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Gene drives in plants: opportunities and challenges for weed control and engineered resilience

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Plant species, populations and communities are under threat from climate change, invasive pathogens, weeds and habitat fragmentation. Despite considerable research effort invested in genome engineering for crop improvement, the development of genetic tools for the management of wild plant populations has rarely been given detailed consideration. Gene drive systems that allow direct genetic management of plant populations via the spread of fitness-altering genetic modifications could be of great utility. However, despite the rapid development of synthetic tools and their enormous promise, little explicit consideration has been given to their application in plants and, to date, they remain untested. This article considers the potential utility of gene drives for the management of wild plant populations, and examines the factors that might influence the design, spread and efficacy of synthetic drives. To gain insight into optimal ways to design and deploy synthetic drive systems, we investigate the diversity of mechanisms underlying natural gene drives and their dynamics within plant populations and species. We also review potential approaches for engineering gene drives and discuss their potential application to plant genomes. We highlight the importance of considering the impact of plant life-history and genetic architecture on the dynamics of drive, investigate the potential for different types of resistance evolution, and touch on the ethical, regulatory and social challenges ahead.

1. Introduction

Plants play key roles as primary producers and underpin the diversity and functioning of terrestrial ecosystems. However, many natural and agricultural ecosystem systems face critical challenges associated with invasive species, climate change and the evolution of herbicide resistance. Consequently, the development of tools to help manage wild plant populations is urgently needed. Recent conceptual and technological developments relating to the engineering of gene drives place us at the forefront of an era in which the engineering of specific traits of economic or conservation interest into wild plant populations has become feasible [1–3].

While genome engineering has been widely embraced as a tool for the genetic improvement of plant species of economic or cultural value, synthetic tools for the genetic management of wild populations have received relatively little attention [4–6]. Gene drives are selfish genes that are able to distort segregation ratios during meiosis or gamete development [7]. They are thus able to spread through populations, even when they impose a fitness cost on their host [8], and in principle may be engineered to deliver desirable genetic changes in wild populations. The applied potential of gene drives has been long recognized, but the molecular tools required to engineer them for specific species and purposes remained unavailable. Now, new conceptual insights [2,5], the development of new endonuclease-based (e.g. CRISPR) genome engineering technologies

[3,9,10] and proof of concept experiments in animals [11] and fungi [12] have led to a surge in interest for application to plants [1,6]. However, little explicit consideration has been given to the context in which gene drives might be used to manipulate plant populations, how they function at the molecular–genetic level, nor to the eco-evolutionary processes that will influence their spread and impact. For drive to occur, there must be synergy between processes taking place at molecular, cellular, organismal and population scales. This point is of particular importance when assessing the utility of gene drive as tool for the management of wild plant populations, given plants encompass a diverse range of life histories, DNA repair mechanisms and cytogenetic arrangements.

2. Why (not) consider genetic intervention in wild plant populations?

(a) Weed control

Weed control is a dynamic and complex problem involving interacting natural–human systems, and simple solutions for the control of invasive and agricultural weeds alike are increasingly difficult to identify. One rapidly emerging and increasingly urgent issue is associated with the widespread evolution of herbicide resistance [13]. A second urgent issue involves invasive alien weeds, which can be difficult to control because they often invade sensitive, rugged and remote areas, where conventional management strategies may be difficult, impossible or too expensive to implement [14]. In principle, gene drives can be used to control weeds in two fundamentally different ways: (i) population suppression; or (ii) population sensitization [6]. In the first case, by directly targeting key traits (e.g. fecundity, establishment, persistence), suppression drives have great potential to enable low-input control of invasive alien weeds in natural and agricultural environments [1]. In the second case, sensitizing drives could be used to render a target population susceptible to some form of specific management intervention. For example, for weeds that have evolved resistance to herbicides [15], herbicide susceptibility could be restored by using an endonuclease-based drive to disrupt resistance alleles at herbicide target sites and substitute wild-type susceptibility alleles, re-enabling the efficacy of old chemistries for control of key species [6].

(b) Engineering resilience

Gene drives also have potential to be adopted as tools for the conservation genetics of endangered or threatened plant populations. Genetic management of small, declining populations (often termed 'genetic rescue') is typically implemented by transplanting small numbers of genetically discrete immigrant individuals into small populations. The general aim is to introgress beneficial genetic variation into threatened populations from a small number of immigrants 'better adapted' to prevailing conditions. Ideally, adaptive potential is enhanced, but locally adaptive genetic variation is not swamped [16,17]. For example, rapidly driving new, adaptive variation into populations could increase population fitness by enhancing resilience to environmental change and invasive parasites [18]. Predicted advantages over conventional, transplant-based approaches [16] would be the increased rate of spread of beneficial genes, and the capacity to 'future-proof' populations by driving adaptive traits prior to the arrival of the selective agent/threat.

One major challenge for using gene drive for such purposes lies in the identification of genes underlying adaptive traits. Thus, clearly defined traits, controlled by genes of major effect, are likely to represent good targets for initial attempts at genetic engineering. For example, pathogen resistance traits are relatively easily phenotyped, in addition to being relatively well understood at genetic and molecular levels. Furthermore, many plant species and communities are threatened by invasive pathogens. In Australia, the recent invasion of the fungal pathogen *Puccinia psidii* threatens many common and widespread species in the family Myrtaceae (including *Eucalyptus* spp.) [19]. Resistance to *P. psidii* has been identified [20], and work aiming to identify the genetic basis of resistance is under way [19]. The cloning of genes that confer resistance to *P. psidii* will open the possibility of using gene drive to engineer resilience to this pathogen into susceptible populations and species.

(c) Polyploid genetic modification

Synthetic gene drives may also be designed to operate at much smaller, genomic scales. For example, due to genome duplication and the presence of multiple homologous and homeologous alleles, genetic modification of polyploid species is often extremely challenging. While gene editing using CRISPR has been reported in several polyploid plant species (e.g. potato, oilseed rape, strawberries and wheat), outcomes to date are highly variable. These range from reports of only one edited allele [21,22], through various combinations of edited homeologous alleles [21–23], to comprehensive editing of all alleles in a genome [21–24]. This is problematic because gene edits across all alleles may be required to engineer a modified trait of interest in polyploid species. Endonuclease-based gene drives (as opposed to gene editing) may therefore be a valuable tool for working with polyploid species if they can be designed to drive edits across all homeologous alleles and gene copies within a genome.

