CRISPR technology shows promise as an effective vector control method for mosquito-borne diseases.

Using CRISPR to Combat Human Disease Vectors

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The annual incidence of vector-borne disease exceeds 1 billion globally—roughly half of the world's population is at risk of infection.[1] Mosquito-borne diseases account for the majority of cases (WHO 2014), but there are no vaccines for most of them, so prevention, mainly through inefficient vector control of limited effectiveness, is the primary method to reduce disease burden. Furthermore, treatments for most mosquito-borne pathogens are also limited, and those that are effective are under threat from increasing pathogen drug resistance.

The severity of the problem is best exemplified by the repeated development of antimalarial resistance in Southeast Asia. In the 1990s parasite resistance to first- and second-line malaria drugs necessitated the development of combination therapies for treatment (Nosten et al. 1987, 1994). However, high resistance to these combination drugs and their later derivatives resulted in an increase in malaria-related deaths in this region (Dondorp et al. 2009; Ménard et al. 2016; Phyo et al. 2016). Therefore, in most cases, vector control is the best approach for reducing the burden of vector-borne diseases.

[1] World Health Organization, "Vector-borne diseases," October 31, 2017 (https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases).

Vector Control Tools

Chemical insecticides have historically been an important tool for mosquito control, but they have limitations, most notably their limited efficacy due to increasing vector insecticide resistance and their limited species specificity and duration. While insecticide-driven approaches have been successful in some disease prevention programs (Pluess et al. 2010), for a myriad of reasons they have mixed results overall (Esu et al. 2010; George et al. 2015; Maciel-de-Freitas et al. 2014). Even in areas where sustained vector control has been achieved in the past, insecticide resistance has greatly reduced or eliminated the impact of vector control on disease transmission (Hemingway et al. 2002; Liu 2015; Maciel-de-Freitas et al. 2014).

Sterile insect technique is an environmentally friendly method but not feasible for large-scale control of mosquito populations.

Given the widespread use of insecticides and limited number of insecticide families available for vector control programs, insecticide resistance will continue to be a barrier to insecticide-based vector control. New control techniques are therefore being evaluated to complement vector control programs.

Sterile Insect Technique for Insect Control

Sterile insect technique (SIT) is the gold standard for genetics-based insect population control. In classic SIT, insects are treated with ionizing radiation to induce male sterility and then released in high frequency to mate with wild females, resulting in nonviable progeny. Over time, repeated mass releases of sterile males suppress and can even eliminate target populations. This approach was used to eradicate the screwworm fly (*Cochliomyia hominivorax*; Krafsur et al. 1986), the Mexican fruit fly (*Anastrepha ludens*), and the Mediterranean fruit fly (*Ceratitis capitata*) from regions of North America (Hendrichs et al. 2002).

But in mosquitoes irradiation-based SIT causes high male mortality and exceedingly high fitness costs. For example, field studies show that the release of irradiated, sterile male *Aedes albopictus* led to very limited population reduction (Bellini et al. 2013) likely for these reasons.

So although irradiation-based SIT presents an environmentally friendly method of local population suppression, it is not feasible or scalable in its current form for large-scale control of mosquito populations.

Novel Vector Control Methods

In recent years innovative genetic vector control methods, such as the release of insects carrying a dominant lethal (RIDL) (Thomas et al. 2000), have demonstrated large reductions in wild vector populations (Carvalho et al. 2015; Harris et al. 2012). Other novel disease or vector control methods, such as Dengue and Zika virus transmission-blocking *Wolbachia*-infected *Aedes aegypti* and the *Wolbachia* incompatible insect technique (IIT), respectively, are being evaluated in the field (Schmidt et al. 2017). While effective, these methods require large numbers of mosquitoes to be raised, manually sexed, and released as adults in the field near target sites.

Building mosquito mass rearing factories in local disease endemic areas is costly and labor intensive and current procedures are error prone (Gilles et al. 2014; Papathanos et al. 2009). Female release, even in small numbers, is particularly problematic to the *Wolbachia* IIT technology as the release will immunize the target population to the incompatible *Wolbachia* strain and ultimately lead to the failure of the approach. Some studies even indicate that in some contexts, *Wolbachia* actually enhances pathogen infection (Dodson et al. 2014; Hughes et al. 2014) or can have large vector fitness costs, which can be problematic (Joshi et al. 2014).

