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Cleave and Rescue gamete killers create conditions for gene drive in plants

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Georg Oberhofer, Michelle L. Johnson, Tobin Ivy, Igor Antoshechkin & Bruce A. Hay

Gene drive elements promote the spread of linked traits and can be used to change the composition or fate of wild populations. *Cleave and Rescue* (*ClvR*) drive elements sit at a fixed chromosomal position and include a DNA sequence-modifying enzyme such as Cas9/gRNAs that disrupts endogenous versions of an essential gene and a recoded version of the essential gene resistant to cleavage. *ClvR* spreads by creating conditions in which those lacking *ClvR* die because they lack functional versions of the essential gene. Here we demonstrate the essential features of the *ClvR* gene drive in the plant *Arabidopsis thaliana* through killing of gametes that fail to inherit a *ClvR* that targets the essential gene *YKT61*. Resistant alleles, which can slow or prevent drive, were not observed. Modelling shows plant *ClvR*s are robust to certain failure modes and can be used to rapidly drive population modification or suppression. Possible applications are discussed.

Gene drive occurs when genetic elements—genes, gene complexes, large chromosomal regions or entire chromosomes—are transmitted to viable, fertile progeny at rates greater than those of competing allelic variants or other parts of the genome. There has long been interest in the idea that genetic manipulation of wild populations via gene drive could be used for beneficial purposes. Transgenes or alleles of endogenous loci can be linked with a drive element. The results of modelling and lab experiments show this can result in spread of these 'cargo' genes to high frequency in an extant population. Alternatively, drive can result in population suppression or elimination if the spread of the element pushes the population towards an unfit set of genotypes (for example, all male, all females sterile), reviewed in refs. 1–3.

A number of applications of gene drive in plants have been proposed[4–6]. In the context of agriculture, gene drive has been discussed as a way to spread desirable agronomic traits and as a possible tool for weed management, by sensitizing the population to some other form of intervention or by suppressing it directly. Ecosystem engineering/conservation is another possibility. This could take the form of suppressing invasive species. Alternatively, population modification could be used to engineer pathogen resistance or other forms of resilience (a form of evolutionary rescue) into native species in the face of current stresses—or anticipated novel stresses due to climate change.

A variety of selfish genetic elements have been considered for bringing about gene drive. These include transposons and homing endonucleases, which spread through over-replication; multigene complexes that produce female meiotic drive or sperm post-meiotic segregation distortion; and toxin–antidote combinations that spread by causing the death of those (cells, spores, gametes or progeny) that fail to inherit them from a carrier. Toxin–antidote gene drive elements (TA elements) are particularly interesting as they are found throughout all domains of life: prokaryotes, fungi, animals and plants, and the wide distribution of some of these elements in nature shows they can spread and persist in complex natural environments[7–9]. In this Article, we focus on eukaryotes and drive associated with sexual reproduction.

A TA element sits at a fixed chromosomal position and consists of one or more genes that encode linked toxin and antidote functions. The toxin, typically a protein, has the potential to kill or impair the development of those in which it is present, while the antidote, a protein or RNA, suppresses the activity or expression of the toxin[7,10–12]. The toxin is trans-acting and is distributed to all meiotic products or progeny of a TA-bearing parent. However, only those that inherit the TA cassette express the antidote, which counteracts the toxin in cis. In consequence, TA elements ensure their presence in the next generation by causing the death of those that fail to inherit them (post-segregational killing) from a parent, a form of genetic addiction. The death of those lacking the TA cassette can result in a relative increase in frequency of those carrying it. Modelling shows that TA elements in sexually reproducing eukaryotes can (depending on the fitness costs associated with

Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. e-mail: haybruce@caltech.edu

carriage of the element and introduction frequency) spread to high frequency even if they do not confer any advantage to their hosts[13–23].

TA elements in nature[7,8,10–12,24], including those in plants[9,12,25–33], evolved in specific genomic, organismal and ecological contexts, and it is often unclear whether the mechanisms of action, associated gene regulation and species-specific information on development (timing and levels of gene and protein expression and localization) can be easily transferred to bring about drive in other species. Similar considerations apply to synthetic *Medea* TA elements engineered in *Drosophila* in which the toxin is an engineered transient loss of function (LOF) of a maternally expressed gene, the product of which is essential for embryogenesis, and the antidote is a zygotically expressed transgene that restores this missing function in a just-in-time fashion[34–36].

Recently, in an effort to create a chromosomal TA-based gene drive system that utilizes a LOF toxin and consists of a simple and extensible set of components that can plausibly be implemented across diverse species, we developed the *Cleave and Rescue* (*ClvR*) element[19,37–39], also referred to as Toxin Antidote Recessive Embryo (TARE)[20,40] in related implementations (hereafter referred to as *ClvR*, a name that captures the key mechanisms involved). A *ClvR* element encodes two activities. The first component, the Cleaver/Toxin, is a DNA sequence-modifying enzyme such as CRISPR-associated protein 9 (Cas9) and multiple guide RNAs (gRNAs). These are expressed in the germline or cells that will become the germline. Cas9 and its associated gRNAs disrupt—through cycles of cleavage and end joining that continue until the target site is destroyed—endogenous versions of a haplosufficient (and in some contexts haploinsufficient or haplolethal) essential gene, wherever it is located. Inaccurate repair at multiple positions in the coding region of the essential gene creates LOF alleles. These are the potential toxin. The second component of *ClvR*, the Rescue/Antidote, is simply a recoded version of the essential gene resistant to cleavage and gene conversion with the cleaved version, expressed under the control of regulatory sequences sufficient to rescue the LOF phenotype. LOF alleles of the essential gene, which segregate and exist independently of *ClvR*, perform their toxin function when they find themselves (potentially many generations later) in homozygotes that die because they lack the *ClvR*-derived source of essential gene function. By contrast, those that inherit *ClvR* and its associated Rescue survive. In this way, as with TA-based selfish genetic elements found in nature, *ClvR* increases in relative frequency by causing the death of those that lack it. This results in cells, organisms and ultimately populations becoming dependent on (addicted to) the *ClvR*-encoded Rescue transgene for their survival.

In *Drosophila*, autonomous *ClvR*/TARE elements have been created and shown to spread in wild-type (WT) populations to transgene fixation (all individuals carry at least one copy)[19,37,39,40]. Other features, such as the ability to create strong but self-limited drive[38], engage in multiple cycles of population modification that replace old content with new[37] and achieve population suppression using a conditional Rescue[39], have also been demonstrated. Multiple other configurations of the components that make up *ClvR*/TARE have been proposed, and modelling predicts they can give rise to drive with a diversity of interesting characteristics for population modification or suppression[21,41].

Engineering TA drive based on killing and rescue of gametes is also of great interest because gametic drive can be much stronger than zygotic drive. It will typically drive the element to allele fixation (as opposed to transgene fixation with many zygotic TA elements, which includes heterozygotes), and it can be used to bias sex ratios if the driver is linked to a sex chromosome and has its effects during post-meiotic spermatogenesis. These latter two features are important for several proposed methods of population suppression[13,21,42]. A number of naturally occurring gametic drive systems (most often biasing sperm genotypes) in animals have been characterized, but the information available does not yet provide guidance as to whether or how they can be used as tools[43,44]. Engineering *ClvR*-based gametic drive in animals is challenging for several reasons. In females the gamete is chosen through differential segregation of one of the products of meiosis within the common cytoplasm of the oocyte, and thus there is no opportunity for the antidote to select for carriers. In spermatogenesis the haploid spermatid products of a meiosis are connected by cytoplasmic bridges until late in development, and active content sharing of many but not all products (for example, ref. 45) limits opportunities for bringing about differential killing and survival[46].

In our original description of *ClvR* we noted that gametic drive could be implemented in sexual organisms such as fungi and plants (Supplementary Fig. 1 (ref. 19) and ref. 47), in which sibling gametes do not share components and require haploid gene expression for development and/or survival. In plants, meiotic products undergo additional rounds of mitosis, developing into multicellular haploid gametophytes (the female megagametophyte and male microgametophyte) that produce ovules or pollen. This requires extensive expression of the haploid genome[48]. These features of plant gamete development are reflected in the many recessive mutants (no somatic phenotype in the heterozygous diploid or polyploid parent; the sporophyte stage) that cannot be transmitted through one or the other sex, often identified through sex-specific transmission ratio distortion (for example, refs. 49,50). Mutations in other genes cannot be transmitted through either sex due to a requirement in both gametophyte types[51–53]. These characteristics make plants an ideal system in which to implement gene drive based on a *Cleave and Rescue* mechanism in which gametes die if they fail to inherit *ClvR* from a *ClvR*-bearing parent (Fig. 1a–d).

Arabidopsis thaliana is a good system in which to test self-sustaining gene drive constructs in plants because it is a self-fertilizing hermaphrodite in which fertilization typically occurs before flower opening, thus limiting opportunities for pollen/gene flow. In addition, *A. thaliana* is not naturally wind pollinated, and lab and field experiments show that outcrossing rates are very low[54–58]. Thus, transgene containment is straightforward. However, for these same reasons, population-level gene drive experiments of the type carried out in insects—mixed populations of transgenic and non-transgenics allowed to mate freely and followed for changes in genotype frequency over multiple generations—cannot be carried out. Here we show, using manual mating between parents of different genotypes, the key features required for *ClvR* drive: a high frequency of LOF allele creation, a high, non-Mendelian rate of *ClvR* inheritance in progeny and the absence of resistant alleles (mutated, uncleavable, but functional) that would slow or subvert the intended goal of drive, population modification or suppression. Modelling shows that elements with the features we demonstrate experimentally have the potential, in diploid obligate outcrossing species (dioecious), to bring about population modification or suppression. Possibilities for drive in hermaphrodites (male and female reproductive organs in the same flower) and monoecious species (male and female flowers on the same plant), and the consequences of inbreeding, are also discussed. Together our observations, along with those of Liu and colleagues in related work[59], suggest possible applications, as well as challenges, for use of *ClvR* gamete killer gene drive in plants.

Results

Components of a *ClvR*-based gamete killer

The strength of a gene drive—its ability to spread from low frequency and in the presence of substantial fitness costs—is increased when it biases inheritance in its favour in both sexes, something that is of particular importance when trying to bring about population suppression. Engineering *ClvR*-based gamete drive with this feature (Fig. 1a) requires targeting a gene whose expression during the haploid stage is required for the survival and/or development of the microgametophyte (referred to as pollen, which contains sperm) and megagametophyte (referred to as ovule, a sporophytic structure in the ovary within which each megagametophyte, which includes the egg, develops). Mutations in many ubiquitously expressed housekeeping genes (such as were

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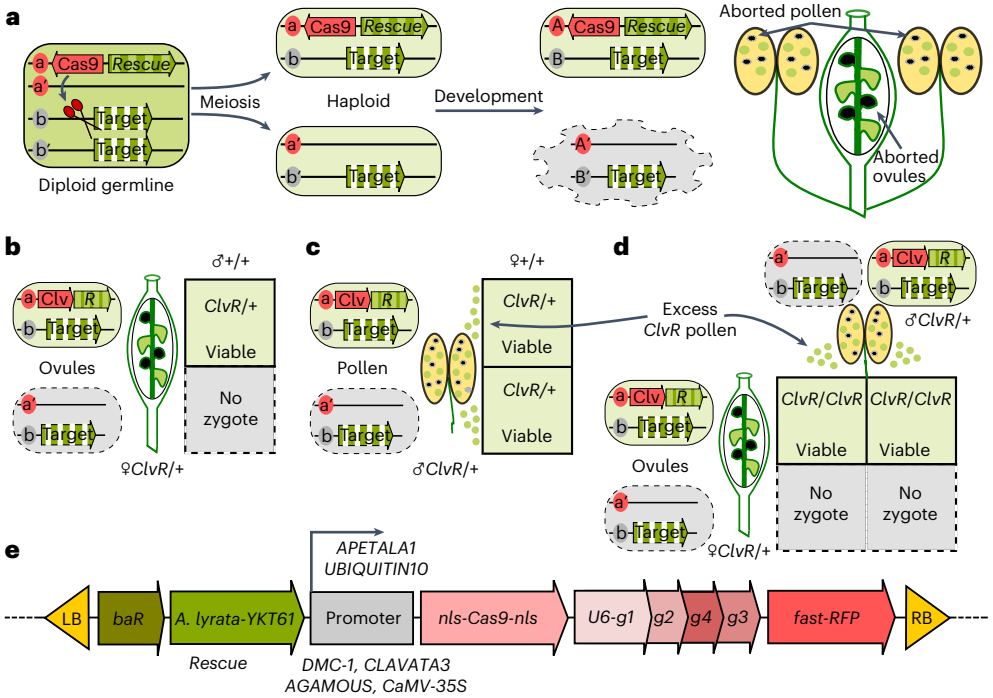

Fig. 1 | *ClvR* behaviour in a diploid plant and construct design. a, Cas9/gRNAs located on chromosome a of an a/a′ *ClvR* heterozygote cleave an essential gene located on chromosomes b and b′ during the diploid parental stage, creating LOF alleles. Diploid cells survive this because the *ClvR* carries a recoded rescuing version of the essential gene, which produces a functional product (light green background). During haploid stages, expression of the essential gene is required for gamete/gametophyte development/survival. Gametes/gametophytes that fail to inherit *ClvR* lack a functional copy of the essential gene and die (indicated with dashed outline and grey background). The hermaphrodite plant to the right has anthers with *ClvR*-bearing pollen (green circles) and dead non-*ClvR*-bearing pollen (dark circles) and an ovary containing *ClvR*-bearing ovules (large green shapes) and dead non-*ClvR*-bearing ovules (large dark circles). **b**, Cross of a heterozygous *ClvR*-bearing female with WT (+/+) pollen. Non-*ClvR*-bearing gametophytes die and do not undergo fertilization (grey no zygote square). Thus, all progeny are *ClvR*-bearing heterozygotes (green square). **c**, Cross in which pollen from a heterozygous *ClvR*-bearing male fertilizes ovules of a WT (+/+) female. Pollen is produced in large excess over ovules. Thus, death of the 50% non-*ClvR*-bearing pollen (dark circles) still allows all ovules to be fertilized, resulting in all progeny being *ClvR*-bearing heterozygotes. **d**, Cross of a heterozygous *ClvR* female to a heterozygous *ClvR* male. Only *ClvR*-bearing ovules and pollen participate in fertilization, resulting in all progeny being homozygous *ClvR/ClvR*. **e**, Genetic makeup of the *ClvR* drive element. From left to right these are a Basta herbicide resistance marker (baR); a *YKT61* rescue transgene derived from *A. lyrata* (*A. lyrata-YKT61*); one of six different enhancer/promoters (*APETALA1* and *UBIQUITIN10*) resulted in substantial transmission ratio distortion) used to direct Cas9 expression; Cas9 (one of two different versions, discussed in text); four gRNAs designed to base pair with DNA for the *YKT61* coding region (U6-g1–4), with each expressed under the control of an independent U6 promoter; and a fluorescent seed transgenesis marker (fast-RFP). Repeats required for transgenesis using agrobacterium (left border (LB) and right border (RB)) flank these elements.

targeted for LOF allele creation in insect *ClvR*s[19,37,40]) likely have such a phenotype in plants, given the extensive gene expression that occurs in gametes, but are challenging to identify as such mutations cannot be passed through the germline. Their identity is sometimes inferred by their absence in mutant collections (for example, ref. 51). Alternatively, with the advent of methods for clustered regularly interspaced short palindromic repeats (CRISPR)-based mutagenesis, genes whose mutation results in loss of male and female gametes can be identified through reverse genetics approaches that incorporate a rescuing transgene into the mutagenized genetic background (for example, refs. 52,53). Here we focus on one such gene, *YKT61*, a ubiquitously expressed R-SNARE protein involved in fusion between vesicle and target membranes[52]. Formally, it is not known whether the *YKT61* LOF phenotype in sporophytes is recessive lethal as crosses between heterozygotes do not produce viable LOF gametes[52]. That said, *YKT61* is expressed ubiquitously and recent work shows that partial LOF of *YKT61* using RNA interference has strong effects on root development[60]. Thus, its LOF is likely to be at least deleterious in the sporophyte.

The components that make up our *ClvR* gamete killers are illustrated in Fig. 1e. As a Rescue we utilized a genomic fragment containing the *Arabidopsis lyrata YKT61* gene (in which some amino acid coding region differences were recoded back to those of *A. thaliana*; Extended Data Fig. 1). For the Cleaver, four gRNAs targeting conserved regions within the *A. thaliana YKT61* coding sequence (see also Fig. 4) were expressed ubiquitously using individual U6 Pol-III promoters[61]. Several versions of Cas9 were tested. One lacks introns and carries a mutation (K918N) shown to increase Cas9 catalytic activity[62], while a second one contains 13 introns, which are thought to increase expression[63]. Regulatory sequences from six different genes were used to direct Cas9 expression. *Arabidopsis DMC1* is primarily expressed during meiotic stages[64]. Sequences from the *CLAVATA3* (also known as *CLV3*, early stem cell identity[65]), *APETALA1* (also known as *API*, flower meristem identity[66]) and *AGAMOUS* (also known as *AG*, reproductive floral organ primordia[67]) genes direct expression in adult sporophyte tissues that include the future germline. The *CaMV35S*[68] (also known as *ERF53*) and *UBIQUITIN10*[69] promoters direct expression broadly, in many if not all cell types. The *DMC1* promoter was used in combination with both versions of Cas9, while *AGAMOUS*, *CLAVATA3*, *APETALA1*, *CaMV35S* and *UBIQUITIN10* sequences were used to direct expression of the version of Cas9 lacking introns.

