What are the benefits and risks of gene drives for population management and conservation biology?

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Abstract:

Gene drive has recently been proposed as a promising technology for population management, including in conservation genetics; it is based on the release of genetically engineered individuals designed to rapidly propagate a desired mutation or transgene to high frequencies in wild populations. Potential applications in conservation biology include the control of invasive pest populations that threaten biodiversity (eradication and suppression drives), or the introduction of beneficial mutations in endangered populations (rescue drives). We examine the challenges posed by the evolution of resistance to gene drives and review the various environmental risks associated with gene drives. Contrary to suppression and eradication drives, the evolution of resistance should not prevent the fixation of rescue drives, while countermeasures to stop their spread are likely to fail. For eradication and suppression drives, minimizing the chances of resistance evolution requires targeting genes whose sequences have low polymorphism in natural populations (e.g. that are functionally constrained), which might however increase the odds of gene drive propagation to non-target populations/species. Conversely, targeting sequences that are present in the target population/species, but absent in non-target populations/species, might increase the odds of resistance evolution due to the introgression of resistant alleles from non-target populations/species. Once a gene drive has fixed in a target population, the time it persists before being inactivated by mutations influences its risk of spread to non-target populations/species. Finally, ethical values along with a clear regulatory framework for risk assessment should guide gene drive research.

Introduction

Using synthetic biology technologies, such as gene drives, to stop the rapid ongoing erosion of biodiversity, has recently sparked interest among conservation biologists (Piaggio et al. 2017), but also many concerns (SymBioWatch 2016). Population management using gene drives is based on the release of genetically engineered individuals designed to propagate a desired mutation or transgene to high frequencies in natural populations (Fig. 1, Box 1). Gene drives could be used to control invasive species (i.e. non-resident species, that are able to maintain, spread, and reproduce in a new habitat following their introduction; Blackburn et al. 2011) or to rescue endangered species that are deemed important for conservation. There are three different types of gene drives in conservation biology: eradication drives, suppression drives and rescue drives (Fig. 2). This typology focuses on the fitness effects of drive constructs, an important parameter for gene drive spread. Another typology considers whether or not the drive confers a new function to the organism (e.g. alteration or replacement drives, that provide resistance to malaria in mosquito; Gantz et al. 2015).

Eradication and suppression drives are designed to extirpate or decrease the size of a population, respectively. These drives are primarily developed for their applications for human health and for the control of agricultural pests, but they could also be applied in conservation to target invasive species that threaten biodiversity, such as plants (e.g. spotted knapweed, common ragweed, yellow star thistle, palmer amaranth; NASEM 2016), insects (e.g. mosquitos, wasps; NASEM 2016; Dearden et al. 2018), tunicates (e.g. *Ciona intestinalis*; Gandhi et al. 2017), mammals (e.g. rodents, possum or stoat; Dearden et al. 2018) or fishes (catfish, common carp, sea lamprey; Thresher et al. 2014). Using gene drive for population management could have lower health, economic and environmental costs than traditional control methods (Harvey-Samuel et al. 2017).

Rescue drives, on the other hand, could be designed to save endangered populations by introducing beneficial mutations or removing deleterious ones (Fig. 2; Esvelt et al. 2014). Due to the non-Mendelian inheritance of gene drives, these mutations would spread more quickly in target populations than with natural selection only. Rescue drives could alleviate an important dilemma traditionally faced by conservation geneticists: should one introduce individuals from other regions that bring in useful genetic variability, or locally adapted individuals? Introducing individuals from a distant source population into an endangered population might inadvertently introduce deleterious alleles that could result in outbreeding depression or in the overall maladaptation of the population (Bucharova 2017). When only a single or a few loci with large fitness effects provide adaptation to a specific environmental factor, rescue drives would allow locus-specific assisted gene flow, by providing beneficial alleles for some adaptive traits, while maintaining alleles for other adaptive traits at high frequencies. Rescue drives could increase stress tolerance (e.g. using drought-tolerance genes in eastern white pine; Tang et al. 2007), or increase resistance to pathogens (e.g. using immunity genes conferring blight resistance in American chestnut; Kubisiak et al. 2013; Newhouse et al. 2014). Rescue drives could also be used in other contexts than conservation. In agriculture, they could make honey bees and other important pollinators less susceptible to neonicotinoid insecticides; while for human health applications, rescue drives could make bank voles more resistant to the tick-borne pathogen *Borrelia afzelii*, which is responsible for Lyme disease in humans (e.g. using Toll-like receptors; Tschirren et al. 2013).

Previous reviews on gene drives have focused either on the different types of drive systems (e.g. Gantz and Bier 2016; Champer et al. 2016; Harvey-Samuel et al. 2017; Marshall and Akbari 2018), on their applications for human health or for pest control in agriculture (e.g. Macias et al. 2017;

Godfray et al. 2017; Scott et al. 2018; McFarlane et al. 2018) or on the challenges of their development in terms of biosafety (Benedict et al. 2018), regulation (Oye et al. 2014; Caplan et al. 2015; Meghani and Kuzma 2018) and ethics (Courtier‐Orgogozo et al. 2017; Thompson 2018). Although a few reviews presented some gene drive applications in conservation (Esvelt et al. 2014; Zentner and Wade 2017; Esvelt and Gemmell 2017; Min et al. 2018), the differences between the risks associated with rescue drives and those associated with suppression and eradication drives were not considered. In this paper, we fill this gap and review the use of gene drives for population management with a special emphasis on conservation biology. We focus on CRISPR-cas9-mediated gene drives (Box 1), which, unlike other drive systems, can be applied to a wide variety of organisms. For readers interested in other drive systems, we recommend other publications (e.g. Gantz and Bier 2016; Champer et al. 2016; Harvey-Samuel et al. 2017; Marshall and Akbari 2018), including reviews on t-haplotype gene drives in invasive mice (Leitschuh et al. 2018; McFarlane et al. 2018).

Table 1 Glossary	
Synthetic selfish genetic elements	Artificial genetic elements that favor their transmission to the next generation and thus bias Mendelian inheritance (Fig. 1)
cas9 [(a)] gene	Bacterial gene coding for the Cas9 protein, an endonuclease
Guide RNA (gRNA)	Engineered ribonucleic acid (RNA) molecule used by Cas9 to recognize and target a specific sequence of DNA
Cas9 [(a)] endonuclease	A RNA-guided enzyme that can virtually cut any sequence of DNA that matches the sequence of the associated gRNA
CRISPR-cas9 gene drive cassette	Fragment of DNA containing the *cas9* gene, a gene coding for a gRNA, flanking sequences and potentially a cargo gene (Box 1)
Homing endonuclease gene	A special class of natural selfish genetic element coding for an enzyme capable of cutting a specific DNA sequence, which can result in the replacement of the target sequence by the selfish genetic element (Box 1)
Homology directed repair (also known as gene conversion)	Repair by homologous recombination without crossing-over
Non-Homologous End-Joining (NHEJ)	Pathway to repair DNA double-strand breaks by ligating the two non-homologous ends. This repair pathway competes with gene conversion to repair DNA double-strand breaks (see Box 1)

[(a)] We follow the bacterial genetic nomenclature using italicized *cas9* for the gene and first-letter capitalised and upright Cas9 for the protein.

