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Pest management by genetic addiction

Fred Gould[a,b,1], Sumit Dhole[a], and Alun L. Lloyd[c,d]

In the PNAS article "Cleave and Rescue, a novel selfish genetic element and general strategy for gene drive," Oberhofer et al. (1) describe an exciting new mechanism for enabling a transgenic sequence to increase in frequency within a sexually reproducing population, even if the transgenic sequence causes individuals bearing it to have somewhat lower fitness than those without it. The authors liken the mechanism to the "gene addiction" that can maintain a useless plasmid in a bacterium. The work of Oberhofer et al. (1) adds substantially to a growing field within genetic engineering, often termed gene drive research, in which selfish genetic elements overcome the rules of Mendelian inheritance and push transgenes into a population. While no engineered gene drives have been released into wild populations, that is the ultimate goal, and both the technical and cultural roads toward that goal have been tortuous at times.

Gene drive projects are categorized based on having one of two aims. The first is to physically link a desirable gene to a gene drive mechanism and engineer both into a viable strain of the target organism. If individuals of the strain are released into a sexually reproducing field population of that species, the DNA sequence of the drive mechanism is predicted to increase in frequency in the population and the linked, desirable gene should "hitchhike" along with it. If the population is a mosquito that transmits dengue virus, the desirable gene could be one that codes for an RNA interference molecule targeted to prevent the virus from replicating in the mosquito—thus interfering with its transmission to a person whom the mosquito subsequently bites.

Projects with the second aim are designed to suppress or eliminate a pest species, be it a mosquito, rat, roach, or crop pest. Here, the gene drive mechanism itself or a linked sequence disrupts the functioning of an essential gene of the targeted species or decreases the ratio of females to males, thus reducing offspring survival or production.

Gene drive projects are also differentiated along two other axes, the geographic area over which they are designed to spread and whether they are expected to remain in the population or to decrease after a period of time. When a gene drive project is designed to have no geographic limits and also designed to eliminate a pest, voices from both the public and the scientific community bring up concerns over potential irrevocable environmental harm that could ensue. A pest to one person is a delight to another, and what may be a pest in a city may have an important ecological role in a natural habitat.

The technical field of gene drive research has a long but frustrating history dating back at least to the 1960s when researchers considered physically linking desirable genes to naturally occurring gene drive elements such as meiotic drive and translocation-based underdominance (2). This early work foundered, but researchers moved on to excitement over the potential for utilizing the natural power of transposons (i.e., jumping genes) that are ubiquitous in most genomes and had been documented to spread rapidly throughout populations of some species without conferring an evolutionary advantage (2). Years of creative research efforts failed to domesticate the complex biology of these selfish genetic elements, but even with these setbacks, researchers pushed along because one potential outcome—eradication of malaria—was so important.

In the first decade of this century, progress was made as some researchers, including one author of the Oberhofer et al. (1) paper, pinned their hopes on manipulating or reconstructing the promising selfish genetic element *Medea*. When the *Medea* element that naturally occurs in *Tribolium* beetles is found in a heterozygous state in a female beetle, the ~50% of her progeny that inherit the element survive, but those without it die. This selfish behavior increases the frequency of the *Medea* element in the population by killing off the alternate allele. An artificial version of *Medea* was developed in *Drosophila* and demonstrated to increase in frequency within laboratory populations (3). Unfortunately, repeated attempts to engineer a similar,

[a]Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695; [b]Genetic Engineering and Society Center, North Carolina State University, Raleigh, NC 27695; [c]Biomathematics Graduate Program, North Carolina State University, Raleigh, NC 27695; and [d]Department of Mathematics, North Carolina State University, Raleigh, NC 27695

Author contributions: F.G., S.D., and A.L.L. wrote the paper.

The authors declare no conflict of interest.

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See companion article on page 6250.

[1]To whom correspondence should be addressed. Email: fred_gould@ncsu.edu.

Published online March 15, 2019.

