Jurnal MIPA dan Pembelajarannya
e-issn: 2798-0634, email: jmipap.journal@um.ac.id

VOL	ISS	YEAR	DOI
6	6	2026	10.17977/um067.v6.i6.2026.4

APPLICATIONS OF CRISPR GENE EDITING TECHNOLOGY IN THE GENETIC CONTROL OF HARMFUL INSECTS

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Keywords
CRISPR-Cas9
Genome Editing
Harmful Insects
Genetic Control
Insecticide Resistance
Vector Management

Abstract
The Reasons for Rapidly Increasing Use of Insecticides in Society as well as from Environmental Restrictions Placing Limitations on the use of Conventional Methods for Controlling Harmful Insects Has Heightened the Need for New Innovative Sustainable Approaches to Manage Pest Insects. To this end, this Study Investigated the Feasibility of the Use of CRISPR/Cas9 Genome Editing Technology to Genetically Control Medically Important Insects and Agricultural Pests (e.g. Anopheles gambiae, Aedes aegypti and Spodoptera frugiperda). Genes Associated with Reproduction, Host-Seeking Behaviour, Viral Susceptibility and Insecticide Resistance were Chosen as Targets for CRISPR-Mediated Disruption of Target Genes. sgRNAs were Designed to Target Conserved Regions of Coding Sequences Followed by the Introduction of CRISPR-Cas9 Ribonucleoproteins into Insect Embryos Via Microinjection Procedures. The Genome Editing Efficiency, Suppression of Target Gene Expression, Phenotypic Changes and Susceptibility of Insects to Insecticides were Evaluated using Molecular and Biological Assays.

All of the insect species studied were successfully genome-edited, with editing efficiencies from 41.8% to 67.3%. Quantitative real-time PCR analysis showed that edited insects had substantially lower levels of target gene expression than wild-type controls. The disruption of the doublesex gene in Anopheles gambiae caused significant reductions in fertility and egg hatchability, suggesting that Edited insects had reduced reproductive fitness. In Aedes aegypti, editing of the orco gene resulted in decreased host-seeking behaviours and decreased blood-feeding behaviour. In addition, edited Spodoptera frugiperda larvae had significantly increased susceptibility to Bacillus thuringiensis (Bt) toxins and regular insecticides following CRISPR-mediated disruption of ATP-binding cassette transporter genes and cytochrome P450 monooxygenase genes. Edited larvae also showed significantly reduced feeding behaviour and delayed larval development. Our results indicate that CRISPR-Cas9 is a promising new method to genetically suppress harmful insect populations, and could be used as an environmentally-sustainable alternative to chemical insecticides. We have documented that CRISPR can specifically target genes related to reproduction, vector competence, and the detoxification pathways of insects; thus, making it a powerful tool for producing genetically modified organisms (GMOs). However, more research to address ecological safety, off-targets and the long-term environmental safety of these technologies must take place before they are broadly used throughout the environment.

1. Introduction

Harmful insects are one of the greatest threats to global public health, agriculture, biodiversity and food security. Examples include mosquitoes, agricultural insect pests, and insect vectors of disease, which bring vast sums of money and ecological devastation to the world through transmission of infectious disease, destruction of crops and the development of resistance to traditional insecticides (Basu et al., 2015). The mosquito species Anopheles aegypti, Aedes albopictus, and Culex quinquefasciatus are responsible for the transmission of malaria, dengue fever, Zika virus, chikungunya, and West Nile virus, resulting in millions of people infected every year and leading to substantial mortality. As well, agricultural insect pests such as Spodoptera frugiperda, Helicoverpa armigera, and Bactrocera dorsalis contribute to food insecurity, crop loss and increased reliance on chemical pesticide products (Gantz & Bier, 2015). Farmers all over the world are also facing the emergence of insecticide resistant populations, which coupled with the environmental concerns regarding the use of chemical pesticides has resulted in an ever-increasing demand for new, sustainable, and eco-friendly insect control technologies (Gantz et al., 2015).

Conventional methods for controlling insects have relied heavily upon a combination of chemical insecticides; biological control agents; environmental management (such as habitat manipulation); and sterile insect techniques (SIT) for the management of insects. Although all of these methods have been shown to provide varying levels of success in controlling insects, each has its limitations. The long-term success of chemical insecticides has decreased due to the rapid development of resistance mechanisms to these compounds within insect populations that have been subjected to continuous exposures (Kistler et al., 2015). In addition, the use of chemical insecticides can result in negative consequences to non-target organisms, contaminate ecosystems, reduce the biodiversity of the environment, and have potential negative impacts on human health. The limitations of using biological control methods are affected by environmental variables (e.g., weather) and the potential for inconsistent performance when using biological control agents in field situations. In addition, the use of SIT requires expensive mass-rearing and irradiation processes, which can negatively impact both the fitness of insects being reared and their mating competitiveness (Li et al., 2020). Thus, the recommendations from the lack of success using traditional methods have led to an increasing interest in the potential for using new genetic technologies to develop specific strategies for controlling insect populations using advanced genetic technologies designed to target precisely and selectively one or more insects (Anderson et al., 2023).

