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Molecular manipulation of insect genomes via CRISPR/CAS9 and their applications

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Abstract
CRISPR technology has revolutionized the field of genetic engineering and insect research, offering unprecedented insights into gene function, functional genomics, and pest control. In model organisms like *Drosophila melanogaster*, CRISPR knockouts have allowed for the study of specific gene functions, contributing to advances in insect toxicology and resistance mechanisms. Gene drives have emerged as a potent tool for pest management, enabling the rapid spread of traits such as reduced fertility or pesticide susceptibility through wild populations. This has potential applications for controlling disease vectors like *Aedes aegypti* mosquitoes, which transmit dengue and Zika, and *Anopheles* mosquitoes responsible for malaria. CRISPR is also facilitating the development of biopesticides and overcoming insecticide resistance in agricultural pests. Beyond pest control, the technology holds promise for conservation, industrial biotechnology, and entomophagy. Insects are being engineered as biofactories for pharmaceutical production and optimized as sustainable food sources. However, concerns about off-target effects, ecological risks, and public acceptance pose significant challenges. Advances in CRISPR precision, alongside ethical and regulatory frameworks, are essential for the responsible deployment of this transformative technology in environmental and agricultural applications.

Keywords: Biopesticide, *Drosophila*, gene drive, malaria, vector

Introduction

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR associated protein 9), a powerful gene-editing tool, is revolutionizing biomedical research. It allows for the quick, cost-effective, and relatively straightforward correction of genetic errors, as well as the ability to activate or deactivate genes in cells and organisms (Yin *et al*., 2014) [62]. This technology has numerous laboratory applications, including the rapid creation of cellular and animal models, functional genomic screening, and real-time imaging of the genome within cells (Asokan *et al*., 2022) [1]. The CRISPR-Cas9 system, adapted from the bacterial immune response, has emerged as a powerful tool for gene editing in a variety of organisms, including insects. First demonstrated in 2012, CRISPR-Cas9 enables targeted modifications with high precision by using a guide RNA to direct the Cas9 enzyme to specific locations in the genome, allowing for the deletion, insertion, or replacement of DNA sequences (Jinek *et al*., 2012). CRISPR/Cas9 gene-editing tool that consists of two key components: a guide RNA, which directs the system to the specific target gene, and Cas9, an enzyme that creates a double-stranded break in the DNA, enabling genome modifications.

Insect pests are a significant constraint on crop production, causing farmers worldwide to lose billions of dollars annually. While synthetic chemicals remain the primary method of pest control, they pose serious environmental and health risks. To lessen reliance on these chemicals, scientists are continuously exploring new, more sustainable strategies for managing pests. Recent advancements in genomics, molecular biology, and bioinformatics have empowered researchers to develop innovative tools, such as genome editing and gene drive technologies, for more effective and eco-friendly insect pest management.

The adoption of genome editing (GE) systems has yielded significant advancements in crop genetic improvement. Genetic engineering revolutionized biological research by introducing *in vivo* genome editing techniques. These methods result in base substitutions or insertions/deletions (indels) in the target DNA. Several GE approaches, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the more recently developed CRISPR/Cas9 system, have been employed (Puchta *et al*., 1993; Upadhyay, 2021) [48, 59]. Unlike TALENs and ZFNs, the CRISPR/Cas9 system is simpler and more efficient, as it requires only a single guide RNA (gRNA) to direct the Cas9 nuclease to the target. Recently, there has been a shift from breeding insect-resistant crop varieties to using CRISPR/Cas9-mediated modifications for improving agronomic traits or targeted mutagenesis in insect genomes (Cong *et al*., 2013) [13].

Its simplicity and cost-effectiveness have made CRISPR the tool of choice for genetic manipulation in entomology, supplanting older techniques such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (Cong *et al*., 2013) [13]. This review explores how CRISPR is being used in insect research, pest management, vector control, and biotechnology, as well as the challenges associated with its application.