Additionally, the antibiotic drugs required during rearing of RIDL mosquitoes have high male fitness costs (about 5 percent that of wild-type male fitness) based on RIDL field trials in the Cayman Islands (Harris et al. 2011) and Brazil (Carvalho et al. 2015), due to the loss or alteration of gut microbiome or symbiotic bacteria as well as toxicity to mitochondrial cell functions (Chatzispyrou et al. 2015; Moullan et al. 2015). Therefore, there is still an urgent need for new vector control technologies for the suppression of wild vector populations.

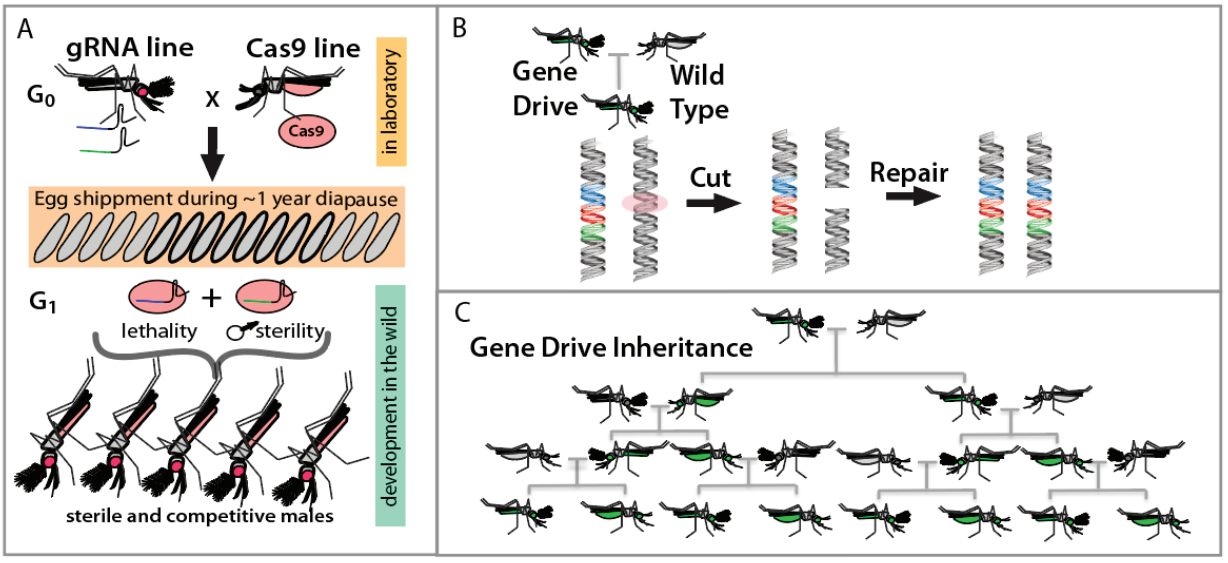

FIGURE 1 Precision-guided sterile insect technique (pgSIT) and homing-based gene drives (HGDs). pgSIT relies on mass rearing two separate strains: the first expresses two guide RNAs (gRNAS) designed to target female viability and male fertility genes, the second expresses the CRISPR-associated protein 9 (Cas9) endonuclease. When crossed, the only surviving progeny are sterile males, which can be repeatedly released as eggs into the environment, resulting in population suppression as they compete with wild males for females (A). HGDs convert heterozygotes to homozygotes using a cut/repair process (B) resulting in biased inheritance and rapid spread into a population (C; green denotes individuals with the gene drive, grey denotes wild-type mosquitoes).

Using CRISPR

The advent of CRISPR[2] technology has excited the potential to engineer new game-changing technologies and innovative systems that can be used to control wild populations of mosquitoes. Two developments of particular interest are a self-limiting system termed *precision-guided sterile insect technique* (pgSIT) (Kandul et al. 2019) and a *homing-based gene drive* (HGD) (Champer et al. 2016; Esvelt et al. 2014). The unique features of these systems can make them valuable in the future to control mosquitoes, as elaborated below.

pgSIT

The novel CRISPR-based pgSIT mechanistically relies on a dominant genetic technology that enables simultaneous sexing and sterilization, facilitating the release of eggs into the environment and ensuring that only sterile adult males emerge. Importantly, for field applications, the release of eggs will eliminate burdens of manually sexing and sterilizing males, reducing the time and effort involved and increasing scalability. Moreover, the release of eggs should reduce the need to build factories near release sites as eggs could be shipped to release locations from a centralized facility and hatched directly in the environment.

