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Genetic Control Of Malaria Mosquitoes

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Abstract

Experiments demonstrating the feasibility of genetically modifying mosquito vectors to impair their ability to transmit the malaria parasite have been known for well over a decade. However, means to spread resistance or population control genes into wild mosquito populations remains an unsolved challenge. Two recent reports give hope that CRISPR technology may allow such challenge to be overcome.

Genetic modification of wild insect vector populations to suppress their ability to transmit human pathogens is a longstanding scientific dream. The advent in 1982 of transgenesis in the fruit fly *Drosophila melanogaster* [1] gave impetus to the idea of genetically manipulating mosquitos to render them incompetent vectors of pathogen transmission. The feasibility of this strategy to block transmission of the malaria parasite by an anopheline mosquito was first demonstrated in 2002 [2] and followed by a number of other studies. However, translation of these successes to the field faced a major challenge: the difficulty of spreading transgenes into wild vector populations.

This problem can be understood considering simple Mendelian genetics. When a mosquito carrying an anti-*Plasmodium* transgene mates to a wild mosquito, only 50% of the resulting progeny will carry the transgene. When these progeny outcross again to wild mosquitoes, the transgene will drop down to only 25% frequency in the next generation, meaning that the transgene is gradually diluted out (Figure 1A, left). Should the transgene bear a fitness cost, it will disappear even more rapidly. The only way to overcome this dilution is to introduce transgenic males at an extremely high frequency (i.e., at numbers exceeding the wild population) and to continually repeat the process. This approach cannot be realistically implemented for a mosquito as abundant and widespread as the principal malaria vector *Anopheles gambiae*.

These barriers could be surmounted by engineering the vector such that the transgene is transmitted at a frequency greater than the 50% predicted by Mendelian inheritance thus ensuring an increase in gene frequency with each generation. This process is known as genetic drive and occurs in nature by a variety of mechanisms. In the 1960s, Curtis [3] proposed the theoretical principle for one such mechanism based on the use of translocation

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chromosomes coupled to a pathogen-resistant gene. However, conversion of such proposal into practice would be a herculean task.

One of the best-studied examples of genetic drive found in nature comes from a family of elements known as homing endonuclease genes (HEGs). These elements encode a highly specific endonuclease that recognizes a unique, long target sequence (15~20 bp) at the position of the wild-type chromosome where the nuclease gene is inserted. In an heterozygous organism, when the nuclease produces a double-strand break at the target sequence on the sister chromosome, the HEG-chromosome locus is used as a template for homology-directed repair, resulting in the introduction of the HEG sequences into the wild-type chromosome. In this way, the heterozygote HEG-carrier (HEG+/HEG-) is converted into a homozygote (HEG+/HEG+). The same process will convert the progeny of this homozygote from heterozygotes into homozygotes (Figure 1A, right).

The first attempt to engineer a synthetic HEG-like genetic drive system using custom zinc-finger and transcription activator-like effector nucleases (TALEN) in *D. melanogaster* was moderately successful. However, the repetitive sequences of the nuclease genes led to deletion and rearrangement events that decreased the efficiency of genetic drive [4]. The recent advent of the simpler, user-friendly [Cas9 nuclease]-[guide RNA (gRNA)] elements derived from the bacterial CRISPR system has provided an opportunity to overcome these difficulties

In April 2015, the first demonstration of a [Cas9-gRNA]-based drive was reported for *Drosophila* and termed mutagenic chain-reaction (MCR) [5]. Now, two groups have ported MCR to malaria vector mosquitos generating a synthetic drive mechanism similar in principle to the HEG approach [6,7]. The MCR cassette consists of a Cas9 nuclease gene driven by a germline-specific promoter (*vasa*) and a gRNA-encoding gene integrated into the mosquito genome at the locus of the gRNA target site, eliminating the site in the process. When the Cas9 nuclease is produced in the germline, it associates with the gRNA and generates a double-strand break at the homologous locus of the sister chromosome. If the break is resolved by a homology-directed repair mechanism, the [Cas9-gRNA] MCR cassette from the homologous chromosome will be used as the template for repair, rendering the germ cell homozygous for the MCR cassette. All gametes arising from such an event will pass on the [Cas9-gRNA] cassette to their progeny, just as with naturally occurring HEGs (Figure 1B). In both mosquito studies, the crossing of a heterozygous MCR-carrier to a wild-type individual resulted in transmission of the allele to over 90% of the progeny, demonstrating strong genetic drive compared to the 50% inheritance predicted by Mendelian genetics.

Working with the Indian malaria vector *Anopheles stephensi*, Gantz *et al.* [6] included two additional genes in their MCR cassette that code for monoclonal antibodies targeting surface proteins of the malaria parasite, both of which have been previously demonstrated to block parasite development [8]. Despite dramatically increasing the size of the MCR cassette (to 17 kb!), strong genetic drive was still observed.

Hammond *et al.* [7] chose a distinct, but equally promising approach. Using the main African malaria vector *An. gambiae*, they identified recessive genes that render female mosquitoes sterile as homozygous knockouts. When any one of these genes is used as targets for MCR, the [Cas9-gRNA] copies itself into the gene in the homologous chromosome, effectively knocking out the gene from the germline. Consequently, nearly all gametes carry the mutant gene and nearly all female carriers of the MCR cassette are infertile, while males remain unaffected (the gene is required for female fertility only). Males continue to transmit the drive element, creating a self-perpetuating sterile-insect release system.

The two strategies differ in an important way. Gantz *et al.* [6] use a ‘population replacement’ strategy that aims at converting a transmission-competent to a transmission-impaired mosquito population while Hammond *et al.* [7] use a ‘population suppression’ approach. The latter has the drawback of leaving an empty biological niche prone to mosquito repopulation.

While both strategies show much promise, some technical issues remain to be resolved, such as decreased spreading due to non-homologous end-joining and decrease of mosquito fitness by gene disruption. However, the high efficiency of both approaches suggests that MCR-driven transgenes may be ready for field trials in a foreseeable future. It is important then, to address the regulatory, environmental and ethical issues for practical implementation. Recently, a study in yeast [9] demonstrated a method for recalling an MCR cassette to return a population to wild type. Creative thinking of this nature will help the dream come true.

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Figure 1. Mendelian Inheritance versus Genetic Drive

(A) The two diagrams illustrate gene frequencies at each generation (bold downward arrows) when no gene drive exists (left diagram; Mendelian inheritance) or under the action of a homing endonuclease gene (HEG)-like gene drive (right diagram). Note that in the absence of a gene drive, the transgene (orange box) becomes ‘diluted’ as the individuals mate with wild-type insects, while the HEG-like gene drive maintains the transgene at a high frequency even when crossing with wild-type individuals. (B) A [Cas9-gRNA]-based HEG-like gene drive. The upper diagram illustrates a heterozygous cell where one of the chromosomes carries a gene cassette encoding a Cas9 nuclease (orange oval), a guide RNA (gRNA; green line) and a ‘cargo’ (gene of interest, for instance, encoding an antiparasite product). The sequence marked in red on the gRNA is complementary to the red target site on the upper chromosome. The middle diagram illustrates how the gRNA leads the Cas9 nuclease to the target site, resulting a double-strand cleavage of the chromosome. Pairing of the homologous sequences (left red and right blue rectangles) promotes introduction of the gene cassette at the break point, resulting into a homozygous state where both chromosomes carry the cassette (lower diagram). Note that the cassette-containing chromosomes have lost the target site.