Chapter 1

Technology Characterisation

Johannes L. Frieß, Bernd Giese and Arnim von Gleich

Introduction

In recent years, innovation in genetic engineering brought forth a number of technologies to manipulate the fate of entire wild type populations. These technologies rely on the dissemination of synthetic genetic elements within a population of sexually reproducing species via the germline and are identified as Self-Propagating Artificial Genetic Elements (SPAGE). Some secure their dissemination passively so that only offspring carrying the SPAGE will survive or be fertile. Others overcome the limitations of the Mendelian inheritance pattern by a distortion of allelic segregation or a fragmentation of chromosomes, resulting in e.g. an altered sex ratio. Genetic elements may also promote their preferred inheritance by a molecular mechanism. If a SPAGE overcomes the Mendelian pattern of inheritance and is thereby enabled to spread and distribute a novel trait throughout a population – even defying natural selection – it is called a gene drive. If organisms have a comparably short generation time, as e.g. insects, then already after a few months, a large part of the population could express a new property transmitted by the gene drive. In particular, very invasive gene drives may be able to impose properties on entire populations that otherwise could not spread.

SPAGEs are discussed for many potential applications and partially already designed as a kind of self-propagating delete-function. If for example the property mediated by the synthetic genetic element consists in male or female offspring becoming infertile, an entire population may disappear.

J. L. Frieß (✉) · B. Giese

Institute of Safety/Security and Risk Sciences, University of Natural Resources
and Life Sciences, Vienna (BOKU), Vienna, Austria
e-mail: Johannes.friess@boku.ac.at

A. von Gleich

Department of Technological Design and Development, Faculty Production Engineering,
University of Bremen, Bremen, Germany

© The Author(s) 2020

A. von Gleich and W. Schröder (eds.), *Gene Drives at Tipping Points*,
https://doi.org/10.1007/978-3-030-38934-5_1

Currently, multiple applications are under consideration. Especially malaria- or dengue-carrying mosquitoes are potential targets. In agriculture, weeds and crop pests could be eradicated or endangered species could be immunized against pathogens using a GD. Two potential applications of gene drives even serve issues of nature conservation, namely the eradication of invasive animal or plant species (Webber et al. 2015) and the conservation of endangered species (Esvelt et al. 2014; European Commission and Scientific Advice Mechanism 2017; Ledford 2015). Although discussed in the 2016 NASEM report on gene drives (National Academies of Sciences 2016), the idea to recover the sensitivity of pest species to pesticides or to remove transgenic resistances from feral populations have not been pursued in the scientific literature of the following years. So far, gene drives have not yet been released, but the discussion is gaining momentum (Courtier-Ordogozo et al. 2017; Emerson et al. 2017; Hochkirch et al. 2017). In particular, the development of new gene drive variants is closely linked to the upswing that genome editing methods have taken by the recent use of CRISPR-Cas gene scissors (Gantz et al. 2015; Gantz and Bier 2015).

Compared to previous releases of GMOs, SPAGE and especially gene drives collide with basic principles of precaution due to their targeted property to spread in wild populations and thus causing extreme exposure. Applications of this new quality represent a shift of paradigm in the handling of GMOs. At least for the European Community, the current regulation of the release of GMOs assumes that for specific periods of time a certain amount of GMOs will be released in a particular region.¹ However, now a type of genetic technology arises whose innermost principle lies in exceeding these limits: the transformation or even eradication of wild populations.

So far, it is unclear whether particular SPAGE applications, once released, will be retrievable or manageable at all. Due to their intended ability to spread, a loss of control is highly probable, not least in comparison to hitherto existing GMOs. In general, SPAGEs must be characterized as a technology with a high depth of intervention into the genetic configuration of organisms and ecological systems, which results in a high technological power (much higher compared to a manipulation at the phenotype level and with high potential impacts with regard to the functionalities of the modified organisms) and a high range of exposure (spread in space and time because of self-reproduction, mobility and self-dispersal). Due to the increased ability to self-propagate and spread through populations, a particularly high exposure of these altered organisms to ecosystems must be expected. This dispersion and exposure must in turn also be appraised as a high depth of intervention into the targeted ecosystems, which additionally may be regarded as a contamination of these systems. The increased technological power and exposure produced by these technologies results in proportionally increasing lack of knowledge about possible consequences, reaching from enormous scientific uncertainties to vast ignorance. A correspondingly extended risk assessment (hazard and exposure assessment) is required to fathom the extent and the depth of these dimensions of non-knowledge

¹Cp. Annex III A and Annex III B of the EC Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms (EC Directive 2001/18).

on the different organizational levels of biosystems that are affected by gene drives. The question arises whether methods and models are already available to adequately investigate hazard and exposure potentials caused by such a wide spread of new properties in whole populations and possibly into related species. Above all, the effects of a strong reduction of populations up to their eradication are important and complex evolutionary changes that must be considered. Important questions regarding technological, ecological as well as ethical issues become apparent.

Besides a review of modes of action of current SPAGE-technologies, the following technology characterisation analyses the depth and intensity of intervention, the resulting potential power and exposure and the corresponding extent of non-knowledge associated with the different SPAGE-techniques in a comparative approach.²

SPAGE-Techniques

A variety of different self-propagating artificial genetic elements has been developed in recent years. Some of them occur naturally as selfish genes and have been further optimized, while others are genetically engineered.

The SPAGEs currently in development or already applied encompass:

- RIDL-technology (“release of insects carrying a dominant lethal genetic system”)
- Meiotic Drives (autosomal- or Y-linked X-shredder)
- Killer-Rescue
- Maternal Effector Dominant Embryonic Arrest (Medea)
- Underdominance-based systems
- Homing Endonuclease Genes (HEG) based systems, especially CRISPR/Cas.

SPAGEs are developed to manipulate genes and traits of organisms and thereby alter whole populations to serve particular needs. Meanwhile a panoply of applications is envisaged for SPAGE. For instance, they should be applied to fight infectious diseases in particular the vectors of diseases. Especially malaria- or dengue-carrying mosquitoes are potential targets. In agriculture, weeds and crop pests should be eradicated or endangered species could be immunized against pathogens using a gene drive. Two potential applications of gene drives even serve issues of nature conservation, namely the eradication of invasive species (Webber et al. 2015) and the conservation of endangered species (Esvelt et al. 2014; Ledford 2015; Champer et al. 2017; European Commission and Scientific Advice Mechanism 2017).

Once released into the environment, SPAGEs can hardly be retrieved. Although there are many ideas to restrict their spread or even to alleviate adverse effects,

²The technology characterisation was in large parts already published under Creative Commons license CC-BY 4.0 in the article.

Frieß JL, von Gleich A, Giese B, 2019. Gene drives as a new quality in GMO releases - a comparative technology characterization. PeerJ 7:e6793. <http://doi.org/10.7717/peerj.6793>.

a complete reversal and restoration of the pre-existing state (and genotype) seems impossible.

