divergent mating preferences and eventual program failure (Eberhard, 1999; Lance et al., 1998; McInnis et al., 1996; Robinson and Hendrichs, 2005).

Next, we consider separate populations of males and females to account for different mating systems. We found that the final evolutionary outcome of the spread of the gene drive (distortion and Medea drive) for a polygamous mating is the same as that of the monogamous system. Even the species with a higher degree of polygamy will converge to the same evolutionary fate for a given gene drive system. However, the time needed for the spread of the drive gene is smaller for a higher degree of polygamy in the absence of any fitness cost. When there is a moderate fitness cost, increasing the degree of polygamy decrease the spreading time of the gene drive up to a point but later increases it. This nonlinearity is because the production rate of drive homozygote first increases but later decreases with an increase in the degree of polygamy for moderate fitness cost (Fig. 50). Hence, the drive gene is expected to spread faster for species with intermediate levels of polygamy when there is an associated fitness cost of the drive allele.

Considering a finite population on a network allows us to understand the probable outcomes of gene drive release. A finite population leads to stochastic fluctuations in the frequencies of the genotypes resulting in different outcomes for the same initial conditions. We found that the spread of transgenic release is lowered when individuals, on average, have more mating opportunities and intra-sexual competition. Thus, the fixation time for the transgenic increases with an increase in the average degree of the mating network. Concerning the question of how the connectivity of the mating network is varied, we know the adaptation shapes that network structure among various species regarding the environment that those species live in and the selective pressures under which they evolved (Pinter-Wollmann et al., 2014). Hence, change in environmental conditions such as resource availability, seasonal effects, or selective pressure, and life-history traits can vary the network structure. Within a species itself, variation at the individual level can also lead to heterogeneous connectivity. Hence, species with sparsely connected individuals on the mating network have a higher chance of fixing drive

genes and require less time. We also observe that the success in fixation of drive homozygotes can be mitigated by releasing more transgenic individuals. Furthermore, when there is a high fitness cost associated with carrying drive allele payload, releasing drive heterozygotes instead of homozygotes would result in a higher chance of gene drive fixation.

Researchers developing gene drives need to be cautious while selecting the target gene of the drive, possibly estimating the pleiotropic effects, especially regarding mate-choice. We conclude that evaluating mate choice preferences qualitatively and quantitatively is a prerequisite for modelling gene drive efficacy.

In this study, we have decided to focus on three factors related to the mating complexities of the target species, but many other ecological factors can impact the efficacy of the spread of gene drive. Previous work has quantified the effect of life history traits, age structure, spatial landscape and seasonality etc. on gene drive dynamics (Eckhoff et al., 2017; Huang et al., 2011, 2009; North et al., 2020, 2019; North et al., 2013). When deployed, a drive will eventually face the above mentioned and other ecological stressors together with the evolutionary pressures of resistance and mutational decay. Navigating this ecological complexity might often seem insurmountable (Levin, 2003). For any technology aiming at intervening in complex systems, we will be facing a similar control problem. It is unfeasible to design *insilco* all possible ecological and evolutionary pressures and scenarios that an engineered system will face in the real world (Denton and Gokhale, 2019; Lindvall and Molin, 2020). Identifying and collecting necessary information on the effect of primary ecological and evolutionary pressures will be thus crucial to access the risk before any field deployment (James et al., 2018; Long et al., 2020; National Academies of Sciences Engineering and Medicine, 2016). Our next aim would be to take an integrative approach by including multiple ecological complexities at the same time for a specific drive system given a specific location.

5.5.5 **Appendix A: Additional Methods**

Invasion condition for Medea drive with Mate choice (h)

Let us consider the case of Medea gene drive with fertility selection. The rate of production of the three genotype is given by the combination of (5) and (7),

$$\begin{aligned} F_{WW} &= f_{WW}^2 x_{WW}^2 + (1-h)(1-0.5d) f_{WW} f_{WD} x_{WW} x_{WD} + (1-d) f_{WD}^2 \frac{x_{WD}^2}{4} \\ F_{WD} &= (1-h) f_{WW} f_{WD} \frac{x_{WW} x_{WD}}{2} + f_{WD} f_{DD} x_{WD} x_{DD} + \\ &\quad 2(1-h) f_{WW} f_{DD} x_{WW} x_{DD} + f_{WD}^2 \frac{x_{WD}^2}{2} \\ F_{DD} &= f_{DD}^2 x_{DD}^2 + f_{WD} f_{DD} x_{WD} x_{DD} + f_{WD}^2 \frac{x_{WD}^2}{4} \end{aligned} \quad \text{(A 1)}$$

The rate of change of frequencies of each genotype is still given by equation $\dot{x}_\alpha = F_\alpha - x_\alpha \overline{F}$. (2). Using the constraint on the frequencies of the three genotype in equation $x_{WW} + x_{WD} + x_{DD} = 1$(4), the population dynamics of the three genotype is reduced to two variables after replacing $x_{WD} = 1 - x_{WW} - x_{DD}$ in equation $\dot{x}_\alpha = F_\alpha - x_\alpha \overline{F}$. (2). The drive will not be able to invade the wildtype population if both the eigenvalues of the dynamical system are negative. Eigenvalues can be deduced from the Jacobian matrix (J_d) of the system at $(x_{WW}, x_{WD}, x_{DD}) = (1,0,0)$,

$$J_d = \begin{pmatrix} f_{WD}f_{WW}(1-h) - f_{WW}^2 & f_{WD}f_{WW}(1-h) - 2f_{DD}f_{WW}(1-h) \\ 0 & -f_{WW}^2 \end{pmatrix} \quad \text{(A 2)}$$

Hence, Medea gene drive can invade a population of wildtype if

$$(1-h)f_{WD} > f_{WW} \quad \text{(A 3)}$$

Note that the above invasion condition is independent of the efficiency of Medea gene drive (d).

Invasion condition for Distortion drive with Mate choice (h)

Consider the scenario of distortion-based gene drive with fertility selection. The rate of production of the three genotypes will then be governed by the combination of equation (6) and (7),

$$\begin{aligned} F_{WW} &= f_{WW}^2 x_{WW}^2 + 2(1-h)(1-p)f_{WW}f_{WD}x_{WW}x_{WD} + (1-p)^2 f_{WD}^2 x_{WD}^2 \\ F_{WD} &= 2(1-h)pf_{WW}f_{WD}x_{WW}x_{WD} + 2(1-p)f_{WD}f_{DD}x_{WD}x_{DD} \\ &\quad +2(1-h)f_{WW}f_{DD}x_{WW}x_{DD} + 2p(1-p)f_{WD}^2 x_{WD}^2 \\ F_{DD} &= f_{DD}^2 x_{DD}^2 + 2pf_{WD}f_{DD}x_{WD}x_{DD} + p^2 f_{WD}^2 x_{WD}^2 \end{aligned} \quad \text{(A 4)}$$

Similar to the Medea gene drive scenario, the population dynamics of the above system can be written in the form of two variables x_{WW} and x_{DD} using $x_{WW} + x_{WD} + x_{DD} = 1$(4). The Jacobian matrix (J_m) of the system at $(x_{WW}, x_{WD}, x_{DD}) = (1,0,0)$ is given by

$$J_d = \begin{pmatrix} 2f_{WD}f_{WW}(1-h)p - f_{WW}^2 & 2f_{WD}f_{WW}(1-h)p - 2f_{DD}f_{WW}(1-h) \\ 0 & -f_{WW}^2 \end{pmatrix} \quad \text{(A 5)}$$

From the condition on the eigenvalues, the gene drive can invade wildtype population if,

$$2(1-h)pf_{WD} > f_{WW} \quad \text{(A 6)}$$

Note that when there is no mate choice ($h = 0$) the above condition reduces to the invasion condition derived by (Noble et al., 2017) for CRISPR gene drive.

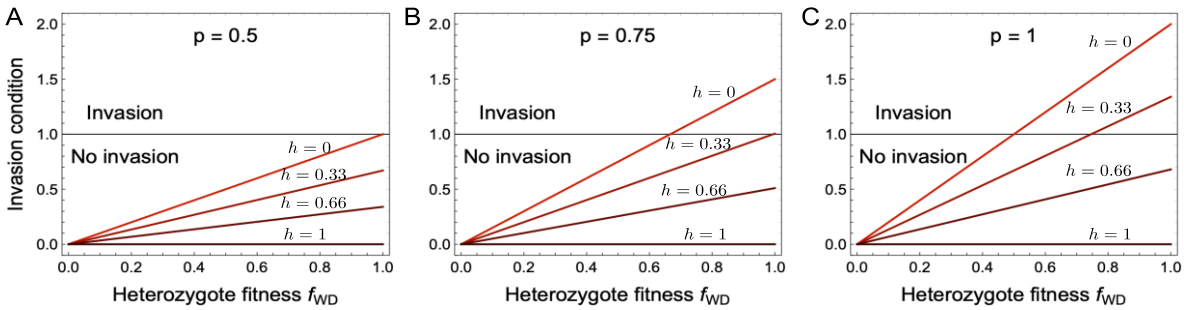


Fig. A 1: Invasion condition with varying mate-choice bias (h) against heterozygotes fitness f_{WD}.
(A) Medea drive or no distortion, $p = 0.5$. Wildtype population cannot be invaded for any value of mate choice bias, h. **(B)** Distortion based gene drive with $p = 0.75$. Wildtype population can be invaded if there is no-mate choice bias $h = 0$ and $f_{WD} > 2/3$. **(C)** Distortion based gene drive with $p = 1$. Wildtype population can be invaded if mate choice bias not very high i.e. for $h = 0$ and $h = 0.33$.

$M_k(\alpha, \beta_j)$ in equation (8) for Medea and Distortion Based Gene Drive

Medea Gene Drive

$$M_{WW} = \begin{bmatrix} 1 & 0.5(1-d_m) & 0 \\ 0.5 & 0.25(1-d_m) & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad \text{(A 7)}$$

$$M_{WD} = \begin{bmatrix} 1 & 0.5(1-d_m) & 0 \\ 0.5 & 0.25(1-d_m) & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad \textit{(A 8)}$$

$$M_{DD} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0.25 & 0.5 \\ 0 & 0.5 & 1 \end{bmatrix} \quad \textit{(A 9)}$$

$$M_{WW} = \begin{bmatrix} 1 & (1-p) & 0 \\ (1-p) & (1-p)^2 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad \textit{(A 10)}$$

$$M_{WD} = \begin{bmatrix} 0 & p & 1 \\ p & 2p(1-p)) & (1-p) \\ 1 & (1-p) & 0 \end{bmatrix} \quad \text{(A 11)}$$

$$M_{DD} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & p^2 & p \\ 0 & 0 & 1 \end{bmatrix} \quad \text{(A 12)}$$

5.5.6 Appendix B: Supplementary Figures

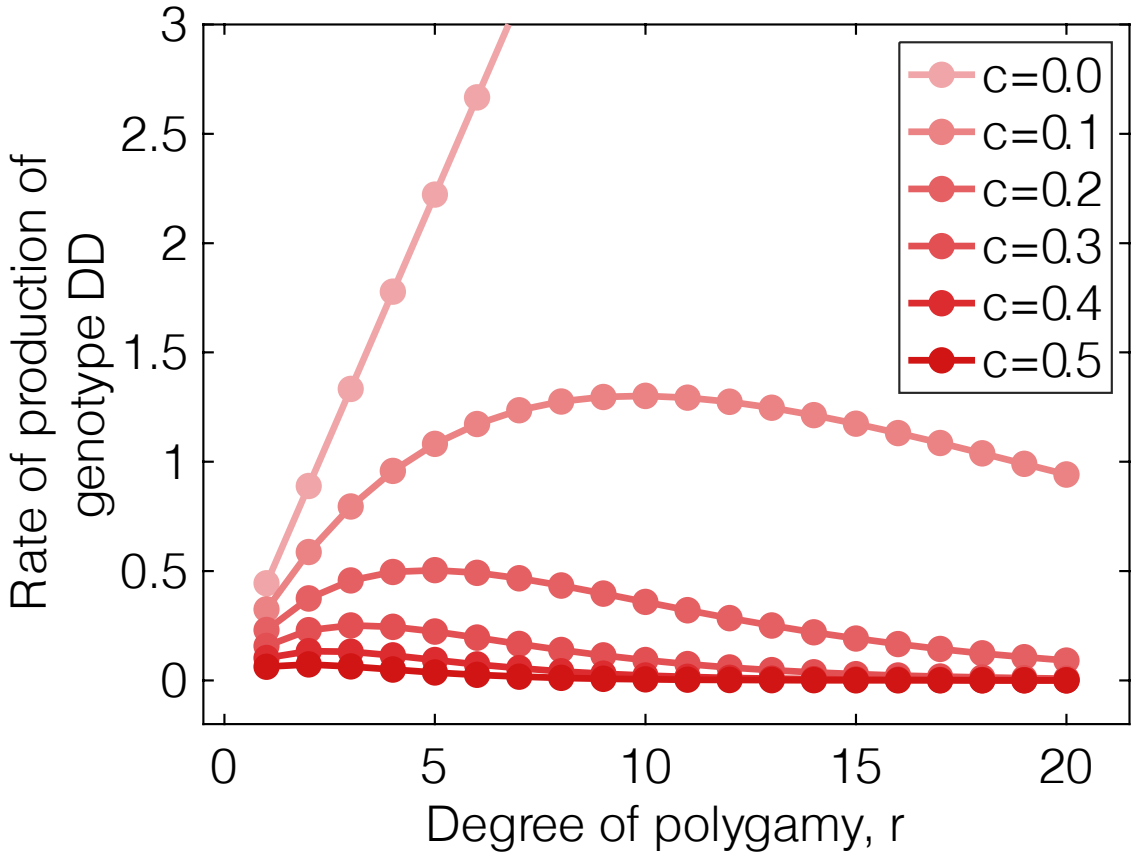


Fig. B 1: Effect on the rate of production of DD genotype with increases in degree of polygamy (r) for different fitness cost (c).

We start from a population with an equal abundance of all three genotypes with 100% drive efficiency of distortion-based gene drive for different fitness costs. In essence, we plotted equation (9) for varying r and c keeping $x_{WW} = 1/3, x_{WD} = 1/3, x_{DD} = 1/3$ and $p = 1$. Increasing the fitness cost of the drive allele decreases the overall production of the DD genotype. For a moderate level of fitness cost, production of genotype DD first increases up to a point for species with a higher level of polygamy but then started to decrease.

6 Part B – Evaluation of Ecological and Conservational Impacts

Margit Seiberl, Bernhard Splechtna, Harald Meimberg

Gene drive organisms (GDOs) are viewed as a potential tool to reduce the population size or specifically change a population of a target organism. Examples include vectors for carrying diseases, agricultural pests, or invasive species. However, the release of GDOs into ecosystems will lead to intended and unintended effects and potentially harm the ecosystem (Rode et al., 2019). The overall goal of Block B1 is to identify ecological and nature conservation effects of GDOs. Therefore, we review current approaches used to define and assess risk and work on suggestions how GDOs can be integrated into risk assessment (Fig. 53).

After reviewing relevant environmental risk assessment (ERA) documents from EPA and EFSA we identified the GMO risk assessment of EFSA (EFSA GMO Panel, 2013) as the current practice. We characterize the steps that are used to set up a risk assessment, streamline the technical jargon for the report and provide the sources. We included a general evaluation of how and whether the ecosystem service approach according to EFSA (EFSA Scientific Committee, 2016) can assist the ERA of GD to formulate specific protection goals. We also outline the use of the risk assessment framework developed for invasive species to assess GDOs.

The research revealed some shortcomings of the current risk assessment of ecotoxicological stressors and of GMOs that make it especially difficult to translate the current system into a GD relevant approach. This applies especially to the discussion about the equivalency of the ecosystem service argument with biodiversity conservation. We therefore investigate also criticism about the use of the ecosystem services concept for the formulation of general or specific protection goals with focus on service providing units. We are highlighting in the report the putative analogy of ecosystem services with biodiversity protection and definition of harm. Additionally, during the last phase, as part of our examination of the EFSA expert opinion draft regarding the “adequacy of existing EFSA guidelines for the risk assessment of gene drive modified insects” (EFSA, 2020), and related literature we refine our view about the applicability of the ERA paradigm to gene drive applications. Our comment that has been submitted in response to EFSA 2020, is included here as supplement.

Although there are some similarities, the release of GDOs is substantially different from the release of GMOs, the application of pesticides, or the spread of invasive alien species, for which risk assessment guidelines already exist. GDOs are designed to be released in wild environments and, in contrast to all other stressors, the characteristic of GDOs are intended to spread through a population. We therefore work towards a framework for a risk assessment of GDOs based on the risk assessment for invasive species.

In GDO application the effect on the ecosystem is not defined by a direct effect of a stressor on non-target organism but by the reduction or change of the target organism population. This affects other organisms and the consideration of such ecological cascades, the chain of events resulting from a change of population size of one organism group that can sequentially influence the other organisms in an ecosystem, is important in the context of GDO. One goal of the study is to suggest ways of how this can be included into risk assessment, including suggestions about which type of models might be useful and what biological information is necessary to be implemented into such an approach.

Therefore, we worked towards a framework for ERA using a simple conceptual model differentiating between target and non-target regions and different effectors. We also looked at possibilities for ecological modeling with ERA and developed the conceptual model further using partial scenarios. We included two different case studies; one for *Drosophila suzukii* in an agricultural (pest control) context, and the other for *Rattus norvegicus* in a conservational setting. We applied different approaches for these case studies. For *D. suzukii* we used system

analysis (Vester, 1999) and outline the network of interactions in more detail in a socio-economic system. For *R. norvegicus*, we derived risk hypotheses following a more descriptive approach. Finally, we elaborate on the suitability of the ERA paradigm for gene drive application.

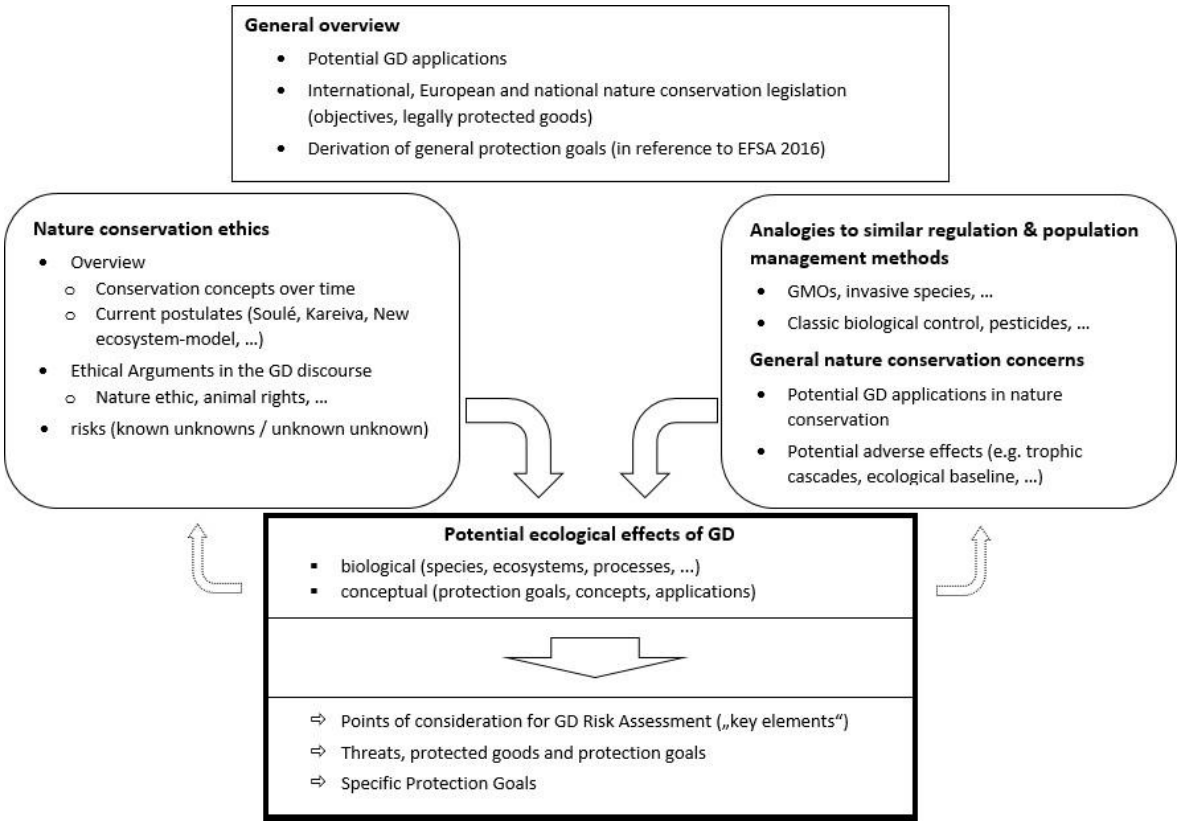


Fig. 53: Schematic overview about the topics and their links covered by work packages B1 and 2, like it had been envisaged at the start of the project. We concentrated on general considerations, start to develop a view about conservation concepts (ecosystem service arguments) and ecological effects.

6.1 Part B.1 – Ecological Risk Assessment and Protection Goals

6.1.1 Ecological Risk Assessment – Key Elements in the US and EU

To summarize and to understand the current practice of ecological risk assessment (ERA) regarding ecotoxicological stressors and GMOs (Genetic Modified Organisms), we performed a literature review of current guidelines of the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA). "Guidelines for Ecological Risk Assessment" have been published from EPA in 1998, which is based on the previously published EPA report "Framework for Ecological Risk Assessment" (EPA, 1992). In general, ecological risk assessments consider human-induced changes in the environment by trying to "*evaluate the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors"* (EPA, 1992).

Data and information are organized to show the relationship between stressor and ecological effects. Stressors are generated by anthropogenic activity and cause adverse ecological effects. Stressors can be chemical, physical or biological, often multiple stressors act together and cause adversity. Because effects can be neutral to one component of the ecosystem but adverse to another part, adversity must be defined. Adversity can be described by type, intensity, scale of the effect, and the potential for recovery. A risk assessment process is

gathering quantitative information, but also qualitative conclusions and associated uncertainties (known unknowns) are important. Risk assessments are used to predict the likelihood of future adverse effects but can also evaluate the effects caused by past exposure to stressors.

The characterization of hazards and the characterization of exposure are two major elements of an ecological risk assessment process. To substantiate these elements in a structured manner, EPA, (1998) describes three important phases in a risk assessment: (1) Problem formulation; (2) Analysis phase and (3) Risk characterization.

The European Food Safety Authority (EFSA) also presents guidance on environmental risk assessments, e.g. of genetically modified plants (EFSA GMO Panel, 2010), of genetically modified microorganisms and their products intended for food and feed use (EFSA, 2011), of food and feed from genetically modified animals and animal health and welfare aspects (EFSA Panels on GMO and AHAW, 2012), of plant production products on bees (EFSA, 2013), of genetically modified animals (EFSA GMO Panel, 2013) or to develop specific protection goals for environmental risk assessments (EFSA Scientific Committee, 2016).

The guidance document for an environmental risk assessment of genetically modified animals (EFSA GMO Panel, 2013) to be placed on the European Union (EU) market is in accordance with Directive 2001/18/EC. This EFSA guidance assesses adverse effects of genetically modified animals (namely fish, insects, birds and mammals) on the environment but also on animal and human health and addresses the following areas of risk:

- **persistence and invasiveness of the GM animal, including vertical gene transfer (VGT)**
- **horizontal gene transfer**
- **interactions of the GM animal with target organisms**
- **interactions of the GM animal with non-target organisms (NTOs)**
- **environmental impacts of the specific techniques used for the management of the GM animal**
- **impacts of the GM animal on biogeochemical processes**
- **impacts of the GM animal on human and animal health**

In this document, EFSA suggests six steps for an environmental risk assessment: (1) problem formulation including hazard and exposure identification; (2) hazard characterizations; (3) exposure characterization (4) risk characterization; (5) risk management strategies; and (6) an overall risk evaluation. These processes are suggested for an environmental risk assessment for GM animals, but as for now GDOs (Gene Drive Organisms) are also included.

Because the meaning of a number of key terms in the literature appears to be not always straightforward and the meaning regularly differs from colloquial language, we provide a list of the most common key terms and their definitions as used throughout the report below.

a. Key Terms

In addition to the key terms we defined in earlier reports: protection goals, specific protection goals, stressor, assessment endpoints, measurement endpoints, and its synonym measure of effect, we add here risk, hazard, and uncertainty.

Risk

"In colloquial use, the term risk is synonymous with threat, harm, or hazard." (National Academies of Sciences, 2016). In the context of risk assessment, risk has a probabilistic meaning (EFSA GMO Panel, 2013; EPA, 1998, 1992; National Academies of Sciences, 2016). Risk can be defined as the hazard times the probability of occurrence (de Jong, 2017), but

probability can also be interpreted in terms of "*the probability of the severity of adverse effects, given their occurrence"* (Haimes, 2009). In our use, we only differentiate between hazard and risk when probability is necessary to be considered, otherwise we use the term risk.

Hazard

Potentially adverse (ecological) outcomes. The Royal Society (1983) defines hazard as a situation that under circumstances could lead to harm. This definition emphasizes that hazard analysis must identify the circumstances that lead to harm, i.e. the causal pathway rather than simply identifying potential adverse outcomes. Hazard can also be equated with impact (Hayes et al., 2018).

Uncertainty

According to the European Environmental Agency (EEA, 2001) uncertainty differs from risk such that with uncertainty the likelihood of occurrence of an impact (or hazard) is unknown. They also define ignorance in this context as the situation, when both, the impact and the likelihood of its occurrence are unknown (EEA, 2001). EFSA (EFSA GMO Panel, 2013) addresses three types of uncertainty, the linguistic uncertainty, the variability and the incertitude. Linguistic uncertainty is caused by different understandings of language; variability arises by fluctuations in different processes (e.g. birth rates…) and incertitude or epistemic uncertainty (National Academies of Sciences, 2016) derives from a lack of scientific knowledge caused by measurement error, systematic error, natural variation, inherent randomness, model uncertainty and subjective judgment (Regan et al., 2002).

Protection goals

Environmental Protection goals are objectives defined in law or legislations regarding the environment, natural resources or natural resource services. As stated in EFSA (EFSA Scientific Committee, 2016), general protection goals can be summarized as "biodiversity" and "human well-being". Although some legislation in the context of nature conservation define more specific goals (e.g. Council Directive 92/43/EEC), these two goals can be confirmed as overarching goals based on a review of the relevant nature conservation legislation.

Tab. 12: Comparative overview of international, European and national conservation legislation (potentially) relevant in the context of GD applications.

International Agreements	*Year (adoption)*	*Protection Goal*	*Legally protected good*
Convention on Biological Diversity (CBD)	1992	Conservation and sustainable use of biological diversity and fair and equitable sharing of the benefits arising out of the utilization of its components	Biological diversity (variability within and between species and of ecosystems)
Cartagena Protocol on Biosafety	2000	Prevention of potential adverse effects resulting from the transfer, handling and use of living modified organisms, especially focusing on transboundary movements	Biological diversity; human well-being
Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress	2010	Provision of liability and compensation regulations in case of potential adverse effects of the transboundary movement of GMOs	Biological diversity; human well-being

International Agreements	*Year (adoption)*	*Protection Goal*	*Legally protected good*
Strategic Plan for Biodiversity 2011–2020 & the Aichi Targets	2010	Conservation and sustainable use of biological diversity, as well as strengthening of the mainstreaming process and international cooperation	Biological diversity
Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)	1973	Provision of international trade regulations of listed species	Listed animal and plant species threatened by trade
Convention on the Conservation of Migratory Species of Wild Animals (CMS)	1979	Conservation of listed migratory species	Listed threatened migratory species (and their habitats)
Ramsar Convention on Wetlands	1971	Establishment, conservation and „wise use“ of wetlands of international importance	Wetlands of international importance
World Heritage Convention (WHC)	1972	Identification, appreciation and protection of natural and cultural heritage	Natural and cultural heritage
Man and Biosphere Program (MAB)	1970	Conservation and sustainable use of ecosystems in accordance with social and economic well-being	Biological diversity; human well-being
European Agreements			
Convention on the Conservation of European Wildlife and Natural Habitats	1979	Conservation of listed species and their habitats	Listed European wild animal and plant species (and their habitats)
Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora	1992	Conservation of listed species and habitats of community interest at a favorable conservation status	Listed European wild animal and plant species (excl. birds) and their habitats
Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds	2009	Conservation of listed bird species of community interest at a favorable conservation status	Listed European wild bird species and their habitats
Alpine Convention	1991	Protection and preservation of the Alps and its regions, as well as the sustainable use of its resources	Alpine animal and plant species, ecosystems and genetic resources; natural and cultural heritage

International Agreements	*Year (adoption)*	*Protection Goal*	*Legally protected good*
Gesetz über Naturschutz und Landschaftspflege (BNatSchG)	2009	Conservation of biological diversity, its potential of service provision and its beauty and singularity	Biological diversity and landscapes
Nationale Strategie zur Biologischen Vielfalt	2007	Conservation and sustainable use of biological diversity and fair and equitable sharing of the benefits arising out of the utilization of its components	Biological diversity

In Tab. 12, the general protection goals as indicated in the respective directives are listed. In most cases, biodiversity and human wellbeing are indicated as protected good with protection goals in some cases adapted to the scope of the agreement.

Workshops on a problem formulation exercise for gene drive mosquitos, held in different parts of the world and with a diverse set of participants frequently identified the same broad protection goals – namely, human health and biodiversity (Roberts et al., 2017; Teem et al., 2019).

Specific protection goals

In the documents of EPA (1998; 1992) the term “management goal” has been used instead of “specific protection goals” (SPG). Management goals describe the *“statements about the desired condition of ecological values of concern”*. In the EFSA document (EFSA Scientific Committee, 2016), the SPG is an *“explicit expression of the environmental components that need protection”*. They refer to assessment endpoints as the ecological entity and their attributes. In the EFSA document, SPGs are used interchangeably with “assessment endpoints”. For defining SPGs, the EFSA´s (EFSA Scientific Committee, 2016) approach is based on the concept of ecosystem services as SPGs are derived from service providing units (SPU).

Stressor

In the documents of EPA (1998) and EFSA (EFSA Scientific Committee, 2016) stressor is similarly defined as “*any physical, chemical or biological entity that can induce an adverse response in a receptor*”. In relation to the GDMO-project, the stressor is the gene drive modified organism, which has the potential to induce adverse effects in the entire ecosystem or parts of it.

Assessment endpoints

The definition of SPGs in the EFSA document 2016 is similar to the assessment endpoints in the EPA documents (EPA, 1998; 1992). Assessment endpoints lie at the core of any risk assessment and are a main result of the problem formulation phase. From broad statements of a desired condition (protection goals), assessment endpoints are specifically defined based on these protection goals and the concrete ecosystem. They are “an explicit expression of the environmental value to be protected, operationally defined as an ecological entity and its attributes.” (EPA, 1998). Such an entity can be defined at different levels; e.g., organism-, population- or community and ecosystem-level (EPA, 2003).

Measurement endpoints

For ERA, the effect of the stressor on the assessment endpoints ought to be quantified. If an assessment endpoint is readily measurable, assessment endpoint and measurement endpoint may be identical (EPA, 1998). However, in many cases a measure needs to be defined. EFSA (EFSA GMO Panel, 2013; EFSA Scientific Committee, 2016) refers to measurement endpoints

as a quantitatively measurable indicator of change in the assessment endpoint. Thereby the endpoint concerns a *"response to a potential stressor that is related to the specific protection goal"* (e.g. measurement of mortality). Within the context of population suppression, this measurement refers to the population density of the wild population(EFSA GMO Panel, 2013).

Measure of effect

In the documents of EPA (1998; 1992) the term "measure of effect" is used equivalently for measurement endpoint, which is defined as *"a change in an attribute of an assessment endpoint or its surrogate in response to a stressor to which it is exposed"*. Since EPA (EPA, 1998) argued that this definition was too narrow two additional measures were introduced: the terms "measure of ecosystem and receptor characteristics" and "measure of exposure" supplementing measure of effect. Both measures are accounting for effects that are not direct stressor – assessment endpoints effects but represent "real world" variations in space and time (including life-history characteristics). Here we use the term measurement endpoint.

b. Problem Formulation

The problem formulation phase provides the basic information for an environmental risk assessment. Therefore, a thorough problem formulation is vital for a meaningful ecological risk assessment (EPA, 1998). It is the phase where clear goals have to be formulated, meaningful endpoints are found and hypothesis about the impacts of a stressor are set. It includes a preliminary description of exposure and the resulting effects (Raybould, 2006; Wolt et al., 2010). However, this phase is an iterative process and new information can be integrated during the whole process (EPA, 1998). Problem formulation results in three products: (1) assessment endpoints that adequately reflect management goals and the ecosystem they represent, (2) conceptual models that describe key relationships between a stressor and assessment endpoint or between several stressors and assessment endpoints, and (3) an analysis plan (EPA, 1998; Raybould, 2006; Wolt et al., 2010). Only clear goals and unambiguous and measurable endpoints identified within this phase will lead to a meaningful risk assessment (EPA, 1998).

This phase includes the definition of the problem, the elaboration of a plan for analyzing and characterizing risk and the provision of information concerning *"sources, stressors, effects, ecosystem and receptor characteristics"*(EPA, 1998). Knowledge gaps should be addressed as well as the existing scientific knowledge. Further, potential exposure pathways have to be identified.

In relation to GDO´s knowledge gaps have been categorized in eight thematic areas (National Academies of Sciences, 2016):

- **Life history and fecundity data (age-specific and sex-specific)**
- **Reference genome**
- **Gene flow**
- **Density dependent reproduction and mate selection**
- **Border biosecurity pathways**
- **Community interactions**
- **Invasiveness of a species**
- **Fertility control**

The problem formulation scheme of EFSA (EFSA GMO Panel, 2013) is overall in line with the outlined steps of EPA. Information about the stressor, the sources of the stressor, the effects to the receiving environment, exposure pathways and hazards must be gathered.

With regard to GMOs EFSA (EFSA GMO Panel, 2013) recommends in the problem formulation phase:

- **to identify the characteristics of the GM animal, that can cause adverse effects to the environment, to animal and human health**
- **to identify relevant aspects of the receiving environment that needs to be protected, according to protection goals outlined in legislations.**
- **to define the intended uses of the GM animal, because this are in relation to the exposure pathways (Exposure pathways describe how the stressors are getting in contact with the environment, including unintentional release)**
- **to identify the adverse effects (Adverse ecological effects alter important structural or functional characteristics or components of ecosystems and can be evaluated in form of the type, intensity, scale of the effect and potential for recovery (EPA, 1998).**

If adverse effects are identified, EFSA (EFSA GMO Panel, 2013) suggests amongst others defining measurement endpoints for hazard and exposure, to set limits of concern for each assessment endpoint or to consider uncertainties as knowledge gaps or methodological limitations.

EPA (1998) recommends several questions, which help with problem formulation, specifically with the identification of assessment endpoints. These questions concern source and stressor characteristics, the exposure characteristics, the ecosystems potentially at risk and ecological effects. These questions can be adapted for the context of GDOs and thereby provide a general starting point for the problem formulation. In the appendix we present a selection of relevant questions.

The problem formulation had been extended to be able to include additional factors as the "Problem Formulation and Options Assessment (PFOA)" (Hilbeck et al., 2020). PFOA is a framework which identifies key social needs, in the center are the people and their needs. Furthermore, precaution is addressed as fundamental guiding principle. Alternative models for problem formulation – such as the PFOA approach – suggest assessing choices between alternative technological options. The different concepts of problem formulation promise a higher number of options to integrate the new developments, like GDO in an assessment framework.

c. Specific Protection Goals in Analogy to EFSA 2016

"Living in harmony with nature" is a main goal first formulated at the Convention on Biological Diversity in Rio de Janeiro in June 1992, which came into effect as from December 1993. Many legislations and conventions outlined "biodiversity" and "human well-being" as general protection goals. Because "biodiversity" is to general for policy makers, in the document of 2016 (EFSA Scientific Committee, 2016) the European Food Safety Authority worked out a concept to specify these goals.

In that document the term "specific protection goal" (SPG) is used synonymous to "assessment endpoint" and is an essential part of the environmental risk assessment and a result of the problem formulation phase. The specific protection goals are derived by the ecosystem service concept. The identification of the relevant ecosystem service is the first step. This is followed by identifying the service providing unit (SPU), which delivers the selected ecosystem service: Then the level of protection can be based on five interrelated dimensions: (1) the ecological entity (e.g. individual birds, populations of earthworms), (2) the attribute or characteristics of the entity (e.g. behaviour, survival, reproduction/growth, population density, processes,

biodiversity), (3) the magnitude of effect, (4) the temporal scale of effect (e.g. duration of the effect, frequency of effects, interval between effects), (5) the spatial scale of effect.

EFSA expanded the classification of the ecological entity to: *Individual –(meta)population – functional group – community – ecosystem – habitat* (see Luck et al., 2009). Then, every ecological entity can be linked with the attribute of that protected entity (Tab. 13). Options for the attributes are: *behaviour, survival, growth, reproduction, abundance, biomass, process, within- and between-species diversity, landscape or habitat structure.*

Tab. 13: Linkages between ecological entity and its attributes according to EFSA (EFSA Scientific Committee, 2016)

Ecological entity	Attribute
individual	behaviour, survival, growth, reproduction
(meta)population	abundance, biomass, population growth
community	within- and between-species diversity, biomass
functional group	process (primary production, decomposition, nutrient cycling), abundance, biomass
ecosystem	process, within and between species diversity
habitat	landscape or habitat structure

The next step is to elaborate the magnitude of biologically relevant effects that can be tolerated for the attributes to be measured (Tab. 14). The options to describe these effects are: *negligible, small, medium, large.*

Tab. 14: Classification of biologically relevant effects, from EFSA (EFSA GMO Panel, 2013)

The classifications are extracted from the Commission Decision 2002/623/EC (European Commission, 2002)	
High-level consequences	might be significant changes in the numbers of one or more species of other organisms, including endangered and beneficial species in the short or long term. Such changes might include a reduction in or complete eradication of a species leading to a negative effect on the functioning of the ecosystem and/or other connected ecosystems. Such changes would probably not be readily reversible and any recovery of the ecosystem that did take place would probably be slow
Moderate consequences	might be significant changes in population densities of other organisms, but not a change which could result in the total eradication of a species or any significant effect on endangered or beneficial species. Transient and substantial changes in populations might be included if likely to be reversible. There could be long-term effects, provided there are no serious negative effects on the functioning of the ecosystem
Low-level consequences	might be non-significant changes in population densities of other organisms, which do not result in the total eradication of any population or species of other organisms and have no negative effects on functioning of the ecosystem. The only organisms that might be affected would be non-endangered, non-beneficial species in the short or long-term
Negligible consequences	would mean that no significant changes had been caused in any of the populations in the environment or in any ecosystems

Some considerations, to justify, for example, the selection of the magnitude of biologically relevant effects are (EFSA Scientific Committee, 2016):

- **Ecological properties of the SPU**
- **Ecological and structural properties of the receiving environment**
- **Level of endangerment**
- **Legal and pragmatic considerations**

Ecological properties considered for the SPU are for example: “the duration of the life cycle, the growth and reproduction rate, individual home range, habitat or food preference, mobility and dispersal ability and the potential for ecological recovery.” (EFSA Scientific Committee, 2016).

EFSA also mentions the need to define the spatial and temporal scale of the biologically relevant effect, or the effect, that can be tolerated. These points help to formulate the specific protection goal.

An SPG may be formulated as follows (EFSA Scientific Committee, 2016): “…not more than 1 % reduction in abundance of adults of any non-target species over the temporal scale of a single year at the spatial scale of a region.”

d. Limitations of Current Approaches when Assessing Gene Drives Ecosystem Services for Risk Assessment

As described above, we consulted (EFSA Scientific Committee, 2016) to determine specific protection goals. This document focuses on the ecosystem service concept as a base to

formulate specific protection goals. EFSA specifies hereby three steps based on the identification of relevant ecosystem services, service providing units (SPUs) are derived and the level of protection of these SPUs is defined. The level of protection is guided by five interrelated dimensions, the ecological entity, the attribute of characteristics, the magnitude of effect, the spatial scale of effect, and the temporal scale of effect.

Within the EFSA approach the use of the ecosystem service concept could be traced back to the year 2010 (EFSA GMO Panel, 2010). At this point in time a distinction between ecological functions and ecosystem services is introduced for emphasizing the direct benefits to humans. However, the two concepts still coexist in some of the current EFSA schemes (e.g. (EFSA GMO Panel, 2013). Nevertheless, the ecosystem service concept has gained much more momentum over the past years, especially as the fundament of the definition of specific protection goals (e.g. EFSA Scientific Committee, 2016) and outlines somehow the basic logic behind the current risk assessment approach.

In contrast, previous concepts (e.g. EPA, 1998) have built their risk assessment approaches mainly on the concept of ecosystem functions. Although the functional endpoints are already theoretically connected to the provision of “services to humans or other ecological entities” (EPA, 2003), the ecosystem services approach is not the basic concept.

