Can CRISPR Help Control Locust Populations to Reduce the Impact of Plague Outbreaks?

Neha Ramesh¹ and Sadaf Khan#

¹King Henry VIII College, Malaysia
#Advisor

ABSTRACT

Desert locusts (Schistocerca gregaria) have been threatening food security since time immemorial and affecting human lives by destroying agriculture. Ravaging plague outbreaks are a reality and cause massive devastation across farmlands and pastures to this present day. Locust attacks and outbreaks globally affect vast areas and millions of people resulting in billions of dollars of economic loss. Although controlling the locust population through chemical pesticides is the primary method currently used, it unfortunately has not been highly effective in managing these outbreaks. Advancements in genetic engineering using CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its successful experiments on arthropods provide a huge opportunity that can be extended to design similar genetic behavioral changes in locusts. CRISPR has become one of the most accurate, quick, and cost-effective techniques that could potentially be very effective in managing locust populations by editing specific genes, particularly those responsible for uncontrolled reproduction during their gregarious phase. Experiments conducted on Mosquitos (Aedes aegypti) to silence microRNA-309 (miR-309), permitting stage-specific degradation of its ability to reproduce in their blood-feeding triggered phase indicates that these interventions could also be performed successfully on desert locusts. It should be possible to achieve similar results in female locusts by targeting the miR-309–6 gene cluster, impairing their ovarian development and potentially controlling locust populations.

Introduction

The Desert Locust

There are over 10,000 species of short-horned grasshoppers and over a dozen serious locust species discovered until now. However, the desert locust is responsible for some of the most dramatic plague outbreaks in history. Although the desert locusts are beneficial to the ecosystem, while they are in their solitarius phase, they bring havoc when they enter a gregarious phase. This is usually triggered by favorable climatic conditions with warm temperatures coupled with excessive rains in semi-arid regions. These incidents, although far and few, are significant when they occur and result in huge economic damage in many countries (across Sub-Saharan Africa, Arabian Peninsula and South Asia) (Figure 1).
Chemical pesticides still remain the main approach in controlling desert locusts, however this adversely impacts human health, our environment and is usually expensive and not very effective (Food and Agriculture Organization of the UN, 2005). The search for environmentally safer options and alternatives to expensive control measures are really the need of the hour to reduce their impact, especially because these outbreaks affect our valuable food sources.

The new gene-editing tool: CRISPR

Over the last 7-8 years innovative genome modification technologies have been developed to manipulate genes that regulate different traits of organisms. While traditional methods of genetic engineering involve the transfer of DNA from one organism to another, new techniques in molecular biology have made it possible to edit the genome of an organism directly. The most efficient tool available today is the CRISPR-Cas system and gives scientists the ability to edit an organism’s DNA. It was originally discovered as part of the bacterial immune system against attacking bacteriophages and viruses. Before the CRISPR-Cas system was discovered, Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) technologies were used for genome modifications that were both complex and labor intensive with limited accuracy. CRISPR-Cas in comparison is way faster, cheaper, accurate, and a more efficient gene editing method. In a nutshell, gene editing in organisms is to first understand what genes trigger which traits in them through genome sequencing and to identify those that are responsible for certain traits and behaviors that could be edited to bring about a desired change. CRISPR can precisely edit the DNA sequence hence impacting the traits that need to be changed, mutated, silenced, induced, or replaced and could well be inherited by the organism’s offspring. A basic CRISPR-Cas system consists of two key molecules that introduce a change into the DNA sequence through an enzyme called Cas and a piece called guide RNA (gRNA- ribonucleic acid). The enzyme acts as a pair of ‘molecular scissors’ that cuts the two strands of DNA at a specific location in the genome so that nucleotides (A, C, T, G) can then be added or removed. The gRNA consists of a small piece of pre-designed RNA sequence located within a longer RNA scaffold, guiding the Cas to the right part of the genome, and making sure that the Cas enzyme cuts at the right point to make the edit. Scientists now use this DNA-altering machinery to introduce changes to one or more genes in the genome of the cell of interest in the organism they want to make a change in.
Researchers from Institute of Zoology, Chinese Academy of Sciences, BGI (Beijing Genomic Institute) have managed to fully sequence the locust genome in 2014 (BGI Shenzhen, 2014). This makes it a lot easier to identify the location as well as the function of certain target genes within the locust genome (yourgenome.org, 2016). Moreover, as CRISPR has been proven to successfully bring desired changes in other arthropods, if genes can be identified in female desert locusts’ genome that can induce uncontrollable reproduction in its gregarious phase, there is an amazing opportunity to alter their ability to breed, hence controlling locust populations.

