Using CRISPR to Combat Human Disease Vectors

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Burden of vector-borne disease

The annual incidence of vector-borne disease exceeds 1 billion globally with mosquito-diseases comprising the majority of the global vector-borne disease burden (World Health Organization 2014). Roughly ½ of the world's population is presently at risk of infection and unfortunately there are currently no vaccines for most mosquito-borne diseases, so prevention, mainly through inefficient vector control, is the primary method to reduce disease burden. Treatments for most mosquito-borne pathogens are also limited, and those that are effective are under threat due to increasing pathogen drug resistance. The severity of this 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 malaria treatment (Nosten et al. 1987, 1994); however, high resistance to these combination drugs and their later derivatives has continued resulting an increase in malaria related deaths in this region (Phyo et al. 2016; Dondorp et al. 2009; Ménard et al. 2016). Therefore, in most cases, vector control is the best approach for reducing the burden of 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 in addition to their limited species specificity and duration. While many insecticide driven approaches have been successful in some disease prevention programmes (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 (Liu 2015; Hemingway, Field, and Vontas 2002; Maciel-de-Freitas et al. 2014). Due to the widespread use of insecticides and the limited number of insecticide families available to vector control programs, insecticide resistance will continue to be a barrier to insecticide-based vector control. Due to the problems associated with chemical insecticides and other conventional control methods, new control techniques are being evaluated to complement vector control programs.

SIT for insect control

Sterile insect technique (SIT) is the gold standard for insect population control. In classic SIT, insects are irradiated with ionizing radiation to induce male sterility. The sterile males are then released in high frequency to mate with wild females resulting in non-viable progeny. Over time, repeated mass releases of sterile males suppresses and can even eliminate and eradicate 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).* Notwithstanding, in mosquitoes irradiation-based SIT causes high male mortality and exceedingly high fitness costs.

For example, recent field studies release of irradiated, sterile, male *Aedes albopictus* led to very limited population reduction (Bellini et al. 2013) likely for these reasons. While the classic irradiation-based SIT presents an environment-friendly method of a local population suppression, it is not currently feasible or scalable in its current form for wide-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 virus (DENV) and Zika virus (ZIKV) transmission blocking *Wolbachia* infected *Aedes aegypti* and the *Wolbachia* Incompatible Insect Technique (*Wolbachia* IIT), respectively, are currently 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, 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* technology as this 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* enhances pathogen infection (Hughes, Rivero, and Rasgon 2014; Dodson et al. 2014) or can cause large vector fitness costs (Joshi et al. 2014). Additionally, the antibiotic drugs required for RIDL mosquitoes have high male fitness-costs (~5% that of *wt* male fitness) based on RIDL field trials in the Cayman Islands(Harris et al. 2011) and Brazil(Carvalho et al.

2015) resulting from the loss or alteration of gut microbiome or symbiotic bacteria as well as toxicity to mitochondrial cell functions (Moullan et al. 2015; Chatzispyrou et al. 2015). Therefore, there is still an urgent need for new vector control technologies for the suppression of wild vector populations.

Using CRISPR

The advent of the CRISPR technology has excited the potential to engineer new game changing technologies that can be used to control mosquitoes. In fact, in the last several years many innovative systems have been engineered in insects using CRISPR which have the potential to control wild populations. Two systems of particular interest include a self-limiting system termed precision guided sterile insect technique (pgSIT)(Kandul et al. 2019) and a system referred to has a homing based gene drive (HGD)(Champer, Buchman, and Akbari 2016). Each of these systems has unique features which can make them valuable in the future to control mosquitoes which are elaborated further below.

pgSIT

Recently a novel CRISPR-based technology termed precision guided SIT (pgSIT) was described. PgSIT mechanistically relies on a dominant genetic technology that enables simultaneous sexing and sterilization, facilitating the release of eggs into the environment ensuring only sterile adult males emerge. Importantly, for field applications, the release of eggs will eliminate burdens of manually sexing and sterilizing males, thereby reducing overall effort 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% sterile males that could suppress populations. The system functions by mass producing two strains - one expressing the Cas9 endonuclease - and the other expressing two guide-RNAs (gRNAs) one targeting a gene important for female viability and the other targeting a gene important for male fertility. When these two separate strains are crossed together the only surviving progeny are sterile males which can be directly deployed (Figure 1A). Efforts are currently underway to transfer this technology to mosquitoes, and in the coming years we may see this system deployed in the field.

Homing Gene Drives

Replacement of wild insect populations with genetically modified individuals unable to transmit disease provides an environmentally friendly, sustainable, and self-perpetuating method for disease prevention. However, transgenes that mediate disease refractoriness are unlikely to confer an overall fitness benefit on insects that carry them. Additionally, wild populations are large, partially reproductively isolated, and dispersed over wide areas. Therefore, population replacement requires a gene drive mechanism in order to spread linked genes mediating disease refractoriness through wild populations at greater than Mendelian frequencies. To address this problem, recently the CRISPR revolution accelerated the development of HGD's in model systems in addition to mosquitoes and even mammals (Gantz and Bier 2015; Gantz et al. 2015; Grunwald et al. 2019; Kyrou et al. 2018; Li et al. 2019; Windbichler et al. 2011; A. Hammond et al. 2016; Yan and Finnigan 2018; DiCarlo et al. 2015; Champer et al. 2017, 2018; KaramiNejadRanjbar et al. 2018; A. M. Hammond et al. 2018). HGD's function by encoding the Cas9 endonuclease and an independently expressed guide RNA (gRNA) responsible for mediating DNA/RNA base pairing