The use of ecosystem services to define protection goals has some implications for risk assessment for GDOs. On one hand, there are general problems with the approach, for example the estimate of functional redundancy (Silvertown, 2015) or how to include biodiversity in this concept. On the other hand, they might be less readily implemented when the impact of GDOs is estimated after description of cascading effects. When specific goals have to be expressed in terms of ecological cascades, they are less well defined than goals, which can be associated with clearly measurable quantitative values. Since ecosystem service as a goal is similarly difficult to define, this might enhance the uncertainty. Additionally, the use of ecosystem services to formulate goals might lead to declaring the impact on a species as acceptable, if the allocation to a service is not obvious. This might result in definition of impact as negligible even though it might have long-term effects.

We also want to discuss the ecosystem service concept for elaborating specific protection goals, in principle. On one hand, the results of this concept are easier to use for policy maker, but on the other, this concept can be misused to accept the eradication of species, because a special service can be redundant, or the service can be delivered also by another species. In a time of excessive species extinction (Hallmann et al., 2017), the argument that another species can also deliver a special service appears problematic.

e. Ecosystem Services as General and Specific Protection Goals

The specific use of ecosystem service approaches to define goals in risk assessment had been developed under the assumption that the result is analogous to the use of conventional arguments, centering on biodiversity or ecosystem function, but is better and more convincingly to argue. Therefore, the approach resembles foremost a communication strategy and not a tool to facilitate the assessment itself. The analogy to conventional approaches, in particular biodiversity, is hereby generally treated as self- evident. That when ecosystem services are secured also biodiversity is retained has its roots in the origin of the Ecosystem service concept because this formed the base for its formulation by Ehrlich and Ehrlich (1981). In the beginning, it was used to illustrate the need to protect biodiversity. Ergo per definition when all biodiversity is protected than all Ecosystem services are. It is implied that this also works the other way around. However, scientific treatment of this question is rare. We performed a literature review to determine which scientific findings relevant for this question exist and what they are supporting. The outcome of this search is described here.

There is a wealth of literature concerning ecosystem services and their application. However, few studies address the issue of biodiversity or Ecosystem service arguments (sensu Deliège and Neuteleers, 2015). In Nienstedt et al. (2011) and the statements therein, a summary of risk assessment steps is given and related to the use of ecosystem services for assessment. It seems to form the published, peer reviewed, scientific justification the approach in EFSA Scientific Committee, 2016 is based on. The argument is twofold: first, using ecosystem services rather than biodiversity or ecosystem function appears to reflect better the goal to preserve human wellbeing. The service is necessary for human wellbeing, so using services is more relevant for humans than using one of the other measures. Second, when ecosystem services are preserved, all biodiversity is preserved, too. This means that for practical reasons, results are the same regardless of biodiversity or ecosystem services are being used as the argument. For this it is necessary that the variables measured for assessment are the same in both cases; for example, population size of a species. However, when defining service providing units for formulation of specific goals this might be not the case.

The supporting literature cited in Nienstedt et al. (2011), to verify this approach is limited. Essentially only Goldman et al. (2008) is cited as support for equivalence of biodiversity and ecosystem service arguments.

f. Scientific Base for Ecosystem Service versus Biodiversity Arguments

The team around Goldman *et al*. published two papers in this context (Goldman et al., 2008; Goldman and Tallis, 2009). These studies use the same dataset and had been undertaken to define goals in the context of project-based conservation, but not as support for environmental protection. The data presented by Goldman *et al.* are comparing a rather high number of conservation projects performed by NGOs that are formulating specific goals within the project. The dataset is based on two groups, projects with biodiversity background and projects with an ecosystem services background. After interviews it had been found that regardless of how the goal is formulated a-priori, conservation of biodiversity is similarly expressed. Therefore, it supports the use of an approach that brings other advantages, in this case better stakeholder involvement. The publication is in the line with arguments led by authors affiliated to the nature conservancy to include human wellbeing into conservation concepts; however, all these studies are in the context of project related conservation and have the increase of acceptance by the human population as a goal. The conception (new conservation, conservation science, (Kareiva and Marvier, 2012; Mace, 2014) has therefore a characteristic of a communication strategy that is used to communicate goals. There are a number of critical replies to this approach (Miller et al., 2014). Problems include the difficulty to circumscribe goals, the potential conflict between goals, and the slow but foreseeable replacement of conservation goals with anthropocentric and ultimately economic ones.

The data sets shown in the papers of Goldman et al. (Goldman et al., 2008; Goldman and Tallis, 2009) have illustrative qualities in this context but do not constitute proof. The biggest problem that we see so far is the difficulty to define the categories biodiversity project versus ecosystem service project. The categories seem not to be completely independent. Biodiversity projects as defined by the authors have species-related goals, while ecosystem service projects are defined as ones that have several goals, including, among others, also species-related goals. Problematic is here that according to these definitions, ecosystem service projects also include biodiversity goals. The goals are therefore not clearly separated; therefore, the two categories cannot be clearly separated also. Conservation projects that do not aim for conservation of biodiversity do not exist.

Regardless, Goldman et al. do not conclude that biodiversity will be prevented from declining with a purely anthropocentric interpretation of risk. They only conclude that in conservation practice there is no difference of outcomes regardless if management is based on

anthropocentric arguments or on classical conservation ethics. In this respect, the slight circularity in the argument might become excusable. The criterion for an ecosystem service project is a higher number of goals than a biodiversity project. Therefore, they should also have a higher number of different stakeholders and their positions. Ergo the notion that an ecosystem service project attracts more stakeholder interest and funding is circular because these interests had already been used defining the group. This illustrates the aim of these publications not as scientific argument but as contribution to a discussion about communication of conservation goals. The use of this citation as proof for equal quality of biodiversity or ecosystem service arguments as protection goal to assess risk of an adverse impact seems therefore misleading.

g. Definition of Harm

Related to the point outlined above, is the definition of harm. Harm (sensu Carstens et al., 2012) describes the adverse effects the prevention of which is addressed as SPG. It concerns the question how the consequences of a biological effect (according to Tab. 14) are rated, or, more specifically, what level of an effect is regarded as harmful or negligible, respectively. Under the ecosystem service argument, harm would be defined as reduction in services measured as impact on service providing units. With gene drive and the respective differences to conventional systems, this impact might be especially difficult to assess. However, also the current application in risk assessment should be revisited. In particular, the magnitude of an impact on, for example, non-target arthropods of plant protection products or GMOs designed for insecticide activity, is difficult to define.

The difficulty stems from the definition of harm as deviation from a certain baseline. Even if quantitative values like individual numbers of a species are used, it is difficult to determine the changes empirically. Individual numbers are fluctuating from year to year and these fluctuations have to be disentangled from the response to a stressor. This might not be possible; so, all data that are collected can only describe a component or a simplified version of the system. If ecosystem services are used as a goal an additional level of interpretation is introduced in the system. It might be difficult to estimate if a change affects the provision of a service adversely or not.

A second difficulty in determining change, is the problem that the absence of significance of an effect is not proof that there is no effect. There is an ongoing discussion mainly in the context of human medicine that has also implications in the context of risk assessment. In a typical experimental setup for testing the effect of a product, one group of organisms with exposure are compared to a control group without exposure. The “dichotomous” interpretation of results, significant difference means “effect” while no significance means “no effect” is not exactly accurate. While significance supports the existence of an effect, a non-significant result means that no effect could be measured, but not that it is absent. This is a difference. This can also be expressed as a bias in science to avoid false positives (Type I error), which may lead to create false negatives (Type II error) (EEA, 2001). In many circumstances, this might be the better (conservative) choice, but this is clearly not the case, when testing adverse effects of a stressor.

There is a twofold argument how current practice might be insufficient when applied to assessment of an emerging technique. First, the lack of impact is very difficult to test statistically, because the lack of a significant difference does not necessarily mean a lack of effect. Second, when fluctuations for example of population sizes exist, an effect within the range of the fluctuation does not mean necessarily that the effect is negligible. A stressor might cause an effect that overlays the effects causing the original fluctuations. Even though these arguments are an expression of the simplification in experimental systems, which cannot be overcome, they should be taken into consideration.

That risk assessment not necessarily is aware of such possibilities shows the following example we came across during our literature review: In the study of Carstens et al. (2012) that looks at risk assessment and the necessary experiments in the context of GMOs, thresholds for mortality for non-target arthropods are presented that are completely counter intuitive. The study states that “For example, under the US EPA framework, a threshold of 50% mortality or a 50% effect on growth or reproduction has been accepted for early tier studies, because effects that do not surpass this threshold would be unlikely to cause significant population level effects under realistic environmental conditions” (Rose, 2007). This statement could be interpreted that this mortality rate means not necessarily a significant effect on the long-term or next-year population size. This seems quite unlikely, because even if mortality for most insect species were very high, the reduction of population size by the toxic effect would constitute a factor in addition to all other factors causing natural mortality still in effect. The reference supporting this is a white paper, summarizing the approach of different tiers to test insecticide activities of GMOs (Rose, 2007). The different tiers constitute different subsequent experimental set ups that refine results in a predicable way. When in tier one, for example a laboratory experiment of toxicity, no effect can be measured, a higher tier experiment can be omitted. If toxicity exists, a higher tier community experiment can proof, for example, if this result is relevant for field conditions. In Rose (2007), we did not find presented data that would justify the extensive conclusion of Carstens et al. (2012). Exposure to the insecticide proteins is described, with the notion that a 50% mortality at MHD, the maximum hazardous dose, at tier one, is an indication that a tier two test with more realistic conditions is necessary (Rose, 2007). This necessity does not result from the lack of effect, like indicated by Carstens et al. (2012) but from the experimental design of the different tiers providing different information at each tier. The interpretation as biological characteristics, as a result derived from observation is not justified. It supports a narrative of high resilience of populations.

The resilience narrative is reflected by the definition of harm as in “not measurable” or “negligible” effect. The high fluctuation that insect populations can show, makes it difficult to estimate any impact. Therefore, also effects that seem to be insignificant, because they do not differ from natural fluctuations, might add up to a visible effect on the long run. In Fig. 54, we include a stochastic simulation that shows a population that changes between 0 and 5% each year, resulting in a more or less stable population that fluctuates around a long-term average. If we add towards this stochastic factor another factor, e.g. of between 0 and 1% and estimate that as potential negative impact, then over a long term the population is likely to shrink. This is also true, if the effect is much smaller than the standing fluctuation. The relevant characteristic is that the additional factor is between 0 (no effect) and a value that is low (negligible) but always negative (Fig. 55). Even though very simplified, this simulation illustrates a very well understood mechanism: even if effects are small, when they are only in one direction this will add up to a significant effect in the long term. It treats the changes in population size as stochastic effect and describes the population size in the sense of a dynamic fluctuation. In such a model the second factor modifies the dynamics, in this case as directing it to a negative trajectory. Similarly, the additive effect of several factors can lead to accumulation of small or negligible effects to a visible impact (Van Den Brink et al., 2016).

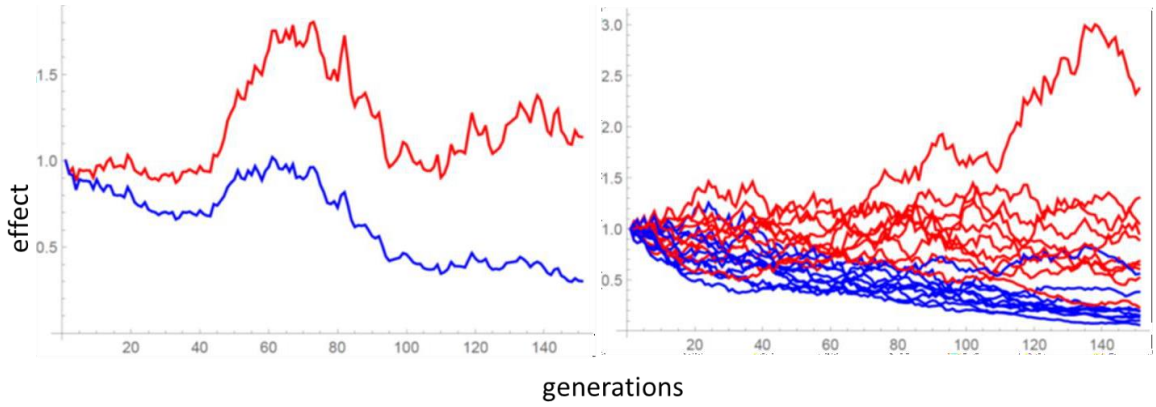


Fig. 54: Stochastic simulation of population development illustrating the additive effect of a small adverse effect and a regular fluctuation.
Shown is a positive or negative change between two generations of 0 to 5% (red line) and the same values overlaid by an adverse effect between 0 and 1% (blue line), left as single iteration and right as 10 iterations. (Analysis from Klaus Scheicher, Institute for Mathematics, Boku).

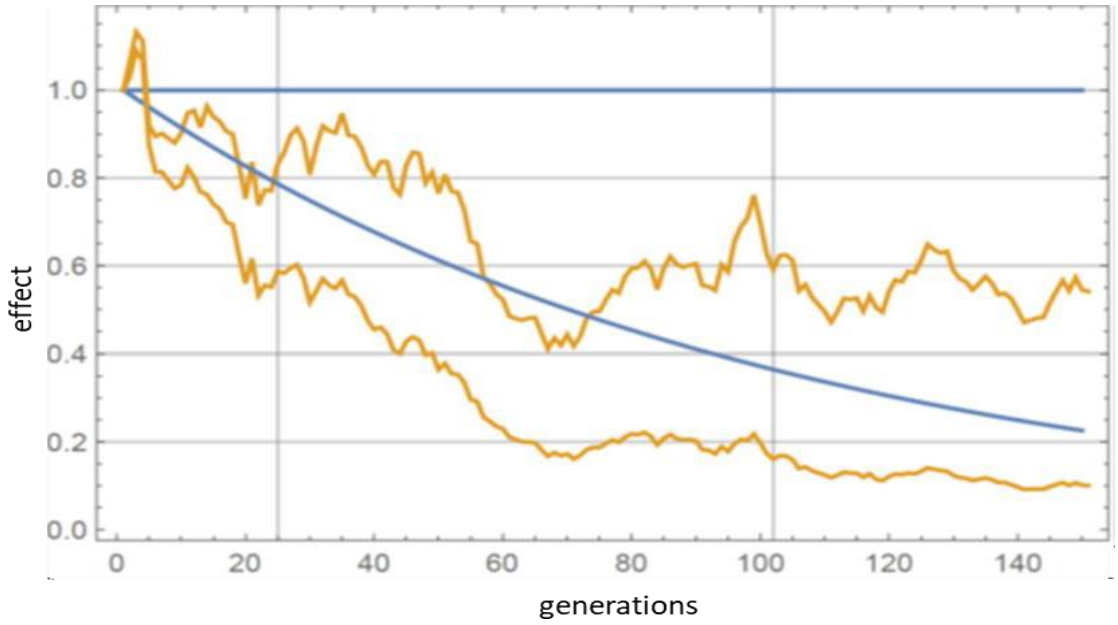


Fig. 55: Stochastic simulation of population change between two generations of 0 to 5% analogous to Fig. **54**.
The iteration average as expectation value, straight line at y=1 the 5% fluctuation and the declining graph for the additive adverse effect. Expectedly, the adverse effect will change the trajectory of the graph towards a decline. This results from the assumption in the simulation that the adverse effect is always between 0 and slightly negative, similar to a negligible effect that is between no effect and slightly negative. Analysis from Klaus Scheicher, Institute for Mathematics, Boku.

h. Precautionary Principle

Unlike genetically modified organisms (GMOs), gene drive organisms (GDOs) represent a technological tipping point and pose a new dimension on risk assessment, because they are intended to spread (please see also the chapter “The suitability of the Environmental risk assessment paradigm for GDOs” below). They intend to bring a permanent change to a population and therefore to the entire ecosystem. Although these might happen rarely, GDOs will likely spread unintentionally through space, across species and across barriers. Because many of this is uninvestigated, a thorough uncertainty analysis will be mandatory.

Within the crucial step of problem formulation, all the questions that merit risk assessment have to be asked. It includes the identification of potential adverse effects (hazards) and needs to identify all possible exposure pathways including unintended ones. Included in the problem formulation is also the identification of measurable assessment endpoints, i.e., specific protection goals, which can be transferred into measurement endpoints. A clear description of how these surrogate measures are related to the protection goals is required. Because of the nature of GDOs it will be difficult to derive such measurement endpoints. The impact on ecosystems must consider cascade effects and a modeling approach appears, therefore, to be necessary. However, ecological modelling is also very much limited by the availability of data and knowledge about the ecosystem.

After identifying potential hazards as first step, in a second step the magnitude of the hazards is specified. In a third step the likelihood of the exposure should be estimated including unintended exposure. Within these last two steps may lie the biggest problem for ERA for GDOs, since either likelihood of the occurrence of the adverse effect, or magnitude and likelihood of occurrence of the adverse effect, are very likely unknown. If this is the case, we are clearly entering uncertainty analysis (EEA, 2001).

The authors of the report for the European Environmental Agency (EEA, 2001) about the precautionary principle stress that it is important to distinguish between risk, uncertainty and ignorance on one side and prevention and precaution on the other side. They define ignorance as the state, when both impact and probability of occurrence is not known, uncertainty, when the impact is known but not the probability of occurrence; risk is the only category when knowledge about both impact and probability of occurrence exist (EEA, 2001).

The EEA (2001) concludes with 12 “late lessons from early warnings”. In the problem formulation we will therefore keep in mind the 12 lessons and examine their relevance to the application of the GD technology.

12 Lessons (EEA, 2001)

1. **Acknowledge and respond to ignorance, as well as uncertainty and risk, in technology appraisal and public policymaking.**
2. **Provide adequate long-term environmental and health monitoring and research into early warnings.**
3. **Identify and work to reduce ‘blind spots’ and gaps in scientific knowledge.**
4. **Identify and reduce interdisciplinary obstacles to learning.**
5. **Ensure that real world conditions are adequately accounted for in regulatory appraisal.**
6. **Systematically scrutinize the claimed justifications and benefits alongside the potential risks.**
7. **Evaluate a range of alternative options for meeting needs alongside the option under appraisal, and promote more robust, diverse and adaptable technologies so as to minimize the costs of surprises and maximize the benefits of innovation.**
8. **Ensure use of ‘lay’ and local knowledge, as well as relevant specialist expertise in the appraisal.**
9. **Take full account of the assumptions and values of different social groups.**
10. **Maintain the regulatory independence of interested parties while retaining an inclusive approach to information and opinion gathering.**
11. **Identify and reduce institutional obstacles to learning and action.**
12. **Avoid ‘paralysis by analysis’ by acting to reduce potential harm when there are reasonable grounds for concern.**

i. European Rabbit as Example for Unnatural Escapes

The introduction of European rabbits to Australia and later attempts of biological control serve as a famous and well-suited analogy to the application of GDO as biological control of invasive species. A number of biological control measures had been performed for the rabbit that have an effect on population size similar to what can be expected from a gene drive. The release of a virus causing myxomatosis, a presumably population size controlling disease for the species, caused a temporary decrease of numbers, later resistance and recovery, and was one of several similar attempts. The specificity and the impact on population size, constitute an analogy to gene drive applications. A huge amount of literature exists also about the discussion of negative effects, which can serve as example for the expected discussion about gene drive. Although there are clear differences between viral diseases and GDO applications, lessons can be learned among other things regarding the field trials and subsequent problems with quarantine as well as with the danger of illegal spread.

In 1859 Thomas Austin introduced the first wild European rabbits into Australia for hunting (Cooke, 2014). The rabbits spread rapidly and by 1950, there were 500 million to a billion rabbits in Australia present despite rabbit control using fences, hunting, trapping, fumigation and habitat destruction (Kerr, 2008). The rabbits had negative impacts on agriculture and landscape; they damage pastures and crops and compete with native animals for food and habitat (Kerr, 2008).

The viral disease, myxomatosis is a well-known example of application of biological control to an invasive species. Field trials started in 1930s and after world war II the virus escaped. The virus was lethal at first but after a decade, the virus developed into less virulent strains and rabbits had developed genetic resistance.

Another viral disease (RHD – rabbit hemorrhagic disease) was also considered as biological control in Australia. Field trials were carried out at Wardang Island and the virus escaped to the mainland in 1995 through flies. As a result, many rabbits were killed at first. The spread occurred through aerosol (locally) and through flies (over large distances). After the fact, the application was approved for Australia; RHD virus was declared “agent” and rabbit “target organism”. In New Zealand legal approval was not granted but the virus was brought to New Zealand illegally. Farmers used food blenders to spread infected tissue to other areas in New Zealand (Cooke, 2014). However, the virus is changing, and young rabbits are immune, when they are infected they develop antibodies.

The example of the rabbits in Australia and New Zealand shows, that quarantine was not working, and, in both cases, unintended escape occurred because of vectors that had not been anticipated. Illegal spread was real and immanent. The virus worked well at the beginning, but resistance developed, and additional measures are necessary to keep rabbit populations in check. The discussion on biological control of European rabbits in Australia and New Zealand has many more details that may be interesting as analogy to GDO release.

j. Current ERA is Developed for Ecotoxicological Stressors

In the first part of the report basic principles of an ecological risk assessment were examined. Following an extensive literature review, we identified EPA (Environmental Protection Agency) guidelines and EFSA (European food safety Authority) documents as standards for traditional ecological risk assessment. Ecological risk assessments consider human induced changes to the environment and try to evaluate the likelihood of ecological adverse effects resulting of the exposure to one or more stressors. We pointed out the pitfalls of this approach even for ecotoxicological stressors in the chapter about “definition of harm” above. With fluctuating population from year to year, the response to the stressor may be unclear. Small negative effects on a fluctuating population will eventually lead to an overall decline. Similarly, the

additive effect of several small effects can sum up to a visible impact (Van Den Brink et al., 2016). However, these quantitative risk assessment approaches with measurable endpoints and the need to comparison with a baseline may be most useful with ecotoxicological stressors. These quantitative approaches may not be readily applicable to biological stressors though (Andersen et al., 2004).

Although risk assessments for GMOs (EFSA GMO Panel, 2013) stress that potential invasive spread of GMOs must be addressed, they follow in principal the ecotoxicological approach. During problem formulation the factors that influence persistence and invasiveness of a GMO shall be considered, e.g. life history of the organism, characteristics of the receiving environment, and the potential rate of introduction(EFSA GMO Panel, 2013). However, the invasion is treated as if it could be prevented, which might be feasible with GMOs, but appears to be unlikely when GDOs are concerned.

k. The Difficulty of Defining Protection Goals in GDO Risk Assessment Processes

In the “Framework for ecological risk assessment” (EPA, 1992) and the “The Guidelines for ecological risk assessment” (EPA, 1998) the U.S. Environmental Protection Agency use the term “management goal” or “assessment endpoints”. Basic characteristics to derive assessment endpoints are ecological relevance, susceptibility and the relevance to management goals (EPA, 1998). This terms were later summarized as “protection goal” first used by EFSA in an environmental risk assessment in the (EFSA GMO Panel, 2010; EFSA Scientific Committee, 2016). They differentiated between general and specific protection goals and pointed out, that *“General protection goals are stated in European legislation but specific protection goals (SPGs) are not precisely defined.”* (EFSA Panel on Plant Protection Products and their Residues, 2010).

General protection goals can be derived e.g. from the definition of environment of the EU Regulation (EC) No 1107/2009 (European Union, 2009), which includes: *“waters (including ground, surface, transitional, coastal and marine), sediment, soil, air, land, wild species of fauna and flora, and any interrelationship between them, and any relationship with other living organisms.”* (EFSA Panel on Plant Protection Products and their Residues, 2010). According to the CBD and other legislatives, biodiversity and human health are global protection goals valid all over the world. Also, in legislations of the European Union environmental protection goals are conservation of biodiversity and ecological functions. Biodiversity is defined in article 3 (29) as *“variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this variability may include diversity within species, between species and of ecosystems;”* (EFSA Panel on Plant Protection Products and their Residues, 2010). It was summarized that general protection goals includes all terrestrial and aquatic ecosystems *“including their relationships with other living organisms.”*

This broad formulation cannot be implemented by risk assessors and policy makers; nevertheless, general protection goals build the basic of the derivation of specific protection goals. EFSA stated that a relevant part of an environmental risk assessment is to define specific protection goals, risk assessors should know what to protect, where to protect it and over what time period (EFSA Panel on Plant Protection Products and their Residues, 2010). Due to the ecosystem service concept, in the guidance of EFSA GMO Panel (2013) specific protection goals are now related to ecosystem services and focus on natural resources (e.g. arthropod natural enemies, bees) or natural resource services (e.g. regulation of arthropod pest populations, pollination) as set out by EU legislations(EFSA GMO Panel, 2013).

Further recommendations of EPA and EFSA to find assessment endpoints or to define specific protection goals are to work out relevant ecological entities and their attributes. The idea of

this concept is the comparative approach and therefore the definition of harm (useful for ecotoxicological risk assessments). But the comparative safety assessment for risk assessment in the EFSA guidance is a re-introduction of the “substantial equivalence”, which means that familiar foods that have long been known to be safe are used as a benchmark for the safety assessment of novel foods. EFSA also suggested the ecosystem service concept to work out specific protection goals. We have already elaborated on the arguments why we consider these approaches problematic.

Because of the ecological relevance of protection goals, it seems to be useful to conduct an ethical discussion about nature. In the debate of environmental ethics, there is a distinction between intrinsic and instrumental value of nature. The instrumental value belongs to the usefulness of nature to humans, the intrinsic value of nature is independent of human needs (Wickson, 2014). Wickson (2014) argues, that environmental risk assessments are inherently entangled with ethical discussions. She argues that environmental risk assessment depends on what we value in the environment and this depends on our socio-cultural relations with the land or the environment. She further concludes that Europe has many different ecosystems and therefor there would be many different values or socio-ecological aims. It can also be argued that social and ethical discussions are important for the decision-making in the context of use of gene drive (Roberts et al., 2017).

In relation to GDOs biodiversity will be always a pertinent broad protection goal, pertinent according to (Roberts et al., 2017). In their “Perspective Piece” of a workshop to conduct a problem formulation for the use of gene drive mosquitoes, a result of problem formulation was to define pertinent environmental/ecological protection goals. Scientific participants discussed broad areas of environmental protection to work out pertinent and non-pertinent broad protection goals. By this distinction pertinent protection goals could be scrutinized more precise.

Pertinent broad protection goals in relation to malaria vector mosquitos were identified: human health, biodiversity, animal health (i.e., livestock), water quality. Non-pertinent broad protection goals are soil quality, air quality, natural resources (other than biodiversity), agricultural protection (excluding animal health). Biodiversity as protection goal in risk assessment is complex and it´s important to identify “*what aspects of biodiversity are considered valuable, and what changes in biodiversity are considered to be harmful or undesirable.“* (Roberts et al., 2017).

Like illustrated in Noss (1990) biodiversity can be differentiated in several aspects like structure, composition and function. Additionally, each aspect is divided in levels on which biodiversity can be assessed, for example the level of genes, populations, habitats and landscapes. Because GDOs can change diversity at each of these levels, these general protection goals form the basis for scrutinizing more specific protection goals.

Wickson (2014) argued, that there should be a linguistic development away from environmental protection goals towards socio-ecological promotion aims. The term “protection goal” implies that human activities have negative consequences for the environment, which has to be protected. Wickson (2014) stated that socio-ecological promotion aim is the better term because it allows to imagine a healthy environment. An alternative to protection goals could be the definition of “sustainable development goals” which also integrate social, economic and biological dimensions. SDGs can focus on national and regional levels and they can be developed with broadly participatory approaches. According to Wickson the key question would be what kind of relationship we want to build with the life on earth and which kind of technologies we want to accept or tolerate.

Based on our literature review, we see many difficulties for defining specific protection goals for ERA in general and even more so for ERA of GDOs. For nature conservation, maintenance of biodiversity is the most important general protection goal. We could show that the ecosystem service concept is rather a communication strategy for general protection goals but does not

work as concept for defining specific protection goals. Although most people agree that biodiversity is the base for ecosystem services and therefore our well-being, the link between a concrete ecosystem service and biodi-versity is not always straight forward. The contribution of a species to an ecosystem ser-vice may be simply unknown or ecological redundancy may lead to the assessment that extirpation of species A can be accepted because species B does also provide the ser-vice. Because the complete role of a given species in an ecosystem remains unknown the effect of eliminating or drastically reducing a species from an ecosystem may have unknown indirect effects and is therefore from an ecological viewpoint problematic. This is even more true from the perspective of nature conservation, if an intrinsic value is considered for every living organism. Consequently, from the point of view of nature conservation, the use of ecosystem services and service providing units for defining specific protection goals is not a viable option. We postulate that even though effects on specific ecosystem services are relevant they cannot serve as measurement endpoints if biodiversity is a general protection goal.

To avoid these issues with ecosystem services, a logical step is going back to all compo-nents of biodiversity as specific protection goals. In many cases measurable endpoints will therefore be a change in population size of a given species. However, we illustrated the potential problem that negative population trends may be masked by natural fluctua-tions (see Fig. 54 and Fig. 55) and elaborated on the problems to assess changes in population sizes correctly, because of difficulties to separate natural fluctuation from negative effects of the stressor.

For finding specific protection goals and pathways to harm, knowledge about the ecosys-tems of the target area is very important. However, GDOs resemble in many ways inva-sive species adding more challenges for defining specific protection goals and pathways to harm within and outside the target area, which we will explain in more detail below.

6.1.2 **Similarities Between Invasive Species and Gene Drive Modified Organisms**

The procedures of traditional ecological risk assessment (ERA) dealing with ecotoxicological stressors is a first step towards an ecological risk assessment for gene drive organisms. These provide very good examples of well-structured and organized guidelines for assessing the probabilities of impacts of single stressors on quantitatively measurable endpoints.

By overriding Mendelian inheritance, gene drive organisms are designed to spread and possibly persist in the environment even when coming from low frequencies in the population (National Academies of Sciences, 2016). The intentional or unintentional spread of invasive species illustrates that local containment of GDOs in a globalized world may be unrealistic. In addition, experience from failed containment of biological control (e.g., Rabbit Haemorrhagic Disease was brought to New Zealand by farmers) shows that GDOs will likely be deliberately brought into other regions. GDOs have much in common with invasive species, both, in terms of spread and how they change ecosystems (Esvelt and Gemmell, 2017). Therefore, GDOs have aspects of different concepts for risk assessment, related to their effect on populations and risk of spread.

Like invasive species GDOs may alter biological interactions within an ecosystem leading to cascade effects within and outside the ecosystem they were originally released in. For example, known effects of eradication of predators include mesopredator release, herbivore release, disruption of predator social systems, and compensatory immigration (Doherty and Ritchie, 2017; Caut et al., 2007).

These different aspects of GDO are difficult to implement within one conceptual framework. For the continuation of the project we are considering three aspects of risk of GDOs:

Risk field 1) the effect of population declines on ecosystem and ecosystem services. This includes effect on species interacting with the target species, other cascading ecological effects, and not desired effects related to population size development of the target species.

Risk field 2) the risk of escape of the GDO into other geographical regions, i.e. overcoming geographical barriers. This is mainly relevant for applications were gene drive should be restricted to parts of a global range of species.

Risk field 3) the risk of transfer of the gene drive to non-target populations or other species by hybridization independent from geography.

The division in these three aspects of risk is a preliminary overview for which analogies to current risk assessment schemes will be outlined. By the subdivision we are able to identify analogies between different aspects of gene drive and different risk assessment schemes creating a more detailed picture than with ecotoxicological based risk assessment applied to GMOs. Practically this means that we can link the current practice of GMO risk assessment and risk assessment for invasive species within one framework. We link with these EFSA approaches on GMO risk assessment (EFSA GMO Panel, 2013) and pest risk assessment (EFSA PLH Panel, 2010).

Because synthetic GDOs are notwithstanding genetically modified, risk assessment for GMOs is the obvious choice when we look for analogies to already established assessment concepts. However, like we outlined above, many GMO applications relevant for risk assessments had been done in the context of modifications that had toxicological relevant persistence mechanisms against insects. Because of similarities of GDOs with invasive species, frameworks for risk assessments of invasive species appear appropriate to serve as guidelines for ecological risk assessments for GDOs (National Academies of Sciences, 2016). Therefore, we review such approaches, identify the generic steps within these frameworks and describe the analogies between invasive species and GDOs in relation to these generic steps.

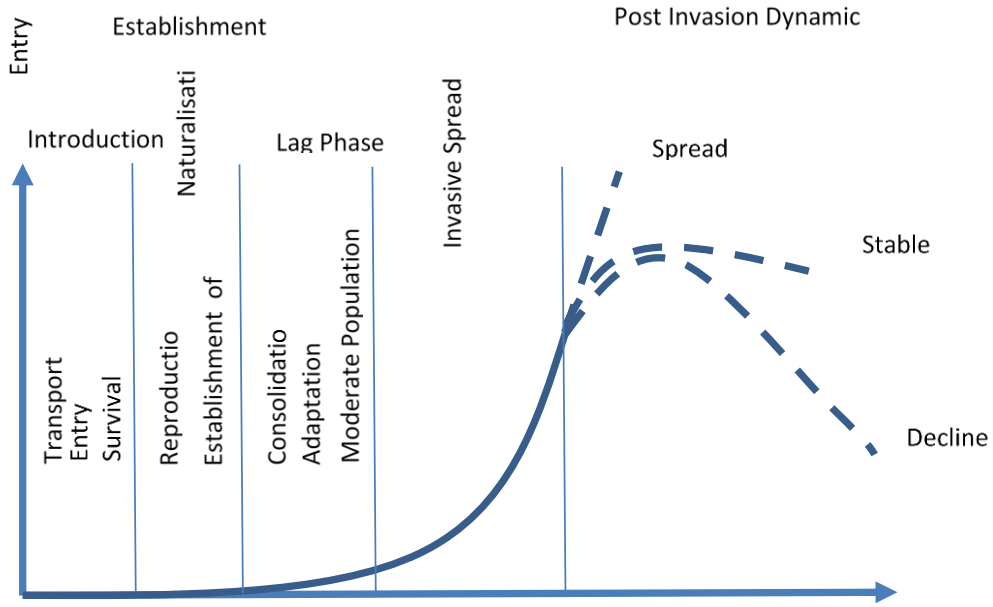


Fig. 56: Population size development (schematic) of an invasive population.
The invasion is defined by several phases upper row (entry etc., according EFSA PLH Panel, 2010), middle row (introduction etc.) generally used in invasion biology. During each of the phases several processes can be identified to play a role during invasion (e.g. transport etc.) each of which can be.

Fig. 56 shows a schematic representation of population size development of an invasive population during different phases. The phases can be roughly divided into entry, the colonization process of the invasive species, establishment, the development of persisting and reproducing populations in the new range and the invasive spread, the rapid increase of population size typical for a biological invasion. Establishment can be further subdivided in naturalization, the establishment of the first populations itself and the lag phase during which populations persist and presumably adaptation or acclimatization via plasticity increases performance in the new range. This can be a prerequisite for the invasive spread. Each of the phases are related to barriers or filters because only a subset of individuals will be able to transgress from one phase to the other. For example, this applies to the transition between entry and establishment, because not all transported individuals will be able to establish and not all established populations will become invasive.

The phases are related to processes, e.g. transport, survival, or reproduction, some of which are interacting. They can be conditions for subsequent processes, for example transport as a process determines the number of individuals introduced, which might decrease minority disadvantage of newcomers and support establishment. This constitute propagule pressure one major recognized mechanism to support invasions (Roman and Darling, 2007). Each process and its likelihood of occurrence can be used for risk assessment on invasive species in the different assessment approaches or systems.

In accordance, the change of population sizes related to a gene drive organism release can be assigned to phases and processes. In Fig. 57A, this is shown for a successful application, when organisms are released, and start to affect population size during what we call here also a lag phase. During this phase the target population is not yet decreasing rapidly, and the effect might still be reverted by other processes. The effect itself constitutes the population decline that is the desired outcome of the drive. Subsequently populations might recover, e.g. by developing resistances, might persist or get extinct. The decline and post drive characteristics mediate the impact, which is the effect of population size change of the target population on ecological processes.

The risk of escape of GDOs into populations or related species (risk field three) or to other regions (risk field two) can then be illustrated in accordance to the phases identified for biological invasions. In Fig. 57B the relative increase of GDO individuals in a population and the expected development of a population are indicated. Escape of GDO to a new area where it could interact with populations of conspecifics which should not be part of the drive, would respond to similar processes related with the introduction phase of biological invasions. Lag phase and phase of increase can be less clearly defined like biological invasions, but some processes might also apply. Impact will here also be mediated by decrease of non-target population, even though any interaction of non-target population with GDO would be related to a certain risk and has to be excluded.

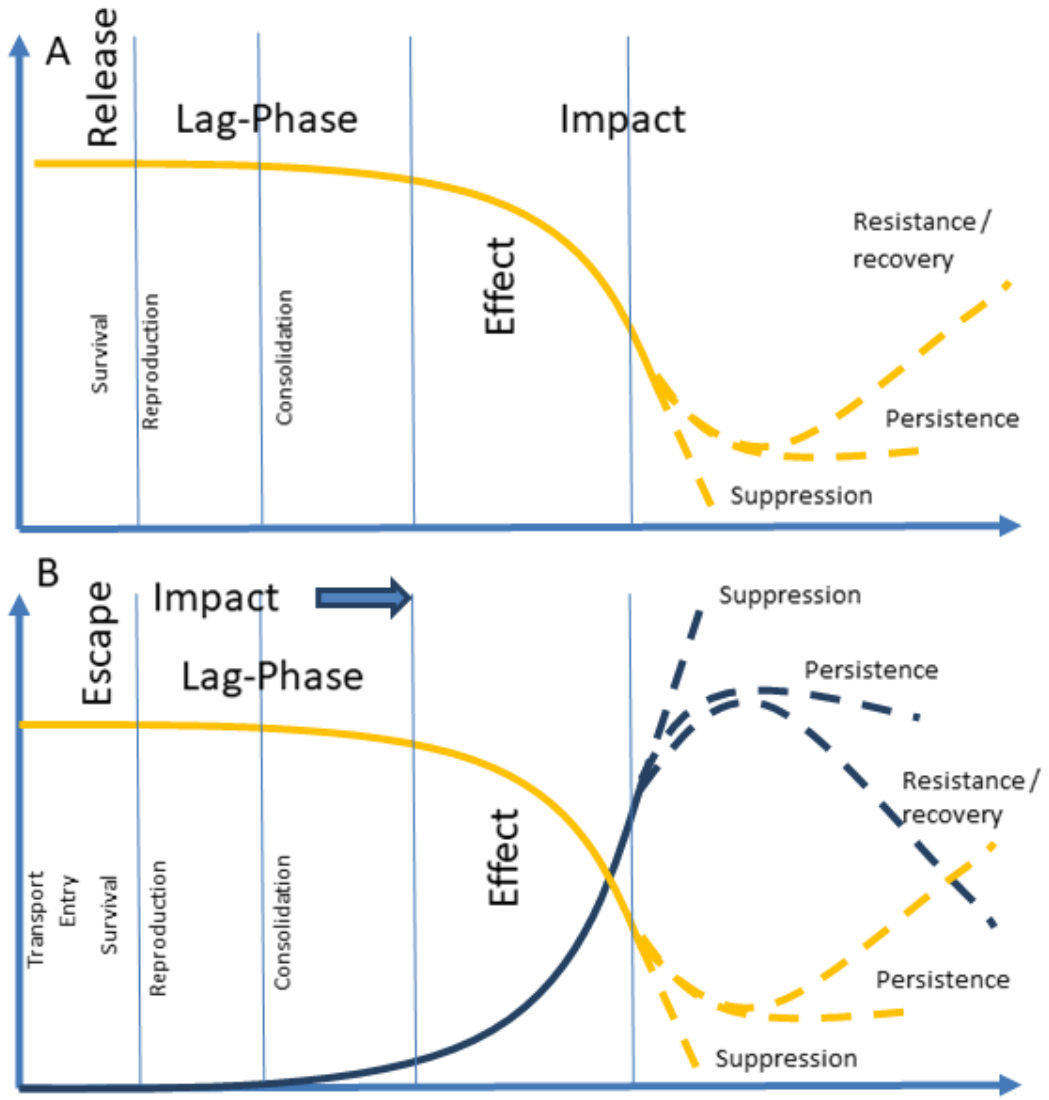


Fig. 57: Population size development of GDO influenced populations (schematic, Yellow).
Indicated are the different phases in analogy to invasion biology, where applicable. In **A**, phases are indicated as a "successful" gene drive and **B**, as an escape, a drive in a non-target population. In B the proportion of GDO in the population is indicated in blue.

a. Review of Risk Assessment Approaches for Invasive Species

To examine essential elements of an environmental risk assessment for invasive species, we have investigated and compared several risk assessment guidelines and protocols. During this preliminary analysis we looked into the guideline of IMO (International Maritime Organization)(IMO, 2007), the IPPC (International Plant Protection Convention) (FAO, 2004) and OIE (Office International des Epizooties)(OIE, 2019). Also, the protocols of the UK risk assessment scheme (Baker et al., 2008), which is based on the EPPO (European and Mediterranean Plant Protection Organization) framework (EPPO, 1993) and the Belgian Harmonia+ protocol (D'hondt et al., 2015; D´hondt et al. (2014) have been examined. An example for an application delivers the EFSA Paper (EFSA PLH Panel, 2010) which is also following the EPPO framework. In the meanwhile, the paper published by (Srèbalienè et al., 2019) was following a similar approach.