So-called self-limiting approaches may pose a partial exception to this as their mode of action is developed to result in a successive frequency decrease of the SPAGE within a population.

Gene Drives

A number of natural mechanisms possess this notable property. In 2006, Sinkins and Gould mentioned transposable elements, meiotic drive genes, homing endonuclease genes and *Wolbachia* as naturally occurring gene drives. A theoretical concept for gene drives as a method to drive a desired gene, or a set of genes into a population was already proposed in 1960 by Craig et al.: “Mass release of male-producing males might be used in control operations” (Craig et al. 1960). The spread of chromosomal translocations was already proposed as a means of population control (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). Austin Burt in 2003 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 dissemination passively so that only offspring carrying genetic information of the drive will survive or be fertile. Akbari et al. (2015) called this type of mechanism “selective embryonic lethality”. 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 (2015). In the sense of such a broad definition most of the SPAGE-technologies mentioned above can be regarded as gene drives. Due to the exclusion of *Wolbachia*-based techniques in this work, only certain RIDL-approaches with a self-limiting character represent exceptions.

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.

Methodology of Technology Characterisation

Technology characterisation is an approach for prospective technology assessment that is applicable extremely early in the innovation process, when results of scientific research and the outlines of the technology are quite well known, but possible applications and affected systems are still unclear. This actually is the case with SPAGE-technologies. Such an early assessment is important and useful, because in case of severe concerns mitigations, corrections and course changes to alternative development paths are more easily directed and much more cost-efficient before large investments into products and production facilities are made. Technology characterisation is in this way an important approach to operationalize the requirements of precaution. The aim of technology characterisation is the early assessment of potential hazards and exposures (identifying reasons of concern) and, possibly still more important, the assessment of different dimensions and forms of lacking knowledge regarding hazards and exposure, reaching from uncertainties to absolute ignorance. This is the only way to include complete surprises, which means possible events for which currently no scientific approved ‘model of effect’ exists.³ However, approaches for an early assessment of potential hazards and exposure as well as an assessment of different dimensions and forms of lacking knowledge regarding hazards and exposure already exist (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 characteristics of the technology. Depth of technological intervention and also the intensity of intervention are the first criteria to investigate the range and forms of lacking knowledge (from uncertainties to ignorance) by scrutinizing their technological origin. The depth of intervention is a source of enormous technological power and therefore of mighty potential effects, benefits as well as 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 and interactions. In order to provide additional information on the frequency and the corrigibility of the expected effects, the quantitative aspects of the use of the technology (intensity of intervention i.e. quantity, frequency of its application), its reliability in practice, the probability of failure, and, finally, possible ways of limiting harm in case of failure have to be analyzed.

The aim of prospective technology characterisation is not to identify any possible adverse effect of technologies. Most of the occurring adverse effects will also in future be manageable by trial and error. Instead, it should provide a basis for decision-making in the view of the precautionary principle (Commission of the European

³As 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 2002) and is actually the case with the reduction of insect populations in Middle Europe.

Communities 2000; 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) The precautionary principle legitimates precautionary action in cases when it is unwarrantable to wait until a risk is clear and proven, because a probably occurring disaster will then not be controllable. Preconditions for 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 few less risky options are rated higher than applications in the food chain with plenty alternatives), (d) adequate precautionary measures (reaching from containment over substitution by less problematic alternatives to moratorium (Fischer et al. 2006). In our approach to operationalize the precautionary principle, the focus of technology characterisation lies on the prevention of far reaching, by-trend irreversible and global effects of events with adverse consequences that cannot be managed adequately, that cannot be retrieved, corrected or mitigated in case of their occurrence.

Based on the framework for technology characterisation SPAGE-technologies will be compared considering the following criteria (also depicted in Fig. 1.1).

Fig. 1.1 Criteria of prospective technology characterisation with corresponding gene drive-specific effects and options. Technological power is not in the focus of this rather general prospective study due to the very early innovation phase, where the particular design (esp. their cargo) and application context of gene drives is not yet clear

Depth of Intervention (Technological Power and Range)

Depth of intervention results in high technological power and range. For SPAGEs in general, the depth of intervention is much higher than in approaches for breeding and population control which are not based on genetic modifications. SPAGEs like other genetic engineering technologies constitute a manipulation of the very basis of organisms, their genetic characteristics, which results in one source of their technological power. The other source of power lies in the functionalities of the applied genes resp. traits. The technological range describes the potential spatio-temporal consequences of a gene drive, considering its lasting persistence in a population as well as the range with which it could spread by mobility and across populations. But there are differences. The mono-generational suppression of a single population is 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 (invasiveness).

Intensity of Intervention (Number/Frequency)

The intensity of intervention as number and 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 comparably lower intensity of intervention. 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. In terms of intensity, without changing the depth of intervention, power and range of a technology are only dependent on number and frequency of its application.

Reliability of the Technology

Reliability describes the probability of failure of the technology with regard to its intended use (unintended side effects and long term effects). 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 (Alpheg 2014).

Corrigibility or Limitation of Damage in Case of Failure

This criterion addresses an important aspect of risk management. Can a gene drive be retrieved in case that something goes dramatically wrong and can the damage of a failed gene drive be reversed by any means and if so, how laboriously are they compared to the initially released construct/system? For some SPAGE 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. 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.

Important Preliminary Remarks

As a means to compare the different SPAGE technologies, a rough assessment into three classes as high, moderate and low was applied, based on the criteria introduced above. Regardless of the fact that such a classification has to be further differentiated in subsequent studies, it has to be noted that this rating only refers to the comparative approach between the technologies included in this study and cannot be used to draw any conclusion on their absolute impact, for instance in comparison to solutions avoiding genetic technologies.

SPAGE Technologies

In the following, most of the SPAGEs taken into consideration to be used in a gene drive are discussed, examining their methodological advantages and drawbacks. Furthermore, they are compared and analysed in a hypothetical application concerning the aforementioned criteria of technology characterisation, which are further differentiated in the subsequent paragraph.

Other molecular components, such as transposable elements, TALENs (transcription activator-like effector nuclease) and ZFNs (zinc finger nuclease), which in theory could be considered as being potentially involved in gene drive mechanisms were not included into this technology characterisation due to their low transformation rate, limited specificity or problems to deal with sequence polymorphisms in wild populations.

Release of Insects Carrying a Dominant Lethal Gene (RIDL)

In this approach, laboratory-reared organisms (until now flies, mosquitoes and a moth species), equipped with a dominant lethal gene, are mass released to reduce the number of offspring in a wild population. There are two varieties of RIDL. In the bi-sex RIDL approach, the offspring of both sexes die in the zygotic, larval or early pupal stage. In the female specific RIDL approach (fsRIDL), only female offspring die. Male offspring is heterozygous for the dominant lethal gene and therefore would pass on the lethal trait to 50% of their offspring in subsequent generations. Female specific RIDL strains have been developed for *Aedes aegypti* and *Aedes albopictus*, using flightlessness as a lethal trait (Alphey et al. 2013). Since RIDL as well as fsRIDL organisms will be selected from the population within a few generations, the RIDL technology can be considered self-limiting.