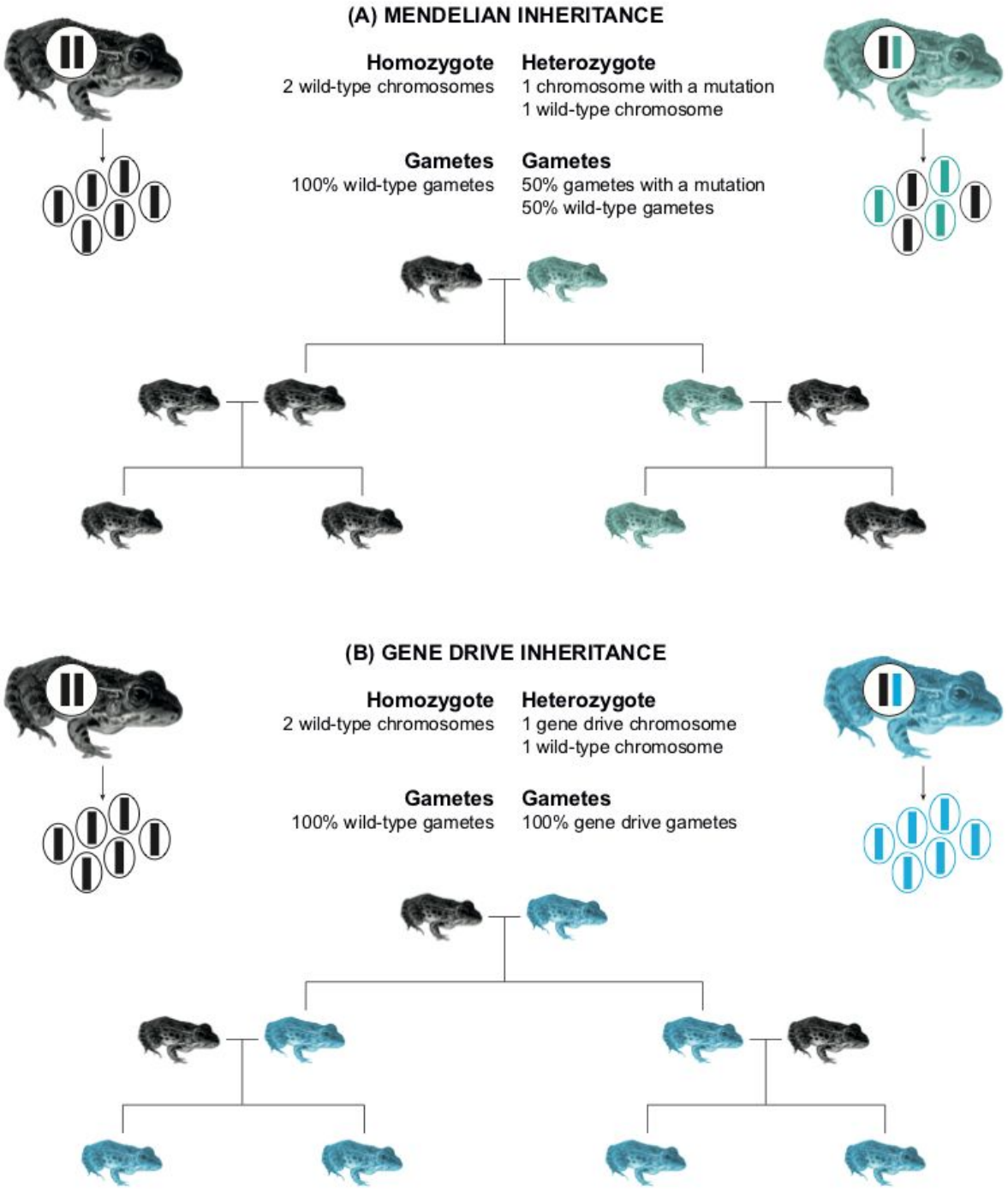


Fig. 1 (A) A classical mutation is transmitted to 50% of the gametes of heterozygous individuals (in green; Mendelian inheritance). (B) A synthetic gene drive element can be transmitted to 100% of the gametes of heterozygous individuals (in blue; non-Mendelian inheritance). A single pair of chromosomes is presented (black rectangle: wild chromosome, green rectangle: chromosome carrying a regular mutation, blue rectangle: chromosome carrying a gene drive). In this example, gene conversion takes place in the gonads. Original frog picture by Brian Gratwicke-Flickr.

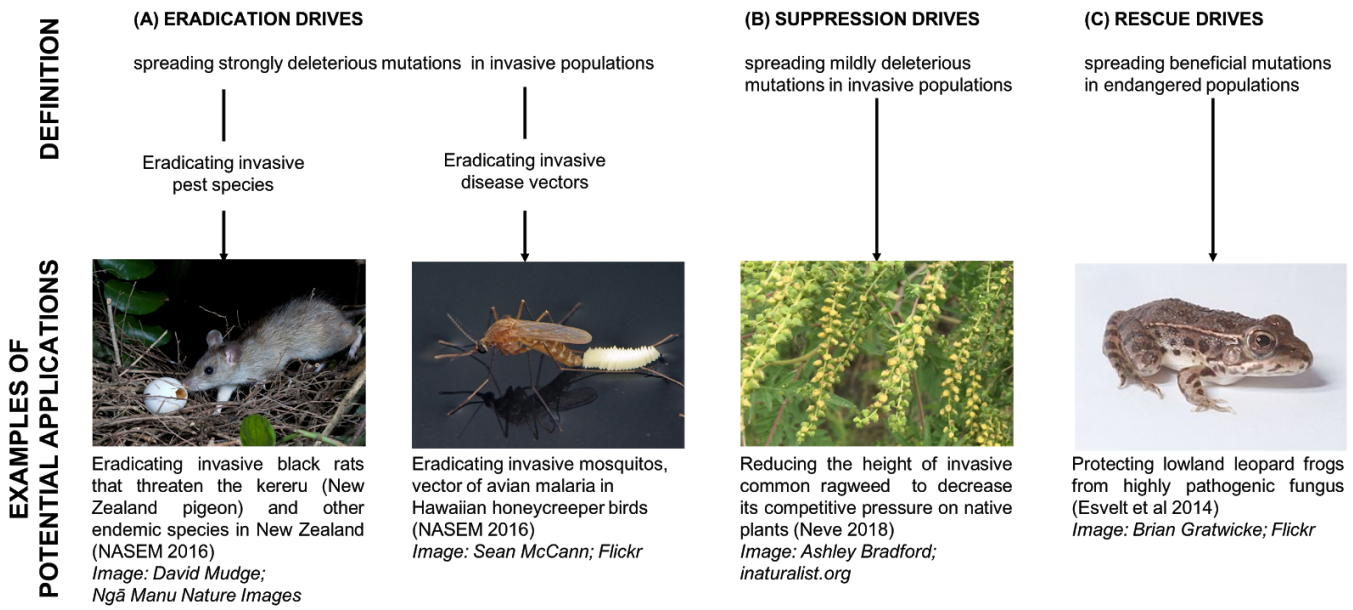
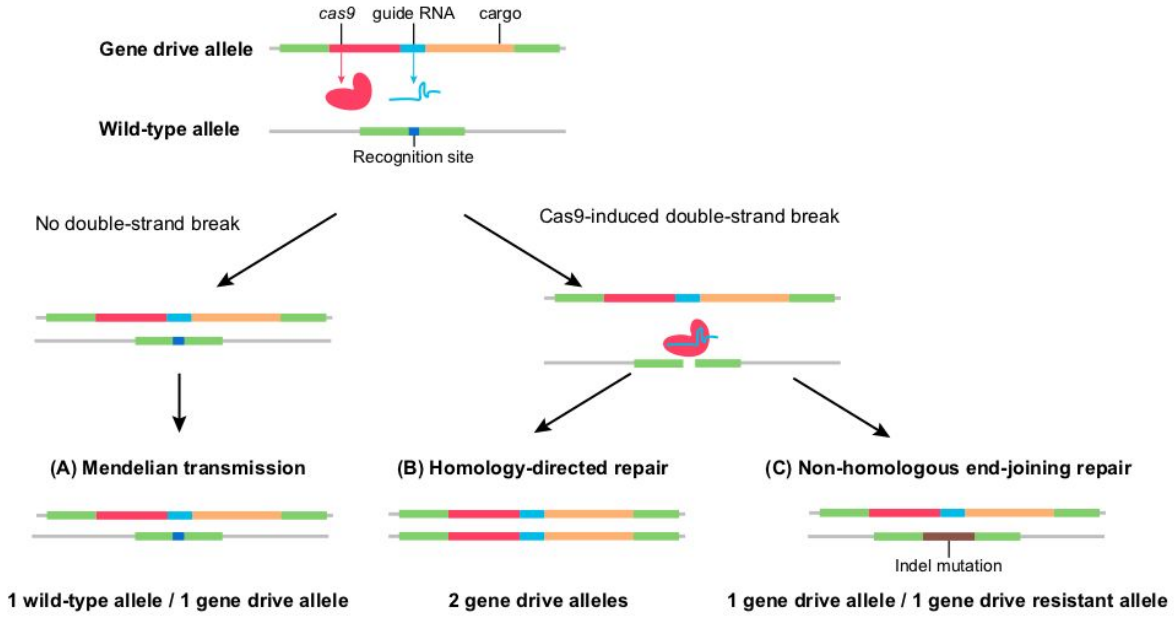


Fig. 2 Three different types of gene drives and their potential applications in conservation biology. See text for details.

Box 1 CRISPR-cas9 gene drives: definitions and rates of conversion

By propagating a mutation (or transgene) of interest in a population, gene drives offer the possibility of a new type of transgenesis, no longer at the level of the individual, but at the level of the population. This technique is based on the use of synthetic selfish genetic elements, which, like their natural counterparts, such as homing endonuclease genes, invade populations through the conversion of a wild-type allele into a gene drive allele. Hence, gene drives bias Mendelian inheritance and can spread a mutation conferring a character of interest in a population, even if this mutation is otherwise deleterious for carrier individuals. The Cas9 enzyme is part of the Clustered Regularly Interspaced Short Palindromic Repeats (hereafter CRISPR) immune system in bacteria, and can target virtually any sequence of DNA (Jinek et al. 2012). A CRISPR-cas9 gene drive cassette is a fragment of DNA that comprises different sequences:

- the *cas9* gene, that codes for the Cas9 endonuclease enzyme
- a guide RNA (hereafter gRNA) that recognizes a target sequence on the homologous wild-type chromosome
- flanking sequences homologous to sequences on both sides of the targeted region
- optionally, a cargo (or "payload") gene that codes for a trait of interest (e.g. malaria resistance).



In a heterozygous individual that carries both a wild-type allele and a CRISPR-cas9 gene drive cassette, there are three different fates for the wild-type chromosome. (A) It can remain intact, for example when the sequence on the wild-type chromosome is different from the sequence recognized by the gRNA and not cleaved by Cas9. (B) The wild-type chromosome can be recognized by the gRNA and cut by Cas9, which activates the DNA double-strand break repair machinery. The break can be repaired by homology-directed repair (homologous recombination without crossing over) using the chromosome bearing the CRISPR-cas9 gene drive cassette as a template. This process, called homing, represents a mechanism of biased gene conversion, whereby the gene drive allele is preferentially transmitted to the offspring (Burt and Trivers 2006). (C) When

the break is not repaired by homology-directed repair, it can be repaired by non-homologous end-joining (NHEJ). Conversion rates can vary from 14% to 100% and depend on the organism, the genetic background, the sex of the heterozygous individual, the type of construction (one vs. several gRNAs), the timing of expression (expression in the gonads or in the zygote), the location within the genome (autosomes vs. sex chromosomes) and the experimental protocol (Noble et al. 2017a; Champer et al. 2018b).

Key features affecting gene drive propagation

Gene drive timing of expression

The timing of expression of the Cas9 endonuclease and gRNA (i.e., timing of conversion) is an important parameter for the successful propagation of a gene drive that is not fully dominant (Fig. 3; Deredec et al. 2008). For eradication and suppression drives, wild-type/drive heterozygotes have higher fitness than drive homozygotes, so that gene conversion late in life (in the gonads) favors drive spread more than early gene conversion (in the zygote) does (Fig. 3A). Conversely, early conversion (in the zygote) favors the spread of rescue drives (Fig. 3B). The timing of conversion can be adjusted experimentally by using the appropriate promoters for the gene drive cassette (e.g. Champer et al. 2017; Hammond et al. 2018).