(d) Risks of gene drive

Much of the scientific discussion has focused on the technical and biological aspects of enabling effective gene drives. However, it is important to note that the release of a synthetic gene drive could have downsides [1,25], and that there are serious ethical issues and potentially unforeseeable ecological consequences associated with the genetic alteration of populations of wild organisms [26]. Plants are not exempt from these concerns. For example, within the context of this review, many invasive weeds remain key components of the flora and play important roles in ecosystems in their native range (e.g. Australian *Acacia* spp. [27]). Likewise, in agriculture, many weeds of crops are also important pasture species, sometimes even on the same farm (e.g. *Lolium* spp.). Such context dependence raises ethical, regulatory, management and ecological concerns, because suppression drives could, in theory, lead to unwanted declines and species extinction if they were left to spread unchecked [28]. Such concerns are exacerbated by the potential spread of the drive to non-target species via hybridization [29].

Different drive designs offer different levels of risk of unintended spread to non-target populations [30], and several strategies have been proposed for limiting the spatial and temporal spread of gene drives (e.g. [31,32]). While such technical

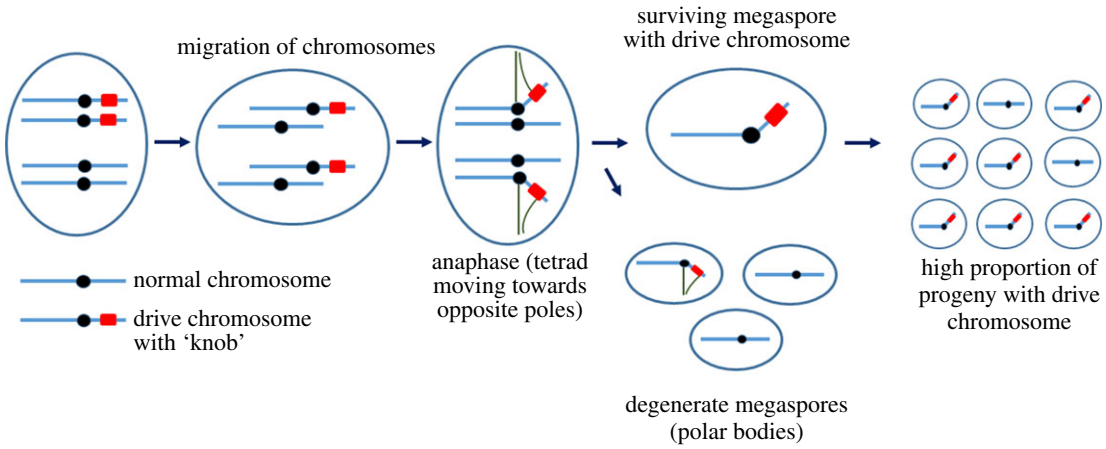

Figure 1. Example of mechanisms facilitating pre-gametic drive during female meiosis, modelled on Maize Ab10 [36]. During meiosis, the drive element (shown in red) can induce non-random chromosome segregation to the cell that ultimately develops into the female gamete. Non-drive alleles are relegated to the polar bodies (along with one of the drive alleles), and segregation ratios in gametes are strongly skewed towards the drive allele. (Online version in colour.)

advances will be important for engineering gene drives that can potentially be released into the wild, comprehensive ecological and socio-economic investigations will ultimately be required to rigorously assess risks in an integrated context [33]. Researchers, managers and regulators must carefully weigh the risks of a release with the potential benefits, and clearly and transparently communicate scientific outcomes with the broader community [25].

3. What can we learn from nature?

Much of the current focus on synthetic gene drives has been on the design and engineering of systems based on Cas9 nucleases, or synthetic toxin–antidote systems (e.g. [1,6,18]). However, there are many types of naturally occurring selfish genetic elements with capacity to distort segregation ratios described in plants [8,34]. Understanding the mechanisms that facilitate the drive of naturally occurring selfish genetic elements, and the factors that influence drive frequency within populations can potentially offer insight into optimal ways to design and use synthetic drive systems.

One general way to mechanistically classify different types of drive elements [34] is to distinguish whether they distort transmission ratios before or after the formation of gametes.

(a) Pre-gametic drives

Pre-gametic (or meiotic) drives distort transmission ratios during meiosis, so that gametes carrying the drive allele have a higher probability of being produced [8]. In plants, the characterized meiotic drive systems have evolved mechanisms that bias chromosome segregation to the cell that ultimately develops into the female gamete (i.e. 'female meiotic drive' [8]). This is possible because for most species of flowering plants, only one product of female meiosis forms a viable megaspore, which will develop into the female gametophyte containing a fertile egg cell. The other three undergo programmed cell death [35] (figure 1). This genetic asymmetry in meiosis facilitates the evolution of selfish genetic elements, because it rewards competition among homologous chromosomes for presence in the single surviving egg cell (figure 1).

One of the best-characterized meiotic drive systems in plants is the abnormal 10 (Ab10) meiotic drive system in maize (*Zea mays*) [36]. Ab10 is a selfish form of the 'normal' chromosome 10 (N10). Relative to N10, Ab10 has large blocks of repetitive DNA called knobs on the long chromosome arm. While the mechanisms that facilitate biased transmission are multipartite [37], in essence, the knob structures bias transmission by enabling Ab10 to actively migrate along meiotic spindles to the fertile egg cell ahead of N10 chromosomes. This results in strong preferential segregation, such that Ab10 is transmitted to 60–80% of progeny [38]. A second well-characterized example of female meiotic drive in plants is the monkey flower (*Mimulus guttatus*) centromere-associated distorter (D) locus [39,40]. Initially identified via observations of non-Mendelian inheritance in hybrids of *M. guttatus* and the closely related *M. nasutus*, the D locus is similar to Ab10, in that it promotes preferential transmission of chromosomes carrying the drive element to the egg cell during female meiosis. This results in a strong transmission bias, with 98% of progeny resulting from heterospecific crosses carrying D, and 58% in conspecific crosses (with the difference likely to be due to suppressor elements in *M. guttatus*) [41].

Perhaps the most well-characterized class of selfish genetic elements in plants are B chromosomes. These are supernumerary chromosomes that accumulate in a non-Mendelian fashion. They typically have deleterious effects on their hosts and have been identified in more than 1000 species of flowering plants [7,42]. The drive of B chromosomes is made possible by a variety of irregular mitotic and meiotic mechanisms that allow them to distort transmission ratios and accumulate selfishly in the germline (for comprehensive reviews see [42,43]). However, like the meiotic drives described above, B chromosomes in plants often achieve drive by manipulating genetic asymmetries during gametogenesis (typically pollen mitosis).

