Controlling the transmission of Zika and other mosquitoe-borne diseases by using genetically engineered mosquitoes

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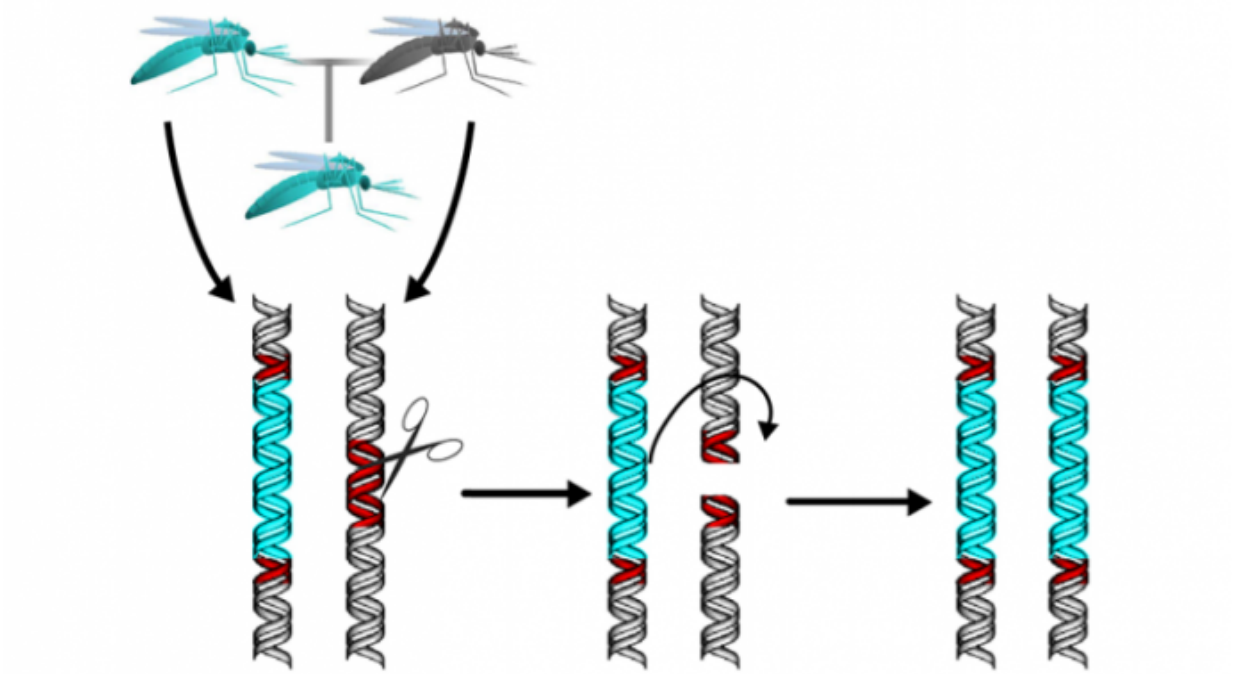

CAPTION: In organisms that inherit one drive-containing and one wild-type chromosome, the drive cuts the wild-type chromosome, causing the cell to copy the drive when it uses the drive-containing chromosome as a template to repair the damage. Because it now has two copies of the drive (and whatever alteration the drive is spreading), all of the organism's offspring will inherit a drive-containing chromosome to repeat the process.

In the past few weeks, Zika virus infection has become headline news and on February 1st, it was declared as a Public Health Emergency of International Concern by the World Health Organisation, this was due primarily due to the yet to be confirmed link with abnormal births (microcephaly) and neurological (Guillain Barre Syndrome) disorders.

Zika virus is mosquito borne disease related to dengue, yellow fever or West Nile Virus. The virus is transmitted by Aedes aegipti or Aedes albopictus mosquito bites. Despite the fact the infection gives asymptomatic or mild symptoms for over 80% of infected population, and results in less severity and death than Chikungunya or Dengue virus infections, there are serious concerns for the health outcomes of the severe cases as the outbreak is spreading at a rapid pace in South and North America. Sadly, as with most of emerging infectious diseases, there is no cure for the infection and the development of a vaccine could be a lengthy process. There are many suggested strategies to control the transmission of the infection, one of which is the release genetically modified mosquitoes, which relies on the CRISPR/cas9 technology described below.

CRISPR/Cas9 gene drive: a novel avenue to combat mosquito-borne diseases

The rapid progresses in the field of genome-engineering has considerably changed our perspective on how to tackle and control the transmission of emerging infectious diseases. Novel avenues such as CRISPR/Cas9 gene drive strategy have raised hopes that they will stop the transmission of the virus and eradicate the mosquitoes in many media outlets. While the gene drive strategy was developed for many years using transposable elements or meiotic drive, the association of a CRISPR/Cas9 system has considerably improved the efficiency of the system. The CRISPR/Cas9 gene drive consists of a combination of a

Cas9 enzyme associated with a guide RNA and 2 homology arms of a gene of interest. This strategy enables an efficient and rapid spread of deleterious alleles within a population only in few generations.