*ClvR*s targeting *YKT61* show features required for gene drive

We used floral dipping with agrobacterium to transform a number of primary transformed (T0) WT plants with the above constructs (Fig. 2a). A number of independent transformants, identified as red transgenic seeds of the T1 (seeds coming from T0 plants) generation, were

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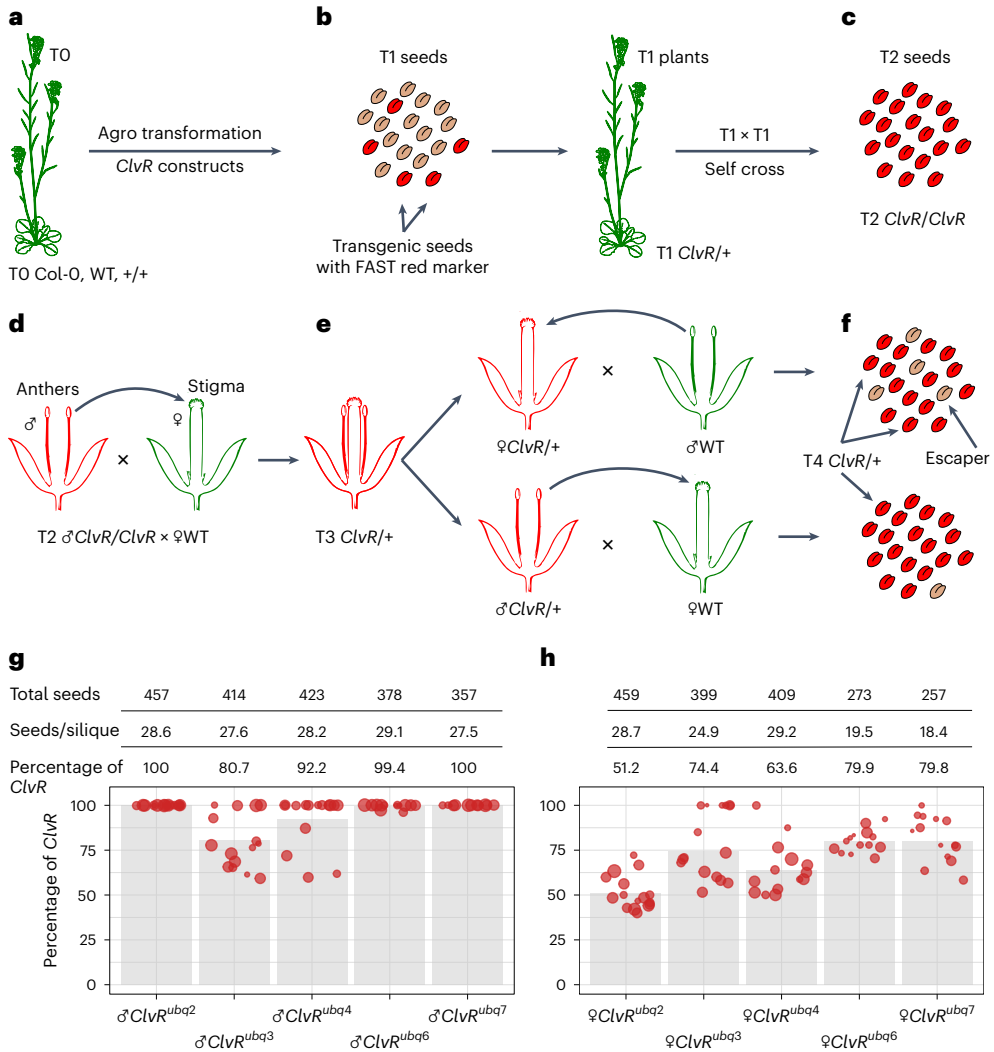

Fig. 2 | Genetic evidence for *ClvR*-based gamete killing and rescue. **a**–**f**, Crosses used to establish independent *ClvR* insertions (**a**–**d**) and test for gametic drive through the male and female germline (**e**,**f**). WT plants (T0, Colombia-0 (Col-0)) (**a**) were transformed using agrobacterium. T1 seeds produced by these plants (**b**) include some bearing a transgene (red). T1 plants derived from transgene-bearing seeds were self crossed to generate T2 progeny (**c**). **d**, Pollen from T2 transgenics was used to fertilize WT ovules. **e**, T3 progeny (left) must be heterozygous for *ClvR*. Transgene-bearing pollen and ovules from T3 plants were outcrossed to WT, giving rise to T4 seeds (**f**). **g**,**h**, Frequency of *ClvR* transmission through the male (**g**) and female (**h**) germlines. We counted the total number of seeds and the number of seeds per silique. Each circle represents an individual silique. The size of the red circle scales with the number of seeds in the silique. Grey bars represent mean *ClvR* inheritance values from all seeds in a cross. Note that for the female crosses in **h**, in general as the frequency of *ClvR* inheritance goes up, the number of seeds in the silique goes down. This is expected as the number of functional ovules determines the maximal seed output, and a *ClvR* with efficient killing and rescue would only be expected to produce half the WT number of functional ovules/seeds. Seed and silique counts are in Supplementary Table 1 (*ClvR*^{ubq} Crosses). *Arabidopsis* icons adapted from (BioRender (2023), Structure of *A. thaliana*).

collected from these plants (Fig. 2b) and characterized in the crosses outlined in Fig. 2c–f. T1 seeds (heterozygous for one or more *ClvR* elements) were grown to adulthood and allowed to self cross (T1 × T1; Fig. 2b). T1 self crosses that produced progeny siliques (a seed pod, which contains progeny from the ovules of one flower) containing all or primarily red seeds—the T2 (seeds coming from T1 plants) generation, possibly *ClvR/ClvR* homozygotes (Fig. 2c)—were characterized further as this is the expected phenotype if gametic drive occurred in one or both sexes in a cross between heterozygotes (Fig. 1b–d). Based on the results of these experiments (a substantial fraction of non-*ClvR* seeds), constructs utilizing regulatory sequences from the *DMC1*, *AGAMOUS* and *CLAVATA3* genes were not considered further.

T2 seeds carrying constructs that utilized the *APETELA1* and *UBIQUITIN10* regulatory sequences were grown to adulthood, and pollen from these plants were used in an outcross to WT to produce T3 *ClvR*/+ seeds (Fig. 2d). Finally, in the key outcross to test for gametic drive, T3 seeds were grown to adulthood, and pollen and ovules were used in outcrosses to WT (Fig. 2e). The frequency of *ClvR* inheritance (*ClvR*/+) in progeny T4 seeds provides a measure of gamete killing and rescue (Fig. 2f). *ClvR* inheritance rates in T4 seeds from T3 pollen, shown for five different insertions using *UBIQUITIN10* sequences (*ClvR*^{ubq} lines) to direct Cas9 expression (Fig. 2g), were generally very high, with three of the five showing inheritance rates greater than 99%. Inheritance rates of *ClvR*^{ubq} in T4 seeds from T3 ovules were also substantially above 50%, but a number of non-*ClvR* seeds (generically referred to as escapers) were observed (Fig. 2h). Similar results were obtained for *ClvR*s utilizing *APETALA1* regulatory sequences (Extended Data Fig. 2). Crosses with *ClvR*s using the *CaMV35S* promoter showed inheritance that was modestly *ClvR*-biased (Extended Data Fig. 3). These were not considered further. The basis for the differences between drive through pollen and ovules is addressed further below.

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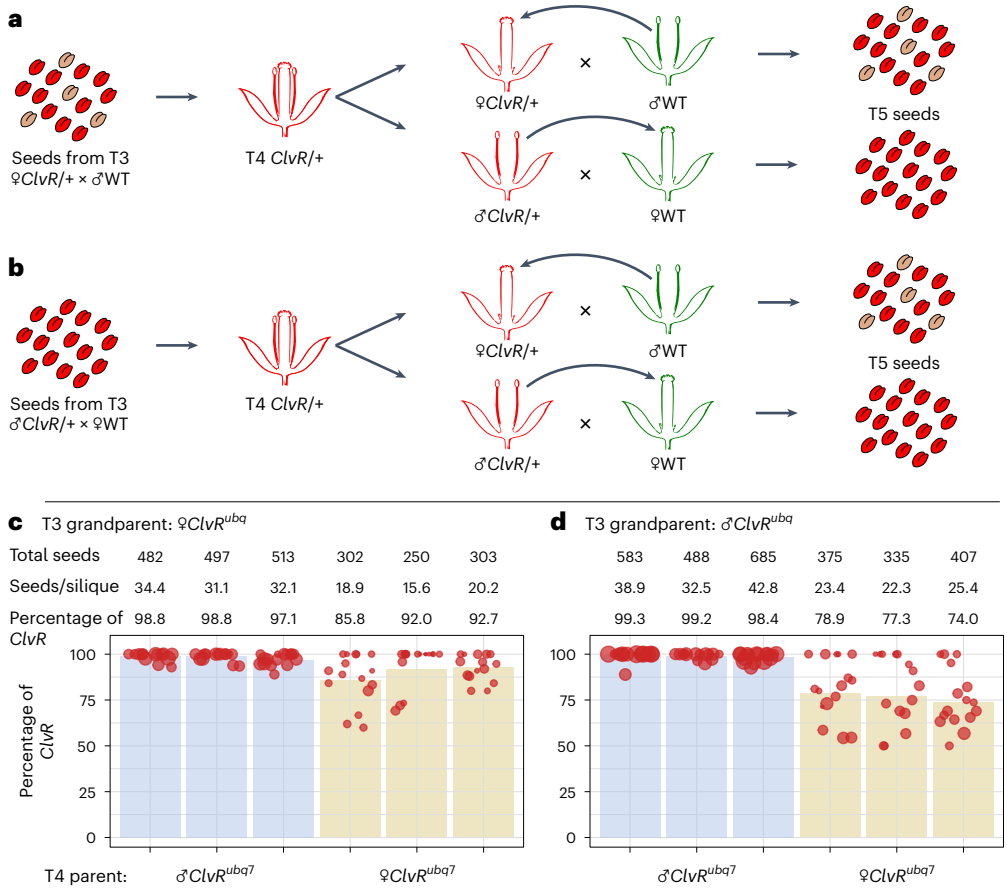

Fig. 3 | *ClvR*-based gamete killing and rescue is stable over multiple generations. a, A T3 cross between *ClvR*^{ubq7}/+ females and WT was used to generate T4 *ClvR*^{ubq7}/+ heterozygous plants. Pollen and ovules from these T4 individuals were used in outcrosses to WT to generate a *ClvR*^{ubq7} heterozygous T5 generation. **b**, A T3 cross between *ClvR*^{ubq7}/+ males and WT was used to generate T4 *ClvR*^{ubq7}/+ heterozygous plants. Pollen and ovules from these T4 individuals were used in outcrosses to WT to generate a *ClvR*^{ubq7} heterozygous T5 generation. **c**, Frequency of *ClvR*^{ubq7} inheritance in crosses in which the T3 grandparent was a *ClvR*^{ubq7}/+ heterozygote female and T4 parents were either a *ClvR*^{ubq7}/+ female (left six columns) or male (right six columns). **d**, Frequency of *ClvR*^{ubq7} inheritance in crosses in which the T3 grandparent was a *ClvR*^{ubq7}/+ heterozygote male and T4 parents were either female (left six columns) or male (right six columns). Seed and silique counts are in Supplementary Table 1 (*ClvR*^{ubq} Crosses). A description of crosses is presented in Extended Data Fig. 5. *Arabidopsis* icons adapted from (BioRender (2023), Structure of *A. thaliana*).

ClvR killing and rescue is stable over multiple generations

The above results show that *ClvR*s designed to kill and rescue a gamete essential gene can bias inheritance in their favour in *Arabidopsis*, satisfying the key requirement for TA-based gametic gene drive. We focused our characterization on the *ClvR*^{ubq7} line as it is associated with a single insertion, it showed a high frequency of inheritance through pollen and ovules, and heterozygotes and homozygotes were otherwise healthy (Extended Data Fig. 4). To determine whether the bias in *ClvR*^{ubq7} inheritance was stable and whether it had any dependence on the sex through which the drive element was inherited, we characterized the drive of *ClvR*^{ubq7} alleles present in T4 *ClvR*^{ubq7}/+ individuals (Fig. 2f) that came from either a male or female *ClvR*^{ubq7}/+ T3 parent (Fig. 3). *ClvR*^{ubq7}-bearing T4 seeds derived from male or female *ClvR*^{ubq7}/+ T3 parents crossed to WT were grown to adulthood, and pollen and ovules from *ClvR*^{ubq7}-bearing T4 individuals were used in outcrosses to WT, giving rise to a T5 generation of seeds whose *ClvR*^{ubq7}/+ grandparents (the T3 generation) and parents (the T4 generation) were either both female (ovules), both male (pollen), or one (T3) and then the other (T4) (Fig. 3a,b). As shown in Fig. 3c,d inheritance rates remained comparable—very high when transmitted through pollen and high but with more non-*ClvR*^{ubq7}-bearing escaper seeds when transmitted through ovules—regardless of the parental and grandparental sex.

Mutation sequences and basis of escape from gamete killing

The high frequency of *ClvR*^{ubq7} inheritance when transmitted through *ClvR*^{ubq7}/+ pollen argues that rates of cleavage and LOF mutation creation are high and that rescue is efficient. The *UBIQUITIN10* regulatory sequences drive expression broadly throughout development, from the embryo onwards[70], long before the male and female germlines form. This suggests that rates of cleavage and LOF allele creation in female gametes are high as well. To understand the molecular events associated with drive, and the unexpectedly high numbers of non-*ClvR*^{ubq7} progeny observed when a *ClvR*^{ubq7}/+ individual was the female parent, we sequenced the endogenous *YKT61* locus in leaves of several genotypes: *ClvR*^{ubq7}/+ T4 heterozygotes and *ClvR*^{ubq7}/*ClvR*^{ubq7} T5 homozygotes derived from a T4 self cross; and non-*ClvR*^{ubq7} escapers from crosses of *ClvR*^{ubq7}/+ to WT, in which the *ClvR*^{ubq7}/+ was the female or male parent (Fig. 4). See Supplementary Table 1 (Cleavage events) for the details of sequence alterations at each gRNA target site. In *ClvR*^{ubq7}/+ individuals, all four target sites were cleavable and mutated to LOF (frameshifts) at high frequency, with at least four sites being altered in all five plants. In *ClvR*^{ubq7}/*ClvR*^{ubq7} homozygotes, all four sites were altered in all four sequenced individuals. In outcrosses using *ClvR*^{ubq7}/+ pollen, a very small number of non-*ClvR*^{ubq7} escaper seeds was observed (~1% of all seeds; Figs. 2 and 3). Six T5 escapers were grown to adulthood and sequenced. All were WT at all four gRNA target sites (Fig. 4). Thus,

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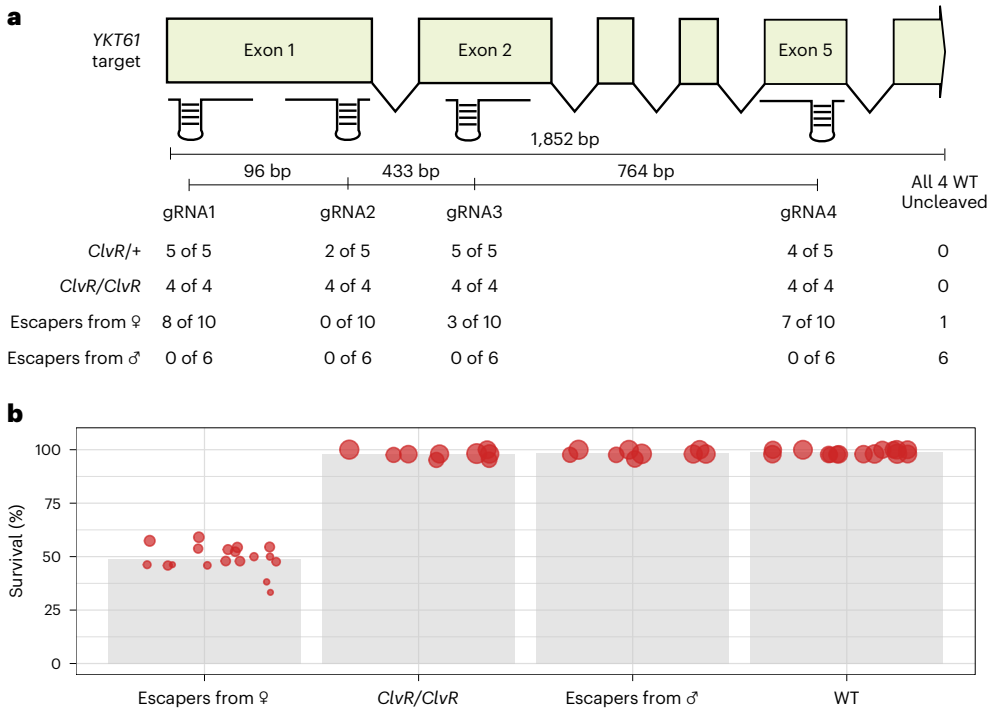

Fig. 4 | Characterization of the target locus following exposure to *ClvR*^{ubq7}, and genetic behaviour of LOF mutations found in non-*ClvR* progeny of a female *ClvR* parent. a, The genomic region containing the *YKT61* gene is shown, along with the approximate locations of four gRNA target sites (see Extended Data Fig. 1 for exact sequences). The table summarizes how many of the target sites were altered to likely LOF (frameshifts or large deletions) in *ClvR*^{ubq7}/+, *ClvR*^{ubq7}/*ClvR*^{ubq7} (progeny of heterozygous *ClvR*^{ubq7}/+ inbred for one generation), escapers coming from a *ClvR*^{ubq7}/+-bearing mother and escapers from a *ClvR*^{ubq7}-bearing father. One out of ten escapers from *ClvR*^{ubq7} mothers were WT at all four target sites. Six out of six escapers from *ClvR*^{ubq7} fathers were WT at all four target sites. **b**, Fraction of total ovules in individual siliques (red circles) that developed into seeds in self crosses from four different genotypes. The size of the circle scales with the number of seeds in a silique. Escapers derived from a female *ClvR*^{ubq7}/+ parent aborted ~50% of ovules, consistent with the known LOF phenotype of *YKT61* mutants[52]. Escapers derived from a male *ClvR*^{ubq7} parent (all WT at the *YKT61* locus) showed high seed production as did *ClvR*^{ubq7}/*ClvR*^{ubq7} homozygotes.

escape from death by non-*ClvR*^{ubq7} pollen is due to lack of cleavage and/or sequence alteration following cleavage, at all four sites. By contrast, in crosses with females as the parent, nine out of ten non-*ClvR* progeny of a *ClvR*^{ubq7}/+ parent carried one or more sequence alterations at gRNA target sites that create LOF alleles (frameshifts) in the *YKT61* coding region (Fig. 4 and Supplementary Table 1 (Cleavage events)). The frequency of mutation at each target site in escapers from a female *ClvR* parent is reduced compared with that observed in *ClvR*/+ and *ClvR*/*ClvR* genotypes. However, this is expected as mutagenesis is ongoing in the *ClvR* carriers but not escapers. Finally, the other escaper was WT at all four target sites. No resistant versions of endogenous *YKT61*—mutated but likely to be functional—were observed.

