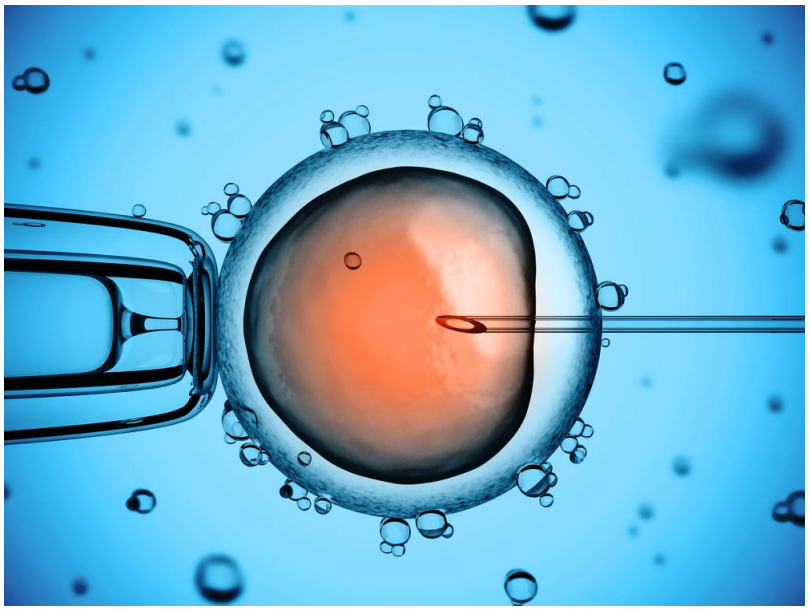
Malaria-blocking mosquitoes



1. Preface:

After a fair amount of research on a multitude of projects I came across the University of California's project of malaria-blocking mosquitoes. It immediately caught my attention and struck me as being very interesting. I find the project is quite different from a majority of other genetic engineering projects because it has the potential to actually help save human lives and doesn't only serve as a luxury. There is still work being done on the project by the professors at UC Irvine and UC San Diego and the genome editing procedure is a recent discovery. Therefore the project is not yet very widely known and it was new to me as well. I wanted to write about something that I didn't know about before in order to learn more in the process.

The questions I hope to be able to answer while working on this paper are:

-How exactly does the CRISPR-Cas9 technique work? This is important to me because it can be used in many different fields of genetic engineering.

-What are the main pros and cons of this project?

2. Introduction:

For almost 20 years Professor Anthony James and his team at UC Irvine have been working on engineering anti-disease mosquitoes. There were two main goals that had to be reached in order to create such mosquitoes. Firstly genes capable of interfering in pathogen development had to be either identified or created in the laboratory. Secondly a system to put those genes into the mosquitoes had to be developed. The team at the James lab did succeed in developing a first basic system to stably insert such genes into the *Anopheles stephensi* mosquitoes. This in turn led to contributions from a number of other labs and collaborators. These contributions included the technique to engineer mosquitoes to make them completely resistant to dengue and malaria parasites. This technology was adapted by the James lab to generate single-chain antibodies which led to the production of effector genes that disabled malaria parasites. The key gene to make the phenotype ''flightless female'' was identified. This is significant because it is the females that feed on blood which makes them the transmitters of pathogens. This gene is lethal to females because it makes them unable to mate, feed or fly. Because the gene does not affect the males in such a way, they can spread the gene. Cage trials with these engineered mosquitoes showed that they can effectively cause large mosquito populations to die out.

Earlier this year Professor James collaborated with Professors Valentino Gantz and Ethan Bier of UCSD who had developed the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (Cas9)-mediated gene-drive system in their work on fruit flies. This system allows the transmission of mutations through the germ line with an inheritance rate of 95%. They were able to combine anti-malaria genes with the Cas9 enzyme and a guide RNA which led to the dual anti-plasmodium falciparum effector genes, a marker gene and autonomous gene-drive components to be introgressed in 99.5% of the progeny of outcrosses between transgenic lines and the wild type mosquitoes.