In the plant systems for which data are available, pre-meiotic drives are typically maintained at intermediate frequencies. Fitness costs associated with carrying the drive element might help explain the maintenance of polymorphisms and why drives do not spread to fixation, despite their transmission advantage. For example, in theory, the maize Ab10 system can achieve an 83% transmission rate in heterozygotes, and empirical estimates of transmission range between 60 and 80% [38]. However, in wild teosinte populations, Ab10 is present only at low–intermediate frequencies (sampled in 75% of populations, at a mean frequency of 15%) [44], probably due to reductions in pollen viability, seed viability and seed size in Ab10 homozygotes [38]. Similarly, the D locus in *Mimulus* can

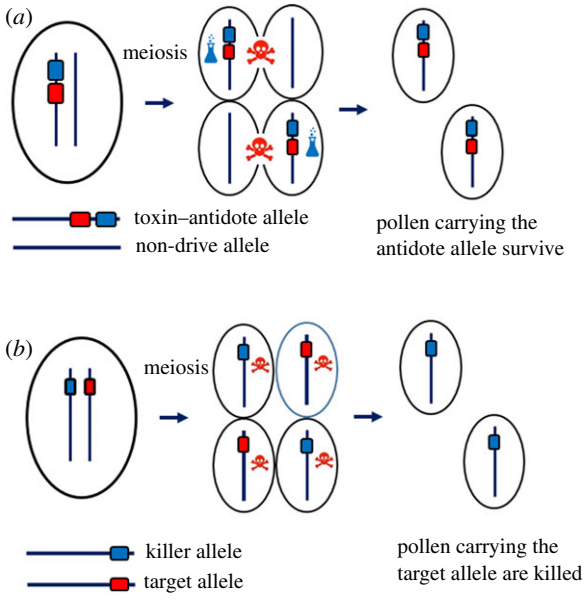

Figure 2. Example of mechanisms facilitating post-gametic drive (i.e. gamete killers). In (*a*), the drive acts by producing a *trans*-acting toxin and a *cis*-acting antidote. Non-drive alleles are killed by the toxin, whereas drive alleles are 'rescued' by the antidote. In (*b*), the drive acts via direct interference with gametes carrying non-self alleles, for example, by producing a molecule that is only harmful to gametes carrying the non-drive allele. (Online version in colour.)

attain nearly 100% transmission rate in interspecific crossing experiments, but when present in natural populations segregates at frequencies ranging between 30 and 40% [41], reflecting significant (20%) pollen fertility costs [39] and a reduced (21%) seed [41].

The population dynamics of B chromosomes likewise may reflect the opposing effects of segregation distortion and deleterious effects on the host. At low numbers, B chromosomes seem to have limited deleterious effects, but as they accumulate plants generally experience reduced growth and fertility [42]. Such additive deleterious effects presumably contribute towards limiting B accumulation and stabilize frequencies within populations [45]. While the precise mechanisms underlying fitness costs in plants remain to be determined, from a physiological perspective, negative effects may simply reflect (at least in part) costs associated with replicating and maintaining non-essential DNA associated with B chromosome accumulation [46].

(b) Post-gametic drives

Post-gametic drives accomplish segregation distortion via mechanisms that render gametes inviable after meiosis has taken place. In order to achieve this, post-gametic drives must be able to perform two general functions. First, the driving allele must have the ability to distinguish competing meiotic products (i.e. distinguish self from non-self). Second, the driving allele must be able to render the competing meiotic product inviable [34] (figure 2).

In plants, most post-gametic drives have been discovered via observations of hybrid sterility, following crosses between crop species and wild relatives. Subsequent genetic dissection of sterility traits has in many cases revealed loci that bias allele transmission by acting as 'gamete killers' [47]. While the different post-gametic drives described so far encompass a wide range of molecular mechanisms, many segregate as a single locus containing multiple tightly linked genes, which either enable direct interference with gametes carrying non-self-alleles, or enable production of both a toxin and an antidote [48]. For example, the qHMS7 locus found in *Oryza sativa* drives via a poison–antidote type system that acts on male gametes [49]. In *sativa*/*meridionalis* backcrossed hybrids, pollen that inherits the *meridionalis* qHMS7 haplotype is rendered infertile. The *sativa* qHMS7 locus contains two tightly linked genes; ORF2 encodes a pollen-killing toxin, whereas ORF3 encodes an antidote to the ORF2 toxin. By contrast, the *meridionalis* qHMS7 locus encodes a non-toxic form of ORF2 and lacks ORF3.

The population dynamics of gamete killers in plants are largely obscure (although more is known for some animal systems [8]). In all described cases, post-gametic drive loci have been fixed within a species, with the phenotype only observable via interspecific crosses. One interpretation of this pattern is that following their emergence, these loci (at least the successful ones) are highly efficient at driving through populations. The strong transmission advantage conferred by the killing competing gametes means that, in theory, they may become fixed in populations relatively quickly. However, the transmission advantage is likely to be offset by costs; the most obvious of these should be reduced fertility, which has strong potential to influence the population dynamics of pollen-killing post-gametic drives [50]. How such limitations might be overcome within the context of synthetic gene drives requires further investigation, including investigating the role of selfish evolution in driving the emergence of these loci [48].

4. Engineering gene drives in plants

Demonstrations of a synthetic gene drive in plants are currently lacking, but design principles of synthetic gene drives from other kingdoms are undoubtedly a good starting point and some of these will be discussed briefly below.

(a) Nuclease-based drives

Although the idea of using selfish elements for the genetic control of biotic problems is not new [51,52], an important concept for designing a synthetic gene drive was proposed more recently [2], based upon homing endonucleases engineered to target an essential gene in which the homing endonuclease is inserted. The underlying principle is that when the endonuclease cuts its target sequence, the DNA break is repaired by homology directed repair (HDR) using the engineered homologous allele, in which the nuclease is embedded, as a template. Endonuclease drives essentially act pre-gametically, in that cutting and repair processes, and hence drive, take place during meiosis.