To date, the gene drive strategy using a CRISPR/Cas9 system to stop the transmission of infectious diseases has been tested only on Anopheles stephensi or gambiae, the vectors for the malaria parasite Plasmodium falciparum, and only in a laboratory setting. Two scenarios were tested. The first approach in Anopheles gambiae targeted several candidate fertility genes leading to a disruption of these genes and a recessive female sterility phenotype (Hammond, Galizi et al. 2016). The reported success ranged from 85 to 99% in the laboratory. Another alternative was to target not the mosquito reproduction, but genes that are critical for the transmission of the parasite (Gantz, Jasinskiene et al. 2015). This was done by knocking out a receptor in the mosquito required for the parasite to replicate within the salivary glands. The reported efficiency of the gene drive using this approach was ~ 98%.

Is the CRISPR/Cas9 gene drive that promises to cure

mosquito-borne diseases overstated?

While there is excitement about the use of the CRISPR/Cas9 gene drive technology and its use into the eradication agenda, there are many unknown, as well as many know hurdles that could prevent the success of this strategy. Firstly, the gene drive was performed only in laboratory conditions for anopheles and drosophila species and to date this hasn't been done in Aedes mosquito species. Importantly, none of the CRISPR/Cas9 gene drive system has been tested in the field. To date it is impossible to predict how many edited mosquitoes founders are needed for a sufficient penetration of the genome modification into the population or for instance the coverage of the mosquito release. To complicate the matter, Aedes mosquitoes have an extraordinary capacity to adapt to various conditions. The eggs are laid on every possible indoor or outdoor surface as long as the water is available, this includes trees holes, flowers vases, plastic bottles etc, and this may prevent the successful penetration of the edited mosquitoes. While there is enthusiastic hype for CRISPR/Cas9 gene drive, extensive field trials are no doubt needed to assess the likelihood of success of this strategy. This would certainly require a few years to perform the trials and to get approval from authorities before the first release of the genetically modified mosquitoes.

Wolbachia infection: an alternative way to combat mosquito-borne diseases

Another recently publicized avenue as an alternative to gene-edited mosquitoes is the infection of Aedes mosquitoes with the endosymbiotic bacteria Wolbachia. Wolbachia pipentis is an intracellular bacteria that infects the Aedes or Anopheles host and prevent any transmission of the virus or malaria. The bacteria can spread rapidly into the mosquito population affecting the transmission of the infectious agents. Field trials

showed successes in using Wolbachia to stop the transmission of dengue virus, chikungunya, yellow fever virus or the malaria parasite (Frentiu, Zakir et al. 2014). However there is published evidence that the Wolbachia infection induces resistance to malaria and dengue virus (Bian, Xu et al. 2010). More importantly, we don't yet know if Wolbachia infection would stop the transmission Zika virus.

How to combat mosquito-borne diseases?

The CRISPR/Cas9 gene drive, as well as Wolbachia or alternative strategies, could certainly help to combat Zika and other emergent mosquitoes-borne diseases and possibly eradicate the mosquitoes, and therefore mosquito-borne diseases. While this is conceptually realistic, there are many unknown factors involved, and to date, there is no evidence this would actually be possible in the field. The viruses could adapt to a new host that is not targetable by the current strategies, the penetration of the eradication methods are unknown, and the impact of the suggested strategies is almost impossible to predict. Viruses, and in general pathogens, have the ability adapt to a new niche in response to the host or environment; or a proposed approach could select a pathogen to arise and spread in the same way we have seen before with the malaria parasite in response to a mutation in Duffy antigen variant in Africa (Prugnolle, Rougeron et al. 2013) Numerous concerns have been raised about the impact on the ecological and environment using CRISPR/Cas9 gene-drive technology.

Releasing CRISPR/Cas9, as well as Wolbachia infected mosquitoes, is certainly a tempting approach to stop the spread of the virus infection and eradicate the mosquito-borne diseases. However, while the expectations are high, a more measured approach combined with the explorations of novel research avenues, and importantly, a better comprehension of the biology of these infections, is a necessity to avoid a similar disappointment and disillusion such as that observed before use

of DTT (Riveron, Chiumia et al. 2015) . For current outbreak such as Zika virus, preventive measures are crucial in the approach to the disease, and these include household protection, waste disposal or community participation, following WHO recommendations to combat the transmission of the infection.

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