Recently, the James lab was given approval to conduct cage studies in a disease-endemic country.

Currently the attempt to suppress disease-transmitting mosquito populations and lower the risk of being bitten by infected mosquitoes is being sought by the use of pesticides and bed-nets. But these technologies are increasingly becoming less effective due to insecticide resistance or insufficiency. Due to climate change and increasing demand for water more breeding sites for mosquitoes are created. This makes the matter of developing a way of controlling viral diseases like malaria all the more urgent today.

3. Description of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (Cas9)-mediated gene-drive system:

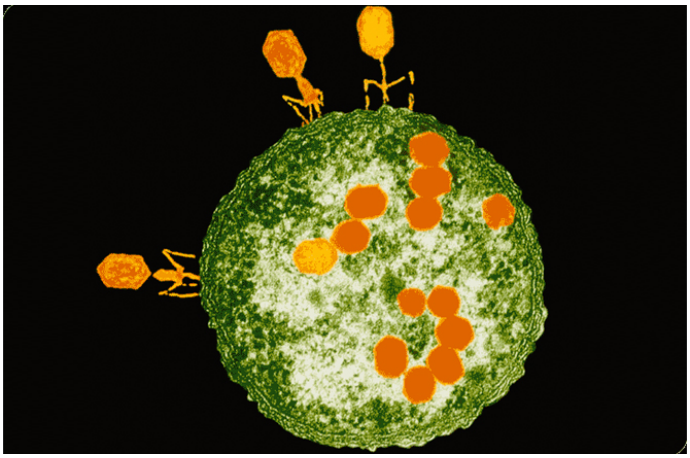


Image 1: Virus attacking E.coli bacteria

This system of genetic engineering is derived from the natural system used by bacteria to defend themselves from viral infections. In the presence of a virus, bacteria form two RNA strands. One of these strands is complementary to a target-sequence of the virus. This is the so-called guide RNA. Together with an enzyme called Cas9 these strands form a complex. Thanks to the guide RNA this complex finds the target sequence in the viral DNA. The Cas9 enzyme then disables the virus by cutting the DNA.

In recent years researchers studying this system realized that it could be applied to any chosen DNA by changing the sequence of the guide RNA (gRNA) to match the targeted sequence. Now the Cas9 and gRNA complex latch onto the DNA at the Protospacer Adjacent Motif (PAM) sequence. The Cas9 unzips the DNA double helix allowing the guide RNA to attach to it. Then the Cas9 cuts the target DNA with its so-called ''scissors''. When the DNA tries to repair itself, mutations occur leading to the disablement of the gene. Thanks to this process, scientists can further explore the functions of choice genes. Earlier this year it was discovered that the CRISPR Cas9 method could also be used as a tool for gene editing, by introducing a chosen DNA sequence. This sequence will attach to the gap formed in the DNA where it was cut by the Cas9 enzyme.

