A BIOLOGICAL WAR AGAINST TROPICAL DISEASES

WITH THE MAIN EMPHASIS ON MALARIA

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1. Preface

One of the worst tropical disease is malaria. The half of the world population is endangered from it. Every year, 250 million catch an infection and 1 million of them dies.
Our motivation to work on this topic is that malaria is a widespread disease which affects many human being every day. As early as in 1907, a French physician called Charles Louis Alphonse Laveran has received the Nobel prize for his studies about the pathogen of malaria. He has found out that *plasmodium* is the causative organism and the anopheles the carrier.
So humans have known many information about malaria for years but are still not able to solve totally this huge problem today. We know that science has made progresses in the field of combating malaria but have never heard that it has lead to success. A good example is the ineffective vaccination for humans against malaria.
Recently, we have read in a newspaper article that scientists have found a new biological method to fight against malaria, which refers to genetic engineering. We will have a closer look at that method.
Our focus will be on the following questions:
How does this engineering technique work?
Are there any advantages and/or disadvantages in comparison to conventional treatments against malaria?

2. Introduction

Over half of the worlds population is in danger of a tropical diseases. One of the most common is Malaria. Tropical diseases are on the rise. Only on Malaria, over 200 million people are infected. Recent researches showed that the mortality rate is even much higher then expected. Last year, 2014, 584 000 people died from Malaria.
However, we have to be careful with this data: the numbers of dead and infected people from the WHO differ a lot from alternative sources. American scientists are talking about 1.2 Million deaths in 2010, that's nearly twice as much as declared from the WHO.
But Malaria is not a new or hardly known disease. Already the ancient Egypt had experiences with Malaria and the Chinese 2000 BC even discovered medication (Artemisinin). Later on, Malaria was widespread also in Europe around the Mediterranean Sea. From the middle ages until to the 20th century Malaria was common in Middle Europe. In the 1960 one tried to combat the disease with pesticides to avoid the Malaria transmitter, the Anopheles mosquito. This system led also in tropical areas to a decreasing amount of infected people. One thought the decrease would continue up to the extinction of the insect, like it in Europe. So after a while the WHOs focus moved on to other projects. But the mosquitoes survived and population stayed approximately at a level as it is today. Scientific progress to combat malaria are comparable to other tropical diseases.

The discussed technique of gene manipulated male Anopheles mosquitoes (sex ratio distortion) was neither tested in the field nor legitimated from any government. The technique is still in research. Oxitec, the leading company in GM mosquitoes is currently making field experiments with transgenic mosquitoes on test areas in Brazil. At the moment the so called "sterile insect technique" is only set up with Egypt Tiger mosquitoes. These tests are already analysed and showed a decrease over 80% of the mosquitoes. However, further tests, also with Anopheles mosquitoes are planed. At the moment the field tests only take place on controllable and therefore zoned areas (for example islands).

Since Malaria is a known and a common disease, people always made research for treatments. The most common ones today are the following.
Infected people can take medication (ingredients: Artemether/Lumefantrin, Atovaquon/Proguanil, Chloroquin) to the disease.
There are also a handful of medications to prevent Malaria, so called chemoprophylaxis, such as Mefloquin, Atovaquon/Proguanil or Doxycyclin. Preventions could also be a net over the sleeping area or walls, which are treated with pesticides.
Like already mentioned, there is no effective vaccine against Malaria.
The disease is treatable and doesn't lead to any further consequences. However, if the disease is not or not correct medically treated, Malaria usually leads to death.

Malaria is a tropical disease, which is caused by a unicellular parasite from the genus plasmodium. The parasite is normally transmitted to human via a sting from the female Anopheles mosquito. The symptoms are high and periodic fever, seizures and gastrointestinal disorder. The untreated disease can lead to death.

3. Description of the engineering techniques

The genetic engineering techniques which are presented are performed on the carrier of malaria to the humans. The carrier of the pathogen *plasmodium* is the female anopheles mosquito. There are many techniques to fight against them. The most researched one is the **'sex ratio distortion'** method which has not yet been tested on the field.