To summarize, rates of cleavage and mutation to LOF at the *YKT61* locus are very high in male and female gametes. In males, death of non-*ClvR*^{ubq7} pollen, coupled with efficient rescue of those inheriting *ClvR*^{ubq7}, leads to a very high frequency of *ClvR*^{ubq7} inheritance in progeny. In females, inheritance of *ClvR*^{ubq7} is also high, but there are also substantial numbers of non-*ClvR*^{ubq7} progeny. The results of sequencing show that most of these have a LOF mutation, probably created by *ClvR*^{ubq7} at a much earlier stage in the diploid sporophyte. The construct was not present in plants grown from escaper seeds (Extended Data Fig. 6), arguing against transgene silencing playing a role. In earlier work, Cas9-induced LOF alleles of *YKT61* were uniformly not passed to progeny through female gametes, resulting in abortion of 50% of ovules in a self cross[52]. Given this, our observations suggest that in the *ClvR*^{ubq7}/+ diploid, the *YKT61* rescue transgene from *A. lyrata* provides *YKT61* transcript and/or protein that is carried over from the mother into the non-*ClvR*^{ubq7} haploid ovules (maternal carryover rescue) and that this is sufficient to rescue the survival of some gametes carrying a LOF *YKT61* allele. A strong prediction of this hypothesis is that with LOF alleles generated by *ClvR*^{ubq7} and present in LOF/+, non-*ClvR*^{ubq7} heterozygotes should, as they lack the *ClvR*^{ubq7} Rescue, not be transmitted to the next generation. In a self cross of female escapers (most of which are LOF/+ heterozygotes based on the results of sequencing; Fig. 4), this should manifest itself as 50% abortion in progeny siliques. As illustrated in Fig. 4b and Supplementary Table 1 (Escaper crosses) this is the phenotype we observed for a number of female escapers tested. By contrast, and as expected, the ovule abortion rate in self crosses of homozygous *ClvR*^{ubq7}/*ClvR*^{ubq7}, male escapers and WT was very low.

The mechanism by which maternal *ClvR* rescues some female gametes from death requires further exploration. Recoding associated with use of *A. lyrata YKT61* may have created a messenger RNA with an extended half-life. Alternatively, position effects based on chromatin structure and/or nearby transcriptional regulatory sequences may lead to increased expression and/or extend expression of *A. lyrata YKT61* further into meiosis, resulting in carryover into non-*ClvR*-bearing gametes carrying a LOF mutation in *YKT61*. Incorporation of chromatin insulators[71] and/or targeting of other essential genes can minimize such effects.

Modelling of *ClvR* gamete killer drive in diploid plants

Plants, being immobile, have limited control over who they mate with, a process mediated by wind, water or pollinators. Gene drive can only occur in the presence of outcrossing, which provides an opportunity for different alleles to compete for transmission to viable and fertile progeny. Thus, the potential for gene drive is minimal in self-fertilizing hermaphrodites and maximal in those that engage in obligate outcrossing. Seed-bearing plants (gymnosperms and angiosperms) have a variety of mating systems[72], which can also vary among populations[73]. Most

flowering plants (angiosperms; ~95%) produce male and female gametophytes on the same plant. Many of these are hermaphrodites, with male and female gametophytes in the same flower (perfect flowers), while others are monoecious, with male and female flowers (imperfect flowers) on the same plant. For both these systems (in some but not all species), anatomical features (herkogamy), differences in the time of maturation of male and female gametophytes (dichogamy) or genetic forms of incompatibility can reduce the likelihood of inbreeding[74,75]. Finally, a modest number of species (both gymnosperms and angiosperms) have separate sexes (dioecy), with male and female flowers on separate plants. Here we focus our modelling on dioecious species, in which outcrossing is obligate. Similar principles will apply to hermaphrodites and monoecious species, although inbreeding will always work to slow or prevent drive by reducing the frequency of outcrossing, which allows *ClvR*-associated fitness costs to accumulate and prevents them from being counterbalanced by the relative fitness increase gained from killing of non-*ClvR*-bearing alleles. These last points notwithstanding, it is noteworthy that a number of protein-based TA elements have been identified in rice[9,12,25,26,28–33], a monoecious species with a high inbreeding coefficient[76], and that one of these, the DUYAO-JIEYAO element, has spread to high frequency over the past 50 years[9].

To explore the utility of *ClvR*-based gamete killers for population modification and suppression, we used a stochastic model (a dioecious panmictic population with non-overlapping generations that considers individuals and gametes; see Methods for details) to explore *ClvR* behaviour in several scenarios. This type of model is often used to gain insight into population genetic processes and provides a format that allows comparison of gene drive methods with respect to their basic population genetic features. However, it provides only heuristic guidance and is not predictive for any particular species or environment as it does not include consideration of many other environment- and species-specific variables. These include the mating system, level of inbreeding, overlapping generations (including the presence of seed banks), spatial structure, pollen and seed flow throughout that structure, whether pollen is in excess or limiting for fertilization, and the details of density dependence. Temperature sensitivity of DNA-sequence-modifying enzymes such as Cas9 and how this interfaces with climate and the timing of gamete development and cleavage will also be important to consider.

In animals, gene drive is often modelled using a paradigm in which matings are monogamous and male gametes (sperm) is not limiting. However, in many plants of interest (crops, weeds, targets of conservation) polyandry (fertilization of a female with pollen sourced from multiple males, which is in excess) and polygyny (fertilization by a male of multiple females) is likely to be more relevant[77]. In other contexts not explored here (which will also decrease drive strength) pollen limitation can occur[78]. These aspects of the mating system are important to consider as the relative benefit in transmission frequency that *ClvR*-bearing gametes (or those of any other chromosomal TA element) gain in fertilizing ovules, due to loss of competing non-*ClvR* gametes from the same individual, decreases as the number of competing non-*ClvR* gametes from other males increases. Here we provide some representative examples of outcomes when a pan-gamete killing *ClvR* is introduced into a population and the mating system is monogamous—a best case scenario in which *ClvR* gametes from a single male monopolize the ovary of a female—or polyandrous, with 5 or 20 males each contributing 1/5 or 1/20 of their pollen to a female. We also consider the role of gamete fitness costs, as might arise due to incomplete rescue or cleavage-induced aneuploidy[79] that manifests itself as death during the haploid stage. The effects of maternal carryover of Rescue activity are also explored.

We first consider population modification. Figure 5a–c shows examples in which *ClvR* is introduced at a frequency of 10% into a WT population. The LOF allele creation rate is set to 95%, somewhat lower than the rate inferred from the results of our experiments with *ClvR*^{ubq7} (Figs. 3 and 4). Gametic fitness costs (dominant because they are in the haploid stage) were varied between 0% and 15%. Maternal carryover was set to 0 as exploration of other scenarios shows it has very little impact on population modification. With monogamous mating, *ClvR* spreads rapidly over a range of fitness costs (Fig. 5a). In the presence of polyandry, drive is slowed and fails for some higher fitness costs (Fig. 5b,c). However, spread to high frequency can be restored if the introduction frequency is increased (Extended Data Fig. 7). When drive does occur, *ClvR* spreads to allele fixation. This is because whenever a non-*ClvR* homologous chromosome is present with *ClvR* (and the LOF allele creation rate is high) the former has a very high probability of being eliminated from the viable gamete pool as it lacks a Rescue transgene.

For population suppression, we consider two scenarios, in which *ClvR* is located in a gene (thereby disrupting it) whose recessive LOF in the sporophyte results in female (Fig. 5d–f) or male (Fig. 5g–i) infertility (as originally outlined in ref. 21). As above, gamete killing and rescue occurs in both sexes, and some level of maternal carryover rescue of LOF allele-bearing gametes may be present. In both scenarios, a gamete killer can drive the population towards a homozygous male or female sterile state, resulting in population extinction. A 30% maternal carryover rescue of LOF alleles has a modest negative effect on drive towards a homozygous female sterile state (Fig. 5d–f), while 100% maternal carryover rescue prevents suppression (Extended Data Fig. 8). These negative effects arise because rescue of LOF alleles allows WT alleles at the female fertility locus to persist for some time in non-*ClvR* progeny of a *ClvR*-bearing mother. As with population modification, polyandry and fitness costs can slow or prevent drive and suppression (Fig. 5d–i), but increasing the introduction frequency can be used to compensate (Extended Data Fig. 7).

Effect of mutations in cis and trans on *ClvR* drive behaviour

TA-based drive depends on the creation of the toxin—in the case of *ClvR* the LOF alleles created by Cas9/gRNAs—and thus is dependent on Cas9/gRNA activity and the presence of cleavable target sites. As detailed in our earlier work, population modification by *ClvR*s that spread by killing specific zygote genotypes is relatively insensitive to the presence of a high frequency of Rescue/Cargo/gRNA-only alleles lacking Cas9 function[19]. This fact can be utilized to create versions of *ClvR* that show strong but ultimately self-limited drive for population modification when Cas9 and Rescue/Cargo/gRNA constructs are located at different positions in the genome[38]. *ClvR*-based gamete killers behave similarly. This can be inferred from the results shown in Fig. 6a,b in which a split *ClvR* (Cas9 at one location and Rescue/Cargo/gRNAs at another nearby) is introduced at a frequency of 10%, and Cas9 (which is assumed to be the source of any fitness cost to carriers) recombines away from the Rescue/Cargo/gRNA at a frequency of 1%. Cas9 and gRNAs drive the accumulation of LOF alleles, which select for Rescue/Cargo/gRNAs (Fig. 6a) and initially the tightly linked Cas9, which also increases in frequency (Fig. 6b). However, over multiple generations, recombination onto a non-*ClvR* chromosome leads to loss of Cas9 (analogous to a very high mutation rate) when it finds itself alone in LOF gametes lacking Rescue activity. This, in conjunction with Cas9 loss due to natural selection when its presence results in a fitness cost, brings about an eventual end to drive potential (no new LOF alleles can be created) but often not before the combination of intact elements and Rescue/Cargo/gRNA has spread to fixation. A second example that illustrates the resilience of a *ClvR* gamete killer for population modification to loss of Cas9 activity is shown in Fig. 6c,d, in which *ClvR* is introduced at a starting frequency of 10%, with 20% of these elements lacking Cas9 function. Drive of Rescue/Cargo/gRNA-bearing elements (intact elements and Rescue/Cargo/gRNAs) proceeds to allele fixation with only modest delays compared with a 10% introduction of intact *ClvR*s (Fig. 6c; compare with Fig. 5b,c). The frequency of intact elements,

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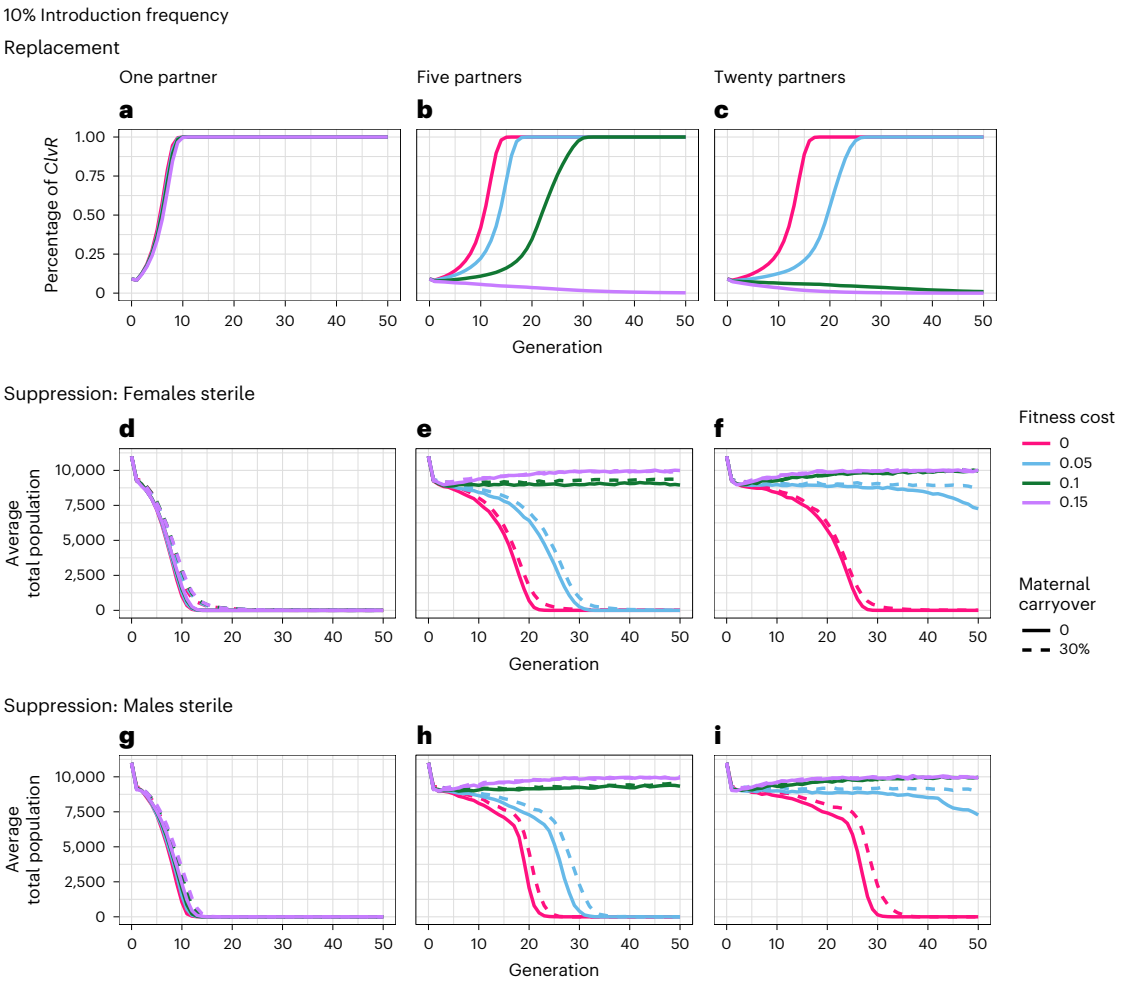

Fig. 5 | Predicted behaviour of *ClvR* for population modification and suppression. a–**c**, Population modification. *ClvR* is introduced as homozygous males at a frequency of 10% of the starting population, which is, at carrying capacity, 10,000 individuals. The mating system is monogamous (**a**), polyandrous with 5 males each providing 1/5 of the pollen needed to fertilize all ovules of an individual female (**b**), or 20 males each providing 1/20 of the pollen needed (**c**). Fitness costs are incurred by gametes (a probability of not being able to participate in fertilization, if chosen by the model). Maternal carryover is set to 0. Lines represent the average of 10 runs. **d**–**f**, Population suppression with a transgene inserted into a recessive locus required for female sporophyte fertility. *ClvR* is introduced as above, at a frequency of 10%. The mating system is monogamous (**d**), polyandrous with 5 males each providing 1/5 of the pollen needed to fertilize all ovules of an individual female (**e**), or 20 males each providing 1/20 of the pollen needed (**f**). Fitness costs are as above. Maternal carryover is set to 0 or 30% (the approximate value observed in our experiments with $ClvR^{ubq7}$). **g**–**i**, As with **d**–**f**, but with the *ClvR* inserted into a locus required for male sporophyte fertility. For these simulations, homozygous females were released into the population as homozygous males are sterile. Lines represent the average of 10 runs.

which also reflects the frequency of Cas9, initially goes up. However, once the combination of intact elements and Rescue/Cargo/gRNAs reaches fixation, the former begins to be eliminated as its presence is associated with a fitness cost that Rescue/Cargo/gRNA elements lack (Fig. 6d).