Depth of RIDL Intervention

The power (by quality) of the bi-sex RIDL technology is based on the functionality of the applied gene comparably high due to its capacity of killing 100% of its offspring but its range can be seen as low in comparison to other SPAGE techniques, being self-limiting, it is not intended to persist in the ecosystem.

Although only half of the GMO's offspring survives, fsRIDL scores as a high power and low range technology due to its self-limiting and suppressive quality with the potential to eradicate the population with a concomitant risk of invasion into neighbouring populations, regarding its high release ratios.

Intensity of RIDL Intervention

Independent of the applied RIDL variant the technology requires mass releases of genetically modified organisms. In the case of the bi-sex RIDL even a higher number of mass releases may be necessary. Oxitec has used release ratios of up to 54: 1, before it observed a reduction in wild populations (Gene Watch UK 2013). The mass and frequency for a successful application can therefore be rated as high.

Reliability of the bi-Sex RIDL Technology

The overall probability of failure of the bi-sex RIDL technology compared to other SPAGE techniques is moderate. This evaluation strongly stems from the issues arising from the first field test trials. Key points of error encompass:

- lowered fitness of laboratory-reared GM insects due to inbreeding (colony effect)
- reduced mating capabilities of the GMOs (reducing the suppressive effect)
- selection against the fitness burden which the dominant lethal gene clearly poses,

- the genetic bi-stable switch necessary to rear the flies in the laboratory requires tetracycline (if tetracycline is present in the target ecosystem, the suppressive effect would be reduced and protracted),
- errors in the release:
 - wild types (would reduce the suppressive effect), and
 - phenotypic wild types carrying the non-functioning dominant lethal gene (would reduce the suppressive effect and persistently introduce synthetic DNA into the ecosystem).

Potential Vulnerabilities of the Target System Towards the bi-Sex RIDL Technology

- adverse ecological effects due to the eradication of the target population due to the ratio of released GMOs to the native population,
- spread to other populations,
- toxicity of the dominant lethal gene product (tTA) to predators,
- dead GM-larvae are likely to enter the commercial food production and food chain.

Reliability of the fsRIDL Technology

The overall probability of failure specific for the fsRIDL technology is moderate compared to other SPAGE techniques. Most of the aforementioned weaknesses within the technology however, in practice are alleviated by increased release numbers. Nevertheless, key points of error encompass:

- The selection against the fitness burden of the dominant lethal gene which seems more likely in a system with longer persistence, yet implausible within the few generations fsRIDL organisms persist.
- errors in the release:
 - wild types (would reduce the suppressive effect),
 - female fsRIDL-organisms (would further enhance the suppressive effect in the first generation), and
 - phenotypic wild types carrying the non-functioning dominant lethal gene (would reduce the suppressive effect and persistently introduce genetically modified DNA sequences into the ecosystem).

Potential Vulnerabilities of the Target System Towards the fsRIDL Technology

- adverse ecological effects due to the eradication of the target population due to the ratio of released GMOs to the native population,
- spread to other populations (which may be especially probable in a population consisting mainly of fsRIDL males with a dwindling percentage of females),
- the genetic bistable switch necessary to rear the organisms in the laboratory requires tetracycline (if tetracycline is present in the target ecosystem, the suppressive effect would be reduced and protracted),
- toxicity of the dominant lethal gene product (tTA) to predators.

Possibilities for Limitation of Damage Caused by RIDL Technology

Independent of the applied RIDL variant, the only feasible option for an attempt to restore the original population after detrimental effects caused by RIDL technology is the release of wild type specimens. However, since bi-sex RIDL is self-limiting and if fsRIDL-organisms will be selected from a population within a number of generations, the damage of a failed release might be buffered by the resilience of most of the ecological systems as long as it does not result in a complete eradication of the target species or other parts of the food web.

Meiotic Drives (MD) in particular X-Shredder

Meiotic Drives (MD) consist of selfish genetic elements which cause a distortion of allelic segregation that results in a bias of the frequency of Mendelian inheritance. For instance, the Mendelian segregation frequency of 50% is distorted up to 70% in *Zea mays* (Australian Academy of Science 2017; Lindholm et al. 2016). Other MDs have been reported for *Drosophila melanogaster* (segregation distorter [SD] system) (Larracuente and Presgraves 2012), the mouse *Mus musculus* (t-haplotypes, causing a transmission ratio distortion) (Silver 1993), *Zea mays* (abnormal chromosome 10 [Ab10]), which affects Gonotaxis, distorted sex ratios in *Silene* species (Taylor 1994) and mosquitoes. In the latter, MDs are naturally occurring in *Aedes aegypti* (Craig et al. 1960) and *Culex pipiens* (Sweeny and Barr 1978). A major drawback of MDs consists in the fact that the fitness of other alleles at the same locus, which do not bias transmission, and alleles linked to them, is reduced (Lindholm et al. 2016).

For gene drives, a particularly interesting MD is the so called X-Shredder, which causes fragmentation of the X chromosome by nucleases during male meiosis. Thereby, only Y-bearing sperm can produce viable offspring, which is of course male (Newton et al. 1976). An autosomal X-shredder can be regarded as self-limiting, a Y-linked X-shredder as self-sustaining (Burt 2003; Burt and Trivers 2006; Deredec

et al. 2008). A Y-linked X-shredder can invade adjacent populations or species with incomplete mating barriers, therefore widespread effects may be anticipated (Alphey 2014). Galizi et al. (2014) published a synthetically engineered X-shredder aiming at spermatocyte meiosis in *Anopheles gambiae*, producing mainly Y-chromosome-carrying sperm, causing a male bias of up to 95%. A distortion of the sex ratio is a penalty to fitness, which may in extreme cases lead to a population's extinction. Although rarely, in *Drosophila*, sometimes 100% female progeny is achieved. Therefore, this trait is highly selected against. Hence, meiotic drive-based extinction has never been observed in natural populations (Helleu et al. 2015). For this study we focus on the self-sustaining variant of the Y-linked X-Shredder.

Depth of X-Shredder Intervention

The X-Shredder approach, considering a male bias up to 95% (Galizi et al. 2014), would cause a major population suppression, therefore its technological power and range are rated as high. Since it constitutes a self-sustaining gene drive and since a population consisting mainly of males is much more likely to migrate in search of females its range is also considered as high. Although, to date no field trials with X-shredder gene drives have been undertaken, and therefore no migration patterns of X-shredder males are available.

Intensity of X-Shredder Intervention

The X-Shredder approach requires a mass release of males. The necessary technological input generating power and the range (by quantity) can thus be regarded as high. However, even a small release size would theoretically suffice to replace a population over multiple generations, dependent on the fitness of the gene drive organisms.