The sex ratio distortion technique has the aim to eliminate the whole mosquito population by modifying the sperm that it won't produce female offspring anymore. If there are no female mosquitoes, the humans couldn't be bitten and infected. It's not possible for the male offspring to reproduce themselves and consequently the population will shrink till it dies out.
To enable that, you have to insert a DNA cutting enzyme called I-Ppol into the male insect.

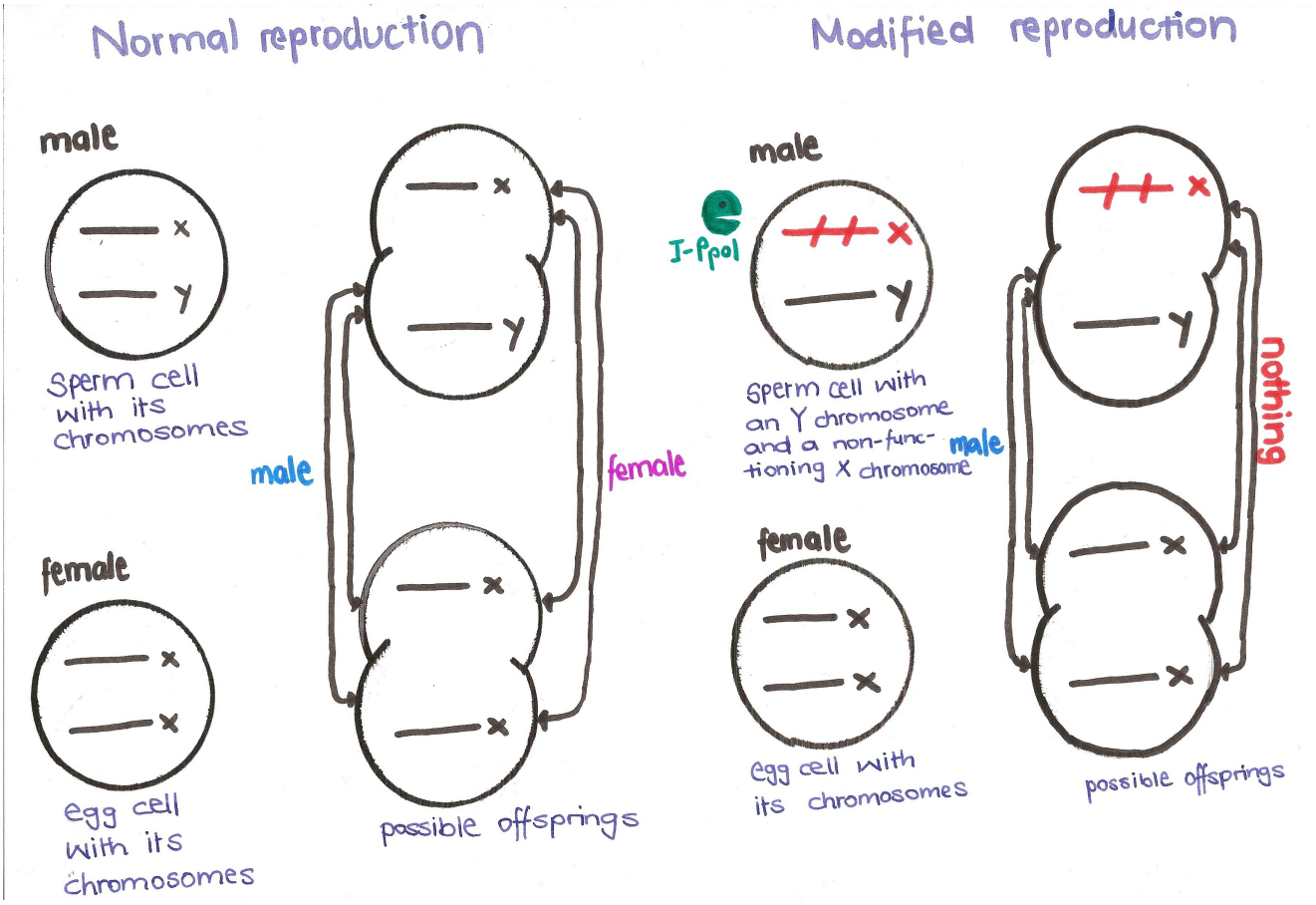

Figure 1. *Comparison of normal and by sex ratio distortion modified reproduction.*