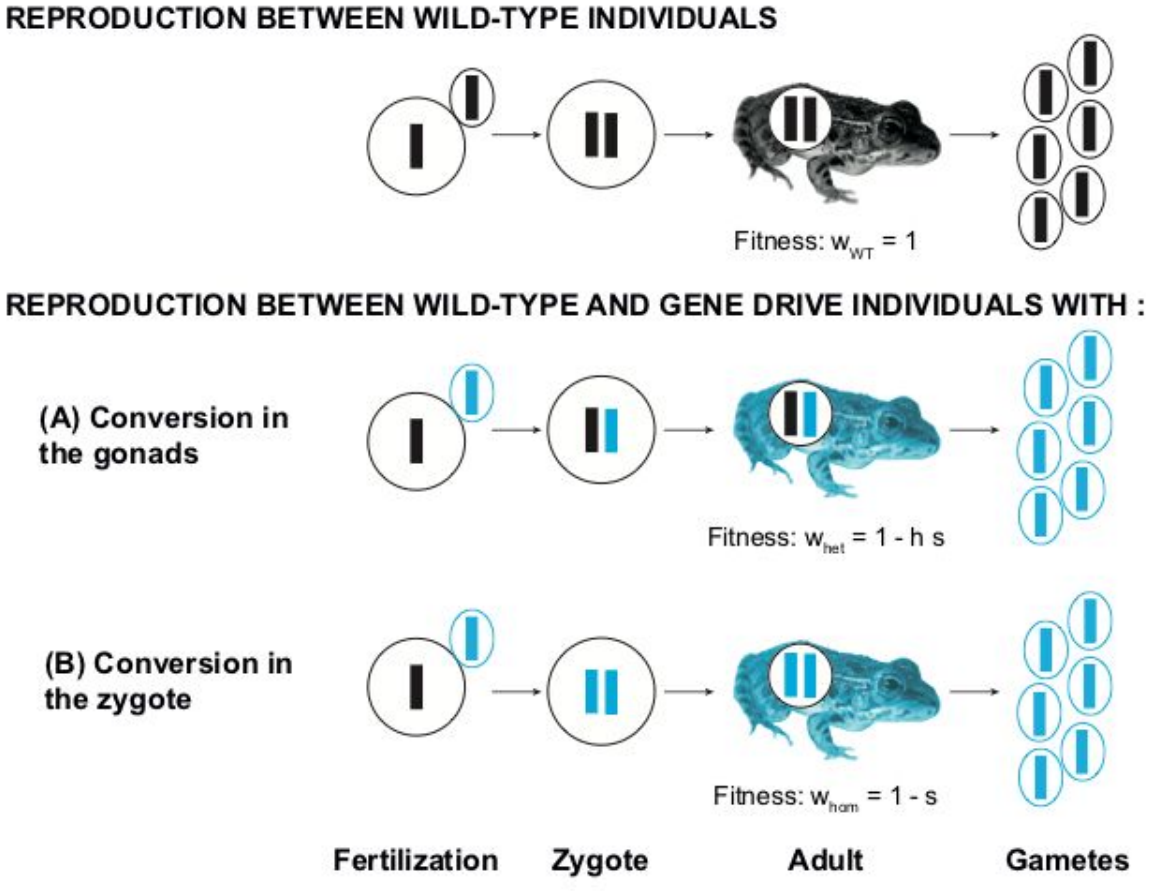


Fig. 3 Conversion of wild-type allele into a gene drive allele can occur either (A) in the adult gonads (i.e. in its germline only) or (B) in the zygote. The selection coefficient, *s*, represents the fitness difference between wild-type homozygous vs. drive homozygous individuals ($s > 0$, for eradication and suppression drives, $s < 0$ for rescue drives). When the fitness of wild-type/drive heterozygotes equals the fitness of wild-type homozygotes, the gene drive allele is completely recessive (dominance coefficient, h=0), whereas when the fitness of wild-type/drive heterozygous equals the fitness of drive homozygous individuals, the gene drive allele is completely dominant (h=1, as represented here). For eradication and suppression drives ($s > 0$), the fitness of heterozygous individuals is higher than the fitness of homozygous individuals ($w_{het} \geq w_{hom}$). For rescue drives ($s < 0$), the fitness of homozygous individuals is higher than the fitness of heterozygous individuals ($w_{hom} \geq w_{het}$). Original frog picture by Brian Gratwicke-Flickr.

Gene drive genetic parameters

Theoretical studies have investigated the influence of different parameters on drive dynamics (e.g. Deredec et al. 2008; Unckless et al. 2015; Noble et al. 2018). Here we distinguish between deterministic models (that assume very large population size and ignore gene drift) and stochastic models (that take chance into account).

In a deterministic model, a gene drive allele can go to fixation, disappear, but also reach an intermediate equilibrium frequency (see Fig. 4, illustrating results from a model where gene conversion takes place in the gonads; see Supplementary Information for model detail). For eradication or suppression drives, the final state depends on parameters such as the probability of

successful gene conversion, the dominance coefficient (*h* in Fig. 4, defined as in Fig. 3), and the coefficient of selection (*s* in Fig. 4) of the drive allele. For some parameter combinations, the final state also depends on the introduction frequency of the drive ("WT or Drive" area in Fig. 3A and red curves in Fig. 3B-C). For rescue drives (*s<0*), the drive allele is predicted to always fix eventually.

These results mostly hold true for stochastic model, but the fixation -- or loss -- of the drive allele is not always guaranteed whenever chance events (genetic drift) are taken into account. Stochasticity can indeed play an important role for populations of small sizes and in fragmented populations, but its effects in such populations is currently under-investigated (or, to our knowledge, completely lacking for rescue drives). Still, it has been confirmed that a higher introduction frequency of the drive allele (i.e., the release of a higher number of drive-carrying individuals) increases the chance of eventual fixation of a drive allele (Unckless et al. 2015).

To our knowledge, theoretical models have so far exclusively focused on fitness differences between gene drive and wild-type alleles during the diploid phase of the life cycle, ignoring potential differences acting also during the haploid phase (e.g. in the pollen for plants or in males of haplodiploid species such as invasive wasps). We expect such fitness differences to favor even further the spread of rescue drives, but to disfavor the spread of suppression and eradication drives.

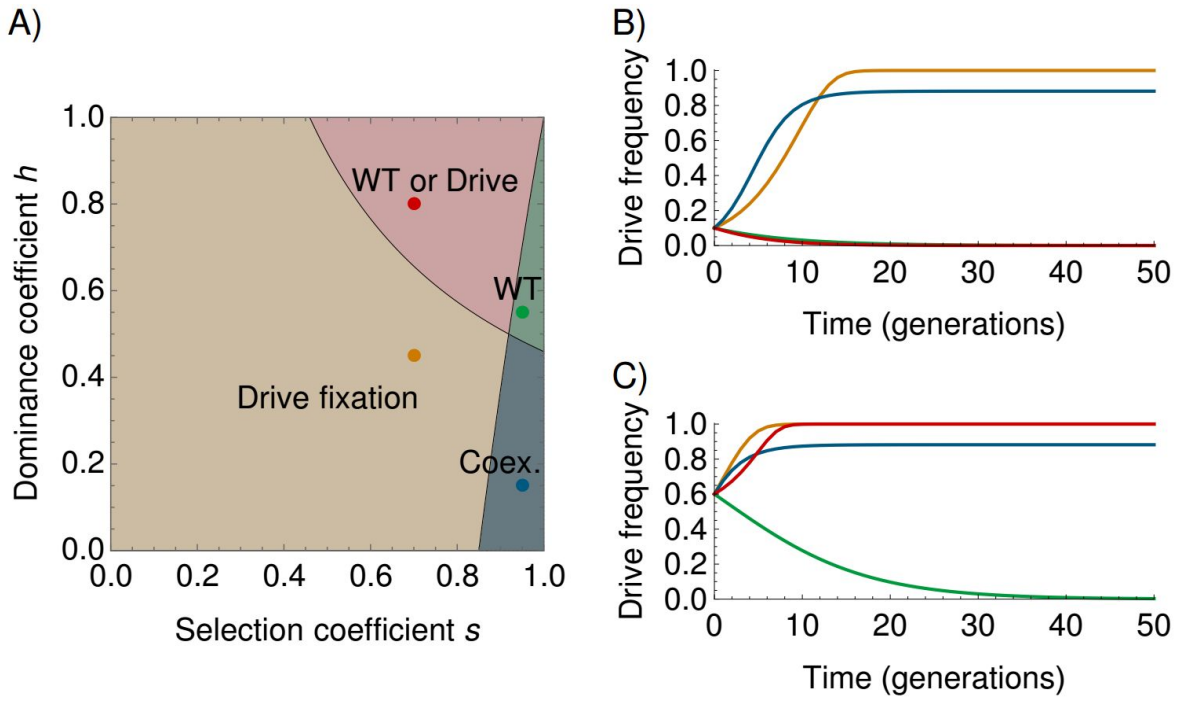


Fig. 4 Effect of selection intensity (*s*) and dominance coefficient (*h*) on drive dynamics, when conversion takes place in the gonads (*s* and *h* are defined as in Fig. 3). (A) Parameter ranges of the different outcomes ("Drive fixation", "Coex": coexistence between drive and wild-type (WT) alleles, "WT": fixation of the WT allele, "WT or Drive": bistability, fixation of either the drive or the WT allele, depending on drive initial introduction frequency). Rescue drives (s < 0) always fix and are not represented. The conversion efficiency *c* is here set to 85%. (B) Dynamics of the frequency of the drive in the population, for four different sets of (*s, h*) parameters; the parameters corresponding to the line colors are shown with colored dots in panel A. The frequency of introduction of the drive allele is 0.1. (C) Same as (B), but with a higher frequency of introduction of the drive allele (0.6); note the different outcome for the red curve (bistability conditions). See Deredec et al. (2008) and Supplementary Information for details.

