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Regulating gene drives

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Regulatory gaps must be filled before gene drives could be used in the wild

Genes in sexually reproducing organisms normally have, on average, a 50% chance of being inherited, but some genes have a higher chance of being inherited. These genes can increase in relative frequency in a population even if they reduce the odds that each organism will reproduce. Aided by technological advances, scientists are investigating how populations might be altered by adding, disrupting, or editing genes or suppressed by propagating traits that reduce reproductive capacity (1, 2). Potential beneficial uses of such "gene drives" include reprogramming mosquito genomes to eliminate malaria, reversing the development of pesticide and herbicide resistance, and locally eradicating invasive species. However, drives may present environmental and security challenges as well as benefits.

Gene drives are subject to two fundamental limitations. First, drives will only function in sexually reproducing species, so they cannot be used to engineer populations of viruses or bacteria. Second, a newly released drive will typically take dozens of generations to affect a substantial proportion of a target population, unless drive-containing organisms are released in numbers constituting a substantial fraction of the population. The process may require only a year or less for some invertebrates, but centuries for organisms with long generation times.

Studies have evaluated the possibility of releasing transgenic mosquitoes to combat the spread of malaria, dengue, and other mosquito-borne diseases, including requirements for containment, testing, controlled release, and monitoring of mosquito gene drives. This work will need to be replicated and extended for proposed gene drives seeking to alter other species (3, 4). It is crucial that this rapidly developing technology continue to be evaluated before its use outside the laboratory becomes a reality.

Technical developments

One promising method for creating a gene drive uses targeted endonuclease enzymes to cut a specific site in the DNA of the organism. In organisms that inherit one chromosome with this enzyme's gene and one without it, the endonuclease will cut the latter, inducing the cell to copy the endonuclease and surrounding genes onto the chromosome that previously lacked them (see the figure) (Fig. 1). Ten years ago, Burt proposed using endonuclease drives to spread traits that would control diseases borne by insect vectors (2). He suggested that drives could be designed to add or delete genes and suppress populations, potentially to the point of extinction. However, no drive capable of spreading efficiently through a wild population has yet been developed. A major reason has been the difficulty of programming drives to cut desired sequences at high efficiency.

Scientists recently developed a powerful and efficient tool for genome engineering that uses the CRISPR nuclease Cas9 to cut sequences specified by guide RNA molecules (5, 6). This technique is in widespread use and has already engineered the genomes of more than a dozen species. Cas9 may enable "RNA-guided gene drives" to edit nearly any gene in sexually reproducing populations (1).

To reduce potential negative effects in advance of construction and testing, Esvelt et al. have proposed several novel types of drives (1). Precision drives could exclusively affect particular species or subpopulations by targeting sequences unique to those groups. Immunizing drives could block the spread of unwanted gene drives by preemptively altering target sequences. Reversal drives could overwrite unwanted changes introduced by an initial drive or by conventional genome engineering, even restoring the original sequence. However, ecological effects would not necessarily be reversed. These and other RNA-guided gene drives have yet to be demonstrated in the laboratory.

Environmental and security aspects

A recent workshop examined key questions concerning effects of development and use of gene drives in varied species and contexts (7, 8).

Targeted wild organisms. Scientists have minimal experience engineering biological systems for evolutionary robustness. Drive-induced traits and altered population dynamics must be carefully evaluated with explicit attention to stability. For example, a drive may move through only part of a population before a mutation inactivates the engineered trait. In some cases, preferred phenotypes might be maintained as long as new drives encoding updates are periodically released. The effects of a strategy dependent on repeatedly releasing drives to alter a population should be thoroughly assessed before use.

Nontargeted wild organisms. In theory, precision drives could limit alterations to targeted populations, but the reliability of these methods in preventing spread to nontarget or related populations will require assessment. To what extent and over what period of time might cross-breeding or lateral gene transfer allow a drive to move beyond target populations? Might it subsequently evolve to regain drive capabilities in populations not originally targeted? There may also be unintended ecological side effects. Contained field trials should be performed before releasing organisms bearing a drive that spreads the trait.

Crops and livestock. A technology capable of editing mosquito populations to block disease transmission could also be used to alter populations of agricultural plants or livestock by actors intent on doing harm. However, doing so surreptitiously would be difficult because many drive-containing organisms must be released to alter populations within a reasonable time span. Moreover, drives are unlikely to spread undetected in contract seed production farms and animal breeding facilities that test for the presence of transgenes. It would thus be difficult to use drives to affect food supplies in the United States and other countries that rely on commercial seed production and artificial insemination. Developing countries that do not use centralized seed production and artificial insemination could be more vulnerable.