As you can see in the figure above, the enzyme cuts the DNA of the X-chromosome during the sperm production that it stops functioning. If the sperm containing the one intact Y chromosome fertilises the egg cell, only male mosquitoes could be produced. There are no other possible gender. The experiments in the lab showed that this technique is self-sustaining. The manipulated gene is passed to the next generations.

Another way to eradicate the malaria carrier is provided by the British biotech company *Oxitec*: The **Sterile Insect Technique,** short **SIT.** It has been tried on the Tsetse fly and screw worm in the wilderness with success. Now, they are going to adapt it to the mosquitoes.

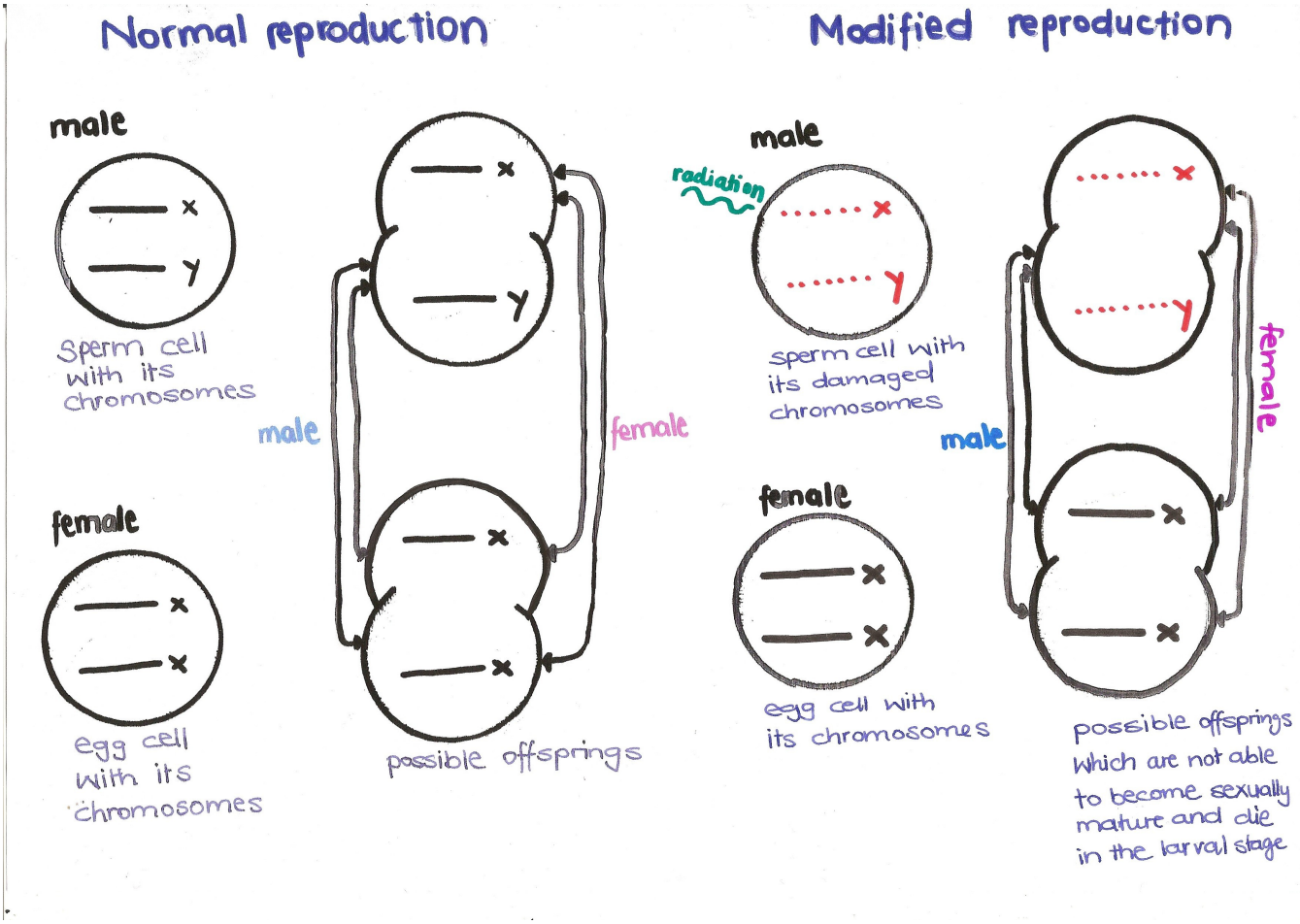

Figure 2. *Comparison of normal and by SIT modified reproduction.*

The male insects are irradiated with gamma emission. Their chromosomes and the DNA of the sperm cells are damaged. After that, they are sterile. That means that they are still able to fertilise female eggs. But the filial generation will die in the larval stage.
In comparison to the 'sex ratio distortion' method, the SIT is not self-sustaining because the offspring of the sterilised insect die before reproducing themselves. You have to release permanently new sterilised ones to keep up this technique.

4. Interview with Pie Müller from the Swiss TPH

Dr Pie Müller is medical entomologist and head of vector control at the Swiss TPH (Swiss Tropical and Public Health Institute).

1. ***What methods exist to combat the mosquitoes? And which ones are in your opinion the most effective?***

In principle mosquitoes are what we call vectors. They are just vehicles to transport pathogens from one person/or animal to the other.
So the most effective strategies are those who interrupt this interaction. In that sense the best thing is to kill them.
That brings us to methods to kill mosquitoes:
The most vector control these days is based on insecticides. I
n malaria the mosquitoes are mainly night active. There we have very effective tools, which are insecticide treated nets. There the people can sleep under the nets. The net provides on one hand a barrier and on the other by landing on the net it takes up insecticide.