Discussion

Desert locusts belong to phylum Arthropods within the insect class (Figure 2), with close semblance to grasshoppers. Most times they lead solitary lives just like a grasshopper and are identified as green harmless insects that live alone for years. However, when conditions are ideal - usually when there is a lot of rainfall and moisture - they dramatically increase in numbers. This is the main reason why they stand out as the most destructive pests with the ability to make a switch in their development. This stage is called the gregarious phase and here they undergo remarkable transformation in their physiology (phenotypic plasticity) that results in changes to their brain, body size and a distinct change in coloration (to yellow).

![Taxonomic classification of the desert locust phases, solitary and gregarious.](image)

<table>
<thead>
<tr>
<th>Taxonomic ranks</th>
<th>Scientific classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superkingdom</td>
<td>Eukarya</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Metazoa</td>
</tr>
<tr>
<td>Phylum</td>
<td>Arthropoda</td>
</tr>
<tr>
<td>Superclass</td>
<td>Hexapoda</td>
</tr>
<tr>
<td>Class</td>
<td>Insecta</td>
</tr>
<tr>
<td>Subclass</td>
<td>Neoptera</td>
</tr>
<tr>
<td>Infraclass</td>
<td>Orthopteroidea</td>
</tr>
<tr>
<td>Order</td>
<td>Orthoptera</td>
</tr>
<tr>
<td>Suborder</td>
<td>Caelifera</td>
</tr>
<tr>
<td>Superfamily</td>
<td>Acridoidae</td>
</tr>
<tr>
<td>Family</td>
<td>Acridae</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Cyrtacanthacridinae</td>
</tr>
<tr>
<td>Genus</td>
<td>Schistocerca</td>
</tr>
<tr>
<td>Species</td>
<td>Schistocerca gregaria</td>
</tr>
</tbody>
</table>

In this phase, locusts get attracted to one another - and if these conditions persist, they start to group together and form swarms, which is identified as a plague outbreak (Figure 3). During this gregarious phase, they ravage farms and practically eat anything in sight including fodder and pastures. Because they are polyphagous, if their population is not controlled, they will cause a huge problem. Locusts can eat up to 2.5 gm in a day (as much as its own body weight) and this trait makes them extremely destructive. These swarms are huge and can range anywhere from one square km to 100 square km or more, with 60-80 million locusts in over half a square km. They bulldoze crops, pastures, and fodder in dark clouds the size of small cities. In northern Kenya, for example, one swarm was reported
to be 35 km long by 50 km wide that could blanket a city the size of Paris 20 times over. In this phase, a regular adult can fly up to 150 km in one day and due to this, they are also called International Transboundary Pest (Baskar, 2020).

Figure 3. The formation of the desert locust’s gregarious phase.

The lifespan of a desert locust is typically about 4-6 months long, although this entirely depends on weather conditions. The locust life cycle comprises three different stages: egg, hopper, and adult. An adult female can lay up to 80-100 eggs in a single egg pod at a time and usually can lay eggs thrice in their lifetime. Eggs usually hatch in about 14-20 days, and hoppers develop over a period of 5-6 weeks and become mature adults in 2-4 months (Figure 4), all depending on ecological conditions (mainly temperature). Adults are initially sexually immature, but eventually become more active and start copulating (World Meteorological Organization, Food and Agriculture Organization of the UN, 2016). Usually, locust outbreaks are seen by the time kharif crops are harvested during the monsoon and by this time the locust eggs have hatched and are now adults.