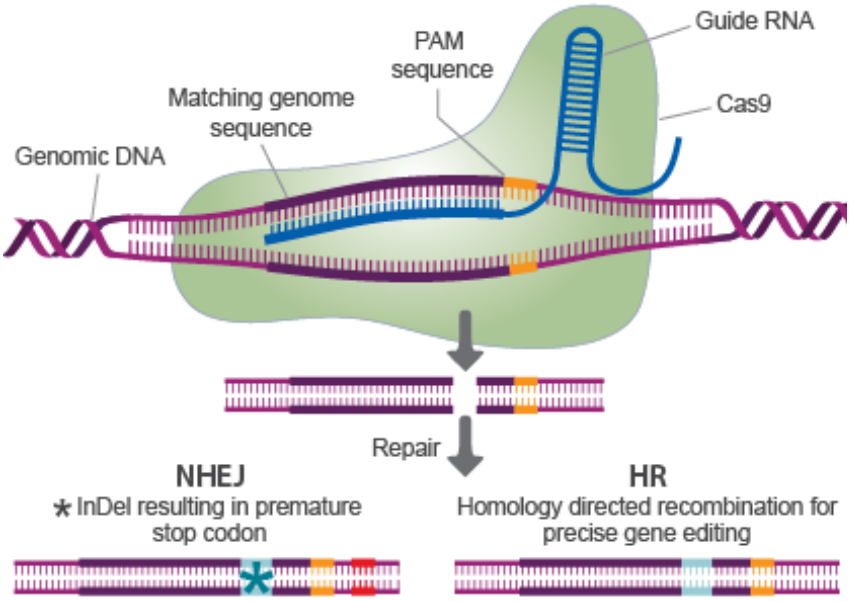


Image 2: CRISPR-Cas9 explained

This method was used in the development of malaria-blocking *Anopheles stephensi* mosquitoes. Professor Gantz of UCSD combined antimalarial antibody genes and a guide RNA with a Cas9 enzyme creating a complex. When inserted into the mosquito embryo this complex went on to target a very specific location on the germ line DNA to insert the antimalarial antibody genes. To ensure that the antimalarial genes had reached the chosen spot, a protein was included in the complex which gave the offspring fluorescent red eyes.

4. Interviews with researchers:

I wrote to the three main researchers Professor Anthony James (UC Irvine), Professor Ethan Bier and Professor Valentino Gantz (UC San Diego) with some interview questions. In addition I wrote to Tom Vasisch the director of research communications at UC Irvine asking for photographs of the labs. I was very happy when all of them replied sending useful material. Professor James sent me a document entitled Research History and Contributions, which I used for the paper. Professors Bier and Gantz both answered my questions (shown below). Mr. Vasisch sent me two pictures.

Image 3: Prof. A. James in the lab with mosquitoes

Interview:

-What led you to collaborate on the malaria-blocking mosquito project with Professor Anthony James? What is most interesting to you?

Valentino Gantz: While I interacted with Dr. James earlier during my PhD career, it wasn't until I re-visited his office to talk about the Anopheles project that I realized it. What led my boss Ethan, and me to contact him the second time was the last statement of one of his recent publications (Isaacs et al 2012, attached): *"If coupled with a mechanism for gene spread, ... malaria-resistance transgenes could become a self-sustaining disease control tool."*

Ethan Bier: We knew of the excellent work done by the James lab from reading the scientific literature. We had also met him previously for a different reason and knew he was a great person.

-What still needs to be improved? What are your future research steps?

VG: That publication was a proof-of principle, now what needs to get done is develop a final product and perform a field study to show that the technology can be used effectively in an affected area.

EB: We need to find the best way for making sure that the gene-drive process is confined to the male germline since in the egg (the end product of the female germline) this process is partially blocked in cells of the fertilized embryo prior to producing the next generation of germ cells.

-Many environmentalists and other critics claim that the release of such genetically modified mosquitoes will lead to unpredictable ecological consequences. What is your standpoint on this subject? Do you agree?

VG: I think that if you weigh the very low probability of unpredictable consequences to what is known to happen for sure every year, which is: (1) tons of pesticides released in the environment decimating indiscriminately all kinds of insects and their predators, and (2) the fact that almost 1 million people die of malaria, it is fairly easy to reach a sensible conclusion.

EB: There are two main reasons that I do not think there are likely to be unpredictable ecological consequences from using gene drives that modify mosquito populations to render them unable to propagate the malarial parasite. First, the system is very specific, so we can be almost certain that the genetic element will not jump from one species to another. Second, our strategy is not to harm the mosquito but rather to prevent it from carrying the parasite, so we should not alter any aspect of the ecology that depends on the mosquito.