Mating systems

Gene drives require sexual reproduction for their transmission. Mate limitation during the establishment phase of an invasive species is thought to favor species with mixed mating systems (i.e. species that can both outcross and self-fertilize or that can reproduce both sexually and asexually; Baker 1967; Pannell et al. 2015). Gene drives are likely to be less efficient in invasive species with such mixed mating systems (Bull 2017). For example, a gene drive that targets a species with high rates of selfing and low inbreeding depression is unlikely to reach high frequencies (Noble et al. 2018). More generally, many of the important challenges in conservation genetics involve species that reproduce exclusively through selfing or asexual reproduction. For instance, despite the recent discovery of potential candidate genes to mitigate coral bleaching through locus-specific assisted gene flow (Jin et al. 2016), asexual reproduction of many coral species (e.g. Highsmith 1982) or of their endosymbionts (Andras et al. 2011) represents a major limitation to their management using gene drives. Similarly, gene drives cannot be used to modify asexually reproducing micro-organisms and increase their biodegradation of pollutants (Joutey et al. 2013) or to modify the asexual cells causing transmissible cancer in Tasmanian devils (Siddle et al. 2013).

Generation time

Although gene drives can invade populations in a few dozens of generations, this process could take several centuries in long-lived species. For instance, fixation of a gene drive within 20 generations in eastern white pine would take about 600 years, considering a generation time of 30 years (Nijensohn et al. 2005). As climate change occurs on a much shorter time scale (Pachauri et al. 2014), it is likely that rescue drives could not prevent extinction in this particular case.

Population structure in space, time and age

In spatially-structured populations, dispersal is another important parameter to consider to predict gene drive propagation. Population fragmentation with low dispersal rates between populations, as often observed in endangered species, would likely affect gene drive dynamics in a metapopulation. Very low dispersal rates could slow down the spread of a rescue drive in a metapopulation (Noble et al. 2018).

The presence of resting stages (e.g. seed bank in plants) might result in a constant inflow of wild-type individuals for years or decades (i.e. dispersal in time rather than space), which might reduce the spread of gene drives (NASEM 2016).

While conversion rates can vary with age (e.g. as observed in *Drosophila melanogaster*; Preston et al. 2006), the influence of this factor on drive propagation remains to be investigated.

Potential applications in conservation

Eradication of invasive black rats in New Zealand

Black rats have been introduced to New Zealand following European colonization during the 19th century (Atkinson 1973). Their introduction resulted in the suppression of many endemic plants

(through seed predation) and in the extinction or decline of several insect, snail, spider, reptile and bird species (Towns et al. 2006; Towns 2009).

Different population control methods can be used against rats, such as physical removal (e.g. trapping), pesticide treatments (e.g. anticoagulant toxicants), biological control (e.g. releasing cat predators) or female sterilization (e.g. contraception; NASEM 2016). In 2016, the New Zealand government financed a plan (Predator Free 2050) to eradicate black rats along with other invasive species by 2050 (Norton et al. 2016). Predator Free 2050, together with universities and non-profit organizations, is developing an international program on gene drive research against rodents (Genetic Biocontrol of Invasive Rodents program; Hall 2017). Thanks to the advances of CRISPR-cas9 genome editing in rat (Remy et al. 2017), gene drives currently developed in house mice (Grunwald et al. 2018) could be tested in rats in the future (Fig. 2A; Prowse et al. 2017).

Finally, targeting rats in New Zealand using gene drives could have potential unintended consequences. Dispersal of gene drive rats to neighbouring countries would be an important international issue. Black rats can hybridize with the Asian rat, *R. tanezumi* (Chinen et al. 2005), which poses a risk of propagation to *R. tanezumi* native populations. In addition, removing rats (with any control method) could have some negative consequences for native species. For example, rats might have replaced native species originally responsible for dispersing seeds of native plants (Shiels and Drake 2011) or spores of native fungi (Vernes and McGrath 2009).

Protection of honeycreepers in Hawaii

Honeycreepers and other endemic birds in Hawaii have evolved in the absence of avian malaria and, consequently, are particularly susceptible to the invasive malaria parasite *Plasmodium relictum* (Lounibos 2002). The main vector of this parasite, the southern house mosquito, *Culex quinquefasciatus*, is invasive in Hawaii since the beginning of the 19th century (Lounibos 2002). An eradication drive targeting mosquito populations could protect endemic birds (Fig. 2A; NASEM 2016).

Potential methods include the disruption of mosquito genes that are required for female fertility (e.g. *doublesex* gene; Kyrou et al. 2018) or for the propagation of malaria parasites (e.g. a *Culex* gene equivalent to the *FREP1* gene in *A. gambiae*; Dong et al. 2018). An alternative to eradication drives includes that introduction of cargo genes that code for antibodies preventing the reproduction and transmission of the parasites (Gantz et al. 2015). Note that strategies alternative to gene drives based on the sterilization of females with irradiation (Sterile Insect Technique) or using the bacteria *Wolbachia* (Incompatible Insect Technique) are currently being developed in different mosquito species (Lees et al. 2015; Ritchie et al. 2018).

Spreading *Rht* dwarfing alleles in invasive common ragweed

Common ragweed (*Ambrosia artemisiifolia*) is an annual plant, native to North America and invasive in South America, Europe, Africa, Asia and Australia (Smith et al. 2013). Ragweed produces different allelochemical metabolites and suppresses the growth of native plant species, hence reducing plant biodiversity (Smith et al. 2013). As ragweed causes important allergies in humans (Smith et al. 2013) and is considered as a weed in agriculture (Bassett and Crompton 1975), many countries and the European Union have launched eradication programs (Smith et al. 2013). However, control methods based on mechanical or herbicide treatments can have a negative impact on biodiversity (Alberternst et al. 2016).

In a recent perspective on gene drive applications for weed management, Neve (2018) suggested developing a suppression drive targeting homologues of Reduced height (*Rht*) genes, responsible for dwarfism in cultivated wheat. Height is an important trait for competition for light in plants, including ragweed (Coble et al. 1981). A gene drive targeting genes orthologous to *Rht* genes found in related sunflowers (Ramos et al. 2013) could reduce ragweed competitive ability (Fig 2B). The evolution of selfing would not be an issue, as ragweed is an obligate outcrossing plant with separate sexes (Friedman and Barrett 2008). As ragweed is wind-pollinated, the decline of its population should not affect pollinator communities. However, ragweed populations are characterized by large seed banks (Brandes and Nitzsche 2006), which might impair the spread of suppression drives in this species.

Introducing MHC resistance alleles in endangered amphibian species

The chytrid fungus, *Batrachochytrium dendrobatidis*, has emerged as a global threat for up to 50% of amphibian species (Fisher et al. 2009). The fungus reproduces mostly asexually (Fisher et al. 2009), and cannot be targeted with a gene drive. However, resistance to *B. dendrobatidis* infection varies both within and among amphibian species (Fu and Waldman 2017). Major histocompatibility complex (MHC) peptides play an important role in the innate immune system of vertebrates, by presenting antigens to lymphocytes. A specific MHC allele has been shown to increase the chance of survival of infected individuals in the lowland leopard frog *Lithobates yavapaiensis* (Savage and Zamudio 2011).

CRISPR-cas9 genome editing is advancing in clawed frogs, Xenopus spp. (e.g. Aslan et al. 2017), so the development of rescue drives using resistant MHC alleles could be considered for amphibian populations at risk (Fig 2C; Esvelt et al. 2014). However, mounting an effective immune response against *B. dendrobatidis* might also carry immunity trade-offs (Fu and Waldman 2017). Although replacing an endogenous MHC gene by a resistant MHC allele would increase resistance to this fungus, it would also reduce allelic diversity at the MHC locus which could in turn increase population susceptibility to other pathogens. An alternative would be to insert a gene drive cassette with a cargo including a resistant MHC allele at a locus unlinked to the endogenous MHC locus. This strategy might preserve MHC variability but is likely to affect the stability of the gene drive cassette due to the risks of recombination with the endogenous MHC locus.

Issues associated with a lack of efficacy of gene drives

Cas9 activity outside of the germline/zygote

As described above, the optimal timing for gene conversion differs between eradication and suppression drives (gonads) vs. rescue drives (zygote; Fig. 3). For gene drives with conversion in the gonads, conversion can occur unexpectedly during development either in somatic cells or in the zygote due to the maternal carryover of the Cas9 protein and the gRNA in the egg cytoplasm (Champer et al. 2018a; Oberhofer et al. 2018). Such conversion events can result in mosaic heterozygous individuals that have reduced fitness compared to normal heterozygotes, which would decrease the spread of eradication and suppression drives (Champer et al. 2018a; Oberhofer et al. 2018). Indeed, some somatic cells that are normally drive/wild-type heterozygous can become either

homozygous for the drive allele (when homology directed repair predominates) or heterozygous with a drive chromosome and a resistant chromosome with an indel (when NHEJ predominates, Box 1).

For rescue drives with conversion rates in the zygote below 100%, the effect of conversion outside of this optimal time window depends on the relative importance of NHEJ vs. homology directed repair. If NHEJ predominates, the indels carried by resistant chromosomes are likely to be deleterious, so that mosaic heterozygous individuals would have reduced fitness compared to fully heterozygous ones. In contrast, if homology directed repair predominates, mosaic heterozygous individuals could have increased fitness compared to full heterozygotes. Conducting experiments with rescue drives will be necessary to quantify the relative importance of NHEJ and homology directed repair when conversion occurs outside of the zygote.

Evolution of resistance to gene drives

Chromosomes that cannot be recognized or cleaved by Cas9 confer gene drive resistance (Box 1C). Such resistance can occur either through standing genetic variation (i.e. polymorphism at the target site) or through evolution by *de novo* mutations (Unckless et al. 2017).

Alleles that confer resistance to a gene drive through standing genetic variation are already present and segregate in the target population (Drury et al. 2017). Such resistant alleles could originate from mutations in the past or be acquired through hybridization and introgression with a related drive-resistant species (as observed for anticoagulant resistance in house mouse; Song et al. 2011). The risk of resistance via standing genetic variation can be reduced by targeting sequences that are functionally constrained and hence present low polymorphisms in natural populations (e.g. Kyrou et al. 2018). This would, however, increase the risks of gene drive propagation to non-target species (see below).