Figure 4. Life cycle of the desert locust.
CRISPR’s ability to edit only targeted DNA and avoid off-target editing is called ‘specificity’. Achieving high levels of specificity is possible only through the right combination of nuclease and guide RNA. There are many precise interventions within CRISPR such as Non-Homologous End Joining (NHEJ), homologous recombination, silencing with antagonirs etc., which all have different effects on the targeted gene. Many experiments have been performed to explore specific gene functions in arthropods to understand the impact of modification in their behavior. Some of these interventions have been used to change certain traits to find solutions to insect-related problems like pest control and disease outbreaks. Some of the arthropods where CRISPR has been used are Drosophila, Aedes aegypti, Lepidoptera, Bombyx mori, etc. An example of successful CRISPR usage was performed in Anopheles gambiae, a major vector of malaria, where researchers used CRISPR/Cas9 technology to target three genes related to a female-sterility phenotype. CRISPR has also been used on some other locust species including the Locusta migratoria where researchers were trying to understand the pheromone signals in their brain that directed behaviors such as foraging, feeding, mating, and spawning. This allowed modification of their genome by targeting the Odorant Receptor genes (Orco) by micro-injecting the Cas9-sgRNA and Orco sgRNA into Locusta migratoria eggs. The Orco gene is a receptor gene that allows locusts to detect different smells and by removing this gene, locusts lose an attraction response to aggregation pheromones under crowding conditions. This successfully established Orco homozygous and heterozygous mutant lines in the subjected locust embryos (Li Y, Zhang J, et al, 2016). This indicates that CRISPR if performed on other hemimetabolous insects like Schistocerca gregaria will yield similar results.

Analysis and Results

Genome-scale phylogenetic analysis (a DNA sequencing method) using 122 single-copy genes from 10 sequenced arthropod genomes (Figure 5) revealed that the locust is the basal taxon for the other insects sequenced so far, and this supports the paraphyletic status (being the last common ancestor) of locusts (Wang, Fang, et al, 2014). This is an important discovery and the basis to assume that locusts share similar genome to other arthropods and genes successfully edited could be identified and targeted in desert locusts to achieve similar desired genetic outcomes.

Figure 5. Arthropod ancestry, indicating that the locust is the basal taxon of multiple arthropods.

Recently, a cluster of fast-evolving miRNA, miR-309–6, was identified undergoing particularly dynamic evolutionary events within the arthropod phylum and ancestry, including duplication, gain, and loss of miRNA parts within it. In some arthropods like fruit flies (Drosophila), this cluster of miRNA is expressed more often in developing eggs and promotes messenger-RNA (mRNA - a strand of RNA complementary to a gene and read by a ribosome to
synthesize a protein) turnover during the maternal-to-zygotic transition. These fast-evolving genes could show evolution which would have led to species divergence as well as different physiological traits in different arthropods. Therefore, from an evolutionary standpoint, it can be concluded that miR-309~6 gene clusters have an important role in regulating the physiology of arthropod reproduction (Zhang Y, Zhao B, et al, 2016).

One of the initial CRISPR experiments to prove this, was conducted in the Aedes aegypti, the mosquito. In 2015, scientists used CRISPR Cas9 to alter a gene (within the miR-309~6 cluster) in its embryo that induced multiple mutations in them and conducted experiments in female mosquitoes to reduce their fertility rates and therefore the likelihood of them spreading diseases. The scientists targeted a specific microRNA called microRNA-309 (miR-309) that was responsible for the rapid egg development during the blood feeding-triggered phase of ovarian development. They found that the levels of this microRNA in the ovaries was extremely high after feeding and was mainly seen when eggs were maturing. This shows that the microRNA is mainly responsible for efficient ovarian function. The researchers used Antagomirs (a CRISPR technique wherein a small synthetic guide-RNA complementary to a microRNA is specifically used to target and silence it) - Ant-309 - on the gene in order to silence the microRNA in vivo. As this gene was silenced, its expression reduced and hence caused pre-stage degradation of the ovary. The miR-309 gene works as a switch which targets a specific mRNA for the SIX homeobox 4 protein (SIX4) - this allows for stage specific degradation of the ovarian mRNA. This clearly shows that knocking the miR-309 gene down compromises their reproduction (Zhang Y, Zhao B, et al, 2016).

Since the gene cluster of miR-309~6 was discovered in all arthropods and affects their reproductive physiology (as proven with mosquitos), it can be derived that there might be similar effects of these genes in desert locusts as well. With this, we can derive that silencing the miR-309 will result in stage-specific degradation of the female locust’s ovary, causing a depletion in the number of eggs produced and the viability of ova during the gregarious phase (Zhang Y, Zhao B, et al, 2016). Also, in mosquitoes, an abundance of miR-309 was found in the ovaries during their blood feeding-triggered phase. This phase change in the female mosquitoes was triggered as they fed on blood. This could be compared to locusts when they similarly change from a solitary to gregarious phase, (due to pheromones released when they are under crowding conditions like Locusta migratoria) triggering the mutation and leading to degradation of their reproductive physiology (Sun D, Guo Z, Liu Y, Zhang Y, 2017).