Steps of an invasive species risk assessment

The main steps in invasive risk assessment frameworks are

1. **Providing basic information about the invasive species**
2. **The assessment of the probability of introduction and spread of the invasive species**
3. **The evaluation of the impacts**
4. **The assessment of risks**

Ad 1) To identify the steps for the basic information, we took a detailed look into the IPPC guideline (FAO, 2004) and in the EFSA document (EFSA PLH Panel, 2010). The basic information seems to be similar to the problem formulation phase of the EPA ERA concept (EPA, 1998, 1992), where the key features of the problem formulation are the source of the stressor, the stressor itself, the receiving environment, the identification of potential adverse effects, and the exposure pathways. In the IPPC guideline (FAO, 2004), the basic information for invasive species including living modified organisms (LMOs) consists of:

- **the identity of the pest, which means in relation to LMOs, the characteristics of the recipient or parent organism, the characteristics of the donor organism, the genetic construct, gene or transgene vector and the nature of the genetic modification**
- **the presence or absence in the pest risk analysis (= PRA) area and the regulatory status of the area**
- **potential for establishment and spread: which means changes in adaptive characteristics resulting from genetic modification that may increase the potential for establishment and spread; the gene transfer or gene flow that may result in establishment and spread; the genotypic and phenotypic instability that could result in the establishment and spread of organism with new pest characteristics.**
- **potential for economic and environmental consequences in the pest risk analysis (= PRA) area**

In the EFSA document (EFSA PLH Panel, 2010) the basic information is about:

- **Taxonomy and biological characteristics of the invasive species**
- **Occurrence, distribution and prevalence of the pest in various geographical areas; environmental data that could affect establishment and spread; farming practices and crop characteristics**
- **Transport and storage conditions; trading patterns and other pathways relevant to spread of pests.**

Ad2) The next step is the assessment of the probability of introduction (= entry and establishment) and spread of the invasive species. The process of invasion follows the typical phases entry, establishment, spread and impact.

Entry contains information about intentional and unintentional pathways of the species. To describe and assess establishment, biological information of the pest, such as life cycle, host range and survival is needed, further the suitability of the environment, information about the genotypic and phenotypic instability of the pest and also cultural practices and control measures (FAO, 2004) (Fig. 58).

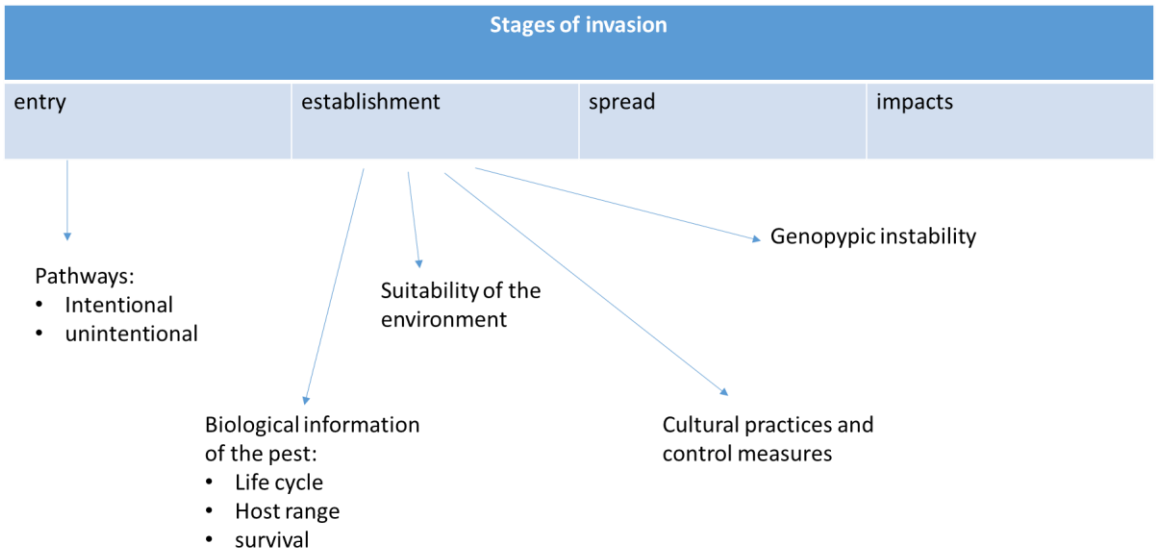


Fig. 58: Stages of invasion and important factors influencing entry and establishment suggested for risk assessment.

The assessment of the probability of spread needs information about the suitability of natural and/or managed environment for natural spread of the pest, the presence of natural barriers, the potential for movement with commodities or conveyances, the intended use of the commodity and potential natural enemies of the pest in the pest risk analysis area (Fig. 59).

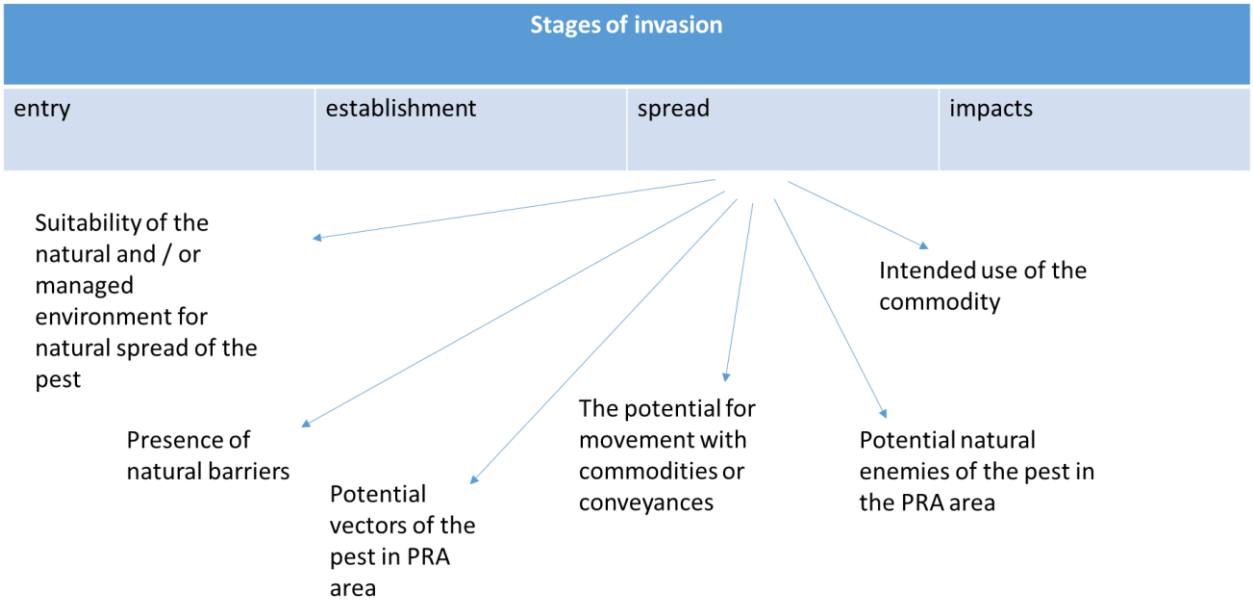


Fig. 59: Stages of invasion and important factors influencing spread suggested for risk assessment.

The Belgian Harmonia+ protocol (D'hondt et al., 2015) consists of 30 questions that refer to different components of invasion, the stages of introduction, establishment and spread and different kinds of impacts.

Example: The Questions for introduction:

1. **The probability for The Organism to be introduced into Area´s wild by natural means is low / medium / high]**
2. **The probability for The Organism to be introduced into Area´s wild by unintentional human actions is low / medium / high]**
3. **The probability for The Organism to be introduced into Area´s wild by intentional human actions is low / medium / high]**

For different questions diverse predetermined answers are possible. They belong from low, medium, high to non-optimal, sub-optimal, optimal, or can differ from very low to very high. Experts should provide answers "…as much as possible based on evidence, and not on a purely hypothetical or speculative basis." (D´hondt et al., 2014, p. 5) Afterwards answers have to be scored and the estimation of risk can be figured out. (D'hondt et al., 2015, 2014). For organisms normally qualitative scales are chosen, because of the difficulty to collect quantitative information (Moeed et al., 2006).

Ad3) Impacts

In the publication "A comparison of impact and risk assessment methods based on the IMO Guidelines and EU invasive alien species risk assessment frameworks" (Srèbalienè et al., 2019) are listed 4 types of impact categories, the human health, the economical category, the environment and social-cultural impacts. For the environment categories, 20 different impacts of invasive species have been identified from different risk assessment methods.

Environment impacts:

Pest on native species, Pathogen on native species, Parasite on native species

Pest vector, Pathogen vector, Parasite vector

Habitat change or loss

Biodiversity alteration

Species abundance

Keystone species

Threatened or endangered species

Toxicity on native species

Predation

Herbivory/grazing

Competition

Hybridization

General ecosystem services

Nutrient regime alteration

Hydrological cycle changes

Food web changes

A general overview of the key features in an invasive species risk assessment provides the paper "A comparison of impact and risk assessment methods based on the IMO Guidelines and EU invasive alien species risk assessment frameworks" (Srèbalienè et al., 2019) (Fig. 60). The comparison provides a basic concept for the evaluation procedure of several bio-invasion impact and risk assessment methods and amalgamates elements of both RA frameworks.

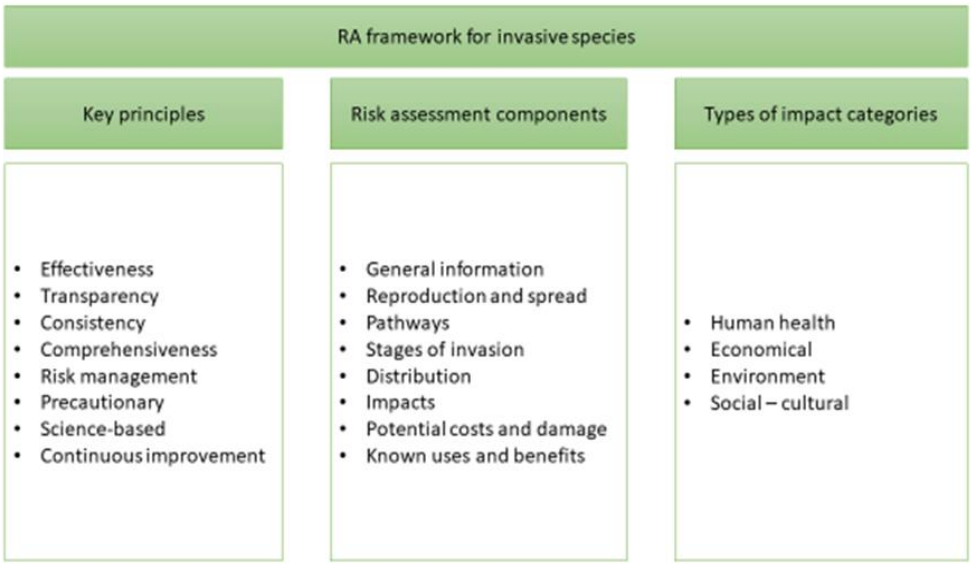


Fig. 60: Risk Assessment framework for invasive species (according to Srèbalienè et al., 2019).

6.1.3 **Towards a Framework for an ERA for Gene Drive Modified Organisms**

In previous parts of this report, we summarized ecotoxicological risk assessment and risk assessment for invasive species. We also identified similarities and analogies between invasive species and GDOs. However, the well-structured and comprehensive process of problem formulation may be helpful in the beginning of an ERA of GDOs. Therefore, in the following, we will describe ways of combining ecotoxicological approaches with the insights of risk assessment for invasive species to work towards a framework for ERA for GDOs.

A meaningful problem formulation needs an investigation of the wild type of the engineered organism, the gene drive organism, the pathways to harm, the possible receiving environments and the adverse effects. This is valid for both GDOs and invasive species.

Following, aspects of the problem formulation for GDO risk assessment referring to the wild type organism and GDOs are summarized (Tab. 15). This is a result reviewing and analysing various risk assessment guidelines (EFSA PLH Panel, 2010; EFSA GMO Panel, 2013; FAO, 2004) and papers concerning risk assessments (Moro et al., 2018; Rode et al., 2019; Andersen et al., 2004; National Academies of Sciences, 2016).

Tab. 15: Background information of potential scientific field relevant for the problem formulation in comparison to their aspects. Content of the different fields are detailed below.

aspects of the problem formulation	*problem field*
wild type	life history
	reproductive biology
	habitat requirements
	spatial ecology
	biotic interactions
	genome
GDO	intended use
	GD technique and intention
	potential for entry
	barriers: ecological barriers, abiotic barriers
	potential for establishment
	barriers: survival and reproduction barrier, technical barrier, environmental barrier
	potential for spread
	barriers: dispersal barriers, environmental b.; biotic and abiotic stressors at all development stages
pathways	VGT
	HGT
	transport
receiving environment	native environment
	accessible ecosystems
	management systems
	new accessible environment because of the modification
	potential of the GDO to exploit new niches or environments
adverse effects	trophic cascades
	community processes

a. Wild type organism:

Life history:

Life history refers to the age and generation time. The information is necessary because the gene drive spread can be increased by short lifecycles. Furthermore, population structure and social structure needs to be investigated. Population structure gives information about population size, population density and ability to migration, which are influenced by social structure i.e. age distribution and sex ratio. Population structure in time refers to a constant inflow of wildtype individuals because of resting stages (e.g. seed bank in plants) that might affect the gene drive spread (Rode et al., 2019; National Academies of Sciences, 2016).

Moreover, life stages have to be worked out, especially the development rate and viability of larvae and pupae and the proportion reaching adult maturity (EFSA GMO Panel, 2013).

Reproductive biology:

With regard to reproductive biology the fertility rate, fecundity (EFSA GMO Panel, 2013) and the numbers of offspring (Moro et al., 2018) have to be investigated. These aspects are influenced by mating systems as female mating success, the male mating competitiveness (EFSA GMO Panel, 2013), polygamous versus monogamous mating systems (Moro et al., 2018). Breeding seasons and breeding structures (Moro et al., 2018) also belongs to reproductive biology.

Habitat requirements:

Habitat requirements for all development stages (EFSA GMO Panel, 2013) may be inferred from the native environment including abiotic factors such as climate factors (e.g. temperature, precipitation seasons) and biotic factors (e.g. vegetation, landscape structures, food resources, disease, predation, competition).

Spatial ecology:

The population structure in space includes dispersal and distribution of the population. A low dispersal can affect gene drive dynamics (Rode et al., 2019), long distances between populations affect gene drive spread. Therefore, a distribution map would be helpful and provide information to identify potential barriers to breeding and gene flow.

Biotic interactions:

Information about the trophic level of the species, the role in the predator system with natural enemies and competitors but also the symbiotic system with host plants or host animals have to be investigated.

Genome:

Information about the characteristics currently available for a reference genome (Moro et al., 2018) is neccessary. In addition, information about within population and within species genetic diversity as well as potential population differentiation in the species is required.

b. Gene drive organism:

For the gene drive organism, first of all, the intended use (disease control, agriculture, conservation) and the gene drive technique and intentions have to be elaborated.

Because of the spreading characteristics of GDOs, aspects influencing the risk of the GDO to become invasive must be considered. These are amongst others changes of the gene drive organism and its hybridized offspring in relation to the wild relatives in fitness, reproductive potential, and the potential to exploit new environments because of the modification.

The steps of invasion are grouped into entry, establishment and spread, aspects that influence these processes are listed below.

Potential for entry:

First step is the deliberate release or escape of the GDO.

Regarding the release, the threshold of the release influences the potential of getting invasive. The threshold refers to“…the quantity, timing, frequency, duration, and routes of exposures as well as the numbers, species, and other characteristics (e.g. susceptibility) of the populations exposed” (Andersen et al., 2004).

The escape can happen with intended or unintended pathways. This can happen through transport or storage conditions of commodities, trading patterns (EFSA PLH Panel, 2010) but also through abiotic factors as wind (EFSA GMO Panel, 2013) and water. Also, socioeconomic goals can influence transport possibilities. Vertical and horizontal gene flows also have to be considered.

Ecological and abiotic barriers have to be overcome to reach the potential for establishment.

Potential for establishment:

To assess the potential for establishment, the biological information of the wild type organism is required (see section about the wild type organism above) respectively life stages (larvae, pupae, adult maturity), their development rate, but also the reproductive biology (fertility, fecundity development, sexual maturity), their fitness (EFSA GMO Panel, 2013, p.76; Rode et al., 2019) and their ability to survive (e.g. disease, predation, competition, food availability (EFSA GMO Panel, 2013). Also, the host range and habitat requirements for survival (= suitability of the environment) (FAO, 2004) and other ecological requirements (EFSA GMO Panel, 2013; Moro et al., 2018) are necessary. Afterwards, the differences of the biological characteristics between the wild type and the GDO organism due to the modification can be worked out. For example, there exist new accessible ecosystems because of the modification of the GDO. To assess the potential for establishment, also control measures and cultural practices have to be considered.

The barriers to overcome these phases are the survival and the reproduction barriers, the technical barrier, environmental barriers, minority disadvantage & metapopulation dynamics (= population growth barrier).

Potential for spread:

To be able to spread, the GDO needs different pathways (see also pathways) including the potential for gene flow, but also can move on its own (EFSA GMO Panel, 2013). The suitability of the environment (FAO, 2004; EFSA GMO Panel, 2013) is an important factor, especially the potential to exploit new niches in the environment due to modification (e.g. temperature and drought tolerance).

Barriers are dispersal barriers, environmental barriers (Richardson et al., 2000; Blackburn et al., 2011), biotic stressors (disease, predation, competition food availability) and abiotic stressors (e.g., temperature, humidity, and radiation) (EFSA GMO Panel, 2013).

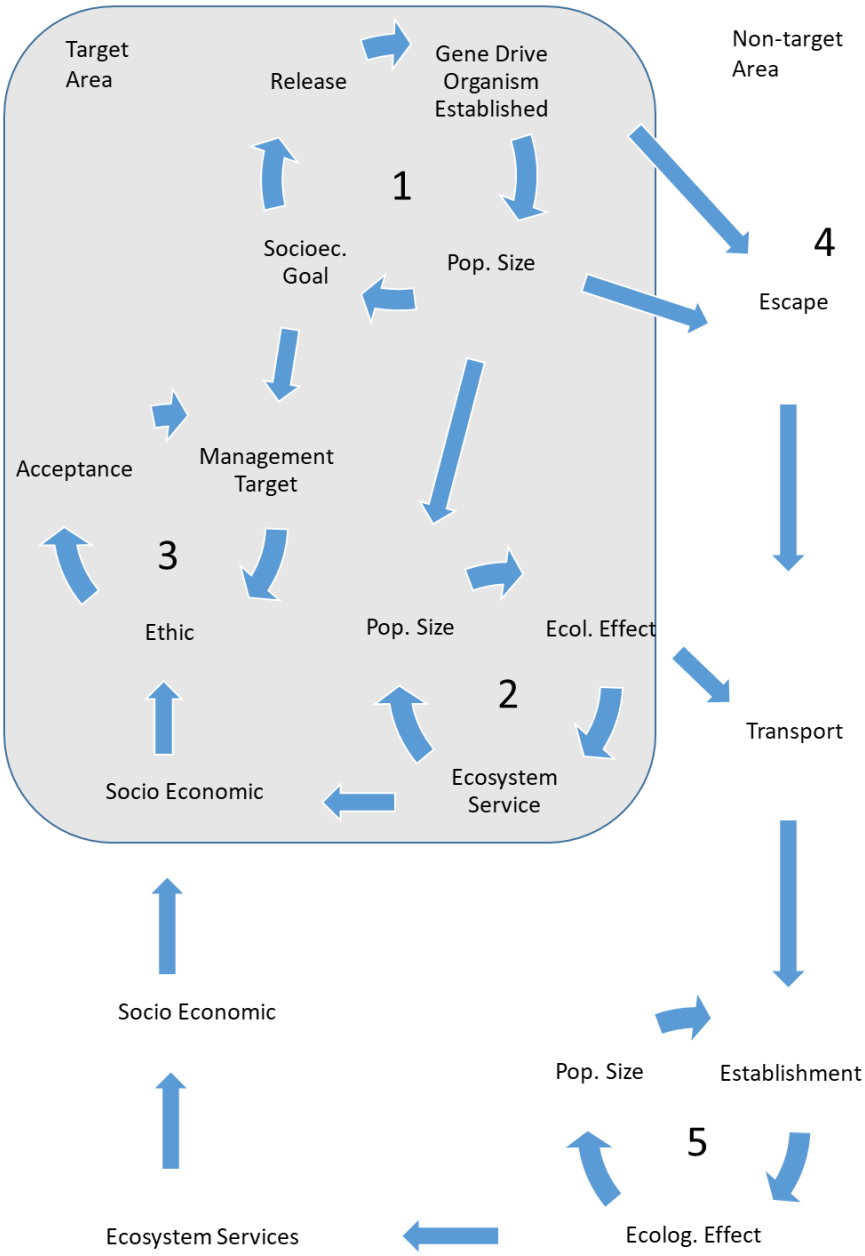


Fig. 61: Draft of a framework of elements determining population sizes and constituting potential effectors to develop risk hypotheses within and outside the target area (after escape) for a geographically restricted suppression drive.
We identify five basic pathways: 1 in target area effect of the release of GMOs, 2 effect of population size on Ecosystem services in the target area, 3. Effect of goal and hazard observation on the management and socio ecological parameters 4. Escape, including all mechanism to accidently overcome the restrictions of the drive, 5. Effect on population size on Ecosystem services in non-target area, we expect here a feedback between population size and establishment.

Fig. 61 shows the framework in a single figure to visualize how different feedback loops affect the population sizes within the target area and – following escape – potentially outside the target area. As gene drive application is as much a political and socio-economic as an ecological endeavour, we included also socio-economic and ethical aspects. There are five basic-, however, interconnected pathways. (1) the direct effect of the GDO in the target area on the wild type (intended effect), (2) the effect of the reduced population size on the

ecosystem and on ecosystem services within the target area, (3) the effect of (1) and (2) but also (4) and (5) on socio-economy and ethics including the resulting effect on the acceptance of the gene drive technique and the management target (4) the escape including all mechanisms to accidently overcome the restrictions of the drive, finally leading to (5) the effect on the population size and following ecological effects and effects on ecosystem services in the non-target area – we expect here a feedback between population size and establishment. The framework is expressed for a geographically restricted suppression drive, other forms of escape, e.g., horizontal gene transfer can be treated analogously.

The proposed example for a framework for an ERA for Gene Drive Modified Organisms combines the existing ecotoxicological framework and elements of the risk assessment for invasive species. In a general way, this is expressed in Fig. 61. However, to be able to assess the ecological effect (pathway 2), all the information listed in Tab. 17 must be available. Because it is rare that enough data of adequate quality are available beforehand, the formulation of specific protection goals and specific hypothesis about pathways to harm may lead to ignoring important ecological effects. Therefore, we propose an open framework in an (eco)system approach, which we explored in a case study of *Drospohila suzukii* using the sensitivity analysis sensu Vester and the provided computer tool (Vester 1999).

6.2 Part B.2 - Priority of Risks and Case Studies

6.2.1 Choice of Organisms

Aim of the project is to use case studies to illustrate the assignment of general and specific protection goals and exemplify a risk assessment approach. We compiled a list of organisms that are under discussion or mentioned as potential target to be subjected to a gene drive (Tab. 16). By January 2019, around 270 publications had been listed in the web of science that concern gene drive. In these publications we counted 43 species that had been mentioned, among these 10 for application in disease control, all of them mosquito species as vectors of human or animal disease. Eleven species are considered in the context of agricultural pest control and the largest group of 22 species in the context of environment and conservation, most of them invasive species, which should be subjected to a gene drive outside their native range.

The choice of the case study considers different aspects of potential GDO application. We selected one species from the field of agriculture (*Drosophila suzukii*) and one from the field of environment and invasive alien species (IAS) (*Rattus norvegicus*). *D. suzukii* is an important pest species in orchards in Europe and North America and therefore there is a high pressure to find new measures to control it. Furthermore, the group of fruit flies is very well studied and therefore gene drive applications in the not-so-distant future appear realistic. *R. norvegicus* serves as an example for a species that is invasive in parts of the world causing nature conservation problems and is widespread in most areas.

Tab. 16: Species considered as target organisms in different application fields.

Disease Vector Species	Common Name
Aedes aegypti	Yellow fever mosquito
Aedes albopictus	Asian tiger mosquito
Aedes fluviatilis	Aedes fluviatilis mosquito
Aedes vigilax	Aedes vigilax mosquito
Anopheles albimanus	Malaria mosquito
Anopheles coluzzii	Malaria mosquito
Anopheles funestus	Malaria mosquito
Anopheles gambiae	Malaria mosquito
Anopheles stephensi	Malaria mosquito
Culex quinquefasciatus	Southern House Mosquito
Agricultural Pest Species	
Ceratitis capitata	Mediterranian fruit fly
Cochliomyia hominivorax	Screw worm
Drosophila melanogaster	Vinegar fly
Drosophila suzukii	Spotted wing Drosophila
Halotydeus destructor	Redlegged earth mite
Jacobaea vulgaris	Common ragwort
Listronotus bonariensis	Argentine stem weevil
Lucilia cuprina	Australien sheep blowfly
Lymantria dispar	Gypsy moth
Plutella xylostella	Diamondback moth
Tribolium castaneum	Red flour beetle
Invasive Alien Species	
Boiga irregularis	Brown tree snake
Bufo marinus	Cane toad
Centaurea maculosa	Spotted knapweed
Cyprinus carpio	Common carp
Cytisus scoparius	Common broom
Dreissena polymorpha	Zebra mussel
Felis catus	Domestic cat
Halyomorpha halys	Brown marmorated stink
Mus musculus	House mouse
Mustela erminea	Stoat
Oryctolagus cuniculus	European rabbit
Pueraria montana	Kudzu
Rattus argentiventer	Ricefield rat
Rattus exulans	Polynesian rat
Rattus norvegicus	Brown rat
Rattus rattus	Black rat
Sturnus vulgaris	Common starling
Trichosurus vulpecula	Common brushtail
Vespa velutina nigrithorax	Asian hornet
Vespula germanica	German wasp
Vespula vulgaris	Common wasp
Vulpes Vulpes	Red fox

6.2.2 **Drosophila suzukii**

The goal of the case study is to evaluate if ecological effects are expected and if this can be outlined with the available information. In the following we describe ecological charactereistics and in the absence of concrete quantitavie data, we explored system analysis of Vester as a tool to gather and organize the knowledge about the system of a landscape with orchards that are suffering from the agricultural pest *D. suzukii* outside its native range.

a. Ecological Characteristics

In the master thesis from Carina Roberta Lalyer (2019) advised by Bernd Giese from the ISR the current state of knowledge on *Drosophila suzukii* was accumulated. In the following, we summarize the ecological characteristics of the species.

According to (Ometto et al., 2013) *Drosophila suzukii* originally evolved in montane temperate forests of Tibet and its native range in Southeast Asia spans Japan, China, South Korea, India and Thailand (Asplen et al., 2015; Hauser et al., 2009). However, very likely through fruit transports, *Drosophila suzukii* has invaded many places in the world and occurs now in Europe, USA, Brazil and Hawaii. This was facilitated by a wide range of host species of the genera *Prunus*, *Rubus, Ribes,* and *Vaccinium* (roughly 80 species in Europe alone (Kenis et al., 2016)), including several crop species, e.g., cherries, blueberries, strawberries, raspberries, and blackberries. Unlike most other fruit flies *Drosophila suzukii* can lie eggs into ripening fruits, which makes it to a severe agricultural pest in Japan (Kanzawa, 1939 cited in (Asplen et al., 2015)), USA (Bolda et al., 2010), and Europe (Lee et al., 2011). With a female's capability of laying up to 600 eggs and the fact that *D. suzukii* can produce 7 to 15 generations a year (Cini et al., 2012), the population can grow quickly and damage crop species severely. The fruits are affected not only through the larval feeding but also because the initial piercing of the fruits provide a gateway for other species or yeasts (Bernardi et al., 2017; Hamby et al., 2012).

Adults of *D. suzukii* are fruit flies of 2-3 mm in length with red eyes, a brown thorax, and black stripes on the abdomen (Cini et al., 2012). Females can be distinguished from males by the enlarged ovipositor with many sclerotized teeth (Hauser, 2011), which enables the species to lay eggs into fruits with intact skin. Males feature a dark spot located on the top edge of each of their wings and can also be distinguished by two black combs on their tarsus (Hauser, 2011). The species develops a summer and a winter morph, the latter being adapted to cooler temperatures by a larger body, larger wings and darker pigmentations (Shearer et al., 2016; Stockton et al., 2018). Individuals of the summer morph are most active at 20°C and at 30°C activity becomes reduced (Walsh et al., 2011) while they die at temperatures of 40°C (Zerulla et al., 2017). Below 7.5°C the summer morph cannot develop (Zerulla et al., 2017). *D. suzukii* can migrate in the summer to higher elevation to use different resources (Mitsui and Kimura, 2010). Emiljanowicz et al. (2014) found that one individual lived for 86 days (154 days maximum). The time of development from egg to adult varies dependent on temperature between 8-10 days (at 25°C) and 21-25 days (at 15°C) (Lee et al., 2011).

It appears that, at least in Japan, *D. subpulchrella* may act as a competitor of *D. suzukii* as this species has a similar seasonal cycle and resource use (Mitsui and Kimura, 2010). As parasites act mainly wasp species that lay their eggs into the larvae of *D. suzukii* (Girod et al., 2018; Mitsui and Kimura, 2010), however it appears that parasitoid wasp species in North America and Europe cannot as successfully parasitize *D. suzukii* as similar species in its native range (Poyet et al., 2013). Several arthropods predate on larvae and pupae of *D. suzukii,* i.e., kissing bugs, ants, staphilinids, carabids and spiders (Gabarra et al., 2015; Walsh et al., 2011). However, the biological interactions of the species and its functional role in the ecosystem are still not completely understood.

The ecological characteristics of *Drosophila suzukii* like outlined of the thesis of Carina Layler can now be used to identify above mentioned phases and processes related to effects of population size fluctuation, transport and other parameters that would define the risk related to ecological role of the species and escape of GDOs to non-target populations.

b. Exploring Sensitivity Analysis of Vester for Risk Assessment

The sensitivity analysis of Frederic Vester is a method to describe and analyse systems using quantitative and qualitative variables. The advantage of the approach is that within the process of a guided system description a set of variables must be agreed on between stake holders. We used this guided process rather as expert system as project participants agreed on the important parts of the system.

The computer program that was developed to aid this process can integrate both, quantitative and discrete variables but also qualitative or general difficult to quantify socio-economic variables like displeasure, anger, fears, consensus, quality of life and more. Not the amount of information is important, but the right choice of variables. Therefore, the description and delimitation of the system and understanding the relationships of the components of the system are crucial for this sensitivity analysis. After describing the system, the interactions can be visualized, and new characteristics may be discovered during the process, like feedback effects, thresholds, self-regulations, and overturning effects.

Important for any system description is the formulation of the right goal. According to Vester (1999) the relevant goal always is to increase and secure the viability of a system. If managers and experts follow the idea that all economic, social, and ecological harms can be repaired using technology, this may draw consequential damages and can be very expensive. This thinking also concerns the repairing environment protection, because it allows to act as before, if harm can be repaired (Vester, 1999).

The specific characteristic of the sensitivity analysis of F. Vester is that it allows the investigation of feedback effects, threshold values, self-regulation and tipping points for different kinds of systems (e.g. biological, technical, sociological systems) (Vester, 1999). As part of the analysis, different levels of investigation are suggested. First the complexity of a system is reduced to a manageable but critical variable set. Second, the interactions of the variables are investigated and graphically visualized. Third, the analysed system can be assessed considering the optimising of the viability of the system (Vester, 1999).

c. Sensitivity Analysis *Drosophila suzukii*

Recently, gene drive technology has been proposed as a control measure against the severe pest *Drosophila suzukii*. It is important to consider facts about *D. suzukii* as proposed in the framework (see Tab. 15). This information concerns the wild form as well as the gene drive form of *D. suzukii*, the pathways that are crucial for the spread of the invasive species, the presence of accessible ecosystems and the possible negative impacts.

If the gene drive *D. suzukii* has been deliberately released, it can hardly be limited in space and time, the global level with all its effects and interactions over space and time have to be considered. Therefore, it is difficult to define specific protection goals. The aim of the sensitivity analysis is to work out the resilience of the system and how it can be strengthened, or which aspects would weaken it. In this case, the habitat in which *D. suzukii* occurs should be strengthened so that the pest has fewer opportunities to reproduce and cause damage. One of the major risks in the use of gene drive *D. suzukii* is the escape in space or/and time and in the worst case, the re-introduction to Japan, where it can have adverse effects in the ecosystem as a native species, such as being absent from the food chain or as competitor.

The analysis was done according to the following general procedure: (1) System description, (2) Definition of variables, (3) Impact matrix, (4) Role allocation of the variables, (5) Causal networks, (6) Partial scenarios and simulations.

An essential aspect of the sensitivity analysis is the definition and delimitation of the sys-tem. For this purpose, about 20 variables have to be worked out, which cover certain are-as of the system such as the economic or ecological aspects. A characteristic of the sys-tem analysis of Vester is that soft facts such as fears in the population about genetically modified organisms can also be included in the evaluation. Following questions are also recommended to define the system: Where are the problems? What could be done about it? What is connected to it? What are the limits to this? Who is against it and why? What must be preserved? What are its peculiarities?

First, a half-day workshop with the BOKU members of this project was organized to describe and delimitate the system and to examine manageable variables. To define the system, it was helpful to define and answer some questions according to the problem of the system *D. suzukii* and to consider the most important areas of life in a holistic, networked view.

After an extensive and iterative decision process, 22 variables were identified and assessed considering their relevance for the system. Both quantitative and qualitative (e.g. uncertainty) variables were incorporated, because the variables shall represent economy, ecology, feelings, infrastructure, members of the system, land use, rules and regulations (Tab. 17). Then three persons separately worked out the effects from one variable to the other in impact matrices. Afterwards the results were combined to one impact matrix. This process required intensive discussions so that everyone could finally be satisfied with the result (Fig. 62). Subsequently the automatic analyse of the program was used for the investigation of the role in the system (Fig. 63). The next steps were the graphical visualisation of direct interactions of the variables (causal networks – Fig. 64) and of separate scenarios (Fig. 65). These steps facilitate the perception of direct effects and interactions as well as feedback loops within a system. With the partial scenarios, different simulations can be shown by describing the variables with different starting values and defining direction and strength of effects. The program allows for the input of curves on how one variable affects the other. These curves can consider a change in effect with changing quantities of the effector (non-linear effects). For example, if the effect of the GDO on the wild type is increasing with higher population size of the GDO. However, this non-linear behaviour is not defined by mathematical equations but graphically. For the start of any simulation starting points for all the variables have to be chosen. The simulations are therefore based on the knowledge on relationships between two variables and visualize the resulting network. The simulations are carried out in several rounds (i.e. years) and as with every round the starting values change for the variables, the simulation is progressing. It is important to note that these simulations do not make exact predictions about the future but can be used to visualize trends.

d. Definition of variables

During the workshop 22 variables were identified and agreed on to describe and delimitate the system of *D. suzukii*, which are listed and described below in Tab. 17.

Tab. 17: List and description of the variables of the system D. suzukii. Numbers correspond to the nodes of the program as shown in figures Fig. 62and Fig. 63

Number	*Variable*	*Description of the variable*
1	*Drosophilla suzukii*	Native range in Southeast Asia. Through fruit transports Drosophila suzukii has invaded many places in the world and has become a severe agricultural pest in Europe.
2	Gene drive *Drosophila suzukii*	In this system analysis we assume that Drosophila suzukii is modified with a suppression gene drive with the goal to eradicate the fruit fly in a certain area. The variable reflects the actual use of GDDS.
3	Predators	Insectivores, birds and other animals feeding on insects, particularly D. suzukii.
4	Biological control	A biological pest control for D. suzukii are kissing bugs, ants, staphilinids, carabids, spiders and may be used by farmers instead of insecticides. Biological control means also using traps for monitoring D. suzukii population for efficient application of pesticides.
5	Competitors	They have similar seasonal cycles and compete for same food and niche resources. Related species are in risk of hybridization.
6	Parasites	Parasites of Drosophila suzukii are certain wasp species that lay their eggs in their larvae.
7	Orchards	This variable represents the plants with its fruits in orchards, which can be infested by D. suzukii. In the system analysis this variable does not show the damage of D. suzukii for the farmers, but the orchards as place, where D. suzukii can reproduce.
8	Host plants	Host species of the genera Prunus, Rubus, Ribes, and Vaccinium facilitate the “natural” dispersal of D. suzukii.
9	Crop	The tradable fruits including the revenue for farmers.
10	Consumer satisfaction	Consumer satisfaction represents the expectations of consumers to a steady provision with “good” fruits.
11	Concern	The concerns of conservationists about risks and hazards of gene drive organisms for the environment and human health.
12	Insecticides	They have adverse effects on D. suzukii and other insects. Insecticides can negatively impact pollination or human health.
13	Structural diversity	This variable represents structural diversity and diversity of natural habitats. Diversity of structures and habitats probably reduce the population size of D. suzukii.
14	Genetic diversity	Genetic diversity is part of biodiversity and can be reduced by gene drive applications.
15	Species diversity	Species diversity is part of biodiversity and should be preserved by activities of governments. It supports ecosystem services like pollination and pest control.
16	Drosophila suzukii in Japan	D. suzukii has its native range in Japan and Southeast Asia. From there, she has invaded many places in the world.
17	Commodity transport	This variable represents the transport of fruits on the global trading market that facilitates the spreading of D. suzukii across the world.

Number	*Variable*	*Description of the variable*
19	GDDS dispersal	GDDS dispersal represents the possibility and risk of an unintended dispersal of gene drive D. suzukii.
20	Uncertainty	Gene drive applications cause many uncertainties. We don´t know much about the impacts nor about their likelihood of occurrence.
21	Pollination	Pollination is an important function in ecosystems and necessary for crop production in agriculture.
22	Pest control	Pest control can be supported by species diversity, it is an important ecosystem service and necessary in crop agriculture.

e. Impact Matrix

In the impact matrix direct effects are shown and assessed with values of 0, 1, 2 or 3. 0 means no effect, 1 describes that a strong change of variable A causes a slight change of variable B. 2 means that a change of variable A causes the same strong change of Variable B and 3 means that a weak change of variable A causes a very strong change of variable B. Three persons separately worked out the effects from one variable to the other in impact matrices. Afterwards the results were combined to one impact matrix. This process required intensive discussions so that everyone agreed to the final result (Fig. 62).

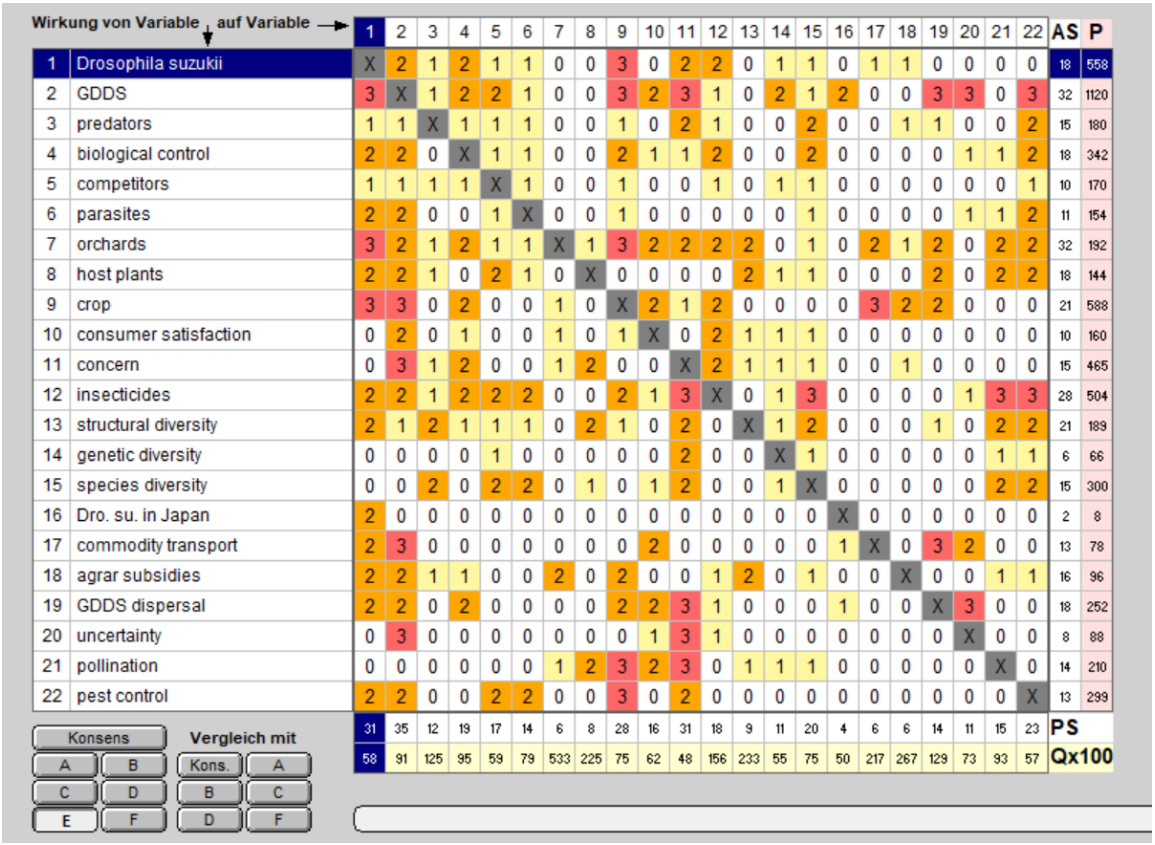


Fig. 62: Impact Matrix showing anticipated direct effects from one variable on another from 0 (no effect) to 3 (very strong effect).

f. Role Allocation of the Variables

Subsequently the automatic analysis of the program was used for the investigation of the role in the system (Fig. 63). The output of the impact matrix is shown as role allocation of the variables (Rollenverteilung). The variables are positioned between the cybernetic fields “buffering”, “reactive”,” active” and “critical”. Variables in the “active” area have a leverage function; they can stabilize a system again after changes. Variables in the “critical” area function are catalysts or accelerators, they can get things going in the first place and potentially cause the system to spiral out of control and tip over. For variables at this position highest caution is required. For variables in the “buffering” range interventions and controls are not necessary. Similarly, interventions on variables in the “reactive” area would only be symptom treatments, but these variables work well as indicators. Variables in the middle of a system are difficult to control, but they are good for the self-regulation of a system. The variables at the upper left side influence the variables on the bottom right.