The evolution of *de novo* resistance represents an important risk, especially for eradication and suppression drives. Non-homologous end-joining (NHEJ) repair often results in an insertion or a deletion that makes wild-type chromosomes resistant to further cleavage by the Cas9 endonuclease (Box 1C). A recent study in *D. melanogaster* shows that the probability of occurrence of such indel mutations in the germline could be several orders of magnitude higher in drive/wild-type heterozygotes compared to wild-type homozygous (e.g. 20% vs. 10^{-8} %; Sharp and Agrawal 2016; Champer et al. 2017). In addition, genetic variation in NHEJ repair both among and within *D. melanogaster* populations could select for increased resistance to gene drives (Champer et al. 2017, 2018b). Indel mutations conferring resistance are selected for when their fitness costs are lower than the fitness costs associated with gene drive alleles (Unckless et al. 2017). Studies suggest that, in plants, NHEJ repair predominates over homology directed repair (Gorbunova and Levy 1999; Li et al. 2013). The high occurrence of such indel mutations in target species where NHEJ predominates would impair the spread of suppression or eradication drives.

The emergence of resistance within a few generations is currently one of the main causes of failure in gene drive experimental evolution studies (Hammond et al. 2017; KaramiNejadRanjbar et al. 2018; Oberhofer et al. 2018). Using several gRNAs that target multiple sites is predicted to decrease the rate of emergence of resistance alleles (Noble et al. 2017b). This strategy is similar to multi-drug therapy, whereby targeting multiple sites makes the evolution of resistance simultaneously at all sites less likely (Rex Consortium 2013). Two experimental studies found that targeting multiple sites decreases the appearance of alleles resistant to cleavage (Champer et al. 2018a; Oberhofer et al. 2018). However the constructions differed and Oberhofer et al. (2018) found many instances of incomplete homology-directed repair where the CRISPR-cas9 cassette was only partially copied (e.g.

without the *cas9* gene). Individuals carrying partial copies of the cassette incur important fitness costs, which can prevent the spread of such gene drive constructs (Oberhofer et al. 2018).

Resistance, on the other hand, maybe not be a problem for rescue drives: alleles resistant to cleavage are expected to be more deleterious than the drive allele, and would therefore not prevent the spread of a drive-propagated beneficial mutation.

Risks of unintended effects

Using gene drive in conservation biology could result in potential hazards at different scales, from molecular to ecosystem levels.

Molecular off-target mutations

To our knowledge, all gene drive experimental studies so far have focused on conversion at the target site, without testing for potential off-target effects outside of the targeted genomic region. In both heterozygous (wild-type/drive) or homozygous (drive/drive) individuals, the presence of the Cas9 endonuclease could induce double-strand breaks in genomic regions different from the target site. For example, a random mutation in the gRNA could lead to the cleavage of off-target sites (i.e. gRNA "retargeting"; Scharenberg et al. 2016). A drive construct can therefore be considered as a mutagen, whose off-target effects will depend on the specificity and on the susceptibility to potential retargeting mutations of the gRNA. Such off-target double-strand brakes could be repaired by homology directed repair (Box 1B) or NHEJ (Box 1C). NHEJ repair could result in indel mutations with potential fitness costs (Barton and Zeng 2018), while homology directed repair would result in a local loss of heterozygosity (i.e. in the replacement of the chromosomal regions surrounding the off-target regions by corresponding regions in the homologous chromosomes; Gorter de Vries et al. 2018). Furthermore, near-target mutations can also occur through homology-directed repair in regions flanking the target site (Cho et al. 1998), potentially resulting in extensive loss of heterozygosity. The tract length of these near-target mutations will likely depend on the length of homologous flanking sequences. In some extreme cases, loss of heterozygosity in near-target or off-target regions could even extend over entire chromosome arms as demonstrated by a recent study in yeast (Gorter de Vries et al. 2018). Such loss of heterozygosity would result in increased inbreeding depression. Hence, these near- and off-target effects would globally increase the mutation load in targeted populations. They would represent an important risk of failure for rescue drives. In contrast, they would accelerate population decline for eradication or suppression drives. Further studies are needed to determine the extent of near- and off-target mutations induced by gene drives.

Transposition of the gene drive cassette within the genome

Phylogenetic analyses have shown that naturally occurring endonucleases can insert into new positions within the host genome (Dalgaard et al. 1997). Transposed gene drive cassettes would likely be lost due to genetic drift, unless they have non-Mendelian inheritance (Fig. 1), which may occur when the cargo gene is also endogenously present in the host genome. The endogenous sequence could be converted into a gene drive cassette following the repair of a double-strand break naturally occurring in its vicinity (Box 1B). The risk of these ectopic recombination events (Lee et al. 2016) is likely to be affected by the position of the cassette relative to the endogenous copy.

Propagation to non-target populations

Several strategies could limit the spread of a gene drive from targeted populations to non-target populations. First, so-called "precision drives" (Esvelt et al. 2014) would target a gene or sequence specific to a target population. A second strategy consists in first recoding an allele by propagating a gene drive with no fitness effect, and then releasing a second drive that would target the recoded allele only, and so forth with several successive drives (Esvelt et al. 2014). This approach would then limit the probability of spread of suppression and eradication drives to non-target populations. For rescue drives however, the final drive is selectively favored and could still propagate to non-target populations. Overall though, implementing this strategy would take a very long time, as each intermediate gene drive would need several generations to fix. Although the effect of resistance on drive dynamics has been studied in a metapopulation context (Noble et al. 2018), no theoretical model has investigated whether precision drives could fail due to resistance through adaptive introgression with non-target populations (see above).

Propagation to non-target populations could also be avoided by the use of self-limiting drives, such as drives that only spread if introduced above a given frequency (e.g. a drive with the parameters of the red curve in Fig. 3, underdominance systems, or a combination of gene drive and underdominance systems; Dhole et al. 2018a). A strategy, called "daisy-chain drive" (Noble et al. 2016), has been proposed to achieve self-limitation. This method involves a linear chain of unlinked genetic elements, such that gene conversion at locus $i+1$ can only occur if a drive allele is present at locus i. Each of the lower elements confers some fitness cost, so that they are all sequentially lost from the population (Noble et al. 2016). So far, no laboratory report using daisy-chain drives has been published, but proof-of-concept experiments using nematodes have been proposed (Min et al. 2017). While temporally self-limited, daisy drives are not spatially self-limited, as they can easily spread to non-target populations (Dhole et al. 2018b).

Propagation to non-target populations is a key concern for the use of gene drive on islands. Islands are the primary biogeographical systems in which gene drives might be developed for conservation (e.g. 80% of the world's islands now have invasive rodents; NASEM 2016). Dispersal may be rare, but a drive can spread with just one introduced individual. In addition, the deliberate unauthorized transport of gene drive individuals to non-target populations represent an important risk (Esvelt and Gemmell 2017). Eradication drives planned to be released to control invasive black rats and house mice in New Zealand could spread to the native range of these species (Leitschuh et al. 2018). Large-scale population genetics studies to estimate gene flow between New Zealand and other countries could help better estimate the risks of gene drive individuals escaping to other countries, and the risk of reinvasion of New Zealand following eradication.

The propagation of a transgene to non-target populations has been reported in genetically modified plants. For example, the dispersal of a transgene up to 3.8 km away from a test site has been observed following a field trial of glyphosate-resistant bentgrass in the USA (Reichman et al. 2006). While originally present in *D. melanogaster*, the *P*-element is now spreading in *D. simulans* populations (Hill et al 2016). Transfer might have occurred through hybridization with *D. melanogaster* (as some crosses between the two species can produce fertile hybrids; Davis et al. 1996) or horizontally (Kofler et al. 2015), but an unintended escape of a few laboratory-raised *D. simulans* flies genetically engineered to carry a *P*-element cannot be ruled out.

Conservation geneticists might help better assess the risks of propagation to non-target populations by developing further theoretical models and by precisely estimating gene flow between

target and non-target populations. They could also make use of previous studies aimed at estimating dispersal and contamination of genetically modified organisms.

Propagation to non-target species

The effects of gene drives are expected to be specific to the target species, as they spread through sexual reproduction. We can predict that the risk of propagation of a gene drive to non-target species depends on the probability of hybridization (or of horizontal transfer), on the fitness of hybrids (or the fitness of individuals carrying the transgene) and on the similarity between the sequence targeted by the gRNA and its flanking sequences with the homologous sequence in the non-target species. Targeting a sequence that is conserved within a species to reduce the risk of resistance due to standing genetic variation (e.g. Kyrou et al. 2018) could increase the risk of spread to related species where this sequence might be also conserved. Many invasive species have a wide geographic distribution and can occur in sympatry with closely related native species with which they can hybridize (e.g. ragweed; Vincent and Cappadocia 1987; *Culex* mosquitos; Smith and Fonseca 2004; house mouse; Song et al. 2011; black rats; Lack et al. 2012). A gene drive could spill-over to a related species through hybridization. While introgression to non-target species has been observed for genetically modified plants (e.g. Zapiola and Mallory-Smith 2012), the risks of spread of a gene drive to non-target species have not yet been investigated neither theoretically nor empirically. The risks of transfer of a gene drive to non-target species could be estimated using population genomic approaches (e.g. by testing for potential gene flow between target and non-target species and for the presence of the target sequence and flanking sequences of the gene drive cassette).

In addition to hybridization, DNA can be naturally transferred from one species to another through horizontal gene transfers. Such transfers are rarely detected, as most newly inserted DNA sequences are likely to be lost by genetic drift unless they confer strong fitness advantages (e.g. adaptive introgression of genes responsible for carotenoid biosynthesis in pea aphids; Moran and Jarvik 2010), or have a transmission advantage (self-replicating genetic elements). Horizontal gene transfers can occur via parasites, pathogens or endosymbionts (e.g. viruses, bacteria, fungi and either sap-sucking insects in plants; Cho et al. 1998; or parasitoids in animals; Gilbert et al. 2010, 2014) and between extremely distantly related species (e.g. the *BovB* element moved at least 11 times between snakes, lizards, ruminants and marsupials; Ivancevic et al. 2017; and the *Steamer* element was transferred from molluscs to fish; Metzger et al. 2018).

