Genetically modified mosquitoes

Term paper 2.5.2016 Noah and Laurent

Content

1. Preface

Our motivation to the topic & what we find especially interesting

After considering countless topics for this biology paper, a news article flew by us discussing the idea of genetically modifying mosquitoes. With the a new outbreak of the Zika virus in Brazil this topic became as urgent, importing and interesting as never before, we found the perfect topic for our semester work. Through that, a personal interest in the complexity of this topic gave us the perfect motivation. After reading into more details we realized how impressive the results are and still how different the opinions to this topic split in the public opinion. A detailed understanding of this technique would then allow us to create our own, fact-based opinion in regard to this topic.

What struck us with interest was the realization of the power this tool promised and the simultaneous, seamless transition to the danger it preserves.

Questions we had with respect to the chosen topic

Our questions in regard to the topic evolved in the history of writing this paper, a question like how it works got more and more complicated as we began to understand the complexity of this topic. At first we asked ourselves what the basic advantages and dis advantages are in regard to sustainability are, but also if there are any much so feared risks to the environment or the entire ecosystem.

After we realized the effectiveness this tool promised we asked if this technique would be the final resolution in the fight of mosquito-born diseases and or how this idea could be perfected in further detail.

Some questions like what it would cost and if it can compete with its complexity in a third world country in comparison to alternative treatments remained unanswered to us, as we couldn't find any information to that regard.

2. Introduction

Context/ recent events

As temperature rises across the globe and travelling of people, cargo and goods reaches to every corner of this planet, one insect follows. The mosquitoes. Carrying disease from malaria to dengue fever and the now newly dominant Zika, is this small creature creating a rising threat to the nations of this world. To this day no vaccination has been found to counteract any of these most deadly illnesses. With a recent outbreak of the Zika-Virus in Brazil, additional attention has aroused to this topic, in this paper we will present you a way to minimalize the threat, which is created by the *Aedes* mosquitoes.

Scientific history

The basic idea to release a great number of sterile insects into the wild, which then mate, without creating any offspring, isn't an entirely new idea. As early as 1954 did the first researchers use this technique to successfully eradicate screwworms from an island in Venezuela. But now there is a new approach by a UK firm called Oxitec, their idea is to not only sterilize these insects but much further alter their genetic data, to ensure that their offspring never surpasses the larva stage.

Where and why used

A pilot project in a city of the Brazilian state of São Paulo launched in April 2014, approximately a year after a big epidemic of dengue fever which causes more than 1.5 million cases in Brazil. This project presented incredible and promising results in a fight against these tiny insects. After 10 months' time the number of dengue cases among 5'600 residents dropped from 133 to only one. A similar project is thought to be launched by the FDA in Florida this year, though it still strikes against a great resistance of the public health opinion.

Alternative treatments

Alternative treatments against the rise of mosquito populations in a big area, are limited, their effects leave room for efficiency and sustainability.

The most effective alternative way to kill mosquitoes is by posing the water they lay their eggs in, two main problems arise there; first, big reserves of water can't be treated with chemicals without influencing the quality of the drinking water it supports and secondly it is logistically not possible to treat every single poodle of water in an urban environment. The local mosquito population in both outdoor and indoor area can be dammed more efficient by mosquito fogging. Through a special gun different pesticide like permethrins are spread into the environment. They attack the nervous system and paralyze the mosquito and all other bugs in effective range.

3. Description of engineering technique

Explanation of the applied technique

The basic principle is to release sterilized males from the lab to compete with the wild males for mates in order to shrink the population over time.

Oxitec developed the first genetically modified mosquito back in 2002 and then had a long road of researches to follow before it could be tested in action. Its mosquitoes aren't actually sterile. Instead, a specific lab created gene is inserted to the targeted organism (*Aedes* mosquitoes). That engineered gene codes for the overproduction of a specific protein called tTAV (tetracycline repressible activator variant). This protein acts as a switch to manipulate the activity of other genes. In these modified insects the non- toxic tTAV protein binds up the organism's cell machinery, which results in a lac of the proper expression of other genes what then leads to the death of that particular insect. This chain reaction leading to the death of the organism needs about 3 to 4 days' time.