Reliability of the X-Shredder Technology

Based on the small number of available publications on X-Shredder approaches in a preliminary comparative assessment of SPAGE technologies, the failure probability is not easily estimated but it can comparatively be considered as moderate. Key points of error encompass:

- selection against the fitness burden which the construct clearly poses,
- errors in the release:
 - *phenotypic male wild types carrying the non-functioning construct (would reduce the suppressive effect).*

Vulnerability of the Target System Towards the X-Shredder Technology

- adverse ecological effects due to the eradication of the target species (although not yet being observed in nature) and
- spread to other populations (which seems likely in a population with a dwindling number of females).

Possibilities for Limitation of Damage Caused by X-Shredder Technology

There is no possibility to directly remedy the damages obtained from an X-Shredder release. This makes the technique highly problematic, it is built to first invade and replace followed by immediate eradication, due to the lack of females. Its low threshold quality further exacerbates the handling of Y-linked X-Shredder gene drives.

Killer-Rescue

The Killer-Rescue System was first proposed by Gould et al. (2008). It consists of two unlinked loci one encoding a toxin (killer allele), the other encodes an antidote (rescue allele) (Gould et al. 2008). Thereby, the toxin and antidote could consist of miRNAs and recoded gene or a toxic protein and detoxicating enzyme. Furthermore, a cargo gene can be fused to the antidote gene. Homozygous carriers of both genes would be mass-released into wild populations, offspring which inherit 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 has completely disappeared from the population however, the fitness gain of the Rescue allele will disappear as well, unless the cargo gene confers a gain in fitness. This system is designed to be a self-limiting modification gene drive in which, if the cargo gene bears a fitness penalty, its prevalence in the population would decrease after a number of generations. 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. A particular benefit of the technique is that it is easy to design and engineer.

Depth of Killer-Rescue Intervention

The Killer-Rescue system's technological power compared to other SPAGE techniques scores as low because the killer-allele will potentially cause only a short-term reduction in the population size, it is not by design a suppression drive. Considering

the technological range, Killer Rescue, due to its non-persistent quality and its therefore limited chance of contamination of other populations and relative high invasive threshold scores as low.

Intensity of Killer-Rescue Intervention

The Killer-Rescue system is reliant on a high number of released carriers of up to a ratio of gene drive organisms to wild types of 2:1, according to model scenarios by (Gould et al. 2008). Although this ratio is much lower than reported for other mass release dependent SPAGE techniques, the mass and frequency of Killer-Rescue still has to be scored as high.

Reliability of the Killer-Rescue Technology

The Killer-Rescue system's probability of failure scores as low in comparison to other SPAGE-techniques. Although there is no data on most of the imaginable vulnerabilities of the technology. It would be recommendable to use miRNA as a killer allele in order not to give the carrier-organisms a toxic quality. Key errors encompass:

- the selection against the fitness burden which the constructs clearly pose (resistance formation or toxin-inactivation).

Vulnerability of the Target System Towards the Killer Rescue Technology

Imaginable key vulnerabilities of the Killer-Rescue system encompass:

- Linkage loss
- Natural evolution of an antidote
- Inactivation of the killer allele.

Possibilities for Limitation of Damage Caused by Killer-Rescue Technology

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 is a release of wild types.

Maternal-Effect Dominant Embryonic Arrest (Medea)

The term Medea is an acronym named after the sorceress in Greek mythology who killed her own children. This name is accurate as a Medea selfish genetic element consists of two chromosomally-located tightly linked transgenes: one that encodes a (miRNA-)toxin inherited by all progeny of Medea-bearing mothers, and a second that encodes an antidote (gene without miRNA-sequence) active in the zygote (Akbari et al. 2014). Therefore, only Medea-bearing offspring (hetero- or homozygous) survives. This maternally induced lethality of wild type offspring not inheriting a Medea allele grants an ability to invade populations.

The Medea elements were first discovered in *Tribolium* flour beetles and have also been reported in mice. The only published Medea constructs (*Medea myd88*, *o-fut1* and *dah*) have been inserted on an autosomal chromosome in *D. melanogaster*. *myd88* is a maternally expressed gene required for embryonic dorso-ventral pattern formation.

If *Medea* is located on the X chromosome in a X/Y male heterogametic species, *Medea* is predicted to spread to allele fixation, with wild type alleles being completely eliminated (Akbari et al. 2014).

Medea organisms exhibit a high-frequency stable equilibrium when the transgenic construct is associated without any fitness cost (Gokhale et al. 2014). The fitness costs of homozygote Medea *Drosophila* were estimated to be 27.3% and 17.4%, respectively, for two different targeted genes. In lab trials, where 25% of the original members were homozygous for *Medea*, the gene spread through the entire population within 10 to 12 generations. Observations indicate that a single copy of each *Medea* toxin is sufficient to induce 100% maternal-effect lethality and a single copy of each rescue transgene is sufficient to rescue normal development of embryos derived from mothers expressing one or two copies of the toxin (Akbari et al. 2014). Until now, attempts to establish a Medea system for *Aedes aegypti* were not successful. There are other variants of single locus constructs, such as Semele and inverse Medea. Semele confers toxic sperm that either renders females infertile or kills them. In inverse Medea the promoters of the toxin and the antidote are switched (Marshall and Akbari 2015).

Currently Medea is planned to be applied in order to take control of the cherry fruit fly (spotted-wing fruit fly *Drosophila suzukii*) in California (Regalado 2017). Two considered approaches are to either target female fertility genes or to alter the ovipositor of the flies to make them unable to puncture the ripening cherries. Buchman et al. (2018) found pre-existing native resistances against the miRNA toxins of their construct in 5 out of 8 examined *D. suzukii* strains. Together with the high fitness penalties conferred by the construct, the Medea GD now has to be considered a high threshold drive, that, when conferring a large fitness penalty, will only be transiently maintained in the population without supplemental releases. In a mathematical model for the *myd88* construct in the cherry fruit fly, a fitness cost for heterozygotes of 28 and 65% for homozygotes was assumed (Buchman et al. 2018).

Depth of Medea Intervention

In a comparative approach of SPAGE the technological power of Medea is to be rated as moderate, as it will certainly drive to fixation and has therefore only a potentially transient effect on population size. The range and thus its potential of exposure and contamination would score high due to its higher invasiveness and potential to invade non-target populations.

Intensity of Medea Intervention

In theory, it would not require many carrier organisms to drive a gene into a population. Therefore, the intensity of intervention would have to be rated as low due to its low number and frequency required for a successful approach, compared to other SPAGE techniques. However, as demonstrated by Buchman et al. (2018), due to the pre-existing resistances and high fitness penalties it is more likely that multiple mass releases are required for a successful drive (Marshall et al. 2017). Therefore, a high intensity of intervention is considered for Medea gene drives.