In recent years, a variety of options for engineering such nucleases have been explored including transcription activator-like effector nucleases (TALEN), zinc finger nucleases (ZFN) and Cas9-guide-RNA constructs [53]. TALEN and ZFN-based drives have been engineered in insects [54] and Cas9-guide-RNA-based nucleases in yeast, insects and mice [12,51,52,55]. A highly desirable property of these nucleases is their high specificity to the targeted sequence, which could allow the design of drives restricted to a subpopulation of the target species. However, the underlying requirement for this repair to occur via HDR may limit the generality of nuclease-based drives, as DNA repair mechanisms can vary

Box 1. The influence of seed banking on gene drive.

The impact of life-history traits on the spread and dynamics of gene drives is complex and interactive. In general, high rates of drive will be enabled by host species that are highly dispersed, allow high homing rates and where the drive has a positive impact (or limited cost) on fitness. Therefore, understanding how life-history factors influence these key parameters could facilitate the screening of diverse plant species to identify their potential as targets for genetic management. Theoretical modelling, based on population demographic and genetic dynamics, provides a means of predicting the general influence of life-history traits when a drive is introduced into populations. Here, we develop a simple model to illustrate how a single, potentially key life-history trait might influence the spread and maintenance of drive in a plant population. The storage of seeds in persistent seed banks is a common strategy in many plant species [64], and it can be predicted that seed banks will influence the spread and persistence of a gene drive either by acting as a reservoir of non-drive alleles or as a demographic buffer against population extinction.

We demonstrate the importance of a seed bank on the dynamics of a nuclease-based gene drive with a simple model (detailed in electronic supplementary material, text S1). This model tracks the dynamics of a single locus, diallelic gene drive (resistant alleles are ignored). The population represents an idealized annual species where the aboveground population is well mixed, strictly outcrossing and replaced on an annual basis by germinating seeds from the seed bank. We describe the dynamics of a perfect gene drive (i.e. with a homing and conversion rate of 1) carrying a recessive lethal cargo, therefore aiming for population suppression (figure 3). The results of the simulations show that a deeper seed bank (i.e. increasing average time to germination) has two negative impacts on this gene drive strategy. First, the spread of the drive allele in the population is delayed, a consequence of the existing reservoir of wild-type alleles in the seed bank. Second, the resulting fitness impact and population suppression effect is similarly delayed. In this example, for a species with a short-lived seed bank, a drive might be considered a viable solution for reducing a target population below 10% of its original levels in approximately 10 generations. However, for a second species with a deeper seed bank, the drive is predicted to take up to 50 generations to have a similar impact and would therefore likely be considered unsuitable as a control option.

More generally, with this model, we demonstrate that the existence of a seed bank, and its precise profile, will have significant impacts on the dynamics of all types of gene drive, and will therefore be a significant factor to determine the suitability of genetic control methods for species with significant seed banks. It should additionally be noted that this simple model considers seed banks in an otherwise idealized species. Many other traits, relating to life history, behaviour or mating patterns, are likely to prove just as impactful for gene drives. Eventually, a comprehensive modelling effort will be necessary to determine the feasibility of gene drive in an integrated context.

among species and cell type. This was highlighted in mice where HDR and drive was only observed in the female germline, despite cutting and repair by non-homologous end-joining (NHEJ) occurring in both sexes [55]. In most plants, an understanding of DNA repair processes that predominate during meiosis is lacking. For synthetic drives in plants, this knowledge will be crucial for predicting the utility of nuclease-based gene drives.

(b) Toxin–antidote systems

In species where HDR processes are not favourable to nuclease-based drives, other options may be possible. In *Drosophila* spp. [56,57], synthetic maternal effect dominant embryonic arrest (MEDEA) drives have been engineered based upon a maternal oogenesis-expressed micro-RNA (miRNA) toxin that silences a gene essential in embryo development. The developmental defect is rescued only in embryos carrying an early embryogenesis-expressed miRNA-insensitive version of the target gene. These two components are placed adjacent to each other in the genome and provide very efficient drive that could be linked to cargo genes. A large suite of genes essential for embryo development in plants are known and could form the basis of synthetic plant MEDEA drives [58]. Specificity to a target species or subpopulation may be possible via appropriate selection of the miRNA (or siRNA) target sequence(s).

Male gamete killer drives linked to a desirable cargo trait would also be a possible route to a reengineered plant gene drive. A number of natural elements which mediate interspecific hybrid sterility in rice have recently been cloned (reviewed in [48]) which could function in other species. However, using male gamete killers will not provide the sequence specificity afforded by the nuclease-based and MEDEA type drives.

5. The importance of plant life history and genetic architecture

Plants are an extremely heterogeneous group of organisms that show a remarkable diversity of life-history strategies and genetic architectures. Modes of reproduction, for example, can be highly variable, spanning a continuum from asexual through to completely sexual [59], including hermaphroditic plants ranging from primarily self-fertilized through to highly outcrossed [60]. Similarly, plants vary widely in seed and pollen dispersal modes (e.g. wind, animal, water), traits which are likely to have a strong impact on dispersal distances [61]. Generation times also vary widely; longevity can range from weeks through to millennia [62], while many plant life cycles include dormant stages (seedbanks) [61,63] (box 1). In addition, plants can vary extensively in terms of genetic architecture. Chromosome number can range from 2 to over 600, and ploidy from 1 to over 20 [65]. These and other sources of biological heterogeneity are likely to be important determinants of adaptive potential, demographic dynamics and population genetic structure, and hence have significant impacts on the genetic and demographic dynamics of gene drives [66].

While in most cases the effects of life-history on gene drive are little studied and their potential effects are generally not well understood, the effects of inbreeding have been subject to some investigation. Gene drives rely on sexual reproduction

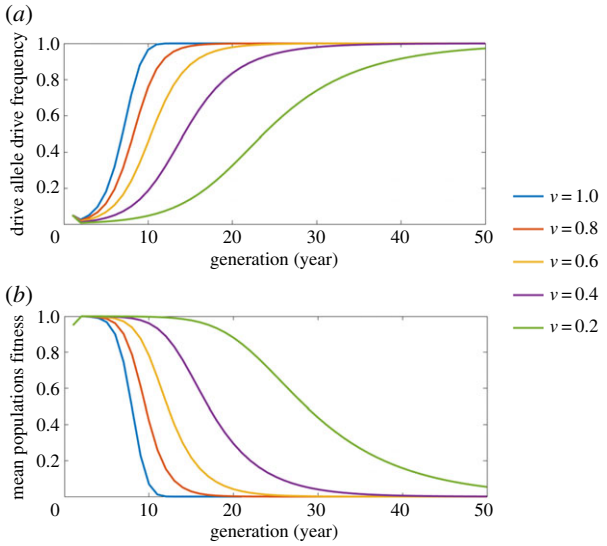

Figure 3. Impact of seed bank structure on the dynamics of a gene drive carrying a recessive lethal cargo. The model of gene drive dynamics in seed banks is presented in electronic supplementary material, text S1. This figure presents the frequency of the drive allele D in the population (*a*) and the reduction in the mean fitness of the population (*b*) following the introduction of the recessive lethal gene drive in the population at time 0 (initial frequency of 5%). ν represents the yearly seed germination rate (and consequently the average time spent in the seed bank is $1/\nu$). Genotype-specific fitness is set as $c_{DD} = 0$, $c_{Dd} = 1$, $c_{dd} = 1$. (Online version in colour.)

