Population genetics

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Driving evolution in wild plants

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Two groups of scientists independently engineer gene drives in *Arabidopsis thaliana*, demonstrating the possibility for spreading fitness-reducing genetic modifications through wild populations of plants for population suppression.

Imagine a future where yield-robbing agricultural weeds or biodiversity threatening invasive plants could be kept on a genetic leash, or where the evolutionary rescue of extinction-threatened plant species could be super-charged. Synthetic gene drives can subvert the normal rules of evolution by spreading harmful (or beneficial) mutations and/or genes through wild plant populations to achieve these goals. In a recent issue of *Nature Plants*, Oberhofer et al. and Liu et al. make exciting advances that bring the theory closer to reality.

In agriculture, plant breeding (genetic modification) enables the introduction of agronomically important traits into domesticated crops. Often, these novel traits incur fitness trade-offs, meaning they would not proliferate through wild populations. In crops, this is desirable; annual crops are re-sown each year so that traits need not spread or persist, agronomic management minimizes trade-offs to maximize crop yield and the potential for crop 'ferality' is limited. However, genetic modifications of wild plant populations is more difficult.

Genetic modification of weedy and invasive plants seeks to introduce a genetic load that would modify (for example, nullify herbicide resistance) or directly suppress populations, enabling their management and control[1,2]. However, in wild populations, Darwinian selection would prevent the spread of this genetic load, necessitating repeated introductions of load carrying individuals at high frequencies. In extinction-threatened species, the spread of beneficial modified genes may be too slow to track environmental change. These are the genetic impasses that synthetic gene drives seek to overcome. Synthetic gene drives are made possible by the existence in nature of several types of selfish genetic elements that circumvent the normal rules of Mendelian inheritance to ensure their rapid spread through populations[3] (Fig. 1).

To date, most discussions of synthetic gene drives have focused on using CRISPR technology to create homing-based drives[3] in insect pests, particularly in insect disease vectors[4]. These homing-based drives harness homology-based DNA repair (HDR) mechanisms to copy themselves and an associated genetic cargo into targeted locations on the homologous chromosome during meiosis, thus biasing inheritance. The application of homing-based drives in plants is thought to be limited, as plants often use a non-homologous end joining (NHEJ) DNA repair mechanism that generates target-site mutations and the emergence of gene drive resistance alleles.

The notable methodological advance reported by both Oberhofer et al. (*ClvR*, Cleave and Rescue) and Liu et al. (*CAIN*, CRISPR assisted inheritance using *NPG1*) is to develop a synthetic drive element in plants based on the "toxin-antidote" (TA) principle[5]. TA-based gene drive systems avoid the requirement for homology-directed