To ensure a mass production of these genetically modified insects an antidote is given to the organisms in their rearing facility. That antidote is an antibiotic called tetracycline its purpose is to bind to the tTAV protein and in that matter prevent it from working, in that case it is possible for the insects to survive and reproduce as long as it is given this specific antidote. This so-called self-limiting gene occupies tetO (tetracycline Operator) sites, which attract tTAV(tetracycline repressible Trans-Activating factor Variant) protein that then bind to the operators. Those proteins are a basic promoter and coding sequence for tTAV. Without the antibiotic antidote the tTAV protein is produced, which then simultaneously binds to the attracting tetO sites and the transcriptional machinery. Therefor promoting the expression of the self-limiting gene. This cycle produces large amounts of tTAV, which then, without needing to bind to any more tetO, binds to more transcriptional machinery. As a result of that over occupation the transcription machinery eventually overloads and is unavailable for the expressions of other vital genes. The repression of the essential gene expression inside a cell leads to a cell depletion and initially to cell death and death of the entire organism/insect before it can reach adulthood.

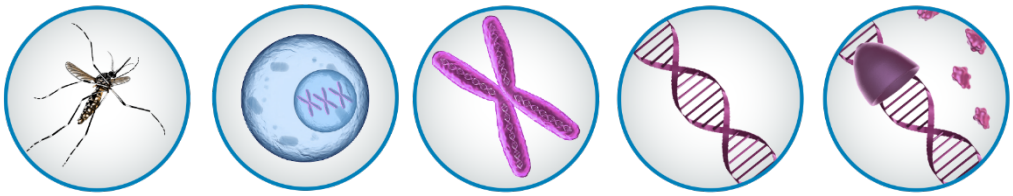
Inside the production facility the antidote (tetracycline) is absorbed by the mosquitoes while they are as larva in their second developmental stage. There it binds and inactivates tTAV and as long as the organism is supported by the antidote, breaking the endless cycle. In that case the transcriptional machinery is not dissipated as only a minimal amount of the tTAV is produced, which does not affect the cell functions in any negative way. The Insects survive and reproduce naturally before released out of the production facility.

SELF-LIMITING GENE
HOW IT WORKS

OXITEC

Introduction

The self-limiting gene is an environmentally friendly way to control insect pests like dengue mosquitoes. It works by preventing them from developing without using toxins or pesticides. It is species-specific so the genes do not spread, and the released insects and their genes do not stay in the environment.



Mosquito | Mosquito cell nucleus | Chromosome in cell nucleus | DNA in chromosome | Normal gene expression (transcription)

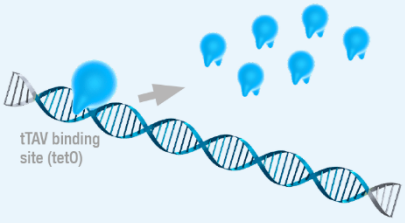
Components

Self-limiting gene codes for tTAV protein | tTAV protein from expression of self-limiting gene | Transcriptional machinery | tTAV ties up transcriptional machinery so it cannot operate | Tetracycline antidote to self-limiting gene | tTAV inactivated by tetracycline antidote | Essential genes for mosquito survival | Essential proteins

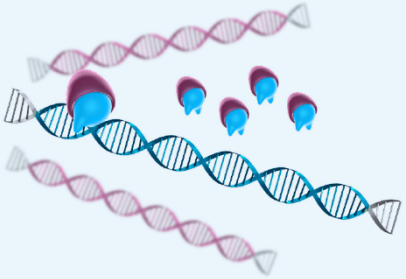
Mosquito Releases

Self-limiting gene at work controlling dengue mosquitoes

tTAV binds to special site (tetO operator) so more and more tTAV is produced



tTAV ties up transcriptional machinery so essential genes are not expressed



Without essential gene expression mosquitoes cannot develop

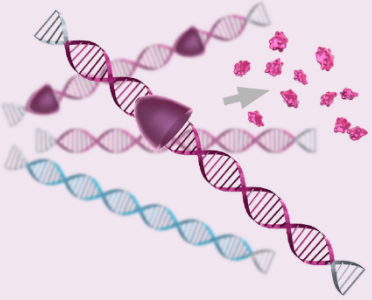
Pest mosquito offspring die before they can reproduce and before they can transmit disease

Mosquito Production

Self-limiting gene inactivated by antidote to produce Oxitec mosquitoes

During the Oxitec mosquito production process, the antidote 'switches off' the self-limiting gene by preventing tTAV from binding to the tetO operator

With the self-limiting gene inactivated, normal gene expression takes place



Transcriptional machinery works to express essential genes for essential proteins the mosquito needs to develop and reproduce

Oxitec mosquitoes are produced for release

4. Interview

For our interview we wanted to interview a scientist involved with Oxitec but sadly we couldn't get in contact with one. So we interviewed Tobias Junt an immunologist from Novartis in Basel. He had worked on the dengue fever before but never did anything related to genetically modified mosquitoes. Nevertheless he could answer some of our questions, especially the dengue fever related, really well.