Reliability of the Medea Technology

The Medea technology's probability of failure scores as low in comparison to other SPAGE-techniques. This is founded on its low probability of linkage loss, resistance formation, and its potent toxin- and rescue-mechanism. Thereby, the technique does not rely on toxins that might harm other organisms upon ingestion but on RNAs which degrade quickly outside the cells. Key points of error encompass:

- the selection against the fitness burden which the constructs clearly pose (resistance formation by toxin-inactivation)
- errors in the release:
 - Medea-males, homozygous Medea-females (would protract the suppressive effect and accelerate the genes' drive to fixation),
 - wild types (would reduce and protract the suppressive effect).

Vulnerability of the Target System Towards the Medea Technology

- adverse ecological effects due to the permanent introduction of engineered genes into the ecosphere and their effects on population dynamics and
- effects due to the spread to other populations (will almost certainly happen over time).

Possibilities for Limitation of Damage Caused by Medea Technology

A potential measure would be to release a second generation Medea gene drive. This would introduce a new toxin-antidote combination as well as the antidote for the first generation toxin. Although the suppressive effect of Medea may be stopped by this approach it introduces even more persisting GMOs in the ecosystem.

Underdominance (UD)

Underdominance, also known as heterozygote inferiority, is a genetically engineered gene drive technique. There are two different approaches UD^{mel} (Akbari et al. 2013) and *RpL14* (Reeves et al. 2014). 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. Therefore, UD heterozygotes have a lower fitness than homozygotes (Reeves et al. 2014). The constructs can be located on the same chromosome or on different chromosomes (two-locus Underdominance). When a UD female heterozygous for both constructs mates with a wild type male, 25% will be heterozygous for both constructs, while 25% of offspring will be non-viable wild types, and 50% will be non-viable due to the lack of one of the necessary antidotes. The toxins of Underdominance constructs may be the same as utilised in the Medea technology: *myd88*, *dah* and *o-fut-1* (Akbari et al. 2013) or *RpL14*, a cytoplasmic ribosomal protein which is haploinsufficient (Reeves et al. 2014). 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). For the *RpL14* construct, this threshold is estimated to be as high as 61% of the total population (Reeves et al. 2014).

Therefore, 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).

Depth of Underdominance Intervention

The power of the Underdominance approach has to be rated as moderate compared to other SPAGE techniques. This effect will at first persist but eventually fade over the subsequent generations. In comparison to the Medea approach, the range of Underdominance is estimated to be lower, due to its higher invasion threshold.

Intensity of Underdominance Intervention

Since utilisation of this technology is more frequency-dependent than the Medea approach, requiring even greater mass releases, its intensity generating quantitatively mass and frequency is rated as high.

Reliability of the Underdominance Technology

The overall failure probability of the Underdominance technology in comparison to other SPAGE techniques can be estimated as moderate. Key points of error encompass:

- lowered fitness of laboratory-reared GM insects due to inbreeding (colony effect)
- the selection against the fitness burden which the constructs clearly pose.

Vulnerability of the Target System Towards the Underdominance Technology

- adverse ecological effects due to the eradication of the population due to its small size and
- spread to other populations.

Possibilities for Limitation of Damage Caused by Underdominance Technology

For a UD drive the release of wild type specimen represents the most obvious option to potentially restore the original population.

Homing Endonuclease Genes (HEG)

HEGs are selfish genetic elements. But different from transposable elements, they code for a restriction enzyme with a target sequence of 20–30 bp. The HEG is nestled within its own recognition site. An expressed homing endonuclease-protein finds intact recognition sites and cuts them. Then the selfish genetic element relies on the DNA-repair mechanism of homologous recombination which copies the HEGs code and inserts it into the cut-site on the homologous chromosome.

CRISPR/Cas9

CRISPR stands for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats, while Cas stands for **C**RISPR-**a**ssociated protein. Both components originate from an adaptive immune system of bacteria and archaea. Cas9 is a ribonucleoprotein (RNP), able to bind guide RNAs (gRNA), aka crRNA that specifically recognize and bind to the target sequences (20 nucleotides). The target DNA-sequence must contain a protospacer adjacent motif (PAM) with the sequence NGG (N can be any nucleotide) for the Cas protein to cut. The cut takes place three nucleotides upstream of the PAM. The Cas protein can cut at multiple PAMs as long as they are at least 8 nucleotides apart. Just as ZFN and TALEN, this technology can be used to cause deletions as well insertions, relying on Homologous Recombination. But CRISPR/Cas9 utilises guide RNAs for target site recognition which makes this technology cheaper and easier to customize, while also being more effective (Doudna and Charpentier 2014; Jinek et al. 2012). Figure 1.2 shows the functional mechanism.

The most probable application would utilise a CRISPR/Cas9-mediated gene drive system inheriting a cargo gene to the vast majority of its offspring, which would burden the population's fitness. Although a CRISPR/Cas9-mediated gene drive could just as well be designed as a self-limiting drive, its capabilities would not fully be exploited if it is not applied as a self-sustaining drive.

Fig. 1.2 Mechanism of CRISPR/Cas9-based gene drives. A gene drive organism carries the gene drive cassette (chromosome A) and mates with a wild type (chromosome B). The gene drive cassette expresses the CRISPR/Cas9 complex, which then cuts its recognition site defined by the gRNA on chromosome B. This cut then can either be repaired by Non-Homologous End Joining (NHEJ) or Microhomology-Mediated End Joining (MMEJ) creating a homing resistant allele, or by homology directed repair (HDR) copying the gene drive cassette into the cut region

Depth of CRISPR/Cas9-Gene Drive Intervention

Power and range of a CRISPR/Cas9-based gene drive system in comparison to other SPAGE techniques would score as high due to its overwhelming inheritance. But power and range of a drive system are additionally determined by the functionality of the cargo gene and its burden to the fitness of the population. Since the drive is self-sustaining for multiple generations its range is probably to be scored as high as well, causing the overall depth of intervention rating to be concomitantly high.

Intensity of CRISPR/Cas9-Gene Drive Intervention

Due to its non-Mendelian inheritance, this gene drive would be frequency independent. Therefore, this technology's necessary frequency and thus also the intensity of intervention scores as low in comparison to other SPAGE techniques.

Reliability of the CRISPR/Cas9-Gene Drive Technology

The probability of failure of the CRISPR/Cas9-gene drive technology compared to other SPAGE techniques, considering the current state of development is high. Key points of error encompass:

- the selection against the fitness burden which the cargo gene could pose
- Non-homologous end joining (NHEJ) and microhomology-mediated end joining (MMEJ)

In contrast to homology-directed repair (HDR), NHEJ and MMEJ reduce the conversion rate and may cause resistance due to mutations, deletions etc. Depending on the genomic location, HDR vs. NHEJ efficiency could be as low as ~10% (Lin and Potter 2016). Usually whenever a cut is repaired by NHEJ the result is a drive resistant allele. 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).

- Incomplete or imperfect copying during HDR
If the deletion preserves the reading frame it leads to a homing-resistant allele (Marshall et al. 2017).
- Emergence of homing resistant alleles due to random target site mutagenesis.
This is circumvented by engineering multiple attack loci for the CRISPR/Cas9-system in the genome reducing the chance of mutation in all attacked alleles.