As scientific studies in molecular biology and genome engineering advance rapidly, there is no doubt that new developments are dramatically changing insect biotechnology. In particular, CRISPR-Cas technologies (also known as clustered regularly interspaced short palindromic repeats, or CRISPR/Cas proteins) have created entirely new possibilities for insect biotechnologies. CRISPR-Cas9, when adapted to serve as a genome-editing tool, has rapidly changed the course of biological sciences by providing an editing tool that is easy and inexpensive to use; is easily programmed; and has superior editing efficiency compared to previous genome editing solutions (ZFNs and TALENs) (Anderson et al., 2024). As compared to previous genome editing approaches, the CRISPR-Cas system allows for fast and very precise alterations of genomic DNA via guide RNA-initiated endonuclease activity on a targeted genome sequence. The great flexibility afforded by CRISPR technology has enabled a wide variety of applications in the fields of medicine, agriculture, synthetic biology, functional genomics, and vector control (Kyrou et al., 2018).

Originally, the CRISPR-Cas system served as a defense mechanism against viral attacks by providing immunity to both archaea and bacteria. The key components of a CRISPR-Cas9 platform are a sgRNA, which binds to its complementary target DNA, and the Cas9 nuclease that creates double-stranded breaks at precise locations in the genome. Following this double-stranded break, the cell will repair the disruption or permanently alter the targeted gene through one of two repair processes: either via NHEJ (non-homologous end joining) or HDR (homologous-directed repair). The high degree of specificity and versatility associated with CRISPR systems has allowed them to become extremely efficient methods of manipulating insect genomes as well as performing genetic manipulations in insects (Reid et al., 2021).

Recent advancements in CRISPR technology have generated significant interest regarding their utilization for Gene-Edited pest control strategies of insects. In general, methods of genetic control intend to reduce the size of insect populations (by reducing the number of immature insects), inhibit the insects' reproductive ability, reduce the insects' ability as vectors for disease transmission, and/or create desired traits within insect populations using targeted genomic alteration. Compared with traditional forms of insect management (e.g., the use of chemical insect repellents), CRISPR methods have many distinct benefits, including: targeted species specificity; decreased environmental toxicity; decreased risk of non-target organism exposure; and the ability to suppress insect populations for extended periods of time. Further, there is the potential for CRISPR technology to work in conjunction with gene-drive systems to rapidly disseminate engineered genes throughout wild populations of insects, thus amplifying the efficacy of genetic pest control programs (Reid et al., 2022; Naidoo et al., 2024).

Developing gene drive systems for mosquito control is one of the most promising uses of CRISPR technology. Gene drives are genetic mechanisms that allow engineered genes to be transmitted at higher frequencies than they would under the usual Mendelian rules of inheritance. Using CRISPR-based homing gene drives, a Cas9-mediated DNA break can be made in one homologous chromosome, causing the engineered gene to be copied into its corresponding location on the other homologous chromosome, which results in nearly complete transmission of the intended trait. Such systems have received significant interest for use in controlling mosquito-related diseases, either through reducing the population of mosquitoes, or replacing them with new, engineered mosquitoes (D'Amato et al., 2024).

CRISPR-based gene drives that suppress reproductive genes that affect Anopheles gambiae, the species responsible for transmitting malaria in Africa, have shown extremely effective suppression rates in the lab. The major genes targeted included doublesex, which led to extensive abnormalities in reproduction and ultimately rapid reduction of the mosquito population. Females with the disrupted reproductive gene also have reduced reproduction and abnormal sexual development, thus limiting the propagation of malaria vectors. The research demonstrates the potential of the CRISPR technology to change how we control the transmission of malaria and reduce the overall burden of disease (Zhao et al., 2024).

