Opinion: Is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat?

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Scientists have recognized the potential for applying gene drive technologies to the control of invasive species for several years (1, 2), yet debate about the application of gene drive has been primarily restricted to mosquitoes (3). Recent developments in clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 technology have restarted discussions of using gene drive for invasive species control (4).

The implications are potentially remarkable: for the first time we may genuinely have a tool with the power to permanently eliminate a target species from the planet (Fig. 1). The question is no longer whether we can control invasive species using gene drive, but whether we should. Here we explore the implications of recent developments in CRISPR-Cas9 gene drive technology from a biosecurity perspective, through broad comparison with classical biological control (CBC).

Researchers, policymakers, and resource managers must carefully weigh the risks of implementation that could threaten rather than assist a given ecosystem. To assess the technology's potential and avoid the considerable pitfalls, we must look at the many successes, failures, and cautionary tales born of traditional control methods: they must be precise, must be cognizant of ecosystem-wide implications, and must be wary of and anticipate unintended consequences.

Fig. 1. CRISPR-Cas9 gene drive technology has vast potential as a tool for controlling invasive species. From top, left to right: Giant African snail, kudzu, black rat, and zebra mussels. Images courtesy of (*Top*, *Left* to *Right*) Wikimedia Commons/Thomas Brown, Shutterstock/J. K. York, CSIRO ScienceImage, US National Oceanic and Atmospheric Administration.

Driven to Extinction

Gene drive technologies provide the ability to disperse engineered genes throughout target populations much more quickly than would be possible via simple genetic inheritance (5). In nature, selfish genetic elements use a similar strategy, generating multiple copies across the genome to improve the chances that they are inherited (6).

CRISPR-Cas9 is a bacteria-derived endonuclease system that cuts a target DNA sequence based on complementarity to a 20-bp guide RNA (gRNA) (7). When included as part of a gene drive, a CRISPR-Cas9–generated mutation will make copies of itself anywhere the genome sequence is complementary to the gRNA (7), and hence will convert individuals heterozygous for the mutation into homozygotes (4). Unlike previous technologies, CRISPR-Cas9 offers simplicity, flexibility, and precision in gene targeting, such that any standard molecular biology laboratory could adopt the technology with little additional specialist equipment or training (7). Perhaps most importantly, CRISPR-Cas9 is efficient and inexpensive compared with all previously proposed gene drive technologies (4).

A CRISPR-Cas9 gene drive approach to invasive species control would be based on a laboratory strain with a deleterious trait (e.g., distorted sex ratio, reduced fertility, chemical sensitivity) being mass-reared and released into the field in sufficient numbers for the engineered mutation to spread and control the target population within a desired time frame (1). With careful selection of an appropriate coding region, the promulgation of severe deleterious traits could rapidly remove entire populations comprised of individuals with short generation times from both managed and natural environments. In principle, this approach could serve as a "silver bullet" solution for the management

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of highly threatening invasive alien species. But clearly there are several important factors to take into account, and CBC offers important insights in this context.

Lessons from the Past

To date, CBC has been the only cost-effective management option for controlling widespread and abundant introduced organisms (8). Sterile insect technology has also proven to be an effective control option for some target species (9), but without gene drive sterile insect technology is not self-propagating and for most targets is cost-prohibitive. We contend that the implementation of a gene drive control strategy against invasive alien species would be highly analogous to a CBC program. The logistics of release will be similar, with stringent prerelease risk assessment of nontarget effects to prevent unintended ecological consequences, followed by large-scale rearing and a distribution strategy based on sound ecological understanding of population dynamics. As such, practitioners of CRISPR-Cas9 gene drive should consider the lessons learned from decades of carefully regulated CBC research if we are to apply this technology to biosecurity challenges. We focus on three relevant priorities: (*i*) the importance of understanding target specificity, (*ii*) the implications of population connectivity, and (*iii*) the need to carefully consider unintended cascades for community dynamics.

First, the strictly regulated process required to approve the introduction of CBC agents requires a systematic testing of agent specificity and the consideration of off-target impacts in the context of phylogenetic relatedness, biogeographic overlap, ecological similarity of a range of nontarget species, and the ecology, evolution, and behavior of the agent (8). Any evidence of off-target impacts for CBC represents a risk for agent introduction and is rarely tolerated.

Nontarget effects also need to be an essential consideration for a CRISPR-Cas9 gene drive approach to controlling invasive species (4). Off-target mutations in the target genome can be common if the gRNA is poorly designed, because of a tolerance for mismatches (reviewed in ref. 7). Because the CRISPR-Cas9 gene drive technology remains fully functional in the mutated strain after it is created, the chance of off-target mutations also remains and the likelihood increases with every generation pre- and postrelease. If there is any risk of gene flow between the target species and other species, then there is also a risk that the modified sequences could be transferred and the adverse trait manifested in nontarget organisms. If gene drive technology is to be used for biocontrol purposes, we need to have a robust understanding of gene flow networks involving the target species, and an improved understanding of potential specificity limitations for the gRNA.