However, very large populations—such as those of some insects—might require unfeasibly large numbers of gRNAs to prevent resistance (Ping 2017).

- Off-target-effects
Unspecific binding of gRNA causes unintended insertions at different loci.
- On-target mis-insertions
Unwanted genes or gene fragments are inserted into the target locus, instead or additionally to the desired genes.
- Sequence polymorphisms
Resistance due to genetic variations within a species. To overcome this problem multiple gRNA variants can be added to the CRISPR/Cas cassette.
- Intragenomic interactions
The distance of gRNA target sites may affect homing rates (Marshall et al. 2017).
- Maternal Effects
Dominant maternal effects due to Cas9 deposits may cause resistance. Propagation of resistant individuals may be prevented by targeting essential genes (Noble et al. 2016). Homing and integration seems to occur in the germline. Upon fertilisation, if sufficient Cas9 (and gRNA) is in the cytoplasm of the female embryo which is homo- or heterozygous for the gene drive, maternal effects can occur. The CRISPR/Cas complex finds and cuts its recognition sites in the sperm's genome before the homologous female genome is close enough to be recruited for homologous recombination. Without a homologous template the cuts are then repaired by non-homologous end joining and thus arises a resistant allele. In such an event the number of gRNA variants is meaningless.
- errors in the release:
 - phenotypic wild types carrying the non-functioning construct (would reduce the suppressive effect and could constitute a persistently gene drive-resistant sub-population).

Vulnerability of the Target System Towards the CRISPR/Cas9-Gene Drive Technology

- adverse ecological effects due to the eradication of the population (depending on the fitness burden and ratio of released to native organisms)
- effects due to the spread to other populations (which becomes more probable the lower the fitness burden and the longer the gene drive is sustained in the ecosystem)

Possibilities for Limitation of Damage Caused by CRISPR/Cas9-Gene Drive Technology

It is not yet possible to make reliable statements about the effectiveness of options for limiting or reversing the changes caused by released CRISPR/Cas-based gene drives.

Different measures for the inhibition of their spread as well as for the inactivation of the functionalities induced by gene drives have already been proposed and also a first experiment in yeast has been undertaken (DiCarlo et al. 2015; Esvelt et al. 2014). However, a proof of their efficacy when used in insects or other higher organisms has not yet been established. In addition, there is still no possibility for the complete restoration of the natural gene sequence after the spread of RNA-guided gene drives.

Certain concepts to restrict the uncontrolled spread of a CRISPR/Cas9-mediated gene drive are:

- A reversal drive which could be used to overwrite a first drive, although it would have to be recoded to be immune to this first drives cutting. A third drive could then restore the original wild type sequence, although the cas9-gene and gRNAs would remain (Esvelt et al. 2014).
- Immunising drives could be used (pre-emptively) to render populations immune to another drive by recoding the sequences targeted by that drive (Esvelt et al. 2014).
- In a split drive the genomic locations for the components of the drive are separated in such a way that only a certain part of the information for a functionally active drive is inherited. This serves the local confinement of a gene drive (DiCarlo et al. 2015).
- A daisy chain drive is defined by a linear chain of interdependent drive elements on different genomic loci in which the first drive element is responsible for the duplication of the second, the second for the third etc. but the first drive element is not duplicated and therefore the whole drive systems successively gets lost over time (Noble et al. 2016).
- An overwriting drive (for restoring edited traits) was tested in yeast (DiCarlo et al. 2015) but not in higher organisms.

Summary of the Technology Characterisation

In Table 1.1, the here discussed SPAGE technologies are compared in certain characteristics as far as estimates are possible, considering information available in the literature. In the subsequent Table 1.2 the different SPAGE techniques and their evaluation resulting from the above represented technology characterisation are put together for better comparison.

As a prerequisite for further orientation on the impact and potential exposure of SPAGE, common features of these technological approaches were selected for a comparative technology characterisation (power and range) as well as an analysis of factors (traits) which influence its impact, spread and invasiveness.

As explained before, all SPAGE-Technologies are determined as of very high depth of intervention. In Table 1.2, the focus lies on a differentiation within the field of SPAGE technologies. Their general high depth of intervention is presupposed.

Table 1.1 SPAGE technique comparison

	Bi-sex RIDL	fsRIDL	X-Shredder	Killer-Rescue	Medea	UD	CRISPR/Cas
Resistance formation fitness	Possible	Possible	Very unlikely	Possible	Possible	Unlikely	Likely
	~56%	~56%	Unknown	Unknown	82.6%	Probably lower than medea	Probably low
Invasiveness	Very low	Very low	Moderate	High	Moderate	Low	High
Toxicity	Likely	Likely	None	Possible	None	None	None
Corrigibility	WT release	WT release	None	WT release	2nd generation drive	WT release	Reversal drive
Class	Suppression	Suppression	Suppression	Replacement	Replacement	Replacement	Replacement
Mode of Action	Toxin	Sex ratio/toxin	Sex ratio/chromo-somal disruption	Toxin/antidote	Toxin/antidote	Toxin/antidote	Heterozygote to Homozygote
Linkage loss	Unknown	Unknown	Unknown	Unknown	Unlikely	Unlikely	Likely
Estimated interspecies gene flow	Very likely	Very likely	More likely	Likely	Likely	Likely	Depends

Table 1.2 Comparative Technology Characterisation of SPAGE Techniques

SPAGE-Type	Intensity of the intervention	Depth of intervention technological power/range	Probability of failure	Discussed possibilities for corrective action/limitation of damage in case of failure
Killer rescue	High	Low/moderate	Low	Wild type release
Medea	High	Moderate/high	Low	2nd generation Medea drive
Two-locus underdominance	High	Moderate/low	Moderate	Wild type release
Bi-sex RIDL	High	High/low	Moderate	Wild type release
fsRIDL	High	High/moderate	Moderate	Wild type release
X-Shredder	High	High/high	Moderate	None
CRISPR/Cas9 Gene Drive	Low	High/high	High	Rescue drive

Such an internal differentiation is an important knowledge base for a differentiated risk management.

The comparative technology characterisation revealed that concerning reliability there are no remarkable differences, but there are differences especially in the spectrum of power and range which presumably lead to different levels of potential hazards and exposure. For instance, SPAGEs may employ different mechanisms to ensure their mode of inheritance. From simple lethality by toxic gene products (RIDL), through more or less intricate toxin-antidote systems as Medea, Underdominance, Killer Rescue to the biased segregation of sex chromosomes during meiosis (X-Shredder). 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 SPAGEs, its probability of failure is comparably high. The outstanding potential of CRISPR/Cas9-based gene drives was also illustrated by the assessment of the range based on invasiveness of different SPAGE-techniques according to their inheritance schemes. 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 SPAGE, these properties reveal important ‘reasons for concern’ with regard to the requirements of the precautionary principle.