and the generation of heterozygotes for transmission. Hence, variation in any trait that leads to the development of population structure or increased rates of inbreeding is likely to be critical for predicting the population dynamics of drive [67]. Theoretical investigations confirm that population structure [67] and increasing rates of inbreeding are likely to inhibit the spread and frequency of gene drives within populations [67–69]. Importantly, even relatively low rates of self-fertilization from a plant perspective ($t = 0.15$) have potential to cause gene drives introduced into populations to go extinct [70]. Consistent with these predictions, Burt & Trivers [68] found that predominantly selfing (5.5%) and mixed-mating (6.8%) species hosted B chromosomes at much lower frequency than dioecious and self-incompatible species (29%).

6. Resistance evolution

Too often, society has shown boundless enthusiasm for new technologies without giving due consideration to the potential for unintended evolutionary responses from targeted organisms [13,71]. Gene drives will not be immune to the evolution of such resistance mechanisms, and it is important these be well understood so that resistance can be managed. In the case of synthetic gene drives, two main avenues of resistance evolution can be distinguished: resistance that slows or prevents drive and resistance against the genetic control mechanism itself.

The mechanisms of resistance to a gene drive, impeding its ability to be preferentially inherited, have been identified for a variety of gene drives [2,72,73]. In particular, for drives relying on site-specific nucleases, the mechanisms of emergence of resistant alleles and subsequent consequences on population dynamics have been well elucidated [28,74]. These dynamics are determined in large part by DNA repair pathways activated by the site-directed nuclease. Nuclease-based drives inherently rely on HDR pathways, as described above. Alternative repair mechanisms such as NHEJ not only prevent drive, but also generate novel alleles when the repair is erroneous [74]. Because of the sequence specificity of the nucleases, these alleles are resistant to drive. In instances where the drive allele is associated with a fitness cost, resistant alleles are expected to be positively selected, and therefore quickly impede the spread of the gene drive in a population.

Our current understanding of DNA repair pathways suggests that the engineering of nuclease-based drives may prove relatively difficult in plants. Details about DNA repair processes are scarce, in part because they are difficult to obtain and highly variable [75]. In plants, however, NHEJ appears to be the preferred repair pathway [76,77], seemingly an obstacle to the design of nuclease-based gene drives. However, as for other organisms, HDR seems to be more prevalent during meiosis [77], indicating that nuclease-based drives that are active in the germline at meiosis might be less affected by DNA repair errors. Ultimately, drive dynamics will depend on the error rate when the drive is expressed, which will almost certainly need to be empirically determined.

Life history, in addition to influencing the dynamics of the gene drive itself (see above), can also affect the evolution of resistance [67]. For example, just as a seed bank can impede the spread of a drive by providing a reservoir of wild-type alleles (box 1), it can in theory slow down the spread of resistant alleles even when they are under positive selection [64]. In polyploid species, the relative fitness of a given genotype (combining drive, wild-type and resistant alleles) will depend in a complex fashion on dominance issues and expression patterns of specific chromosomes [78]. Consequently, the dynamics of drive and resistance will be significantly harder to predict. Strategies to mitigate resistance issues have been proposed, such as guide-RNA multiplexing or targeting of essential genes [3,79]. Further theoretical and experimental work is needed to understand and predict the applicability of these approaches in plant populations.

Finally, resistance may also appear to the mechanism that the drive is carrying into the target population. In the case of a genetic cargo, this would typically consist of modifying, inactivating or losing the cargo altogether. Even an intact drive would then carry an inefficient genetic load into the population.

7. Concluding remarks and future perspectives

The development of innovative tools to help manage wild plant populations in both natural and agricultural systems is urgently needed. Gene drives offer one potential technical solution to some of the plant-associated problems in both natural and agricultural ecosystems. However, key questions remain, including the efficacy of engineered drives at the molecular–genetic level, the potential for resistance evolution, and how plant life history and genetic architecture may influence their spread and impact. In addition, for many potential target organisms, a lack of established genomic and molecular tools represents a significant challenge to feasibility.

The existence of diverse, naturally occurring gene drives in plant populations suggests that synthetic drive systems can probably be engineered for some plant species. However, demonstrations of a functional synthetic drive in a plant population are currently lacking. Key questions include the suitability of CRISPR and other endonuclease-based drives given potential

for NHEJ, and the precise cell types, organs or developmental stages in which to target expression [80]. In addition, it is important to understand if naturally evolved toxin–antidote systems can be repurposed for synthetic drive purposes, and the relative benefits of the alternative strategies.

For synthetic drives to work, there must be synergy between often complicated processes taking place at molecular, cellular, individual and population scales. Ultimately, however, gene drive transmission relies on population-level processes (e.g. mating, dispersal, plant population structure), and plant species show a remarkable diversity of life-history strategies, most of which influence population-level genetic and demographic processes. Furthermore, the effects of the drive on plant fitness may vary considerably. How such sources of variation might interact to differentially influence the spread of gene drives and the potential for the evolution of resistance is currently unknown. Considerable research effort into the autecology, population biology and fitness consequences of the drive will be required in order to determine the likely success of a gene drive programme for any given species.

Most of the scientific discussion, in this manuscript and elsewhere, has focused on the technical and biological aspects of making gene drives work in different organisms. However, the regulatory context will ultimately determine how this technology is able to be deployed in different jurisdictions and the extent to which societies (and markets) accept the use of gene drives [25]. Risk–cost–benefit (RCB) approaches will ultimately be vital to guide the development, regulation and deployment of gene drives in plants; discussions in this regard are already under way [81]. In order to guide such an RCB approach, models addressing the ecological and evolutionary context of gene drives are needed to enable planning for different scenarios. Such models, coupled with a clear and transparent communication of the science, will be key to securing the social licence and market acceptance to apply this important scientific tool on plants to achieve meaningful agricultural and environmental outcomes [82,83].