In the system of *Drosohila suzukii*, the allocation of variables shows that the orchards (7) have the most influencing character, whereas variables as concern (11) or pest control (22) are more reactive. In the critical area lie the gene drive *Drosophila suzukii* (2), the *wild Drosophila suzukii* (1) and the crop (9), but *D. suzukii* and crop have a more reactive role as GDDS. Buffering variables of the system are at the left side. Most of the variables lie in the neutral range and can only control the system with difficulty, but they can have a regulating function.

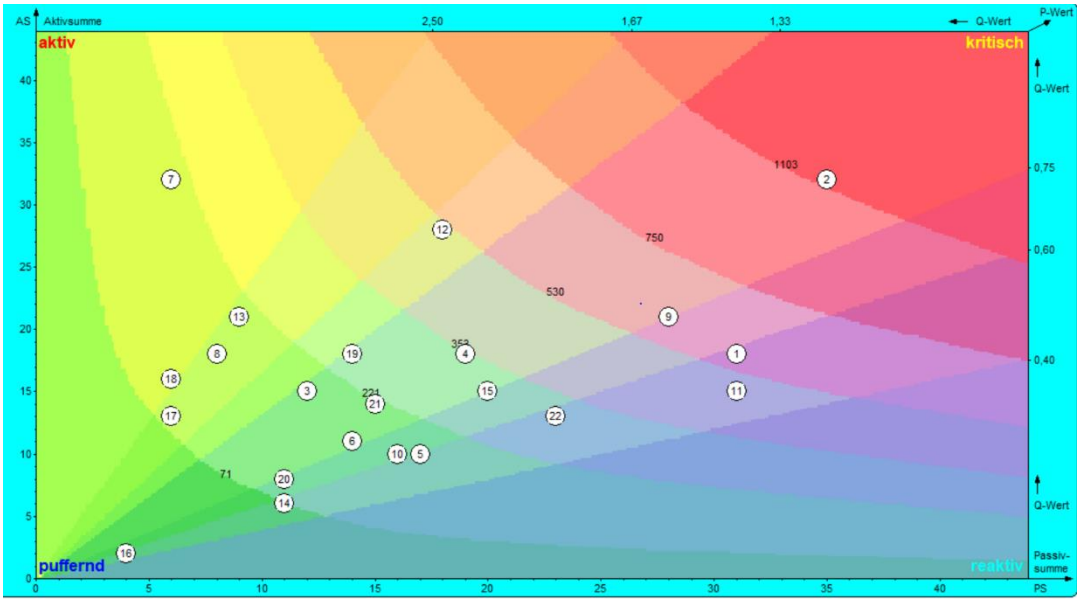


Fig. 63: Allocation of the variables according the Vester procedure (in German Rollenverteilung). Note that expectedly the variable 2 (GDDs individuals) is positioned in the critical area, 7 (orchards) is for example positioned as active. This means changes in this variable effect other variables in the system.

Owing to the wealth of information we had to restrict the analysis to the most important or eye-catching variables. The GDDS (2) is the variable that is most critical. The description given by the program states for these variables to be “powerful accelerators and catalysts”. This is expected as the GDDs individuals will initiate the drive. This means also that the application of gene drive *Drosophila suzukii* can dangerously affect the system and should, if at all, only take place under the strongest of precautions. Please see also the simulations of the partial scenarios below for how the system is affected. The use of GDDS as a solution to control *D. suzukii* cannot be presented lightly after interpretation of the distribution of roles. The objective of any system - to increase and ensure viability - cannot be implemented with critical variables.

The wild type of *D. suzukii* (1) is a variable that is only “slightly critical”. The program description says: „The already strong reaction of this slightly critical component to changes in the system (even if caused by itself) makes it unsuitable for targeted controlling interventions. An unreliable, but - because it is easy to handle - also seductive lever.” According to the evaluation of the sensitivity analysis, combating *D. suzukii* is not the solution of the problem. The shift levers for stabilization are on the orchards and agrarian subsidies.

(7) orchards and (18) agrarian subsidies

“Suitable as a shift lever, which, if the right approach to its operation is found, can stabilize the system again after modification (plastic stability).”

(9) crop

“By interventions into components of this area, often pendulum movements occur, which compensate corrections within the system relatively soon. This momentum, which brings some development to a standstill, can rather be handled from outside of the system.”

(11) concern

“Quite mobile reactive component, in which interventions succeed relatively easily and superficially lead to the desired result, which however is soon neutralized by the repercussions from the system.”

(12) insecticides

“Slightly active component that can be used for minor corrections and switch settings without causing too much feedback.”

g. Causal Networks

A causal network shows the variable relationships that are currently actually active. A solid arrow indicates a direct correlation; a dashed arrow indicates an inverse relationship. In this way, the effects and feedbacks of the system are made visible, and the current reality is represented in its multidimensional network. Furthermore, control loops can be shown. There is a distinction between negative and positive control loops. Negative control loops indicate self-regulation and are represented by an equidirectional and an opposite relationship (one arrow is solid, the second is dashed) or the control loop consists of an odd number of opposite relationships. Positive control loops represent self-amplifying feedbacks (both arrows are solid or there is an even number of equidirectional or opposite relationships) and can lead to a build-up in the system (Fig. 64).

To increase readability for the causal network, the variables “comparators” and “parasites” were summarized to the variable “antagonists”, the variables “structural diversity”, “genetic diversity” and “species diversity” were summarized to “biodiversity”.

Fig. 64 A shows all incoming and outcoming effects concerning *D. suzukii*. Fig. 64 B shows a self-amplifying loop with GDDS. The more GDDS is used, the more genetic diversity is reduced. Less genetic diversity means less pest control, which in turn results in more D. suzukii and more application of GDDS. Fig. 64 C shows the stabilizing control loop of the variable “concern”. This illustrates that conservation activities have a stabilizing factor in this system. If concerns of nature conservationists are very strong, the possibility of the application of GDDS decreases.

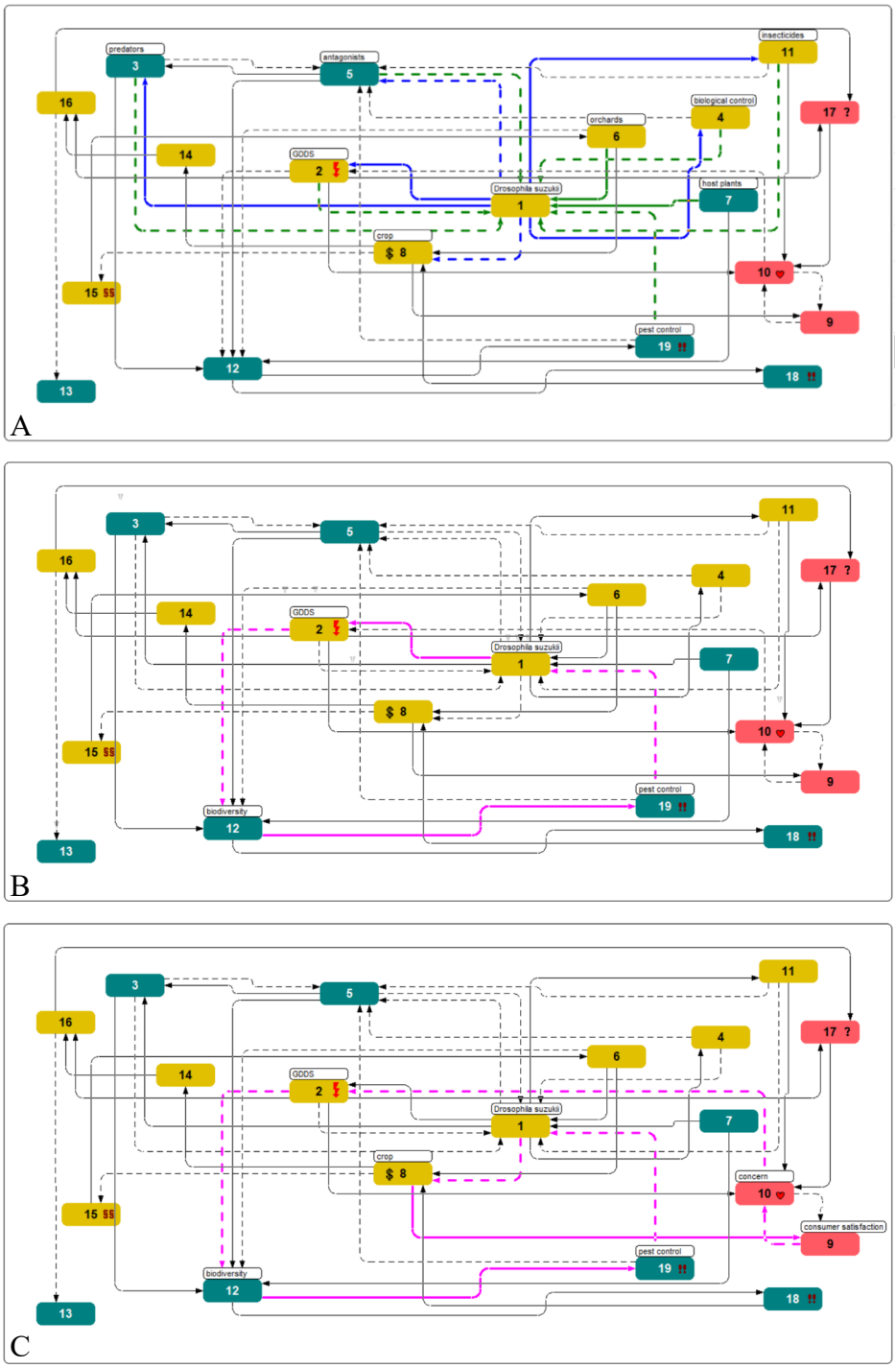


Fig. 64: Causal network as base for the simulations showing the single variables and their connectivity indicated as asrrows.
A solid line indicates a positive effect, a dashed line a negative effect. In **A**, variable "*D. suzukii*" is highlighted and their incoming (green) and outgoing effects (blue) are shown. In **B**, one self-amplifying control loop that links *D. suzukii* population size and GD carrying individuals (GDDS) is highlighted. In this case GDDS results in a loss of biodiversity, reduces pest control and consequently increases the population size of *D. suzukii* and results in increase of GDDS. In **C**, the same loop is shown in addition to a stabilizing control loop including the variable "concern". Conservation activities have a stabilizing factor in this system. If concerns of nature conservationists are very strong, the possibility of the application of GDDS decreases. All variables are summarized in Tab. 18

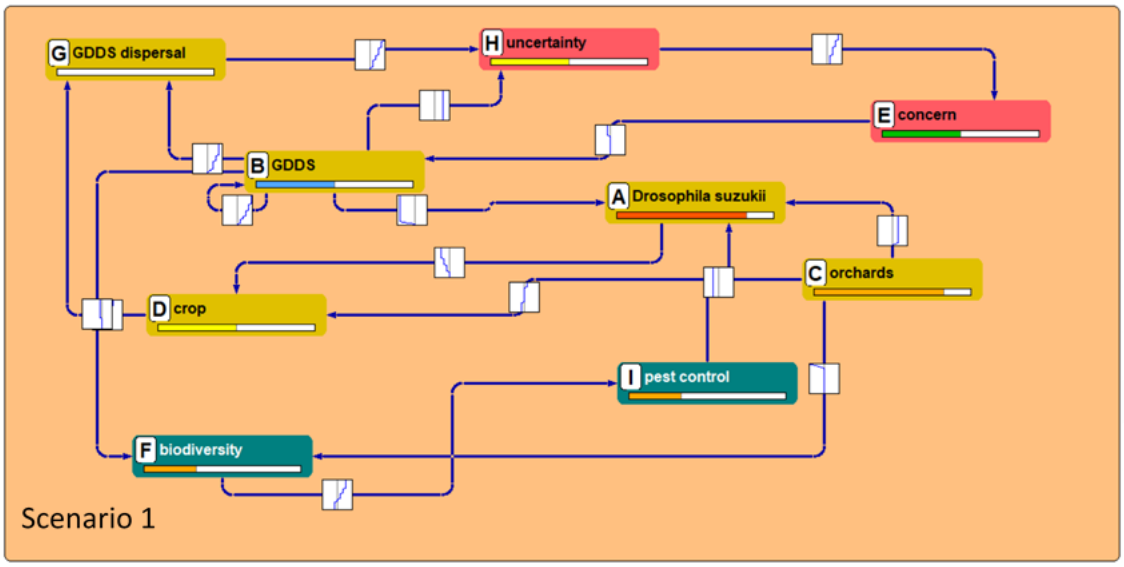
Tab. 18: Variables for the causal network

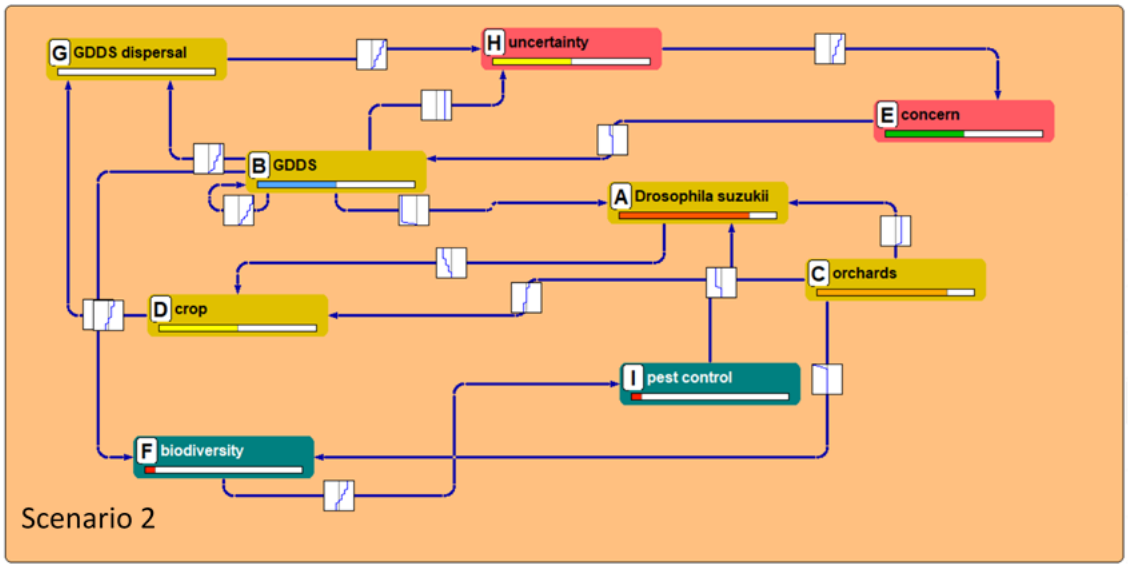
#	*Variable*
1	*Drosophila suzukii*
2	Gene drive *Drosophila suzukii*
3	Predators
4	Biological Control
5	Antagonists
6	Orchards
7	Host Plants
8	Crop
9	Consumer satisfaction
10	Concern
11	Insecticides
12	Biodiversity
13	*Drosophila suzukii* in Japan
14	Commodity transport
15	Agrarian subsidies
16	GDDS dispersal
17	Uncertainty
18	Pollination
19	Pest Control

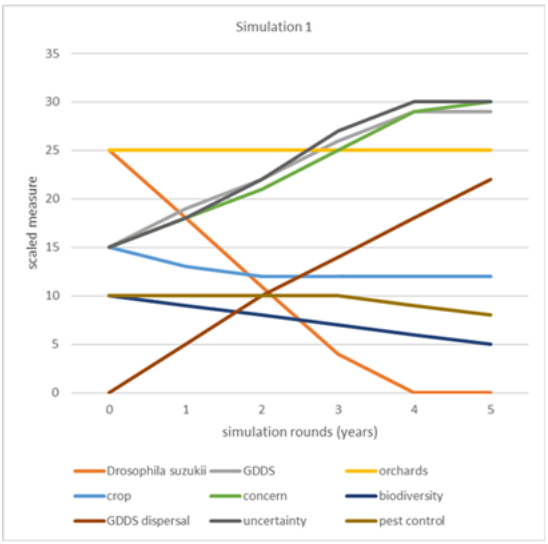
h. Simulations of Partial Scenarios

Partial scenarios are parts of the causal network, where the variables are specified with changeable values defined as starting points for the simulations. New relationships of the variables can be added and other – less important connections for this specific partial scenario – can be deleted. The strength and direction of effects of one variable onto the other have to be defined. Therefore, sufficient information of the strength of the effect is important, but no concrete data are necessary. Nevertheless, relevant expert opinions and profound discussions are considered and have to be merged into one result. For the simulation, the deciding factor and starting point of effects (the deliberate release of GDDS) and the order of the sequence of effects still need to be determined.

Following, two partial scenarios (Fig. 65: Scenario 1 and Scenario 2) are considered, where the variables are the same, but for some variables different starting values are used. In all partial scenarios, *"Drosophila suzukii"* occurs frequently and causes reduced "crop" yield. The starting values of "GDDS", "concern" and "uncertainty" are in the middle of their ranges, the value of "GDDS dispersal" is set to zero at the beginning. In the partial scenario 1 (Fig. 65) it is assumed that there are so many "orchards" in the hypothetical region of the system, that "biodiversity" is no longer as high. In the partial scenario 2 (Fig. 65) "biodiversity" and thus "pest control" are much more reduced. The corresponding simulations 1 and 2 (Fig. 65) are the results of the partial scenarios, after the computer programm of Vester has been started and 5 rounds have been run through. The simulations show what happen with *"Drosophila suzukii"* or general with pests in agricultural areas, where "biodiversity" cannot be maintained.


G GDDS dispersal
H uncertainty
E concern
B GDDS
A Drosophila suzukii
C orchards
D crop
I pest control
F biodiversity
Scenario 1


G GDDS dispersal
H uncertainty
E concern
B GDDS
A Drosophila suzukii
C orchards
D crop
I pest control
F biodiversity
Scenario 2


Simulation 1
scaled measure
simulation rounds (years)
Drosophila suzukii
GDDS
orchards
crop
concern
biodiversity
GDDS dispersal
uncertainty
pest control

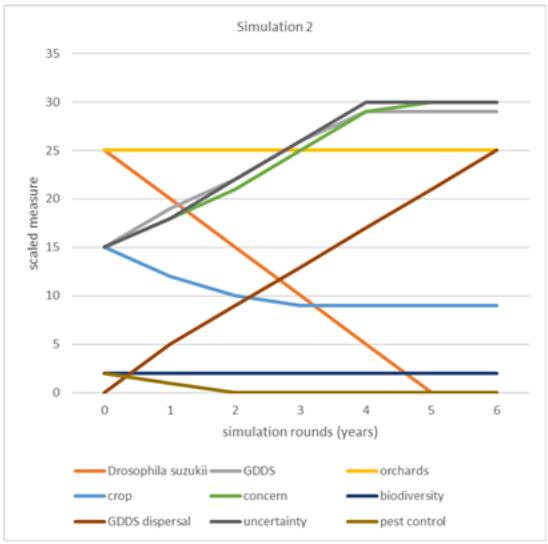

Simulation 2
scaled measure
simulation rounds (years)
Drosophila suzukii
GDDS
orchards
crop
concern
biodiversity
GDDS dispersal
uncertainty
pest control

Fig. 65: (previous page): An example of partial scenarios and corresponding simulations of the causal network of the system "Drosophila suzukii".
The scenarios indicate connections as arrows, strength of effect in the center of the arrow, and variable settings for the simulation. The scenarios differ in the relative settings for F, "biodiversity" as protection goal and related ecosystem service of natural I, "pest control". In scenario 1 a moderate high level of "biodiversity" is assuemed and a proportional level of "pest control", both is reduced in scenario 2. The simulation differs in the effect of the gene drive on biodiversity: In scenario 1 biodiversity declines about 50 % in the five year period of the simulation, while in scenario 2 it remains stable on the low level. The related ecosystem service "pest control" shows a reverse trend: in scenario 1 it is lost less severe then biodiversity (on 75% after 5 years) while in scenario 2 it is lost completely after 2 years. The rational for the initial settings for the other variables are as follows: A, *Drosophila suzukii*: The the gene drive target population is high; B, Gene Drive: *D. suzukii* is modified with a very effective suppression drive with an enhancing effect on itself; C, Orchards: high number of orchards are assumed with a stable increasing effect on *D. suzukii*, a stable effect on the crop and a negative effect on biodiversity; D, Crop: set to the median of the range because of the negative impact of *D. suzukii*. E: Concern: a medium effect of nature conservationists with little impact on the release of GDDS or on GDDS itself is assumed. G: GDDS dispersal: starts at zero and influences uncertainty. H: Uncertainty: medium starting point and affected by GDDS and GDDS dispersal.

The simulation 1 (Fig. 65) shows, that the elimination of the wild type *D. suzukii* can be successfully reached after some years (4 years in this simulation), but the risk of escape – the GDDS dispersal – is very likely. "GDDS" increases and the dispersal of GDDS cannot be stopped. Although conservationists "concern" and the "uncertainty" of this technique increase, these variables cannot influence or stop the development of the "GDDS dispersal". Additionally, "biodiversity", the main goal in the context with gene drive technique and the resulting ecosystem service "pest control" decreases continuously.

In partial scenario 2 (Fig. 65) and the corresponding simulation 2 (Fig. 65) it is assumed that biodiversity and pest control are very much reduced and therefore have no impact on the reduction of the population size of *D. suzukii*. Here, the ecosystem service "pest control" decreases fast and disappears after two rounds (years). "Biodiversity" still exists at a very low level. Because "pest control" does not exist anymore, crop also decreases more as in the simulation 1 where the impact of "pest control" is stronger.

Discussion of the partial scenarios and simulations:

The partial scenarios show the development of the system after the deliberate release of a suppression gene drive modified *D. suzukii*. The goal of the GDO release is the elimination of the wild type of this fruit fly. In the partial scenarios, GDDS has an enhancing effect on itself due to the gene drive organism's ability to override Mendelian inheritance. The population of GDDS and also the risk of escape respectively the GDDS dispersal increases.

In all simulations *D. suzukii* could be eliminated after some years. The problem of the gene drive application is the increasing population of the gene drive organism which probably cannot be stopped. Also, the risk of escape, in this case the dispersal with crop or through a high amount of host plants is very high and consequential there is high risk of dispersal of the gene drive *D. suzukii* in the native habitat of the wild form of *D. suzukii* in Japan.

The main protection goal in the system "*Drosophila suzukii*" in non-native regions, where orchards are common, is biodiversity, whereby the term biodiversity summarizes genetic diversity, structural diversity and species diversity. The evaluation of the ecological effect of the release of *D. suzuki* via the simulations show clearly that biodiversity is very important for sustainable agrarian land use. The absence of biodiversity causes the loss of ecosystem services, in this case the ecosystem service pest control cannot be maintained, which in turn results in crop failures. Roughly summarized species diversity and structural diversity enable the ecosystem service pest control. Genetic diversity can be affected through hybridization between genetically engineered and wild organisms which can change evolutionary effects in ways we don´t know and cannot influence. To use terminology of Vester (1999): the relevant

goal to increase and secure viability of the system, where *D. suzukii*. Occurs in orchards outside its native range, is biodiversity.

In the absence of concrete numerical data, we explored system analysis of Vester as a tool to gather and organize the knowledge about the system of a landscape with orchards that are suffering from the agricultural pest *D. suzukii* outside its native range. Although the description of the model ended up being very general, it was very well suited to derive risk hypotheses, which are implicitly described in the previous paragraph. However, the approach quite obviously cannot overcome the general problems of ecological risk assessment that knowledge about ecological processes is incomplete and data for testing risk hypotheses are lacking.

6.2.3 **Rattus norvegicus**

A second case of potential gene drive applications is provided by the genus *Rattus.* As rodents, members of the genus can play an important ecological role as prey and predator, which can cause high impact in areas where they are invasive. In particular on Islands they can cause severe nature conservation concerns by preying on native species which causes many of them to exist in endangered remnant populations. Gene drive is suggested as a conservation measure to minimise the impact of the introduced predator. Three species of the genus, *R. rattus* (black rat, ship rat, roof rat or house rat), *R. norvegicus* (brown rat) and *R. exulans* (Pacific rat or Polynesian Rat) can be invasive and constitute a major threat to biodiversity on islands where they are introduced (Campbell et al., 2015). *R. exulans* is probably native in South-East-Asia (Csurhes, 2016) and has its main distribution in tropical areas. Its occurrence on islands in the pacific can be traced back to the expansion of Polynesians. Although *R. exulans* has damaging impacts in certain areas, *R. rattus* and *R. norvegicus* are considered more harmful (Varnham, 2010). Hereby *R. rattus* has been identified as the most damaging rodent to island ecosystems, as a species that was very easily accidently displaced on ships (Banks and Hughes, 2012; Ruffino et al., 2009; Traveset et al., 2009). *R. rattus* is native to tropical and subtropical forests in South Asia (Jenrich et al., 2010) but also thrives in human-dominated areas (Shiels et al., 2014). In Europe *R. rattus* occurs mainly in the Mediterranean but rarely in free-living populations, where the native habitat requirements are trees and bushes. It is mainly associated with humans, where it lives in the houses or the roof of the houses and barns. Here, we focus on *R. norvegicus* because it is an important commensal species of humans with now worldwide distribution, and it has an important role in the ecosystem in Europe. It is the biggest of these three congenerics and has a worldwide distribution. It is up to 215 mm long and its average weight is about 240 g.

In the following we outline an example for a possible risk assessment, considering aspects of the framework worked out in this report. We summarize basic information about the wild type of *R. norvegicus*, information in relation to the gene drive application like the intended use and the gene drive technique. We outline the general and specific protection goals and risk hypotheses, which include – as proposed in risk assessments for invasive species – the risk of introduction and spread considering the possibilities to overcome barriers. Next to possible pathways to escape, receiving environments and impacts are elaborated. Pathways to harm are considered as mediated by population declines on ecosystems and ecosystem services in target areas and non-target areas, as spread in non-target areas with the risk of hybridization or unintended gene transfer. Endpoints relevant for assessment are considered and the possibility of thresholds and priorisations are discussed.

a. Ecological Characteristics

To conduct risk assessment that is related to the change of the ecological role of an organism's detailed information about the wild type *R. norvegicus* are needed, i.e. life history, reproductive biology, habitat requirements, spatial ecology, biotic interactions and genome characteristics. The average lifespan of *R. norvegicus* in the field is less than one year (Dieterlen, 2005). According to Telle(Telle, 1966) 45 % of the packs in Germany consist of more than 60 individuals mostly due to a family association with at least one initial pair. Immigrant rats may also live in the group (Dieterlen, 2005). However, they can also live as solitary animals and groups form in particular when food availability is high. *R. norvegicus* can live in the field but it prefers to live as commensal with humans. Female rats move for foraging in the fields up to 349 meters, male rats move up to 660 meters. In urban surroundings they only move 30 to 50 meters (Roguin, 1995). If the population's density is too high, the packs disperse, and new areas are colonised trough migration (Jenrich et al., 2010). The distribution of the Norwegian rat is explained by human accidental transport which can be interpreted as adaptation to humans as migration partner by which the commensal species could spread worldwide (Dieterlen, 2005).

Reproduction is possible during the whole year, especially in animals that are not exposed to strong temperature fluctuations, e.g. in sewer systems. The main period of reproductive activity is between March and June and between September and October (Dieterlen, 2005). The gestation period is 22-24 day. On average a female animal can have up to 5 litters per year with around 7 till 8 cubs. In extreme cases a female can have up to 55 cubs per year. After 20 days, the cubs can leave their nest, sexual maturity is reached at around 50 to 60 days of age. (Roguin, 1995).

R. norvegicus can be found everywhere, provided there is water nearby. In Europe, the brown rat lives near humans, especially in sewer systems, cellars and storage systems, in haystacks, riverbanks and lakeshores (Roguin, 1995). They can also live in the open field if climate and ground conditions are favourable. There they live in shallow underground burrows (Dieterlen, 2005). As omnivore species it has a high demand for water. Food sources are grains and fresh plant parts, they like fish and meat meanly from dead animals, but that is not so easily available. As predator the species catches young of free-living birds but also from poultry like chickens and ducks. They also eat eggs, nesting mammals like rabbits, insectivores, small rodents, amphibian, snakes, mussels and many other animals, especially invertebrates (Dieterlen, 2005). The displacement to formerly mammal free island where biota are dominated by birds can be therefore very damaging. It constitutes the introduction of a predatory species.

The Norwegian rat was originally native to Southwest Siberia and northern China (Long, 2004). It has now a worldwide distribution except the Arctic and polar regions. In more temperate regions as New Zealand the distribution is patchy, there *R. rattus* or *R. exulans* are more common. *R. norvegicus* is widespread throughout Europe with the exception of the Mediterranean and the high mountain regions (Jenrich et al., 2010). It mainly lives in underground systems as sewer systems and near humide/wet biotops (Quéré and Le Louarn, 2011). Natural enemies of the brown rats are cats and dogs. Furthermore, marten species such as stoats *(Mustela erminea)*, weasels *(Mustela nivalis)*, polecats *(Mustela putorius)* and stone martens *(Martes foina)*. Also, owls, especially the eagle owl *(Bubo bubo)* (Dieterlen, 2005).

b. Intended use of Gene drive and proposed techniques

One of the main drivers of extinctions and ecosystem changes on islands is the introduction of exotic rodents (Doherty et al., 2016). Recently, gene drive techniques were emphasized as conservation tool to control invasive species (Newcomb et al., 2017;Royal Society Te Aparangi

Gene Editing Panel, 2017;(Backus and Gross, 2016); Campbell et al., 2015; Leitschuh et al., 2018; Piaggio et al., 2017). For example, New Zealand considers using the gene drive technique to eliminate the mammalian pests that threaten its unique fauna and flora. The government of New Zealand has adopted a goal of being predator-free by 2050 that creates pressure on developing new methods (e.g., gene drive) for eradication. This concerns especially brushtail possum *(Trichosurus Vulpecula)*, mustelids (*Mustela ermine, M.nivalis, M. furo*), rats (*Rattus exulans, R. norvegicus, R. rattus*), and feral cats (*Felis catus*) (Russell et al., 2015)(Tompkins, 2018). Thus, the intended use of the gene drive technique is to reduce or eliminate the population of *R. norvegicus* on islands, where it is an invasive species and pest especially preying on endemic and endangered birds. The most promising potential gene drive technique for vertebrate pest control is the "daughterless" approach (Campbell et al., 2015). However, In the context with a male-biasing gene drive, there are knowledge gaps for sex-determining genes in black rats in Australia, and it is recognized that multiple copies of male-determining genes exist in the genome of the brown rat (Moro et al., 2018b). Especially the self-propagating CRISPR/Cas9 technique is considered very efficient.

c. The potential to assess risk of Gene Drive application in *R. norvegicus*

A gene drive in Rattus has potential impact on general and specific protection goals also if released in an environment where the species is not native. Here it is investigated how the RA framework can be applied to gene drive of R. norvegicus. This is mostly but not only related to the potential for entry a new environment, establishment and spread, the pathways, receiving environment and possible adverse effects.

General protection goals apply also to the system where gene drive is used for a conservation goal. This is the general protection of Ecosystem services and human well being. Specific protection goals and risks are related to the escape of the gene drive from the area of release. However, there are also specific goals like the retainment of a certain population size of the species for intrinsic reasons, like outlined by Shiels et al., (2014) who describes the cultural significance of rats and related value.

Most importantly, specific protection goals in the case of a gene drive that is conducted as a conservation measure against an invasive species, is the effect that the activity has on the environment where the gene drive is released. Also, here effect on general protection goals via e.g. biodiversity exist, but also the effect on the target of the activity. In case of gene drive application in nature conservation a specific protection goal exists in terms of the conservation target that might have a relative value compared to specific goals related to the gene drive (Moro, 2018). Especially for GDO release in geographic well-defined areas like islands protection goals can apply to the area of release and to an area where the drive can spread unintentionally. In case of control of invasive species, like in case of the brown rat, the ecological effects of eradication are part of the intent of the release. They are not in the native range of the species, so one major factor in RA is the estimate whether GDOs can escape or not. Prevention of escape can be hypothesised therefore as a major mechanism how protection goals might be affected.

d. Risk hypotheses for Gene Drive on *Rattus norvegicus*

There are a number of hypotheses what risks might be related to gene drive release on rats, which can be divided in two larger parts, first the risk of escape of GDOs from the target region and the other as adverse ecological effects after population size change in the target area or the non-target area. In case of rats, where gene drive is suggested as a control agent for non-native, invasive populations on islands, this corresponds to the invasive range and the non-invasive range. Within the draft of a framework this applies to adverse effects in the target area

(Fig. 54, step 2) and the non-target area (Fig. 54, step 5), and also include the risk of escape or accidental as well as intentional transport (Fig. 54, step 4). Within the protection goals ethical aspects (Fig. 54, step 3) can be hypothesised.

The general literature search in Web of Science used in paragraph 6.2.1 with the key words “gene drive”, was refined to subgroups, i.e. environment, agriculture, general and other methods and human health. To find risk hypotheses for the genus *Rattus*, publications were searched in the group of environments, refined for the keywords risk and invasive species. In particular eight publications were used, which are cited below in the context of description of the single risk hypotheses.

Risk hypotheses related to potential for entry, establishment and spread as important pathway to harm

Esvelt & Gemmel (2017) pointed out that creating a gene drive system is likely to be equivalent to creating a new, highly invasive species, which can spread to any ecosystem where they are viable and can cause ecological change. Factors, which influence the spread and persistence of a GDO are the population structure, potential barriers to breeding and gene flow, climate and resource availability, existing biocontrol, translocation stress in the presence of established conspecifics (Moro et al., 2018b). Even though not specifically investigated, it can be assumed that the outlined life history characteristics of *R. norvegicus* from its native range also apply for the species on islands. Due to the fact that many populations of *R. norvegicus* are invasive, it seems likely that also in the future it will be able to overcome potential barriers for introduction and spread. Such barriers are either ecological or abiotic, like survival and reproduction barrier, technical and environmental barrier, dispersal barriers, biotic and abiotic stressors at all development stages.

There are several ways gene drive rats could spread, although long distance dispersals of rats (black rat and brown rat) are uncommon and are mostly a result of resource limitations, high intraspecific competition and/or drastic environmental change (Feng and Himsworth, 2014; Gardner-Santana et al., 2009; Storer and Davis, 1953). Nevertheless, *Rattus norvegicus* can cross water gaps by swimming up to two kilometres (Bassett et al., 2016). Also, aircrafts and ships are transport possibilities and vectors for repeated introductions of rats, especially on routine routes travelled and regulatory of transport schedules (Shiels et al., 2014).

Esvelt and Gemmell (2017) assumed that *“invasive and self-propagating gene drive systems are likely to spread to every population of the target species throughout the world.”* When a gene drive rat escapes from an island, the risk that it occurs wherever the wild type of the rat occurs is high at least after a certain period of time. As a commensal species, the brown rat is distributed worldwide. The gene drive could spread all over the world causing adverse effects, e.g. decreasing populations of the wild type in Europe and cascading adverse effects in the food chain.

Hypothesis 1: Unintentional spread of the drive from the released site.

The first risk hypothesis related to transport and escape of a GDO rat is the possibility of unintentional spread. The application of a gene drive technique always includes the risk of escape or transport of the GDO to non-target areas. Due to multiple ways of connections in an ecosystem, ramifications to non-target areas are likely (Esvelt and Gemmell, 2017). Also on islands gene drive organisms would be present for several years and the possibility to escape or to hitch a ride to other islands and continents increase (Esvelt and Gemmell, 2017).

This can be can be caused by an accidental translocation or natural dispersal (Piaggio et al., 2017). “Rats are very good invaders, disperse well, and hybridise with closely related species, making the accidental release and spread of gene drive modified rats a serious consideration.”

(Royal Society Te Aparangi Gene Editing Panel, 2017). *R. norvegicus* can cross water gaps by swimming (Bassett et al., 2016) or have the potential for movement with commodities and conveyances, especially on routine routes (Shiels et al., 2014).

A suppression drive can spread from the invasive population in a target area back into the native habitat (Esvelt et al., 2014) because gene drives in their existing form are highly invasive (Moro et al., 2018b). *"….invasive and self-propagating gene drive systems are likely to spread to every population of the target species throughout the world."* (Esvelt and Gemmell, 2017). Considering the wide distribution of the species, this requires an assessment of effects of a worldwide impact on rat populations. Risks can emerge in association with the spread and persistence of a transgenic animal, influenced by breeding seasons, mating systems and reproductive biology (Moro et al., 2018b). In association with *R. norvegicus*, spread can arise quickly, because this animal has a high reproductive biology.

Hypothesis 2: Intentional spread of the drive from the release site

Similar in effect to unintentional transport or escape cases of intentional release especially with species that are considered a nuisance in their native range, gene drive carrying organisms could be illegally translocated and released. This potential is outlined in several publications as part of illegal intentional transportations by humans (O'Hara, 2006) or other intentional human actions (Esvelt et al., 2014).

Hypothesis 3: Risk of transfer to other species and hybridization

A special form of gene drive escape is the potential of horizontal gene transfer, the case that a gene drive is transferred to a closely related species by hybridization (Piaggio et al., 2017). There exists the potential of a horizontal gene flow or interspecific breeding – the transfer of genetic material from a donor organism to a recipient organism that is not its offspring, and the vertical gene flow – the unintended intraspecific breeding with conspecifics outside the target area (Moro et al., 2018b). Rare mating events increase the possibility of the drive to affect closely related species (Esvelt et al., 2014).

Risk hypotheses related to adverse ecological effects, like changed populations sizes and effects on ecological interactions

Ecological effects of a decline in population size of the species after a gene drive can be visible in the target area of the drive and in the non-target area after successful escape. Adverse ecological effects can thus consist of changes in interactions of species affecting specific protection goals in the target are, or more general impact on ecosystem function in the native range when the species is accidently impacted there.

Hypothesis 4: The elimination of the population of *R. norvegicus* on bigger islands has adverse ecological effects in the target area

Adverse ecological effects are e.g. negative changes to community processes, when a targeted invasive species is removed from the ecosystem (Moro et al., 2018) or other unanticipated ecosystem effects after a successful removal of an invasive species (Piaggio et al., 2017). Removal or eradication of invasive species can lead to unintended effects, as the establishment or increase of other invasive species (Zavaleta et al., 2001). For example, the removal of an exotic prey can lead to increasing predation on native preys by exotic predators (Zavaleta et al., 2001). Zavaleta et al. (2001) developed a conceptual framework to detect secondary effects of removing invasive species. Areas of considerations have been established: 1) the trophic cascade, 2) predator-prey interactions and 3) herbivore plant

interactions. Zavaleta et.al. (2001) give examples of adverse effects of removing invasive species on islands. The first example is about removing feral cats on Stewart Island, New Zealand, where they prey on the native parrot kakapo *Strigops habroptilus*, that would lead to the mesopredator release of rats, which also prey on the endangered kakapo. Conversely, if rats would be removed, cats would prey more on the endangered native flightless parrot kakapo. Mesopredator release can alter ecosystem-scale properties as well as native populations (Zavaleta et al., 2001). The second example concerns the exotic rats *R. rattus* and possums *Trichosurus vulpecular* in New Zealnd, which are part of the diet of the exotic stoats *Mustela ermina*. The removal of only rats or possums would result in an altered diet of the stoats to native birds and bird eggs (Zavaleta et al., 2001). Another adverse ecological effects could be a temporary rodent (e.g. *R. norvegicus*) population increase when releasing GD rodents (e.g. GD *R. norvegicus*), which can lead to permanent ecological consequences (Caroline M. Leitschuh et al., 2018).