Drive to allele fixation for population modification can be maintained in the presence of a high frequency of Rescue/Cargo/gRNA-only elements because most of the elements (containing Cas9 and gRNAs) continue to push non-*ClvR* alleles out of the population through creation of LOF alleles. As this occurs in gametes, not zygotes, the Rescue/Cargo/gRNA-only element can only provide a respite from killing until the non-*ClvR* allele finds itself in a heterozygote carrying an intact *ClvR* element, whereupon it is fated to die in a LOF gamete. Similar considerations will apply to gamete killers that work through a traditional protein-based TA mechanism. Antidote-only alleles can provide a respite from killing. But so long as outcrossing brings the non-element-bearing chromosome into regular contact with intact elements, it is fated to be lost. That said, if the presence of the Toxin (or in the case of *ClvR* the Cas9 and gRNAs that create the LOF toxin) results in a cost to carriers, then its mutation to inactivity will ultimately lead to a population composed of Cargo-bearing Rescue/Antidote-only elements (Fig. 6d). These, if they also carry a fitness cost and are not already at allele fixation, will ultimately be lost through natural selection, returning the population to a WT state, a process originally described in modelling of *Medea*, a maternal-effect zygote-killing TA element[16].

Population suppression by a *ClvR* (or other TA element) inserted into a recessive sporophyte fertility locus is also able to tolerate some level of Rescue/Antidote-only alleles. This behaviour is illustrated in Fig. 7 for a population in which a *ClvR* located in a gene required for sporophyte female fertility is introduced at a starting frequency of 10%, with 1% of these elements lacking Cas9 (Rescue/gRNA only elements). Under conditions in which population elimination occurs when an intact *ClvR* is introduced at a frequency of 10% (Fig. 5d–f), elimination also occurs when some Rescue/gRNA-only elements are present. This occurs, as in the case of population modification, because the high frequency of intact elements continues to create LOF alleles, which push the population towards allele fixation for Rescue-bearing

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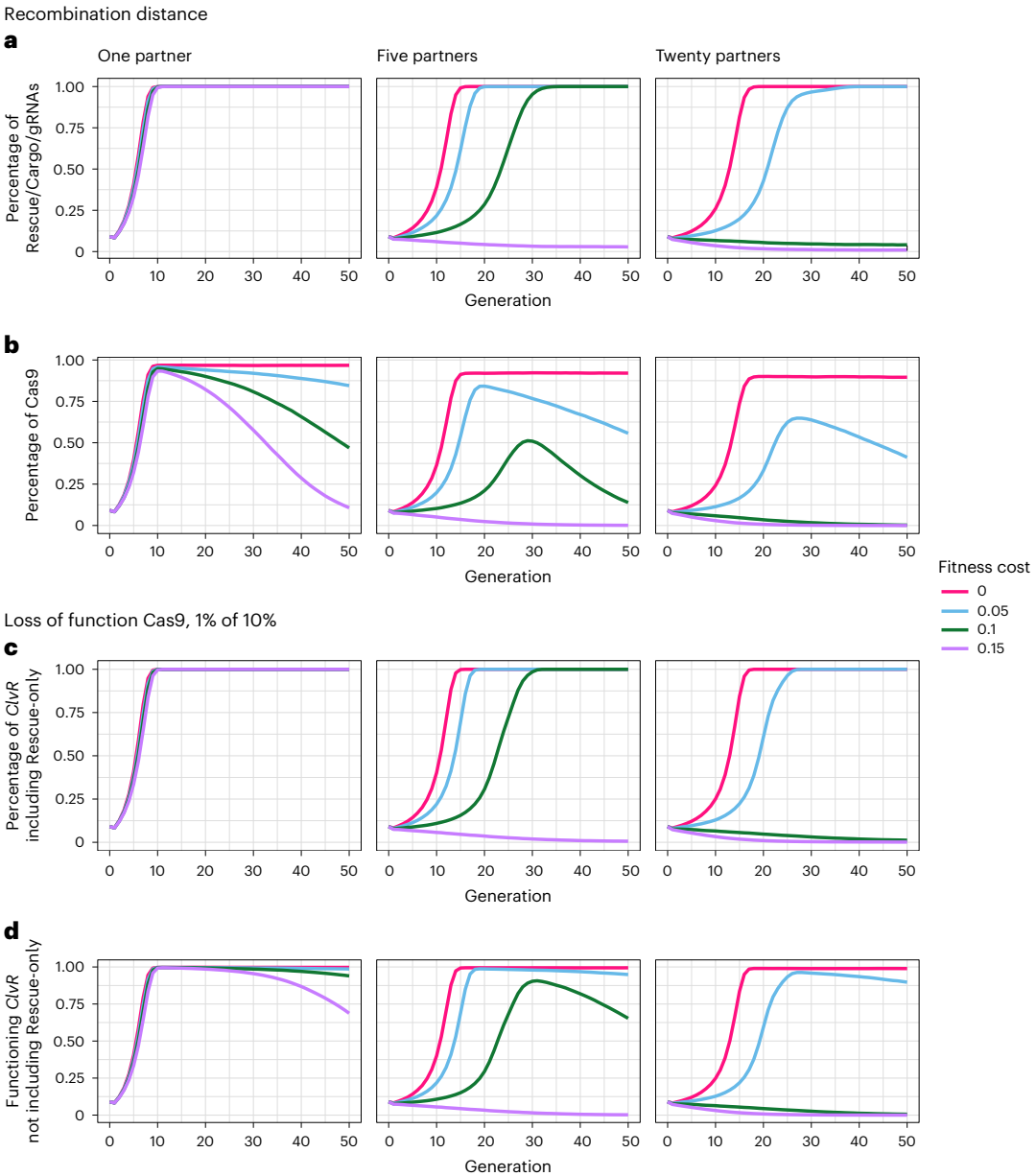

Fig. 6 | *ClvR* gamete drive for population modification tolerates the presence of substantial frequencies of Rescue-only elements lacking Cas9 or gRNA function. a,**b**, *ClvR* is introduced at an allele frequency of 10%. Recombination distance/frequency between Cas9 and Rescue/Cargo/gRNAs is 1%. **a**, The frequency of the combined genotypes carrying a Cargo (intact elements and those consisting of Rescue/Cargo/gRNAs) is shown for 1, 5 and 20 mating partners. **b**, The frequency of Cas9 (which largely reflects its frequency in intact elements) initially increases due to linkage Rescue/Cargo/gRNAs. Recombination into LOF gametes lacking a source of Rescue and natural selection then cause Cas9 to be lost over time. **c**,**d**, *ClvR* is introduced at a frequency of 10%, with 20% (a very high frequency) of these elements lacking Cas9 (Rescue/Cargo/gRNA). **c**, The frequency of the combined genotypes carrying a Cargo (intact elements and those consisting of Rescue/Cargo/gRNAs) is shown for 1, 5 and 20 mating partners. **d**, The frequency of intact elements (which also reflects the frequency of Cas9), goes up initially but then falls as Cas9 is lost, leaving the population (when drive is successful) consisting primarily of Rescue/Cargo/gRNA-only elements. Fitness costs are associated with Cas9 and are thus absent in carriers of Rescue/Cargo/gRNA-only elements. Lines represent averages of 10 individual runs.

elements (intact and Rescue/gRNA elements), a homozygous infertile state for one of the sexes. By contrast, when the fraction of Rescue/gRNA-only elements is 20% of the 10% introduction frequency, or a split *ClvR* is utilized, with a 1% recombination distance between Cas9 and Rescue/Cargo/gRNA, more runs fail (Extended Data Fig. 9). Failures occur in small populations that contain at least one WT allele at the essential gene and fertility loci and few or no intact elements able to create LOF mutations that select against the WT fertility locus. Other features of the population such as inbreeding and spatial structure can further decrease the probability of successful suppression. Inbreeding decreases the mean fitness of TA element-bearing individuals, while spatially structured populations can allow for local extinction and repopulation with viable and fertile genotypes through migration[80–83].

Sequence polymorphisms (naturally occurring or arising through inaccurate DNA repair) that create uncleavable but functional versions (resistant alleles) of a target gene are generally detrimental to gene drive (reviewed in refs. 1,3). Resistant versions of the essential gene can allow non-*ClvR* chromosomes to survive in gametes produced by *ClvR* carriers. However, selection against the non-*ClvR* allele is still very strong in most gametes, which have or will have LOF alleles in the future. In the context of population modification, this can allow *ClvR* alleles to spread to fixation. This is shown in Fig. 8a for a *ClvR* introduction

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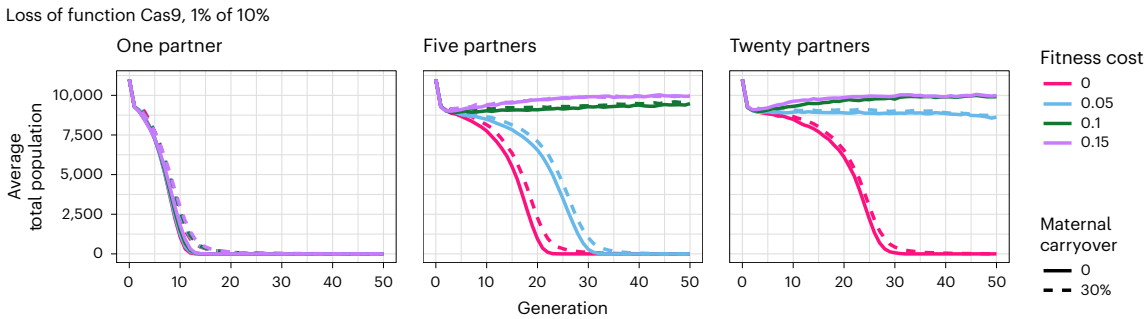

Fig. 7 | *ClvR* gamete drive for population suppression tolerates the presence of modest frequencies of Rescue-only elements lacking Cas9 or gRNA function. Population suppression occurs when 1% of *ClvR*, located in a female fertility gene and introduced as transgenic males at a frequency of 10%, consists of elements that lack Cas9 (Rescue/gRNA). Outcomes are comparable to those observed when *ClvR* is introduced at a frequency of 10%, and all elements are intact (Fig. 5d–f). Fitness costs are associated with Cas9.

frequency of 10% into a wild population carrying resistant alleles at a frequency of 1% (compare with Fig. 5a–c). The frequency of the resistant allele increases along with that of *ClvR* (Fig. 8b). In those populations in which the *ClvR* allele ultimately reaches fixation, the frequencies of LOF and resistant alleles stabilize because there is no longer a fitness difference between carriers of one versus the other (Fig. 8b). By contrast, when drive does not proceed to allele fixation, there is strong selection against the LOF allele, which is then lost along with the *ClvR* allele through natural selection.

Resistant alleles pose a much greater challenge to population suppression. *ClvR*-based selection against the non-*ClvR* allele is generally outweighed (in small populations experiencing density-dependent growth) by the large fitness benefit associated with being a fertile heterozygous *ClvR* sporophyte, made possible by the presence of resistant alleles. An example of suppression failure for a *ClvR* introduction frequency of 10%, with 5×10^{-7} of these (100 heterozygous individuals in a population of 100 million) being resistant, is shown in Fig. 8c. The population undergoes a large initial drop in numbers. This is associated with an initial rise in the frequency of LOF and *ClvR* alleles (Fig. 8c, right panel). The population then rebounds as resistant alleles and non-*ClvR* alleles, which allow survival and fertility respectively, are selected for and accumulate (Fig. 8c, right panel). Thus, as with other cleavage-based population suppression drives such as homing, prevention of resistant allele formation is essential. Results from work in *Drosophila* on *ClvR* by ourselves and others[19,20,37,39,40], as well as this study and that of Liu et al. in *Arabidopsis*[59], along with the results of modelling[84], suggest that multiplexing of gRNAs (four or more) may be able to achieve this goal (however, see the section on polyploidy in the 'Discussion' for a context in which this may not be the case).

When *ClvR* or other TA gametic drive elements are located in a recessive sporophyte fertility gene, there are several other possible mechanisms by which population suppression can be defeated. These elements drive against the WT allele at the fertility locus, suppressing the population as sterile homozygotes accumulate. Movement of the TA cassette or the WT allele of the fertility locus to a new location can disrupt this relationship. Thus, if the TA cassette moves elsewhere—through transposition or some other very rare event occurring probably in a single individual—this creates a new allele that can rescue the survival of gametes lacking a TA element at the original location. Because the new TA allele sits at a neutral location, it can perform this rescue function without risk of infertility (as would occur in homozygotes for the original TA cassette), thereby preventing population extinction, although not drive. This is shown in Extended Data Fig. 10, in which *ClvR*s are introduced at a frequency of 10%, and 1×10^{-5} of those *ClvR* individuals have one copy of *ClvR* translocated to an unlinked neutral position. An initial population suppression is followed by a rebound. A similar effect is observed when rare individuals in a wild population carry a WT version of the fertility locus at an unlinked position (Extended Data Fig. 10). As all eukaryotic chromosomal TA elements (as well as other chromosomal drive elements, such as engineered underdominance) spread by driving against counterpart alleles on the homologous chromosome, and drive meant to bring about population suppression strongly selects for suppressor mutations, it will be important to understand the frequency with which genes and multi-gene cassettes move to new locations and/or are present at multiple locations in existing populations.

The above suppression failures due to cassette/gene movement to a new location can be prevented if the TA drive cassette sits at a neutral location and uses a site-specific nuclease to create LOF alleles in the fertility locus (wherever it is located) only after the time during which the gene product is needed for sporophyte fertility, perhaps during meiosis. In this configuration the TA element mediates gametic drive towards homozygosity, while cleavage and LOF allele creation in the fertility gene during meiosis (which must still avoid resistant allele formation) ensures that all homozygous progeny (males or females), but not heterozygotes, are sterile. However, transcriptional regulatory sequences/Cas9 variants that can achieve this goal with high efficiency have yet to be identified.

Finally, we note that in the context of population modification, the Cargo will also mutate to inactivity at some frequency in any gene drive system. It may also lose effectiveness due to evolution of the host or pathogen it is meant to counter. Recombination between chromosomal TA drive elements and WT homologous chromosomes can also—in some configurations but not others[19,34]—lead to the creation of Rescue-only elements lacking Cargo. For chromosomal TA drive elements, the consequences of Cargo loss/failure can be ameliorated by carrying out a second round of population modification in which a first-generation element, at its original location, competes against a next-generation element (carrying a new Cargo, Toxin and Antidote, and a copy of the first-generation Antidote) at the same location. The latter drives itself in while driving the first-generation element (and any remaining WT alleles) out of the population[34,37]. Next-generation gamete killers are expected to be particularly efficient as they spread to allele fixation, which leads to complete elimination of first-generation elements.