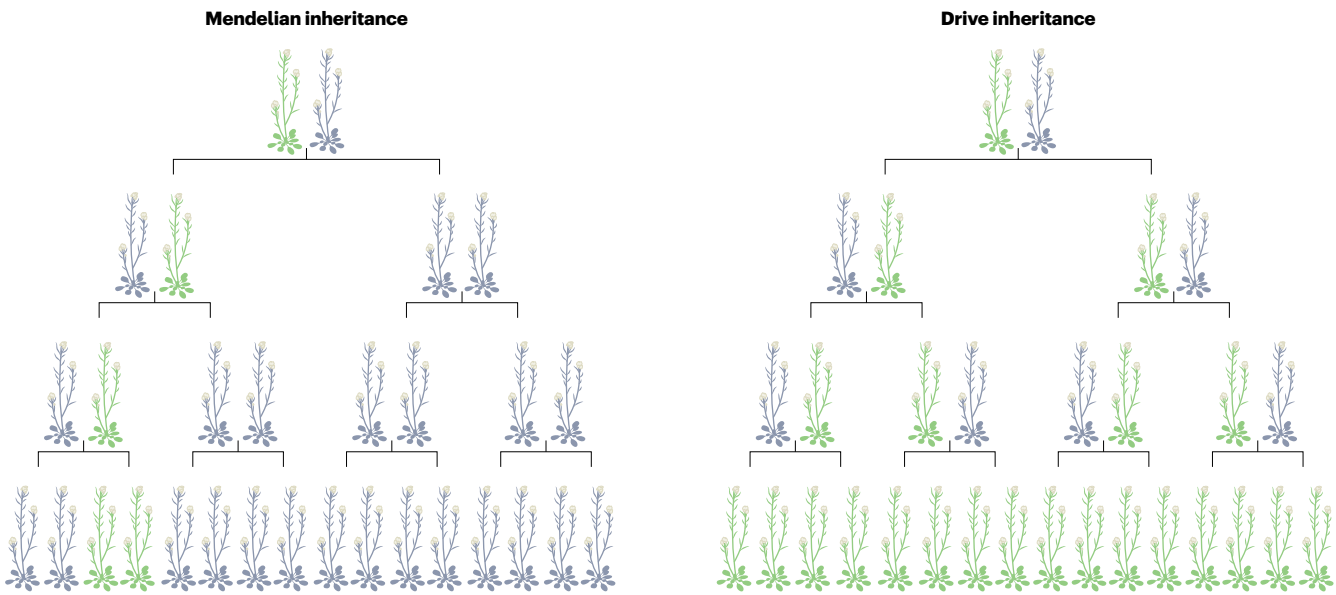

Fig. 1 | Comparison of Mendelian versus gene drive inheritance patterns. In each case, a low frequency of transgenic individuals (green) are introduced into a population of wild plants (grey). With no or very low fitness costs, under Mendelian inheritance the transgene is, at best, maintained at the frequency at which it was introduced. Synthetic gene drives enable rapid proliferation of the transgene via mechanisms that favour its inheritance.

DNA repair and so alleviate the NHEJ resistance issues associated with homing drives. Both groups develop these drives in the self-pollinating model plant, *Arabidopsis thaliana*, which limits the potential for escape and proliferation of the drive into wild populations. In both cases, a multiplexed gRNA/cas9 construct is used to disrupt an essential target gene (the toxin) while also providing rescue (the antidote) using a non-cleavable version of the target as part of the drive construct. Any gametes carrying wild-type targets are disrupted by the toxin and rendered nonviable, thereby increasing the relative frequency of the drive allele. In these proof-of-concept papers, the drives are not designed to enable population suppression or control, but instead carry fluorescent markers as cargo.

Functionally, the *ClvR* drive of Oberhofer et al. targets YKT61, a housekeeping gene whose expression is essential for the survival of both male and female gametes. In this case, the toxin cleaves the YKT61 gene with subsequent inaccurate repair, creating loss-of-function alleles. The antidote is a cleavage-resistant form of YKT61 cloned from *A. lyrata*. In contrast, the CAIN drive described by Qian et al. targets a gene (NPG1) essential for the survival of male gametes only (that is, pollen viability), while rescuing drive-carrying pollen cells with a modified, cleavage resistant version of the *A. thaliana* target gene.

In both papers, several versions of the drive were tested, using different promoters and versions of Cas9. While transmission ratios in experimental crosses varied depending on the drive version, significant distortion was achieved in most cases, although inheritance rates were consistently higher with certain promoters and in seeds derived from pollen transmission. Impressively, the most efficient iterations of *CAIN* and *ClvR* displayed very strong drive (>99%) over multiple generations. Importantly, resistance alleles were not observed. Modelling studies were used to extrapolate these experimental results and simulate proliferation of *ClvR/CAIN* through a single, outcrossing population. Simulation results indicated that the drive might be fixed in between 10 and 30 generations, depending on assumptions about mating system, inbreeding and gametic fitness costs. These results indicate that drives should be able to increase in frequency in plant populations from low initial frequencies, even under a scenario where drives impose a significant fitness penalty (that is, population suppression).

The results reported by Oberhofer et al. and Liu et al. represent a significant advance in technical feasibility and highlight the future potential for using synthetic toxin-antidote gene drives for the genetic modification of wild plant populations. Innovative tools are desperately needed to for the sustainable control of weeds in agriculture and the environment, and the genetic rescue of threatened and endangered species. Nevertheless, much work remains to be done before gene drives can be considered as desirable, safe and effective tools for these purposes.

There is currently no clear governance framework for the development and use of gene drive technologies. However, there will likely be significant ethical and regulatory barriers to the introduction of gene drives into natural and agroecosystems in most jurisdictions. Pertinent questions about social license, ecological risk assessment, freedom to operate when using CRISPR technology for gene drive and safeguarding strategies remain[6,7].

Ultimately, gene drives will need to spread and persist under natural conditions. In this respect, population and meta-population level processes (for example, dispersal, inbreeding and seed bank longevity) will be critical. Considerable research in ecology and population biology will be required to determine the likely success of a gene drive program for any given species. In an agricultural context, when managing large weed populations, introduction rates of 1–10% will be hard to attain and the timeframes of population modification or suppression will be long. Also, the probability of the evolution of resistance to drives in large, wild populations over multiple generations cannot be properly quantified in lab studies.

Notwithstanding the challenges associated with regulation, societal acceptance, licensing and gene drive transmission in wild populations, the work by these research teams in China and the USA represents a major advance in demonstrating the technical feasibility and application of synthetic gene drives.

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Competing interests

The authors declare no competing interests.