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EDITORIAL

Transgenic mosquitoes: Is that a reality?

The real battle against mosquitoes started in 1897, when Sir Ronald Ross from the Liverpool School of Tropical Medicine found malaria parasites in the gut of an anopheline mosquito, confirming that malaria is a mosquito-borne disease. With the expansion of our knowledge on the transmission of mosquito-borne diseases, control of mosquitoes became a major strategy in controlling these diseases, especially in the absence of effective vaccines for disease prevention. Introduction of DDT in the 1940s marked a milestone in the war against mosquitoes. Incidence of malaria and other insect vector borne diseases sharply decreased with the application of DDT. However, the situation reverted with the appearance of DDT resistance in insects. Despite the subsequent introduction of novel groups of synthetic insecticides such as organophosphates, carbamates and pyrethroids replacing DDT in many mosquito control programmes, resistance development was detected eventually against all groups of synthetic insecticides. Also, it became obvious that these chemicals pollute the environment thus causing harmful impacts on all living organisms. Although eco-friendly methods such as the use of Bt crystals, polystyrene beads, various biological agents, were also developed, their use in field operations were not successful due to heavy costs, operational difficulties and storage issues. Even today, use of synthetic insecticides is the major way of controlling mosquitoes during disease outbreaks.

Despite the heavy use of insecticides, the disease incidence still prevails. Every year, more than 500,000 deaths occur due to mosquito-borne diseases worldwide mainly due to malaria and dengue. Climate change, increased globalization, rapid urbanization, vector resistance to insecticides and ineffective vector control programmes have significantly contributed to the increased vector populations. Therefore, failures in vector control methods need to be addressed urgently with novel and effective strategies with the help of emerging technologies. Release of transgenic mosquitoes was proposed as one of such novel strategies for effective control of vector mosquito populations. Two different endpoints are targeted by these interventions; population reduction or suppression and population replacement with strains that are refractory to pathogen development and transmission.

Release of insects carrying a dominant lethal (RIDL) gene, a promising approach for population suppression, was pioneered by the Oxitec (www.oxitec.com). In this system, transgenic insects carry a female-specific lethal dominant and repressible gene system (Thomas et al., 2000). Only males are released into the environment to mate with wild females so that the subsequent female progeny die in their immature stages. The lethal gene is repressed using an antidote (tetracycline) so that female mosquitoes can be reared to adulthood in the laboratories in the presence of tetracycline for reproduction. Although this has been successful in reducing the dengue mosquito population up to 95% in field trials, high costs, need for mass rearing and frequent release, and low fitness shown by modified mosquitoes have hindered its success.

Incompatible Insect Technique (IIT) uses mosquitoes infected with the bacterium *Wolbachia* to prevent viable progeny production leading to a population reduction. An IIT approach resulted in up to 98% reduction of the wild type population of *Aedes aegypti*. *Wolbachia* infection could result in an almost complete blocking of transmission of DENV (Dengue viruses) in field dengue vector populations in Australia and Vietnam (Nguyen et al., 2015). The public concern of releasing transgenic organisms is largely less debatable in this particular instance due to the facts that the genome of the mosquitoes is not changed and the bacteria is already widely present in all environments.

The term ‘transgenesis’ implies ‘introduction of exogenously-derived genes’ whereas `gene drive’ refers to the ‘ability to inherit at a greater rate leading to rapid increase in frequency’. The existence of an almost complete database of the genome of vector mosquitoes has now allowed scientists to identify highly conserved potential target sequences across species to produce mosquitoes refractory to pathogen development and transmission. These genes can block pathogenic development inside the mosquito by altering its genome with anti-pathogen effects or deleting mosquito’s host factors required for pathogen development. Using CRISPR/Cas9 system, a highly effective tool for precision genome-editing, scientists have now developed transgenic mosquitoes with gene drive systems for population replacement. Here, a gene-driven cargo, consist of a `gene of interest’ and a `CRISPR/Cas9 system’, is introduced to mosquitoes, where the gRNA component of the CRISPR/Cas9 system identifies the target mosquito DNA and Cas9 enzyme does the alteration required to insert or repair the gene of interest, replacing the wild type allele in the population. A fascinating CRISPR gene-drive system developed in mosquitoes has the ability to be transmitted in a highly-efficient “super-Mendelian” fashion to >99% of progeny and carries a gene cassette conferring resistance to malarial parasites (Grants et al., 2015).

These transgenic mosquitoes are still tested at laboratory level and their efficacy in the field level is yet to be tested. Although researchers have carried out experiments with large indoor cages to show that transgenic mosquitoes with gene drive systems can make the whole mosquito population driven to extinction within one year, to which

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extent these experimental cages can mimic complexities of nature is questionable. Once mosquitoes are released, the ecological consequences that they cause could not be reversed. Acceptance of transgenic mosquitoes for field application in regulatory and social domains is yet to be resolved. Potential ecological, ethical, and social impacts should be thoroughly evaluated, and should satisfy the requirements of the World Health Organization and Cartagena Protocol on Biosafety to the Convention on Biological Diversity. This practice has to be safe and spillover of genetic elements to non-target populations should be minimized. Once released, there is a chance that transgenic mosquitoes may cross boundaries to other states/countries where they are not accepted. So far, the experience shows that genetically modified mosquitoes are prone to reduce their fitness in the field. There are instances where mating discriminations have been shown by wild females towards genetically modified mosquitoes. Theoretical work has indicated that resistance to CRISPR invasion is likely to evolve in populations. Laboratory studies using *Drosophila* have shown rapid resistance evolution against gene drive systems (Powel, 2022), thus indicating that mosquitoes may soon develop resistance to mechanisms employed for genome repair. Long term refractoriness of mosquito vectors can not be anticipated, in the light of high rates of mutability and adaptability shown by pathogens and vector mosquitoes for their survival!

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