Hypothesis 5: The reduction of the population of R. norvegicus in non-target areas leads to reduced ecosystem services

The risk of impacting ecosystem functioning by accidently eradicating rat populations by escapes is very closely related to the question whether GD carrying individuals will be able to translocate to the non-target area. In this case though, effects could be severe. The difference to the range where the species is not native is that it can be considered as an integrated part of the ecosystem and thus changes in population size might affect biodiversity related ecosystem services. It is difficult to estimate the effect of a declining population of rats in a natural environment. Highlighted could be the role of the species as part of the food web where it provides prey for a number of species like mustleids or owls (Dieterlen, 2005). However, it is also a predator, and an eradication could release some species from predator pressure. Contrary, the fast eradication of a prey species can lead to an overabundance of predator species and increases the pressure on alternative prey species. These effects are difficult to estimate. Although rats are pests to many people of the world, in other regions of the globe they deliver ecosystem services (e.g. pollination or critical elements of ecosystem food webs (Royal Society Te Aparangi Gene Editing Panel, 2017).

e. Endpoints and Assessment

The risk hypotheses imply certain endpoints that might be used for an assessment. Endpoint related to the first group of hypotheses should define or observe escape, or furthermore, excluding escape by monitoring or observation. In principle it has to be shown and proven that gene drive rats are not escaping. Practically, this can be only done indirectly, for example by observing natural populations and using the population size and their fluctuations as measure. The second possibility is the implementation of a genetic monitoring to find gene drive elements in the genepool of the native range. Genetic monitoring is also the only option to determine escape of a gene drive element across species borders by hybridisation.

In the target areas, obvious endpoints are population size of the target species and the population size of interacting species. General ecological endpoint like species composition and population density that are considered for mechanisms of recovery according to EFSA (2016), can here also be used to assess success of the measure and unwanted results. Functional endpoints according EFSA (2016) in form of ecosystem services as part of an assessment do not seem to be feasible with rat species, no clear services besides some specialised cases (Royal Society Te Aparangi Gene Editing Panel, 2017) can be related. This also impedes a clear definition of what level of population decrease can be considered harmful.

Knowledge gaps will hinder the future progress of gene drive work on invasive species (Moro et al., 2018). Moro et al. (2018) have identified knowledge gaps in association with rats, here shown for *R. rattus* within the Australian context, in the areas of spatial structure, population regulation and translocation biology (translocation into existing populations of conspecifics), and fertility control.

While it seems possible to monitor populations of interacting species in the invasive range, it is difficult to implement monitoring of rat population size and genetic elements. It is therefore also difficult to define standardisation of assessment endpoint at this stage of the scientific discussion.

6.3 Part B.3 - Potential of Ecological Models for Risk Assessment

6.3.1 Ecological Modelling for Risk Assessment of GDOs – Literature Research

Ecological models have been developed for many different purposes, but mainly for answering specific research questions rather than for practical applications, e.g. the prediction of the effect of management practices on the ecosystem (Schuwirth et al., 2019). Models can be in principle of two kinds – process-based (mechanistic) or data-driven (empirical), the latter relying on correlation. While process-based models can be better transferred from one area to the other, because they rely on explicit cause-effect relationships, they require a good knowledge about the system and the need for estimating many parameters regularly asks for data that are difficult to obtain (Dormann et al., 2012; Schuwirth et al., 2019). Empirical models rely on available data that does not necessarily reflect causal relationships but may lead to precise predictions, on the other hand may be misleading and are not considered reliable outside the range of calibration (Schuwirth et al., 2019).

Galic et al. (2010) presented a review of population models in the ERA of chemicals, which can serve as a starting point for exploring the potential of ecological models for ERA of GDOs. They reviewed 90 ecological models and categorized them according to the level of organization – individual, population or ecosystem level (Tab. 19). For ecological risk assessment of chemicals, population models are favored as in many cases the effect of a stressor on a non-target population is of interest. Population models can be further divided into individual-based models when the entity is the individual and the result of all the intra- and interspecific interactions and the interactions with the environment of many individuals sum up to the population development. Such models are cumbersome to build and acquiring data for fitting parameters may be very costly and time-consuming but have the potential of being closer to reality. A generally less data-consuming approach are models that use populations as model units, in many cases these populations are structured further in subunits that act or react differently to the stressor, e.g. different life stages (Galic et al., 2010).

Tab. 19: Number of ecological models reviewed by Galic et al. (2010) by level of organization and model type.

Level of organization and model type	*Number of models reviewed*
Individual-level	8
Population level	68
Individual-based models	17
Matrix and other stage structured models	44
Unstructured population	7
Ecosystem level	10
Individual-based models	1
Unstructured	9

Population models have been suggested and evaluated for ecological risk assessment of chemicals in 5 areas (Galic et al., 2010; Hommen et al., 2010), i.e. for extrapolation from the individual to population level, extrapolation to other exposure patterns, estimation of recovery processes of a population, prediction of indirect effects on populations of other species, and prediction of bioaccumulation. Even in the rather simple case of ecotoxicological risk assessment the models would need extensive data about the life history, the environment, spatial heterogeneity, and the interactions between these components. This information is lacking or scarce under most circumstances hampering the application for ecological risk assessment.

For ERA of chemicals, mainly population models were applied to predict the effect of the stressors on non-target populations (Galic et al., 2010). In the case of genetically engineered insects, David et al. (2013) argue to distinguish between two phases: the transitory phase, when the target population is changing rapidly and a steady-state phase, when the target population is stable. Population models may be applied to the target population to predict the success of the gene drive during the transitory phase but can also be applied to non-target organisms that may be adversely affected by the GDO during the steady-state phase, e.g. the removal of one species (wild type) from the ecosystem might have effects on several other species. However, such effects are not as straight forward and knowledge about species interactions in complex ecosystems is still scarce (Baker et al., 2019; Ballari et al., 2016; Estes et al., 2011; Säterberg et al., 2013). For example, eliminating invasive predators from the ecosystem, led to unwanted effects such as meso-predator or herbivore release (Doherty and Ritchie, 2017). Therefore, the need for an ecosystem approach in managing invasive species is increasingly acknowledged (Ballari et al., 2016). As we have pointed out the many analogies between invasive species and GDOs before, the need for applying an ecosystem approach for ERA of GDOs becomes obvious.

Galic et al. (2010) reviewed ten ecosystem models that have been applied to ERA and these were basically food-web models applied to freshwater systems (Tab. 19). A recent review article (Geary et al., 2020) acknowledges the chances but also the challenges for applying ecosystem models to management. Based on a conceptual model of interactions (interaction network), Geary et al. (2020) differentiate between three different mathematical approaches to tackle the modelling problem: Bayesian belief networks (interactions are represented as a chain of probabilistic events), Network theory (when the conceptual model is parametrized in a mathematically simple way), and Dynamical systems theory (when the interactions of the conceptual model is transferred into deterministic formulas using large data sets). The authors also clearly state that the choice of model depends on the model objective, i.e. the modelling approach must be appropriate for the decision or management problem. Uncertainty must be

considered and handled explicitly in ecological models. In fact, models provide an opportunity to deal with uncertainty. However, the more complex the model the higher the uncertainty related either to the structure of the model or the parameters (Geary et al., 2020).

Schuwirth et al. (2019) propose six requirements for models to support management decisions.

1. **There exists a basic mechanistic understanding of the system regarding causality, which is considered in the model.**
2. **The model input and output variables are aligned with the management question.**
3. **The model has an appropriate spatial and temporal resolution to address the management question.**
4. **The model uncertainty can be quantified.**
5. **The model has a sufficient predictive performance to be useful for the management problem.**
6. **The modelling procedure, its assumptions, and its deficits are transparently communicated**

The first requirement of a basic mechanistic understanding is crucial for any model (Schuwirth et al., 2019) but seems particularly important if the goal is to quantify risk. While much of the benefit of a model is that during the process of modeling much can be learned about the system (Wang and Grant, 2019a; 2019b; Geary et al., 2020), this benefit might not apply to the case of ecological risk assessment. When uncertainty becomes high in complex models, even if it can be quantified, it does not allow for an informed decision about ecological risk and the precautionary principle must be applied. The highest complexity and need for data have so-called end-to-end ecosystem models. These attempt to incorporate all the major parts of ecosystems, including biophysical, economic and social parts. Because of their complexity, the results of such models are usually not intended to be prescriptive management advice, but rather tools to understand ecosystem development based on different scenarios (Geary et al., 2020).

(Wang and Grant, 2019a) agree with (Walters, 1986) that the primary value of modeling in ecology and resource management is not to make precise predictions, but is rather to create representations of the true world against which the experience can be tested. Although ecological models can aid in natural resource management as they can structure our knowledge, data, and assumptions in a disciplined way, it is a myth that ecological models can substitute field studies in cases when these are too expensive or too dangerous (Wang and Grant, 2019a). Models can also be good tools to involve all stakeholders in the modeling process. So that the non-modelers understand the assumptions and uncertainties related to model predictions (Wang and Grant, 2019a).

After reviewing the literature of ecological modelling, we must conclude that the use of models in risk assessment remains, at least, problematic. In line with the conclusions drawn in chapters A2 and A3 (this report) the main obstacle is the lack of reliable data for a specific case in a specific environment. Furthermore, the basic mechanistic understanding of complex systems is rather poor and incomplete, i.e. only a few of the important ecological processes are understood. This is also shown by the fact that most of the reviewed ecosystem models concentrated on food webs. On the other hand, models that were used to describe gene drives did not consider biotic interactions with non-target organisms (see also A2 of this report).

Another issue with ecological models is clearly the interaction of processes acting at different levels of organisation. Even the relatively simple case of generic modelling the population development of a given gene drive and the wildtype (shown in A3 of this report) remains very far from accurate predictions in the real world. The expansion to ecological models that could aid ecological risk assessment would need at least the further consideration of spatial heterogeneity together with the integration of interactions with non-target organisms. The calibration of such a model for a given GDO in a given ecosystem appears very ambitious

given the lack of ecological data. Furthermore, such a model can hardly reach the predictive power required for risk assessment.

6.3.2 **Conceptual model to understand ecological risk**

In principle, the visualisation of the framework we presented in Fig. 61, constitutes a conceptual model differentiating between target and non-target area and different effectors showing five pathways and how they are interconnected. Within this model the effects do not act in a single direction but are organized in (feedback-) loops. As gene drive application is as much a political and socio-economic as an ecological endeavour, we included also socio-economic and ethical aspects. There are five basic-, however, interconnected pathways. (1) the direct effect of the GDO in the target area on the wild type (intended effect), (2) the effect of the reduced population size on the ecosystem and on ecosystem services within the target area, (3) the effect of (1) and (2) but also (4) and (5) on socio-economy and ethics including the resulting effect on the acceptance of the gene drive technique and the management goal, (4) the escape including all mechanisms to accidently overcome the restrictions of the drive, finally leading to (5) the effect on the population size and following ecological effects and effects on ecosystem services in the non-target area – we expect here a feedback between population size and establishment. The framework is expressed for a geographically restricted suppression drive, other forms of escape, e.g., horizontal gene transfer can be treated analogously.

To further explore the conceptual model, we modified it slightly and transferred into a causal network using the Vester program (Fig. 66). The figure shows effects of a deliberate release of a suppression gene drive in form of feedback loops.

a. Conceptual Model using a Causal Network of the Vester Model

To transfer the conceptual model into the Vester program, we had to define all the variables within the model (Tab. 20).

Tab. 20: List and description of the variables.

#	Variables	Description of the variables
1	GDO Established	The gene drive organism has been established.
2	Population Size 1	The population size of a target species of the GDO application.
3	Management Target	This variable represents the specific goal of the release of the gene drive organism. If the population size of the targeted species decreases, the management target has been reached.
4	Release	The deliberate release of a suppression gene drive.
5	Adverse Ecological Effect 1	An adverse ecological effect caused by the reduction of the population size of a target species.
6	Ecosystem Service 1	A certain ecosystem service influenced of an adverse ecological effect caused by the reduction of the population size of a target species.
7	Ethic Concern	This variable describes common ethical or conservationists' concerns.
8	Acceptance	Acceptance of deliberate releases of GDOs
9	Socioeconomic Goal	The socioeconomic goal behind the management target (e.g. a good harvest…).

#	Variables	Description of the variables
11	Establishment	Establishment and spread of a GDO in a non-target area.
12	Population Size 2	Population size of a non-target species in the non-target area.
13	Adverse Ecological Effect 2	Adverse ecological effects caused by changes in population sizes of non-target species in the non-target area.
14	Socioeconomic Effect 1	Impacts on economy and society caused by changes (reduction) in population size of target species and the consequences.
15	Socioeconomic Effect 2	Impacts on economy and society caused by changes in population sizes (reduction or enhancement) of non-target species and their consequences.
16	Ecosystem Service 2	Decreasing ecosystem service as effect of adverse ecological effects in non-target areas.

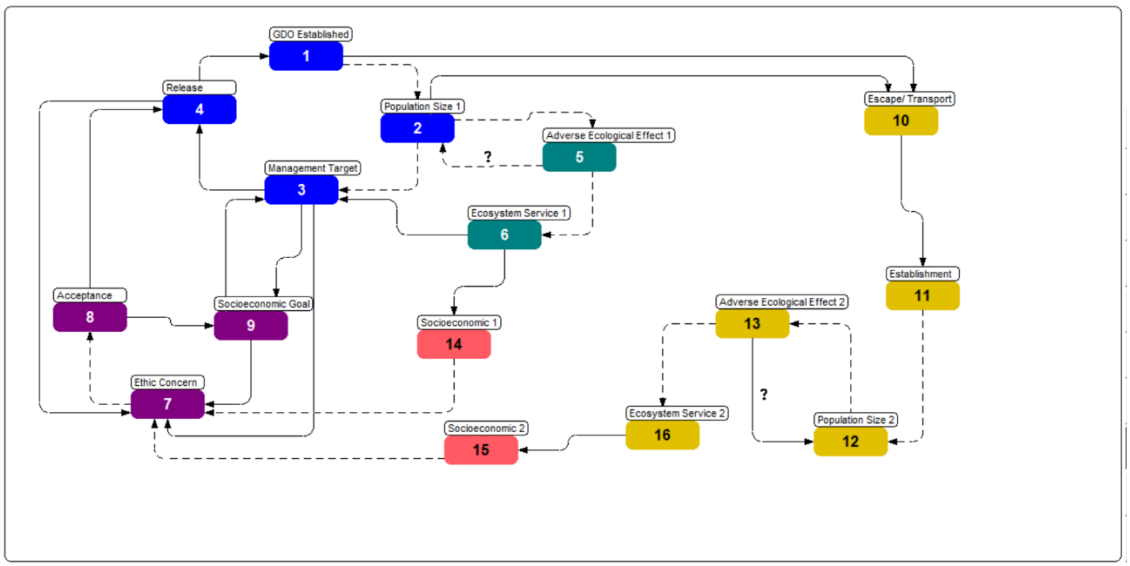


Fig. 66: Conceptual model determining population sizes and constituting potential effectors to develop risk hypotheses, shown as a causal network with outgoing and incoming vectors and feedback loops:
A solid arrow indicates a direct relationship, a dashed arrow indicates an inverse relationship, The different coloured elements (blue, green, violet) indicate different loops in the target area as described in figure 9 (see pathways 1, 2 and 3), yellow elements refer to variables showing a cascade effect in the non-target area (see pathways 4 and 5 in Fig. 61), the red variables describe socioeconomic effects.

The blue loop describes the release of a suppression gene drive and as result the established GDO, which causes a reduction of the population size of a target species. The decreasing population size means that the management target has been reached. Reaching the management target ensures the ongoing release of the GDO. The blue loop is therefore the “management loop” for reaching the desired objective. The green loop illustrates potential consequences for the environment of the target area, if the decreasing population size leads to adverse ecological effect(s), which potentially reduce specific ecosystem services. An unstable or reduced ecosystem service in turn has negative effects on the actually desired management target.

The conceptual model furthermore shows that reduced ecosystem services have negative impacts on economic and sociological life and raising ethical concerns, although ethical

concerns exist from the beginning because of the conservationists' basic attitude towards GDOs. If concerns arise, the acceptance of the release of GDOs declines which in turn affects the socioeconomic goal.

If the GDO has been established, the risk of an escape or a transport exists. This also includes the risk of vertical or horizontal gene transfer. The potential of establishment/spread rises and also cascade effects in the non-target area, beginning with changes in population sizes of non-target species which entails adverse ecological effects and fragile ecosystem services. This again influences economic and sociological life which in turn has effects on the ethic concern and acceptance of the GD release.

The conceptual model can be used to derive the following hypotheses about pathways to harm:

1. **The decreasing population size of the targeted species has adverse ecological effects.**
2. **The ecosystem service is decreasing because of the increasing adverse ecological effect.**
3. **A reduced ecosystem service has negative impacts on the management target.**
4. **Reduced ecosystem services negatively impact economic and social life.**
5. **The risk of escape or transport of a GDO increases with the establishment of the GDO.**
6. **The establishment/spread of a GDO in the non-target area increases with the higher risk of transport or escape of a GDO.**
7. **The establishment of a GDO in the non-target area impacts population sizes of non-target species.**
8. **Changes of population sizes of non-target species cause adverse ecological effects.**
9. **Adverse ecological effects in non- target areas cause unstable, fragile or decreasing ecosystem services.**

b. Simulation and Partial Scenario within the Target Area

To further illustrate potential pathways to harm, we used the tool for simulating partial scenarios within the Vester program. Fig. 67 shows the elements we chose for a partial scenario within the target area. The program allows for the input of curves on the effect of one variable onto the other. These curves can consider a change in effect with changing quantities of the effector (non-linear effects). For example, if the effect of the GDO on the wild type is increasing with higher population size of the GDO. However, this non-linear behaviour is not defined by mathematical equations but graphically. For the start of any simulation starting points for all the variables have to be chosen. The simulations are therefore based on the knowledge on relationships between two variables and visualize the resulting network. The simulations are carried out in several rounds (i.e. years) and as with every round the starting values change for the variables, the simulation is progressing.

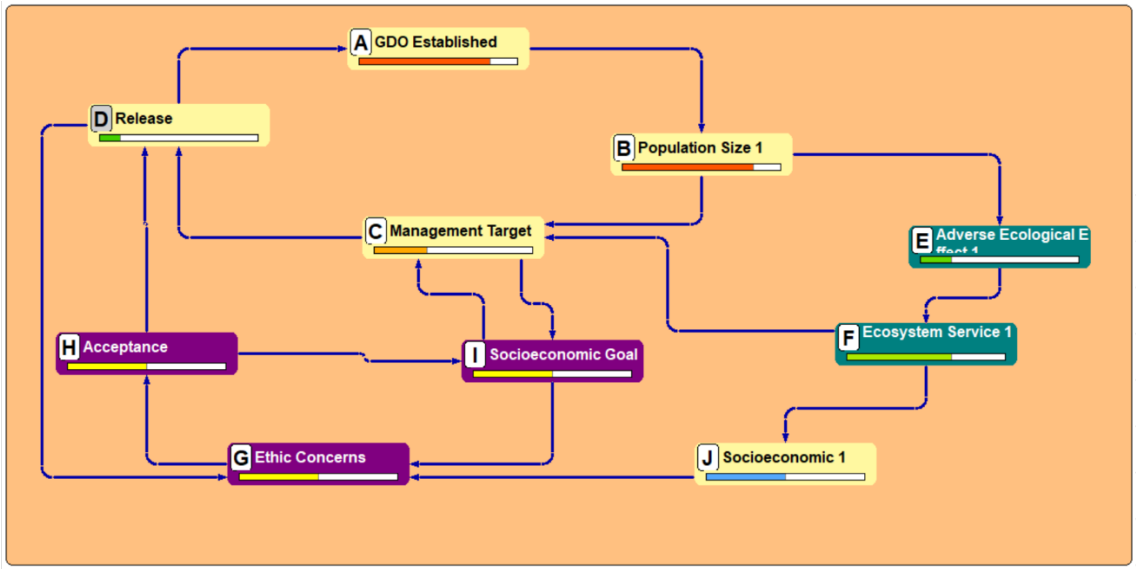


Fig. 67: Partial scenario of the conceptual model (Fig. 66).
A: GDO Established: In this simulation it is supposed that the established GDO starts at a high level and will stay at a high level during the following six rounds. B: The starting point of the population size of the wild type is also very high but decreases quickly and disappears after two rounds, because the GDO is very effective. C: The management target starts at a low level because at the beginning of the simulation the management target is not achieved. D: The deliberate release of a suppression gene drive starts at a low level and stops after two rounds (it´s not necessary anymore because of the GDO established). E: At the beginning, the adverse ecological effect is low. F: The starting point of the ecosystem service lays at a high level. I, G, H: The starting points of the socioeconomic goal, the ethical concerns and the acceptance start in the middle. J: The socioeconomic effects were started in a middle range.

In our scenario, after a few rounds, the population size of the target species is zero and the release of the GDO has stopped, but the GDO established is at a very high level (Fig. 68). First it seems that the management target has been reached, but after the second round, the management target begins to decrease, because it is also negatively affected by the decreasing ecosystem service. The ecosystem service decreases quickly as result of increasing adverse effects; the simulation should show the hard effects in a living system of disappeared ecosystem services (synonymous for ecosystem functions or biodiversity). The socioeconomic life is influenced and therefore, the ethical concerns arises and the acceptance of the socioeconomic goal, which should be reached with the GDO release also decreases. But at this moment, there is no chance of stopping the development.

Since the simulation is based on our very general conceptual model the simulation is only meant to give an example of how our conceptual model could be used to assist in organizing the knowledge about a system. It is not intended to substitute lacking knowledge but should rather aid in identifying knowledge gaps and creating hypotheses.

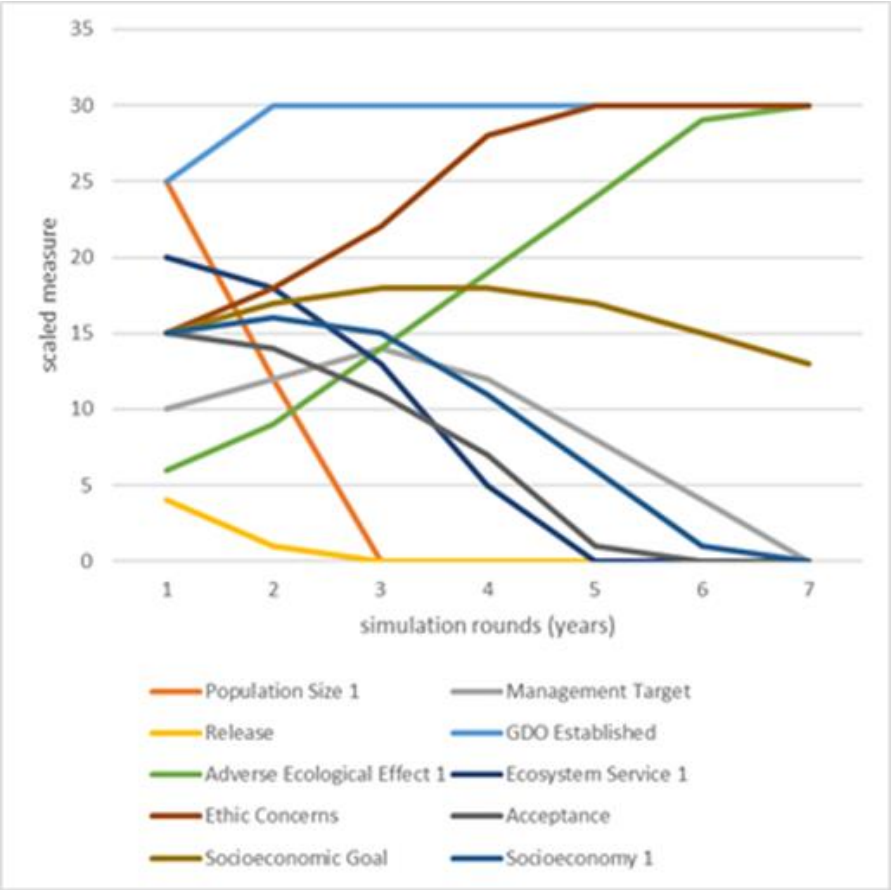


Fig. 68: Simulation of the partial scenario.
Development of variables during the simulation period of 7 years.

c. Further Aspects to Consider

The conceptual model should be expanded also to effects related to time, i.e. the temporal scale has to be included. We agree with David et al. (2013) who differentiate in the case of genetically modified insects (but the same does apply to GDOs) between the transitory phase and the steady state phase. In the transitory phase, the target population changes rapidly in density. This phase can cause ecological interactions but also evolutionary effects (David et al., 2013). Ecological interactions can result of a GDO that takes on ecological roles, e.g. as resource, as consumer, as competitor or as disease vector (David et al., 2013). Evolutionary effects could be the resistance to a control tactic, e.g. at the SIT program, wild female melon flies evolved to reject mating attempts by released sterile males (Koyama et al., 2003). Further evolutionary effects can evolve because of interspecific gene flow through mating, hybridization or introgression between genetically engineered and wild organisms or intraspecific gene flow. Ecological and evolutionary effects also occur in the steady state phase, a phase, which represents the effects after a situation has established. Evolutionary effects concern community genetics caused by changes in selection pressures (Myers and Knoll, 2001), evolution after species invasion (Sax et al., 2007), altered evolutions of virulence and transmission or higher pathogen virulence caused by suppressing vector populations.

In this respect we would like to point out that for many ecological interactions on the individual, community, or ecosystem levels, data are scarce. Because of the many ecological interactions also unknown ecological effects are likely to occur (unknown unknowns). Therefore, decisions on managing ecosystems have always to be made with a certain degree of uncertainty, and therefore, uncertainty has to be explicitly dealt with. If the degree of uncertainty becomes too

high, the precautionary principle must apply. Within the case studies, we have shown that the uncertainty regarding the application of GDOs is very high. Below, we elaborate on the new dimension GDOs are adding to ecological risk.

6.3.3 The Suitability of the Environmental Risk Assessment Paradigm for GDOs

a. Established Assessment Schemes as Comparator

During preparation of this report we became aware of a new publication that link GDMI release to known insect eradication activities, eradication meaning the suppression of populations over a wide geographical area (Romeis et al., 2020). The experience with these measures and the observed outcomes could provide comparators for GDOs and could be used to inform Environmental Risk Assessment (ERA) procedures to be developed for GDMI release. The paper focused on agricultural pests and their control but could also be used for vectors for human diseases. Many examples for eradication come from mosquito species (e.g. *Aedes* and *Anopheles* mosquitos). The study also triggered press coverage summarizing that no new environmental risks are expected when applying gene drives (e.g. Standard form 23.4.20). Hereby, the known risks constitute more precisely risk hypotheses, some of which might reflect a rather high impact on the environment. The general result is quite similar to the EFSA Expert opinion draft which we discuss in the following.

Several activities are identified that had been related to eradication of insect populations using insecticides, hereby counting the chemicals used to area wide eradications and also biological agents like viruses, classical biological control as the release of a predator or parasite specific to the species to be controlled, genetic control methods including sterile insect technique SIT and cytochrome incompatibilities induced by Wolbachia, the use of pest-resistant GM crops describing the effect of bt Maize on eradication of populations, and the use of GM insects. The latter example, citing an eradication program using a repressible lethal genetic construct to eradicate *Ae. aegypti* populations. Insecticides are covered with ERA procedures and the eradication aspect is only a minor concern while the ecotoxicological effects are in the foreground. With GM crops, like bt Maize, the eradication is also a side effect of the activity. Therefore the paper considers, biological control, genetic control and GM insects and discusses the consideration of factors that had been used for risk assessment or provided observations that can be considered in a risk assessment for GDMI (Romeis et al., 2020).

There is a significant overlap between this paper and the latest EFSA draft for how GDMI should be treated and the identification of comparators in the review (Romeis et al., 2020). Related to this are the results of the stakeholder workshop reported in (Devos et al., 2020). The draft of the expert opinion is consequently mainly on the risk assessment of GM insects and to what extent the ERA developed in this context is applicable (EFSA, 2020). The comment we were directing to the draft is provided in this report below. Intriguing for these analogies is that similarly to gene drives, the eradication is reached not by the release of a chemical or mechanic device like trapping or a land use change but by the release of organisms. This makes the systems look similar but is a technicality considering the quality of gene drive organisms. We will elaborate on this later.

The review, (Romeis et al., 2020) and the draft, (EFSA, 2020) indicate the state of the art as envisaged by EFSA for the treatment of GDMI. Future development of suggestions how to treat GDMIs are likely to be based on this. Authors of the review were members of the expert panel and the review provides similar conclusions, even if much more detailed and scientifically funded. What we can take out of these documents is that the similarities to invasive species does not play a role in the newest developments. In addition, we can outline the protection goals, the envisaged risk hypotheses, and the pathways to harm.

Generally, four types of harm must be considered: 1) direct effect on humans, either by the released organisms, comparably to an toxicological effect, or by the pathway of their construction especially for sterile insect technique that used radiation; 2) direct effect on the environment by the released organisms, and 3) effect of the eradication, means the effect on the environment once the goal of the activity is reached, and 4) effect of an unintended eradication and the possibility that this happens. The last point includes all risk hypotheses that are related to an escape of a restricted gene drive, be it geographically, genetically, or temporally.

To clarify the last two points, we need to differentiate more clearly between eradications of invasive and native populations. The differences have to be considered between the ecological role of an organism within its native range, including areas where it can potentially expand its range to naturally, and its ecological role within the range where the species had been introduced to and is invasive. We would, therefore, like to make the following differentiation: eradication of populations of invasive species in their non-native range, and eradications of populations of species in their native range. The latter can be further divided into intended eradication and unintended eradication, for example of an eradication attempt in the non-native range that spread to the native range. We refer to it in the following as eradication of invasive populations, native populations and source populations (as in source for the invasion).

b. Similarities between the assessment of GM-Insect and GDO

The highest level of similarity of gene drive organisms is seen with genetically modified insect (GMI). Examples mentioned are *Aedes aegyptii* and the example of modified mosquitos that had been reported to hybridize and outcross unexpectedly (Evans et al., 2019). Risk in this context is hybridization and horizontal gene transfer, in addition, it had been hypothesized that outcrossing of the genetic construct might lead to a fitness increase of the recipient population. This is one example where impact on evolutionary parameters are taken into consideration. However, the long-term effects are still difficult to estimate, and fitness increase can experimentally only be tested for a few generations and only for a few fitness components and traits. The influence of the genetic background from the population used for the modification might also play a role here (Evans et al., 2019).

Problem formulation, risk hypotheses and pathways to harm identified for GMI are considered as applicable for GDOs and have consequently been regarded as in principle suitable as base for risk assessment for GDOs as result of the expert opinion (EFSA, 2020) forwarded for commenting in April 2020. The introduction specifically outlined as one of the questions to be answered by the experts if the current frameworks developed for GMI would be applicable for GDO and if the procedure should be adapted and to what extent. The general conclusion was that there are in principle no new qualities of risk associated with gene drive. Even if the framework provides a blueprint on how risk might be evaluated, there remains the question of scale as also mentioned in (Romeis et al., 2020): Under the worst-case scenario, the easy implementation of a low threshold suppression drive, a high number of eradication programs might be suggested. Successful eradication programs in the past were very elaborate. The screw worm eradication has been a several decades long effort involving propagating sterile individuals with controlled releases. The latest programs of modified insects like mosquitos in Brazil (e.g.Carvalho et al., 2015) were expensive programs and unlikely to be widely applied. The possible socioeconomic benefit that can be generated by the eradications is too low for species that are not having a wide economic impact. Therefore, so far vectors of diseases are considered and invasive species like *D. suzukii* that were already under investigation as target for the already established methods (e.g. SIT). The facilitation eradication programs could experience with low threshold suppression drives might result in an increase in scale that might suggest a different approach for risk assessment. In this respect, it had been highlighted that

a cost benefit analysis is necessary and should be included in the regulation process (Romeis et al., 2020).

When scale is considered, the question is, if current treatment of risk is appropriate. Therefore, the framework and paradigm have to be adjusted and challenged. Also, when the existing frameworks are accepted as applicable, several questions remain that should be taken into consideration:

First, are the risk hypotheses that had been applied to the traditional methods already used to formulate risk mitigation, thus, are they already taken into consideration in any form of risk assessment?

Second, are the risk hypotheses that had been outlined for the traditional methods complete? i.e. are there some observations in nature that can be used to develop hypotheses that not yet had been taken into consideration for the traditional methods?

And third, is there a quality of the upcoming techniques that, even if fitting into established risk hypotheses, allow alternatively to formulate new ones?

Below, the argument is made that the expected change in scale requires the formulatation of new risk hypotheses and also make the traditional ERA paradigm difficult to apply.

c. Implications of the origin of the current ERA Paradigm

The current tradition of developing risk hypotheses, also applied to the impact of GMOs, has its origin in the ecotoxicological assessment of compounds released into the environment. Above we outlined that already, with the main argument of inability to experience additive or synergistic effects when monocausal small effects are looked at individually. One of the basic metaphors in conservation is illustrating this and forms the base for the so-called rivet-popping hypothesis. It is, like the name suggest, about somebody popping rivets from the structural elements of a plane during flight. When observed that this might affect the safety of the trip, the answer “Don t worry, this had been done the whole day and nothing happened”, is not assuring. The risk results from the additive effect of small, singly insignificant incidents, that only in its entirety causes a threat to the system. The hypothesis had been formulated in the context of species extinction and was and still is challenged by arguments about prioritizing species with important functions over others to increase stability or resilience in an ecosystem. This argument (redundancy hypothesis, e.g. Walker, 1992) seems to be still used as a base of the ecosystem service arguments, however, it is only valid in a conservation context when resources are restricted and have to be applied to the most effect. An important part of the rivet metaphor is the uncertainty that is related to the role of the rivets. From time to time also an essential one will be removed (Ehrlich and Walker, 1998).

Regardless, the deliberate removal of species is a completely new quality that had not been taken into consideration in these arguments. Contrary to the rivet or redundancy discussion which can also be extended to populations, the eradication using GDOs is not collateral damage of management, but target. So, the question is not if extinction of one or the other species, has to be accepted on some occasions but how it can be organized as a goal. This context is not explicitly considered in the publications we reviewed so far. The definition of eradication effect that is in all publications noted as negligible, the impact of the removal, mainly outlined for mosquito species as native populations could not be seen as a clearly negative impact and therefore no argument could be found against it. One formulation about the eradication of *Ae. aegyptii* using a GMI strategy was that the species has a negligible role in the ecosystem because it is rather poor in individuals, and a specialist parasitic species on humans. Because it is no keystone species it has no role that would not be taken over by another species. Not only can this be debated, because the effect of a keystone species is specifically a relatively high impact compared to its biomass, but also only because the single

effect of the species is difficult to assess (Romeis et al., 2020 and references therein). Also, the quality of keystone species of such groups is difficult to assess. The ecological role of the species certainly goes beyond their participation within food-webs. The founding of Rome on seven hills, instead of the wet hollows between them and the riparian areas around the river might be very well related to vector borne diseases depending on the ephemeral wetlands, making the vectors a keystone species role model: a very small biomass with a very large effect.

The novelty is furthermore underlined because the eradication might be easily reached with the promised facilitation of the technical procedure, as worst-case scenario the low threshold suppression drives, or replacement drives that affect fitness. When we look at the comparators and the effort that previous programs demanded to successfully perform an eradication, the application frequency of such techniques was always supposed to be low. In this context the question of the ecological role of the species is appropriate: when only a few species are eradicated then a distinction between their ecological role makes sense. It might prevent the accidental removal of a keystone species, but in most cases the removal of single species will be regarded as having no visible impact. Using the rivet metaphor: with the removal of only the one or the other rivet during a trip, the chance of removing a very essential one is quite low, and nobody might get too nervous. With eradication in terms of suppression or replacement is more frequent or even becomes the standard in pest management, it might be different. Not only the chance to remove a rivet that is unnoticed essential increases, but also the chance that the amount of removed rivets causes overall damage. The risk involved is therefore not defined by the single activity but by the sum of activities planned, involving among other things the establishment of a baseline at time of implementation of the new technique.

The critique we formulated above about the ecotoxicology focus of risk assessment implies that the problems with additivity are not restricted to gene drive applications and also apply to other forms of management. The study of Hallmann et al. (2017) was very prominently illustrating that the current view of how the environment is impacted has to be questioned. The study, known as the Krefeld study, shows a biomass decline of insects in conservation areas. This indicates that effects from other areas, ecological cascades, or implications for food webs influence the populations in an unprecedented way. Even though, the study and subsequent similar studies and reviews tried to pinpoint the observation on specific drivers or causes (e.g. pesticides, climate change) the main message is that many factors that are part of an environmental impact assessment like land use patterns and agrochemicals obviously failed to predict this development. Eradication programs based on gene drive will add here an additional factor and constitute “new rivets to be pulled”. In this light, the current risk assessment paradigm does not seem to be suitable to reflect the risk that is associated to environmental impacts that can be explained by these models. The Krefeld study marks the initial point of a paradigm shift in biodiversity conservation that now allows to critically challenge traditional procedures. When taking this shift into consideration we should try to formulate more informative risk hypotheses.

Above, we already outlined the impact of additive small effect and the inability to reliably take negligible effects into consideration (Fig. 54 and Fig. 55). We now try to translate this into suggestions to include this into risk hypotheses, hazard descriptions and problem formulations. When we look at it more inclusively, the risk of the additive effect of small steps can stem from a scenario of a tipping point after which a certain ecosystem service is not available anymore (for the sake of the argument we stay with the measurable endpoint of ecosystem services, even though we are quite critical about its applicability, as outlined in a previous chapter). Alternatively, it can stem from a gradual degradation of the service, making it difficult to detect if no baseline is defined upfront. Both pathways will not detectably be impacted by the removal of a single species if it does not have a high visibility and obvious ecological interactions. Of course, risk hypotheses will have to include the effect by the released individuals and the effect of the suppression / replacement of the target species. Nevertheless, when the removal of a

species constitutes a potential hazard and the probability that the hazard causing harm constitutes risk, the risk will increase with every gene drive application within the species, geographical area, areas where transport occurs into, or whatever escape scenarios we can imagine. This adds a new variable to current risk assessment which probably would have been very well to be included also in traditional approaches and in particular in the ecotoxicological derived paradigm.

6.4 Evaluation of Ecological and Conservational Impacts – Summary

The main goal of Block B was to evaluate potential adverse effects the release of Gene Drive Organisms (GDOs) poses on the ecosystem and biodiversity. Therefore, we reviewed current approaches used to define and assess risk and worked on suggestions how GDOs can be integrated into risk assessment. The task was divided into three parts, (i) reviewing approaches to define protection goals, (ii) finding ways of framing Ecological Risk Assessment (ERA) for GDOs and applying it to two case studies, and (iii) exploring the potential of ecological modelling as tool used in ERA of GDOs. Finally, we concluded on an evaluation of how the current paradigm of ERA is applicable to the case of GDOs.

The definition of general protection goals is relatively straight forward and can be derived from legal documents of international, European, and national treaties. Based on the analysis of all the relevant agreements, there are two general goals; biodiversity and human well-being. More difficult is the identification of specific protection goals that are measurable, which is needed for ERA. In the existing, mainly ecotoxicological framework of ERA, these are also called measurement endpoints. Because the link between biodiversity and human wellbeing can be explained well by the ecosystem service concept, the recent tendency to define specific protection goals goes towards using concrete ecosystem services to derive measurement endpoints. We criticize this tendency because i) although through the ecosystem service concept it can be argued that maintenance of all biodiversity is providing all the ecosystem services, it does not necessarily work the other way round; ii) ecosystem redundancy could be used to argue that a concrete species could be removed from the system without losing a specific service; iii) unknown cascading effects of species removal are not taken into account; iv) a slight but regular adverse (non-significant) effect over a short period of time might still sum up to a negative impact over longer periods. The latter argument questions the definition of harm used in ERA in general and does apply to all specific protection goals, e.g. population size of any species. We provide a simulation for a hypothetical example.

The framework of current ERA the problem formulation phase is playing a crucial role, as it is this phase, when all the important information is gathered to assess potential adverse effects of the stressor on the environment. However, GDOs in many ways resemble invasive species as they are designed to spread and how they influence the ecosystems. Therefore, we explored the analogies between invasive species and GDOs.

The intentional or unintentional spread of invasive species illustrates that local containment of GDOs in a globalized world may be unrealistic. In addition, experience from failed containment of biological control (e.g., Rabbit Haemorrhagic Disease was brought to New Zealand by farmers) shows that GDOs will likely be deliberately brought into other regions. Therefore, GDOs have aspects of different concepts for risk assessment, related to their effect on populations and risk of spread. Like invasive species GDOs may alter biological interactions within an ecosystem, leading to cascade effects within and outside the ecosystem they were originally released in. For example, known effects of eradication of predators include mesopredator release, herbivore release, disruption of predator social systems, and compensatory immigration. These different aspects of GDO are difficult to implement within one conceptual framework. Therefore, we identified three different fields of risk:

(1) the effect of population declines on ecosystem and ecosystem services. This includes effect on species interacting with the target species, other cascading ecological effects, and not desired effects related to population size development of the target species. (2) the risk of escape of the GDO into other geographical regions, i.e. overcoming geographical barriers. This is mainly relevant for applications where gene drive should be restricted to parts of a global range of species. (3) the risk of transfer of the gene drive to non-target populations or other species by hybridization independent from geography.