Naturally occurring selfish endonuclease elements (so-called homing endonuclease genes) are in essence similar to gene drive constructs. A natural homing endonuclease is an enzyme that recognizes and cuts a specific target site on the homologous chromosome. Homology-directed repair converts the wild-type sequence into the homing endonuclease gene. Transfers of naturally occurring homing endonuclease genes have been documented between closely-related species (most likely through hybridization) and between distantly-related species (horizontal gene transfers). For example, the *omega* element has been transferred among different yeast species at least 15 independent times (Goddard and Burt 1999). Phylogenetic analyses in plants indicate that a class I intron homing endonuclease gene, that specifically targets the *cox1* mitochondrial gene, has been transferred independently 70 times between 162 taxa belonging to 45 different families (Sanchez-Puerta et al. 2008). This element is also present in the genome of several species of fungi, green algae and liverworts, which suggests extensive horizontal gene transfers (Cho et al. 1998). However, this view may be biased as endonucleases that are easier to characterize are those that cut conserved sites that are shared among distantly related species, and hence more likely to be horizontally transferred.

Whether the spread of gene drive cassettes in non-targeted populations directly compares to the non-Mendelian propagation of natural endonuclease genes remains to be determined.

A target population fixed for an eradication drive goes extinct. For suppression or rescue drives that are fixed in a target population, there is no selective pressure to maintain a functional endonuclease, so that the CRISPR-cas9 cassette can eventually accumulate mutations (e.g. stop codons). These mutations have normal Mendelian inheritance (Fig. 1) and can spread in target populations either through genetic drift (if they are neutral) or through natural selection (if they are beneficial, e.g. if the constitutive expression of Cas9 is costly). The risk of propagation to non-target species depends on the relative frequency of functional and non-functional gene drive cassettes and on the time before the non-functional CRISPR-cas9 cassette reaches fixation. The persistence time of gene drives is currently unknown. Whether it is the same order of magnitude as chemicals used for population management (several decades for many persistent organic pollutants such as DDT; Jones and De Voogt 1999) remains to be investigated.

Consequences for ecosystems

Removing invasive species might have unanticipated negative impacts on ecosystems through indirect effects on food webs (Zavaleta et al. 2001). Eradicating an invasive population might lead to the subsequent reinvasion by the same species or a different species with the same ecological niche. Other potential indirect effects depend on the position of the invasive species in the food chain. Invasive prey species can be heavily consumed by predators so that their sudden removal might result in increased predation on endemic species (Courchamp et al. 2003). For example, poisoning of black rats in a New Zealand forest resulted in invasive stoats (*Mustela erminea*), one of the main rat predators, switching their diet to native birds and bird eggs (Murphy and Bradfield 1992). Conversely, removing an invasive predator or an invasive herbivore might cascade down the food chain. For example, the eradication of feral goats and pigs on Sarigan islands (a US territory in the northwestern Pacific) led to the release of a previously undetected invasive vine (*Operculina ventricosa*) that subsequently covered most of the native forest and surrounding grassland (Kessler 2002). When two invasive species compete for the same niche (e.g. rats and mice), targeting only one competing species can result in an increase of the population of the other (Caut et al. 2007). Invasive species can also create new habitats or provide a food source for native species. For example, the worldwide invasion of the American brine shrimp *Artemia franciscana* has led to the extinction of many native *Artemia parthenogenetica* populations in Southern France (Rode et al. 2013). Contrary to *A. parthenogenetica*, *A. franciscana* is present throughout the winter in the area and represents a food source for native birds, including the greater flamingo (*Phoenicopterus roseus*). Eradicating the invasive *A. franciscana* might negatively affect native bird communities. More generally, eradicating an invasive species can move the ecosystem further away from its equilibrium without returning to its pre-invasion state, sometimes even making the system more susceptible to new invasions (David et al. 2017).

All of the risks listed above are not specific to population management using gene drives. As the pace of population suppression/eradication is likely to differ between gene drives and other control strategies, theoretical models could help anticipate and mitigate potential negative effects (David et al. 2013). For instance, the release of gene drive individuals might transiently increase population size with potentially long-lasting ecological consequences (David et al. 2013). Finally, rescue drives could destabilize food webs, for example by turning an endangered species into an invasive one.

Risk of failure of countermeasures to stop an ongoing drive

A key issue with the genetic modification of wild organisms is the reversibility of the modification. A first straightforward method to stop an ongoing gene drive is to release drive-resistant individuals that carry a functional copy of the targeted gene without the recognition sequence (Box 1C; Vella et al. 2017). This approach is expected to be effective for eradication drives, which impose strong fitness costs, but not for rescue drives or suppression drives imposing mild fitness costs (see above).

A second method consists in stopping the spread of a gene drive using a so-called gene drive brake (hereafter "brake"; Wu et al. 2016). Depending on whether the brake allele includes the *cas9* gene, one can distinguish "immunizing reversal drives" and "reversal drives". The former are used to replace both the initial drive and wild-type alleles with a second drive immune to the initial drive (Esvelt et al. 2014). Immunizing drives include the *cas9* gene and have two different gRNAs that target either the wild-type sequence or the sequence of the initial gene drive (Esvelt et al. 2014). The latter do not possess the *cas9* gene and only target the sequence of the initial gene drive (Gantz and Bier 2016; Wu et al. 2016). In gene drive/brake heterozygotes, the gRNA binds with Cas9 to disrupt the functional copy of the *cas9* gene (Fig. 5). In wild-type/brake heterozygotes, the brake has a regular Mendelian inheritance. A laboratory experiment in *D. melanogaster* showed that a reversal brake can inactivate a gene drive with a high efficiency (> 90%; Wu et al. 2016). Both immunizing reversal drives and reversal drives can in theory include a functional copy of the gene(s) disrupted by a prior suppression or an eradication drive, and thus have a fitness similar to that of the wild-type allele (Esvelt et al. 2014).

Brakes are not a silver bullet against drives: the presence of a drive allele in a population during the time between brake release and drive elimination can have long-lasting effects on the recovered populations, including inbreeding depression due to a temporary decrease in population size or to the presence of off-target mutations. Moreover, countermeasures against rescue drives are likely to fail, as drive alleles have a higher fitness than wild-type alleles.

A recent theoretical study shows that the release of brake-carrying individuals can lead to the fixation of the brake allele, the restoration of the wild-type allele or oscillations around a polymorphic equilibrium where both wild-type, gene drive and brake alleles are maintained through time (Vella et al. 2017). The polymorphic equilibrium is observed when the brake has a lower fitness than the wild-type. Overall, immunizing reversal drives are better at stopping an ongoing gene drive than reversal drives, as they target both the wild-type and drive alleles (Vella et al. 2017). However, the population remains genetically modified with the *cas9* gene and the continued presence of the Cas9 protein can increase the risks of potential negative off-target mutations (Gantz and Bier 2016; see above). Populations fixed for a reversal drive are also genetically modified, as they express the gRNA directed against the *cas9* gene. Finally, the probability of stochastic elimination of an ongoing gene drive decreases with the cost of the brake allele (Vella et al. 2017).

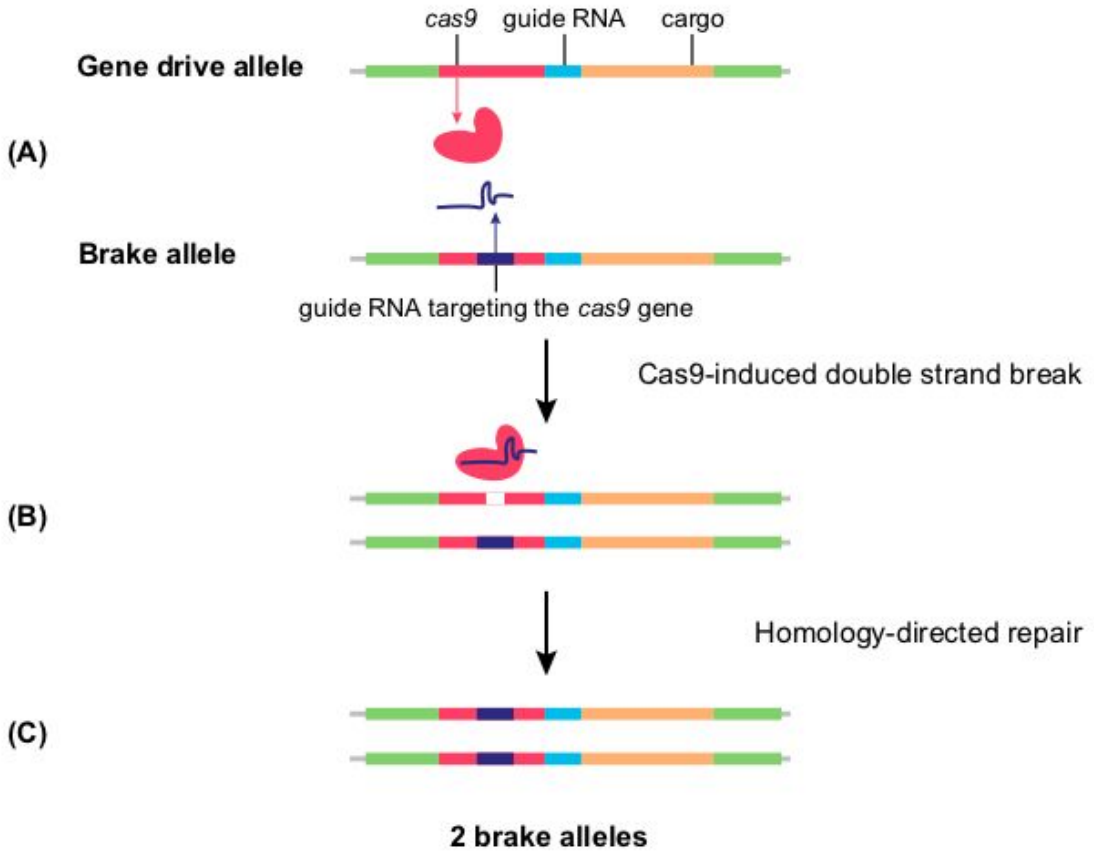


Fig. 5 Transformation of a chromosome carrying a CRISPR-cas9 cassette into a chromosome carrying a reversal brake. (A) The reversal brake includes a gRNA recognizing a sequence of the *cas9* gene on the gene drive cassette. (B) The Cas9 endonuclease produced by the gene drive allele can bind to the gRNA produced by the brake allele to recognize and cleave the *cas9* gene. (C) The double-strand break is repaired by homology-directed repair using the brake chromosome as a template, resulting in the conversion of the gene drive allele into a brake allele.