This system was recently systematically engineered in an insect fly model system and was shown to be extremely efficient at generating 100 percent sterile males that could suppress populations. The system functions by mass producing two strains, one expressing the CRISPR-associated protein 9 (Cas9) endonuclease and the other expressing two guide RNAs (gRNAs), one targeting a gene important for female viability and the other a gene important for male fertility. When the two separate strains are crossed the only surviving progeny are sterile males, which can be directly deployed (figure 1A).

Efforts are underway to transfer this technology to mosquitoes, and in the coming years it may be deployed in the field.

Homing-Based Gene Drives

Replacement of wild insect populations with genetically modified individuals unable to transmit disease provides an environmentally friendly, sustainable, and self-perpetuating method of disease prevention. How-

[2] CRISPR (clustered regularly interspaced short palindromic repeats) is a family of DNA sequences in the genomes of prokaryotic organisms such as bacteria.

ever, transgenes that mediate disease resistance to treatment (refractoriness) may inadvertently compromise the fitness of insects that carry them. Furthermore, wild populations are large, partially reproductively isolated, and dispersed over wide areas.

Population replacement therefore requires a gene drive mechanism to spread linked genes that mediate disease refractoriness through wild populations at greater than Mendelian frequencies. In an effort to achieve this, CRISPR methods have been used to accelerate the development of HGDs in model systems in addition to mosquitoes and even mammals (Champer et al. 2017, 2018; DiCarlo et al. 2015; Gantz and Bier 2015; Gantz et al. 2015; Grunwald et al. 2019; Hammond et al. 2016, 2018; KaramiNejadRanjbar et al. 2018; Kyrou et al. 2018; Li et al. 2019; Windbichler et al. 2011; Yan and Finnigan 2018).

HGDs function by encoding the Cas9 endonuclease and an independently expressed gRNA responsible for mediating DNA base pairing directing Cas9-mediated cleavage at a predetermined site (Champer et al. 2016; Esvelt et al. 2014; Gantz and Bier 2016; Marshall and Akbari 2018). When the HGD is positioned in its target site in a heterozygote, double-stranded DNA breakage of the opposite chromosome can cause the drive allele to be used as a template (i.e., donor chromosome) for DNA repair mediated by homologous recombination. This can result in copying, or "homing," of the HGD into the broken (receiver) chromosome, thereby converting heterozygotes to homozygotes in the germline, which can bias Mendelian inheritance ratios and lead to an increase in HGD frequency in a population (figure 1B,C).

Given recent progress toward developing HGDs in pest species such as mosquitoes (Gantz et al. 2015; Hammond et al. 2016, 2018; Kyrou et al. 2018; Li et al. 2019), there is significant enthusiasm for their potential use to control wild populations. For example, release of HGDs linked with effector genes that inhibit mosquito pathogen transmission (Buchman et al. 2019a,b; Isaacs et al. 2011; Jupatanakul et al. 2017) may lead to replacement of disease-susceptible mosquitoes with disease-resistant counterparts, thereby reducing pathogen transmission (i.e., population modification drive). Alternatively, HGDs targeting genes that affect the fitness of female mosquitoes could also lead to gradual population declines and potentially even elimination (i.e., population suppression drive) (Kyrou et al. 2018; Windbichler et al. 2008, 2011).

Conclusion

Both genetic SIT systems and modification and suppression drives have the potential to transform mosquito population control measures (Burt 2003; Champer et al. 2016; Esvelt et al. 2014), and therefore have excited discussions about their potential use, regulation, safety, ethics, and governance (Adelman et al. 2017; Akbari et al. 2015; NASEM 2016; Oye et al. 2014). Field testing of these systems over the next 5 to 10 years will help illuminate the efficacy and safety concerns of these systems.

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