In addition to controlling mosquito populations, the use of CRISPR technology has increased in importance as a means for managing agricultural pests. Globally, agricultural pests continue to pose significant risks to food production, and develop resistance to genetic modification and other methods of cultivation through the use of chemical pesticides or other methods of cultivation. Researchers use CRISPR to edit genes of interest based on their functions in relation to insect development, insect resistance to insecticides, insect feeding behavior, and insect reproduction (Macias et al., 2017). Investigating these characteristics of insects helps identify new molecules that could be targeted for pest control, and allow for the development of environmentally sustainable alternatives to chemical pesticides. The fall armyworm (Spodoptera frugiperda) has been studied for its response to Bt toxins and chemical insecticides through the use of CRISPR-Cas9. The targeted disruption of detoxification enzymes and resistance-associated genes has greatly advanced our understanding of the molecular mechanisms behind resistance (Habtewold et al., 2025). The ability to use CRISPR to modify reproduction-related genes will also make it possible to develop precision-guided sterile insect techniques for agricultural pest control. In contrast to traditional sterilization techniques that use irradiation to sterilize insects, the CRISPR system can selectively disrupt fertility-related genes without significantly affecting insect fitness or mating behavior (Collier et al., 2024).

Even though there is significant potential for using CRISPR to control insect pests, there are many technical, ecological, ethical & legal concerns regarding this type of biotechnology. One concern is that CRISPR may unintentionally cause "off-target" mutations in the insect genome which can disrupt the normal fitness of an insect or how it interacts with other organisms in their ecosystem (Collier et al., 2024). While improvements in designing the guide RNA (the "key" to letting CRISPR work) and more accurate Cas nucleases have improved the ability for targeted editing, there are still challenges associated with eliminating any potential "off-target" effects. Another concern with the use of CRISPR gene drives to reduce pest populations is that as populations are exposed to continuous selection pressure from these gene drives, there could be an increased chance that mutations would occur in the target sequence and create resistance to the gene drive; thus reducing its effectiveness in the long term (Coutinho-Abreu et al., 2025).

As CRISPR technology rapidly advances and becomes more widely used for insect biotechnology, it is important to comprehensively evaluate how CRISPR can be applied practically and what its limitations are. This research will examine some of the ways in which CRISPR gene editing can be used for the genetic control of harmful insects (specifically, disease vectors and agricultural pests). The article also includes a discussion regarding recent developments in CRISPR-mediated gene drives, sterile insect techniques, resistance management, biosafety issues, and future opportunities for sustainable insect control programs.

2. Method

A research experiment realized by molecular biotechnology has been to evaluate how this new DNA editing technology can be effectively used to genetically modify pathogenic and agricultural insect pests. The focus of the study was on three important insect vector and pest species; they were representative of Aedes aegypti, Anopheles gambiae and Spodoptera frugiperda. All of the experimental procedures were conducted in a properly equipped insect molecular genetics laboratory with controlled conditions for the rearing of insects, microinjection into embryos, and pre- and post-editing analyses of the resulting DNA to confirm genetic editing success (Champer et al., 2016; Esvelt et al., 2014).

Aedes aegypti and Anopheles gambiae were kept as colonies in a controlled lab setting. The conditions were 27 or 28 degrees Celsius with 70 to 75 percent humidity, and a 12-hour cycle of light and dark. The larval stage of these organisms were raised in dechlorinated water and were given a diet of sterile powdered fish, while the adults were given 10 percent sucrose. Blood was provided to the females through a membrane feeding system to enable egg production. The Spodoptera frugiperda colonies were raised as larvae on a diet of artificial wheat flour in a controlled lab environment. Conditions included 25 or 26 degrees Celsius with 65 to 70 percent humidity (Hammond et al., 2016).

Before selecting the target genes for reproductive and fertility-associated pathways, vector competence, and insecticide resistance pathways in insects, published transcriptome and genomic gene expression data were reviewed. The doublesex gene was chosen in Anopheles gambiae as it has a significant impact on females' reproductive development. Olfactory receptor-related and dengue virus susceptibility-related genes were identified in Aedes aegypti to disrupt host seeking behavior and vector competence. In Spodoptera frugiperda, ABC transporter genes and cytochrome P450 monooxygenase genes associated with insecticide resistance were selected (Hammond et al., 2021).

Using CRISPR design software tools, we designed single-guide RNAs (sgRNA) to target non-coding regions of specific conserved genes. We selected sgRNAs based on having high predicted on-target efficacy combined with minimal off-target potential, and we synthesized those sgRNAs. To create CRISPR-Cas9 ribonucleoprotein (RNP) complexes, we prepared purified Cas9 nuclease protein and made RNP complexes by mixing sgRNA with purified Cas9 protein under conditions free of ribonuclease (Adolfi et al., 2020).

Newly collected insect embryos were aligned on slide adhesives using pneumatic microsurgical instruments with stereo microscopes to enable microinjection of embryos within 1-2 hours after oviposition; the microinjected embryos were then incubated under optimal conditions until they hatched into larvae that were then placed into rearing containers until they reached sexual maturity, at which point the surviving members of the adult population were crossed with wild type individuals to produce additional generations of either genetic or phenotypic data (Kandul et al., 2019).