Discussion

Our results, along with those of Liu and colleagues[59], argue that gamete killers based on a *Cleave* and *Rescue* mechanism provide a general strategy for achieving gene drive-mediated population modification or suppression in outcrossing diploid plants. *ClvR* elements utilize a simple toolkit of components that should be available in many species: a site-specific DNA-modifying enzyme such as Cas9 and the gRNAs that guide it to specific targets, sequences sufficient to direct gene expression in cells that will become the germline (which, as in our work,

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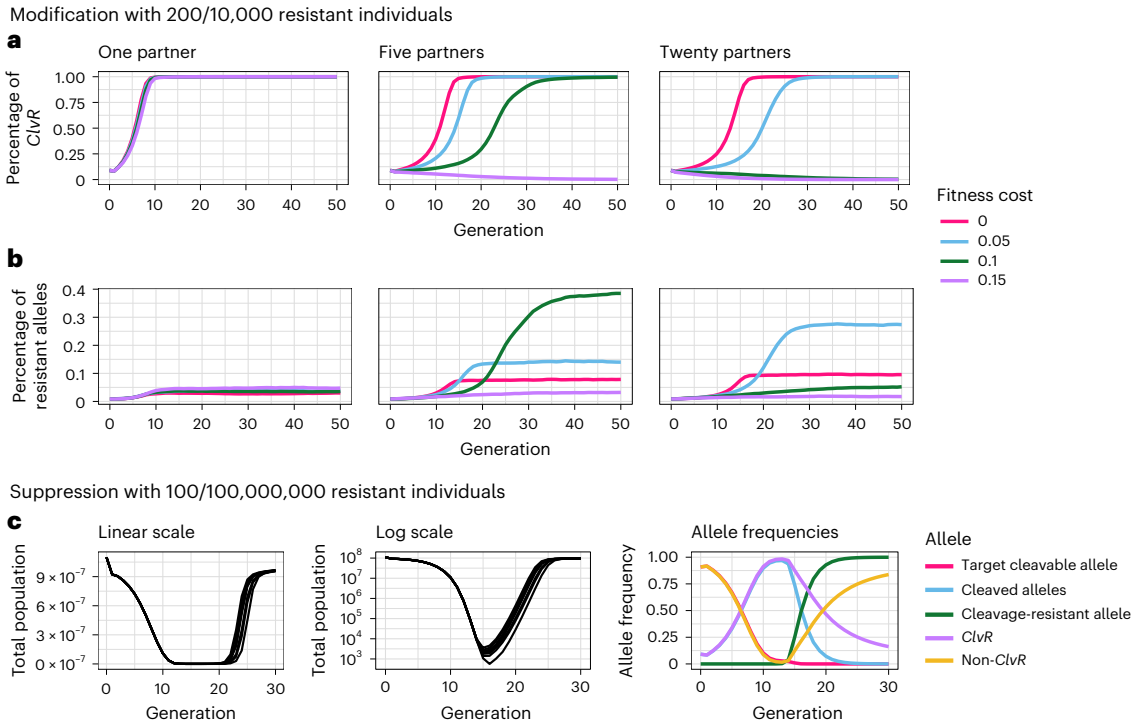

Fig. 8 | Population modification but not population suppression can occur in the presence of resistant alleles. a,**b**, Population modification in the presence of a resistance allele frequency in the wild population of 1% and a *ClvR* introduction frequency of 10%. **a**, *ClvR* spreads to allele fixation under conditions that also support drive when no resistant alleles are present (Fig. 5a–c) but with some delay. **b**, The LOF alleles created by *ClvR* select for the presence of resistant alleles, which increase in frequency. However, *ClvR* can still reach allele fixation in many cases **a**) because the large number of LOF alleles created from cleavage sensitive essential gene alleles still select against the non-*ClvR* allele. If *ClvR* reaches fixation, the frequency of resistant alleles stabilizes because these alleles no longer have a selective advantage. **c**, *ClvR* is introduced at a frequency of 10%, with a resistance allele frequency in the wild population of 5×10^{-7} (100 resistance allele heterozygotes in a population of 100,000,000 diploid individuals). Total population over time is plotted on a linear scale (left) and a log scale (middle). Right: the dynamics of alleles at the *ClvR* and essential gene loci over time.

need not be germline-specific), an essential gene to act as target and a recoded version of the essential gene resistant to sequence modification and able to rescue the LOF condition. For population modification, these components are best located at sites distant from the target essential gene. For several strategies for population suppression, the element needs to be located in a gene whose recessive LOF in the sporophyte results in either male or female infertility. Many such genes are known[85–88], particularly for male fertility. Alternatively, if LOF alleles of the sporophyte fertility gene can be efficiently created late in germline development, after the time when gene function is needed for fertility, then the element can be located anywhere in the genome.

An alternative strategy for population suppression in some plants is suggested by modelling and experiments in animals focused on the creation of sex-linked gamete killers. The goal of these efforts is to create Y-linked killers of X-chromosome-bearing sperm, resulting in males that only produce male progeny (reviewed in refs. 1,3). Such a system can be used to drive population suppression or elimination (towards an all-male state) when sperm is not limiting and females mate with one or a few males[13,42]. Many dioecious plants lack sex chromosome with well-defined regions whose presence is sufficient to confer a specific sex on carriers. However, for those that do carry such regions[89,90], it may be possible to engineer a similar behaviour. For example, if a pan-gamete killing *ClvR* (or a killer of non-*ClvR* gametes only in pollen) is tightly linked to a gene or genes that are sufficient for male sex determination (for example, refs. 89,91) then *ClvR*-bearing individuals (by definition, males) will only produce *ClvR*-bearing sons and pollen that gives rise to male progeny. As discussed in the context of Fig. 5, drive towards population extinction becomes weaker as the level of polyandry increases, but may still occur in a timely manner if the introduction frequency is increased. Notwithstanding these points, it is important to note that even in outcrossing species, inbreeding and spatial structure can work to prevent elimination of a population[80–83].

The *Cleave* and *Rescue* mechanism could also be used in a non-gene drive strategy for population suppression. Modelling and experiments in insect systems show that periodic releases of males carrying an autosomal transgene that gives rise to fertile males and inviable or sterile females can bring about population suppression or elimination by driving a progressive decrease in the number of fertile females[92–94]. Such an element does not show self-sustaining drive because it finds itself in dead-end females half the time. However, its persistence over multiple generations in fertile males provides an ongoing force that contributes to a reduction in the number of fertile females. Such an element could be created in diploid plants (dioecious, monoecious or hermaphrodite), although self-fertilization will always reduce effectiveness in hermaphrodites and monoecious species that lack strong incompatibility systems. There are several possible approaches. In one a pan gamete *ClvR* (or other protein-based TA element) is located at a neutral position and carries a transgene that dominantly blocks female gamete development. However, this system is not evolutionarily stable as, if the transgene needed to block female fertility is inactivated by mutation, one is left with a self-sustaining gamete killer drive element. A more stable strategy involves locating a pan gamete killing *ClvR* element within (thereby disrupting) a gene whose expression in female gametes is required for their survival. Reproductive structures of individuals carrying this construct only produce *ClvR*-bearing pollen. Female gametes that inherit the *ClvR* die because they lack the female gamete essential gene while those that lack the *ClvR* die because they lack a functional copy of the pan gamete essential gene targeted by *ClvR* for LOF allele creation. Loss of Cas9 function through mutation

in a small fraction of the suppression strain can allow the survival of some Rescue/gRNA-only individuals, but their presence does not block suppression because they cannot be transmitted through the female germline.

Any gene drive method, when it does not provide an unalloyed fitness benefit to carriers, is sensitive to mutational inactivation. Second site suppressors may also be selected for that block drive or its intended consequences. Our modelling suggests that population modification is relatively insensitive to mutation of Cas9/gRNA to LOF or the presence of a modest frequency of resistant alleles at the essential gene locus. Population suppression can also occur in the presence of a modest frequency of elements that lack Cas9 but is very sensitive to the presence of resistant alleles, as with homing-based strategies. Suppression through some mechanisms is also sensitive to movement of the TA element or a WT allele of the fertility gene to a new chromosomal location. Finally, while the Cargo can also undergo mutational inactivation or loss of efficacy, population modification and suppression strategies with chromosomal TA elements can be made resilient—able to recover from breakdown—using next-generation elements that drive old elements out of the population while driving themselves in.

Our experiments focused on cleavage and rescue of the ubiquitously expressed R-SNARE *YKT61* gene. It is likely that many other ubiquitously expressed housekeeping genes can be targeted to similar effect. Alternatively, drive can be limited to one sex or the other by targeting genes required more specifically for gametogenesis in only one sex (for example, refs. 49,50). Liu and colleagues used just such an approach, targeting the *NO POLLEN GERMINATION 1* (*NPG1*) gene for cleavage and rescue in *A. thaliana*. Carriers of this construct show high levels of *ClvR*-biased segregation distortion through pollen but not ovules[59]. Ideally, Cas9 expression, cleavage and LOF allele creation would be limited to cells of the appropriate reproductive organ or meiosis, to minimize fitness costs associated with Cas9 expression or heterozygosity for LOF mutations in the target gene (haploinsufficiency) and allow for targeting of fertility genes after the time in development they are needed. These were the reasons we tested regulatory sequences from genes with restricted expression patterns that include the future germline: *DMC1* and *APETELA1*, *CLAVATA3* and *AGAMOUS*. Among these, only *APETELA1* sequences showed evidence of strong drive in males. Low levels of drive were observed in females. Based on the results of experiments discussed above, we speculate this may be because of particularly strong maternal carryover rescue of gametic LOF alleles. Even when using the *UBIQUITIN10* sequences to drive Cas9 expression, providing many opportunities throughout development to cut and create LOF alleles, we observed a low frequency (~1%) of uncut/unmodified alleles at the *YKT61* locus in escapers. This is not due to transgene silencing as the construct was not present in escaper seeds (Extended Data Fig. 6). Nucleosome structure has been shown to inhibit Cas9 cleavage efficiency[95–97] and could play a role, although it is surprising that all four sites remained uncleaved. Only the results of more experiments with diverse promoters, and other RNA-targeted DNA sequence modifying enzymes that cleave or create LOF mutations through other mechanisms such as base editing, acting on *YKT61* and other target genes, will provide guidance on how best to ensure that all target sites are modified. Regardless, our modelling shows that a low frequency of WT escapers (which are still subject to cleavage in future generations) does not prevent population modification or suppression.

Plants are the backbone of life on earth and the source, directly or indirectly, of most human food. Given this, it is important to consider whether gene drive in plants constitutes a dual use research of concern (DURC). In the context of biology, DURCs are research products that, while designed to provide a clear benefit, could potentially be misused to threaten public health and safety, agricultural crops and other plants, animals or the environment[98]. As discussed in earlier work[99], gene drive in plants and animals in general does not lend itself to DURC applications. First, spread of a drive element to high frequency is very slow, because it requires many generations of outcrossing. In the case of plants, generations tend to be seasonal (yearly) at best, and inbreeding, which slows drive, is common. Drive elements that work indirectly, by killing those that fail to inherit them, are (compared with drives that home at high frequency) particularly slow to spread when introduced at low frequency[1,17,19,20]. Second, in modern agriculture, crop breeding and seed production and distribution typically occur under tightly controlled conditions using specific genotypes, making it unlikely that other genotypes could be introduced into the production/food chain without detection[100]. Related to this last point, gene drive is easily detected if searched for, either through observation of phenotypic changes in a population or through genome sequencing. Finally, gene drive for population suppression can and has been blocked—many times—through the creation of resistant alleles (reviewed in refs. 1,3). It has also been blocked through introduction of a transgene that inhibits Cas9 function[101,102], and it can in principle be blocked using a second modification drive that actively targets key components of the initial drive element of concern. The consequences of population modification (although not necessarily a rapid return to the pre-transgenic state) can also be prevented through the use of next-generation elements that drive a first-generation element out while driving itself in[37].

The above points argue that gene drive in plants is unlikely to constitute a DURC technology. However, the frequent ability of plants to hybridize across species barriers[103,104] calls attention to several competing challenges related to drive, resistance to drive, and gene flow. Gene drive with the *ClvR* system can be limited to a specific species by designing gRNAs that are species-specific. If hybridization does occur in this context, the WT essential gene alleles from the relative are, by definition, resistant. These will block spread in the non-target species but may still allow population modification in the target species, depending on the rate of hybridization and thus the frequency of resistant alleles (Fig. 8a,b). However, as noted above (Fig. 8c), resistant alleles would prevent population suppression in the target species. Alternatively, gRNAs can be utilized that target the essential gene for LOF allele creation in all possible hybridization partners. This should support population modification and suppression in the target species but may also result in modification or suppression in non-target species. Protein-based TA systems, which typically target conserved biological processes rather than specific genomic sequences (for example, ref. 9), may behave similarly. Thus, in considering TA-based gene drive in plants, it will be important to understand the full spectrum of mating partners and possible ecological outcomes associated with drive, both within a target area and in non-target areas connected by migration.

While our experiments and modelling focused on *A. thaliana*, a diploid with a relatively small genome, many plants of interest are polyploid[105]. Large genomes and polyploidy create several challenges. First, large genome size means Cas9 must sample a much larger genomic sequence space in a timely manner[106], which will require increased expression levels or the use of variants with increased catalytic activity. Second, polyploidy may release duplicated genes, even those encoding highly conserved housekeeping genes, from selective pressures that constrain their coding sequence, making it more difficult to identify gRNA target sites that remain unchanged. The design of gRNAs will be particularly challenging in allopolyploids, which have two or more complete sets of chromosomes from different species. The gene dosage needed for rescue (one or multiple copies) also needs to be explored for polyploids. Suppression mechanisms that require insertion of *ClvR* into a gene required for gamete function may be challenging for related reasons. In sum, while our work and that of Liu et al.[59] show that the conditions for *ClvR*-based gene drive in plants can be achieved, much remains to be considered as to species and contexts in which the key mechanisms required for drive (high-frequency creation of LOF alleles and rescue) are most likely to be efficient and evolutionarily robust and in which gene flow can be managed.

How might gene drive be applied in plants? Spread of agricultural traits, weed control and evolutionary rescue have all been suggested[4–6]. As discussed above in the context of DURC research, drive itself is unlikely to be used in spreading agronomic traits into major production crops as seed production (often of hybrids) and distribution is a highly regulated process[100]. Gene drive that causes death of non-carriers (compared with homing, which can immediately create homozygotes from heterozygotes) is also unlikely to dramatically speed the breeding process. Possible exceptions where population modification could provide some utility include wild plants used as forage for livestock on the range or in aquafarming. In these contexts, potential target species will often not have undergone selection by humans and thus might benefit from introgression of genetic changes that enhance food traits and/or resilience in the face of current or impending environmental stresses.

The most proposed application of gene drive in plants is weed control. This could take the form of population suppression or sensitization, in which the goal is to drive a trait into the population that makes the species less fit in a managed agricultural environment or specifically sensitive to some other intervention, such as herbicide application. One species that has been suggested as a good target for gene drive is *Amaranthus palmeri* (Palmer amaranth)[4], an invasive agricultural weed that is very economically destructive and difficult to manage[107]. The features that make Palmer amaranth amenable to gene drive-mediated suppression and/or sensitization are that it is an annual, dioecious and diploid, and a region containing genes associated with male sex determination has been identified[91,108]. Finally, in many locations, Palmer amaranth has become resistant to available herbicides, with a key source of resistance (and thus a good target for mutation) being a large autonomously replicating extrachromosomal circular DNA transmitted through pollen[109,110]. These attractive features for drive that results in suppression and/or sensitization notwithstanding, Palmer amaranth also exemplifies potential challenges and trade-offs. It produces a very large number of seeds (100,000–500,000) per plant and can also hybridize with related species[111], some of which are also weeds. It is also native to Northern Mexico and the Southwestern United States and has cultural significance to Native Americans, who have used it as a food source[107]. These facts highlight the topics of evolutionary stability, gene flow and social acceptability, subjects that have not been explored in plants, particularly in the context of highly managed modern agricultural environments where the goal will be very local rather than global population control.

Gene drive in plants, as well as animals, has also been suggested as a tool for bringing about evolutionary/genetic rescue, the process by which a species threatened with extinction adapts rapidly enough to survive. Evolutionary rescue involves bringing new individuals into a population. This increases population size, buffering it against stochastic fluctuations, while at the same time introducing genetic variants that can decrease inbreeding depression and—when they are present at high frequency—increase absolute population fitness (the ability of the population to increase in size) through adaptation. Here we focus on the role of new adaptive variants. The question evolutionary rescue strategies face is whether modest introductions of these variants can bring about an increase in population fitness before stochastic effects take the population below a critical density that leads to extinction[112,113]. While introduced beneficial alleles will spread through natural selection, the rate of spread (and thus the time the population spends near the critical density) depends on the strength of selection and whether the beneficial alleles are dominant, additive or recessive. We speculate that there may be some contexts in which gene drive can increase the rate of allele spread, thereby keeping average absolute population fitness (and thus population size) higher than it would be otherwise, supporting recovery. However, modelling that tests this hypothesis by comparing the rescue effects of a beneficial Mendelian allele that spreads through natural selection—which requires the death of non-carriers—with that of a similar allele also subject to a gene drive that does not require the death of non-carrier adults (which decreases population size) remains to be carried out. Finally, we note that gene drive, but not Mendelian transmission and natural selection, could be also used in an anticipatory manner to spread genetic variants that do not confer a strong benefit now but that will be beneficial under likely future conditions.

Methods

Synthesis of Arabidopsis *ClvR* constructs

In this study, constructs were assembled using Gibson cloning[114]. The gRNA cassette, composed of four repeats of gRNA with U6 promoters, was cloned with Golden Gate assembly. Enzymes utilized were obtained from New England Biolabs (NEB), and cloning as well as DNA extraction kits were sourced from Zymo. The *A. lyrata* Rescue gene was synthesized by Twist Bioscience.

We began with an intronized Cas9 variant known as zCas9i (pAGM55285, which was a gift from S. Marillonnet (Addgene 153212)[63]. In this Cas9 version, we replaced the RPS5 promoter with that of *DMC1*. In addition, immediately upstream of the start codon, we incorporated 21 base pairs from TAIR10 genome assembly locus AT1G58420, which had previously been demonstrated to enhance translational efficiency[115]. Finally, we integrated the recoded *A. lyrata* Rescue into the construct. A detailed sequence map including all primers used is provided in Supplementary Data 1.

gRNA design and cloning

To assemble the gRNA cassette, we used the shuttle vectors from Stuttmann et al.[61] (pDGE332, pDGE333, pDGE335 and pDGE336, which were a gift from J. Stuttmann, Addgene 153241, 153242, 153243 and 153244). Guides were designed in Benchling to target exon 1 (gRNA1 and gRNA2), exon 2 (gRNA3) and exon 5 (gRNA4) of *YKT61* and cloned into the Bbs1 digested shuttle vectors with annealed primers. The final Golden Gate assembly was performed with the Cas9-Rescue plasmid from above and Bsa1.