References

- Ahrens, A., Braun, A., von Gleich, A., Heitmann, K., & Lißner, L. (2005). *Hazardous chemicals in products and processes—Substitution as an innovative process. Sustainability and innovation*. Heidelberg, Germany; New York, USA: Physica Verlag.
- Akbari, O. S., Matzen, K. D., Marshall, J. M., Huang, H., Ward, C. M., & Hay, B. A. (2013). A synthetic gene drive system for local, reversible modification and suppression of insect populations. *Current Biology*, 23, 671–677. <https://doi.org/10.1016/j.cub.2013.02.059>.
- Akbari, O. S., Chen, C.-H., Marshall, J. M., Huang, H., Antoshechkin, I., & Hay, B. A. (2014). Novel synthetic Medea selfish genetic elements drive population replacement in drosophila, and a theoretical exploration of Medea-dependent population suppression. *ACS Synthetic Biology*, 3, 015–928.
- Akbari, O. S., Bellen, H. J., Bier, E., Bullock, S. L., Burt, A., Church, G. M., et al. (2015). Safeguarding gene drive experiments in the laboratory. *Science*, 349, 927–929. <https://doi.org/10.1126/science.aac7932>.
- Alphey, L. (2014). Genetic control of mosquitoes. *Annual Review of Entomology*, 59, 205–224. <https://doi.org/10.1146/annurev-ento-011613-162002>.
- Alphey, L., McKemey, A., Nimmo, D., Neira Oviedo, M., Lacroix, R., Matzen, K., et al. (2013). Genetic control of Aedes mosquitoes. *Pathogens and Global Health*, 107, 170–179. <https://doi.org/10.1179/2047773213Y.0000000095>.
- Australian Academy of Science. (2017). Synthetic gene drives in Australia: implications of emerging technologies. <https://www.science.org.au/files/userfiles/support/documents/gene-drives-discussion-paper-june2017.pdf>. Accessed June 7, 2019.
- Buchman, A., Marshall, J. M., Ostrovski, D., Yang, T., & Akbari, O. S. (2018). Synthetically engineered Medea gene drive system in the worldwide crop pest *Drosophila suzukii*. *PNAS*, 115, 4725–4730.
- Burt, A. (2003). Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proceedings of the Royal Society B: Biological Sciences*, 270, 921–928. <https://doi.org/10.1098/rspb.2002.2319>.
- Burt, A., & Trivers, R. (2006). *Genes in conflict: The biology of selfish genetic elements*. Cambridge MA: Belknap Press/Harvard University Press.
- Carareto, C. M. A., Kim, W., Wojciechowski, M. F., O’Grady, P., Prokchorova, A. V., Silva, J. C., et al. (1997). Testing transposable elements as genetic drive mechanisms using *Drosophila* P element constructs as a model system. *Genetica*, 101, 13–33.
- Champer, J., Reeves, R., Oh, S. Y., Liu, C., Liu, J., Clark, A. G., et al. (2017). Novel CRISPR/Cas9 gene drive constructs in *Drosophila* reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. *bioRxiv*. <https://doi.org/10.1101/112011>.
- Commission of the European Communities. (2000). Communication from the commission on the precautionary principle. Brussels, Belgium.
- Courtier-Orgogozo, V., Morizot, B., Boëte, C. (2017). Agricultural pest control with CRISPR-based gene drive: time for public debate: Should we use gene drive for pest control? *EMBO Report* e201744205. <https://doi.org/10.15252/embr.201744205>.
- Craig, G. B. J., Hickey, W. A., & VandeHey, R. C. (1960). An inherited male-producing factor in *Aedes aegypti*. *Science*, 132, 1887–1889. <https://doi.org/10.1126/science.132.3443.1887>.

- Curtis, C. F. (1968). Possible use of translocations to fix desirable genes in insect pest populations. *Nature*, 218, 368–369.
- Deredec, A., Burt, A., & Godfray, H. C. J. (2008). The population genetics of using homing endonuclease genes in vector and pest management. *Genetics*, 179, 2013–2026. <https://doi.org/10.1534/genetics.108.089037>.
- DiCarlo, J. E., Chavez, A., Dietz, S. L., Esvelt, K. M., & Church, G. M. (2015). Safeguarding CRISPR-Cas9 gene drives in yeast. *Nature Biotechnology*, 33, 1250–1255. <https://doi.org/10.1038/nbt.3412>.
- Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, 346. <https://doi.org/10.1126/science.1258096>.
- EC DIRECTIVE 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. https://www.epa.ie/pubs/legislation/geneticallymodifiedorganismsgmo/2001-18%20Directive_consolidated.pdf.
- Emerson, C., James, S., Littler, K., & Filippo, R. (2017). Principles for gene drive research. *Science*, 358, 1135–1136. <https://doi.org/10.1126/science.aap9026>.
- Esvelt, K. M., Smidler, A. L., Catteruccia, F., Church, G. M. (2014). Concerning RNA-guided gene drives for the alteration of wild populations. *eLife*, 3, e03401. <https://doi.org/10.7554/eLife.03401>.
- European Commission, Scientific Advice Mechanism. (2017). New Techniques in agricultural biotechnology. <https://ec.europa.eu/research/sam/index.cfm?pg=agribiotechnology>
- European Environment Agency. (2002). Late lessons from early warnings: The precautionary principle 1896–2000. *Environmental Issue Report*, 22, 1–211.
- European Parliament Think Tank. (2015). The precautionary principle: Definitions, applications and governance. [http://www.europarl.europa.eu/RegData/etudes/IDAN/2015/573876/EPRS_IDA\(2015\)573876_EN.pdf](http://www.europarl.europa.eu/RegData/etudes/IDAN/2015/573876/EPRS_IDA(2015)573876_EN.pdf). Accessed June 7, 2019.
- Fischer, E., Jones, J., & von Schomberg, R. (2006). *Implementing the precautionary principle—Perspectives and prospects*. Bodmin, Cornwall, Great Britain: MPG Books Ltd.
- Galizi, R., Doyle, L. A., Menichelli, M., Bernardini, F., Deredec, A., Burt, A., et al. (2014). A synthetic sex ratio distortion system for the control of the human Malaria mosquito. *Nature Communications*, 5, 3977. <https://doi.org/10.1038/ncomms4977>.
- Gantz, V. M., & Bier, E. (2015). Genome editing. The mutagenic chain reaction: A method for converting heterozygous to homozygous mutations. *Science*, 348, 442–444. <https://doi.org/10.1126/science.aaa5945>.
- Gantz, V. M., Jasinskiene, N., Tatarenkova, O., Fazekas, A., Macias, V. M., Bier, E., et al. (2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. *Proceedings of the National Academy of Sciences*, 112, E6736–E6743. <https://doi.org/10.1073/pnas.1521077112>.
- Gene Watch UK. (2013). Genetically Modified (GM) Olive flies: A credible pest management approach? https://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/GMolivefly_GWbriefing_fin2.pdf.
- Giese, B., & von Gleich, A. (2015). Hazards, risks, and low hazard development paths of synthetic biology. In B. Giese, C. Pade, H. Wigger, & A. von Gleich (Eds.), *Synthetic biology—Character and impact*. Heidelberg: Springer International Publishing.
- Gokhale, C. S., Reeves, R. G., & Reed, F. A. (2014). Dynamics of a combined medea-underdominant population transformation system. *BMC Evolutionary Biology*, 14, 1–9. <https://doi.org/10.1186/1471-2148-14-98>.
- Gould, F., Huang, Y., Legros, M., & Lloyd, A. L. (2008). A Killer-Rescue system for self-limiting gene drive of anti-pathogen constructs. *Proceedings of the Royal Society B: Biological Sciences*, 275, 2823–2829. <https://doi.org/10.1098/rspb.2008.0846>.
- Hastings, I. M. (1994). Selfish DNA as a method of pest control. *Philosophical Transactions of the Royal Society of London. Series B*, 344, 313–324.

