Term Paper Biology (GF)

Fighting Malaria with CRISPR/Cas9

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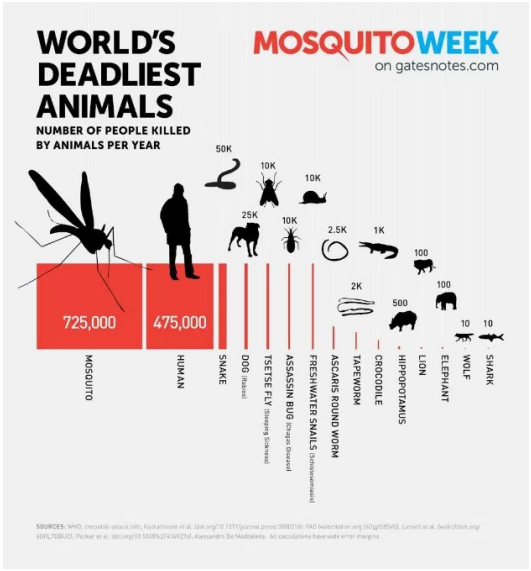
Contents

1. Preface

The animal responsible for most human deaths per year isn't a snake nor any wildcat but rather the mosquito. Mosquitoes or more accurately those within the Anopheles complex such as the *Anopheles gambiae* are pre-dominant carriers of deadly viruses and parasites such as malaria. So far, from the 460 recognized species, only over 100 transmit to humans and there are only 30-40 species which commonly transmit the *Plasmodium* parasite. These viruses, that the mosquitoes carry are the cause for the loss of 725,000 lives every year. So what if we could stop the number one killer of humans? Scientists have already found ways to manipulate the genes of Anopheles to prevent them from transmitting the malaria parasite with the help of the CRISPR system. However, these solutions have not yet been implemented in a real world environment. So what is hindering these solutions from being implemented ? What are the pros and cons of releasing genetically manipulated mosquitoes out into the wild? These ethical questions and the concept of manipulating mosquitoes on a genetic level will be researched, explained and discussed in this term paper.

Fig. 1



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2. Introduction

2.1 What is CRISPR and CRISPR-Cas9?

The abbreviation CRISPR stands for *Clustered Regularly Interspaced Short Palindromic Repeats* and is important for the natural bacterial defence systems of bacteria as well as for the formation of the basis of the CRISPR-Cas9 method which is made up by CRISPR and its produced enzyme called Cas9. Originally the CRISPR comes from bacteria and are often part of their genotype. In bacteria the CRISPR contains snippets of DNA sequences of viruses that have attacked them. The bacterium uses these snippets to detect and destroy the DNA from similar viruses during new attacks. The CRISPR describes a specialized stretch of DNA which has the following two different characteristics: the presence of nucleotide repeats and spacers. Those sequences of nucleotides are distributed throughout the whole CRISPR region whereas the spacers are bits of DNA that are mixed among the repeated sequences. The Cas9 is a CRISPR associated system being an immune system as well as an enzyme, produced by the CRISPR system, which is capable of cutting strands of DNA and is therefore also called a "molecular scissor". The CRISPR-Cas9 method has become a very powerful DNA-targeting gene-editing tool in the area of genetic engineering.[1]

2.2 Recent scientific history

In 1987 Yoshizumi Ishino and his colleagues at the Osaka University accidentally discovered what today is called CRISPR and studied it on *E. coli*. In 1993 researchers independently from each other from the Netherlands and from the University of Alicante in Spain made similar discoveries. In 2001 Francisco Mojica and Ruud Jansen proposed the acronym CRISPR. First discoveries of the new CRISPR-Cas9 method were approached in 2007 and 2008. With the rapid research that was done, in 2013 it was first able to demonstrate the capabilities of the Cas9 genome in eukaryotic species, more precisely in mammal (mouse) cells. This is primarily thanks to studies which showed how type II CRISPR could be successfully engineered from *Streptococcus thermophilus* and *Streptococcus pyogenes*. Since this discovery CRISPR-Cas9 is used in research, medicine and biotechnology.[2]

[1] https://phys.org/news/2018-03-crispr-genetic-big-rna.html
https://phys.org/news/2018-03-crisprcas9-mutation-disease-future.html
https://www.livescience.com/58790-crispr-explained.html
https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answer S-about-crispr
https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

[2] https://en.wikipedia.org/wiki/CRISPR
https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
https://www.sciencedirect.com/science/article/pii/S0092867414006047
https://ars.els-cdn.com/content/image/1-s2.0-S0092867414006047-gr3_lrg.jpg

Formerly the CRISPR-Cas9 procedure is used in stretches of DNA but in recent studies, scientists have found a way to target the RNA instead of the DNA creating a new possibility to repair genetic diseases which are originally caused by the RNA and by its resulting proteins during the transcription of the DNA into the RNA.