Data accessibility. The code used to run the simulation model and generate data is available at https://github.com/legrosmathieu/genedrive.seedbank.

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References

1. Webber BL, Raghu S, Edwards OR. 2015 Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? *Proc. Natl Acad. Sci. USA* **112**, 10 565–10 567. (doi:10.1073/pnas.1514258112)
2. Burt A. 2003 Site-specific selfish genes as tools for the control and genetic engineering of natural populations. *Proc. R. Soc. Lond. B* **270**, 921–928. (doi:10.1098/rspb.2002.2319)
3. Esvelt KM, Smidler AL, Catteruccia F, Church GM. 2014 Concerning RNA-guided gene drives for the alteration of wild populations. *eLife* **3**, e03401. (doi:10.7554/eLife.03401)
4. Hay BA, Chen C-H, Ward CM, Huang H, Su JT, Guo M. 2010 Engineering the genomes of wild insect populations: challenges, and opportunities provided by synthetic Medea selfish genetic elements. *J. Insect. Physiol.* **56**, 1402–1413. (doi:10.1016/j.jinsphys.2010.05.022)
5. Gould F. 2008 Broadening the application of evolutionarily based genetic pest management. *Evolution* **62**, 500–510. (doi:10.1111/j.1558-5646.2007.00298.x)
6. Neve P. 2018 Gene drive systems: do they have a place in agricultural weed management? *Pest Manag. Sci.* **74**, 2671–2679. (doi:10.1002/ps.5137)
7. Burt A, Trivers R. 2009 *Genes in conflict: the biology of selfish genetic elements*. Cambridge, MA: Harvard University Press.
8. Lindholm AK *et al.* 2016 The ecology and evolutionary dynamics of meiotic drive. *Trends Ecol. Evol.* **31**, 315–326. (doi:10.1016/j.tree.2016.02.001)
9. Akbari OS, Matzen KD, Marshall JM, Huang H, Ward CM, Hay BA. 2013 A synthetic gene drive system for local, reversible modification and suppression of insect populations. *Curr. Biol.* **23**, 671–677. (doi:10.1016/j.cub.2013.02.059)
10. Unckless RL, Messer PW, Connallon T, Clark AG. 2015 Modeling the manipulation of natural populations by the mutagenic chain reaction. *Genetics* **201**, 425–431. (doi:10.1534/genetics.115.177592)
11. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, Nolan T, Crisanti A. 2018 A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nat. Biotechnol.* **36**, 1062–1066. (doi:10.1038/nbt.4245)
12. Shapiro RS *et al.* 2018 A CRISPR–Cas9-based gene drive platform for genetic interaction analysis in *Candida albicans*. *Nat. Microbiol.* **3**, 73. (doi:10.1038/s41564-017-0043-0)
13. Gould F, Brown ZS, Kuzma J. 2018 Wicked evolution: can we address the sociobiological dilemma of pesticide resistance? *Science* **360**, 728–732. (doi:10.1126/science.aar3780)
14. Epanchin-Niell RS, Hufford MB, Aslan CE, Sexton JP, Port JD, Waring TM. 2010 Controlling invasive species in complex social landscapes. *Front. Ecol. Environ.* **8**, 210–216. (doi:10.1890/090029)
15. Kreiner JM, Stinchcombe JR, Wright SI. 2018 Population genomics of herbicide resistance: adaptation via evolutionary rescue. *Annu. Rev. Plant Biol.* **69**, 611–635. (doi:10.1146/annurev-arplant-042817-040038)
16. Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA. 2015 Genetic rescue to the rescue. *Trends Ecol. Evol.* **30**, 42–49. (doi:10.1016/j.tree.2014.10.009)
17. Tallmon DA, Luikart G, Waples RS. 2004 The alluring simplicity and complex reality of genetic rescue. *Trends Ecol. Evol.* **19**, 489–496. (doi:10.1016/j.tree.2004.07.003)
18. Novak BJ, Maloney T, Phelan R. 2018 Advancing a new toolkit for conservation: from science to policy. *CRISPR J.* **1**, 11–15. (doi:10.1089/crispr.2017.0019)
19. Butler JB, Freeman JS, Vaillancourt RE, Potts BM, Glen M, Lee DJ, Pegg GS. 2016 Evidence for different QTL underlying the immune and hypersensitive responses of *Eucalyptus globulus* to the rust pathogen *Puccinia psidii*. *Tree Genet. Genomes* **12**, 39. (doi:10.1007/s11295-016-0987-x)
20. Tobias PA, Guest DI, Külheim C, Hsieh J-F, Park RF. 2016 A curious case of resistance to a new encounter pathogen: myrtle rust in Australia: resistance to myrtle rust in Australia. *Mol. Plant Pathol.* **17**, 783–788. (doi:10.1111/mpp.12331)
21. Zhang Y, Liang Z, Zong Y, Wang Y, Liu J, Chen K, Qiu J-L, Gao C. 2016 Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nat. Commun.* **7**, 12617. (doi:10.1038/ncomms12617)
22. Andersson M, Turesson H, Nicolia A, Fält A-S, Samuelsson M, Hofvander P. 2017 Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR–Cas9 expression in protoplasts. *Plant Cell Rep.* **36**, 117–128. (doi:10.1007/s00299-016-2062-3)
23. Wilson FM, Harrison K, Armitage AD, Simkin AJ, Harrison RJ. 2019 CRISPR/Cas9-mediated mutagenesis of phytoene desaturase in diploid and octoploid strawberry. *Plant Methods* **15**, 45. (doi:10.1186/s13007-019-0428-6)