We developed a conceptual model for risk assessment of GDOs based on the analogies to invasive species and the fields of risk. As gene drive application is as much a political and socio-economic as an ecological endeavor, we included also socio-economic and ethical aspects. There are 5 basic-, however, interconnected pathways that are acting in loops. (1) the direct effect of the GDO in the target area on the wild type (intended effect), (2) the effect of the reduced population size on the ecosystem and on ecosystem services within the target area, (3) the effect of (1) and (2) but also (4) and (5) on socio-economy and ethics including the resulting effect on the acceptance of the gene drive technique and the management target (4) the escape including all mechanisms to accidently overcome the restrictions of the drive, finally leading to (5) the effect on the population size and following ecological effects and effects on ecosystem services in the non-target area – we expect here a feedback between population size and establishment.

Further analysis of the conceptual model, also using it for the two case studies showed that many of the data needed are lacking and that much of a potential risk assessment would have to be done with high uncertainty. In addition, many of the processes are not understood well. Ecological modelling could help to increase the understanding of processes but by no means can be a substitute for lacking data. The notion that modeling could be used instead of field studies must be dismissed, as well as the idea that ecological models could provide precise and unbiased predictions for measurement endpoints, i.e. specific protection goals.

Finally, we discuss the applicability of the current ERA paradigm to GDOs referring to a paper from Romeis et al. (2020). We argue that GDOs do bring a new quality, because of the combination of effects they can have: deliberate eradication of a species in the target area, escape to non-target areas and or other species. Above, we already outlined the impact of additive small effect and the inability to reliably take negligible effects into consideration. Given the ongoing biodiversity crisis, any ERA framework should account for ecological effects that may not be obvious but may cause harm on the long run, regardless of the technique used. We do not think that this is the case in any of the current frameworks. However, when the removal of a species constitutes a potential hazard and the probability that the hazard causing harm constitutes risk, the risk will increase with every gene drive application within the species, geographical area, areas where transport occurs into, or whatever escape scenarios we can imagine.

7 Part C - Monitoring of Gene Drives

Kathrin Pascher

7.1 Gene Drives – Relevant Aspects in the Context of Monitoring

Synthetic gene drives (GDs) are currently being developed to minimize population sizes, eradicate whole populations in the wild or to rapidly incorporate and establish targeted traits in wild populations. Using this technique, genetic information is spread with **higher probabilities of inheritance** in comparison to the Mendelian inheritance theory. In contrast to classical genetic modification methods, the use of synthetic gene drives is not intended to modify domesticated crops and livestock, but to **modify wild populations with a focus on animals**. In this respect, target organisms and target locations differ in most cases completely between genetically modified organisms (GMOs) and gene drive organisms (GDOs). Gene drives can either be used to spread artificial or modified traits (*population modification/replacement*) within wild populations or even with the aim of eradication of an entire population (*population suppression*). The targeted traits are obtained either by modification of existing genes or by introducing new genes. In most cases, a **fitness disadvantage** for the organism or the entire population is initiated which is new compared to classical GMOs. Synthetic gene drives rely on sexual reproduction and generation change. For their rapid spread, it is beneficial, if the organism has a **short life cycle** and produces **many offspring**. Gene drives are no new invention by humans. They also occur naturally and have already been proven for several species (e.g. red-brown rice flour beetle *Tribolium castaneum*: Beeman et al., 1992). These naturally occurring drives are largely based on selfish genes. Hastings (1994) considered these genetic elements also for application in synthetic gene drives (see chapters 3.1 and 5.5). In contrast to synthetic gene drives, however, the naturally present gene drives in those species have been **evolutionarily tested** and are already part of the genetic inventory.

The various synthetic gene drive systems, which are currently developed and tested only under laboratory conditions or are still in a theoretical development phase, can be classified as *active* or *passive systems* with regard to their distribution dynamics, depending on their mechanism for achieving disproportionate inheritance of the artificially incorporated traits (see Block A.0: Technical characterization of Gene Drives). Active systems interfere actively with the genome of the organism (e.g. DNA repair process dependent copying of the own sequence). Instead, most passive systems rely on toxin-antidote combinations to ensure that the survival of embryos of a GD carrier is dependent on the drive sequence (Frieß et al., 2019). With regard to the potential of their spread dynamics, gene drives are classified as *self-limiting* or *self-sustaining systems*. The effect of self-limiting gene drives leads to a **reduction in the spread of the gene drive system** within a population. This class of gene drives only persists for a limited number of generations or then disappears completely. By contrast, self-sustaining gene drives persist and thus have the **ability to invade and persist in non-target wild-type populations** (Alphey, 2014). Moreover, gene drives are also categorised according to their *threshold value* which corresponds to the discrimination into local and global gene drives (see chapters 2.1 and 2.3). The threshold reflects the percentage of released GDOs in relation to the total population. In this process of categorisation, different gene drive techniques have **different degrees of freedom** concerning their spatial and temporal spread i.e. **in their invasiveness and their potential or range to spread across target areas, time periods, populations and hybridisation partners**. Due to this '*boundlessness*' and the increasing complexity of genetic, organismic and ecological interactions, the consequences at all these mentioned levels can only be assessed inadequately or even not at all.

In order to be able to experimentally investigate and determine an influence on wild populations, there has not yet been any release of a GDO into the wild, apart from first laboratory tests. Also small scale tests under natural conditions ('lab in the field'; Simon et al., 2018), if realised, would only be able to give initial information and indications, but would not allow to derive any concrete statements and assumptions about potential adverse effects that

could arise at the large scale. Nevertheless, a large number of application areas are already under consideration and high expectations are being placed on them in the future (National Academies of Sciences, 2016):

(1) **Application in farmland**: pest control e.g. spotted wing drosophila (*Drosophila suzukii;* Asplen et al., 2015), mice (*Mus musculus*; Silver, 1993).

(2) **Nature conservation**:

a) Control of invasive species: mainly in Australia [e.g. rabbit (*Oryctolagus cuniculus)*; Australien Academy of Science, 2017] and New Zealand [Australian opossum (*Trichosurus vulpecula)*, German wasp (*Vespula germanica*), rats (domestic rat: *Rattus rattus*; Norway/common rat: *Rattus norvegicus)*; Dearden et al., 2018; Royal Society Te Apārangi, 2019], Super weeds: elimination of herbicide and insect resistance; see Frieß et al., 2019.

b) Protection of endangered species: lowland leopard frog (*Lithobates yavapaiensis*) threatened as a result of anthropogenic spread of pathogenic fungi (Rode et al., 2019).

(3) **Health sector**: control of vector-borne infectious diseases: e.g. decimation of disease-carrying mosquitoes (malaria, dengue; Macias et al., 2017).

Gene drives possess the artificially transmitted ability to intensively influence naturally existing populations and, in extreme cases, have the potential to spread globally, that means, they are regionally unlimited. In comparison to the classically produced GMOs, GDOs in most cases would not be released in a limited time span (e.g. cultivation season) and space (e.g. field unit), but rather in a comparatively unbounded manner in large regions such as islands (e.g. New Zealand). Natural and semi-natural habitats would be the target regions for gene drives. By suppression or eradication of an entire species in a natural ecological system, unoccupied niches could be created in the ecosystem which may have to be filled with new niche occupants with similar behaviour (e.g. pest potential) which in turn could create new problems. As a result, the use of additional gene drives could be necessary due to the still existing problem control not being successfully solved. Furthermore, there is the possibility that the genetic modification could become independent under natural conditions and could also be transferred accidentally to related hybridisation partners. Also, a drive could lose its cargo gene and could spread without a specific function (a variant of a 'shadow drive'; Guichard et al., 2019). Furthermore, other sequences in a piggy-back effect might hitchhike and also be spread by the drive ('*selective sweep*') (Oh et al., 2021). Moreover, the detached cargo gene could also be inherited, mutate, be out-selected or driven to fixation itself independently of the drive. One prominent example of classical GMOs may be the outcrossing of herbicide resistances in volunteer oilseed rape (Hall et al., 2000).

It is still an open question how an appropriate risk management of GDOs could be implemented and ensured, based on the current state of knowledge. As a baseline for such management approaches data would be required on gene drive purpose, distribution dynamics, target organisms but also on potential non-target organisms such as hybridisation partners and the environmental conditions of the corresponding ecosystems. In addition, the intensity of intervention into the integrity of the target species should be characterised in order to be able to estimate hazard and exposure. In order to evaluate the intensity of the intervention, it is crucial to calculate the required number of GD individuals to be released or the repeated release frequency of the gene drives (Frieß et al., 2019). The reliability of the gene drive also needs to be assessed in advance. Options for verification and mitigation/limitation should also be evaluated prior to the release (Giese et al., 2019). Uncertainties and large knowledge gaps ('known unknowns', 'unknown unknowns', see Part A.0; chapter 2.4) resulting from the complexity of the technology and the diverse ecological context as well as the potentially follow-up wide-ranging consequences, represent the major challenges to design and implement an appropriate monitoring of GDOs.

Due to the specific traits, behaviour and impact pathways of GDOs, new challenges are imposed to the monitoring to be implemented for GDOs.

7.2 Post-market environmental monitoring (PMEM) of genetically modified organisms

In the European Union, GMOs may only be experimentally released or placed on the market after an authorisation procedure in accordance with Directive 2001/18/EC or Regulation EC 1829/2003. Applicants must conduct an environmental risk assessment (ERA) and submit a plan for the post-market environmental monitoring (PMEM). The objective of the ERA is to examine, on a case-by-case basis, whether potential adverse (direct or indirect, immediate or delayed, or cumulative long-term) effects could arise from the intended use of GMO. Conclusions made in the ERA affect the dimensions of the PMEM (see below). Following the release of the PMEM a GMO, the implementation of a PMEM is mandatory to monitor potential adverse effects of GMOs and their use on human health and the environment and to control post-approval safety measures. If any adverse effect on human health and the environment are identified, immediate response and action are required to minimise ecological harm. In this respect, PMEM provides the function of an early warning system (Züghart et al., 2011). The **Precautionary Principle** plays a key role for the assessment of hazard and risk (Gene Technology Act: GTA; Bourguignon, 2015). '*It is not defined in the Treaty* […]. *But in practice, its scope is much wider, and specifically where preliminary objective scientific evaluation, indicates that there are reasonable grounds for concern that the potentially dangerous effects on the environment, human, animal or plant health may be inconsistent with the high level of protection chosen for the Community*' (Commission of the European Communities, 2000).

Monitoring approaches for GMOs and adaption necessity for GDOs

'Monitoring of GMOs is the systematic approach for observing, collecting and analysing data on potential adverse effects, based on a risk assessment following a GMO's release' (literal definition originally taken from CSS, 2019, p. 312).

The assessment and monitoring procedures *step-by-step* and *case-by-case* have been in the focus in the early days of European Gene Technology Act (GTA). With the amendment by the Directive 2001/18/EC of the European Parliament and of the Council/Commission, legally binding monitoring has been included as a further instrument in the authorisation and safety procedure for GMOs. It has to be applied in case of experimental release as well as of placing on the market of GMOs. It is targeted in identifying and assessing those impacts that cannot be investigated conclusively or not at all in an experimental setting and in verifying conclusions made in the ERA in reality. These investigations include more complex interactions at population and ecosystem level (Simon et al., 2018), cumulative and long-term effects, and impacts at landscape and regional scale. From a scientific perspective, it is very challenging to identify causal relationships from measured data and subsequently draw correct conclusions. The implementation of an accompanying monitoring of the effects of GMOs and their use/application on human health and the environment is mandatory under the EU Directive 2001/18/EC and EC Regulation 1829/2003. The implementation of the EU requirements on monitoring into national law was performed by the Law on the reorganisation of the GTA in 2005, which for the first time included specific regulations for monitoring in the GTA. Monitoring is intended to contribute to verifying decisions made on approvals and safety precautions in practice. Furthermore, it is required to increase the prediction reliability for future risk assessment. Ideally, it provides the basis for an **early warning system** in order to be able to respond at an early stage in case of identifying adverse effects on the environment and human health (Kleppin et al., 2011). In this way, damage should be identified, prevented or mitigated as quickly and extensively as possible.

Directive 2001/18/EC specifies in detail and comprehensively the parameters for the development and implementation of a monitoring plan of a GMO. For the PMEM of a GMO, two types of monitoring are mandatory, on the one hand monitoring in the context of risk assessment and on the other hand monitoring of unforeseeable adverse effects (Directive 2001/18/EC, Council Decision 2002/811/EC):

(1) **Case-specific monitoring (CSM)**:

The aim of this approach is to evaluate and verify assumptions made in the environmental risk assessment (ERA) about the occurrence and impact of potential adverse effects of the GMO or its use on the environment and human health.

(2) **General surveillance (GS)**:

The objective is to identify indirect, cumulative and long-term effects of the GMO or its use on human health or the environment that were not covered or predicted in the ERA and which are difficult or impossible to be predicted.

General surveillance is largely independent of the outcomes of the ERA. General requirements for its monitoring design are addressed in the Directive 2001/18/EC and in Council Decision 2002/811/EC. According to Article 1, the guidance notes set out in the Annex to this Decision shall be used as a supplement to Annex VII of Directive 2001/18/EC. Council Decision 2002/811/EC recommends using already running ecological monitoring programs for the GS, which has not been implemented so far.

Ecological monitoring, in general, is the repeated systematic collection of significant and representative ecological data and study parameters in a standardised manner at regular intervals over time (Spellerberg, 2005). Data recording has to be carried out at the same predefined sites in order to detect and record changes and trends that have occurred over the last years/decades. Hence, monitoring is to record changes (Goldsmith, 1991). Data collection is mainly performed based on a specific problem or for a specific reason, such as to ensure that a given standard is met, which are consequently the starting points for implementing a monitoring (Spellerberg, 2005).

According to Annex VII of Directive 2001/18/EC the objective of a monitoring plan is to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO or its use in the ERA are correct, and identify the occurrence of adverse effects of the GMO or its use on human health or the environment which were not anticipated in the ERA. The goal is that in case, adverse effects on the environment are detected, appropriate steps have to be taken immediately to mitigate, prevent or reverse ecological damage. In this respect, monitoring provides an essential basis for an early warning system (Kleppin et al., 2011). However, GMO monitoring can only serve to a limited extend as a basis for an early warning system for adverse effects of GMOs. If monitoring served as a tool for damage prevention, its usability would be limited, as ecological harm can only be detected after its occurrence. So to say, monitoring is always running behind. Moreover, methodological limitations and lack of data and comprehensive information (Myhr and Traavik, 2001), limit the detection of potential adverse effects, especially when observing unexpected, indirect and long-term effects. In any case, damage prevention that is avoiding detrimental impact caused by the release of a GMO in the environment, e.g. secondary effects on non-target organisms and unintended gene transfer which could address a global dimension in the case of a GDO, should be the prioritised strategy following the *Precautionary Principle,* which is the basic principle for dealing with potential ecological impacts of GMOs and decision-making process in Europe. The Precautionary Principle also addresses the importance of considering scientific uncertainty and the hazard of irreversible damage when assessing ecological impact of GMOs (Bourguignon, 2015; Freestone and Hey, 1996). According to Article 4 of Directive 2001/18/EC, the Precautionary Principle is a general obligation which applies for all measures. It says that, '*Member States shall, in accordance with the Precautionary Principle, ensure that*

all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release or the placing on the market of GMOs.'

Monitoring used and adapted to evaluate behaviour and impacts of GDOs on semi-natural and natural systems must not only observe the change or elimination of the target organism alone, but also has to consider its role within the ecosystem, its ecosystem function (e.g. pollination, parasitism), interaction with other species (e.g. food chain), its migration and dispersal ability, general adaptability, global occurrence, its population genetics (hybridisation potential) as well as its specific temporal and spatial scale (CSS, 2019). For example, if a GD rat would be released into its invasion areas, it has to be expected that this globally present species could return to its areas of origin as a returnee *via* transport activities in connection with trade and affect the native populations by transferring fitness-reducing or eliminating traits which in turn could lead to major ecological damage there. In their regions of origin, rats have an essential function in the ecological system as part of the food chain (e.g. food source of birds of prey). Moreover, in their role as omnivores they support ecological clean-up processes.

The following chapter 7.3 highlights the specific requirements of GDOs arising from their characteristics and impact pathways. It must be clarified in time, whether and to what extent the legal requirements of a GMO monitoring are also suitable and applicable for the monitoring of GDOs. The law has to be scrutinised in this respect, and the standards of the respective Guidance Documents must be adapted or even supplemented to the requirements of a GDO.

7.3 Specific traits of GDOs in comparison with GMOs and a proposal for a monitoring

Gene drive organisms have a range of specific traits and characteristics which distinguish them significantly from GMOs. As a result, there is a particular initial situation to be considered that renders special requirements for monitoring concepts after a potential release of a GD (see Frieß et al., 2020). There are several differences between GDOs and classical GMOs as well as GMOs produced with genome editing techniques which make a fundamentally new approach for risk assessment and monitoring mandatory. Due to the complex genetic machinery the continuous production of GDOs is not restricted to laboratory conditions but is happening in the wild under natural uncontrolled conditions. Genetically modified organisms are released as a completed and in the lab tested product whereas GDOs are an adjustable tool for genetic modification released into natural ecosystems (Simon et al., 2018). The term '*lab in the field*' (Simon et al., 2018) summarizes the paradigm shift.

A main difference between GDOs in comparison to GMOs is that for the first time, the target genetic modification focuses on interference with wild populations (Reeves et al., 2018). With gene drives, transgenic constructs are released that are intended to spread into wild populations, even when fitness disadvantages will be the result of this intervention. There is a wide technical range of gene drive constructs that have different mechanisms for operation. Hence, the extent of ecological impact highly depends on the genetic construct and the nature of gene drives (see chapter A.0). In most cases, the release of GDOs is not restricted to farming units as it is mostly the case with GMOs, but is intended for the regions where the target species is found. Gene drive organisms are developed to be applied for mainly three purposes: control of agricultural pests, infectious diseases or invasive species with the objective to protect native and in particular endemic species. The applied GD technologies are very complex and their spread is likely to be difficult to control once GDOs are released into natural habitats (see chapter A.1 for confinement and mitigation strategies).

Hence, as this technological approach has far-reaching intrinsic consequences for wild ecosystems, it may conflict with nature conservation demands and standard practice because artificial GDOs are released into natural areas to eliminate invasive species and thus support

the survival of endemic species (e.g. GD rat in New Zealand: Royal Society Te Apārangi, 2019; Dearden et al., 2018, see chapter 6.3.3). This discrepancy with commonly followed classical approaches in nature conservation and goals also leads to a large conflict situation and discussion need regarding the application of GDs due to different perspectives and assessment approaches. In any case, controllability and retrievability of adverse effects in this context will only be possible to be carried out insufficiently, if at all. This raises the question of whether such systems which are accompanied with a high degree of uncertainty should be applied at all in the context of nature conservation measures.

The following list of various relevant features and aspects concerning gene drive specific information, target organisms and specific characteristics as well as information about ecological behaviour and impact on natural habitats emphasises the tremendous requirements for a GDO monitoring, but also the challenges associated with a comprehensive meaningful approach. Therefore, Tab. 21 provides a concise overview regarding individual relevant factors to be considered in the monitoring of GDOs. The special requirements of a 'classical' GMO (with a focus on GM crops) for monitoring are contrasted with that of a GDO. Because of the different implications arising from the two main gene drive types and applications – **population suppression or modification drive** –, it is necessary to subdivide into two separate columns for GDOs for several issues in the table. Suppression drives may eradicate entire populations (e.g. *Anopheles* mosquito, invasive species). Modification drives are less intrinsic. They spread new traits within populations, but do not disrupt the populations (e.g. *Drosophila suzukii*: morphological alteration of the ovipositor). In addition, the table highlights the paradigm shift from 'classical GMOs' (excluding GMOs which are produced using genome editing techniques) to GDOs. The elements in the list are supported with examples from different types of GDs. Moreover, case studies illustrate general requirements that GDs entail. References to the insights into their behaviour and possible effects previously compiled in the case studies of the previous work packages are provided including aspects which were raised from participants during the monitoring workshop within the framework of the current project that was carried out in autumn 2020 (see chapter 7.7). Case studies are provided in the table including literature reference to illustrate the listed traits (e.g. the global spread of GD rats). In the following text passages special requirements for the monitoring are discussed. That means, in which respect the GMO PMEM approach would have to be extended. However, the question arises whether a comprehensive and appropriate PMEM for monitoring GDOs will be possible at all.

In the first place, Tab. 21 should help to illustrate the paradigm shift from a classical GMO to a GDO. However, in some cases a direct comparison of the comparators is challenging. In order to avoid generalisation concerning certain issues, it is attempted to provide individual case studies for illustration and clarification. Genome edited organisms are excluded in the table because the objective of the present study does not focus on the techniques but rather on the specific requirements for monitoring of a GDO.

Tab. 21: List of new key traits, comparators, 'impact pathways' and requirements for monitoring of GMOs in comparison with GDOs.

Trait / condition	Classical GMOs excluding GMOs produced with Genome Editing	Gene drive organisms (GDOs)		Case studies for GDOs
		GDO with modification drive	GDO with suppression drive	
Biology				
Organism	crops, microbes, few animal species	wild living animals (and plants)		
Target species	domesticated, bred or cultured	occurring in natural ecosystems		
Population	GMO is introduced with the intention not to interfere with wild populations	wild populations present in nature, should be affected by GDO		
Generation	one (annual crops) to several (perennial crops, e.g. *Medicago sativa*), animals, microbes)	several		
Target ecosystem	in most cases farmland units	natural habitats		
Effectiveness of modification	application-dependent: primarily fitness promoting (e.g. insect resistant; herbicide resistant e.g. Hall et al., 2000), in some cases fitness neutral or reducing (e.g. production of human breast milk: Yang et al., 2011; Jackson et al., 2010)	fitness reducing / promoting	fitness reducing	fitness promoting: e.g. modification against chytrid fungi in amphibians: e.g. lowland leopard frog (*Lithobates yavapaiensis*; Rode et al., 2019) fitness reducing: GD *Anopheles* mosquito for Malaria eradication (Macias et al., 2017)
Comparison to natural counterparts	transgenes not evolutionary proven	GD not evolutionarily proven		Comparison of the natural selfish genetic element Medea in rice flour beetle with the synthetic MEDEA in *Drosophila* species (Beeman et al., 1992)

Trait / condition	Classical GMOs excluding GMOs produced with Genome Editing	Gene drive organisms (GDOs)		Case studies for GDOs
		GDO with modification drive	GDO with suppression drive	
Hybridisation with related species	possible; with the same species as crop, volunteer and feral plant and closely related species	possible, depends on the availability and contact opportunities		e.g. GD rats in New Zealand (Dearden et al., 2018; Royal Society Te Apārangi, 2019)
Ecological niche	competitive behaviour when the GMO runs feral and establishes populations	competitive behaviour	becomes free in case of a suppression and must be refilled newly	e.g. *Anopheles* mosquitos (Macias et al., 2017)
Potential spread	regionally highly limited	depends on the GD-type and the target organisms, for transcontinental invasive species like *D. suzukii* spread could be global		e.g. comparison of a low threshold vs. a high threshold drive (Esvelt and Gemmel, 2017)
Genetics				
Introduction of genes	single ('species foreign') genes	multiple ('species foreign') or modified genes for GD and its cargo		e.g. flightlessness in mosquito species (Fu et al., 2010)
Application context of introduced trait	farming context: herbicide resistance (R), insect R, combination, cold R, drought R, sterility (GE Salmon), hornlessness (dairy cattle) etc.	agriculture (controlling weeds and pest species), nature conservation (controlling invasive species, aiding threatened species), human health (e.g. pathogens, diseases); mostly inhibiting propagation or skewing offspring sex ratio, in some cases to promote disease resistance)		e.g. controlling *D. suzukii* as an invasive pest species (chapter 6.2.2)
Genetic variability	of the breeding line under lab conditions is low, GMOs are uniform	of GDs in principle is low (development of uniform breeding lines). In contrast, genetic variability of target wild population may be high, there may also be unintended variants (CRISPR error rates). GDs generally reduce genetic diversity in the GD locus of the target species		e.g. global variability of *D. suzukii* (Buchman et al., 2018a)

Trait / condition	Classical GMOs excluding GMOs produced with Genome Editing	Gene drive organisms (GDOs)		Case studies for GDOs
		GDO with modification drive	GDO with suppression drive	
Transfer of genetic information	on maximum 50% of progeny, new traits are generally out-diluted	on up to 100% of the offspring, traits are spread within the population/species		e.g. *D. suzukii* and small-molecule control (Del Amo et al., 2020)
Transfer of the transgene *via* hybridisation	target species-dependent hybridisation potential, possible to the same crop of conventional and organic cultivation as well as to closely related wild relatives	target species-dependent hybridisation potential, possible and intended to the wild target species, unintended for non-target populations as well as to closely related wild relatives		
Frequency of GMO / GDO	limited when cultivated, prevailing in case of successful spread into wild relatives especially in natural habitats	high		
Establishment of the trait in the population	dependent on whether the fitness of the organism is reduced or increased; in the first case, GM trait is likely to become lost through genetic drift due to disadvantageous selection	GD can establish itself in a population despite fitness reduction		For synthetic GDs, only lab experiments have been carried out which demonstrate the establishment of the GD in the test population (Noble et al., 2018). *Wolbachia,* a genus of intracellular bacteria, which is also considered a GDO in a broader sense could provide observation data (Sinkins & Gould, 2006)

Trait / condition	Classical GMOs excluding GMOs produced with Genome Editing	Gene drive organisms (GDOs)		Case studies for GDOs
		GDO with modification drive	GDO with suppression drive	
Technique				
Strategy	defensive increasing resistance to certain stressors	offensive potential to suppress whole wild populations	offensive potential to replace whole wild populations	modelling examples (Frieß et al., 2019; Simon et al., 2018)
Transgene	heredity and spread are unintended	spread and establishment is the goal of GD		
Maturity degree of the GM product when released into the environment	completed and fully tested in the lab	adjustable tool for genetic modification, tested in the lab in cultured populations, becoming effective after release into ecosystems first		'lab in field': Simon et al. 2018, Frieß et al., 2020
Scheduled time span of experimental release	is determined, e.g. one cropping period in the field	GDO release can also be determined, transient activity is targeted (techniques are under development but there is no proof of their efficacy yet), GDs are very likely to leave traces in the genome or transgenes in any case		Alphey, 2014; OECD, 2021
Temporal range	low / limited	high / unlimited / unknown		
Retrievability	in some cases, perhaps / limited	highly debatable (some strategies exist in theory), techniques for clearance of population genomes from GD transgenes (e.g. by split drive approaches) are under development but there is no proof of their efficacy yet		see chapter A.1 - confinement strategies
Controllability / inactivability	assessment and, if required, knowledge of appropriate mitigation measures in place / in some cases also limited	highly limited / unknown to a major degree		Directive 2001/18/EC does not provide legal safeguard against the diffusion of transgenes and their fate into the environment as long as no harmful effects are identified

Trait / condition	Classical GMOs excluding GMOs produced with Genome Editing	Gene drive organisms (GDOs)		Case studies for GDOs
		GDO with modification drive	GDO with suppression drive	
Fields of application	mainly agriculture	nature conservation (e.g. Australian Academy of Science, 2017), agriculture (e.g. Asplen et al., 2015), health issues (Macias et al., 2017)		
Exposure	limited	potentially global; regional to global (depends on GD-type and target organisms) (see “potential spread”)		
Geographic range	Intended distribution of GMOs is in most cases restricted, but potentially possible in case of a positive effect of the genetic modification and after sufficiently long period of time	Intended distribution of GDOs is potentially unlimited; the known approaches aim at a regionally limited application		
Ownership	privatised property (seed companies, farmers)	The GD belongs to the producing company		Simon et al., 2018
Authorization	approval: decision of e.g. EU as well as single countries, then farmer’s decision	assessment and approval according to national regulation of release of GMOs; participation and consent of the public should be considered		
Risk assessment / Regulation / Monitoring				
Ecological consequences	possible reversible / irreversible	potentially unlimited / far-reaching / complex / irreversible		They are heavily depending on the particular case. If e.g. a high threshold GD for *D. suzukii* is not able to establish in other regions as the target area, the ecological consequences may be small to negligible (see chapter 5.1.3)

Trait / condition	Classical GMOs excluding GMOs produced with Genome Editing	Gene drive organisms (GDOs)		Case studies for GDOs
		GDO with modification drive	GDO with suppression drive	
Predictability of effects on ecosystems	knowledge about potential effects already available, but not completely; '*uncertainty*'	insufficient known/unknown 'unknowns'; '*uncertainty*'		
Risk prediction	partly known, scientific knowledge and experience already available	It depends on the individual case. In several cases very difficult, effects are highly complex, long-term experience missing		Considering a modification drive for a plant (National Academies of Sciences, Engineering, and Medicine, 2016: case study 6), we might end up with a similar situation of an unintended spread of e.g. GM oilseed rape (e.g. Schafer et al., 2011)
Procedure for release of a GMO into the environment	stepwise principle: testing order: lab – greenhouse – field	Stepwise principle is insufficient, in several cases: far-reaching ecological interactions cannot be predicted in field trials on a small scale		
Regulation	at the European level: Guideline 2001/18/EC	At the European level Directive 2001/18/EC. At the international level, it would be helpful to define a common guideline which should be established globally in order to be able to prevent the global spread of a GDO		
Monitoring	EU legally regulated and mandatory to be carried out: (1) case-specific monitoring (2) general surveillance	currently at EU level because a GDO is regarded as a GMO: (1) case-specific monitoring (2) general surveillance		

According to Recital (20) of Directive 2001/18/EC, 'it is necessary to establish a common methodology to carry out the environmental risk assessment based on independent scientific advice. It is also necessary to establish common objectives for the monitoring of GMOs after their deliberate release or placing on the market as or in products. Monitoring of potential cumulative long-term effects should be considered as a compulsory part of the monitoring plan.' As a consequence, this principle also has to apply for a GDO. Due to the differences between GM and GD technique used, the target organism, its traits and behaviour and the ecological environment of the inhabited habitats of the target organism and its potential hybridisation partners are of specific concern. Table 21 could be used as a **checklist** to ensure that all relevant and specific factors of the GDO are considered when setting up a monitoring accordingly to be able to survey and identify unintended potential ecological impacts of the GDO on the environment.

Information about ecological behaviour and impact on natural habitats that should be considered in PMEM (see Block A.2: Base Data, list slightly modified):

1. Monitoring area, monitored time span and impact on natural habitats

a) In which area is the GD to be released? According to the Directive 2001/18/EC, the description of the regional distribution and the natural habitat of the released organism is required which includes information on natural predators, prey animals, parasites, competing organisms, symbionts, and host organisms. Also, the range of spread of the GDOs should be evaluated.

b) Is it possible that the GDO might spread to other regions outside the target region? Which regions would be affected in that case (e.g. protected areas)?

c) How large should the monitored area be?

d) Should the complete areas be sampled or should only samples be taken based on a stratified sampling procedure?

e) How long will the area be monitored before GD release? Is it possible to capture the baseline status to be able to monitor and identify effects of a GDO e.g. on the protection good biodiversity? Are there existing national biodiversity monitoring programs that could be used to monitor the impact of a GDO on biodiversity?

f) How long should the area be monitored after GD release dependent on the GD organism to be able to record long-term effects as well?

g) Do the releases in any way rely upon action or omission of action (such as pesticide spraying) from any resident humans living in or around the monitored area?

h) Have (unintended) anthropogenic actions (such as pesticide spraying) been taken into account considering the effectiveness of the gene drive and if so, how?

2. Target organism

a) Taxonomic name of the target organism (TO) species: organism (e.g. animal, plant, microorganism) – family – genus – species – subspecies – cultivar – common name
b) Why does the TO species qualify for a GD application?
c) What is the TO's generation time?
d) What is the TO's maturation time?
e) Are TO species fertile throughout the year or are there specific mating seasons?
f) How long is an individual TO fertile in its life?
g) How many offspring do individuals of the TO species produce in general per generation?
h) Are TO species polygynous or polyandrous?

i) If the TO species are polygamous, how many partners including error margins does the TO have in its life time?
j) List all hybridization partners for each target species, the percentage with which such matings lead to viable offspring and the percentage of fertile offspring.
k) Which of the affected species have overlapping habitats (not only in the planned release area) of the TO species?
l) How far are the TO species known to migrate on average, including error margins?
m) How far do the gene drive-carrying conspecifics migrate on average including error margins?
n) List all other (multicellular) species that interact with the TO species in their natural habitats and what are their relationships?
o) List all other (multicellular) species that interact with the species listed under point n) in their natural habitats and what are their relationships?
p) Are any of the species listed under the former 2 points reliant upon the TO species and to what degree?
q) Which of the species listed under the former 3 points occur within the monitoring area, how will effects of the releases on their populations be monitored?

3. Confinement and mitigation strategies

a) How is the confinement of the TO populations and their GD-carrying conspecifics to the target area ensured?
b) How will be ensured that these confinement strategies are effective during monitoring?
c) What are the appropriate counter-measures in the PMEM, should the confinement strategies prove to be ineffective? How is it expected to last until these counter-measures are effective to mitigate escapees?

The list does not claim to be complete and might be supplemented.

As laid down in the Annex of Decision 2002/811/EC, '*in the first instance, the likelihood of potential direct, indirect, immediate or delayed adverse effects arising from the GMO should be considered in line with its intended use and the receiving environment.*' In this context, the following aspects should be taken into consideration. In regard to GDOs, for example, GD rats released in New Zealand could spread uncontrollably worldwide. In this case, retrievability is very unlikely. This is in contrast to GM crops. For example, feral GM maize plants which have escaped cultivation can be identified and retrieved comparatively easily.

Especially the assessment of possible indirect effects is of importance and sometimes limited due to the lack of predictability. In the Annex of Decision 2002/811/EC it is mentioned that '*indirect effects may arise where reduction in the population of target insects impacts on populations of other organisms that normally feed on these insects*'. The larvae of *Anopheles* mosquitoes play a key role in the food chain as a food resource for many other species of the ecosystem (TargetMalaria, 2021). In case of eradication of *Anopheles* species by the application of a gene drive, key food resources for the ecosystem could be lost, which in turn would affect larvivorousspecies. Ecological niches would become unoccupied due to the eradication of the *Anopheles* mosquito species and would have to be replaced by other species of the ecosystem.

In the respective Annex of Decision 2002/811/EC it is also stated that '*observations of indirect effects are also likely to be delayed. These factors must, however, be considered as part of the strategy. Immediate effects refer to effects on human health or the environment that are observed during the period of release of the GMO. Immediate effects may be direct or indirect.*

Delayed effects refer to effects on human health or the environment which may not be observed during the period of the release of the GMO, but become apparent as a direct or indirect effect either at a later stage or after termination of the release […]'. Some types of gene drives are intended to have a broad long-term effect on target species, e.g. invasive rats in New Zealand. Since, unlike GM crops, they are not restricted to fields but are released into the wild and interfere with natural systems, unintended cumulative long-term effects could still occur years after their release.

As cited in Recital 20 of Directive 2001/18/EC, '*monitoring of potential cumulative long-term effects should be considered as compulsory part of the monitoring plan*'. This is particularly relevant for GDOs - see above - although monitoring to identify and determine cumulative long-term effects will be challenging to set up and to perform.

Finally, it is stated in the Annex of Decision 2002/811/EC that '*it is very difficult if not impossible to predict the appearance of potential unforeseen or unanticipated effects that were not highlighted in the risk assessment. General surveillance for potential unforeseen or unanticipated effects should therefore be considered as a part of the monitoring strategy.*' Similarly to GMOs, in the context of general surveillance a comprehensive survey of the unaffected initial state (baseline) of the ecosystem - into which the GDO is released - is required as a reference in order to detect specific effects of GDOs on the ecosystem and non-target organisms at all.

Another aspect has to be mentioned in this respect, concerning the '*degree of uncertainty*' of possible effects. This principle also has to be considered in an appropriate monitoring plan. There is a high degree of uncertainty of a synthetic gene drive's fate in wild habitats because the gene drives cannot be tested under natural conditions before they are released into natural habitats (see Part B1: chapter 6.1.1).

To summarize, Table 21 does not claim to be complete and reflects the *current state of knowledge* on this topic. It is intended to serve as a *working document* and *should be updated* in the future to reflect the latest state of knowledge. It may also be necessary to correct individual factors in the table which are currently assessed according to the latest state of knowledge. It provides a first orientation and should support an initial assessment of GDOs. The table could serve as a framework for target-oriented discussion and for decision-makers.

7.4 Requirements for monitoring

In this chapter specific requirements for the monitoring of a GDO are compiled and discussed, which are expected to extend not only the currently used monitoring approaches of a classical GMO (e.g. MON 810) but also concepts from the Federal Agency for Nature Conservation (BfN) of Germany, the Federal Office for the Environment (FOEN) of Switzerland and the Environment Agency Austria (EAA) of Austria (EAA 2011), the BAFU (https://www.bafu.admin.ch/) and VDI Guidelines (Zühart et al., 2013) which address current weakness in the legally mandatory monitoring plans according to the Directive 2001/18/EC from a nature conservation and environmental protection perspective. In addition, it must be evaluated whether, considering the new features listed in chapter 4, there is any possibility at present for an adequate and reliable monitoring of a GDO at all.

7.4.1 Comprehensive considerations of the requirements and regulations of a GDO

The current research focus on GDOs is the controlling of agricultural pests and infectious diseases, but also to eradicate invasive species with the aim to protect native species (Godwin et al., 2019; Johnson et al., 2016; Prowse et al., 2017). The particular characteristics of GDOs imply a specific initial situation for monitoring which depends on the type of a GDO (e.g. plant, insect, or mammal). This also results in specific requirements for monitoring a GDO release (see Frieß et al., 2020). One of the main differences compared to GMOs is that the target

populations for genetic modification are **wild populations in natural habitats**. The release of a GD which will affect natural systems because of the inherent functionality of GDOs to spread and invade natural populations is expected to have far-reaching intrinsic consequences for wild ecosystems (see Part B: chapter 6.1.2 Similarities between invasive species and gene drive modified organisms). Thus, GDOs, although intended as a supportive measure, might so far not be compatible with conservation considerations, as GDOs might also have additional uncontrollable, wide-ranging impacts on ecosystems which are unwanted or unforeseeable. Hence, from a nature conservation perspective these unintended consequences on ecosystems make their release highly questionable at all. This has to be clarified based on the data of a comprehensive risk assessment of the GDO according EU law before approval.

The **precautionary principle** plays an important role in dealing with risks of GMOs and GDOs (CSS, 2019). It should be the starting point for handling and regulating any GDO. Before even considering the release of a GDO, it is crucial to develop and implement effective **international and legally binding regulations** for a GDO *per se* (CSS, 2019). Existing biosafety regulations established for classical GMOs are insufficient and not fully adequate to address the inherent risk posed by GDOs. CSS (2019) reviewed the Convention on Biological Diversity (CBD) and related protocols for their suitability and potential application to GDOs. Biodiversity as a protection goal is the main issue of this convention and will provide a general framework also with regard to the regulation of GDOs (see Part D: chapter 8.3.1 Convention on Biological Diversity and its Protocols). For GDOs, however, the requirements are not fully covered by this convention. Biodiversity is in crises, which Hallman et al. (2017) dramatically highlighted with the 'Krefeld Study' on the decline of flying insect biomass by more than 75% in the last 25 years in protected areas in Germany, not only in scientific circles but also for the first time successfully to the public and politicians. Since GDOs additionally might contribute to biodiversity loss in the future (see Part B: chapter 6.1; CBD; CSS, 2019) e.g. due to their ability to eradicate entire species, it is crucial to focus monitoring and impact screening on biodiversity.

Without such mandatory and imposed regulations in place, there should be **no intentional and certainly no unintentional release of any GDO into the environment**, even in the run of small scale field testing experiments of a GDO which have to be performed before placing on the market of a GDO according to Directive 2001/18/EC, Part B. For example, it would be possible to conduct small-scale releases of GD mosquitos in a special tent that protects environment from an unintended escape of the GDO but enables the simulation of a natural environment. In this case, prevention of the spread of the GD insect would be more likely. However, a 1:1 release of GD mosquitoes can hardly be simulated in small-scale experiments, as the spread of mosquitoes is unbounded. This is different compared to GM maize, where regional restrictions on the cultivation of GM maize in a field with protection zones around it are possible as a safety measure for experimental purposes. Consequently, there are limitations in testing the potential boundlessness of a GDO before a large-scale release.

In any case, strict standards are needed for regulation, in the laboratory and in the field. Concerning the contained use of GMOs, the existing EU legislation has to be reviewed to see whether it meets the requirements for working with GDOs. All regulations must operate and need to be adaptable or expandable as needed. Public opinion and acceptance should also be involved and considered in the decision-making process of a possible introduction of GD systems.