Biosafety and gene drive research

The unintentional release of gene drive individuals in the environment represents an important risk for biosafety. Best practice guidelines have been proposed by various groups of experts (NASEM 2016; Krishnan and Gillum 2017; van der Vlugt et al. 2018). Gene drive strains should be managed using an appropriate combination of confinement strategies to mitigate these risks (Akbari et al. 2015):

- ecological confinement, by conducting gene drive research in countries where the target species is not present and cannot establish in the wild;
- physical containment, by using physical barriers (e.g. nets, secured lab facilities, etc.) or animal anesthesia;
- reproductive confinement, by using lab strains that cannot reproduce with wild individuals (e.g. *Drosophila* strain with chromosomal rearrangements; Akbari et al. 2015);
- molecular confinement, by using split gene drives with *cas9* gene and gRNA on different chromosomes, or gene drive targeting an artificial sequence (DiCarlo et al. 2015);
- molecular identification, by tagging gene drive strains with specific phenotypic markers with dominant expression (Benedict et al. 2018).

There are currently no guidelines for the transport of gene drive strains, and some researchers have suggested that they should not be distributed to other laboratories (Akbari et al. 2015). For split gene drives, strains carrying the gRNA could be kept and distributed separately from the strains carrying the *cas9* gene. The safety of gene drive research projects should be assessed by independent experts (e.g. institutional biosafety committees; Benedict et al. 2018). Funding agencies should ensure that appropriate guidelines are followed and enforced when necessary. Finally, we believe that a broad national and international consensus is required before carrying on deliberate release in controlled field trials, provided the high risks of propagation to wild populations.

Ethical and regulatory issues

Besides identifying possible risks, regulating gene drives requires ethical principles considering both human social values and non-human environmental values (NASEM 2016). Scientists should be socially responsible for informing lawmakers and engaging with the "various publics that will use, be affected by, take an interest in, benefit from or be at risk from gene drives" (Thompson 2018). Such engagement is key so that stakeholders and the public can make informed decisions, considering both the benefits and risks associated with gene drives as well as potential alternatives to the genetic engineering of wild populations.

Given the high risks of propagation of gene drive individuals across borders, there is a pressing need to build a strong international regulatory framework. As genetically modified organisms (hereafter GMOs) containing foreign pieces of DNA, gene drives are subject to GMO national and international regulation and their provisions. At the international level, GMOs are regulated under the 2003 Cartagena Protocol on Biosafety, a supplement to the Convention on Biodiversity (ratified by 167 nations with the exception of the United States of America and Canada; CBD 2003) and under two directives of the European Union on GMO legislation. National agencies have also issued more specific recommendations for the safe use of gene drives (e.g., Germany, ZKBS 2016; USA, NASEM 2016; Australia, AAS 2017; France, HCB 2017; the Netherlands, RIVM 2018). However, as the technology is evolving rapidly, some of the international and national GMO regulatory frameworks need to be adapted to the specificities of gene drive organisms (GDOs; Oye et al. 2014; van der Vlugt et al. 2018). In 2016, 160 civil society organizations called for a global moratorium on the development and release of the gene drive technology (ETC Group 2016). The IUCN general assembly has also adopted a petition on the implications of gene drives and related techniques on biological diversity (IUCN 2016).

In particular, GDOs can be seen as an efficient technology for population control but also as potential bioweapons (Gurwitz 2014). The recent $100-million program including gene drive research projects ("Safe Genes program") funded by the American Defense Advanced Research Programs Agency might contribute to these concerns (Reeves et al. 2018). The debate about a potential use of gene drive technology requires the transparency of gene drive research programs (including their funding sources and an appropriate risk assessment) and a broad engagement of evolutionary biologists with the public (Oye et al. 2014; Meghani and Kuzma 2018; Kofler et al. 2018) .

Conclusions

Potential gene drive applications in conservation include the extirpation of invasive pest populations that threaten biodiversity and the introduction of beneficial mutations in endangered populations. We highlight the peculiarities associated with rescue drives compared to suppression and eradication drives. Rescue drives are likely to have different dynamics (e.g. no risk of resistance evolution, but no known countermeasure to recover the wild-type population). Overall, evolutionary and conservation geneticists can help better assess environmental risks associated with gene drives using both experimental (primarily in the lab) and theoretical approaches. Conservation geneticists could identify candidate genes for gene drives, estimate gene flow between target and non-target populations/species using population genomics approaches, and develop custom theoretical models for different drive scenarios. Finally, conservation ecologists could help design appropriate gene drive management policies by quantifying interaction networks, such as food web diversity, structure and functioning. We believe that it is essential that conservation geneticists develop an expertise on gene drive technologies to engage in the current debate regarding their potential applications. This engagement should help stakeholders, policymakers and the public make informed decisions regarding the use and regulation of gene drives.

Compliance with ethical standards:

Conflicts of interest: The authors declare that they have no conflict of interest.

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Supplementary Information

What are the benefits and risks of gene drives for population management and conservation biology?
Rode et al.

This document details the model behind Fig. 4; a similar model was already analysed in Deredec et al. (2008, Genetics).

Model definition and analysis

Model definition

Hypotheses:
- Well-mixed population of large size
- Hermaphroditic individuals
- Gene conversion takes place in the gonads

Fitness effects:
wDD 1-s
wD0 1-h s
w00 1

s is the selection coefficient,
h is the dominance coefficient;
c is the probability of successful gene conversion

We denote by *p* the frequency of the drive allele among the gametes.

At the next generation, this frequency becomes

In[1]:=
$$pp = \frac{(1-s)\,p^2 + p\,(1-p)\,(1-s\,h)\,(1+c)}{1 - s\,p^2 - 2\,p\,(1-p)\,s\,h};$$

The first term of the numerator corresponds to the amount of drive gametes produces by drive homozygotes: the frequency of drive-homozygous zygotes is p^2 (random mating of gametes), and their fitness is (1-s).

The second term of the numerator corresponds to heterozygotes; the amount of drive gametes produced depends on the conversion probability (1/2 if no conversion, 1 if conversion):
(1-c)*1/2 + c*1 = 1/2*(1+c),
which is multiplied by 2*p(1-p) (frequency of heterozygous zygotes) and (1-s h) (fitness of heterozygotes).
Finally, the denominator is the mean fitness in the population.

Equilibria

Let's find the equilibrium value of *p*

In[2]:=
```
sol = Solve[pp == p, p] // FullSimplify
```

Out[2]= $\left\{\{p \to 0\}, \{p \to 1\}, \left\{p \to \frac{c - (1+c)\, h\, s}{s - 2\, h\, s}\right\}\right\}$

There are three possible equilibria:
p=0 (drive extinction),
p=1 (drive fixation),
and p=pmid=$\frac{c-(1+c)\,h\,s}{s-2\,h\,s}$. This last solution is admissible if 0<pmid<1.

Let's define the derivative of *pp* with respect to *p*

In[3]:=
```
Der = D[pp, p] // FullSimplify
```

Out[3]= $\left(1 - h\, s + p\left(-2 + p + h\left(2 + p\left(-2 + s\right)\right)\right) s - c\left(-1 + h\, s\right)\left(1 + p\left(-2 + p\, s\right)\right)\right) /$
$\left(1 + \left(2\, h\, (-1 + p) - p\right) p\, s\right)^2$

Let's define conditions on the different parameters :
1) for negative effects of the drive (s>0, eradication or suppression drive),
2) for positive effects of the drive (s<0, rescue drive).

In[4]:=
```
AF1[x_] := Assuming[c > 0 && c ≤ 1 && s > 0 && s < 1 && h ≥ 0 && h ≤ 1, FullSimplify[x]]
AF2[x_] := Assuming[c > 0 && c ≤ 1 && s < 0 && s < 1 && h ≥ 0 && h ≤ 1, FullSimplify[x]]
```

Let's now investigate the stability of each of the equilibria
(an equilibrium is stable when *Der* evaluated at the equilibrium is lower than 1)

a) Extinction of the drive

In[6]:=
```
Der0 = Der /. p → 0 // FullSimplify
```

Out[6]= $1 + c - (1 + c)\, h\, s$

- Drive with negative effects

In[7]:=
```
ext1 = AF1[Reduce[Der0 < 1, s]]
```

Out[7]= $s > \frac{c}{h + c\, h}$

- Drive with positive effects

In[8]:=
```
AF2[Reduce[Der0 < 1, s]]
```
Out[8]=
```
False
```

This means that drive extinction never happens for a drive with positive effects (in this deterministic model)

b) Fixation of the drive

In[9]:=
```
Der1 = Der /. p → 1 // FullSimplify
```
Out[9]=
$$-\frac{(-1+c)\,(-1+h\,s)}{-1+s}$$

- Drive with negative effects

In[10]:=
```
fix1 = AF1[Reduce[Der1 < 1, s]]
```
Out[10]=
$$(h == 0 \&\& s < c) \;||\; (0 < h < 1 \&\& s + c\,h\,s < c + h\,s) \;||\; h == 1$$

In[11]:=
```
Reduce[s + c h s < c + h s, s] // AF1
```
Out[11]=
$$(h == 0 \&\& s < c) \;||\; (s + c\,h\,s < c + h\,s \&\& h > 0)$$

- Drive with positive effects

In[12]:=
```
AF2[Reduce[Der1 < 1, s]]
```
Out[12]=
```
True
```

This means that drive fixation always happens for a drive with positive effects (in this deterministic model)

c) Intermediate equilibrium (coexistence)

- First, we need to find the conditions for the existence of this intermediate equilibrium

-- Drive with negative effects

In[13]:=
```
midexist1 = AF1[Reduce[0 < (p /. sol[[3]]) < 1, s]]
```
Out[13]=
$$(h == 0 \&\& s > c) \;||\; \frac{c}{1+(-1+c)\,h} < s < \frac{c}{h+c\,h} \;||\; \left(h < 1 \&\& \frac{c}{h+c\,h} < s < \frac{c}{1+(-1+c)\,h}\right) \;||\; (h == 1 \&\& c < s + c\,s)$$

-- Drive with positive effects

In[14]:=
```
midexist2 = AF2[Reduce[0 < (p /. sol[[3]]) < 1, s]]
```
Out[14]=
```
False
```

The intermediate equilibrium does not exist when the drive has positive effects.