It also has the potential to be used to correct genetic defects as well as curing or preventing genetic diseases. Therefore, it is possible to modify genes in living cells and organisms and correct defects and mutations at precise locations permanently.[3]

2.3 Malaria and CRISPR-Cas9

As mentioned in the preface, malaria and other mosquito associated diseases take up a big part of the causes of human deaths every year. In order to decrease malaria, many different possibilities of prevention and cure have been tested but not all of them are suitable for long-time treatment. By discovering CRISPR, especially the CRISPR-Cas9 method, scientists and researchers have gotten a new idea: To genetically engineer mosquitoes against the parasite *Plasmodium falciparum*.
The mosquitoes DNA is changed by adding antibody genes that specifically target *Plasmodium falciparum* making those mosquitoes immune against malaria. If researchers used the conventional method of changing the mosquitoes DNA with CRISPR-Cas9 and then by sexual reproduction between lab type mosquitoes and wild type mosquitoes would take place the offspring of parasite resistant mosquitoes would be half. To prevent this and to ensure that all of the resistant mosquitoes' DNA would be passed on, researchers have used a different additional method called gene drive. These designed genes which are encoding the components of the system were inserted into the mosquitoes' DNA to cause the specific mutation. Then the CRISPR-Cas9 system copies the mutation from one to the other chromosome. In fact 99.5% of the offspring will then be parasite resistant.[4]

2.4 Other uses of the CRISPR-Cas9 method

Overall the CRISPR-Cas9 method is used in research as well as in medicine and biotechnology. In these cases Cas9 can be used to facilitate a big part of genome engineering applications especially in a lot of species that couldn't be treated with traditional genetic manipulation techniques beforehand. It provides an efficient and specific genome modification.

[3] https://phys.org/news/2018-03-crispr-genetic-big-rna.html
https://ars.els-cdn.com/content/image/1-s2.0-S0092867414006047-gr3_lrg.jpg
[4] http://www.pnas.org/content/pnas/112/49/E6736.full.pdf
https://youtu.be/TnzcwTyr6cE
https://www.nature.com/articles/nbt.3439
http://www.pnas.org/content/pnas/112/49/E6736.full.pdf
https://wyss.harvard.edu/staticfiles/newsroom/pressreleases/Gene%20drives%20FAQ%20FINAL.pdf
https://www.nature.com/news/gene-drive-mosquitoes-engineered-to-fight-malaria-1.18858

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The Cas9 from the bacterium *Streptococcus pyogenes* (SpCas9) has recently often been used for genome editing in different species and cell types such as human cell lines, bacteria, zebrafish, yeast, mouse, fruit fly, roundworm, rat, common crops, pigs and monkeys.
Cas9 is also used to investigate gene function or elucidate causal genetic variants by designing a short RNA sequence and therefore retargeting the Cas9 which causes an unbiased genome perturbation. Further, if an assortment of proteins or RNAs are binded to a Cas9 or a sgRNA (single guide RNA; mentioned in the next chapter) it can change transcription states of specific genomic loci, monitor chromatin states or rearrange the three dimensional organisation of the genome.
With genetic screening in humans using CRISPR-Cas9 or just using CRISPR-Cas9, scientists are trying to find a way to cure (genetic) diseases.[5]

2.5 Alternative treatments for Malaria

The first step to avoiding malaria and malaria related anaemia is prevention through medication, mosquito elimination and prevention of bite. Chemoprophylaxis is used for tourists that are not staying in a malaria affected country for a very long period of time. For the local individual, prevention effective insect repellents such as DEET or picaridin are used. Also insecticide-treated mosquito nets and indoor residual spraying are a big part of the traditional malaria prevention. Wearing long sleeved clothing can help as well. Malaria medications such as mefloquine, doxycycline and atovaquone/proguanil are used for prevention as well as for medication, but after a short repeated use of them, the parasite becomes resistant against them. The most effective malaria medication is artemisinins. For further treatment, medication against fever and other symptoms are used.[6]