Monitoring – as a consequence – is in general a useful instrument to identify potential unintended effects of a GDO. But the extent to which monitoring might also function as an early warning system in the case of a GDO, still remains questionable. In some cases, it might be possible that a spread of a GDO which gets out of control is detected quite quickly. With regard to a GMO, the identification of feral GM oilseed rape plants that have escaped from cultivation or got lost during transport activities, for example, can be ensured comparatively quickly (e.g. Schafer et al., 2011; Schoenenberger and D'Andrea, 2012). However, the

subsequent steps of retrieval and damage mitigation, if at all, would then be difficult to be achieved especially in the case of a GDO and are expected to remain insufficient (see Part A1, chapter 3 – confinement strategies). In addition, **modelling** could be applied to provide supporting predictions or evidence for effects of a GDO (see Part A: chapter 4.3.) However, there are limitations to the models in that case, since it is only possible to model effects that are known. Unknown ecological effects will remain unconsidered in that context. Hence, comprehensive modelling would require much more specific data and knowledge about influencing parameters and ecological interaction. For example, in the case of *Anopheles* mosquitos, the drift distance of the insects is strongly dependent on weather conditions. But wind direction and wind strength can only be predicted to a limited extend and therefore, can only be modelled with restrictions. This has already been shown in a field study on a GMO in Spain, where the likely levels of adventitious presence of GM maize plants in non-GM maize crops was estimated in field as a function of wind direction, field size and buffer areas between donor and recipient fields of GM maize (Brookes et al., 2004; Melé et al., 2004). GM maize pollen drift occurred in unpredicted areas in addition to pollen deposition in the main wind directions. In contrast to the modelling approach, a scientific monitoring can produce data on the main ecologically influencing factors directly in the field, i.e. in connection with weather conditions (e.g. current wind situation, temperature and humidity).

The capacity of a monitoring in both time and space must not be overestimated and misused as an operational instrument for a 'safe' release of a GDO. Monitoring is a system for identifying impacts, but not a system for avoiding and controlling negative impacts. It also has its limitations, which are particularly severe in the context of GDOs. Moreover, monitoring is only as effective as the available knowledge and monitoring methods on which the setup, the selection of indicators (study subject) and effect hypotheses and monitoring parameters are based.

7.4.2 **Requirements for a GDO monitoring**

The specific characteristics and aspects that distinguish GDOs and GMOs will require an adapted as well as extended monitoring approach. Unintended impact of a GD on the environment can occur in regions where there are wild populations of the target organism present or closely related cross-breeding species occur. Accordingly, the **methodological approach** applied for an environmental monitoring should be adapted to both, the gene drive technique and the type of environmental effect that could be expected. In addition to the **time span of monitoring** which needs to be much longer in case of a GDO because detection of long-term effects should also be covered in the monitoring, the **organism-specific selection of the parameters** to be investigated, the (complementary) methods and the **observation sites** which will in many cases of GDOs also have a larger range are of key importance. The set-up of the monitoring must be adapted considering the **released particular organism** under investigation and its **artificially introduced characteristics**.

Especially for General Surveillance, where unintended and unexpected effects of a GMO/GDO should be detected, the national as well as international implemented monitoring programs are able to provide broad biodiversity data sets on specific indicators as a starting point for detailed investigations of the ecological behaviour of a GDO as a reference data set. It must be assessed in detail for the requirements of each different organism types of a GDO, if possible adverse effects of a GDO could also be identified using the general approach of these programs. Several impacts of GDOs that have to be expected are still unpredictable or even unknown in their hazard and exposure potential. These programs primarily focus on presence or absence of single species, or species groups including population size or on specific target species which are protected (FFH species). While single locus techniques such as Medea or single-locus underdominance may require monitoring of single, unified loci, multi-locus techniques such as daisy chain and multi-locus homing systems need simultaneous monitoring

of multiple loci and multiple transgenes, a necessity which further complicates investigation and assessment (see chapter A.0). It is to decide, whether monitoring of mutations that could have an effect on gene drive systems should be a fixed part of an ecological monitoring approach of GDOs. In any case, investigations on how they may affect the behaviour of a GDO and cause unintended effects on the environment will be time-consuming and costly. The extent of required extension of a monitoring by a GDO in this context will depend on the configuration of individual gene drive techniques (transgene traits and sequences). For example, a change in gene drive (possibly in the transgene sequence) may occur after release through natural selection. Efforts to adapt monitoring strategies should therefore be based on a comparable approach which is already used for GMOs. That means, hypotheses that have been made in the ERA should be evaluated and verified within the framework of a **case-specific monitoring** approach, complemented by the control of genetic traits with appropriate geographical and temporal scaling. At the same time, a screening of the identified risk potential should take place during monitoring in order to enable an adaptive approach through feedback. In addition, **general surveillance** commonly used for GMOs, should be carried out in parallel, in order to identify impacts of GDOs and GDs on common protected goods such as biodiversity. Mitigation measures should be considered in advance and applied if necessary (see chapter 3.3.2 mitigation strategies).

A GDO monitoring must tackle the following challenges:

I) Monitoring objects:

1) Monitoring has to monitor and detect ecological change and harm on the environment during a long time-period.
2) Monitoring must monitor large areas, possibly worldwide.
   Since only a sample of individuals from the GD population can be realistically monitored, modelling of potential dispersal routes could be applied to better define the monitoring region to be tested. Modelling is in principle possible in this case, but it is equally limited in terms of prediction, as it is only a model.
3) The actual state of biodiversity (*status quo*) in the target area envisaged for the GDO release must already be measured as a baseline prior to the potential GDO release in order to provide comparable data sets and to be able to detect damage caused by the GDO in the first place: In the introduction of the Annex of Dec 2002/811/EC it is stated that '*Monitoring can be defined, in general, as the systematic measurement of variables and processes over time and assumes that there are specific reasons for collection of such data, for example, to ensure that certain standards or conditions are being met or to examine potential changes with respect to certain baselines. Against this background, it is essential to identify the types of effects or variables to be monitored and importantly, the tools and systems to measure them and an appropriate time-period for measurements. Monitoring results may, however, be important in the development of further research.*' Additional information concerning existing historic knowledge e.g. on crops should also be considered (contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19. - 20. November 2020) In parallel to the release of a GDO, there is also the possibility of testing reference plots at the same time on which no GDO has been released, in order to better determine impacts on the environment caused by the GDO according to Decision 2002/811/EC. However, whether this approach is feasible in case of a global spread of a GDO, needs to be further analysed.
4) The monitoring must focus a.o. on the wild target population and its closely related species (non-target populations) with which hybridization is possible to prevent unintended gene transfer of GD systems. Regional occurrence and frequency of these species have to be observed before GD release.
5) As a basis for determining the factors to pay attention to and identifying regional conservation goods, a high level of basic knowledge about species occurrence, composition and interaction must be available before a GDO monitoring concept can be drawn up.
6) Monitoring must be comprehensive and consider multiple determinants (e.g. other influenced taxa) involved. In order to do so, a range of appropriate monitoring parameters need to be identified and surveyed in order to make effective assessments.
7) Monitoring must address comprehensive data collection in the field and cautious data interpretation. Broad baseline data are required for a reliable estimation and assessment of the effectiveness, the spatial and temporal dispersal potential of a GD as well as the potential detrimental effect of a GD on the environment caused by a GDO (see Part A2 chapter 4 Base Data for the Prospective Assessment of Gene Drive Releases). These data include a) data specific to the GD system, b) data specific to the target organism and c) data specific to the environmental conditions of the corresponding ecosystems that will be affected by the GDO. For identification and evaluation of unintended ecological harm of the GD on the ecosystem, ecological knowledge about the receiving environment is necessary. In this context, the collection of information about non-target species, food webs, relevant ecological factors such as weather conditions (e.g. wind) etc. is a mandatory prerequisite to be able to investigate the complex interrelationship and

interactions of GDOs with non-target organisms and ecological factors. However, adverse effects on the environment are also the least predictable. Supportive modelling in this context as well can only be carried out on the basis of a broad range of ecological data.

II) Ecological effects and harm identification:

8) Mainly impacts on natural habitats have to be surveyed and considered for GDOs. There are impacts on ecosystem functions (e.g. vacated ecological niches) and change of habitat use to be considered. It should be checked, which taxa would have the potential to fill these niches? Moreover, impacts on food webs have to be surveyed (e.g. the invaded rats that are the target for eradication by the release of the GD rat do already play a role as a prey for endemic species in New Zealand).
9) Monitoring must be able to identify unintended (expected or unknown) effects of GDOs on natural systems, including biodiversity as promptly as possible.
10) Ecological harm caused by the GDO must be defined in advance. At which effect is a negative impact to be addressed ('*limits of concern*') and which harm is still acceptable in a benefit-harm assessment? Criteria for acceptance must be developed and defined (Bartz et al., 2009; contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020).
11) Monitoring must consider known unknowns, but also unknown unknowns.

III) Required framework conditions and legal regulation of GDO monitoring:

12) In Recital 24 of Directive 2001/18/EC it is stated: '*The introduction of GMOs into the environment should be carried out according to the step by step principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken*'. This step-wise procedure is useful in order to identify suddenly arising effects of GDOs, so that possible harm can then still be prevented in time. Accordingly, in case of a step-wise release of a GDO, a spatially and temporally limited release would have to be carried out and it would be necessary to ensure that all GDOs can be retrieved from the environment. Operators also would not want to take any risk with an initial experimental release of a GDO (contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020). A failure would pose difficulties for the entire technology.
13) A standardised global (at least a European harmonised approach) guideline for GDO monitoring which defines all essential attributes must be established in international coordination and agreement to enable harmonized data collection and global procedures and actions.
14) Monitoring must control and help to prevent unintended transboundary movement of GDOs (Regulation (EC) 1946/2003; Cartagena Protocol).
15) The GD technology carries the potential of large-scale impact on humans and environment. According to Simon et al. (2018), it '*is not fit for practical use at present*.' In this context, it is essential that science must take responsibility for the potential impacts caused by gene drives. Thus, the role of science in the decision-making process should be identified and defined.

In principle, it is a promising first approach to apply the basic framework of a GMO monitoring - CSM and GS – which so far has only been carried out on GM plants - to GDOs as well (Directive 2001/18/EC). However, a monitoring of future GMOs (e.g. Genome Editing GMOs) and GDOs goes far beyond the requirements of a classical GMO monitoring. It is therefore very doubtful whether such a comprehensive monitoring for a GDO can be sufficiently designed and built up at the current stage of knowledge to adequately record and assess the

multifactorial and complex effects a GDO may cause. In any case, a high residual risk remains, since very far-reaching effects are to be expected. The monitoring setup of the CSM as well as the GS is too insecure and insufficient to capture all of these yet known and unknown effects.

7.5 Analyses of the suitability of existing GMO monitoring concepts and programs in the context of nature conservation

In this chapter, the following questions are addressed and discussed: *Can already implemented monitoring concepts for GMOs or biodiversity in Germany be used as starting point for setting up a monitoring concept for GDOs?*

GDOs and gene drives are expected to have complex and long-term effects on biodiversity and entire ecosystems. GDOs are designed to accumulate in the environment and stay there for a period of time – in some cases active spreading is intended depending on the type of GD – in comparison to classical GMOs where spread to the wild has to be prevented. In this context, the question arises which already implemented environmental monitoring programmes in Germany focussing on biodiversity issues could be used as a baseline for identifying detrimental effects of a GDO. Moreover, existing monitoring guidelines as well as concepts provide a framework for adoption to the specific requirements of a GDO.

A monitoring system should enable to identify impacts at an early stage and subsequently and at best, might be used as an early warning system. Another question which should be raised is to what extent these programmes can be used. At present, nationally implemented environmental monitoring programs which collect data on habitats and species diversity and distribution could serve to provide at least baseline data concerning the ecological features of the receiving environment of a GDO including the presence and the distribution of target and non-target organisms and initial concepts as a starting point for assessment of adverse effects on the biodiversity caused by GDOs. However, specific upgrades and extensions will be necessary in the framework to be used for GDOs as for example, the affected spatial and temporal dimensions of a GDO have to be calculated much larger. Moreover, cumulative and long-term effects also have to be expected and considered in the monitoring. For this purpose, new systems as well as new methods have to be identified, developed, put into practice and established. Currently, the already implemented environmental monitoring programs are primarily targeted at selected organisms such as protected species or habitats (Council Directive 92/43/EEC of 21st May, 1992 on the conservation of natural habitats and of wild fauna and flora). Additionally, the occurrence and spread of invasive species (https://neobiota.bfn.de/) or pests is observed in several projects. These concepts also consider status and trend analyses of single species, species groups and habitats.

Environmental monitoring approaches that would be required for GDOs must, in the same way as GMO monitoring, take into account the **target organism** of the gene drive, the **specific impact** of **mechanism of the applied technology** and the resulting **gene drive-specific potential environmental impacts**. In this context, the choice of *suitable study parameters*, the *study time-span* to be defined as well as *affected observation sites/regions* (locally affected range) play a key role for the set-up of a GDO monitoring. GD monitoring must consider, among other factors, the features and behaviour of the respective target organism as well as its introduced trait(s).

Monitoring programs in Germany

Several monitoring programs (e.g. Nationwide Bird Monitoring, Monitoring under the Habitats Directive (FFH-Monitoring, Monitoring of High Nature Value (HNV) Farmland Monitoring) are already implemented and currently carried out in Germany. Currently, several programs are in development, e.g. Ecosystem Monitoring (ÖSM), Nationwide Insect Monitoring, Monitoring on National Natural Heritage Sites (NNE). Monitoring in Germany is performed as a federal responsibility in the Federal Nature Conservation Act, under EU directives, international conventions and Germany's Genetic Engineering Act (GTA). The focus of these monitoring schemes are survey, determination, description and assessment of nature, landscape and species condition, occurring changes in their status and their drivers as well as resulting ecological consequences. The homepages of the BfN and of the national monitoring center for biodiversity (nationales Monitoringzentrum zur Biodiversität) provide detailed description of the monitoring programs (https://www.bfn.de/themen/monitoring.html; https://www.monitoringzentrum.de/monitoringprogramme).

Monitoring concepts and future approaches

The continued list contains further considerations regarding additional observation approaches to be used for identifying GDOs and their spread, e.g. taking possible global routes for unintended future entry of GDOs into Germany into account**:**

1. **Monitoring environmental impacts of Genetically Modified Organisms**

   According to the Directive 2001/18/EC: case-specific monitoring and general surveillance (see Part C, chapter 3).

2. **VDI-guidelines**

   According to Directive 2001/18/EC, PMEM has to apply standard methods that are available and appropriate for effective monitoring. To provide appropriate standardised methods for data acquisition and bio-molecular analyses, VDI guidelines (VDI 4330 – VDI 4333) have been developed by working groups constituted of experts from relevant disciplines and are revised in regular intervals (Zühart et al., 2013). One area of application of the VDI guidelines is standardised post-market monitoring of adverse effects of a GMO on non-target organisms (VDI 4330 Part 1). The aim of the VDI guideline is to enable consistent application of these standards for a harmonised ecological monitoring approach of GMOs.

   Similar to the PMEM for GMOS, the PMEM for a GDO also needs predefined standards to be followed in order to be reliable. Moreover, sampled data need to be comparable between different monitoring regions. The methods prescribed in the VDI guidelines can in general also be applied to GDO monitoring. Since the VDI-guidelines were developed for the monitoring of environmental effects of genetically modified plants, it should be analysed to what extent the guidelines are suitable and where there is need for further development for a monitoring of GDOs.

3. **Indicators for Nature Conservation**

   This is not a monitoring program itself, the indicators - e.g. Indicators for the National Strategy on Biological Diversity, Indicators for the German Strategy for Adaption to Climate Change - are based on the data of the performed monitoring programs. They do not provide their own data bases, but summarise the aspects of several programmes and hence, make a broad data range possible.

4. **International flow of goods**

   In particular, during introduction (transport and handling activities) *via* railways, roads (trucks), ship or air, unintended entry of GDOs is to be expected, in case GDOs have been released somewhere in the world. This has already been shown in the case of GMOs with oilseed rape (e.g. Pascher et al., 2017). Consequently, controls against unintended entry of GDOs could be carried out regularly in the course of commodity control (e.g. GD rats in New Zealand as a potential returner to its area of origin e.g. Europe – transport activities in the course of global trade as a source). Corresponding monitoring approaches could be supplemented to the already existing controls. However, these controls could not be applied to all organisms of GDOs. For example, limitations of this approach are expected for *Drosophila suzukii* and the detection and proof of laid eggs due to their small size and associated detection methods.

5. **Citizen Science programs**

   For single easy identifiable species Citizen Science could be applied as an initial fast data capturing system in order to obtain comprehensive data for assessment of the regional spread of a GDO (e.g. mosquitos: In parts of Germany, for example, a citizen science programme is currently underway in which mosquitoes are collected by citizens who send their samples to assist in taxonomic identification and estimation of mosquito species diversity and distribution; https://mueckenatlas.com/; contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020). In citizen science projects areas are not sampled consistently, on the one hand some of the regions are not covered by data, on the other hand data are overrepresented in highly populated regions. Citizen science programs, however, can give a first rough overview about species diversity and distribution or can be used to support existing scientifically collected data sets.

Single programs are still under conception and development.

The listed monitoring programs and concepts could provide key settings (indicator species, monitored area, etc.) for a monitoring of a GDO depending on the type of the GDO, e.g. small mammal, insect and their area of release.

Application of novel technologies in future monitoring as supporting tools – an example

Additional methods including novel tools such as remote sensing (Dalton et al., 2021) in combination with field data could contribute to ensuring effective and efficient monitoring for unintended detrimental complex effects of a GDO on the environment. In the following section, an example of a novel taxonomic tool is discussed that could support classical monitoring approaches and could help to guarantee high quality monitoring of e.g. GD insects in the future.

Metabarcoding – a tool for assessing target and non-target organism diversity

The application of the new molecular technique of metabarcoding of an environmental DNA sample to identify e.g. aquatic species diversity as quickly and comprehensively as possible, especially in locations where there is still little taxonomic knowledge, could be included in a monitoring process in future (Dalton et al., 2021), also in a monitoring of a GDO. To rapidly check the unknown status of e.g. regional mosquito populations in malaria-affected areas in Africa and to pre-record and assess species diversity in advance, such techniques could be applied. In this context, the usage of operational taxonomic units (OTUs) enables an initial survey of species diversity, even when the species are still taxonomically unknown (Blaxter et al., 2005). This allows for a first assessment of regional species diversity. Applying the approach of operational taxonomic units (OTUs), mosquito diversity could be assessed very roughly. Taxonomic identification of insects in African regions is very challenging because of rich diversity and broad taxonomic knowledge gaps. For example, there are a total of 481 formally recognized species and more than 50 unnamed members within the subfamily Anophelinae, around 30 to 40 of those are functioning as malaria vectors (https://mosquito-taxonomic-inventory.myspecies.info/; TargetMalaria, 2021). A reduction in the population of *Anopheles* species could cause detrimental effects on the entire ecosystem, as their larvae are important parts of the ecological networks. A number of aquatic animals feed on them. If these larvae populations were reduced, this in turn could have a negative effect on populations which feed on the larvae ('cascade effect'; see Part B).

Metabarcoding does not replace classical data collection methods, but can be applied as a supporting methodological tool, as only rough information on species diversity can be obtained with this novel technology (Dalton et al., 2021). Analyses on changes in abundance and trends of single species based on the taxonomic metabarcoding outcome will hardly be possible at all. For example, in aquatic biomonitoring environmental DNA is mainly used for three purposes, which are detection of single species, biodiversity survey (community composition) and biological assessment (biotic indices) (BAFU 2020).

Specific requirements for the set-up of a monitoring of a GDO / GD have to consider the following key challenges:

a) Functional GDs released into the environment remain active in affected ecosystem for a long period of time. Consequently, monitoring of GDOs will have to be carried out for a much longer time span in comparison of GM plant monitoring
b) unlimited, regionally wide-ranging areas invaded by the GDO (no area reference), potential for global spread of the GDO
c) focus on natural areas for release
e) the potential of the impact to be expected in individual cases can hardly be estimated
f) potential side effects of the GDO
g) several uncertainties due to proof of concept studies, experimental testing should only be carried out under safety conditions (e.g. Saran-tent)
h) 'known unknowns', 'unknown unknowns'
i) need of a common worldwide approach and survey strategy for monitoring GDOs as they could occur globally
j) need to harmonize worldwide monitoring data
l) standardized guideline/guidance document and setting of mitigation measures if necessary.

In summary, this implies that the specificity of GDs and GD organisms and their effects is that they have the potential to cause detrimental impact on their wild populations (intended effect), closely related species, natural plant and animal communities, and on natural ecosystems that can be long-term, large-scale and potentially irreversible (UBA 2019). The major challenge in monitoring GDOs is that it is not yet entirely clear in detail which parameters are to be monitored and which investigation hypotheses need to be formulated and tested. In this

respect, critical opinions were expressed on future monitoring systems for GDOs (contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020). Complex interrelationships such as ecological networks or ecosystem functions need to be considered, which is currently mainly done through modelling (Mumford 2021, presentation at the Online-Webinar Gene Drive: 21.-24.6.2021). In addition, cumulative effects must be expected in the future, in case other GMOs but also GDOs were released. The discrepancy between classical nature conservation approaches and the use of GDs to protect species and species communities also makes the assessment more difficult. In Germany, the term '*purity of nature*' has been raised. GDs are a construct that '*crosses a border of artificial borders'* (Simon, 2021, presentation at the Online-Webinar Gene Drive: 21.-24.6.2021). This claim to designability of nature opens up a new dimension of '*instrumentalisation of nature*' which leads to an '*erosion of nature*' (contributions of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020). Before applying GD technologies which are *'not fit for practical use at present'* (Simon et al., 2018), the identification of alternative approaches with comparable benefits but a lower hazard- and exposure potential is a must (see chapter A.1). In any case, the release of a GDO should only be the last option to be considered. The search for alternatives should therefore be supported. Also, independent risk research should be financed for public institutions such as Universities. At present, limited research budgets are invested in baseline research and field data collection, as scientific research is more targeted and focussed on its immediate practical application compared to the past (contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020).

7.6 Recommendations for GDO monitoring

The monitoring procedures, that are already mandatory for GMO monitoring – (1) case-specific and (2) general surveillance – must be incorporated into or should be the basis of the GDO monitoring program that is to be adapted, supplemented or even developed newly. Since GDOs may have a global range of impact, it would be of utmost importance to establish future guidelines for the safest possible handling of GDOs and the requirements for monitoring in a *globally standardised framework*, in order to be able to ensure comparability of a global monitoring which in the case of GDOs is crucial. To be able to assess the risks of gene drives appropriately and to prepare adequate monitoring, broad basic research and independent risk assessment on current developments of gene drive technologies and their impact as well as on natural systems and possible change due GDOs is required. Moreover, research for adequate methods for monitoring GDOs should be enforced. Sufficiently large budgets have to be provided in order to enable GDO monitoring over many years. Additionally, these financial resources must be assured also in future to be able to guarantee long-term implementation and repeated monitoring runs and additionally, to promote gaining of basic knowledge to be able to formulate risk hypotheses.

It is recommended to distinguish between two main monitoring approaches:

1. Monitoring to identify exposure:

GDO monitoring should be able to identify an active gene drive that has been released into the wild (CSS, 2019). There are two main limitations for success of a gene drive which are functionality only in sexually reproducing species and a time span of several generations so that the newly released drive is able to affect a substantial proportion of a target population, unless GD organisms are released in large numbers of a substantial fraction of the population (Oye et al., 2014). The authors address minimal experience in creating biological systems for evolutionary robustness that means the stability of such incorporated systems is still uncertain and mutations inactivating the incorporated/modified trait may occur easily. Different GD techniques possess different possibilities of spreading to non-target or related populations (see Part A.O, chapter 2.3). It is still unclear to what extent and over what time span GD could move

unintendedly. Unexpected ecological side effects could also occur, that are at current state not foreseeable. To distinguish between an active and an inactivated gene drive would require extensive and detailed molecular characterisation. Metagenomics could be used and applied for this purpose (Schwartz et al., 2007). Such a required differentiation could be achieved on the basis of sequencing analyses.

In addition, the incorporation of **artificial genetic markers or the use of inherent unique DNA sequences to identify the GD organism** would also facilitate monitoring. For example, special genomic markers are already in use for insects (contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20.11.2020).

Furthermore, modelling approaches support the evaluation of effects. Ecological modelling is very complex. There are numerous data which have to be put into the modelling system to evaluate e.g. ecosystem functions. However, their reliability still needs to be tested. It is also valuable to learn from natural GDs such as selfish elements in order to understand the population genetics of a GD under natural conditions (see Part A.2; chapter 4.2*;* Simon, 2021, presentation at the Online-Webinar Gene Drive: 21.-24.06.2021).

2. Monitoring to identify adverse effects (hazard) of GDOs on the environment:

Ecological impacts of GDOs on the environment are very complex which require comprehensive survey of relevant parameters and mutual interactions. Monitoring is required in any case for both, intentional and unintentional effects of GD, even once the GDO has already vanished. The conception of a monitoring of ecological effects of a GDO must therefore be designed for long time spans and consider large regional areas, possibly even globally.

For monitoring ecological effects, the following specific factors are recommended:

a) Evaluation on a case-by-case basis should be used for GDOs as an approach in the same way as it is applied with GMOs.
b) From a technical point of view, efficacy of specific reversal drive/ fitness should be checked (Oye et al., 2014). Long-term studies are crucial to investigate the effects of GD use on genetic diversity in target populations (Oye et al., 2014). Drive function and safety should be investigated and evaluated in detail.
c) To provide comparable data of the initial situation, the *status quo* of a natural area, wild populations, the number and frequency of potential cross-breeding species, food chains etc. should be surveyed and assessed as a reference before GDOs are potentially released.
d) Damage is defined differently from various perspectives. A precise definition of harm on the environment ('*pathways to harm*') from an ecological point of view is therefore urgently needed for damage evaluation. According to Annex III/B Commission Directive (EU) 2018/350 the following information – in this case on higher plants – has to be provided for the environmental risk assessment: '*For each of the seven areas of risk referred to in Section D.2 of Annex II the notifier shall first describe the pathway to harm explaining in a chain of cause and effect how the release of the GMHP could lead to harm, taking into account both hazard and exposure.*' As a consequence, this has also to be taken into consideration when implementing the monitoring plan. The identification of protected goods in this context should be the starting point. In the first instance, impact on key organisms, habitats and ecosystem services should be addressed.
e) Transparency regarding release and monitoring approaches of GDOs is another key element which should be encouraged and provided, also for the public. In that context, a wide range of scientific publications are available open-access (own observation).

Moreover, there should be public information and discussions of environmental and security concern, because GDOs affect the global common goods. In the EU the Regulation (EU) 2019/1381 on the transparency and sustainability of the EU risk assessment in the food chain (Transparency Regulation) already exists. Though this regulation is intended for the EU food regulations, some aspects also concern the approval procedure under Directive 2001/18/EC. This could serve as a starting point for further discussions.

f) As a general adaption and extension to a GMO monitoring, GDO monitoring needs to incorporate and apply molecular methods, as the application of metagenomics is necessary for a comprehensive impact assessment and the tracking of their spread. Metabarcoding could be used in a supportive way for biodiversity assessment (see above, chapter 6).
g) As second major extension for GDO monitoring approaches would be the integration of modelling for a comprehensive and more precise investigation and evaluation of the impact of GDOs and GDs on natural populations over years (see Block A.3: Knowledge gain through modelling). In this context, however, it must be emphasised that models are *'only models'* and can thus only be used as a supportive tool for the identification of essential parameters and problem formulation in GDO monitoring. However, they are not capable of representing how the GDs will behave and develop under natural conditions.
h) There are currently neither regulations nor precise ideas of a monitoring plan of GDOs. Before an approval for a release of a GDO is granted, a monitoring plan including *status quo*-surveys needs to be designed and developed, and a set of regulations, e.g. emergency response plans (CSS, 2019), must be in place. Prior to this, large knowledge gaps need to be filled in order to better identify and define all requirements for a GDO monitoring. Another requirement for GD release would be that there are already options to reverse or retrieve GDs. Similar to the GMO moratorium in Europe, a period of time - a moratorium - is required to develop a global guideline for monitoring and handling of GDOs and their potential impacts on humans and the environment.
i) Appropriate cross-border monitoring plans in countries at risk should be implemented before a release of a GDO in order to identify unintended cross-border GD invasion at an early stage (Sustainability Council of New Zealand 2018).
j) In any case, low-risk alternatives to GD technology should be considered before placing on the market of a GDO. The development and success of alternative techniques is often linked to how much money is invested in this research field (contribution of the Online-Workshop '*Monitoring of Gene Drive Organisms*' 19.-20. November 2020).

Referring to the Sustainability Council of New Zealand (2018), **GDO monitoring plans** should consider the following, in summary:

1) Track the movement of gene drive organisms and the potential spread of the trait through populations, and across borders and ecosystems
2) Identify unintended, harmful impacts during and after a gene drive release programme that could lead to a change in or revocation of a gene drive approval
3) It should also fulfil other biosafety functions, such as liability and redress.

In regard to the approval of a GDO, the high potential for ecological damage and the far-reaching consequences of GDs highlight the need for **comprehensive and effective regulation and (global) guidelines for the use of GDOs**. In European regulations, GDOs are currently treated as GMOs. Hence, they are 'addressed' by the Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

The GDO guideline still to be implemented must address the following issues:
a) safety of the construction of the GD system
b) testing of the system under controlled conditions
c) release.

At present, there are no standards available for assessing impact of GDOs on environment. Several international conventions exist which could be used to e.g. control cross-border movements/spread such as the Cartagena Protocol on Biosafety or the Nagoya Protocol (see Part D). But before conducting controls of GDOs, standards for effect assessment, damage estimation and harm mitigation have to be defined in advance (Oye et al., 2014).

7.7 Workshop on synthetic gene drives

An interdisciplinary and international workshop with the topic "*Monitoring of Gene Drive Organisms*" was organised in the course of the GDRA project and took place on November, 19-20, 2020 as an Online-event in accordance with the Covid-19 safety regulations. Scientific researchers, risk assessors, ethicists and regulators from various backgrounds were invited to discuss interdisciplinary aspects of gene drives and ecological consequences with a focus on the specific requirements on a GDO monitoring and further regulations. The workshop addresses the following questions:

(1) *What are the specific characteristics and traits of GDOs and the possible ecological effects caused by GDOs that are both relevant for monitoring, especially in comparison to "classical" genetically modified organisms (GMOs)?*

(2) *What are the special requirements for a monitoring of GDOs?*

(3) *Evaluation of already existing monitoring programs and approaches in Germany with regard to their potential for monitoring and evaluation of possible ecological effects of GDOs.*

The following questions were addressed to the participants for a discussion input:

(1) *According to your opinion, what are the particular challenges of GDOs compared to classical GMOs?*

(2) *In view of the new 'quality' of GMOs achieved with gene drives, is it feasible or appropriate to use already implemented monitoring programs for the GDO monitoring, at least as a starting point?*

(3) *To what extent are existing monitoring programs suitable for recording the environmental impacts / effects of GDOs and in which way do they need to be supplemented or adopted? What do new monitoring systems need to be developed for?*

(4) *In which areas do you see the greatest need for research?*

The outputs of the workshop were summarized in an internal protocol. Key aspects are incorporated into the project report.

7.8 Monitoring of Gene Drive Applications - Summary

Gene drive (GD) strategies aim either at suppressing target populations or at introducing novel or modified traits. These newly developed techniques differ considerably in terms of their efficacy, in particular between *self-limiting strategies*, where the modification is assumed to have limited persistence under natural conditions, and *self-sustaining strategies*, which are supposed to persist indefinitely in the target population and may also invade non-target wild-type populations. Several GD methods with different mechanisms of intervention are under development. However, before a test release or even a large-scale release can be considered,

there is an urgent need to establish an appropriate monitoring plan including investigation hypotheses as well as appropriate indicators and methods to detect possible unintended effects on the environment and human health. The aim of Work Package C was to identify and compile all characteristics and in comparison to a genetically modified organism (GMO), unique features of a gene drive organism (GDO) in order to identify and concretise the specific requirements for a GDO monitoring and the limitations of surveying and controlling potential - in the worst case global - ecological impacts caused by a GDO. Based on these outcomes, recommendations for a future monitoring approach for GDOs are provided. To set-up and develop a monitoring to identify an ecological impact of a GDO on the environment, this report first of all provides a checklist of all the relevant properties and parameters of a GDO that need to be taken into account. In addition, the report presents a table with the comparators between 'classical' GMOs and GDOs in order to better visualise the differences and requirements for the set-up of a required GDO monitoring. Several of the characteristics of GDOs such as their application in natural systems, their temporal and regional unboundedness and the broad efficacy of GDs pose major challenges for the design of an appropriate monitoring scheme. As stipulated in the Convention on Biological Diversity (CBD; https://www.cbd.int/), biodiversity is a prior environmental protection good and must be protected also from harmful interference with GDOs (see chapter 6.1 *Ecological risk assessment and protection goals* and 8.3.1 *Convention on Biological Diversity and its protocols*). Sufficient basic knowledge is still missing to be able to design appropriate monitoring plans. Therefore, it is not yet possible to adequately design and implement monitoring plans, control the invasive behaviour of GDOs and ensure retrievability in case of damage. As a first measure, a moratorium should be implemented to carry out all these necessary steps for a safe handling of GDOs in advance, if this is possible at all. The Precautionary Principle should be at the highest priority. The release of a GDO into the environment poses challenges in legal, environmental, biosafety and governance issues (EU Parliamentary Vote, 8th June, 2021; paragraph 148). PMEM monitoring of a GDO, according to current regulations, must address both approaches case-specific monitoring and general surveillance. In addition, internationally standardised and legally binding regulations (at least a European harmonised approach) for the handling and monitoring of GDOs need to be implemented before a GDO is released. GDO monitoring should be designed to be capable of identifying a) exposure and b) adverse effects (hazard) on the environment. As a general extension, GDO monitoring will become more molecular (metagenomics; e.g. Schwartz et al., 2007, Taberlet et al., 2018) than a monitoring that is currently carried out for 'classical' GM plants and will also include modelling approaches. Existing monitoring concepts and programmes in the context of nature conservation in Germany can currently only provide a starting point for GDO monitoring such as a baseline.

8 Part D - Regulatory Framework for the Deliberate Release of Gene Drive Organisms on the National, European and International Level

Katharina Schreiber, Elisabeth Andersen, Silja Vöneky

8.1 National Law

The national regulation on GMOs is decisive for any deliberate release of GMOs, including gene drive entities (plants and animals),1 in Germany as it lays down the relevant legislation covering various aspects of biosafety. The GMO regulation in Germany is based on European Law and implements the European Biosafety Framework at **Member State level**. Therefore, the German legislation covering gene drive research and development is not spelled out below in detail, referring to more detailed insights into the relevant European provisions in section II.

The *Gentechnikgesetz* (GenTG) governs the deliberate release of Gene Drive Organisms (GDO) into the environment.2 An authorisation is needed for any deliberate release, which is issued as the result of an administrative authorisation procedure.3

The *Gentechniksicherheitsverordnung* (GenTSV) regulates the scientific research with GDO in laboratories by stating relevant biosafety measures.4 The GenTSV was amended in 2019,(Bundesgesetzblatt, 2019, p. 1235ff) which came into force in March 2021. The amendment includes the Biosafety Level 3 (BSL-3, Sicherheitsstufe 3) determination for working with Gene-Drive systems in laboratories.5 These paragraphs are the first rules specifically designed for working with Gene-Drive Organisms in a laboratory under German law. For a decision, the competent authority has to obtain recommendations by the *German Central Committee on Biological Safety* (ZKBS) regarding specific biosafety measures additional to the general requirements of the BSL-3.6

GenTG and GenTSV are relevant for the whole process of working with GDO in Germany as they lay down not only the relevant provisions which need to be adhered by researchers in German laboratories but also the requirements for any deliberate release of GDO in field trials.

8.2 European Law

The European Regulation on GMOs is most pertinent for any deliberate release in the EU and covering various aspects of biosafety. However, the European GMO framework is only applicable if a GDO is a GMO according to European Law and no exemptions apply. If the applicability is determined, different parts of the legislation are relevant, covering deliberate release, contained use and transboundary movement of GDO.

8.2.1 Deliberate Release Directive – Applicability of the European Biosafety Framework on GMO

The GMO definition of Art. 2 No. 2 of *Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms*7 ("Deliberate Release Directive") constitutes the threshold for the applicability of European GMO law in general. It reads as follows:

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1 For a definition what constitutes a GMO, see below at II.2.

2 See § 2 (1) No. 3 GenTG; according to this definition as the implementation of the relevant European provision on Member State level, a GDO constitutes a GMO in the meaning of German national law, see further II.1.

3 See § 10, 14 (1) No. 1 GenTG.

4 See § 1 GenTSV.

5 See § 10 (5) 1, 11 (6) 1 GenTSV.

6 See § 10 (5) 3, 11 (5) 3 GenTSV.

7 Official Journal of the European Communities, L 106, 17.4.2001, 1.

“an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination”

In the case *Confédération paysanne,* the European Court of Justice (“ECJ”) confirmed that a GMO is given if it is primarily the outcome of the use of a genetic engineering technique.8 Organisms that have a gene drive system implemented, e.g. a replacement drive with a cargo gene that causes sterility of males or infertility of females, have been genetically engineered insofar as their genetic material has been altered in a way that does not occur naturally. No exemptions that are laid down in the Deliberate Release Directive or its Annexes are of concern for GDO. Therefore, GDOs constitute GMOs according to European law and the European framework on GMO applies.

Besides the Deliberate Release Directive, Directive 2009/41/EC on the contained use of genetically modified micro-organisms9 (“Contained Use Directive”, see below at II.3.) and Regulation (EC) No. 1946/2003 on transboundary movements of genetically modified organisms10 (see below at II.4.) are of relevance for the deliberate release of a GDO in the environment.

8.2.2 **Deliberate Release Directive – Key Elements**

The Deliberate Release Directive lays down, as written above, the conditions of the deliberate release in the environment and the placing on the market of any GMO11 except for those GMOs that fall within the scope of Art. 13 Deliberate Release Directive. The necessary governmental authorisation procedure is based on an environmental risk assessment (ERA) before a GMO can be deliberately released or placed on the market.12

Art. 4 (1) Deliberate Release Directive is the decisive norm for a potential GDO release in the European Union. It reads as follows:

“Member States shall, in accordance with the precautionary principle, ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the deliberate release or the placing on the market of GMOs. GMOs may only be deliberately released or placed on the market in conformity with part B or part C respectively”

This article includes several legal requirements. These are establishing a high level of protection regarding human health and the environment13, the application of the precautionary principle, the need to undergo an authorisation procedure based on an ERA and the monitoring of GMO after release.

Firstly, the threshold of a high level of protection regarding human health and the environment is in accordance with Art. 191 (2) *Treaty on the Functioning of the European Union* (TFEU) stating that EU policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the EU. Therefore, any release of a GDO in the EU has to be assessed with regard to this threshold, ensuring that no adverse effects on human health and the environment might arise.

Secondly, the norm ensures the stringent application of the precautionary principle as part of the risk regulation of GDOs. The precautionary principle is the *Leitmotiv* of EU risk regulation.

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8 CJEU, Judgement of 25 July 2018 - Confédération paysanne, ECLI:EU:C:2018:583, para. 30, 38.

9 Official Journal of the EU, L 125, 21.5.2009, 75.

10 Official Journal of the EU, L 287, 5.11.2003, 1.

11 See Art. 1 Deliberate Release Directive.

12 See Arts 4, 6, 13-15 Deliberate Release Directive.

13 This follows not directly from the wording of Art. 4 (1) Deliberate Release Directive, but from Art. 191 (2) TFEU as a provision of EU primary law aiming at a high level of protection in EU environmental policy.

According to Art. 191 (2) TFEU, the precautionary principle is enshrined in EU primary law as a key principle for environmental legislation. It is also prominently mentioned as the overall objective of the Deliberate Release Directive in its Art. 1. In general terms, the precautionary principle allows and even obliges States regulating to protect the environment and human health if a plausible risk for the emergence of serious damage to the environment or human health exists even if there is no established link of causation. Principle 15 Rio Declaration, which is part of international (soft) law and referred to at the European and national level, reads as follows: “In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation”. Any release of a GDO in the EU has to be assessed according to this principle, justifying interventions as precautionary measures to ensure the protection of the environment and human health.

Thirdly, Art. 4 (1) Deliberate Release Directive lays down that GMOs are only to be released after undergoing a governmental authorisation procedure based on an ERA. The objective of an ERA is to identify and evaluate potential adverse effects of the GMO, either direct and indirect, immediate or delayed, on human health and the environment, which follows from the deliberate release of a GMO, see Annex II A. The ERA is to be carried out on a case by case basis, meaning that each GMO has to be evaluated against its potential adverse effects on human health and the environment. According to recital 19 and Annex II a case by case basis should also take due account of potential cumulative long-term effects associated with the interaction with other GMOs and the environment. Therefore, for any GDO release in the EU, a case-by-case approach has to be implemented.14

Fourthly, Art. 4 (1) Deliberate Release Directive requires Member States to ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from deliberate release. One relevant aspect is monitoring harmful effects after the release (so-called post-release monitoring) regarding the environment or human health. According to recital 20, post-release monitoring includes the identification of potentially adverse and cumulative long-term effects.15 Therefore, before the release of a GDO, authorities must have tools on how to operate the environmental monitoring and how to adapt risk management procedures once adverse effects occur.16

Hence, the Deliberate Release Directive lays down the essential requirements for releasing GDOs that have to be implemented by Member States. Based on the precautionary principle, any deliberate release of a GDO requires an approval as a result of a governmental authorisation procedure that is based on an environmental risk assessment (ERA). As the applicability of the European Biosafety Framework is assessed on a process-based interpretation of what constitutes a GMO, not only insects, especially mosquitos modified with a gene drive but other applications, such as a suppression drive for invasive species threatening indigenous species, are also considered to be GMOs according to Art. 2 Deliberate Release Directive.