CRISPR applications in basic insect research

1. Gene function studies

CRISPR has significantly advanced our understanding of gene function in model organisms like *Drosophila melanogaster*, commonly used in genetic research including human development and disease. By creating knockouts of specific genes, researchers can study the resulting phenotypes to deduce gene function (Bassett & Liu, 2014) [2]. Given its importance, before being used in other

organisms, the CRISPR/Cas technique was initially tested in these insects. In May 2013, two CRISPR/Cas knockout targets were chosen for the yellow gene, and embryos for both targets were injected simultaneously with two plasmids (phsp-Cas9, pU6-Bbsl-chiRNA) (Gratz *et al*., 2013a). Insertion/deletion sequences between the targets were identified through chimeric genome detection in the G^0 generation. Later, in July 2013, the CRISPR/Cas9 system was confirmed to induce homologous recombination (HR) and create precise mutations in the *D. melanogaster* S2 cell line (Bassett *et al*., 2013) [3]. The study also introduced a strategy that fused crRNA and tracrRNA to create a synthetic guide RNA (sgRNA) linked with a T7 promoter and a guide sequence to target the yellow gene. By injecting sgRNA and Cas9 mRNA into embryos, up to 88% mutant chimera were obtained in the first generation. Additionally, a high-resolution melt analysis (HRMA) method was employed to detect target gene mutations quickly and accurately by comparing the melting temperature of wild-type homoduplexes to that of heteroduplexes containing insertions or deletions (Cui *et al*., 2017) [16].

Many other insects are vital to the environment and human welfare. For instance, mosquitoes transmit the malaria parasite, which in 2022 caused 249 million cases of illness and 608,000 deaths (*Malaria*, 2023). While some insects are not directly harmful to humans, they can damage crops and buildings. Honeybees and silkmoths, for example, hold significant economic value. Additionally, biological control agents, like parasitoid wasps, are essential for pest management.

Advances in genome engineering, such as the CRISPR system, facilitate the study of gene function across various insect species. One example is the water flea, *Daphnia magna*, where in May 2014, injecting Cas9 mRNA into its eggs targeting the eyeless gene resulted in 18–47% of live larvae showing eye abnormalities (Table.1) (Nakanishi *et al*., 2014) [42]. Moreover, 8.2% of the offspring displayed eye deformities, leading to eyeless gene mutations.

CRISPR has been widely used to investigate genes involved in insect development, immunity, and behavior, shedding light on fundamental biological processes.

Table 1: CRISPR/Cas9 gene drive applications in some insects

Targeted genes	Insect Species	Strategy / Therapy	Germline transmission rate %	Mutation rate %	References
yellow, white	*Drosophila sp.*	mRNA injection	0-79	0-34.5	Bassett *et al*. (2013b)
CG4221, CG5961, Chameau		mRNA injection with donor	8.1-26.7	2.7-10.4	Yu *et al*. (2014) [63]
yellow		DNA injection with donor	5.9-20.7	0.25-1.37	Gratz *et al*. (2013) [27]
EGFP, mRFP		Rapid injection	35-71	7.7-24.7	Sebo *et al*. (2014) [54]
yellow		Rapid injection with donor	8-53	15	Gratz *et al*. (2013) [27]
white, neuropeptide gene		Transgenesis	0-100	0-99.4	Beumer *et al*. (2008) [4]
ebony, curled, yellow		Transgenesis with donor	25-100	11.38	Port *et al*. (2014) [47]
eyeless	*Daphnia magna*	mRNA injection	18-47	8.2	Nakanishi *et al*. (2014) [42] Colbourne *et al*. (2011) [11]
ECFP	*Aedes aegypti*	mRNA injection+ DNA injection	0	5.5	S. Dong *et al*. (2015) [17]
BmBLOS2	*Bombyx mori*	mRNA injection	95.5	35.6	Wang *et al*. (2013) [61]
th, re,fl, yellow-e, kynu, ebony		DNA injection	5.7–18.9	Not determined	Liu YuanYuan *et al*. (2014) [39]