The answers from both Ethan Bier and Valentino Gantz were very helpful for me. The answers to the question ''what still needs to be improved? What are your future research steps?'' led me to understand what their future research steps are. And the last question helped me get a better view on the negative critique of the project and what the researchers think about it.

5. Discussion:

The recent progress on the project was to incorporate the CRISPR Cas9 method, binding the antimalarial genes to the germ line and raising the rate of introgression in the offspring. As Professor James says ''this is a significant first step,'' but there are still improvements to be made and more research steps. Such improvements include making sure that the gene-drive system is tied to the male germline as the process can be blocked in the egg as mentioned by Ethan Bier in the interview. This would raise the chances of passing on the antibody gene. As said by Valentino Gantz, another step is to perform field studies to prove that the technology works in practice.

Environmentalists warn that unleashing genetically modified mosquitoes into the wild could have unpredictable ecological consequences. I included a question on that topic in the interviews because I didn't know whether or not this should be treated as a valid argument against the project. Because the strategy focuses on stopping the mosquitoes from passing on pathogens and not on diminishing mosquito populations the ecology dependent on mosquitoes will not be harmed. The system also works very specifically and is geared towards a certain mosquito, the *Anophele stephensi,* which makes the chances of it altering the genetic material of other organisms very low.

The cost of field testing will be very high, which could be viewed as a disadvantage. But it is not uncommon that genetic engineering projects have a high cost, and when weighing out pros and cons it is well worth it.

The main advantage of the genetically modified malaria-blocking mosquitoes is that they have the potential to help lower rates of malaria infections and thus decrease death rates in affected areas. Over 40% of the world's population lives in malaria affected areas. Professor A. James mentions in many press releases that this technology alone will not suffice to completely wipe out malaria. But it will contribute to the fight against malaria.

Another advantage is that it serves as an alternative to the spraying of pesticides. This is good because pesticides can have harmful effects on humans and the environment.

6. Summary:

The James lab at UC Irvine has been working on anti-disease mosquitoes for nearly 20 years. Through recent collaboration with Professors Ethan Bier and Valentino Gantz of UC San Diego they have succeeded in developing mosquitoes of the strain *Anophele stephensi* that carry antibody genes of the malaria pathogen *Plasmodium falciparum* and are able to pass them on to 99.5% of their offspring. The technology of genetic engineering used for this project is called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (Cas9)-mediated gene-drive system, CRISPR-Cas9 for short. This system is based on the natural way that bacteria protect themselves from infections caused by viruses. By including an antimalarial antibody gene, the gene can be edited into the mosquito's DNA at the spot where it was cut by the Cas9 enzyme. After creating a final product the final step will be a field study in an affected country, for which the James lab got permission earlier this year. This new development has the potential to help the fight against malaria without altering the ecology, because its aim is to prevent the passing on of the pathogens rather than killing the mosquitoes. That way it could serve to save many lives by preventing the spread of the malaria pathogen. In addition Professor James mentioned that this new technology could be used to fight other major viral diseases such as dengue or zika.

7. References and sources:

Images:

- Title page photos: *Anopheles stephensi* mosquito on skin from Tom Vasisch, director of UC Irvine research communications

  Embryo: http://www.digitaltrends.com/cool-tech/gene-editing-uk/

- Image 1: http://synbiobeta.com/crispr-cpf1-the-next-crispr-cas9/
- Image 2: http://www.transomic.com/Products/CRISPR-Cas9-for-Genome-Editing.aspx
- Image 3 : From Tom Vasisch

Sources :

- Research history and contribution document sent to me by Prof. A. James
- Interviews with Prof. E. Bier and V. Gantz
- http://universityofcalifornia.edu/news/uc-scientists-create-malaria-blocking-mosquitoes
- http://www.foe.org/news/news-releases/2012-01-genetically-modified-mosquitoes-survival-rate
- https://www.theguardian.com/science/2015/nov/23/anti-malarial-mosquitoes-created-using-controversial-genetic-technology
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All sources were acquired in April 2016.