One can also spray walls inside houses. During digestion the mosquito lands on the wall and gets again infected by the insecticide.
However there are challenges. Not every mosquito goes inside houses. So there is a gap where we need more and new tools. And that is where the GM methods and the repellents come in.

2. ***Which GM methods do exist?***

There are several approaches.
One strategy goes in the direction to produce mosquitoes that either cannot have offspring at all or produce offspring that dies in the larval stage. This would be done with radiation.
One such technology would be the RIDL technology by Oxitec.
Radiated males are released and the offspring these males produce do not survive.
The system includes also that the genetically modified mosquitoes its self does not survive outside the lab. They give them antibiotics to keep them alive.

3. ***What is your opinion about the GM mosquitoes? What are the advantages and the disadvantages?***

I guess that a lot of concerns exist against genetically modified organisms. For example that they could spread genes trough other taxa or something could go wrong one or the other way. But with the RIDL technology you always have to release mosquitoes, which do not propagate through population. The transgenic mosquitoes are therefore under control. So there this risk is minimized or even non-existent. The RIDL system has also been applied to other insects so there is quite a bit of experience there.
The disadvantage with this technology is you do not have a driver who drives the gene through the population. So one has to release GM mosquitoes over and over again to keep their level in the population.
There is something what I personally like about this approach. Some mosquitoes are very hard to find and you do not have a point source (breeding places etc.), where you can combat them. So in this case I guess it is an effective way to target those mosquitoes.

4. ***Have you ever participated in a project on GM mosquitoes?***

The Swiss TPH does not really have plans, which go into the direction of GM mosquitoes. In the whole of Switzerland's research on GM mosquitoes is still quite experimentally in the lab.
But I did several other projects about malaria and the anopheles mosquito.