[5] https://phys.org/news/2018-03-crisprcas9-mutation-disease-future.html
https://www.sciencedirect.com/science/article/pii/S1934590913004621
https://www.sciencedirect.com/science/article/pii/S0092867413010155
[6] http://www.who.int/malaria/areas/treatment/overview/en/
https://en.wikipedia.org/wiki/Malaria

3. Description of engineering technique

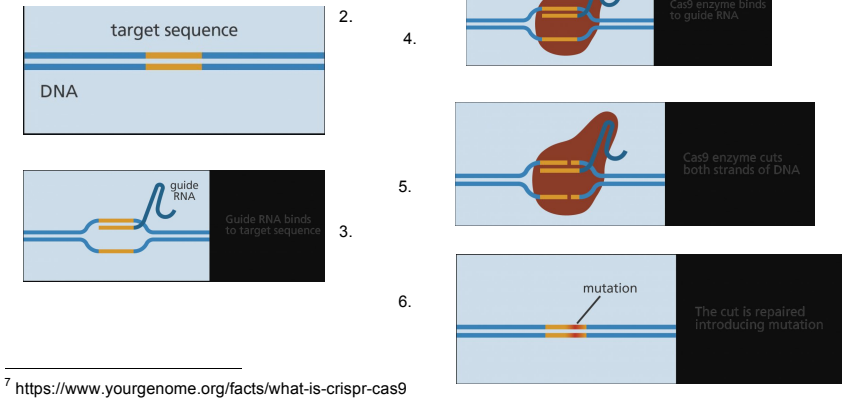
3.1 How does CRISPR-Cas9 work?

For the genome engineering the Type II of the CRISPR-Cas9 complex is used. The following major components are essential for the procedure:

- cRNA: the guiding RNA and locates the target DNA section
- tracRNA: creates an active complex by binding to cRNA
- sgRNA: single guide RNA; consists of tracRNA and cRNA
- Cas9: enzyme; able to modify (and cut) DNA
- Repair template: guides the repair process; allows the insertion of a specific DNA sequence
- Furthermore often a plasmid is required to transfect the target cells.

The CRISPR spacer's sequences are transcribed into short RNA sequences which guide the system to the corresponding DNA sequence and are therefore called guide RNA or crRNA. Is the target DNA found the Cas9 will bind to it and cut it removing the target "defect" gene. With modified versions of Cas9 it is possible for researchers to activate gene expression instead of cutting the DNA. Techniques like this allow researchers to study the gene's function and modify the sequence of the human's genome to treat and heal genetic diseases. The following pictures show this procedure (Fig. 2 - 6) in a visible way.

CRISPR allows scientists to alter and modify DNA sequences and gene functions easily.[7]



[7] https://www.yourgenome.org/facts/what-is-crispr-cas9
https://en.wikipedia.org/wiki/CRISPR
https://www.neb.com/tools-and-resources/feature-articles
/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
https://www.sciencedirect.com/science/article/pii/S0092867413010155

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4. Documentation and pictures of research institution visited

4.1 Interview with PhD. Dipl. sc. nat. Pie Mueller, medical entomologist at Swiss TPH

1.**S**: *What are the risks when changing the genetic structures of an insect through CRISPR? Could the virus become stronger? Could the mosquitoes become more resistant to chemical killers?*
-M: It is very difficult to assess this question. It depends on how you change the genetic structure of the insect. It isn't easy to predict any of the possible outcome. The people working on this would have to do an semi field experiment to get clear results. I don't see any real risks if the changes don't reconstruct the insect.

2. **S**: *When the manipulation would lead to mosquitoes becoming extinct what effect would this have for the ecosystem?*
-M: In every ecosystem there is a balance so when you take something out you have to look what the role of that species was and if this species can get replaced or not. You also need to do a risk reward analysis. Do you want to take the risk of taking one food source of birds away and balance it for less people dying. That's the decision you have to make.