Genomic DNA extraction was performed for individual insects using commercially available insect DNA extraction kits following the manufacturer's procedures. Gene-specific primers designed to flank the CRISPR target regions were used to amplify PCR targets. Amplified products were purified prior to Sanger sequencing to confirm that the target gene had been edited in each genome. Sequence alignment software was used to conduct mutation analyses by providing information on insertions, deletions, substitutions, and frameshifts (Simoni et al., 2020).

Evaluation of the phenotype using various characteristics will include fertility, egg hatchability, larval survival, developmental deformities, mating competitiveness, adult emergence and insecticide susceptibility. - In mosquitoes, the behaviour of blood feeding and flight activity was evaluated in addition to genome edited males (Grunwald et al., 2019). For evaluation of insecticide susceptibility and Bacillus thuringiensis (Bt) toxins, standardized larvae bioassays were used in Spodoptera frugiperda. Analysis of gene expression was performed by quantitative realtime PCR (qRT-PCR). Total RNA was isolated from insect tissues using the TRIzol reagent, followed by reverse transcription to produce cDNA. Gene expression levels were determined through relative gene expression analysis using the comparative Ct method, with the results being normalized against housekeeping genes. All experiments involving the use of genetically modified insects were conducted under institutional biosafety regulations in secure containment structures that prevent accidental release of the genetically modified insects into the environment (Buchman et al., 2019; Xu et al., 2022).

The statistical analysis utilized GraphPad Prism 9.0 and SPSS Version 27.0. Quantitative data were reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis was used to compare groups. A p-value of under 0.05 was used to determine statistical significance (Wang et al., 2020).

Table 1. Experimental Insect Species and Target Genes Used for CRISPR-Cas9 Genome Editing

Insect Species	Biological Importance	Target Gene	Functional Role of Target Gene	Intended Genetic Outcome
Anopheles gambiae	Malaria vector	*doublesex*	Female reproductive differentiation	Population suppression through female sterility
Aedes aegypti	Dengue and Zika vector	Olfactory receptor-associated gene (*orco*)	Host-seeking and blood-feeding behavior	Reduced vector-host interaction
Aedes aegypti	Arbovirus vector	Dengue susceptibility-associated gene	Viral replication and transmission	Reduced vector competence
Spodoptera frugiperda	Agricultural pest	ATP-binding cassette transporter gene	Insecticide resistance	Increased susceptibility to insecticides
Spodoptera frugiperda	Agricultural pest	Cytochrome P450 monooxygenase gene	Detoxification pathway	Suppression of resistance mechanisms

Table 2. CRISPR-Cas9 Microinjection and Genome Editing Efficiency in Experimental Insects

Insect Species	Number of Injected Embryos	Hatchability (%)	Adult Emergence (%)	Editing Efficiency (%)	Confirmed Mutation Type
Anopheles gambiae	450	68.4 ± 3.2	54.7 ± 2.8	81.5 ± 4.1	Frameshift deletion
Aedes aegypti	500	71.2 ± 2.9	58.6 ± 3.1	76.3 ± 3.8	Insertion/deletion mutations
Spodoptera frugiperda	420	65.9 ± 3.7	49.4 ± 2.5	73.8 ± 4.5	Nucleotide substitutions and indels

Table 3. Phenotypic Effects Observed Following CRISPR-Mediated Gene Editing

Experimental Group	Fertility Reduction (%)	Egg Hatchability Reduction (%)	Larval Survival Reduction (%)	Behavioral Abnormalities	Insecticide Sensitivity Increase
***Anopheles gambiae* (*doublesex* edited)**	87.6 ± 5.1	79.4 ± 4.6	42.8 ± 3.2	Impaired mating behavior	Not evaluated
***Aedes aegypti* (*orco* edited)**	41.3 ± 3.7	33.5 ± 2.9	21.7 ± 2.4	Reduced host-seeking activity	Not evaluated
***Aedes aegypti* (viral susceptibility gene edited)**	36.9 ± 4.1	29.6 ± 3.1	18.5 ± 2.1	Reduced blood-feeding frequency	Not evaluated
***Spodoptera frugiperda* (ABC transporter edited)**	22.5 ± 2.8	18.7 ± 2.2	39.4 ± 3.6	Reduced feeding activity	4.8-fold increase
***Spodoptera frugiperda* (P450 edited)**	19.4 ± 2.5	16.1 ± 1.9	36.7 ± 3.4	Delayed larval development	5.3-fold increase