2. Functional genomics

CRISPR is also revolutionizing functional genomics in insects. High-throughput CRISPR screens have been employed to identify genes associated with specific traits, such as resistance to insecticides (Scott & Buchon, 2019) [53]. Ongoing research on the genetic mechanisms behind insecticide resistance has greatly benefited from studies on model species like *Drosophila melanogaster*, which offers a wealth of genetic and genomic resources. The significance of *Drosophila* in insect toxicology has been highlighted in recent comprehensive reviews (Homem & Davies, 2018) [32]. This model system offers numerous advantages, including its remarkable versatility and the ability to conduct cost-effective and reliable toxicity bioassays within a defined genetic background. In recent years, pesticide resistance research has been further advanced by the introduction of genome modification technologies, particularly CRISPR/Cas9 (Scott & Buchon, 2019) [53]. These technologies have transformed various areas of resistance research, enabling the study of insecticide resistance mechanisms in a controlled genetic context and providing a robust framework for exploring the genetic basis of resistance. This has profound implications for understanding the genetic basis of insect adaptations, particularly in pest species that develop resistance to chemical controls.

Pest control and agricultural applications

1. Gene Drives for Pest Management

One of the most promising applications of CRISPR is the development of gene drives, which can propagate genetic modifications or DNA cassette throughout wild populations (Courtier-Orgogozo *et al*., 2017) [15]. This cassette consists of three components: a gene encoding the Cas9 protein from bacteria, a gene that produces guide RNA targeting a specific genome site, and flanking sequences that facilitate insertion at the desired target location (Gantz *et al*., 2015a; Hammond *et al*., 2016a). The system can "copy and paste" itself into the genome, allowing it to spread through the population. Unlike a normal allele, which has a 50% chance of being inherited by offspring, a gene drive cassette has over a 90% chance due to its self-replicating nature. This mechanism overrides the normal processes of evolution, manipulating both heredity and mutations (Gantz & Bier, 2015; Hammond *et al*., 2016) [23, 30]. It ensures the cassette is passed down to the next generation and induces mutations precisely where the genome has been cut, resulting in the intended DNA sequence. Theoretically, introducing just a few individuals carrying the gene drive could lead to its widespread presence throughout the population within 15–20 generations (Burt, 2003a).

This technology has been employed to engineer insect pests such as *Aedes aegypti*, the mosquito responsible for transmitting diseases like dengue and Zika. By introducing a gene drive that reduces female fertility, researchers aim to suppress mosquito populations and thereby reduce disease transmission (Kyrou *et al*., 2018) [36].

Gene drives have also been proposed for agricultural pests, such as *Drosophila suzukii*, a major threat to fruit crops. The CRISPR system can modify the endogenous genes of the water flea, *Daphnia magna* Straus. In May 2014, researchers discovered that when Cas9 mRNA was injected into water flea eggs, targeting the eyeless gene, 18–47% of the live larvae exhibited eye abnormalities (Table 1). Additionally, 8.2% of the offspring displayed eye deformities, indicating mutations in the eyeless gene (Nakanishi *et al*., 2014) [42]. The gene drive ensures that the modified trait (e.g., sterility or susceptibility to pesticides) is inherited by nearly all offspring, allowing for rapid spread through pest populations (Burt, 2003b). However, the ecological risks of releasing gene drives, particularly the potential for unintended effects on non-target species, remain a significant concern (Oye *et al*., 2014) [44].

2. Biopesticides and insecticide resistance

CRISPR is also being used to combat insecticide resistance. By targeting genes associated with resistance in pests like *Helicoverpa armigera* (cotton bollworm), researchers can disrupt these genes, rendering the insects susceptible to previously ineffective insecticides (Halder *et al*., 2022) [29]. CRISPR/Cas9-mediated gene knockouts in the diamondback moth (*Plutella xylostella*) have shown significant resistance to the Bt Cry1Ac toxin, offering *in vivo* evidence for the involvement of these genes in Bt toxin resistance (Gao, 2024; Guo *et al*., 2019) [25, 28] (Fig.1). The brown planthopper (*Nilaparvata lugens*) is one of the most destructive insect pests affecting rice crops. Li *et al*. successfully induced RNA interference (RNAi) by targeting the vacuolar ATP synthase subunit E of *Nilaparvata lugens* through the ingestion of double-stranded RNA (dsRNA). Similarly, in citrus and grapevine trees, *in vitro* transcribed dsRNA was effectively applied by injecting trunks or drenching roots, targeting the arginine kinase of two psyllid species (Hunter *et al*., 2012) [33]. In a separate study, Bolognesi *et al*. first described the mechanism of action of dsRNA aimed at the larvae of the western corn rootworm. The cotton aphid (*Aphis gossypii*), a deadly pest to many key agricultural crops, also contributes to virus transmission and is capable of developing resistance to a wide range of insecticides. However, this resistance to organophosphorus insecticides was significantly reduced by orally administering dsRNA targeting the carboxylesterase gene (Gong *et al*., 2014) [26]. Additionally, transplastomic potato plants (*Solanum tuberosum*) were engineered to express dsRNAs targeting the β-actin gene of the Colorado potato beetle, resulting in strong resistance to this damaging pest (Halder *et al*., 2022) [29].