24. Braatz J, Harloff H-J, Mascher M, Stein N, Himmelbach A, Jung C. 2017 CRISPR-Cas9 targeted mutagenesis leads to simultaneous modification of different homoeologous gene copies in polyploid oilseed rape (*Brassica napus*). *Plant Physiol.* **174**, 935–942. (doi:10.1104/pp.17.00426)
25. Collins JP. 2018 Gene drives in our future: challenges of and opportunities for using a self-sustaining technology in pest and vector management. *BMC Proc.* **12**, 9. (doi:10.1186/s12919-018-0110-4)
26. Hochkirch A *et al*. 2018 License to kill? Disease eradication programs may not be in line with the convention on biological diversity. *Conserv. Lett.* **11**, e12370. (doi:10.1111/conl.12370)
27. Maitre DCL *et al*. 2011 Impacts of invasive Australian acacias: implications for management and restoration. *Divers. Distrib.* **17**, 1015–1029. (doi:10.1111/j.1472-4642.2011.00816.x)
28. Noble C, Adlam B, Church GM, Esvelt KM, Nowak MA. 2018 Current CRISPR gene drive systems are likely to be highly invasive in wild populations. *eLife* **7**, e33423. (doi:10.7554/eLife.33423)
29. Whitney KD, Ahern JR, Campbell LG, Albert LP, King MS. 2010 Patterns of hybridization in plants. *Perspect. Plant Ecol. Evol. Syst.* **12**, 175–182. (doi:10.1016/j.ppees.2010.02.002)
30. Marshall JM, Hay BA. 2012 Confinement of gene drive systems to local populations: a comparative analysis. *J. Theor. Biol.* **294**, 153–171. (doi:10.1016/j.jtbi.2011.10.032)
31. Dhole S, Vella MR, Lloyd AL, Gould F. 2018 Invasion and migration of spatially self-limiting gene drives: a comparative analysis. *Evol. Appl.* **11**, 794–808. (doi:10.1111/eva.12583)
32. Noble C *et al*. 2019 Daisy-chain gene drives for the alteration of local populations. *Proc. Natl Acad. Sci. USA* **116**, 8275–8282. (doi:10.1073/pnas.1716358116)
33. Hayes KR *et al*. 2018 Identifying and detecting potentially adverse ecological outcomes associated with the release of gene-drive modified organisms. *J. Responsible Innov.* **5**, S139–S158. (doi:10.1080/23299460.2017.1415585)
34. Bravo Núñez MA, Nuckolls NL, Zanders SE. 2018 Genetic villains: killer meiotic drivers. *Trends Genet.* **34**, 424–433. (doi:10.1016/j.tig.2018.02.003)
35. Yang W-C, Shi D-Q, Chen Y-H. 2010 Female gametophyte development in flowering plants. *Annu. Rev. Plant Biol.* **61**, 89–108. (doi:10.1146/annurev-arplant-042809-112203)
36. Buckler ES, Phelps-Durr TL, Buckler CSK, Dawe RK, Doebley JF, Holtsford TP. 1999 Meiotic drive of chromosomal knobs reshaped the maize genome. *Genetics* **153**, 415–426.
37. Dawe RK *et al*. 2018 A kinesin-14 motor activates neocentromeres to promote meiotic drive in maize. *Cell* **173**, 839–850.e18. (doi:10.1016/j.cell.2018.03.009)
38. Higgins DM, Lowry EG, Kanizay LB, Becraft PW, Hall DW, Dawe RK. 2018 Fitness costs and variation in transmission distortion associated with the abnormal chromosome 10 meiotic drive system in maize. *Genetics* **208**, 297–305. (doi:10.1534/genetics.117.300060)
39. Fishman L, Saunders A. 2008 Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. *Science* **322**, 1559–1562. (doi:10.1126/science.1161406)
40. Fishman L, Willis JH. 2005 A novel meiotic drive locus almost completely distorts segregation in *Mimulus* (monkeyflower) hybrids. *Genetics* **169**, 347–353. (doi:10.1534/genetics.104.032789)
41. Fishman L, Kelly JK. 2015 Centromere-associated meiotic drive and female fitness variation in *Mimulus*: female fitness costs of meiotic drive. *Evolution* **69**, 1208–1218. (doi:10.1111/evo.12661)
42. Jones RN. 1995 B chromosomes in plants. *New Phytol.* **131**, 411–434. (doi:10.1111/j.1469-8137.1995.tb03079.x)
43. Jones RN. 2018 Transmission and drive involving parasitic B chromosomes. *Genes* **9**, 388. (doi:10.3390/genes9080388)
44. Kanizay LB, Pyhäjärvi T, Lowry EG, Hufford MB, Peterson DG, Ross-Ibarra J, Dawe RK. 2013 Diversity and abundance of the abnormal chromosome 10 meiotic drive complex in *Zea mays*. *Heredity* **110**, 570–577. (doi:10.1038/hdy.2013.2)
45. Werren JH. 2011 Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proc. Natl Acad. Sci. USA* **108**, 10 863–10 870. (doi:10.1073/pnas.1102343108)
46. Lynch M, Marinov GK. 2015 The bioenergetic costs of a gene. *Proc. Natl Acad. Sci. USA* **112**, 15 690–15 696. (doi:10.1073/pnas.1514974112)
47. Endo TR. 2015 Gametocidal genes. In *Alien introgression in wheat: cytogenetics, molecular biology, and genomics* (eds M Molnár-Láng, C Ceoloni, J Doležel), pp. 121–131. Cham, Switzerland: Springer International Publishing.
48. Sweigart AL, Brandvain Y, Fishman L. 2019 Making a murderer: the evolutionary framing of hybrid gamete-killers. *Trends Genet.* **35**, 245–252. (doi:10.1016/j.tig.2019.01.004)
49. Yu X *et al*. 2018 A selfish genetic element confers non-Mendelian inheritance in rice. *Science* **360**, 1130–1132. (doi:10.1126/science.aar4279)
50. Knight TM *et al*. 2005 Pollen limitation of plant reproduction: pattern and process. *Annu. Rev. Ecol. Evol. Syst.* **36**, 467–497. (doi:10.1146/annurev.ecolsys.36.102403.115320)
51. DiCarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM. 2013 Genome engineering in *Saccharomyces cerevisiae* using CRISPR–Cas systems. *Nucleic Acids Res.* **41**, 4336–4343. (doi:10.1093/nar/gkt135)
52. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. 2015 Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. *Proc. Natl Acad. Sci. USA* **112**, E6736–E6743. (doi:10.1073/pnas.1521077112)
53. Rinaldo AR, Ayliffe M. 2015 Gene targeting and editing in crop plants: a new era of precision opportunities. *Mol. Breeding* **35**, 40. (doi:10.1007/s11032-015-0210-z)
54. Simoni A, Siniscalchi C, Chan Y-S, Huen DS, Russell S, Windbichler N, Crisanti A. 2014 Development of synthetic selfish elements based on modular nucleases in *Drosophila melanogaster*. *Nucleic Acids Res.* **42**, 7461–7472. (doi:10.1093/nar/gku387)
55. Grunwald HA, Gantz VM, Poplawski G, Xu X-RS, Bier E, Cooper KL. 2019 Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline. *Nature* **566**, 105. (doi:10.1038/s41586-019-0875-2)
56. Buchman A, Marshall JM, Ostrovski D, Yang T, Akbari OS. 2018 Synthetically engineered Medea gene drive system in the worldwide crop pest *Drosophila suzukii*. *Proc. Natl Acad. Sci. USA* **115**, 4725–4730. (doi:10.1073/pnas.1713139115)
57. Chen C-H, Huang H, Ward CM, Su JT, Schaeffer LV, Guo M, Hay BA. 2007 A synthetic maternal-effect selfish genetic element drives population replacement in *Drosophila*. *Science* **316**, 597–600. (doi:10.1126/science.1138595)
58. Muralla R, Lloyd J, Meinke D. 2011 Molecular foundations of reproductive lethality in *Arabidopsis thaliana*. *PLoS ONE* **6**, e28398. (doi:10.1371/journal.pone.0028398)
59. Barrett SCH. 2015 Influences of clonality on plant sexual reproduction. *Proc. Natl Acad. Sci. USA* **112**, 8859–8866. (doi:10.1073/pnas.1501712112)
60. Goodwillie C, Kalisz S, Eckert CG. 2005 The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* **36**, 47–79. (doi:10.1146/annurev.ecolsys.36.091704.175539)
61. Bakker JP, Poschlod P, Strykstra RJ, Bekker RM, Thompson K. 1996 Seed banks and seed dispersal: important topics in restoration ecology. *Acta Bot. Neerl.* **45**, 461–490. (doi:10.1111/j.1438-8677.1996.tb00806.x)
62. Peñuelas J, Munné-Bosch S. 2010 Potentially immortal? *New Phytol.* **187**, 564–567. (doi:10.1111/j.1469-8137.2010.03360.x)
63. Burnside OC, Wilson RG, Weisberg S, Hubbard KG. 1996 Seed longevity of 41 weed species buried 17 years in eastern and western Nebraska. *Weed Sci.* **44**, 74–86. (doi:10.1017/S0043174500093589)
64. Shoemaker WR, Lennon JT. 2018 Evolution with a seed bank: the population genetic consequences of microbial dormancy. *Evol. Appl.* **11**, 60–75. (doi:10.1111/eva.12557)
65. Bennett MD. 1987 Variation in genomic form in plants and its ecological implications. *New Phytol.* **106**, 177–200. (doi:10.1111/j.1469-8137.1987.tb04689.x)
66. Barrett LG, Thrall PH, Burdon JJ, Linde CC. 2008 Life history determines genetic structure and evolutionary potential of host–parasite interactions. *Trends Ecol. Evol.* **23**, 678–685. (doi:10.1016/j.tree.2008.06.017)
67. Bull JJ, Remien CH, Krone SM. 2019 Gene-drive-mediated extinction is thwarted by population structure and evolution of sib mating. *Evol. Med. Public Health* **2019**, 66–81. (doi:10.1093/emph/eoz014)