5. ***Are there any field experiments going on? What do you know about them?***

Yes, Oxitec has an on going project in Brazil. Their first project took place on the Cayman Island. There is also something going on in Mexico.
These are probably the most advanced projects. The rest is more in the laboratory.
To my knowledge there have no other transgenic mosquitoes been released apart from the ones from Oxitec.
Other people are far less behind Oxitec. They are still searching for genes which code for susceptibility or resistance to parasites etc.

6. ***How long will it take until GM mosquitoes will be released into the wild as a generally used prevention?***

Well I would say that the Oxitec studies are quite advanced. They already release a lot of mosquitoes on larger areas. But I could not say a definite time.

7. ***Is there a high risk that something unexpected happens with GM mosquitoes in nature?***

Well with anything there are risks. You always have to counterbalance risk and advances. The question is are you prepared to take the risks to take the gains.

What would be the risk?

With the GM method of Oxitec it would first of all a financial risk. It is very expensive.

And for the environment?

With the RIDL system it is fairly safe. The GM mosquitoes do not have further impact on other species.

We read about another method where the gene is inherited from one generation to the other (it is called sex ratio distortion) and the population should extinct by some time. What is your opinion about this approach?

That is where I would see a high risk, if something gets really inherited. Because this system is not really under control.

8. ***Could the modified gene be inherited into another species?***

If the anopheles mosquito would interbreed with another species they would most likely have no offspring anyway. And with the RIDL system the offspring would die, so the gene is not propagated through generations.
So I think there is not really a possibility for the gene to spread through any population.
There are other approaches where people try to drive a modified gene trough a population. There one could think that something goes wrong or some kind of selection could happen.

9. ***Are there other methods, which do not include the death of the mosquito?***

Another approach would also be to make the mosquito more resistant to pathogens.

And how would that work?

You do have mosquitoes that express some proteins that inhibit the cycle of the parasite.
There is also a parasitic bacteria, which is called wolbachia, that has two effects. One is called cytoplasmic incapability, which means when the female has the bacteria and the male does not it cannot produce offspring. Only if both have the bacteria they can have offspring. So you do have a driver that drives the bacteria through the population.
At the same time it has been found that mosquitoes that carry wolbachia are less likely to transmit malaria and dengue fever.
There are big projects going on in Brazil and Australia.

10. ***What do you think how future treatments will look like?***

There will not be a single intervention to solve the problem of malaria. There will always be several devices to combat the disease.

But what will be the most effective?

That depends on the species. With malaria mosquito we have the nets etc. which work quite well, because we have a point source.
But then for mosquitoes it is difficult to bring them to a point source. There the GM method makes sense.

You see there different aspects come together, like financial, local and ethical issues. You have to explain to people, why the mosquitoes are dangerous.

11. ***Are GM mosquitoes in your view a good thing to use in affected countries?***

I think we have to differentiate a bit. It is a different approach and you cannot say whether it is good or bad. It depends on the technology and what you can gain from it in terms of saving life.