Humans. Gene drives will be ineffective at altering human populations because of our long generation times. Furthermore, whole-genome sequencing in medical diagnostics could be used to detect the presence of drives. Drives are thus not a viable method for altering human populations. Rare individuals might experience an allergic reaction to peptides

in the Cas9 protein if exposed to an affected organism. Thus, toxicological studies should be conducted to confirm that proposed drive components are safe.

Toward integrated risk management

We recommend the following steps toward integrated management of environmental and security risks:

(i) Before any primary drive is released in the field, the efficacy of specific reversal drives should be evaluated. Research should assess the extent to which the residual presence of guide RNAs and/or Cas9 after reversal might affect the phenotype or fitness of a population and the feasibility of reaching individual organisms altered by an initial drive.

(ii) Long-term studies should evaluate the effects of gene drive use on genetic diversity in target populations. Even if genome-level changes can be reversed, any population reduced in numbers will have reduced genetic diversity and could be more vulnerable to natural or anthropogenic pressures. Genome-editing applications may similarly have lasting effects on populations owing to compensatory adaptations or other changes.

(iii) Investigations of drive function and safety should use multiple levels of molecular containment to reduce the risk that drives will spread through wild populations during testing. For example, drives should be designed to cut sequences absent from wild populations, and drive components should be separated.

(iv) Initial tests of drives capable of spreading through wild populations should not be conducted in geographic areas that harbor native populations of target species.

(v) All drives that might spread through wild populations should be constructed and tested in tandem with corresponding immunization and reversal drives. These precautions would allow accidental releases to be partially counteracted.

(vi) A network of multipurpose mesocosms and microcosms should be developed for testing gene drives and other advanced biotechnologies in contained settings.

(vii) The presence and prevalence of drives should be monitored by targeted amplification or meta-genomic sequencing of environmental samples.

(viii) Because effects will mainly depend on the species and genomic change rather than the drive mechanism, candidate gene drives should be evaluated on a case-by-case basis.

(ix) To assess potentially harmful uses of drives, multidisciplinary teams of experts should be challenged to develop scenarios on deliberate misuse.

(x) Integrated benefit-risk assessments informed by the actions recommended above should be conducted to determine whether and how to proceed with proposed gene drive applications. Such assessments should be conducted with sensitivity to variations in uncertainty across cases and to reductions in uncertainty over time.

Regulatory gaps

The prospective development of drives highlights the need for regulatory reform. Currently, U.S. regulations would treat drives as veterinary medicines or toxins. U.S. policies and international security regimes rely on a listed-agent-and-toxin approach. Neither addresses challenges posed by gene drives and other advanced biotechnologies.

U.S. environmental regulations. Responsibility for regulating animal applications of drives in the United States rests with the Food and Drug Administration (FDA). An FDA guidance issued in 2009 states that genetically engineered DNA constructs intended to affect the structure and function of an animal, regardless of their use, meet the criteria for veterinary medicines and are regulated as such. Developers are required to demonstrate that such constructs are safe for the animal. Approval of new veterinary medicines is to be based on the traditional FDA criterion "that it is safe and effective for its intended use" (9). It is unclear whether these requirements can be reconciled with projected uses of drives, including suppression of invasive species. Nor is it clear how this guidance would apply to insects. The application of existing U.S. Department of Agriculture (USDA) and U.S. Environmental Protection Agency regulations governing genetically modified organisms to gene drives is also ambiguous, with jurisdictional overlaps across the Federal Insecticide, Fungicide, and Rodenticide Act, the Toxic Substances Control Act, and the Animal and Plant Health Inspection Service (10).

International environmental conventions. Existing international conventions cover international movements of gene drives, but do not define standards for assessing effects, estimating damages, or mitigating harms. International movements of living modified organisms are treated under the 2003 Cartagena Protocol on Biosafety, ratified by 167 nations not including the United States and Canada. Article 17 of the Protocol obligates parties to notify an International Biosafety Clearinghouse and affected nations of releases that may lead to movement of living modified organisms with adverse effects on biological diversity or human health. Other provisions empower nations to use border measures to limit international movements, but these measures are not likely to control diffusion of drives. The 2010 Nagoya–Kuala Lumpur Supplementary Protocol calls on Parties to adopt a process to define rules governing liability and redress for damage from international movements. Neither the process nor rules have been defined (11).