In addition, genetically modified biopesticides, such as CRISPR-edited entomopathogenic fungi and bacteria, are being developed to provide more sustainable and targeted pest control options (Fisher, 2013) [20].

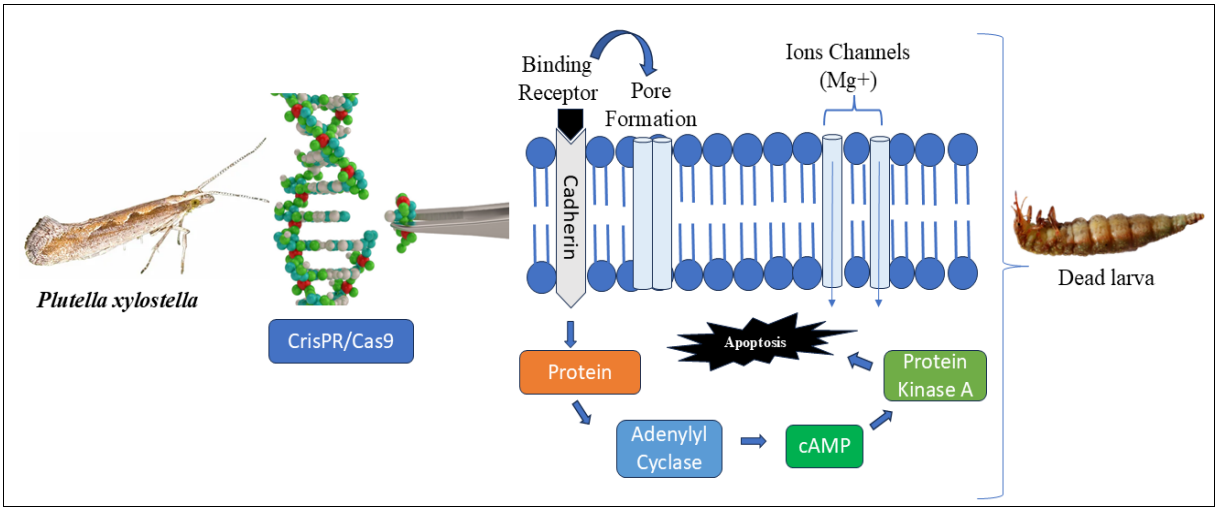

Fig 1: Genes encoding cadherin-like proteins act as receptors for Bt toxins in the insect gut, are crucial Cry1Ac toxin activity in diamondback moth *(Plutella xylostella)*

Vector control and disease prevention

1. Malaria and mosquito control

Vector control plays a crucial role in reducing vector-borne diseases (Bhatt *et al*., 2015) [6]. Once mosquitoes were identified as vectors for pathogens causing diseases like malaria and yellow fever, significant efforts were made to eliminate them from regions where these diseases were endemic (Reed, 1901) [50]. Advances in CRISPR technology and genetic engineering have led to successful campaigns targeting the eradication of *Anopheles* and *Aedes* mosquito species in countries such as Cuba, Panama, and Brazil, eventually expanding across most of the Americas (Kouri *et al*., 1986; Soper, 1963) [35, 56]. The discovery of DDT (dichloro-diphenyl-trichlorethane) in 1939 as a long-lasting insecticide offered the potential for sustainable mosquito control and spurred a new goal: the global eradication of malaria. However, within a decade of its use, resistance to DDT emerged, along with public concerns about its environmental and health impacts.(NIOSH Special Occupational Hazard Review: DDT (78-200) | NIOSH | CDC, 1978) [43]

CRISPR has transformed efforts to control vector-borne diseases like malaria. Mosquitoes from the Anopheles genus, which transmit the Plasmodium parasite, have been a primary focus for CRISPR gene drives aimed at reducing their population or making them resistant to the parasite

(Thomas *et al*., 2000) [58]. In one study, a CRISPR gene drive was used to insert a gene that blocks the parasite from developing within the mosquito, significantly reducing transmission potential (Gantz & Bier, 2015) [23].