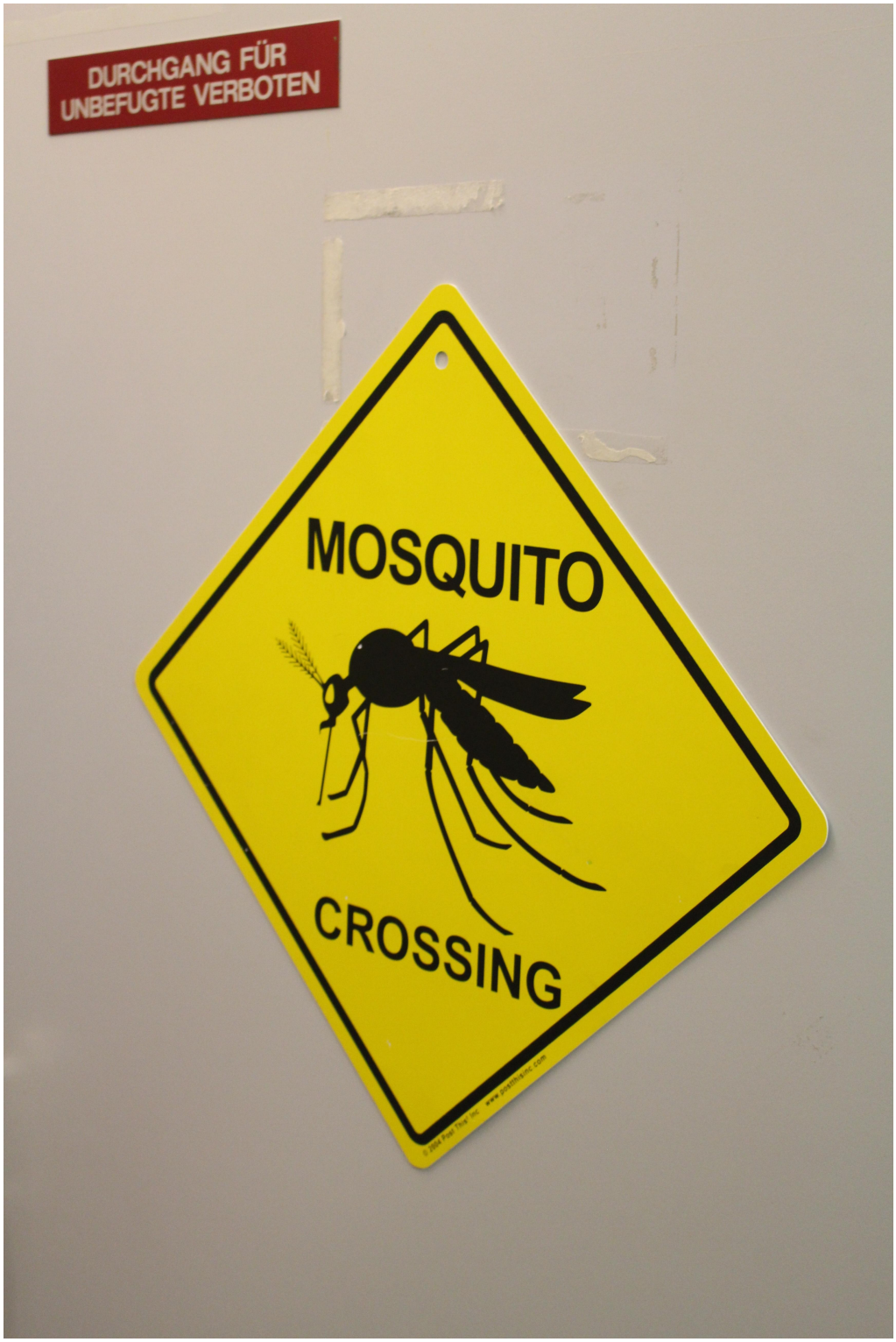

Figure 3. The entrance to the lab where mosquitoes are bred in TPH Basel.

Figure 4. Showing the lab where mosquitoes are bred for experiments.

Figure 5. Here, you can see a lab where experiments with chemicals are performed.

5. Discussion

Progress:

As already mentioned before, there is only one company worldwide which does greater field tests on transgenic mosquitoes to fight tropical diseases. This company is called Oxitec and is a British subsidiary of Syngenta. At the moment, Oxitec has a monopole on researches as well as on any commercial use of described techniques. That means that there is no other institution that does researches in a comparable important way as Oxitec.