Laurent and Noah: What happens to the human body after it's been infected with dengue fever?
Tobias Junt: The virus replicates, the immune system reacts (first innate immunity: cytokine production, then adaptive immunity: antibody production). Fever and flu-like symptoms (particular symptom: bone aches) are produced during the ongoing acute immune response. In most cases the virus is eliminated and immunological memory in the form of virus-neutralizing antibodies is induced. Successive infection with different serotypes – as may happen in endemic areas - may lead to Dengue hemorrhagic fever, which leads to leaky endothelia and hypovolemic shock (low blood pressure) that can be life-threatening. One hypothesis as to why DEN hemorrhagic fever develops is "antibody-dependent enhancement". This means that neutralizing antibodies against one serotype bind to another serotype in a secondary infection, but are poorly neutralizing. Instead of neutralizing, they enhance virus entry into macrophages and other target cells and augment viremia and associated symptoms.

L&N: In your opinion is it possible to successfully eradicate the dengue fever in the next 10 years? If you think so will it be eradicated by a traditional antidote or by genetically modified mosquitoes?

TJ: No, that will likely not be possible, because (at least to my knowledge) there is still no vaccine that effectively protects equally well against DEN1-4. A vaccine by Sanofi has been approved and others are in Phase III, but measurable outcome on public health takes a while. A WHO position paper on this vaccine will be published in July 2016. The WHO's official goal is to reduce dengue morbidity by at least 25% and mortality by at least 50% by 2020. I am not sure what exactly a "traditional antidote" is, and genetically modified mosquitoes are a creative idea but not sure how feasible in the field. My hope is still for an effective vaccine.

L&N: Where you in any way involved in the making of genetically modified mosquitoes because of your work with the dengue fever?

TJ: I currently do not work with Dengue Fever. I was involved in diagnosing the virus from blood at the Pasteur Institute in Cayenne in 1998, and we also detected the virus in mosquitoes by PCR. But I have never been involved with genetically modified mosquitoes.

L&N: Do you have a personal opinion on genetic engineering in general?

TJ: As for any versatile and powerful technology, there is a huge benefit if used right, and if abused there are hazardous or unpredictable outcomes. Therefore this technology needs to be regulated by law, and research institutions have the responsibility to inform the public on how this technology is used.

L&N: Do you think that dengue fever will be a threat to southern Europe/America in the near future?
TJ: The DENV vector Aedes albopticus (not aegypti) has become endemic in Italy and the UA, refugees from Sub-saharan Africas come to Southern Europe in increasing numbers, and there is increasing global travel: Dengue fever cases have increased in Italy recently, and another A. aegypti-transmitted tropical disease (Chikungunya) was also found first time in Italy. So yes, the incidence of DENV will likely increase in Southern Europe, but it is hard to imagine that it will become a public health threat as it is in 3rd world countries, because even if cases of hemorrhagic fever occur, hospitals are equipped to react more quickly.

L&N: If this were the case how can we as people protect ourselves against the illness?

TJ: Vector control, e.g. do not allow puddles to form in your garden (e.g. do not have rain barrels, throw away old bins, no other standing water etc). Sleep under mosquito nets, use repellents. Do not expose skin.

L&N: Can you tell us anything about the advantages of genetically modified mosquitoes over insecticides?

TJ: Not sure whether one will have advantages over another. Not sure how specific an insecticide can be made, and how genetically altered mosquitoes will change the ecosystem. Maybe there is a bacterial parasite or other infectious agent that selectively infects & kills Aedes??

As he had already warned us before we did the interview he could only give us an immunologist's opinion so he wasn't an expert on our topic. But we were lucky to find someone that didn't live on the other side of the world so we could do the interview in person at the Novartis campus in Basel. We weren't allowed to take any pictures but it was an interesting journey going on the Novartis campus and meeting a scientist that showed us around a bit before we did the interview.