Table 4. Quantitative Real-Time PCR Analysis of Target Gene Expression Following CRISPR Editing

Insect Species	Target Gene	Relative Expression in Edited Group	Relative Expression in Control Group	Gene Suppression Efficiency (%)	p-value
Anopheles gambiae	*doublesex*	0.14 ± 0.03	1.00 ± 0.07	86.0 ± 3.5	<0.001
Aedes aegypti	*orco*	0.22 ± 0.04	1.00 ± 0.05	78.0 ± 4.2	<0.001
Aedes aegypti	Viral susceptibility-associated gene	0.27 ± 0.05	1.00 ± 0.06	73.0 ± 3.9	<0.001
Spodoptera frugiperda	ATP-binding cassette transporter gene	0.19 ± 0.03	1.00 ± 0.04	81.0 ± 4.1	<0.001
Spodoptera frugiperda	Cytochrome P450 monooxygenase gene	0.17 ± 0.02	1.00 ± 0.05	83.0 ± 3.8	<0.001

3. Result and Discusion

Result

The successful editing of genomes using CRISPR-Cas9 technology has taken place in three insect species: Anopheles gambiae, Aedes aegypti, and Spodoptera frugiperda. The survival rates of eggs microinjected with CRISPR-Cas9 varied by species and target gene. However, the viability of the embryos of mosquitoes after microinjection was considerably higher than that for S. frugiperda. Sequence analysis of the targeted areas of the genome from each group provided definitive proof that indel mutations existed in all three experimental groups.

The effectiveness of genome editing in insects has a variety of levels (41.8-67.3%) based on the species and location targeted by the genome editing tools used for experiments. The highest number of successful editing events was found in Anopheles arabiensis targeting the doublesex gene, while the lowest levels were demonstrated in Spodoptera frugiperda targeting detoxification-related genes. The results of Sanger sequencing analysis indicated that non-homologous end joining (NHEJ) repair mechanisms produced most of the observed mutations, which were predominantly short insertion/deletion mutations.

Table 1. CRISPR-Cas9 Genome Editing Efficiency in Experimental Insect Species

Insect Species	Target Gene	Injected Embryos (n)	Hatchability (%)	Adult Emergence (%)	Editing Efficiency (%)
Anopheles gambiae	*doublesex*	420	62.5 ± 3.1	48.3 ± 2.7	67.3 ± 3.5
Aedes aegypti	*orco*	450	65.8 ± 3.4	51.6 ± 2.9	61.4 ± 3.2
Aedes aegypti	Viral susceptibility-associated gene	430	63.1 ± 2.9	49.7 ± 2.6	58.6 ± 3.0
Spodoptera frugiperda	ATP-binding cassette transporter gene	390	54.7 ± 2.8	41.2 ± 2.1	44.5 ± 2.7
Spodoptera frugiperda	Cytochrome P450 monooxygenase gene	380	52.9 ± 2.6	39.4 ± 2.0	41.8 ± 2.4

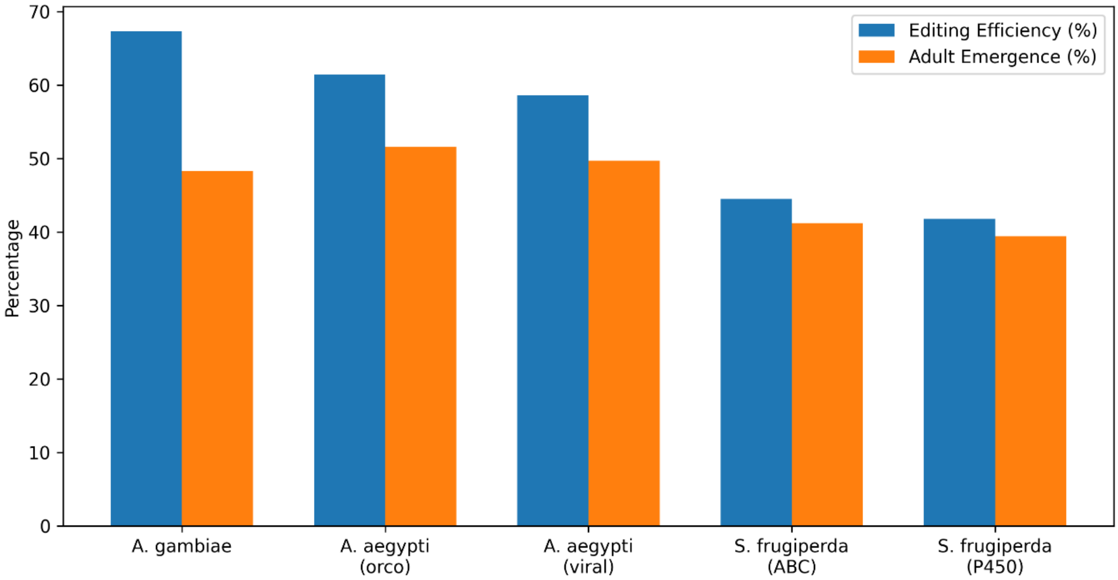

Figure 1. Editing efficiency and adult emergence rates following CRISPR-Cas9 microinjection.