and cleavage at a predetermined site (Esvelt et al. 2014; Champer, Buchman, and Akbari 2016; Gantz and Bier 2016; Marshall and Akbari 2018). When the HGD is positioned within its target site in a heterozygote, double stranded DNA breakage of the opposite chromosome can result in the drive allele being 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 chromosome (i.e. receiver chromosome), thereby converting heterozygotes to homozygotes in the germline, which can bias Mendelian inheritance ratios and result in an increase in HGD frequency in a population (Figure 1B,C).

Given the recent progress toward developing HGDs in pest species such as mosquitoes (Gantz et al. 2015; Kyrou et al. 2018; Li et al. 2019; A. M. Hammond et al. 2018; A. Hammond et al. 2016), there is significant enthusiasm regarding their potential use to control wild populations. For example, release of HGDs linked with effector genes inhibiting mosquito pathogen transmission (Buchman, Gamez, Li, Antoshechkin, Li, et al. 2019; Jupatanakul et al. 2017; Isaacs et al. 2011; Buchman, Gamez, Li, Antoshechkin, Lee, et al. 2019) may lead to replacement of disease-susceptible mosquitoes with disease-resistant counterparts resulting in reduced pathogen transmission (i.e. population modification drive). Alternatively, HGDs targeting genes affecting the fitness of female mosquitoes could also spread, resulting in gradual population declines and potentially even elimination (i.e. population suppression drive) (Windbichler et al. 2011; Windbichler, Papathanos, and Crisanti 2008; Kyrou et al. 2018). Given these features, both modification and suppression drives possess the potential to transform mosquito population control measures (Burt 2003; Esvelt et al. 2014; Champer, Buchman, and Akbari 2016), and therefore have excited significant ongoing discussions involving their potential usage, regulation,

safety, ethics and governance (Adelman et al. 2017; Akbari et al. 2015; National Academies of Sciences, Engineering, and Medicine et al. 2016; Oye et al. 2014). Perhaps in the next 5-10 years we may begin to see the field testing of HGD's which will help illuminate the efficacy and safety concerns of these systems.

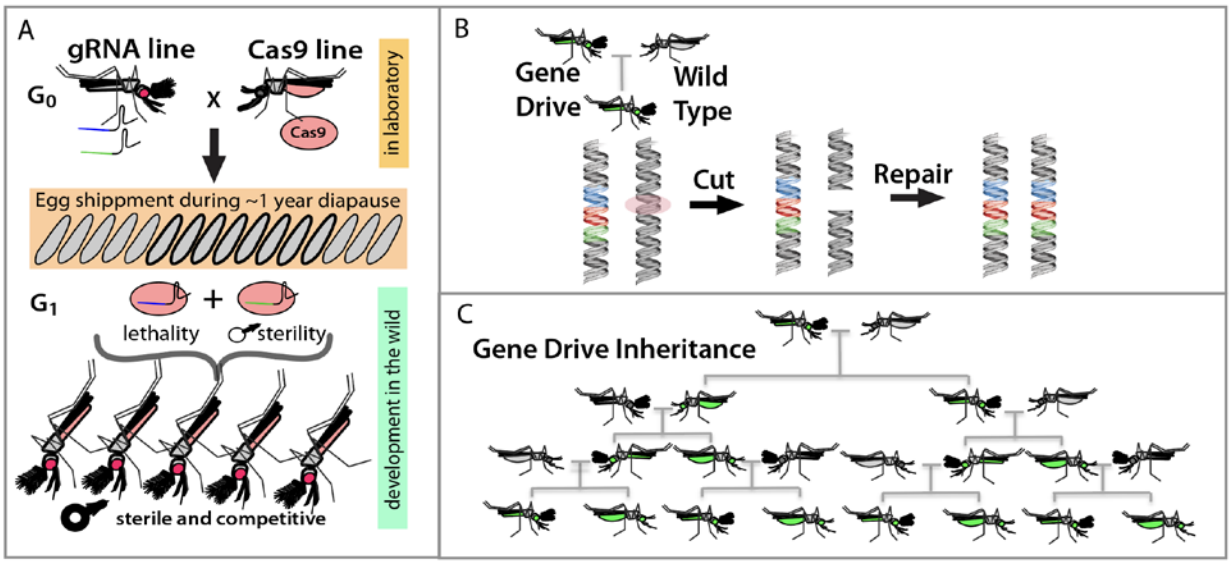

Figure 1. Precision guided SIT and Homing based Gene drives. Precision guided SIT (pgSIT) relies on mass-rearing two separate strains. The first strain expresses two guide RNAs (gRNAS) designed to target female visibility and male fertility genes. The second expresses the Cas9 endonuclease. When crossed together the only surviving progeny are sterile males which can be repeatedly released as eggs into the environment resulting in population suppression (A). A homing based gene drive, converts heterozygotes to homozygotes using a cut/repair process (B) resulting in biased inheritance and rapid spread into a population (C).

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