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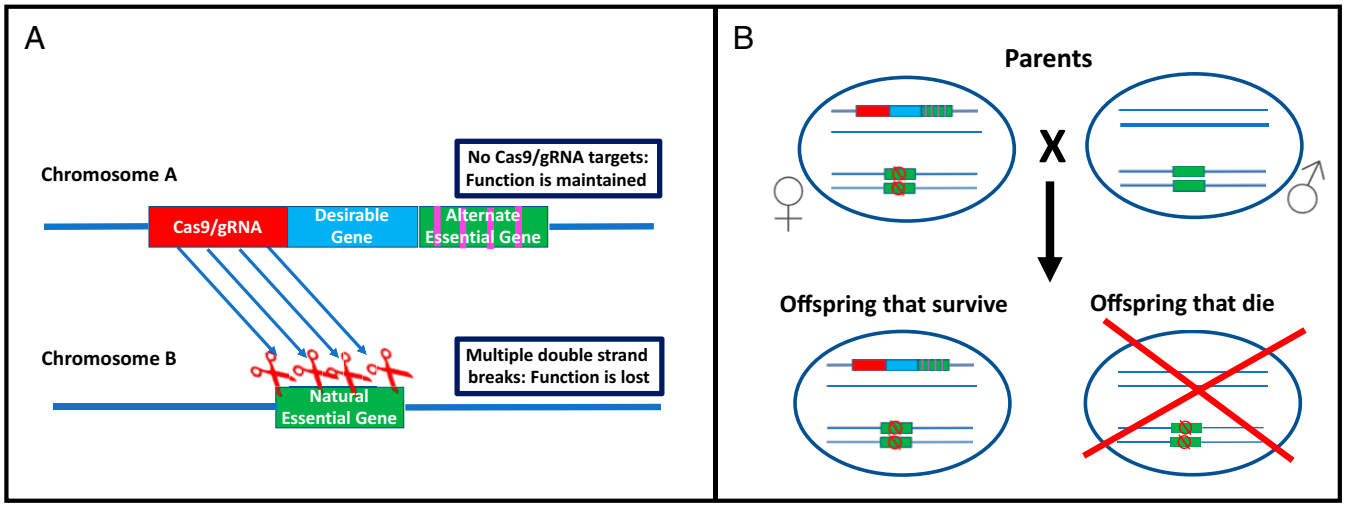

Fig. 1. Simplified view of the Cleaver/Rescue mechanism. (*A*) The Cleaver/Rescue construct on chromosome A produces Cas9 and guides RNAs that target an essential gene on chromosome B, but the alternate form of the essential gene within the construct lacks sequences for binding of the guide RNAs. (*B*) When females heterozygous for the construct mate with wild-type males, the Cas9 and guide RNAs carried over to the embryos cut and disable the natural essential gene from the male, so offspring that don't inherit the Cleaver/Rescue construct die.

artificial *Medea* into a mosquito species failed, likely because the *Medea* function requires very precise, stage-specific expression of its complex molecular components.

Concurrently, other researchers pinned their hopes on the homing endonuclease gene (HEG) selfish genetic elements that were found naturally in taxa such as amoebas and fungi, but not insects or vertebrates. These elements operate in nature by cutting DNA at a specific location in the genome and inserting themselves in that spot by homologous recombination. Individuals that start out heterozygous for a HEG become homozygous (i.e., one copy becomes two), thus increasing the overall frequency of the HEG in the population each generation. Amazingly, a HEG taken directly from a slime mold functioned with high specificity in cutting ribosomal genes on the X chromosome of an *Anopheles* mosquito that transmits malaria (4). Unfortunately, the important next step of using insights from structural biochemistry to alter HEGs to target other desired genomic locations and insert themselves were not sufficiently successful (5).

The current decade brought us CRISPR-Cas gene editing, and in 2014, Esvelt et al. (6) published a conceptual paper describing how this tool might be used to insert the CRISPR-Cas9 constructs themselves into a diversity of locations in the genome. From there, they would function like an artificial HEG with great flexibility due to the feasibility of making guide RNAs that target a great number of genes or other DNA sequences of interest. Less than a year after the Esvelt et al. (6) publication, a team of researchers published proof-of-principle gene drives in *Drosophila* and then in *Anopheles* (7–9). It seemed like the long-awaited breakthrough. But, testing in the laboratory showed that insect strains could evolve resistance to this new gene drive mechanism (10). Recent work demonstrates that there should be ways to prevent such resistance (11), but the homologous recombination process will still rely on polymerase molecules that are error prone, so if the goal is to incorporate a desired antipathogen gene, there is a chance that the final outcome would be a population with a gene mutated to the point of lacking function. Work with CRISPR-Cas9 gene drives for suppressing mouse populations that threaten endangered species on islands has also faced technical challenges (12).