68. Burt A, Trivers R. 1998 Selfish DNA and breeding system in flowering plants. *Proc. R. Soc. Lond. B* **265**, 141–146. (doi:10.1098/rspb.1998.0275)
69. Bull JJ. 2016 Lethal gene drive selects inbreeding. *Evol. Med. Public Health* **2017**, 1–16. (doi:10.1093/emph/eow030)
70. Drury DW, Dapper AL, Siniard DJ, Zentner GE, Wade MJ. 2017 CRISPR/Cas9 gene drives in genetically variable and nonrandomly mating wild populations. *Sci. Adv.* **3**, e1601910. (doi:10.1126/sciadv.1601910)
71. Burdon JJ, Zhan J, Barrett LG, Papaïx J, Thrall PH. 2016 Addressing the challenges of pathogen evolution on the world's arable crops. *Phytopathology* **106**, 1117–1127. (doi:10.1094/PHYTO-01-16-0036-FI)
72. Sinkins SP, Gould F. 2006 Gene drive systems for insect disease vectors. *Nat. Rev. Genet.* **7**, 427–435. (doi:10.1038/nrg1870)
73. Ward CM, Su JT, Huang Y, Lloyd AL, Gould F, Hay BA. 2011 Medea selfish genetic elements as tools for altering traits of wild populations: a theoretical analysis. *Evolution* **65**, 1149–1162. (doi:10.1111/j.1558-5646.2010.01186.x)
74. Unckless RL, Clark AG, Messer PW. 2017 Evolution of resistance against CRISPR/Cas9 gene drive. *Genetics* **205**, 827–841. (doi:10.1534/genetics.116.197285)
75. Britt AB. 1999 Molecular genetics of DNA repair in higher plants. *Trends Plant Sci.* **4**, 20–25. (doi:10.1016/S1360-1385(98)01355-7)
76. Gorbunova V, Levy AA. 1999 How plants make ends meet: DNA double-strand break repair. *Trends Plant Sci.* **4**, 263–269. (doi:10.1016/S1360-1385(99)01430-2)
77. Manova V, Gruszka D. 2015 DNA damage and repair in plants—from models to crops. *Front. Plant Sci.* **6**, 885. (doi:10.3389/fpls.2015.00885)
78. Thompson JD, Lumaret R. 1992 The evolutionary dynamics of polyploid plants: origins, establishment and persistence. *Trends Ecol. Evol.* **7**, 302–307. (doi:10.1016/0169-5347(92)90228-4)
79. Vella MR, Gunning CE, Lloyd AL, Gould F. 2017 Evaluating strategies for reversing CRISPR–Cas9 gene drives. *Sci. Rep.* **7**, 11038. (doi:10.1038/s41598-017-10633-2)
80. Huang T-K, Puchta H. 2019 CRISPR/Cas-mediated gene targeting in plants: finally a turn for the better for homologous recombination. *Plant Cell Rep.* **38**, 443–453. (doi:10.1007/s00299-019-02379-0)
81. Oye KA *et al*. 2014 Regulating gene drives. *Science* **345**, 626–628. (doi:10.1126/science.1254287)
82. Montenegro de Wit M. 2019 Gene driving the farm: who decides, who owns, and who benefits? *Agroecol. Sustain. Food Syst.* **43**, 1054–1074. (doi:10.1080/21683565.2019.1591566)
83. Brossard D, Belluck P, Gould F, Wirz CD. 2019 Promises and perils of gene drives: navigating the communication of complex, post-normal science. *Proc. Natl Acad. Sci. USA* **116**, 7692–7697. (doi:10.1073/pnas.1805874115)