3. **S**: *What are the ethical issues surrounding gene manipulation in regards to CRISPR? What would speak in favor for it is that we would get rid of malaria so that people won't get sick of it anymore and we could also take care of other diseases. But against it would be that would be opening the doors to human experimentation, human modifications and enhancements correct?*
-M: I think that there are two questions. One is related to the genetic change of mosquitoes and the other is the general ethical question of gene manipulation. You need to employ many philosophers to answer the question about general gene manipulation. But to answer the question about the mosquitoes I have to say that they don't have any boundaries so if you would release genetically modified mosquitoes in Basel they would end up in France or Germany so the following question is how can you guarantee that you have the ethical clearance across borders.

4. **S**: *How effective would you believe the CRISPR solution to be in terms of reducing the number of malaria infested insects? Could the changes in the lab possibly be reverted through nature as in dominant or recessive gene?*
-M: To know that you have to do field experiments.

5. **S**: *Let's assume it is possible for genetically modified mosquitoes to have offspring without the malaria disease and these then procreate with malaria infested offspring would it be possible for malaria to fight off the genetically modified versions and therefore infect them as well?*

-M: Yes there have been some counter selections against CRISPR which have been observed. Nature is very creative when it comes to counter selection. If the question is if the solution is bulletproof then I have to say no because there are counter selections. But that stays for every tool it doesn't matter if it is CRISPR, cas9 or an insecticide

6. **S**: *Do you know of any alternatives in terms of eliminating malaria? We have only spoken of CRISPR but in the past we used DDT just as an example of an alternative of course DDT didn't work out as we hoped but do you know any alternatives?*
-M: First I have to say that DDT saved most lives in history.

-S: *Yes of course but it is also affecting us quite harshly nowadays and is considered to have been an inadequate solution at the time. So do you know any alternatives to CRISPR?*
-M: Yes there are many approaches and in the end there isn't only one solution so it is rather a combination of various approaches. There are several tools to kill mosquitoes, insecticides for example. There are also researches about luring mosquitoes away, push pull systems, fungi you name it. There are also vaccines and drugs being developed and health systems being improved so work needs to be done on all of these levels.

-S: *So you believe that for us to effectively eliminate malaria in a large amount we would need a combination of all the approaches we already have currently?*
-M: Yes

7. **S**: *What is your personal take on the ethical issue surrounding the manipulation of the anopheles species through CRISPR because some say that god created the mosquitoes to be mosquitoes and we shouldn't messing with it?*
-M: First of all I don't believe in god but it is a good question. You have to look at the risk reward I would say. Is it ethically correct to do something against mosquitoes to have less people die versus if you release mosquitoes and it gets out of our control. These aspects need to be balanced against each other

-S: *So do you believe that if we decide to eradicate all of the same mosquito specie that nature will do its thing and bring the balance back after we throw a curveball at it?*
-M: Yes to some extent. I mean we have made so many species extinct already not that that would be an argument to eradicate a whole species. But I think the concept of nature fixing the balance isn't right I rather think that there will be evolutions and that other species will fill the gap.

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5. Discussion

From our conducted research, we have been able to gather a vast amount of knowledge concerning the benefits and disadvantages of using the CRISPR system with the Crispr-Cas9 genome. We are henceforth, able to clarify if it is ethically correct to manipulate mosquitoes on a genetic level. For this, a risk-reward check must first be conducted through various field tests, to gain an understanding of what is possible and whether the changes will be more harmful than the present situation. Before scientists are allowed to do actual field trials concerning gene manipulated mosquitoes, research has to be done corresponding to the environmental effects and public health impacts. [8]
Environmental effects are influences that the genetically enhanced mosquitoes have on other species or plants in the ecosystem they get spread out. These environmental effects also concern changes of food sources of the animals that already live in that ecosystem. Public health impact means how the health of the people who live in that environment gets influenced by the new modified species.
After the research has been conducted, scientists can then decide whether the benefits overwhelm the costs/risks. One of the benefits, that we are aware of is that mosquitoes would become incapable of transmitting deadly diseases such as malaria, yellow fever or the zika virus. Therefore a large amount of lives would be saved and one of the deadliest creatures to manl would be stopped.
The costs for such an endeavor lies in the mass production and efficient spreading of these GM mosquitoes. Regardless of the costs, there are also potential risks. These risks stem from a lack of conducted research and field data, as it is yet unknown what kind of impact these genetically altered mosquitoes may have on the ecosystem. Another possibility, is that the malaria parasite would adapt to the modifications made. These could then either adapt to the changes made or it could possibly become even stronger, thus making the malaria parasite an even bigger threat.
Aside from these points, another point is often made which sparks controversy worldwide. Many individuals consider the alterations of genetics to be unnatural or even unholy, often suggesting that if genetic modifications are permitted, it would open the door for performing these modifications/"enhancements" to humans as well. One such controversial topic, is the designer babies debate.[9]