On the flip side, concern has increased that these artificial HEGs may in the future become so effective that when the goal of the gene drive project is to suppress a pest population, there is potential for eradication of the whole pest species and any other species with which it occasionally has a successful mating. Research groups have responded to this concern by trying to develop gene drives that target only specific populations by reexamining the potential of underdominance drives that were considered in the 1960s as well a few other mechanisms, but these have mostly required complex constructs with precise expression patterns.

Enter "Cleave and Rescue." It offers a mechanism for spreading an antipathogen gene or a gene that moderately reduces the fitness of a population. The good news for some applications is that the mathematical models explored by Oberhofer et al. (1) indicate that when the gene being spread reduces fitness, it is unlikely to spread far beyond the general region where it is released. Topping off all of these positive characteristics are the facts that it does not require complicated constructs or precise gene expression and it does not rely on copying of an antipathogen gene through error-prone homologous recombination.

So, how does this work? Conceptually, it is almost embarrassingly simple. What Oberhofer et al. (1) did was to develop a genetic construct that includes three main components, one coding for Cas9, one for guide RNAs designed to target multiple locations of an essential gene, and one coding for a functionally similar essential gene that lacked any of the target sites for the guide RNAs in the construct. The research team inserted the construct into an autosome of a *Drosophila* fly. The Cas9 was primarily produced in the germline, while the guide RNAs were more ubiquitously produced. The working hypothesis was that the expressed Cas9 and guide RNAs would function together to cause multiple double-strand breaks in the essential gene and that even after the action of the cell's DNA repair mechanisms, the gene would have too many deletions and/or insertions to be functional (Fig. 1*A*). The flies would nevertheless survive based on expression of the alternate form of the gene within the inserted construct. This is the key to the gene drive. When the authors released flies with this construct into a caged fly population without the construct, the natural form of the critical gene was permanently disabled in almost all of the offspring of heterozygous mothers that mated with wild-type fathers,

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because the Cas9 and guide RNAs were still active in the zygotes. The ~50% of the offspring that inherited the "Cleaver/Rescue" construct from the mother lived because the normal gene function was maintained by the alternative form of the essential gene, but the half that lacked the construct died (Fig. 1*B*). The cleavage also occurred during gametogenesis in males that had the construct in heterozygous or homozygous form, but without activity of the Cas9 in zygotes. Over time, the higher chance for survival of individuals with the Cleaver/Rescue construct led to it increasing in frequency within the fly population. The population was in essence addicted to the alternate rescuing version of the essential gene.

Even with this simple design, there were lots of things that could have gone wrong. The authors guarded against many. For example, they carefully designed the rescue version of the essential gene to decrease the chance of a process called gene conversion, in which the rescue gene would convert the original gene to a form resistant to cleavage. The authors also used multiple guide RNAs to decrease the chance that a rare, natural cleavage-resistant variant existed, or a resistant mutant would arise. All of these safeguards and more would need to be put in place before a Cleaver/Rescue construct would be robust enough for field release in a pest species. And if mosquitoes could be successfully engineered with the drive construct, we would still need better antipathogen genes to hitchhike with the drive.

So, what does the future hold? Cleaver/Rescue is certainly an important addition to the existing gene drive approaches that are being investigated, and the nascent field of gene drive research has recently been attracting an increasing number of creative researchers. According to the Web of Science, an average of 10 research papers with gene drive as a topic were published per year between 2010 and 2014. In 2018 alone, there were 85 such papers. The future seems bright, but there are at least two hurdles to navigate. One is finding a socially acceptable path forward for assessing the risks and benefits of specific gene drive products. The second is to build a technical pathway to move from the laboratory to the field. History has demonstrated strains that look great in the laboratory but are crippled in the field, and Oberhofer et al. (1) are careful in recognizing that there is a likelihood that their construct could be much less effective under the harsher conditions in the field. Given the potential benefits of gene drives, efforts to overcome these challenges are unlikely to fade.

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