The bacterial endosymbiont *Wolbachia pipentis* was first identified in 1967 as the cause of reproductive phenotypes that influence inheritance patterns in crosses between infected and uninfected mosquitoes (Stouthamer *et al*., 1999) [57]. These reproductive effects allow the bacteria to spread throughout insect populations. *Wolbachia* has been proposed as a potential driver for synthetic gene constructs, but it has not yet been successfully transformed (Collins & Paskewitz, 1995; Sinkins & Gould, 2006) [12, 55]. Nevertheless, Wolbachia's reproductive traits have proven useful in strategies to combat mosquito-borne diseases, facilitating both population suppression and modification. The wPip strain of Wolbachia, isolated from *Culex pipiens* Linnaeus, has been used to infect Aedes albopictus. Infected males are now part of a strategy similar to the Sterile Insect Technique (SIT), as wPip-infected males mate with and sterilize wild females, which are naturally infected with different *Wolbachia* strains. *Wolbachia's* potential to drive population change, combined with the discovery that the wMel strain can block dengue virus development in *Aedes aegypti*, has led to a population modification strategy that pairs pathogen-blocking with gene drive mechanisms (Hoffmann *et al*., 2011) [31]. Releases of *Wolbachia*-infected mosquitoes in countries like Australia, Vietnam, Colombia, and Brazil have been among the most successful examples of large-scale population modification in wild vector populations (Puggioli *et al*., 2016) [49].

Dong *et al*. developed an Anopheles gambiae mosquito with a knockout of the Plasmodium host factor gene FREP1 using CRISPR/Cas9 genome editing. FREP1 is crucial for the parasite's infection of the mosquito midgut (Ross, 1897). Prior studies using RNAi-mediated gene silencing and FREP1-inhibiting polyclonal antibodies had demonstrated FREP1's role as a host factor for *Plasmodium falciparum*, *P. vivax*, and *P. berghei*. However, RNAi silencing only led to a moderate reduction in *P. falciparum* infection, with a 50% decrease in infection intensity and an 11% reduction in prevalence at high infection levels (median oocyst counts of 50 and 20 in two replicates) (Zhang *et al*., 2015) [64]. In contrast, the complete inactivation of FREP1 via gene editing resulted in a much stronger suppression of *Plasmodium*, likely because RNAi-based methods only partially deplete the protein. The resistance level of FREP1 knockout mosquitoes to P. falciparum was comparable to that achieved by transgenically over-expressing the anti-Plasmodium IMD pathway transcription factor Rel2 after a blood meal or by activating the insulin pathway (Corby-Harris *et al*., 2010; Y. Dong *et al*., 2018) [14, 18]. The suppression of parasites at the sporozoite stage in FREP1 knockout mosquitoes suggests that replacing wild-type mosquitoes with these genetically modified ones in malaria-endemic regions could have a significant epidemiological impact (Bhatt *et al*., 2015) [6].

In the study by Hammond *et al*., a CRISPR/Cas9 gene drive system was utilized in Anopheles gambiae to target fertility-related genes. The drive was designed by inserting a CRISPR homing construct into a docking site via recombinase-mediated cassette exchange (RMCE). The gene drive components included a fluorescent marker, a Cas9 nuclease controlled by the vasa2 promoter, and a guide RNA (gRNA) driven by the ubiquitous U6 PolIII promoter. The transmission rate of the gene drive to progeny was estimated to exceed 91%.