This surely suggests that a similar intervention (Antagomirs for knock down), as performed successfully on female mosquitoes, could yield desired mutations in female desert locusts too. However, in locusts, it might prove ineffective to merely silence (knock down) this gene because, given the swarm sizes that go into billions, even a depleted reproductive cycle will potentially result in locust numbers that would still cause huge damage during an outbreak. Hence, it may be better to use Non-Homologous End Joining (NHEJ- a knockout strategy where small indels are induced when a double-strand break joins without the need for a template) technique in female locusts in order to completely remove their ability to reproduce. Instead of using Antagomirs to silence the gene, it would be possible to microinject the Cas enzyme bound with an gRNA complementary to the miR-309 gene into the eggs of locusts (AAT Bioquest, 2020). This would effectively cut out the gene from the genome and render it functionless, leading to the new-born females (mutated hoppers) unable to reproduce due to degradation of the ovary as they mature into adults. This however should be first tested in a laboratory similar to the mosquitos in order to prove the accuracy and efficacy before exercising it widely.

**Conclusion**

The above review concludes that it is indeed possible to deploy CRISPR techniques to degrade the reproductive functions of female Schistocerca gregaria (being an arthropod) thus crippling their population as they enter their gregarious phase. Hence the question, “is it possible to perform CRISPR interventions on miR-309~6 gene clusters in female locusts, impairing their ovarian development and limiting reproduction in its gregarious phase?” raised stands firm based on the literature review and analysis done.
However, the efficacy of NHEJ in desert locusts needs to be validated through targeted experiments by creating a ribonucleoprotein (RNP) complex and delivering it into the target cell (locust egg). A chemically synthesized guide RNA (gRNA) and a Cas nuclease are the necessary reagents to create the RNP complex and this could be administered by microinjection into the cell (as a preferred delivery system) that will intervene to target the miRNA-309 gene. The constituents of the RNP complex, however, need to be checked for whether the gRNA is adenine-thymine- (AT) rich or guanine-cytosine- (GC) rich. This will determine which of the two main Cas nucleases to use – Cas12a (Cpf1) which is more effective for AT-rich sequences, or Cas9 which is more effective for GC-rich sequences (Integrated DNA Technologies, 2020). The success of this testing would confirm that it is indeed possible to perform NHEJ knockout on miR-309 gene in female locusts, impairing their ovarian development and limiting reproduction in their gregarious phase. (Fig.6)

Figure 6. Steps involved in the knockout of miRNA-309 gene in desert locusts: The cut is detected, and the cell repairs it by NHEJ. The repair isn’t perfect, so small indels are induced that should render the gene functionless.

On the other hand, there is also a possibility that this experiment may not yield the desired result as proposed. This could be because the miR-309 gene may not affect desert locusts’ reproductive physiology and cause stage-
specific (gregarious) ovarian degradation. In such an eventuality, the recommendation would be to further continue experimenting with different genes within the miR-309-6 cluster and to try other CRISPR interventions (knock-in, silencing, etc.) to see which specific combination would bring about stage-specific degradation of the ovary. The results however, if achieved as desired, can be deployed within a small wild swarm of a plague infested area to measure the practical efficacy of such an intervention. This essentially should result in much lower population growth despite the locusts entering a gregarious phase, given the inability of female locusts to reproduce.

This clearly shows that CRISPR, if used accurately, has a huge potential as a sustainable pest management technique to successfully control locust populations and dramatically reduce the impact of plague outbreaks.

**Acknowledgments**

I would like to express my sincere gratitude to Dr. Sadaf Khan (Chemistry Teacher, King Henry VIII College), Miss. Jessica Roper (Biology Teacher, King Henry VIII College) and Mr. Jonathan Dancyger (Head of sixth form, King Henry VIII College) for helping me throughout my independent research. Their encouragement has been a great motivator for me to seek more in this field as well as submit my research project.

A special thanks to Dr. Gurion Ang, (Lecturer, School of Biological sciences, Faculty of Science, University of Queensland, Australia) for sparking the deep curiosity in CRISPR through his lecture at our school in 2019.

**References**