U.S. security policies. The draft U.S. Government Policy on Dual Use Research of Concern (DURC) combines a broad definition of concerns with a narrow definition of scope of oversight, the latter focusing on experiments of concern on listed pathogens and toxins (12). The listed-agent-toxins approach is also used in the U.S. Select Agent Rule, USDA Select Agents/Toxins, and Commerce Department export control regulations. Drives do not fall within the scope of required oversight of DURC and other listed-agent-toxin–based policies.

International security conventions. The UN Biological Weapons Convention defines areas of concern in broad terms with the intention of providing latitude to adapt to evolving technologies and threats. Article 1 bans development, production, or stockpiling of all biological agents or toxins that have no justification for prophylactic, protective, and other peaceful purposes and weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes (13, 14). However, national implementation measures defining operational oversight and Australia Group Guidelines governing exports rely on narrow lists of organisms, toxins, and associated experiments (15). Gene drives and most other advanced applications of genomic engineering do not use proscribed agents or create regulated toxins and hence fall beyond the scope of operational regulations and agreements.

Filling the regulatory gaps. We recommend adopting a function-based approach that defines risk in terms of the ability to influence any key biological component the loss of which would be sufficient to cause harm to humans or other species of interest. The agents and targets of concern with a functional approach could include DNA, RNA, proteins, metabolites, and any packages thereof. Thus, suppression drives would be covered because they would cause loss of reproductive capability in an animal population, whereas an experimental reversal drive that could only spread through engineered laboratory populations could be freely developed. Steps taken to mitigate environmental concerns will address security concerns and vice versa. Regulatory authority for each proposed RNA-guided gene drive should be granted to the agency with the expertise to evaluate the application in question. All relevant data should be made publicly available and, ideally, subjected to peer review (16).

Conclusions

For emerging technologies that affect the global commons, concepts and applications should be published in advance of construction, testing, and

release. This lead time enables public discussion of environmental and security concerns, research into areas of uncertainty, and development and testing of safety features. It allows adaptation of regulations and conventions in light of emerging information on benefits, risks, and policy gaps. Most important, lead time will allow for broadly inclusive and well-informed public discussion to determine if, when, and how gene drives should be used.

REFERENCES AND NOTES

1. K. M. Esvelt, A. L. Smidler, F. Catteruccia, G. M. Church, *eLife* 10.7554/eLife.03401 (2014).
2. A. Burt, *Proc. Biol. Sci.* **270**, 921–928 (2003). Medline doi:10.1098/rspb.2002.2319
3. M. Benedict, P. D'Abbs, S. Dobson, M. Gottlieb, L. Harrington, S. Higgs, A. James, S. James, B. Knols, J. Lavery, S. O'Neill, T. Scott, W. Takken, Y. Toure, *Vect. Zoonotic Dis.* **8**, 127–166 (2008). doi:10.1089/vbz.2007.0273
4. World Health Organization, Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission, Geneva, Switzerland, 4 to 6 May 2009.
5. P. Mali, L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, J. E. Norville, G. M. Church, *Science* **339**, 823–826 (2013). Medline doi:10.1126/science.1232033
6. L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini, F. Zhang, *Science* **339**, 819–823 (2013). Medline doi:10.1126/science.1231143
7. T. Kuiken, G. Dana, K.A. Oye, D. Rejeski, *J. Environ. Stud. Sci.* 10.1007/s13412-014-0171-2 (2014).
8. K. Drinkwater *et al*., *Creating a Research Agenda for the Ecological Implications of Synthetic Biology* (MIT Center for International Studies, Cambridge, MA, and Woodrow Wilson International Center for Scholars, Washington, DC, 2014); www.synbioproject.org/library/publications/archive/6685/.
9. Center for Veterinary Medicine (CVM), Food and Drug Administration, U.S. Department of Health and Human Services, *Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs: Final Guidance* (FDA, Rockville, MD, 2009); www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf.
10. S. Bar Yam *et al*., "The Regulation of Synthetic Biology: A Guide to United States and European Union Regulations, Rules, and Guidelines" (Synthetic Biology Engineering Research Center, National Science Foundation, Arlington, VA, 2012); http://synberc.org/sites/default/files/Concise%20Guide%20to%20Synbio%20Regulation%20OYE%20Jan%202012_0.pdf.
11. United Nations, Cartagena Protocol on Biosafety to the Convention on Biological Diversity (2003). https://treaties.un.org/doc/Treaties/2000/01/20000129%2008-44%20PM/Ch_XXVII_08_ap.pdf
12. U.S. Science and Technology Office, United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern, Draft Notice, 2013; https://www.federalregister.gov/articles/2013/02/22/2013-04127/united-states-government-policy-for-institutional-oversight-of-life-sciences-dual-use-research-of-concern.
13. United Nations, 1925 Geneva Protocol; www.un.org/disarmament/WMD/Bio/1925GenevaProtocol.shtml.
14. Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction (1972); www.un.org/disarmament/WMD/Bio/pdf/Text_of_the_Convention.pdf.
15. Australia Group, Guidelines for Transfers of Sensitive Chemical and Biological Materials (2012); www.australiagroup.net/en/guidelines.html.
16. R. G. Reeves, J. A. Denton, F. Santucci, J. Bryk, F. A. Reed, *PLOS Negl. Trop. Dis.* **6**, e1502 (2012). Medline doi:10.1371/journal.pntd.0001502