Cas9 promoters

We could not detect any cleavage with the intronized Cas9 and the *DMC1* promoter, as inferred by the Mendelian inheritance of the full *ClvR* construct in multiple transgenic lines. Based on these results, we replaced the intronized Cas9 with one that had no introns but retained the nuclear localization sequence at the termini (Cas9 without introns from pTX168, which was a gift from D. Voytas, Addgene 89257)[116]. In addition, we introduced a mutation (K918N) in the Cas9 sequence that was shown to enhance its catalytic activity[62]. However, the *DMC1*-Cas9 version without introns also showed no evidence of cleavage. Based on the results obtained with other promoters (*APETELA1* and *UBIQUITIN10*) and this version of Cas9, we inferred that the *DMC1* promoter is likely to be relatively weak. For all additional promoters tested here, we used the version of Cas9 version lacking introns and carrying the K918N mutation.

Using Gibson assembly, we replaced the *DMC1* promoter with transcriptional regulatory sequences from *APETALA1*, *CLAVATA3* and *AGAMOUS* (chosen based on their efficacy in previous work utilizing a Cre/Lox reporter)[117]. Finally, we also built two versions of *ClvR* utilizing regulatory sequences that drive more ubiquitous expression, from the *UBIQUITIN10* gene and the *CaMV35S* promoter. Whole plasmid sequencing was performed by Plasmidsaurus, using Oxford Nanopore Technology with custom analysis and annotation. Genbank files of all *ClvR* constructs with attached Gibson cloning and sequencing primers utilized in this study are in Supplementary Data 1.

Arabidopsis handling

All plants in this study were grown in soil with a 16 h/8 h light/dark cycle. Temperature was 25 °C. All seeds were planted directly in soil and stratified at 4 °C for 3 days. Transgenic plants were maintained

in separate dedicated room in a hood mounted with a screen and surrounded by sticky tape, to minimize airflow around the plants and to prevent potential insect-mediated pollen movement. A floor-mounted sticky surface surrounding the hood performed a similar function. Transgenic plants were disposed of following autoclaving.

Arabidopsis transgenesis

We used the floral dip method with agrobacteria as described previously[118]. *ClvR* plasmids were transformed into GV3101 ElectroCompetent *Agrobacterium* strain from Intact Genomics. T1 seeds were screened for the FAST red seed marker[119] and planted as described above.

Crosses to determine *ClvR* drive activity

Red T1 seeds were grown and allowed to self cross. Siliques (seed pods, with each pod representing the fertilized ovules of a single ovary/flower) of these plants were screened for the FAST red marker again. We looked for plants that showed 100% *ClvR* bearing seeds, suggesting drive activity (Mendelian genetics would result in 75% red seeds). At this stage, we saw that $ClvR^{dmc1}$, $ClvR^{agamous}$ and $ClvR^{clavata3}$ had less than 100% red seeds in the self crosses and decided not to further characterize these lines.

T2 *ClvR* seeds were grown, and pollen from these plants was used in an outcross to WT females to generate heterozygous *ClvR*/+ T3 seeds. T3 seeds were grown into adults again to set up reciprocal crosses with WT. Female *ClvR*/+ for each line were crossed to male WT, and male *ClvR*/+ were crossed to female WT (four crosses per plant, four plants per line). Siliques of these crosses were scored for the *ClvR* marker (results in Fig. 2).

Next, we took T4 seeds from individual T3 crosses and repeated a set of reciprocal (male *ClvR*/+ to female WT and female *ClvR*/+ to male WT) crosses. We crossed four plants with four crosses per plant for each of three individual T3 crosses (results in Fig. 3). We also collected leaf tissue from T4 heterozygous *ClvR* plants and escapers from the female *ClvR*/+ × male WT cross (non-*ClvR* bearing seeds) to extract DNA and sequence the *YKT61* target sites (see 'Molecular analysis of cleavage events'). Sequencing results are in Supplementary Table 1 (Cleavage events). Finally, we grew T5 escaper seeds coming from female *ClvR*/+ and from male *ClvR*/+. Leaves of young plants were again collected and target sites sequenced as described below.

Molecular analysis of cleavage events

DNA from candidate plants was extracted from leaves with the Zymo Quick-DNA Plant/Seed miniprep kit according to the manufacturer's protocol. The *YKT61* target region was PCR amplified using primers ykt-cleaveF1 (TAGCATCTCCGAGTAAGGAATC) and ykt-cleaveR2 (CTTATAGATTTAGTTTCCTTTTTTCCCTGT). The PCR fragment was purified following agarose gel electrophoresis and sequenced by Plasmidsaurus at ~1,000× coverage. The resulting raw reads were mapped to the *YKT61* reference using minimap2 (version 2.17)[120]. The alignment file was sorted and indexed with samtools (version 1.13). The output file variants were then clustered with a Python script from Pacific Biosciences (https://github.com/PacificBiosciences/pbampliconclustering). Mutations were analysed in the output 'variantFraction' file. Results are summarized in Supplementary Table 1 (Cleavage events). Sequencing files are in Supplementary Data 2.

T-DNA insertion site

To determine the transfer-DNA (T-DNA) insertion site of line $ClvR^{ubq7}$, we extracted genomic DNA from two different plants and constructed sequencing libraries using NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB E7805) following manufacturer's instructions. The libraries were sequenced on Illumina NextSeq2000 in paired end mode with read length of 150 nt to the sequencing depth of 35 million paired end reads per sample. Base calls were performed with DRAGEN BCL Converter (version 3.10.12), and structural variant analysis was performed with the DRAGEN Germline pipeline v3.10.12 against the *Arabidopsis* TAIR10 genome[121]. The resulting VCF files contained information on large structural variants (insertions). We identified a single T-DNA insertion on chromosome 3 (Chr3:10231731). The insertion was confirmed with PCRs followed by sequencing of the resulting amplicons (Supplementary Data 2). Supplementary Fig. 1 shows the genomic location.

Modelling

Modelling was performed using a stochastic agent-based model with discrete generations written in python. The model uses various classes to keep track of haploid gametes, diploid individuals and simulation parameters. The lowest level class is diploid, which tracks the genotypes and alleles of a single individual. The next highest class is haploid, which tracks its own alleles and also its diploid parent. The highest class, in which most of the data are stored, is StochasticSim, or FastStochasticSim. This class object contains all the parameters necessary to run a simulation and can store the individuals that are created over the course of a simulation. The parameters stored by StochasticSim include the alleles and genotypes possible, the haploid and diploid fitnesses associated with each genotype, the fecundity of each individual and other parameters used over the course of the simulation. The function stochastic_sim calls on a StochasticSim object to perform the simulation and populate the generations. Each generation starts with a pool of adult individuals (the introduced individuals for generation 0, and the previous generation's mature adults for each following generation). Each adult produces a pool of gametes, and each pool of ovules is matched with one or more pools of pollen, which are congregated together to form one single pollen pool. These gamete pools are then reduced by their haploid fitness costs, which are set as part of the simulation parameters. Surviving pollens and ovules are then matched, with each ovule–pollen pair producing a possible offspring. All possible offspring from these matings are randomly grouped as male or female and are then culled based on diploid fitness and expected survival. Expected survival is based on two factors: the number of offspring per mating and density-dependent growth. At carrying capacity, each offspring in a litter of size N should have a $2/N$ chance of survival, leading to perfect population replacement. This chance of survival is multiplied by a density-dependence function S, which takes in some population size P, the carrying capacity K, and a growth rate g that corresponds to 1/2 the expected maximum number of surviving offspring per mating when the population is at low densities. The function is $S(P) = g/[1 + (g - 1) \times P/K]$. When P is close to K, then $S = 1$ and the chance of survival of each offspring remains $2/N$. However, at low densities, $S = g$, increasing the survival of each offspring to $2 \times g/N$. For our simulations, we used $g = 6$. The offspring that survive the culling become the parents of the next generation. A diagram of this process and more details are included at https://github.com/HayLab/Pigss and in Supplementary Fig. 2.

The function stochastic_sim is called by run_stochastic_sim, which handles doing multiple runs and writing the data out to files. More details are provided on the specifics of stochastic_sim at https://github.com/HayLab/Pigss. For the data shown, we assumed *ClvR* and the target gene were unlinked, that our *ClvR* element had 95% efficiency in creating LOF alleles and that the population had a low-density growth rate of 6.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data are available in the main text and the Supplementary Information files. Illumina sequencing reads were deposited to SRA (bioproject,

PRJNA1074841). The *Arabidopsis* TAIR 10 genome assembly was used in this study. Constructs and seeds of transgenic plants created in this study are available upon request.

Code availability

Modelling code and more information on the model, the scripts and parameters used to generate the data, and the data itself can be found at https://github.com/HayLab/Pigss. Plots were generated in R (version 4.2.3) with the ggplot2 package[122].

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Author contributions

Conceptualization, G.O., T.I. and B.A.H.; methodology, G.O., T.I., M.L.J., I.A. and B.A.H.; investigation, G.O., M.L.J., I.A. and B.A.H.; writing—original draft, G.O. and B.A.H.; writing—review and editing, G.O., T.I., M.L.J., I.A. and B.A.H.; funding acquisition, B.A.H.

Competing interests

The authors have filed patent applications on *ClvR* and related technologies (US application numbers 15/970,728 and 16/673,823).

Additional information

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Correspondence and requests for materials should be addressed to Bruce A. Hay.

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Alyr-recoded      atgaagatcacTGCTTTGTTGGTTTTGAAGTGTgctcccgaaGcttctgatcccgttatc  60
Athal             ATGAAGATCACCGCCTTGCTCGTTCTCAAGTGCGCTCCCGAAGCTTCTGATCCCGTCATC  60
                  *********** ** *** * *** * ***** ************************ ***
                                                  gRNA1
Alyr-recoded      ctcTCTaacgcttccgacgtctctcacttcggtTATTTTCAAAGATCTTCTGTTAAAGAA  120
Athal             CTCTCCAACGCTTCGGACGTCTCTCACTTCGGTTATTTCCAGCGATCTAGCGTCAAGGAG  120
                  ***** ******** ********************** **.*****:  ** **.**.
                                                              gRNA2
Alyr-recoded      ttcgtcgtctttgtcGGAAGAACTGTTGCTTCTAGAACTCCTCCTTCTCAAcgccagtct  180
Athal             TTCGTCGTCTTTGTCGGTCGCACTGTCGCTAGCCGAACCCCTCCTTCCCAACGCCAGTCT  180
                  ****************:.*.***** ***:  .**** ******** ************

Alyr-recoded      gtccaacacgaagagtacaaggttcacgcttacaatagaaatggcctttgcgcggtgggg  240
Athal             GTTCAACACGAAGAGTACAAGGTTCACGCTTACAATAGAAATGGCCTTTGCGCGGTGGGT  240
                  ** ************************************************************

Alyr-recoded      ttcatggatgatcattatcctGTTAGATCTGCTTTTTCTcttcttaatcaggtcctagat  300
Athal             TTCATGGACGATCATTACCCTGTTCGAAGTGCTTTCTCTCTTCTCAATCAGGTCCTAGAT  300
                  ******** ******** *******.**: ****** ******** ***************
                                                  gRNA3
Alyr-recoded      gagtaccagaagagttttggtgagTCTtggaggtctgcaaaagaagactccaAtcagcct  360
Athal             GAGTACCAGAAGAGTTTTGGTGAGTCATGGAGGTCTGCAAAAGAAGACTCCAATCAGCCT  360
                  **************************:*********************************

Alyr-recoded      tggccatacttaactgaagctttaaacaaatttcaggacccagcagaggctgataagctg  420
Athal             TGGCCATACTTAACCGAAGCTTTAAACAAATTTCAGGACCCAGCAGAGGCTGATAAGCTG  420
                  ************** ********************************************

Alyr-recoded      ttgaaaatccagagggagttggatgaAaccaagattatccttcataaaaccattgatagt  480
Athal             TTGAAAATCCAGAGGGAGTTGGATGAGACAAAGATTATCCTTCATAAAACCATTGATAGT  480
                  **************************.**.*****************************

Alyr-recoded      gttctagcccgtGGAGAAAAGTTGGATTCTTTGGTTGAAaagagctcagatttaagcatg  540
Athal             GTTCTAGCCCGTGGTGAGAAGCTGGACAGCCTAGTGGAGAAGAGCTCAGATTTGAGCATG  540
                  **************:**.*** **** :   *.** **.**************.******
                                                  gRNA4
Alyr-recoded      gcatcacagatgttttacaagcaagcgaagaaaacaaattcatgctgtactattctgtga  600
Athal             GCATCACAGATGTTTTACAAGCAAGCGAAGAAAACAAATTCATGCTGTACTATTCTGTGA  600
                  ************************************************************


Alyr-ykt61      MKITALLVLKCAPEASDPVILSNASDVSHFGYFQRSSVKEFVVFVGRTVASRTPPSQRQS    60
Atha-ykt61      MKITALLVLKCAPEASDPVILSNASDVSHFGYFQRSSVKEFVVFVGRTVASRTPPSQRQS    60
*               ************************************************************

Alyr-ykt61      VQHEEYKVHAYNRNGLCAVGFMDDHYPVRSAFSLLNQVLDEYQKSFGESWRSAKEDSNQP    120
Atha-ykt61      VQHEEYKVHAYNRNGLCAVGFMDDHYPVRSAFSLLNQVLDEYQKSFGESWRSAKEDSNQP    120
*               ************************************************************

Alyr-ykt61      WPYLTEALNKFQDPAEADKLLKIQRELDETKIILHKTIDSVLARGEKLDSLVEKSSDLSM    180
Atha-ykt61      WPYLTEALNKFQDPAEADKLLKIQRELDETKIILHKTIDSVLARGEKLDSLVEKSSDLSM    180
*               ************************************************************