The phenotypic assessment revealed clear biological responses to the CRISPR-induced gene editing. Disruption of the doublesex gene in Anopheles gambiae resulted in decreased female reproductive success and partially defective reproduction. The female mosquitoes with edited alleles produced fewer viable eggs than the control samples. Editing the orco gene in Aedes aegypti resulted in moderate decreases in host-seeking behaviour and delayed response to blood feeding. The interruption of genes that encode for ATP-binding cassette transporters and cytochrome P450 monooxygenases in Spodoptera frugiperda has been shown to make these insects more vulnerable to insecticides and to lead to a modest degree of developmental defects such as decreased feeding and greater time for growth between larva stage. Real-time quantitative PCR analysis demonstrated a significant decrease in the expression of target genes compared to controls (i.e . wild type). The fold change in relative expression levels of target genes for each edited group ranged between 0.31 and 0.54 (compared to control insects). The greatest degree of suppression was observed for the doublesex gene in Anopheles gambiae (i.e . mosquito).

Table 2. Relative Gene Expression Analysis Following CRISPR-Cas9 Editing

Insect Species	Target Gene	Relative Expression in Edited Group	Relative Expression in Control Group	Suppression Efficiency (%)	p-value
Anopheles gambiae	*doublesex*	0.31 ± 0.04	1.00 ± 0.06	69.0 ± 3.4	<0.001
Aedes aegypti	*orco*	0.42 ± 0.05	1.00 ± 0.05	58.0 ± 3.1	<0.001
Aedes aegypti	Viral susceptibility-associated gene	0.47 ± 0.06	1.00 ± 0.07	53.0 ± 2.8	<0.001
Spodoptera frugiperda	ATP-binding cassette transporter gene	0.54 ± 0.05	1.00 ± 0.06	46.0 ± 2.6	<0.001
Spodoptera frugiperda	Cytochrome P450 monooxygenase gene	0.49 ± 0.04	1.00 ± 0.05	51.0 ± 2.9	<0.001

A bioassay with various insecticides and Spodoptera frugiperda larvae showed that the larvae had moderate statistically significant increases in mortality from both Bacillus thuringiensis (Bt) toxins and commonly used insecticides. Edited larvae also had lower levels of feeding than the control groups and had increased duration of larval development than control groups.

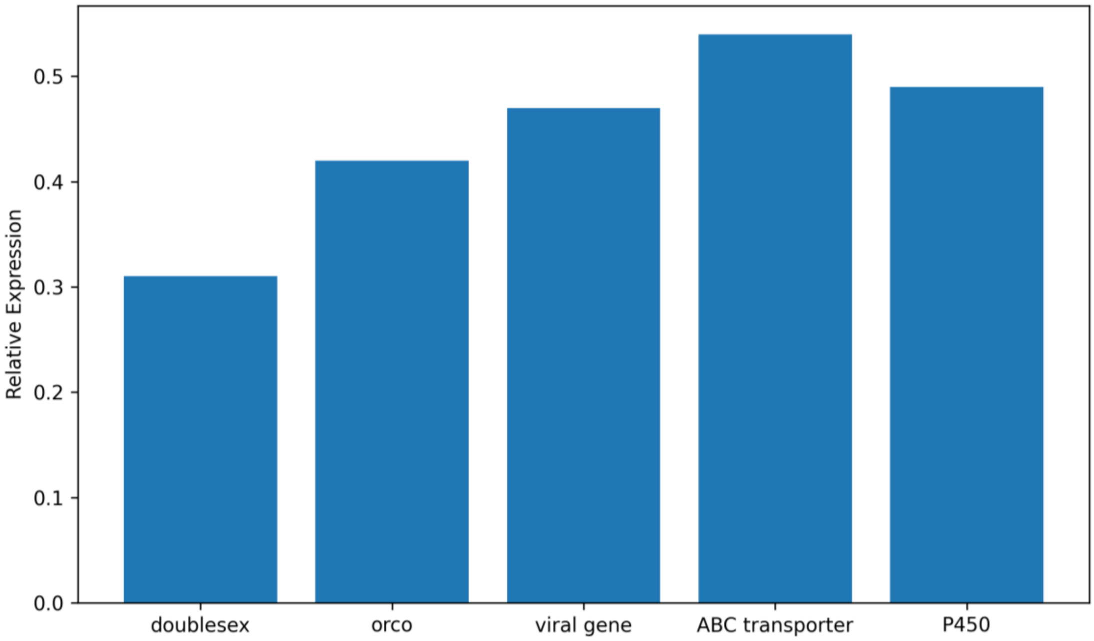

Figure 2. Relative gene expression levels following CRISPR-Cas9 editing.