[8] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3620724/
[9] https://www.theguardian.com/science/2017/jan/08/designer-babies-ethical-horror-waiting-to-happen

6. Summary

Throughout this research paper, we have gained a deeper understanding on a modern controversial issue involving genetic modification. From our conducted research, we have been able to determine the current issues and even the potential of the CRISPR-Cas9 enzyme pertaining to its use against Malaria. Although it does not directly affect the parasite, it could possibly prevent the mosquitoes from transmitting it, decreasing the chance of being infected by a disease vector substantially. Even though, the CRISPR-Cas9 seems promising, due to the lack of conducted research, field studies and trials, a lot of potential problems could arise, making the genome either useless or even detrimental to the current situation. Aside from the possible side effects and problems biologically speaking there are also ethical questions which need to be respected in this regard. The main issue with the genetic modifying of organisms seems to be that people feel that is first of all unnatural and that by permitting genetic modification at any level would lead to opening a doorway for genetic modifications to be done on humans. A popular debate around such a topic is the Designer Babies controversy, in which people believe that parents would not only use genetics to prevent genetic diseases but also to change the appearance or even physically enhance their children. As such genetic modifications are generally frowned upon in today's society. Although we might use methods on the genetic level in the future, as of yet the lack of information concerning the side effects of using GM Mosquitoes is too overbearing, making it not feasible at the current moment. Once studies have been conducted and more information becomes available even the ethical question could be changed to whether or not we should save lives on the basis of a fear, afterall, do we have the right to choose who lives or dies?

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7. References

- **Internet references:**

Agarwala, Vineeta/ Hsu, Patrick D./ Ran, F. Ann/ Scott, David A./ Wright, Jason/ Zhang, Feng. (24.10.2013). Genome engineering using the CRISPR-Cas9 system.
URL: https://www.nature.com/articles/nprot.2013.143#introduction (8.4.2018)

Bai, Meizhu/ Bao, Shiming/ Li, Dangsheng/ Li, Jinsong/ Tang, Wei/ Wang, Yinghua/ Wu, Yuxunan/ Yan, Zhiqiang. (5.12.2013). Correction of Genetic Disease in Mouse via Use of CRISPR-Cas9. URL: https://www.sciencedirect.com/science/article/pii/S1934590913004621 (8.4.2018)

Baker, Dean/ Burt, Austin/ Crisanti, Andrea/ Galizi, Roberto/ Gribble, Matthew/ Hammond, Andrew/ Kyrou, Kyros/ Katsanos, Dimitris/ Marois, Eric/ Nolan, Tony/ Russell, Steven/ Simoni, Alekos/ Siniscalchi, Carla/ Windbichler, Nikolai. (7.12.2015). A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. URL: https://www.nature.com/articles/nbt.3439 (8.4.2018)

Ball, Philip. (8.1.2017). Designer babies: an ethical horror to happen? URL: https://www.theguardian.com/science/2017/jan/08/designer-babies-ethical-horror-waiting-to-happen (8.4.2018)

Bier, Ethan/ Fazekas, Aniko/ Gantz, Valentino M./ James, Anthony A./ Jasinskiene, Nijole/ Macias, Vanessa M./ Tatarenkova, Olga. (26.10.2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*.
URL: http://www.pnas.org/content/pnas/112/49/E6736.full.pdf (8.4.2018)

Bier, Ethan/ Fazekas, Aniko/ Gantz, Valentino M./ James, Anthony A./ Jasinskiene, Nijole/ Macias, Vanessa M./ Tatarenkova, Olga. (8.12.2015). Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*.
URL: http://www.pnas.org/content/112/49/E6736.short (8.4.2018)

Boettner, Benjamin. (26.3.2018). CRISPR/Cas9 mutation prevention system could help prevent and fight diseases in the future.
URL: https://phys.org/news/2018-03-crisprcas9-mutation-disease-future.html (8.4.2018)

Broad Institute. (2015). Questions and Answers about CRISPR.
URL:https://www.broadinstitute.org/what-broad/areas-focus/project-spotlight/questions-and-answers-about-crispr (8.4.2018)