Alyr-ykt61      ASQMFYKQAKKTNSCCTIL*    199
Atha-ykt61      ASQMFYKQAKKTNSCCTIL*    199
*               ******************
```

Extended Data Fig. 1 | Alignment of the recoded *A. lyrata* rescue coding region to the *A. thaliana* target. Guides are indicated as red arrows. Note that the full sequence of the *A. lyrata* YKT61 genomic region used for rescue (Supplementary File 1) contains many additional differences from the equivalent *A. thaliana* sequence, in regulatory sequences, introns and 5′ and 3′ UTR. The amino acid sequences of the two proteins are identical.

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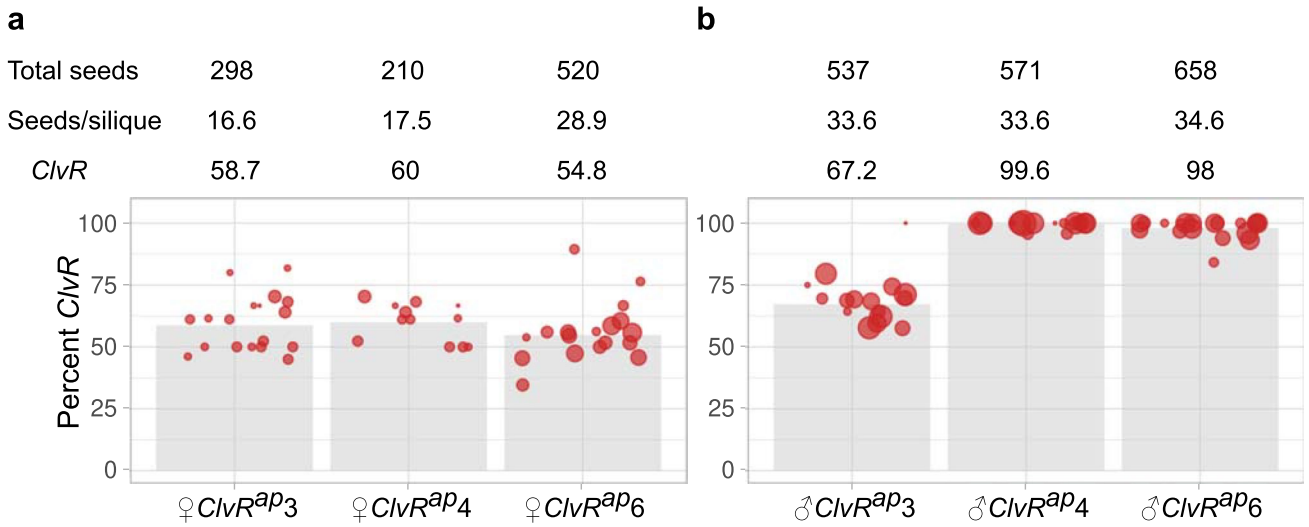

Extended Data Fig. 2 | T3 heterozygous *ClvR* crosses for (a) female *ClvR*^{ap} and (b) male *ClvR*^{ap}. T3 *ClvR*^{ap}/+ heterozygotes were grown to adulthood and their ovules (left three columns) or pollen (right three columns) used in outcrosses to WT. Bar graphs show the number of siliques scored (red circles) and the percent *ClvR* seeds produced in the T5 generation. The number of seeds within each silique scales with circle size. Counts are in Supplementary Table (*ClvR*^{ap} Crosses).

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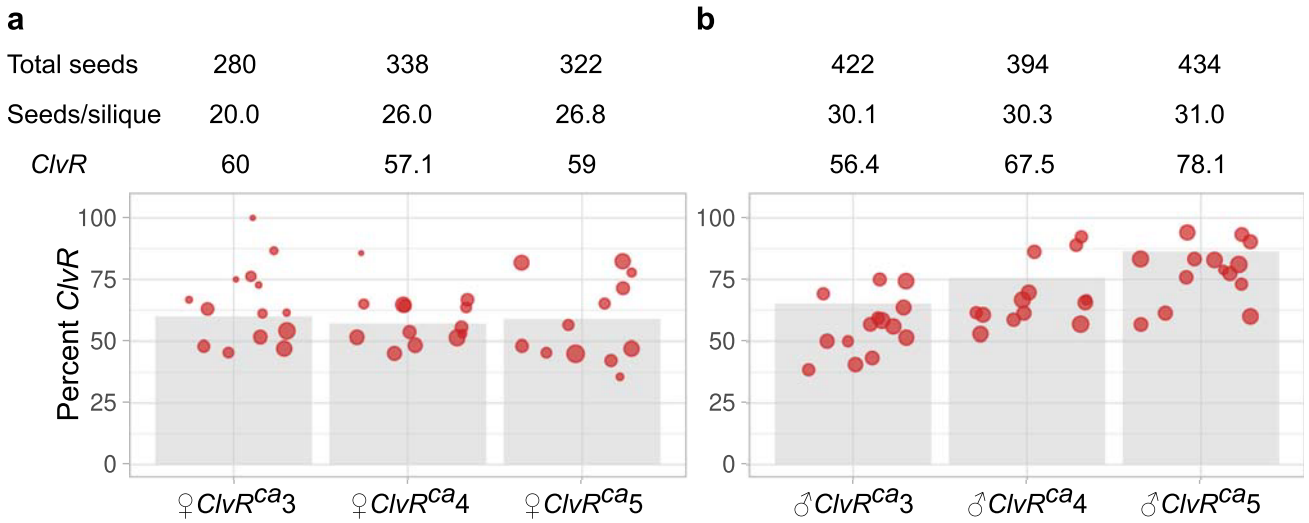

Extended Data Fig. 3 | T3 heterozygous *ClvR* crosses for (a) female *ClvRCaMV35S* and (b) male *ClvRCaMV35S*. T3 *ClvR*^{CaMV35S}/+ heterozygotes were grown to adulthood and their ovules (left three columns) or pollen (right three columns) used in outcrosses to WT. Bar graphs show the number of siliques scored (red circles). The number of seeds within each silique scales with circle size. Counts are in Supplementary Table (*ClvR*^{CaMV} Crosses).

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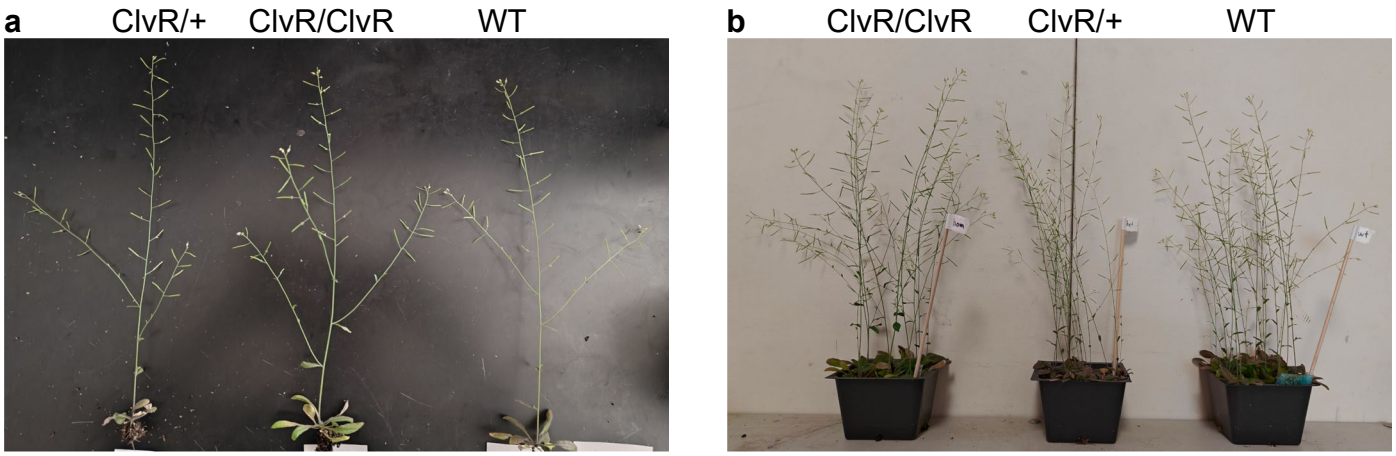

Extended Data Fig. 4 | Images of individual (a) and whole pots (b) of heterozygous *ClvR*^{ubq}, homozygous *ClvR*^{ubq} and WT plants. In **a** individual plants have been removed from their pots and laid flat against a black background. **b** shows pots containing multiple plants.

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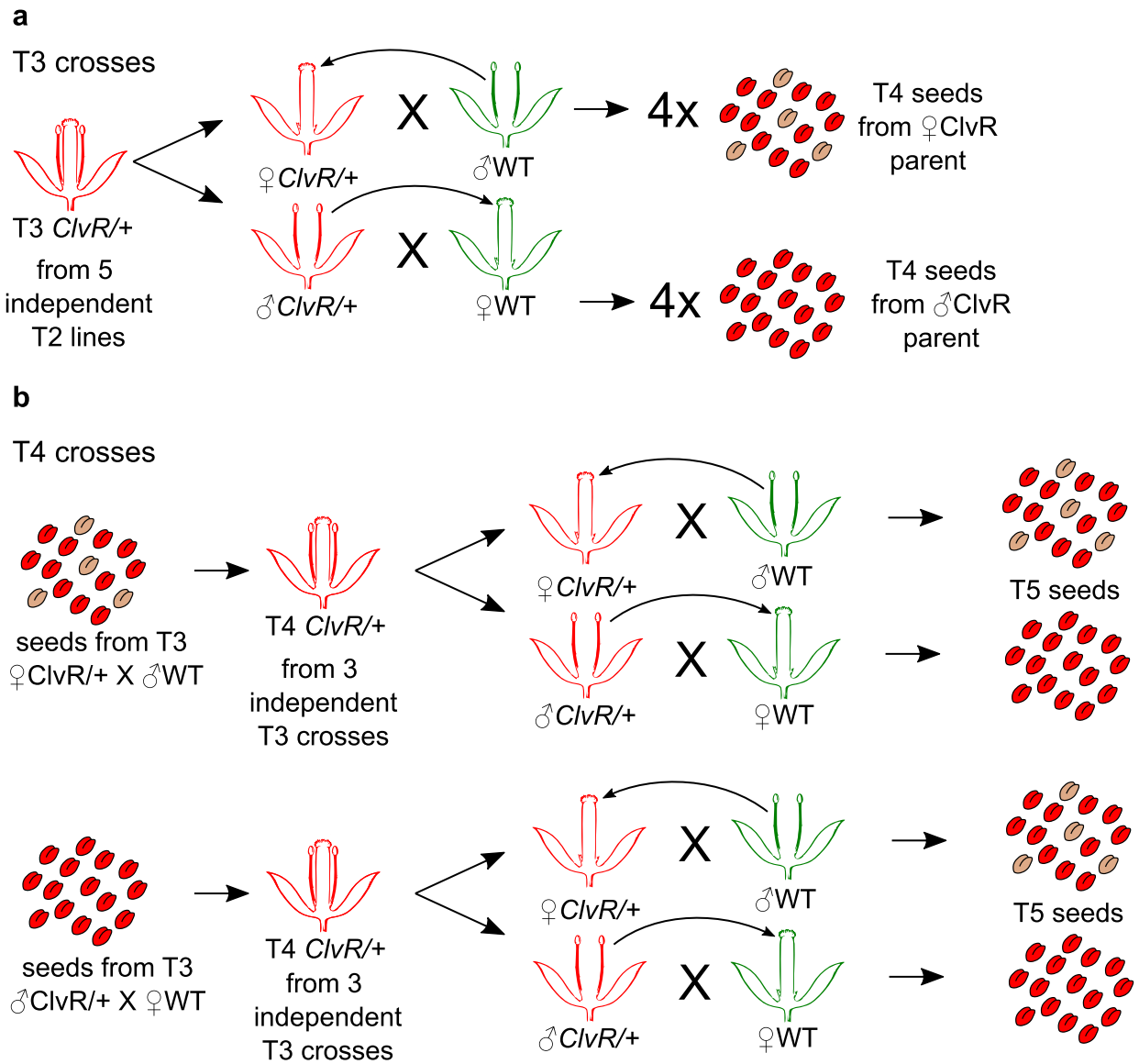

Extended Data Fig. 5 | Crossing scheme for (a) T3 and (b) T4 crosses discussed in text and Figs. 2 and 3. (**a**) We selected 5 independent *ClvR*^{ubq} lines that showed 100% *ClvR* in the T2 self cross. Pollen of T2 plants was outcrossed to WT to generate T3 heterozygotes. For each of these 5 independent lines we set up reciprocal crosses to WT with 4 plants per line (4 crosses/siliques per plant). (**b**) For 1 of the line from (A) *ClvR*^{ubq7} we repeated the reciprocal crosses, with seeds coming from a ♀*ClvR*/+ or ♂*ClvR*/+ parent. For each of these we again crossed 4 plants (4 crosses/siliques per plant). Arabidopsis icons adapted from BioRender (2023), Structure of Arabidopsis thaliana.

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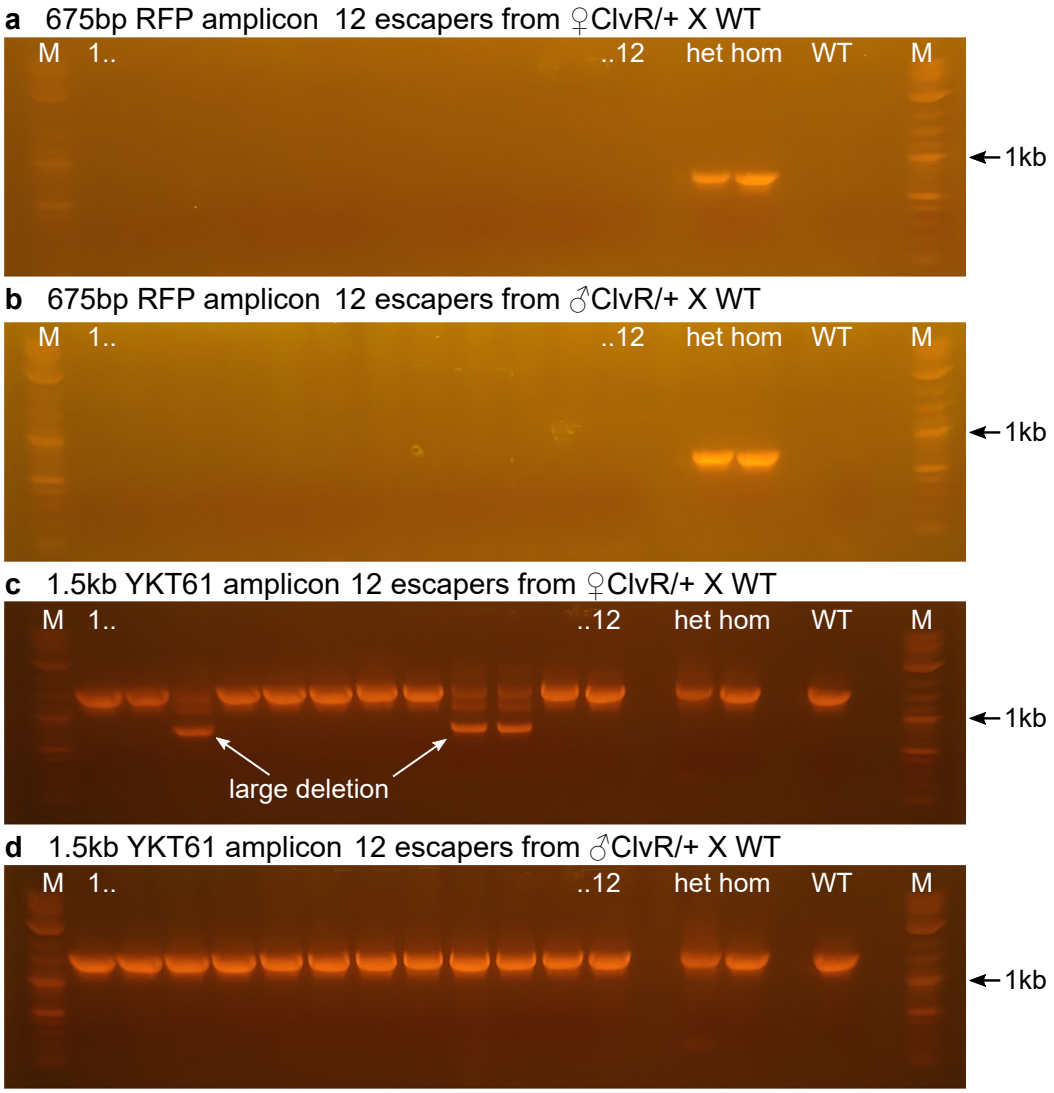

Extended Data Fig. 6 | Escaper genotyping. (**a**-**b**) PCR amplifications of a 675 bp DNA fragment of the RFP marker for escapers from a ♀*ClvR*/+ X WT (**a**) or ♂*ClvR*/+ X WT (**b**) cross. Hetero- and homozygous (het, hom) *ClvR* plants were used as positive controls, WT as negative control. Only *ClvR*-bearing plants showed the RFP band. (**c**-**d**) Control PCRs on the same DNA samples as in **a** and **b**, in which the YKT61 target region was amplified. Note some female escapers in **c** had larger deletions. This experiment was carried out once.

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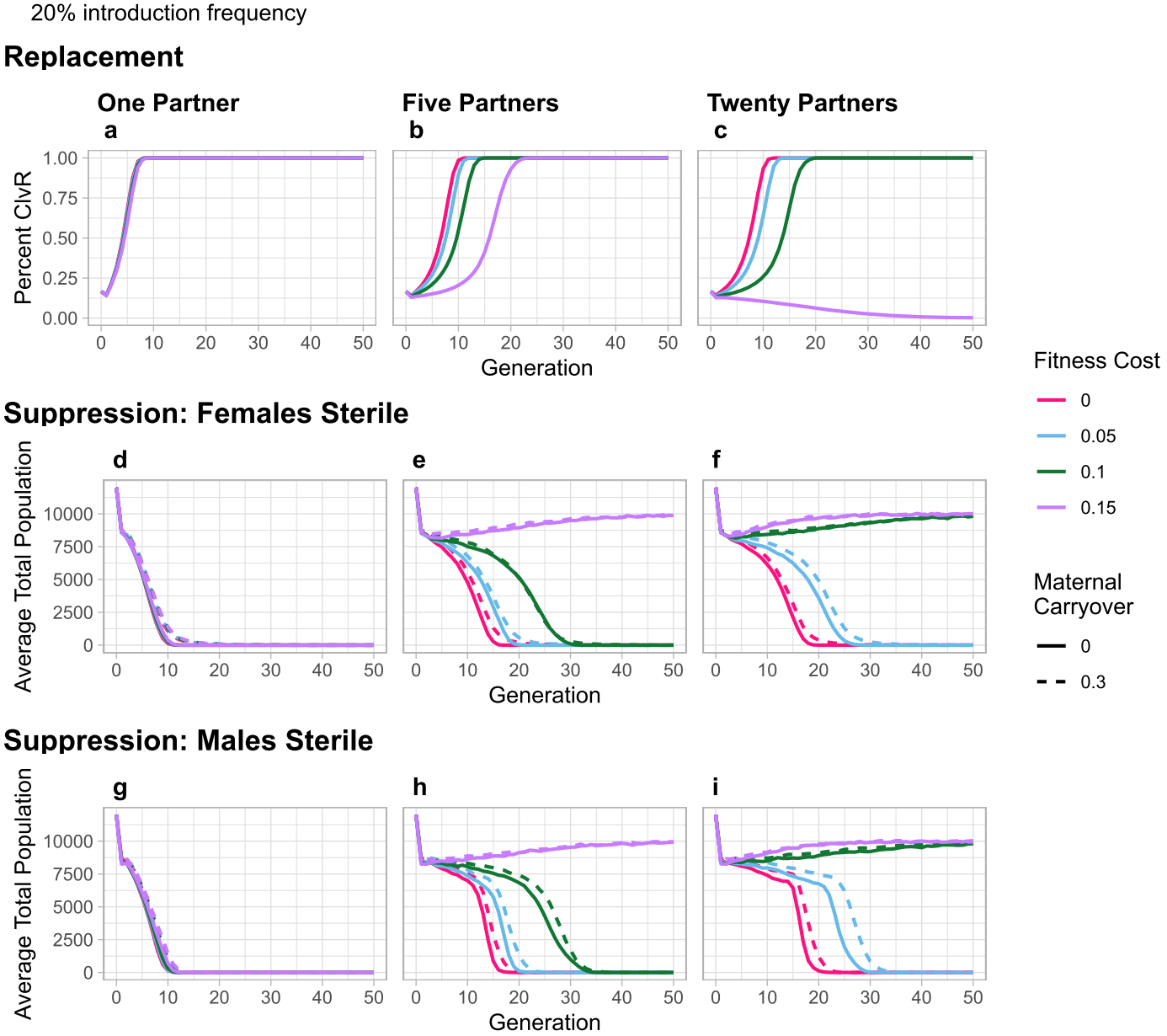
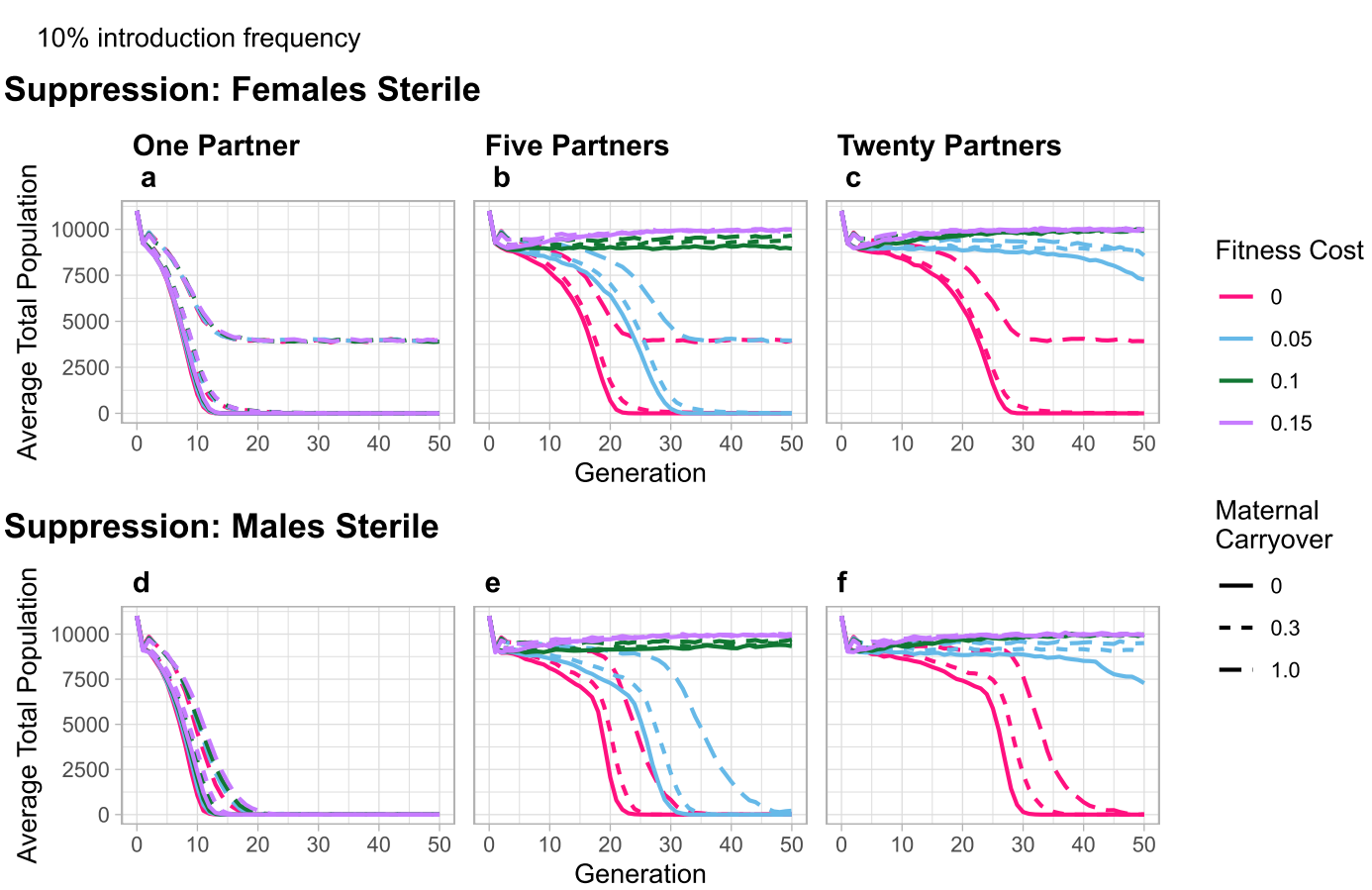


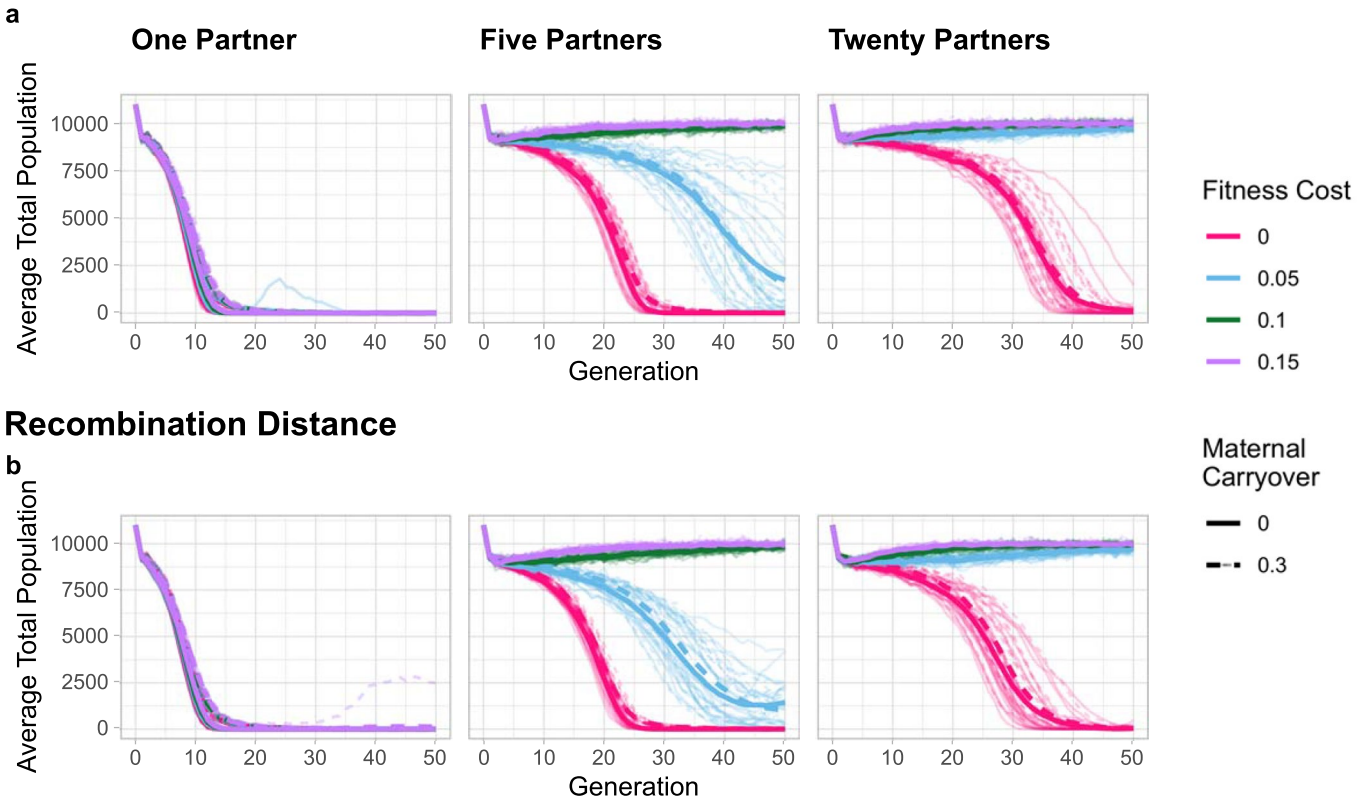
Extended Data Fig. 7 | Predicted behavior of *ClvR* for population modification and suppression. (**a-c**) Population modification. *ClvR* is introduced as homozygous males at a frequency of 20% of the starting population, which is at carrying capacity, 10,000 individuals. The mating system is monogamous (**a**), or polyandrous, with 5 males each providing 1/5th of the pollen needed to fertilize all ovules of an individual female (**b**), or 20 males each providing 1/20th of the pollen needed (**c**). Fitness costs are incurred by gametes (a probability of not being able to participate in fertilization, if chosen by the model). Maternal carryover is set to zero. Lines represent the average of 10 runs. (**d-f**) Population suppression with a transgene inserted into a recessive locus required for female sporophyte fertility. *ClvR* is introduced as above, at a frequency of 20%. The mating system is monogamous (**d**), or polyandrous, with 5 males each providing 1/5th of the pollen needed to fertilize all ovules of an individual female (**e**), or 20 males each providing 1/20th of the pollen needed (**f**). Fitness costs are as above. Maternal carryover is set to zero or 30% (the approximate value observed in our experiments with $ClvR^{ubq}$). (**g-i**). As with **d-f**, but with the *ClvR* inserted into a locus required for male sporophyte fertility. For these simulations homozygous females were released into the population since homozygous males are sterile. Lines represent the average of 10 runs. For all panels compare with 10% introduction frequency data shown in Fig. 5.

https://doi.org/10.1038/s41477-024-01701-3



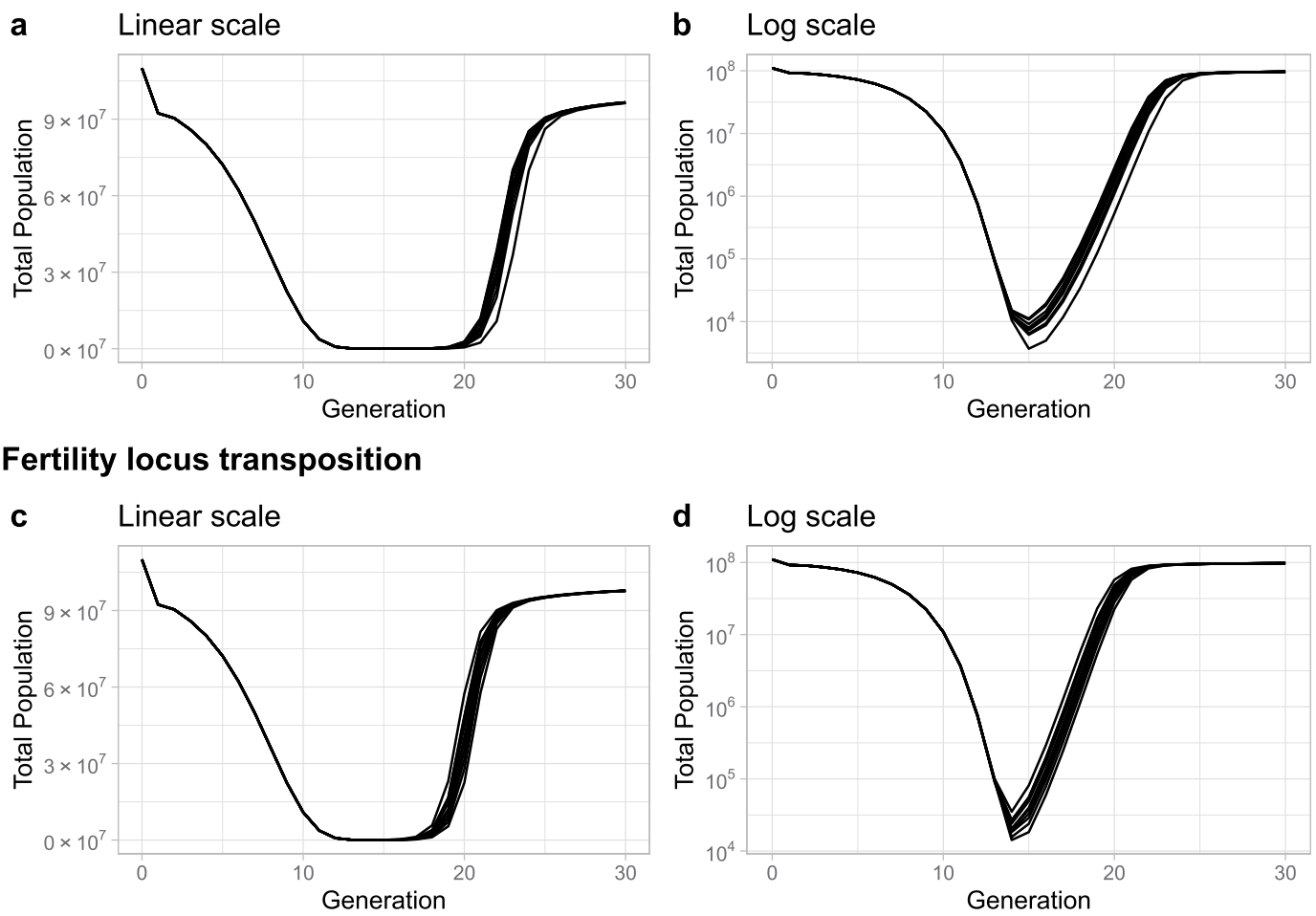
Extended Data Fig. 8 | Predicted behavior of *ClvR* for population suppression with 100% maternal carryover. *ClvR* is introduced at a frequency of 10%, and is present in a female fertility locus (**a-c**) or a male fertility locus (**d-f**), thereby creating a LOF allele. (**a-c**) When *ClvR* is located in a gene required for female sporophyte fertility high levels of maternal carryover prevent population extinction. (**d-f**) In contrast, when *ClvR* is located in a gene required for male sporophyte fertility, population extinction is slowed but not prevented.