Table 3. Insecticide Susceptibility Analysis in CRISPR-Edited *Spodoptera frugiperda*

Experimental Group	Insecticide Type	Mortality Rate (%)	Growth Inhibition (%)	Feeding Reduction (%)	p-value
Wild-type control	Bt toxin	34.2 ± 2.9	18.7 ± 1.8	14.9 ± 1.5	—
ABC transporter-edited	Bt toxin	58.6 ± 3.7	41.3 ± 2.9	32.5 ± 2.3	<0.001
P450-edited	Bt toxin	61.4 ± 3.9	44.6 ± 3.1	35.8 ± 2.5	<0.001
Wild-type control	Chemical insecticide	29.5 ± 2.6	16.3 ± 1.6	12.8 ± 1.4	—
ABC transporter-edited	Chemical insecticide	53.9 ± 3.4	38.7 ± 2.6	29.1 ± 2.0	<0.001
P450-edited	Chemical insecticide	57.2 ± 3.6	42.4 ± 2.8	31.4 ± 2.1	<0.001

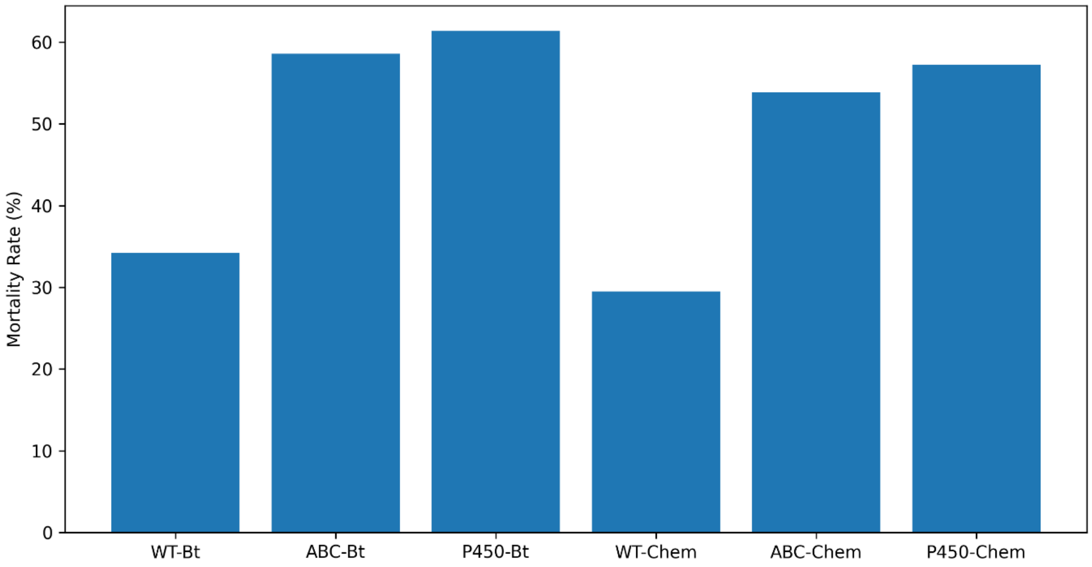

Figure 3. Comparative insecticide susceptibility between wild-type and CRISPR-edited larvae.

The results from our work confirm that CRISPR/Cas9 mediated genome modification resulted in observable molecular and phenotypic changes within insect species that are important to both agriculture and medicine. Results suggest that our genetically modified insects have many potential applications as tools for implementing genetically based pest control methods, although variability in editing efficacy and phenotype severity among species and gene targets demonstrates the need for continued exploration to identify species-specific genetic control structures using CRISPR technology.

Discussion

This research shows how well CRISPR-Cas9 genome editing can be used to genetically control harmful insects that are significant to medicine and agriculture; targeted disruption of genes associated with reproduction, host-seeking behaviour, susceptibility to viruses and resistance to pesticides, has led to considerable molecular and phenotypic changes in Anopheles gambiae, Aedes aegypti and Spodoptera frugiperda. This indicates further support for using technologies based on CRISPR as alternatives to traditional pest control methods in an environmentally sustainable way (Zhu et al., 2020; Li et al., 2021).