- Then we identify conditions under which this intermediate equilibrium is stable (for the drive with negative effects only, since there is no admissible solution otherwise)

In[15]:=
```
Dermid = Der /. sol[[3]] // FullSimplify
```

Out[15]=
$$\frac{s\left(-1+c+2h-(1+c)hs\right)}{c^2\left(-1+hs\right)^2-s\left(1+h\left(-2+hs\right)\right)}$$

In[16]:=
```
midstab1 = AF1[Reduce[Dermid < 1 && midexist1]]
```

Out[16]=
$$\left(h == 0 \&\& s > c\right) \mid\mid \frac{c}{1+(-1+c)h} < s < \frac{c}{h+ch}$$

In[17]:=
```
midinstab1 = AF1[Reduce[Dermid > 1 && midexist1]]
```

Out[17]=
$$\left(h < 1 \&\& \frac{c}{h+ch} < s < \frac{c}{1+(-1+c)h}\right) \mid\mid \left(h == 1 \&\& c < s+cs\right)$$

Conclusions

* Drive with *positive* effects: fixation always stable

* Drive with *negative* effects:

- fixation if $s < \frac{c}{1-(1-c)h}$ || (h==0&&s<c)
- coexistence if $\left(h == 0 \&\& s > c\right) \mid\mid \frac{c}{1+(-1+c)h} < s < \frac{c}{h+ch}$
- bistability if $\left(h < 1 \&\& \frac{c}{h+ch} < s < \frac{c}{1+(-1+c)h}\right) \mid\mid \left(h == 1 \&\& c < s+cs\right)$
- extinction if $s > \frac{c}{h+ch}$

Plotting

Parameters

Colors

In[55]:=
```
colW = Hue[0.4, 1., 0.6];
colD = Hue[0.1, 1., 0.8];
colmid = Hue[0.55, 1, 0.5];
colbi = Hue[0., 1., 0.8];
```

In[59]:=
```
midsat = 0.2;
colW2 = Hue[0.4, midsat, 0.6];
colD2 = Hue[0.1, midsat, 0.8];
colmid2 = Hue[0.55, midsat, 0.5];
colbi2 = Hue[0., midsat, 0.8];
```

Font size of the labels on the plot

In[64]:=
```
thesize = FontSize → 14;
```

Other parameters

In[65]:=
```
c = 0.85; (* Conversion probability *)
```

Plot the different equilibria

Function to homogenize the style of the plots

In[66]:=
```
PlotReg[conds_, color_] := RegionPlot[conds, {s, 0., 1},
  {h, 0, 1}, PlotStyle → color, BoundaryStyle → {Black, Thin},
  AxesOrigin → {0, 0}, PlotRangePadding → 0, PlotPoints → 100]
```

Plotting each region of stability

In[67]:=
```
Pmidstab = PlotReg[midstab1, colmid2];
Pmidinstab = PlotReg[midinstab1, colbi2];
Pfix = PlotReg[fix1, colD2];
Pext = PlotReg[ext1, colW2];
```

Assemble and label the plots

In[94]:=
```
PZones = Show[Pmidstab, Pext, Pfix, Pmidinstab,
   Graphics[Text[Style["Drive fixation", thesize], {0.5, 0.35}]],
   Graphics[Text[Style["Coex.", thesize], {0.9, 0.2}]],
   Graphics[Text[Style["WT", thesize], {0.94, 0.6}]],
   Graphics[Text[Style["WT or Drive", thesize], {0.75, 0.85}]],
   FrameLabel → {"Selection coefficient s", "Dominance coefficient h"},
   LabelStyle → Directive[Larger, Black], AspectRatio → 1, ImageSize → 250];
```

Plot the dynamics as examples

In[72]:=
```
PlotDyn[s_, h_, c_, p0_, style_] := Module[{ppt}, (*
  *)nt = 51;
  ppt = Table[0, {i, 1, nt}];
  ppt[[1]] = p0;
  Do[ppt[[i + 1]] = ((1 - s) ppt[[i]]^2 + ppt[[i]] (1 - ppt[[i]]) (1 - s h) (1 + c))/
      (1 - s ppt[[i]]^2 - 2 ppt[[i]] (1 - ppt[[i]]) s h), {i, 1, nt - 1}];
  ListPlot[Table[{i - 1, ppt[[i]]}, {i, 1, nt}], Joined → True,
   PlotStyle → {style, Thickness[0.0075]}, PlotRange → {{0, nt - 1}, {0, 1.005}}]
 ]
```

In[73]:=
```
p0a = 0.1;
P1 = PlotDyn[0.7, 0.45, c, p0a, colD];
P2 = PlotDyn[0.95, 0.15, c, p0a, colmid];
P3 = PlotDyn[0.95, 0.55, c, p0a, colW];
P4 = PlotDyn[0.7, 0.8, c, p0a, colbi];

p0b = 0.6;
P1b = PlotDyn[0.7, 0.45, c, p0b, colD];
P2b = PlotDyn[0.95, 0.15, c, p0b, colmid];
P3b = PlotDyn[0.95, 0.55, c, p0b, colW];
P4b = PlotDyn[0.7, 0.8, c, p0b, colbi];
```

In[83]:=
```
Pdyn = Show[P1, P2, P3, P4, Frame → {True, True, False, False},
    FrameLabel → {"Time (generations)", "Drive frequency"},
    LabelStyle → Directive[Larger, Black], AxesOrigin → {0, 0}, PlotRangePadding → 0];
```

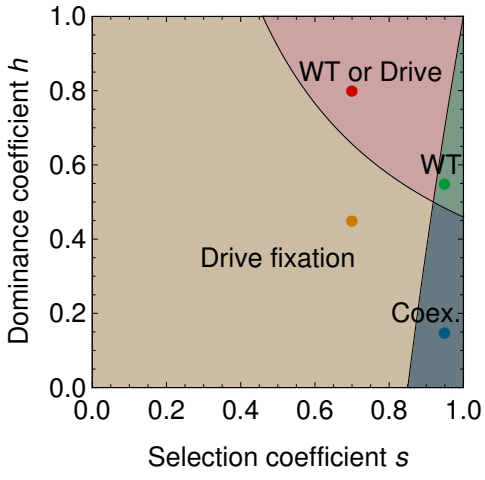
In[84]:=
```
Pdynb = Show[P1b, P2b, P3b, P4b, Frame → {True, True, False, False},
    FrameLabel → {"Time (generations)", "Drive frequency"},
    LabelStyle → Directive[Larger, Black], AxesOrigin → {0, 0}, PlotRangePadding → 0];
```

Assemble the plots

In[85]:=
```
ims = 250;
```

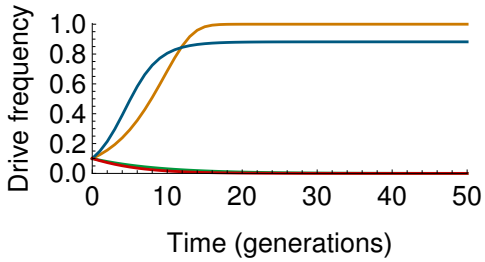
In[95]:=
```
Pzones2 =
 Show[PZones, ListPlot[{{0.7, 0.45}}, PlotStyle → colD, PlotMarkers → Automatic],
  ListPlot[{{0.95, 0.15}}, PlotStyle → colmid, PlotMarkers → Automatic],
  ListPlot[{{0.95, 0.55}}, PlotStyle → colW, PlotMarkers → Automatic],
  ListPlot[{{0.7, 0.8}}, PlotStyle → colbi, PlotMarkers → Automatic],
  ImageSize → ims, AspectRatio → 1]
```

Out[95]=

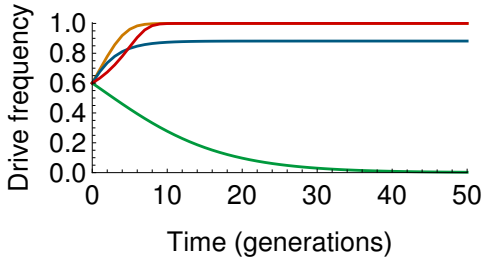


In[87]:=
```
Pdyn2 = Show[Pdyn, ImageSize → ims, AspectRatio → 0.4]
Pdyn2b = Show[Pdynb, ImageSize → ims, AspectRatio → 0.4]
```

Out[87]=



Out[88]=



Export the figures
(the pdfs are probably saved in your home directory)

In[89]:=
```
Export["Fig4A.pdf", Pzones2];
Export["Fig4B.pdf", Pdyn2];
Export["Fig4C.pdf", Pdyn2b];
```