https://doi.org/10.1038/s41477-024-01701-3



Extended Data Fig. 9 | Effects of higher levels of elements lacking Cas9 on *ClvR*-mediated population suppression. (**a**) *ClvR*, located inside a gene required in the sporophyte for female fertility, is introduced at a frequency of 10%, with 20% of these elements lacking Cas9. Individual runs are shown in thin lines and the average as a thick line. (**b**) *ClvR*, located in a gene required in the sporophyte for female fertility, is introduced at a frequency of 10%. Cas9 is located 1 map unit (1% recombination rate) away from the Rescue/gRNAs. Multiple individual runs fail to go to extinction while others that do go to extinction take much longer than under the conditions shown in Fig. 5.

https://doi.org/10.1038/s41477-024-01701-3



Extended Data Fig. 10 | Movement of a population suppression *ClvR* or a WT allele of the sporophyte fertility gene to a new unlinked location negatively affects population suppression. (**a-b**) *ClvR*, located inside a gene required in the sporophyte for female fertility, is introduced at a frequency of 10%, with 1 × 10-6 of the *ClvR* elements having been transposed to a new unlinked locus, which is not required for female fertility. Individual runs are shown here, in a linear scale (**a**) and logarithmic scale (**b**). (**c-d**) *ClvR*, located inside a gene required in the sporophyte for female fertility, is introduced at a frequency of 10%. Additionally, a translocated WT version of the fertility locus is present at a third locus, not associated with *ClvR*, at an allele frequency of 5 × 10-7 (100 out of 100,000,000 individuals are heterozygous for this translocated fertility gene), such that individuals with that gene may be both *ClvR* homozygous and fertile. Individuals runs are shown on a linear scale (**c**) and a logarithmic scale (**d**).

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Corresponding author(s): Georg Oberhofer, Bruce hay

Last updated by author(s): 2024/02/18

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
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☒	☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data
Data analysis	Modeling code:https://github.com/HayLab/Pigss Plots were generated in R (version 4.2.3) with the ggplot2 package Nanopore reads were mapped to the ykt61 reference locus with minimap2 (version 2.17). Illumina NextSeq2000 reads were analyzed with the DRAGEN Germline pipeline (version 3.10.12) against the Arabidopsis TAIR 10 genome

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data is available in the main text and the supplementary information files. Illumina sequencing reads were deposited to SRA (bioproject: PRJNA1074841). The Arabidopsis TAIR 10 genome assembly was used in this study.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	In this study we performed manual outcrosses of different plant genotypes. Details about the number of replicates are in the main text. In general we set up 4 crosses per plant for 3-4 individual plants per line.
Research sample	Samples were Arabidopsis thaliana Col-0
Sampling strategy	No sample size calculation was performed. Sample sizes were based on previously published gene drive papers.
Data collection	Georg Oberhofer recorded the data by scoring siliques under a fluorescent microscope.
Timing and spatial scale	NA
Data exclusions	As described in the main text, certain lines were not further characterized due to poor drive performance in a self cross. Despite repeated efforts we have not been able to identify a single unambiguous location for the Ubq6 element, which is featured in data in Figure 3 and 4. Based on this, and to avoid uncertainty if others want to repeat these experiments with our stocks, we have removed data for this line from the paper for experiments shown in Figures 3 and 4 and in the supplementary materials.
Reproducibility	Outcrosses to determine drive activity were repeated for 2 consecutive generations (T3 and T4)
Randomization	We did not randomize samples because we were interested in the cross outcomes of specific genotypes.
Blinding	Blinding was not performed because only a single person was working on the crossing of plants.

Did the study involve field work? ☐ Yes ☒ No

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☐	☒ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks	The Arabidopsis wildtype strain used to create transgenics was Col-0
Novel plant genotypes	We used the floral dip method with agrobacteria as described previously (ref 81) ClvR plasmids were transformed into GV3101 ElectroCompetent Agrobacterium strain from Intact Genomics.
Authentication	To determine the T-DNA insertion site of line ClvRubq7 we extracted genomic DNA from two different plants and constructed sequencing libraries using NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina (NEB #E7805) following manufacturer's instructions. The libraries were sequenced on Illumina NextSeq2000 in paired end mode with read length of 150 nt to the sequencing depth of 35 million paired end reads per sample. Base calls were performed with DRAGEN BCL Converter and structural variant analysis was performed with the DRAGEN Germline pipeline v3.10.12 against the Arabidopsis TAIR 10 genome. The resulting VCF files contained information on large structural variants (insertions).. Potential insertion sites were verified with custom primers amplifying the insertion sites followed by sequencing.