5. Discussion

Is the genetical engineering of mosquitoes the resolution to dengue fever or the Zika virus and other illnesses transmitted by mosquitoes?

We can't be sure yet but the test results are looking promising. The procedure of killing of a mosquito population in a certain region by genetically modified mosquitoes has been proven to be successful in several cases. Oxitec's *Aedes aegypti* have been tested on the Cayman Islands, in Malaysia, Panama and Brazil (in Brazil before the recent Zika outbreak in 2011) and each time the population has been reduced by up to 90% and there have been no recorded side effects in those regions. The Brazilian government is now allowing the use of the genetically modified mosquitoes in several neighborhoods in order to fight the Zika outbreak. Genetically modified mosquitoes have many advantages over conventional insecticides but the main problem of it being the enormous cost involved in the process of developing the mosquitoes. But still with the phenomenal test results we should be able the have a positive attitude towards the fight against illnesses transmitted by mosquitoes with the help of genetically modified mosquitoes.

On what should the research of the genetically modified mosquitoes focus next?

Experts believe that there should be done more long-term testing in order to definitively tell that there are no negative effects on the ecosystem or any other aspects caused by the genetically modified mosquitoes. The possibility of this form of disease control should be extended more in order to fight a wider range of diseases transmitted by mosquitoes or any other insects. The genes inserted into the mosquitoes have to be changed for each illness and each type of mosquito this is all very expensive but with the success in various test results and in Brazil the companies doing these kind of things should get more and more funding by convinced people and governments.

Advantages and disadvantages of genetically modified mosquitoes

There is a lot of controversy in the media because of those mosquitoes. Some people really are worried about the effects of genetically modified organisms on our life and our planet and those people make some good points. But there are a lot of people that are afraid of genetical engineering because it's not natural or because it's not according to "god's plan". The truth is that while there are some serious reasons to worry about genetic engineering, it's a very helpful tool and it's way too powerful to simply not use in order to save millions of lives.
One of the main counter arguments is that killing of an entire species of mosquitoes is harmful for the ecosystem. With the example of the *Aedes aegypti* you can rest assured that there will be no harm done to the ecosystem because this type of mosquito is an invasive species from Africa which when eradicated will not have a negative effect on the environment.
Some people also say that we should just use insecticides like we do in agriculture but insecticides only kill on touch and in a town or city most mosquito breeding sites are in private homes of the people living there. The government would have to gain access to the homes and the people living there would have to be evacuated from their homes. In short: it's a lot easier to release a bunch of mosquitoes and let them do the killing.
There have been reports that the Zika outbreak in Brazil was caused by the testing of genetically modified mosquitoes but as of now this theory has not been proven and is based on the assumption that the released mosquitoes could have a survival rate of 5%. Another argument often brought up by the contra-side is that the test results that prove that the by Oxitec released genetically modified mosquitoes have the ability to reduce the mosquito population by 90% are faked by Oxitec. Oxitec has released a statement proving that the tests are made by independent laboratories. Those laboratories would not have any benefit in faking test results. But recently there's been a change in public opinion about the topic. More and more people are beginning to realize that genetic engineering isn't the evil thing some media outlets make it out to be but it's the best solution for a worldwide problem.

In conclusion you could say that the opponents are struggling to make sense with their arguments and that the genetically modified mosquitoes really aren't that bad of a thing that could save the lives of millions of people in developing countries. Until there's a vaccine for the diseases the genetically modified mosquitoes probably are our best bet.

6. Summary

Genetically modified mosquitoes are being developed in order to fight diseases transmitted by them. The most common practice is to inject them with a gene that does not let their offspring live past the pupal stage. This method has been successfully tested and is used right now to prevent dengue fever and recently to fight the Zika virus in Brazil. As of right now there are no vaccines for these diseases and the only other option to prevent the disease would be to treat the mosquitoes and their eggs with insecticides which would be a logistically almost impossible procedure.

In the process of creating our term paper we learned a lot about genetic engineering which was really interesting especially because our topic has a lot of relevance in recent news. By spending so much time with this topic we were able to form our own opinion on this slightly controversial topic. Our opinion is that genetically modified mosquitoes are (at the moment) the best way to fight diseases transmitted by them. And while there are some people that are afraid of the process involved, test results show that the mosquitoes really do help and that they aren't harmful in any way. As already said while there is no better option we should stick with what we can do in order to help the billions of people living in areas affected by these diseases.

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