Population Dispersal

Second, high dispersal ability is a common trait for many invasive alien species, and the risk of long-distance introductions is particularly high for species associated with human movement and trade (10, 11). For gene drive, this is seen as advantageous from the point of view of rapid spread of the deleterious mutations throughout the target population. However, many invasive species have been introduced to multiple locations across global trade routes, which effectively act as stepping stones linking alien populations with their native range (12). The spread of CBC agents between populations targeted for control or into nontarget populations depends largely on the dispersal traits of the agent, in addition to the risk of anthropogenic or stochastic dispersal (13). The critical difference between agent-mediated CBC and a gene drive approach to biocontrol is that the focus for gene drive is the target species itself.

Are we are willing to risk the global loss of a species as a result of unintended dispersal of modified individuals back to their native range, to benefit from the control efficiencies that CRISPR-Cas9 gene drive technology could offer?

Thus, the spread of a deleterious trait depends on the dispersal ability of the target, as well as anthropogenic or stochastic dispersal. Dispersal of these traits through populations, including into the native range, would be extremely challenging to detect, reducing the ability and increasing the cost of biosecurity measures to prevent spread of modified agents to areas where they remain unwanted. Importantly, targets of CBC are relatively well adapted to the presence of the released agents in their native range (which is generally the agent source), whereas native populations of species targeted by gene drive would remain highly susceptible.

Community Dynamics

Finally, although it might seem that the targeted removal of problematic populations with gene drive technology should only produce positive outcomes, all biosecurity management options have the potential for unintended or indirect ecological consequences that need to be carefully considered. Given the potential for gene drive approaches for eradicating species, the criticism of elevated risks of indirect effects will be more real for such approaches than has been the case with CBC (14, 15). There is increasing awareness, however, that with a growing pool of invasive alien species, many will have considerable niche overlap, such that if one introduced species was to be removed from the community, another would be likely to rapidly take its place (16, 17). Hence, the overall effect on ecosystem values may remain unchanged even if one invader is eradicated. Furthermore, with long introduction histories also comes change in community structure, with introduced species often fulfilling significant roles, particularly in landscapes with strong anthropogenic modification. As with critical food resources or apex predators filling gaps after earlier human-driven extinctions (18), there remains a risk that removing species with gene drive technology could produce unintended cascades that may represent a greater net threat than that of the target species. Given these issues, a consultative and regulated risk–cost-benefit analysis approach, akin to that used in best-practice CBC (19), may be a prudent step forward in the use of the gene drive approach in a biosecurity context.

Weighing the Risks

The question is this: Are we are willing to risk the global loss of a species as a result of unintended dispersal of modified individuals back to their native range, to benefit from the control efficiencies that CRISPR-Cas9 gene drive technology could offer? There may be situations where the risks identified above are minimal and the use of this technology for controlling invasive species is deemed acceptable after a full risk–cost-benefit analysis (Fig. 1). Isolated islands facing severe threats from alien reptiles or perhaps rodents (e.g., ref. 20), where border control and physical distance could control any outward gene flow, potentially represent a low risk-high gain priority for action. In contrast, marine invasions, where unintended anthropogenic dispersal remains inappropriately frequent (21), or terrestrial invasions of highly effective dispersers such as grasses or volant animals (22, 23), could only be viewed as targets with arguably intolerable risk. More challenging situations would include species that are widely viewed as undesirable throughout both their alien and native ranges; yet, in such cases, assumptions

about other countries' biosecurity priorities should be handled with great caution. There are many cases where the risks could be deemed insurmountable.

Irrespective of how these biosecurity risks are perceived, we caution that without a regulatory framework that provides a mechanism to work through these issues with clarity and transparency for CRISPR-Cas9 gene drive, this putative silver bullet technology could become a global conservation threat. Biosecurity is just one of many areas in which CRISPR-Cas9 gene drive technology is being focused (4). It is encouraging that momentum is building to engage stakeholders and scientists from various disciplines into a public discussion on the potential applications of gene drives (see dels.nas.edu/Study-In-Progress/Gene-Drive-Research-Human/DELS-BLS-15-06). We contend that the extensive experience of regulatory successes (and failures) in the context of CBC can offer an existing framework to provide meaningful guidance for assessing risks and benefits for applications related to invasive species control within this emerging field. The time to develop this regulatory framework is now.

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