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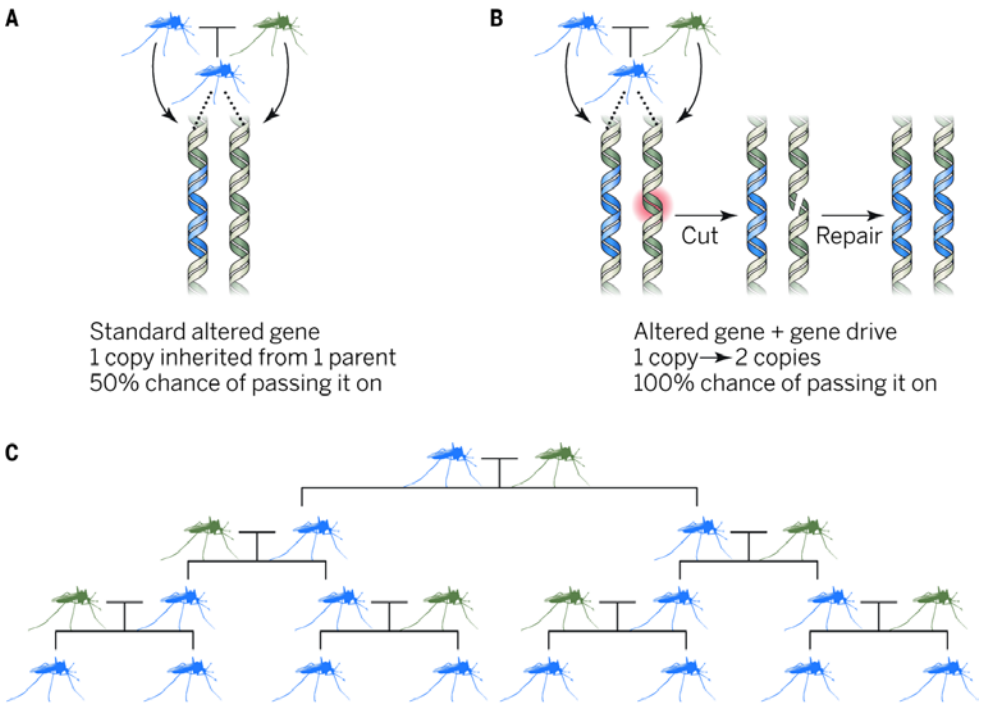

How endonuclease gene drives spread altered genes through populations. (**A**) Altered genes (blue) normally have a 50% chance of being inherited by offspring when crossed with a wild-type organism (gray). (**B**) Gene drives can increase this chance to nearly 100% by cutting homologous chromosomes lacking the alteration, which can cause the cell to copy the altered gene and the drive when it fixes the damage. (**C**) By ensuring that the gene is almost always inherited, the gene drive can spread the altered gene through a population over many generations, even if the associated trait reduces the reproductive fitness of each organism. The recently developed CRISPR nuclease Cas9, now widely used for genome engineering, may enable scientists to drive genomic changes that can be generated with Cas9 through sexually reproducing organisms (*1*).