Callaway, Ewen/ Ledford, Heidi. (23.11.2015). "Gene drive" mosquitoes engineered to fight malaria. Mutant mozzies could rapidly spread through wild populations. URL: http://www.nature.com/news/gene-drive-mosquitoes-engineered-to-fight-malaria-1.18858 (8.4.2018)

Gootenberg, Jonathan S./ Hsu, Patrick D./ Inoue, Azusa/ Konermann, Silvana/ Lin, Chie-Yu/ Matoba, Shogo/ Ran, F. Ann/ Scott, David A./ Trevino, Alexandro E./ Zhang, Feng/ Zhang, Yi. (12.9.2013). Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Editing Specificity. URL: https://ac.els-cdn.com/S0092867413010155/1-s2.0-S0092867413010155-main.pdf?_tid=f860d18f-e98a-44f4-8801-2f40daa451d3&acdnat=1522939003_d25415c4c700bac675ba8d3fc6c7885a (8.4.2018)

Hornblower, Breton Ph. D./ Reis, Alex Ph. D./ Robb, Brett Ph. D./ Tzertzinis, George Ph. D.. (2014). CRISPR/Cas9 & Targeted Genome Editing: New Era in Molecular Biology. URL: https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology (8.4.2018)

Hsu, Patrick D./ Lander, Eric S./ Zhang, Feng. (5.6.2014). Development and Applications of CRISPR-Cas9 for Genome Engineering.
URL: https://www.sciencedirect.com/science/article/pii/S0092867414006047 (8.4.2018)

Huff Post. (27.1.2017). Das ist das tödlichste Tier der Welt: Ein einziger Biss reicht aus, damit du stirbst.
URL: https://www.huffingtonpost.de/2017/01/27/todlich-tier-biss-weltweit_n_14436316.html (8.4.2018)

N. k. (n. k.). FAQs: Gene drives. URL: https://wyss.harvard.edu/staticfiles/newsroom/pressreleases/Gene%20drives%20FAQ%20FINAL.pdf (8.4.2018)

N.k. (n.k.). No title.
URL: https://ars.els-cdn.com/content/image/1-s2.0-S0092867414006047-gr3_lrg.jpg (8.4.2018)

N.k.. (n.k.). What is CRISPR-Cas9?
URL: https://www.yourgenome.org/facts/what-is-crispr-cas9 (8.4.2018)

Nuwer, Rachel. (30.4.2014). Mosquitoes Kill More Humans Than Humans Murderers Do. URL: https://www.smithsonianmag.com/smart-news/mosquitoes-kill-more-humans-human-murderers-do-180951272/ (8.4.2018)

Resnik, David B.. (21.12.2012) Ethical Issues in Field Trials of genetically modified disease-resistant mosquitoes.
URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3620724/ (2.5.2018)

Salk Institute for Biological Studies. (15.3.2018). CRISPR genetic engineering takes another big step forward, targeting RNA.
URL: https://phys.org/news/2018-03-crispr-genetic-big-rna.html (8.4.2018)

Vidyasagar, Aparna. (21.3.2017). What is CRISPR?
URL: https://www.livescience.com/58790-crispr-explained.html (8.4.2018)

Wikipedia. (9.3.2018). Anopheles. URL: https://en.wikipedia.org/wiki/Anopheles (8.4.2018)

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Grundlagenfach
Genetic Engineering
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Sukhraj Bajwa, Florian Pflugi,
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Wikipedia. (8.4.2018). CRISPR. URL: https://en.wikipedia.org/wiki/CRISPR (8.4.2018)

Wikipedia. (30.3.2018). Malaria. URL: https://en.wikipedia.org/wiki/Malaria (8.4.2018)

World Health Organization. (18.1.2018). Overview of malaria treatment.
URL: http://www.who.int/malaria/areas/treatment/overview/en/ (8.4.2018)

- **Pictures:**

Cover picture: URL: https://www.sciencenews.org/sites/default/files/2017/01/main/articles/011917_LH_mosquite-malaria_main.jpg (8.4.2018)

Fig. 1: URL: http://www.respondoj.com/infographic-what-is-the-worlds-deadliest-animal/ (8.4.2018)

Fig. 2 to Fig. 6: URL: https://www.yourgenome.org/facts/what-is-crispr-cas9 (8.4.2018)

- **Video:**

https://youtu.be/TnzcwTyr6cE (8.4.2018)