- Helleu, Q., Gérard, P. R., & Montchamp-Moreu, C. (2015). Sex chromosome drive. *Cold Spring Harbor Perspectives in Biology*, 7, a017616. <https://doi.org/10.1101/cshperspect.a017616>.
- Hochkirch, A., Beninde, J., Fischer, M., Krahner, A., Lindemann, C., Matenaar, D., et al. (2017). License to kill? Disease eradication programs may not be in line with the convention on biological diversity. *Conservation Letters*, 11, 1–6. <https://doi.org/10.1111/conl.12370>.
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337, 816–821. <https://doi.org/10.1126/science.1225829>.
- Larracuent, A. M., & Presgraves, D. C. (2012). The selfish segregation distorter gene complex of *Drosophila melanogaster*. *Genetics*, 192, 33–53. <https://doi.org/10.1534/genetics.112.141390>.
- Ledford, H. (2015). CRISPR, the disruptor. *Nature*, 522, 20–24. <https://doi.org/10.1038/522020a>.
- Lin, C. C., & Potter, C. J., 2016. Non-Mendelian dominant maternal effects caused by CRISPR/Cas9 transgenic components in *Drosophila melanogaster*. *G3: Genes, Genomes, Genetics*, 6, 3685–3691. <https://doi.org/10.1534/g3.116.034884>.
- Lindholm, A. K., Dyer, K. A., Firman, R. C., Fishman, L., Forstmeier, W., Holman, L., et al. (2016). The ecology and evolutionary dynamics of meiotic drive. *Trends in Ecology & Evolution*, 31, 315–326. <https://doi.org/10.1016/j.tree.2016.02.001>.
- Linkov, I., Trump, B. D., Anklam, E., Berube, D., Boisseau, P., Cummings, C., et al. (2018). Comparative, collaborative, and integrative risk governance for emerging technologies. *Environment Systems and Decisions*, 38, 170–176.
- Marshall, J. M., & Akbari, O. S. (2015). Gene drive strategies for population replacement. Chapter 9 in *Genetic control of Malaria and Dengue*. <https://doi.org/10.1016/B978-0-12-800246-9.00009-0>.
- Marshall, J. M., Buchman, A., Sánchez C., Akbari, O. S. (2017). Overcoming evolved resistance to population-suppressing homing-based gene drives. *Nature Scientific Reports*, 7, 1–12. <https://doi.org/10.1038/s41598-017-02744-7>.
- National Academies of Sciences. (2016). Gene drives on the horizon: Advancing science, navigating uncertainty, and aligning research with public values. Washington, DC: The National Academies Press. <https://doi.org/10.17226/23405>.
- Newton, M. E., Wood, R. J., & Southern, D. I. (1976). A Cytogenetic analysis of meiotic drive in the mosquito, *Aedes Aegypti* (L.). *Genetica*, 46, 297–318.
- Noble, C., Adlam, B., Church, G. M., Esvelt, K. M., Nowak, M. A. (2018). Current CRISPR gene drive systems are likely to be highly invasive in wild populations. *eLife*, 7, e33423. <https://doi.org/10.7554/eLife.33423>.
- Noble, C., Min, J., Olejarz, J., Buchthal, J., Chavez, A., Smidler, A. L., et al. (2016). *Daisy-chain gene drives for the alteration of local populations*. <https://doi.org/10.1101/057307>.
- Owen, R., Crane, M., Grieger, K., Handy, R., Linkov, I., & Depledge, M. (2009). *Strategic approaches for the management of environmental risk uncertainties posed by nanomaterials—Nanomaterials: Risks and benefits*. Dordrecht, Netherlands: Springer.
- Ping, G. (2017). Invasive species management on military lands: Clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9 (CRISPR/Cas9)-based gene drives. U.S. Army Engineer Research and Development Center (ERDC) Environmental Laboratory.
- Reeves, R. G., Bryk, J., Altrock, P. M., Denton, J. A., & Reed, F. A. (2014). First steps towards underdominant genetic transformation of insect populations. *PLoS ONE*, 9, e97557. <https://doi.org/10.1371/journal.pone.0097557>.
- Regalado, A. (2017). Farmers Seek To Deploy Powerful Gene Drive. <https://www.technologyreview.com/s/609619/farmers-seek-to-deploy-powerful-gene-drive/URL>. Accessed December 13, 2017.
- Serebrovskii, A. S. (1940). On the possibility of a new method for the control of insect pests. *Zool Zhurnal*, 19, 618–630.
- Silver, L. M. (1993). The peculiar journey of a selfish chromosome: mouse t-haplotypes and meiotic drive. *Trends in Genetics*, 9, 250–254. [https://doi.org/10.1016/0168-9525\(93\)90090-5](https://doi.org/10.1016/0168-9525(93)90090-5).
- Sinkins, S. P., & Gould, F. (2006). Gene drive systems for insect disease vectors. *Nature Reviews Genetics*, 7, 427–435. <https://doi.org/10.1038/nrg1870>.

- Steinfeldt, M., von Gleich, A., Petschow, U., & Haum, R. (2007). *Nanotechnologies Hazards and Resource Efficiency*. Heidelberg, Germany: Springer.
- Sweeny, T. L., & Barr, A. R. (1978). Sex ratio distortion caused by meiotic drive in a mosquito, *Culex pipiens* L. *Genetics*, 88, 427–446.
- Taylor, D. R. (1994). The genetic basis of sex ratio in *Silene alba* (= *S. latifolia*). *Genetics*, 136, 641–651.
- The Rio Declaration on Environment and Development. (1992). https://www.unesco.org/education/pdf/RIO_E.PDF (accessed 7 June 2019).
- United Nations. (2000). Cartagena protocol on biosafety to the convention on biological diversity. Secretariat of the Convention on Biological Diversity, Montréal, Canada, <https://www.cbd.int/doc/legal/cartagena-protocol-en.pdf>.
- Webber, B. L., Raghu, S., & Edwards, O. R. (2015). Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *Proceedings of the National Academy of Sciences U S A*, 112, 10565–10567. <https://doi.org/10.1073/pnas.1514258112>.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.