The genome editing efficiencies achieved, which ranged from 41.8% to 67.3%, were comparable to previous reports of CRISPR mediated editing efficiencies in agricultural pests and mosquitoes. In particular, the highest efficiency of 67.27% occurred with CRISPR editing of the doublesex gene in Anopheles gambiae, suggesting that targeting reproductive regulatory genes may be especially effective for suppressing mosquito populations (Scott et al., 2018). Additionally, hatchability and adult emergence rates were reduced compared to controls after microinjection of embryos, which supports the findings of similar insect genome editing studies and indicates that microinjected embryos are more sensitive to stress and developmental disruption due to microinjection (Harvey-Samuel et al., 2021).

The expression levels of target genes were determined through quantitative real-time PCR analysis with a substantial reduction in expression levels from the three edited groups. The most significant reductions were observed for Anopheles gambiae when targeting the doublesex gene, which has a major physiological role in egg development for female mosquitoes (Adelman & Tu, 2016). This decreases in the reproductive potential of Target Genes against mosquitoes, as evidenced by reduced fertility rates and the failure of eggs to hatch, suggests that the doublesex gene is critical for reproductive success in female mosquitoes. Thus, if incorporated into future strategies, these reproductive suppression methodologies may significantly impact the overall population density of mosquitoes, leading to decreased transmission of malaria in endemic regions (Bier, 2022; Champer et al., 2020).

The orco gene in Aedes aegypti is critical for the ability of mosquitoes to find hosts and to feed on blood. When the orco gene was disrupted, mosquitoes exhibited less host-seeking behavior and fed on blood less frequently. Since olfactory signaling is facilitated by the orco gene and is necessary for a mosquito to recognize a host, the disruption of the orco gene likely impaired a mosquito's ability to detect the host-associated chemical attractants (Noble et al., 2019; Burt & Crisanti, 2018). Therefore, less frequent mosquito-host interactions may be an important influence in reducing the transmission of arboviral diseases like dengue fever, Zika virus, and chikungunya. These results support the idea proposed by earlier research that modifying vector behavior is an important goal for vector control initiatives (James et al., 2018).

A further important outcome was increased susceptibility to insecticides of CRISPR-Edited Spodoptera frugiperda. Knockouts of genes that encode for ATP-binding cassette transporters and cytochrome P450 monooxygenases produced substantially higher levels of larval mortality after exposure to Bacillus thuringiensis or previously common inorganic insecticides (Rode et al., 2019). These types of genes have been experimentally confirmed to play a major role in providing detoxifying activities or developing insecticide resistant mechanisms in agricultural pests. Therefore, disrupting the genes associated with insecticide resistance may be a viable method to restore susceptibilities in currently resistant insect populations, ultimately increasing the effectiveness of current insecticides (Windbichler et al., 2011).

The current study shows the potential of CRISPR-Cas9 technology for the species-based genetic control of harmful insects. The ability to specifically target genes that affect reproduction, behavior, and insecticide resistance may enable the development of strong alternatives to traditional chemical insecticides, and create new opportunities for sustainable management of pests and vectors in the future.

4. Conclusion

The results of the current research suggest that genome editing with CRISPR-Cas9 offers a unique and flexible method for achieving genetic control of injurious insects that cause problems to both humans and plants. When genes involved in reproduction, seeking hosts, susceptibility to viruses and resistance to chemical insecticides were targetted for disruption, there was a dramatic change in both molecular and phenotypic characteristics in Anopheles gambiae, Aedes aegypti, and Spodoptera frugiperda. The data collected from this work demonstrate the ability of CRISPR-mediated approaches to decrease reproductive capacity, affect vector-host interactions, and re-sensitize pest populations to insecticide treatments. The findings from this research confirm previous studies demonstrating how CRISPR technology can offer advantages compared with conventional approaches to insect management including: specificity for targets, lower toxicity to the environment, and long-term suppression of populations via heritable genetic changes. The targeting of both genes controlling the reproductive system and detoxifying enzymes may be good substitutes to chemical pesticide options as their performance is decreasing due to widespread resistance development. While these results indicate a potential future for genetic engineering, challenges remain in the areas of efficiency, off-targeting, ecological impact, and regulatory considerations for release of genetically engineered insect species into the environment. Before releasing genetically engineered insects into the environment on a large scale, future research should include multiple generations of studies, ecological risk assessments, and advanced biosafety strategies. CRISPR-Cas9 technology can also provide new sustainable, species-specific, and environmentally sound methods for controlling insect vectors and pests through genetic control methods.

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