At the moment, there were only two recent affairs where transgenic mosquitoes were used. One of them is only a test, set up in Sansibar, an island in Tansania. Over ten million GM male mosquitoes were released via airplanes over the whole island. This test was set up with transgenic Tse Tse flies (sleeping sickness). Until now, no field tests with the malaria causer were set up. However the test on Sansibar led to quite positive results: the number of infected people was drastically reduced and the disease almost disappeared from the test area.
On the other hand, the test also had quite different effects on the environment than expected. The number of infected animals, mostly cows, decreased and therefore the production of milk and meat increased by approximately 50 per cent. This side effect is based on another disease. The Tse Tse fly spreads also, the cattle disease, BSE.
Of course, side effects like this no one has taken into account during planning and evaluating the use of transgenic mosquitoes.
One of the reasons why these field tests are rather rare, is the need of a closed environment. Sansibar was chosen on one side because of the high occurrence of tropical disease and on the other side mainly because of its geographical state as an island. The island allows the scientists to keep the control over the whole insect population. It is not possible, or at least extremely unlikely, that the mosquitoes leave the island because of the distance cross the ocean. To do the tests on the mainland it would take extreme masses of transgenic mosquitoes to keep an overview over the impact. Furthermore it wouldn't be possible to declare any scientific standardised results on mainland.
Another important reason why the tests are not frequently made are the enormous costs involved.

The other affair, where transgenic mosquitoes were officially used was in Brazil. In April 2014 the government gave green light for the use of Oxitec's transgenic fly in the fight against Dengue fever. During June to December in the year before, the mosquitoes were tried in Jacobina, a city as big as Winterthur in Brazil. This test was successful, 79 per cent of the dangerous mosquitoes disappeared. This result supports the development of the technique to avoid tropical disease. It can be taken as a success and should be kept in mind for the next years.

Future:

We think one of the biggest next steps will be the field test of the sex ratio method. If the tests are successful, the method may be of economical and social importance in the future. The sex ratio method is self-sustainable and could therefore be more efficient then the SIT.
The problem all GM methods bring along are the extremely high costs. As long as they stay on today's level, it will not be possible to cover larger areas with transgenic mosquitoes. The costs will have to be drastically reduced. That means that the future of these methods depends on their economical development.

Ethical Aspects:

Pros:
The GM methods promise a high improvement of the medical situation in tropical countries. It might be possible that areas, which are now not inhabitable because of tropical disease, will be habitable through the use of transgenic mosquitoes. This would lead to an enormous upgrade of these areas for the people and there daily life. It would also change the economical future and may lead to an advantage for the whole country.

Cons:
Scientists all over the world are discordant about Oxitec's statement that there is no risk that transgenic mosquitoes' changed genetic information will be inherited to other species. If that happens, the gravity of the consequences cannot be estimated. Worst case, several species would extinct, which would have extremely negative impacts on the whole food chain of the tropical ecosystem, as well as on man.
Another aspect is the monopole structure of this GM techniques. Oxitec is the only company that could provide transgenic mosquitoes and therefore everybody would depend on them. This could lead to similar problems as with Monsanto's GM seed. The local people on one hand side would be forced to use Oxitec's methods and on the other hand side the state would depend on them in a way which may be dangerous for local politics.

After all, from a sociomedical and economical point of view the new methods would lead to an improvement human life standards in affected countries. But there is a political danger coming from the monopole of Oxitec, which would have to be taken in account by a possible release of GM mosquitoes.

6. Summary

Malaria is one of the worst tropical disease which endangers the half of the world population. Through the development of new genetic engineering techniques, the biological war against malaria has started. The 'sex ratio distortion' method is one of the techniques, which modifies the sperm that it won't produce female offspring anymore. Without females the population will die out after a time. Another method, called the 'sterile insect technique', which damages the chromosomes of the sperm cell by radiation, is being researched, too. After the fertilisation, the offspring will die in the larval stage.
Science and therefore the genetic engineering techniques have positive and negative sides:

+ They save the life of millions of people.
+ Through the mosquitoes endangered places could be cleared. They will be inhabitable.

- The risk that the modified genes are going to be inherited to another species can't be excluded.
- There is only one big biotech company called *Oxitec* which works on transgenic mosquitoes. If the SIT method succeeds, everyone may be forced to use Oxitec's methods and might depend on that company.

7. Sources

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