14 The challenges for implementing an ERA in case of GDO release due to their particularities, for example that the safety cannot be established based on a comparative assessment, are discussed by scholars, see (Dolezel et al., 2020, p. 11ff).

15 Also, in the standard authorization procedure for deliberate release the notifier has to submit a plan for monitoring in accordance with the relevant parts of Annex III in order to identify effects of GMO(s) on human health or the environment, Art. 6 (2) lit. a No. (v) Deliberate Release Directive.

16 This argument is also brought forward by (Dolezel et al., 2020, p. 17f).

8.2.3 **Contained Use Directive**

The Contained Use Directive is complementary to the Deliberate Release Directive as part of EU law. Its scope and aim is to ensure that a GMO is regulated throughout the entire period of its development, from the first laboratory experiments to its storing and transport, and the release into the environment.17

For this purpose, the Contained Use Directive is governing the *laboratory biosafety* of genetically modified micro-organisms (GMMs) and lays down measures for the contained use and the *biosafety* of GMM in order to ensure the protection of human health and the environment.18 According to the definition of Art. 2 lit. a Contained Use Directive a micro-organism is any microbiological entity, cellular or non-cellular, capable of replication or transferring genetic material, including viruses, viroids, and animal and plant cells in culture. Hence, a GDO as part of an animal or plant is a GMM as it is read in concurrency with the Deliberate Release Directive.19

Besides, according to the *World Health Organization*, *laboratory biosafety* can be defined as "containment principles, technologies and practices that are implemented to prevent unintentional exposure to biological agents or their inadvertent release" (Deutscher Ethikrat, 2014; World Health Organisation, 2020). However, as, similar to national constitutional rights, Art. 13 of the *Charter of the Fundamental Rights of the European Union* (CFR) protects the *freedom of science* as a fundamental right20 any restrictions because of biosafety regulations concerning GDO laboratory research have to be necessary, appropriate and proportionate in order to be lawful.21

The most relevant norm for EU laboratory research with GDOs is Art. 4 (1) Contained Use Directive. It read as follows:

"Member States shall ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the contained use of GMMs"

Here, potential adverse effects of GMMs in contained use regarding human health and the environment are acknowledged as part of the rule. This is stressed by recital 8 Contained Use Directive stating the need for evaluation and reduction of the potential risks arising in the course of *all* operations involving contained use.

Nevertheless, the Directive does not provide a generalised view on the potential risk of GMM contained use but rather establishes a differentiated classification. On a general note, the condition is the need to carry out an assessment of the contained use with regard to the risks to human health and the environment.22 This shall result in a final classification of the contained use in four classes23 enabling a differentiated categorisation of the risks that are associated. This risk classification is the basis for assigning the containment levels and protective measures.24 It ranges from class 1 "activities of no or negligible risk' to class 4 'activities of high risk"25. In Germany, the amendment of GenTSV in 2019 included the

17 See Art. 2 lit. c Contained Use Directive. The Directive shall not apply to the storage, culture, transportation, destruction, disposal or use of GMMs, which have been placed on the market in accordance with the Deliberate Release Directive, see Art. 3 (3) Contained Use Directive.

18 See Art. 1 Contained Use Directive.

19 Art. 2 lit. b Contained Use Directive is similar in wording to Art. 2 No. 2 Deliberate Release Directive.

20 Art. 13 CFR reads as follows: "The arts and scientific research shall be free of constraint. Academic freedom shall be respected".

21 Regarding the role of Human Rights in GDO risk regulation, see chapter 8.3.3.

22 See Art. 4 (2) Contained Use Directive.

23 See Art. 4 (3) Contained Use Directive.

24 See Art. 4 (3) Contained Use Directive in accordance with Art. 5 (1) Contained Use Directive.

25 See Art. 4 (3) Contained Use Directive.

determination of the Biosafety Level 3 (BSL-3, Sicherheitsstufe 3) for working with Gene-Drive Systems in laboratories, as mentioned above.26 This was the result of a compromise by the German Government, who argued in its first draft that BSL-2 is sufficient.27 After the Bundesrat voted for the need for BSL-3, the Government accepted this result with regard to need to implement the precautionary principle and protect the environment and human health according to § 1 No. 1 GenTG (Bundesrat, 2019).28

The Contained Use Directive is therefore a key regulation for the contained use and ensuring *biosafety* of GDOs with a view to protecting human health and the environment in laboratory conditions. As the term GMM has to be read in conjunction with the term GMO according to the Deliberate Release Directive, the Contained Use Directive is broadly applicable regarding different applications of GDO in laboratories.

8.2.4 **Regulation (EC) No. 1946/2003 - Transboundary Movements of GMOs**

Regulation (EC) No. 1946/2003 on transboundary movements of genetically modified organisms ensures the coherent implementation of the *Cartagena Protocol on Biosafety* on behalf of the EU in order to contribute to an adequate level of protection in the field of safe transfer, handling and use of GMOs.29 Also, concerning the transboundary movements of GMOs, it stresses the precautionary principle, see Art. 1: "In accordance with the precautionary principle […], the objectives of this Regulation is […]".30

8.3 **International Law**

For the regulation of GDOs at the international level, it is important to keep two points in mind. Firstly, non-state actors, i.e. private actors, companies or research institutions, are not obliged by rules of international law. Obligations laid down in international agreements or under customary international law are only binding upon States and the EU as a supranational entity. Secondly, there is a variety of different legal documents at the international level which have, depending on their source, different binding force. Legally binding in the strict meaning are only those sources of international law enshrined in Art. 38 (1) of the *Statute of the International Court of Justice*.31 These are *inter alia* international conventions, i.e. international treaties, and international customary law. Gene drives are governed by the *Convention on Biological Diversity*32 ("CBD") and its Cartagena Protocol as international treaties that will be discussed below.

26 See § 10 (5) 1, 11 (6) 1 GenTSV.

27 BT-Drucks. 137/19, 89.

28 The statement reads as follows: „Auch bei künftigen Vorgaben für die Risikobewertung und Sicherheitseinstufungen von gentechnischen Arbeiten mit ‚Gene-Drive' - Organismen wird es darum gehen, Raum für Forschung und Innovation zu ermöglichen, wobei gleichzeitig das Vorsorgeprinzip und der Schutz der Schutzgüter des § 1 Nummer 1 des Gentechnikgesetzes gewährleistet sein muss", see Bundesrat (2019, p. 3).

29 See Art. 1 Regulation (EC) No. 1946/2003.

30 For further clarifications of the provisions laid down in the Cartagena Protocol on Biosafety establishing a common system of notification and information in the field of safe transfer, handling and use of GMO see below, chapter 8.3.3.b.

31 Statute of the International Court of Justice (entered into force 26 June 1945) 33 UNTS 933.

32 Convention on Biological Diversity (entered into force 29 December 1993) 1760 UNTS 79.

8.3.1 Convention on Biological Diversity and its Protocols

a. Key Elements

The CBD is a multilateral environmental agreement ratified by more than 190 parties as of May 2021.33 The three main objectives of this international treaty are: the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits of the use of genetic resources (access-and-benefit-sharing), see Art. 1 CBD. "Biological diversity" means the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems, see Art. 2(1) CBD.

For the governance of GDOs, several rules of the CBD can be decisive. Firstly, States are obliged to ensure that activities within their jurisdiction or control do not cause damage to the environment of other States or areas beyond the limits of national jurisdiction (Art. 3 CBD).

Secondly, Art. 8 lit. g CBD calls upon States to

"establish or maintain means to regulate, manage or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health".

In that regard, the CBD states that States shall, "as far as possible and as appropriate", introduce environmental risk assessment procedures. This has to be done where projects are likely to have significant adverse effects on biological diversity in order to avoid or minimise such impacts.34 Furthermore, the *Cartagena Protocol on Biosafety to the Convention on Biological Diversity*35 ("Cartagena Protocol") finds its basis in Art. 19 (3) and (4) CBD, which is also closely linked to Art. 8 lit. g CBD.36

Art. 8 lit. g CBD is the most relevant paragraph regarding the risk regulation of GDOs. It requires State Parties to oversee the risks associated with living modified organisms ("LMOs") resulting from biotechnology before their use or release into the environment. Whether GDOs can be considered as LMOs within the meaning of Art. 8 lit. g CBD has to be determined with regard to the definition of "biotechnology" as provided for in Art. 2 (3) CBD. According to that definition

"'biotechnology' means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use".

The definition for LMOs under the CBD is broader than as part of the Cartagena Protocol since it does not only comprise organisms resulting from *modern* biotechnology but from *biotechnology* in general.37 Since GDOs are qualified as LMOs under the Cartagena Protocol (Convention on Biological Diversity, 2017),38 these organisms also qualify as LMOs under the

33 See Link to United Nations Treaty Collection, last accessed 29.06.2021; the EU and EU Member States are State Parties to the CBD.

34 See Art. 14 (1) lit. a CBD.

35 Cartagena Protocol on Biosafety to the Convention on Biological Diversity (entered into force 11 September 2003) 2226 UNTS 208.

36 Cf. Cartagena Protocol, preamble, para. 1.

37 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.III.

38 See also Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, B.II.e.bb.; for further details on the definition of LMO under the Cartagena Protocol, see Beck, Ch. 3, B.II.

CBD (Glowka et al., 1994)39. Consequently, the CBD demands State Parties to establish or maintain means to regulate, manage or control the risks associated with GDOs. Under Art. 8 lit. g CBD, potential environmental and health risks should be assessed, regulated, managed and controlled in a "rational" and "precautionary manner" (Glowka et al., 1994). This is also supported by Art. 7 lit. c CBD.40 Furthermore, it is suggested to use policy guidance on GMOs as developed by international bodies, such as the OECD, the FAO or the WHO, to formulate approaches to implement Art. 8 lit. g CBD (Glowka et al., 1994). However, the CBD itself does not spell out the details of the design of such a framework. Additionally, Art. 8 lit. g CBD has been rarely addressed by the Conference of the Parties (CoP) to the CBD.41

With regard to the transboundary movement, transit, handling and use of LMOs, Art. 8 lit. g CBD is shaped by the Cartagena Protocol in more detail, which is explained in greater detail in section III.1.b.42

One could also suggest that the obligation to assess the potential environmental effects of GDOs is informed by Art. 14 (1) lit. a CBD, which requires State Parties to

"introduce appropriate procedures requiring environmental impact assessment of its proposed projects that are likely to have significant adverse effects on biological diversity with a view to avoiding or minimising such effects".

However, the International Court of Justice ("ICJ") has stated that this provision does not require State Parties to carry out an environmental risk assessment (International Court of Justice, 2015, para. 164). Nevertheless, the ICJ held that a violation of the obligation to carry out an environmental impact assessment as laid down in general international law was given (International Court of Justice, 2015, paras. 146–162). This implies that the ICJ deems the obligation to carry out an environmental impact assessment to be stronger according to general international law compared to the one that is part of the CBD, which has been criticised.43

Another provision of the CBD that could become relevant in the context of GDOs is Art. 8 lit. h CBD which calls upon State Parties to "prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species". Whether GDOs constitute alien species cannot be assessed in general terms. While GDOs intentionally released into a certain environment cannot be regarded as "alien", they might be considered as such when spreading beyond the initially intentioned geographic range.44 This has also been recognised by the State Parties to the CBD.45

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39 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.III.; see also, 45 on the definition of LMOs under the CBD.

40 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.III.

41 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.III.

42 See also Glowka et al. (1994) who suggest using the mechanisms established under Art. 8 lit. g CBD to fulfil the State Parties' obligations under Art. 19 (4) CBD.

43 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.VI.1.

44 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.V.

45 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.V. with reference to CBD COP, Decision VIII/27, Alien Species that Threaten Ecosystems, Habitats or Species (Article 8(H)): Further Consideration of Gaps and Inconsistencies in the International Regulatory Framework, CBD/COP/DEC/VIII/27, 2006, paras 55, 64.

While the proposal to enact a general moratorium on the further development of GDOs was rejected at COP 13 in 2016,46 the parties to the CBD, at COP 14 in 2018, adopted decision 14/19 (Convention on Biological Diversity, 2018). In this decision, the COP

"[r]*ecognizes* that, as there could be potential adverse effects arising from organisms containing engineered gene drives, before these organisms are considered for release into the environment, research and analysis are needed, and specific guidance may be useful, to support case-by-case risk assessment" (Convention on Biological Diversity, 2018, para. 9).

Also, the decision

"[c]*alls* upon Parties and other Governments, taking into account the current uncertainties regarding engineered gene drives, to apply a precautionary approach, in accordance with the objectives of the Convention, and *also calls* upon Parties and other Governments to only consider introducing organisms containing engineered gene drives into the environment, including for experimental releases and research and development purposes, when:

(a) Scientifically sound case-by-case risk assessments have been carried out;

(b) Risk management measures are in place to avoid or minimize potential adverse effects, as appropriate;

(c) Where appropriate, the "prior and informed consent", the "free, prior and informed consent" or "approval and involvement" of potentially affected indigenous peoples and local communities is sought or obtained, where applicable in accordance with national circumstances and legislation". (Convention on Biological Diversity, 2018, para. 11)47

Hence one can summarize that the CBD provides a general framework with regard to the regulation of GDOs by its Member States which has been further specified to some extent by COP decision 14/19.48 It requires the assessment of risks of GDOs and the establishment of appropriate risk management measure before these organisms are released into the environment. This has to be done in accordance with a precautionary approach as laid down in its Preamble (United Nations, 1992, para. 9). Due to its universal recognition, the CBD is the main international agreement that expressly deals with the regulation of GDOs.

The obligations under the CBD are further elaborated on in the Cartagena Protocol on Biosafety and the Nagoya – Kuala Lumpur Supplementary Protocol on Redress and Liability to the Cartagena Protocol on Biosafety.

b. Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety was negotiated within the framework of Art. 19 (3) CBD and entered into force in 2003.49 173 Parties have ratified it, but a number of key States in the field of biotechnology, such as Argentina, Canada and the United States have not ratified the

46 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 5, B. with reference to IISD Reporting Services, (2016) and Callaway (2016).

47 For further considerations on the requirement on "free, prior and informed consent", see chapter 8.3.3.

48 For details on the legal status of COP 14/19, see Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 5, C.

49 See https://bch.cbd.int/protocol/background/ (last accessed 25.05.2021) with further details on the background of the Cartagena Protocol.

Protocol.50 This is problematic since most of the work on LMOs has been conducted on the basis of the Cartagena Protocol, which has fewer State Parties than the CBD.51

The Cartagena Protocol's objective is to "contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health" (United Nations, 2000, article 1). This shall be done in accordance with the precautionary approach as contained in Principle 15 of the Rio Declaration on Environment and Development (*The Rio declaration on environment and development*, 1992, principle 15; United Nations, 2000, article 1).

In line with this objective, the Cartagena Protocol applies "to the transboundary movement, transit, handling and use of all living modified organisms that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health" (United Nations, 2000, article 4). An LMO in the sense of the Cartagena Protocol is "any living organism that possesses a novel combination of genetic material obtained through the use of *modern biotechnology*" (United Nations, 2000, article 3). Modern biotechnology is further defined in Art. 3 lit. i Cartagena Protocol. The *Ad Hoc Technical Expert Group on Synthetic Biology to the Convention on Biological Diversity* ("AHTEG")

"concluded that most living organisms already developed or currently under research and development through techniques of synthetic biology, including organisms containing engineered gene drives, fell under the definition of LMOs as per the Cartagena Protocol." (Convention on Biological Diversity, 2020 Annex I, para. 42, 2017). 52

While some authors argue that the scope of the Cartagena Protocol excludes LMOs, which are unlikely to have adverse effects,53 this is only the case in accordance with Art. 7 (4) of the Cartagena Protocol. This approach is in line with the precautionary approach, which even subjects LMOs to the Protocol's provision when there is no scientific certainty on their adverse effects, but they have not proven to be safe yet.54

Another main feature of the Cartagena Protocol on Biosafety is the *Advance Informed Agreement Procedure* ("AIA procedure") laid down in Arts 7 to 10 and 12 of the Cartagena Protocol, which regulates the transboundary movement of LMOs. It applies "prior to the first intentional transboundary movement of living modified organisms *for intentional introduction into the environment* of the Party of import" (United Nations, 2000, article 7(1)). However, it does not apply to living modified organisms that are intended for direct use as food or feed or processing. These organisms are regulated by Art. 11 Cartagena Protocol (United Nations, 2000, article 7(3)).

The AIA procedure requires the exporting State Party to notify the competent national authority of the importing State in writing (United Nations, 2000, article 8(1)). In turn, the importing State shall acknowledge the receipt of the notification, also in writing (United Nations, 2000, article 9(1)). Here, it is important to note that a failure to acknowledge the receipt of the notification does not imply the importing State's consent to an intentional transboundary movement

50 See United Nations Treaty Collection, Chapter XXVII Cartagena Protocol under https://treaties.un.org/Pages/ViewDetails.aspx?src=TREATY&mtdsg_no=XXVII-8-a&chapter=27&clang=_en (last accessed 25.05.2021)

51 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, C.III.

52 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, B.I.1.e.bb.

53 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, B.I.2. with reference to Komen (2012); Pavoni (2000); and Ricci (2004).

54 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, B.I.2. with reference to Mackenzie et al. (2003 para. 279).

(United Nations, 2000, article 9(4)). The importing State shall decide on how to proceed with the requested import in accordance with Art. 10 (3) Cartagena Protocol. Such a decision shall be taken following Art. 15 Cartagena Protocol which requires States to carry out a risk assessment in the decision-making process as further elaborated on in Annex III to the Cartagena Protocol. In this context, the "AHTEG [on Synthetic Biology] […] noted that existing risk assessment considerations and methodologies might not be sufficient or adequate to assess and evaluate the risks that might arise from organisms containing engineered gene drives due to limited experience and the complexity of the potential impacts on the environment. The development or further development of guidelines on risk assessment of organisms containing engineered gene drives by the Convention, other international organisations, national governments and professional bodies would be useful in that regard" (Convention on Biological Diversity, 2020 Annes I, para. 42, 2017). The AHTEG on risk assessment therefore "recommended that guidance for the risk assessment on living modified organisms containing engineered gene drives should be developed" (Convention on Biological Diversity, 2020, Annex I, para. 42).

A decision taken on the basis of Art. 10 Cartagena Protocol shall spell out the reasons on which it is based, except for cases of unconditional approval (United Nations, 2000, article 10(4)). Again, in line with the precautionary principle,55 Art. 10 (6) Cartagena Protocol makes clear that "lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, […] shall not prevent that Party from taking a decision, as appropriate, with regard to the import of the living modified organism […] in order to avoid or minimise such potential adverse effects". The decision may be reviewed, either in light of new scientific information on potential adverse effects in accordance with Art. 12 (1) Cartagena Protocol, or on request by the exporting State Party or a notifier following Art. 12 (2) Cartagena Protocol.

In addition to the provisions on the AIA procedure and risk assessment, the Cartagena Protocol also entails provisions on the risk management of LMOs (United Nations, 2000, article 16), their unintentional transboundary movement (United Nations, 2000, article 17), handling, transport, packaging and identification of LMOs (United Nations, 2000, article 18), and on illegal transboundary movements (United Nations, 2000, article 25).

One can conclude that because of the AIA procedure, the Cartagena Protocol provides for specific provisions on how the Member States to the Protocol should proceed and conduct risk assessments in the context of the transboundary movement and deliberate release of GDOs. Also, the Cartagena Protocol lays down specific obligations with regard to risk management and questions arising in the context of the transboundary movement and deliberate release of GDOs.

c. Nagoya – Kuala Lumpur Protocol

The *Nagoya – Kuala Lumpur Supplementary Protocol on Redress and Liability to the Cartagena Protocol on Biosafety*56 entered into force in 2018 and has 49 Parties as of June 2021. Its objective "is to contribute to the conservation and sustainable use of biological

55 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 3, B.II.1.d. with reference to Mackenzie et al. (2003), para. 339; Böckenförde, 'Biological Safety', in: Wolfrum (ed.), MPEPIL (2010), para. 13; Graff, 'The Precautionary Principle', in: Bail et al., The Cartagena Protocol on Biosafety, 2002, 410, 418-419.

56 See https://treaties.un.org/Pages/ViewDetails.aspx?src=TREATY&mtdsg_no=XXVII-8-c&chapter=27&clang=_en (last accessed 29.06.2021)

diversity, also taking into account risks to human health, by providing international rules and procedures in the field of liability and redress relating to living modified organisms".57

Similar to the Cartagena Protocol, the Protocol's applicability is linked to the transboundary movement of LMOs.58 GDOs fall within the term of LMOs defined by reference to the CBD and the Cartagena Protocol.59 Relevant for GDOs, it comprises LMOs destined for contained use and those intended for the deliberate release into the environment.60 But, the Supplementary Protocol also applies to unintentional and illegal transboundary movements.61 Furthermore, the Protocol's applicability requires damage caused by LMOs, which is defined as "adverse effect on the conservation and sustainable use of biological diversity, also taking into account risks to human health"62.63 However, it has to be noted that transboundary damage alone is not sufficient for the Protocol's applicability.64

The Supplementary Protocol lays down rules with regard to administrative and civil liability for damage that can be causally linked to an LMO in accordance with domestic law65. Firstly, it obliges State Parties to require operators, i.e. any person in direct or indirect control of the LMO,66 to take response measures in the event of damage.67 Such response measures may also be taken by the competent authority when the operator has failed to do so.68 Secondly, the Protocol requires State Parties to provide for rules and procedures that address civil liability for damage.69

Accordingly, adverse effects on the conservation and sustainable use of biological diversity that the transboundary movement of GDOs has caused are regulated under the Supplementary Protocol. In the case of damage, operators are required to take response measures and are held liable in accordance with domestic law.

8.3.2 **Law of the World Trade Organisation**

While genetically or living modified organisms are not expressly regulated by the law of the World Trade Organisation ('WTO'), *the WTO Agreement on the Application of Sanitary and Phytosanitary Measures*70 ('SPS Agreement') provides a regulatory framework for Member States' regulations on the protection of human, animal and plant life and health.

The SPS Agreement's scope is defined by Arts 1.1 and 1.2 SPS Agreement in conjunction with its Annex A (1), which lays down the criteria for a sanitary or phytosanitary measure. In

57 See Art. 1 Nagoya – Kuala Lumpur Protocol.
58 See Art. 3 (1) Nagoya – Kuala Lumpur Protocol.
59 See Art. 2 (1) Nagoya – Kuala Lumpur Protocol.
60 See Art. 3 (1) lit. b and c Nagoya – Kuala Lumpur Protocol.
61 See Art. 3 (3) Nagoya – Kuala Lumpur Protocol.
62 See Arts 3 (1) and 2 (2) lit. b Nagoya – Kuala Lumpur Protocol.
63 For an extensive analysis of the Protocol's scope, see Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 6, C.
64 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 6, G.I.
65 See Art. 4 Nagoya – Kuala Lumpur Protocol.
66 See Art. 2 (2) lit. c Nagoya – Kuala Lumpur Protocol.
67 See Art. 5 Nagoya – Kuala Lumpur Protocol; for further details, see Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 6, D.
68 See Art. 5 (4) Nagoya – Kuala Lumpur Protocol.
69 See Art. 12 Nagoya – Kuala Lumpur Protocol; for further details, see Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 6, E.
70 Agreement on the Application of Sanitary and Phytosanitary Measures (entered into force 1 January 1995) 1867 UNTS 493.

this context, it is disputed whether measures to protect biodiversity and the environment (which are not expressly mentioned by one of the alternatives stated in Annex A (1) to the SPS Agreement) also qualify as SPS measures and thus fall within the SPS Agreement's scope of application. Since the SPS Agreement was negotiated in the context of the Agreement on Agriculture, it is said to primarily cover "traditional 'sanitary and phytosanitary' concern[s], such as quarantine risks associated with the entry and spread of pests and diseases via traded agricultural products, or risks posed by toxins, additives or contaminants in imported human foods or animal feed" (Conrad, 2007; Peel, 2006). Nevertheless, the WTO Panel qualified the EU approval procedures for genetically modified organisms to the extent that they protect the environment and biodiversity as SPS measures. Whether provisions regulating import and deliberate release of GDOs fall within the SPS Agreement also depends on the question of whether they qualify as pests or disease-carrying/-causing organisms in the sense of Annex A (1) to the SPS Agreement.

In case that measures regulating the use and handling of GDOs fall within the scope of the SPS Agreement, the States Parties regulatory flexibility is limited by the provisions of the SPS Agreement.

The SPS Agreement calls upon State Parties to base their SPS measures on international standards, guidelines or recommendations.71 These are the standards established by the Codex Alimentarius Commission, under the auspices of the World Organisation for Animal Health and under the auspices of the International Plant Protection Convention and others as identified by the SPS Committee.72 Regulatory measures that conform to international standards, guidelines or recommendations are deemed to comply with the SPS Agreement.73 If international standards exist, a Member State may nevertheless introduce or maintain measures that result in a higher level of protection, as long as these measures are in accordance with Art. 3.3 and any other provision of the SPS Agreement. While one might think of the Cartagena Protocol as an international standard in the context of GDOs, the Protocol has never been identified as such by the SPS Committee.74

Most relevant with regard to GDOs are the science-based obligations of the SPS Agreement. Art. 2.2 requires States Parties to the WTO to base their SPS measures on scientific principles and on sufficient scientific evidence. This obligation is further specified by Art. 5.1 SPS Agreement. Art. 5.7 SPS Agreement provides for a possibility to temporarily bypass these obligations in cases where scientific evidence is insufficient, and a risk assessment cannot be carried out.

According to Art. 5.1, every SPS measure must be based on a risk assessment as defined in Annex A (4) to the SPS Agreement. Such risk assessment requires State Parties to *either* evaluate the likelihood of entry, establishment or spread of a pest or disease within the territory of an importing Member according to the sanitary or phytosanitary measures which might be applied and of the associated potential biological and economic consequences *or* to evaluate the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease-causing organisms in food, beverages or feedstuffs. For GDOs, probably only the first type of risk assessment might become relevant. For this type of risk assessment, it is important to note that it requires the evaluation of the *probability* of risk occurrence in contrast to the mere *possibility* of risk occurrence (Prévost and Van den Bossche, 2005; Scott, 2009). Additionally, State Parties' SPS measures have to *be based on a risk assessment* according to Art. 5.1 SPS Agreement. This requires an objective relationship between an SPS measure and a risk assessment, meaning that the risk

71 See Art. 3.1 SPS Agreement.

72 See Annex A(3) to the SPS Agreement; see also below, section III.5.

73 See Art. 3.2 SPS Agreement.

74 For further details on this suggestion with regard to LMOs, see Böckenförde, Grüne Gentechnik und Welthandel: Das Biosafety-Protokoll und seine Auswirkungen auf das Regime der WTO, 2004, 333-336.

assessment must sufficiently warrant the SPS measure at stake (World Trade Organization, 1998, paras. 189, 193). While an SPS measure can be based on minority scientific opinions coming from qualified and respected sources (World Trade Organization, 1998, para. 194), theoretical uncertainty does not fulfil the requirement of an objective relationship (cp. Scott, 2009). Also, "an unequivocally positive risk assessment will in general not be able to serve as a rational basis for a categorical prohibition on the substance or product in question." (Scott, 2009). These requirements by the SPS Agreement have the potential to limit the regulatory flexibility of Member States to the WTO when it comes to the regulation of the deliberate release of GDOs.

However, Art. 5.7 SPS Agreement provides State Parties with the possibility to provisionally enact SPS measures on the basis of available pertinent information where scientific evidence is insufficient. Scientific insufficiency exists when a risk assessment in the sense of Art. 5.1 SPS Agreement cannot be conducted due to a lack of scientific evidence or inconclusive or unreliable evidence (Prévost and Van den Bossche, 2005, p. 303f). This must not be equated with scientific uncertainty (World Trade Organization, 2003, para. 184). Furthermore, Art. 5.7 SPS Agreement only allows for the temporary application of SPS measures in order to allow State Parties to carry out the risk assessment as required by Art. 5.1 SPS Agreement (Prévost and Van den Bossche, 2005, p. 307). How long States Parties may rely on Art. 5.7 SPS Agreement has not been conclusively clarified yet.

Accordingly, the WTO's SPS Agreement provides for a framework that States have to observe when regulating the deliberate release of GDOs on their territory. While States may introduce a zero-risk policy, such a policy can only be based on a risk assessment that has acknowledged a certain probability of risk occurrence. A zero-risk policy must not be based on theoretical uncertainty with regard to the risks of GDOs. This approach slightly differs from the one taken in the Cartagena Protocol (United Nations, 2000, article 10(6)).

8.3.3 **Human Rights**

Universal human rights treaties and regional human rights treaties are also relevant as they set international legally binding standards for the regulation of biotechnology (Vöneky, 2019, p. 131). The UN-based human rights treaties, the *International Covenant on Civil and Political Rights* (ICCPR) and the *International Covenant on Economic, Social and Cultural Rights* (ICESCR) enshrine relevant human rights for GDO release. As they are in force since 1967, they enjoy universal recognition as legally binding human rights treaties due to their high number of ratifications.

There are several human rights concerned by the proposed release of a GDO. Most particular, Art. 7 ICCPR is a red line for any research on biotechnology and biomedicine, being *ius cogens* for international standard-setting in this area (Vöneky, 2019, p. 135f). It reads as follows:

"[…] no one shall be subjected without his free consent to medical or scientific experimentation"

There is an ongoing discussion about the requirement of consent from a human rights perspective and an ethical perspective. According to the wording of Art. 7 ICCPR, consent of each potentially affected individual participating in scientific experimentation is needed. Also, from an ethical perspective, individual informed consent is a basic requirement for scientific integrity universally recognized in research ethics regulations (for a discussion on public engagement see Annas, 2020; Thompson, 2018; World Health Organization, 2021). However, in the case of GDOs, the seeking of informed consent by any potentially affected individual seems impossible to provide as GDOs are specially designed to spread into a wide geographical range (World Health Organization, 2021).75 Therefore, there is an emerging

75 Beck, Responsibilty and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 5, II.3. b.

consensus for seeking only individual consent if personal data is collected, e.g. in regard to epidemiological endpoints such as incidence of new infections with malaria or if the experiments might be detrimental to participants health.76 Otherwise, so-called community or group consent is sufficient (Vöneky, 2019, p. 138ff). This view is also supported by Decision 14/19 of the 14th COP meeting 2018 explicitly stating the need for free, prior and informed consent (FPIC) of potentially affected indigenous peoples and local communities in case of GDO release (Convention on Biological Diversity, 2018, para. 11).77 The concept of FPIC dates back to the *UN Declaration on the Rights of Indigenous Peoples* from 2007 (United Nations General Assembly, 2007), a soft law instrument providing guidance when interpreting human rights treaties.

Furthermore, *freedom of science* is relevant to any biotechnology or biomedicine research. Art. 15 (3) ICESCR lays down freedom of scientific research as a second-generation human right.78 Besides, Art. 15 (1) lit. b guarantees the right to science as a right of everyone "to enjoy the benefits of scientific progress and its application". In *General Comment No. 25,* the *UN Committee on Economic, Social, and Cultural Rights* ("UN CECSR") (United Nations Committee on Economic and Social Council, 2020) develops the key aspect of participation in scientific progress as a dimension of freedom of science: The UN CECSR clearly extends the wording of Art. 15 (1) lit. b ICESCR to a *right to participate in and to enjoy the benefits of scientific progress and its applications* (United Nations Committee on Economic and Social Council, 2020, para. 11). On the one hand, the legal significance of General Comments as interpretative clarifications by human rights treaty bodies is widely debated;79 on the other hand, at least some scholars award them a high authoritative character.80 Consequently, they might be seen as the most important tool for the interpretation of the ICESCR.81 Finally, this tendency of development towards a right of everybody to participate in scientific progress can stress the importance to ensure that the interests and concerns of people affected by GDO release are heard before.

Regional human rights treaties are another source for human rights-based standard-setting in biotechnology. In the EU, Art. 13 *Charter of the Fundamental Rights of the European Union* (CFR) fundamentally protects *freedom of science* as a human right82. Here, the ECJ is part of the European system of legal protection, where a possible violation of *freedom of science* can be determined.

Universal human rights treaties and regional human rights treaties are of relevance for the deliberate release of GDO as they set international legally binding standards. As GDOs are specifically designed to spread transboundary, this is essential for the risk regulation on the

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76 Beck, Responsibilty and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 5, II.3. b.

77 Convention on Biological Diversity, 2018, para. 11 c) regarding the release of GDO reads as follows: "Where appropriate, the "prior and informed consent", the "free, prior and informed consent" or "approval and involvement" of potentially affected indigenous peoples and local communities is sought or obtained, where applicable in accordance with national circumstances and legislation".

78 It states as follows: "The States Parties to the present Covenant undertake to respect the freedom indispensable for scientific research and creative activity".

79 See for an analysis of the reception of General Comments by state parties and courts (Blake, 2008) in Bundesverfassungsericht (2013) the German constitutional court stresses, that GCs are not legally binding, but cites it for the interpretation of German law.

80 Riedel, 'Committee on Economic, Social and Cultural Rights (CECSR)', in Wolfrum (ed.) MPEPIL (2010), para. 12; Ando, 'General Comments/Recommendations', in Wolfrum (ed.), MPEPIL (2008), para. 10; Roth-Isigkeit, 2012

81 Riedel, 'Committee on Economic, Social and Cultural Rights (CECSR)', in Wolfrum (ed.), MPEPIL (2010), para. 12; BVerwG, Urteil v. 29.04.2009, Az. 6 C 16.08, para. 48, 41; Roth-Isigkeit, 2012, p. 206f

82 Art. 13 CFR reads as follows: "The arts and scientific research shall be free of constraint. Academic freedom shall be respected".

universal level, establishing a level of protection that cannot be undermined by States who are parties to the relevant human right treaties.

8.3.4 **Obligation to Prevent Transboundary Harm**

The obligation to prevent harm to the environment of another State is well established in an international treaty as well as international customary law.83 Under Art. 1 of the ILC Draft Articles on the Prevention of Transboundary Harm from Hazardous Activities, the obligation applies "to activities not prohibited by international law which involve a risk of causing significant transboundary harm through their physical consequences". The "risk of causing significant transboundary harm includes risks taking the form of a high probability of causing significant transboundary harm and a low probability of causing disastrous transboundary harm"84 and "is a combined threshold [including] the potential magnitude of harm and the probability that harm will occur"85. In the context of GDOs, it is questionable whether the transboundary spread of GDOs may violate the obligation not to cause significant transboundary harm to another State's territory. There may be a violation where a GDO causes significant harm through unintended side-effects or deliberately eradicates a whole species in its natural habitat, thereby violating the CBD.86 This could be different if a GDO "exceeds its intended target range but, apart from this, functions as intended and does not cause any injury"87.

8.3.5 **Soft Law**

As already stated above, there are various different legal documents and sources on the international level that have no legally binding force *strictu sensu*. *Soft law* is not legally binding in a strict sense but has normative force since it is agreed upon by subjects of international laws that could establish international hard law.88 Generally speaking, there are relevant *soft law* documents that cover different aspects of biotechnology and genetic engineering in international law.

The *Codex Alimentarius*89 is a collection of standards, guidelines and codes of practices to ensure food safety and quality in international trading drafted and collected by the *Codex*

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83 Cf. Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 4, B. with reference to Principle 21 of the Stockholm Declaration of 1972, Declaration of the United Nations Conference on the Human Environment (16 June 1972), UN Doc. A/Conf.48/14/Rev.1; to Principle 2 of the Rio Declaration 1992, Rio Declaration on Environment and Development (14 June 1992), UN Doc. A/CONF.151/26/Rev.1; to Art. 3 of the CBD; to the ILC, Draft Articles on the Prevention of Transboundary Harm from Hazardous Activities, Yearbook of the ILC 2001, Vol. II, 148 and to international jurisprudence, such as Gabcikovo-Nagymaros Project (Hungary v. Slovakia), 1997 ICJ Rep 7, para. 53; Pulp Mills on the River Uruguay (Argentina v. Uruguay), 2010 ICJ Rep 14, para. 193 and International Court of Justice, 2015, para. 118

84 See Art. 2 lit. a Draft Articles on the Prevention of Significant Transboundary Harm from Hazardous Activities.

85 Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 4, C.

86 See Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 5, E.II. with reference to Hochkirch et al. (2018) and Reynolds (2020).

87 Beck, Responsibility and Liability for Transboundary Harm Caused by Self-Dispersing Biotechnology Under International Law, to be published, Ch. 5, E.II. and Ch. 4, C.VII.2.

88 See the definition in Vöneky, 2019.

89 The numerical Codex standards for food additives, veterinary drugs maximum residue levels and pesticide maximum residue levels are available at http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/en/ (lastly accessed on 29.06.2021).

Alimentarius Commission (CAC).90 Its primary concern is the realisation of food safety standards which are also drafted in regard to the risk assessment of genetically modified plants or food and feed products derived.91 While the application of Gene-Drive Systems is not discussed in terms of food and feed products, the *Codex Alimentarius* offers helpful guidance regarding relevant parameters of GMO risk assessment, which may be adapted for GDO risk assessment.

Moreover, the 1999 *International Plant Protection Convention* is an international *soft law* treaty aiming to protect the world´s plant resources from the introduction and spread of pests (Secretariat of the International Plant Protection Convention, 1997). Therefore, Convention could become relevant if GDOs are considered to be plant pests (Secretariat of the International Plant Protection Convention, 2019).

More specifically, the *World Health Organization* published in 2014 the *Guidance framework for testing of genetically modified mosquitoes,* which was updated in 2021 (World Health Organization, 2021). It covers various aspects of potential GDO release. The report gives *inter alia* an overview of safety evaluation of GDOs and further advice on regulatory frameworks. The role of ethics and public engagement in field testing of GDOs with different public groups is also discussed, stressing the importance of public dialogue and outreach (World Health Organization, 2021).

Soft law is of relevance for the deliberate release of GDOs as it has normative force regarding the development of a regulatory framework. Useful guidance is offered when various relevant aspects of GDO release are discussed, for example, the role of public engagement in field testing or the adaption of GMO risk assessment for GDOs.

90 http://www.fao.org/fao-who-codexalimentarius/about-codex/en/#c453333 (last accessed on 29.06.2021)
91 http://www.fao.org/fao-who-codexalimentarius/thematic-areas/biotechnology/en/ (last accessed on 29.06.2021)

8.4 Regulatory framework for the deliberate release of Gene Drive Organisms – Summary

Various rules and norms are of relevance for the deliberate release of Gene Drive Organisms (GDOs) on the national, European and international level. Most importantly, GDOs fulfil the definition of Genetically Modified Organisms (GMOs) according to the European Biosafety Framework and the definition of living modified organisms (LMOs) according to the Biodiversity Convention and its Protocols mentioned below.

Besides, the German GMO regulation implements the European Biosafety Framework at the member state level. Hence the European Regulation on GMOs is most pertinent for any deliberate release in the EU covering various aspects of biosafety. The Deliberate Release Directive ensures that any deliberate release of a GDO requires approval due to a governmental authorisation procedure based on an environmental risk assessment (ERA), stressing the relevance of the precautionary principle. The Contained Use Directive is governing the laboratory biosafety of GDOs and lays down measures for the contained use in order to ensure the protection of human health and the environment.

At the international level, there are rules and norms that are binding as international law, as the international treaties mentioned below. Due to its universal recognition, the Convention on Biological Diversity is the main international treaty that expressly deals with the regulation of LMOs. It provides a binding international and nearly universal general framework with regard to the regulation of GDOs requiring the assessment of risks and the establishment of appropriate risk management measures before a deliberate release occurs.

Besides, the Cartagena Protocol, a binding international treaty and Protocol of the Biodiversity Convention, provides for specific provisions on how the member states are obliged to proceed and conduct risk assessments as well as specific obligations with regard to risk management in the context of the transboundary movement and the deliberate release of GDOs. Of further relevance is the supplementary Nagoya–Kuala Lumpur Protocol, the third binding international treaty in this area, which regulates the adverse effects on the conservation and sustainable use of biological diversity that the transboundary movement of GDOs might cause.

Moreover, from a world trade law perspective, the WTO´s Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), a binding international treaty, provides a legal framework that states have to observe when regulating the deliberate release of GDOs on their territory. Importantly, a zero-risk policy must not be based on theoretical uncertainty with regard to the risks of LMOs, which is an approach slightly differing from the one taken in the Cartagena Protocol.

From a general human rights perspective, binding universal human rights treaties (such as the International Covenant on Civil and Political Rights and the International Covenant on Economic, Social and Cultural Rights) and regional human rights treaties (such as the Charter of the Fundamental Rights of the European Union) are relevant as they set international legally binding standards for the regulation of biotechnology, and entail the right to freedom of science, even if it is not expressly mentioned.

From the perspective of customary international law, it is questionable whether the transboundary spread of GDOs violates the obligation not to cause significant transboundary harm to another State’s territory. If this rule of international law is violated, the responsible state has to make reparations.

Lastly, so-called Soft law and other guidelines, such as the Codex Alimentarius, are of relevance for the deliberate release of GDOs. These have normative force even if they are not directly binding as law but a violation of these rules does not entail the international responsibility of a state.

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