In a separate experiment, Gantz *et al*. applied the CRISPR/Cas9 system to target the kynurenine hydroxylase (khw) gene locus, which is associated with visible eye phenotypes in *Anopheles stephensi*. The gene drive cassette was successfully transmitted to approximately 99.5% of the progeny after crossing transgenic lines with wild-type mosquitoes. Although the study showed successful results in male-derived transgenic lineages, a high frequency of mutations at the cleavage site of the target gene was observed, likely due to non-homologous end joining (NHEJ) repair in some lineages and progeny (Gantz *et al*., 2015b). In this experiment, wild-type mosquito embryos were injected with Cas9 protein and a gene drive plasmid construct that included: Cas9 DNA under the vasa promoter, a gRNA under the U6A promoter targeting the khw locus, a fluorescent marker, two genes encoding single-chain antibodies against *Plasmodium falciparum* Chitinase 1 and CSP, and ~1 kb homology arms of the khw locus. Additionally, double-stranded RNAs (dsRNAs) were co-injected to suppress the expression of the plasmid-carried Cas9 gene and inhibit NHEJ pathway activity (Gantz *et al*., 2015) [23].

2. Control of other vector-borne diseases

In addition to malaria, CRISPR is being explored as a tool to control *Aedes* mosquitoes, which are vectors for diseases such as dengue and Zika. By engineering mosquitoes to be resistant to these viruses or by introducing gene drives that reduce reproductive success, researchers aim to decrease the incidence of these diseases in affected regions (Carvalho *et al*., 2015) [9].

Aedes aegypti is known to transmit dengue virus, yellow fever virus, chikungunya virus, and Zika virus (Reed, 1901) [50]. Genetic engineering of synthetic phenotypes has led to the creation of population-suppressing mosquito strains, with the most successful to date being the "flightless female" *Aedes aegypti*. This strain carries a genetic element that produces a toxin that destroys the wing muscles of females (Fu *et al*., 2010) [21]. Without functional wing muscles, the females cannot mate, search for food, or locate oviposition sites. The transgene responsible for this phenotype is a repressible, late-acting, sex-specific lethal, allowing for normal rearing and sexing before release, while ensuring larvae survive through subadult stages to compete with wild larvae (Phuc *et al*., 2007) [46]. The gene construct is spread into the population by unaffected male carriers, which are being released as part of control efforts in Brazil and Florida following successful large-cage trials (Carvalho *et al*., 2015) [9]. Similar strains have been developed in *Aedes albopictus* and *Anopheles stephensi*, though they have not yet been employed for wild population control (Labbé *et al*., 2012) [37]. These strains provide a population suppression method in species where traditional SIT or SIT-like strategies have not been successful.

However, the release of genetically modified mosquitoes into the environment poses ethical and ecological challenges. There is a risk of the gene drive spreading beyond the intended target population, potentially disrupting ecosystems (Sciences *et al*., 2016). Moreover, public acceptance and regulatory approval of these technologies vary globally, complicating the implementation of CRISPR-based vector control programs.

Conservation and environmental protection

CRISPR has potential applications in conservation biology, particularly in the management of endangered insect species. By increasing genetic diversity or enhancing resistance to environmental stressors, CRISPR could play a role in preserving threatened populations. Additionally, gene drives could be employed to control invasive insect species that threaten ecosystems, such as the Asian longhorn beetle (*Anoplophora glabripennis*), which has devastated forests in North America (Esvelt *et al*., 2014) [19].

However, the use of gene drives in conservation must be approached with caution. Unintended ecological consequences and the risk of spreading to non-target species could disrupt natural ecosystems (Oye *et al*., 2014b). Regulatory frameworks and ecological risk assessments are crucial before implementing these technologies in the wild.

Insect biotechnology and industrial applications

1. Insects as biofactories

Insects are increasingly being used as biofactories for producing pharmaceuticals, biofuels, and other valuable compounds. CRISPR allows for the precise modification of insect genomes to optimize the production of these substances. For example, CRISPR has been used to enhance the silk production of silkworms, as well as to engineer insects that can produce valuable proteins and enzymes (Khan *et al*., 2020) [34]. In Bombyx mori, the silkworm, CRISPR/Cas9 has been employed to enhance silk production and to engineer the organism to produce recombinant proteins like human collagen (Fig.2). The Fib-H gene, which encodes the heavy chain of fibroin, has been modified to increase silk yield and introduce additional proteins into the silk (Park & Maenaka, 2019) [45].

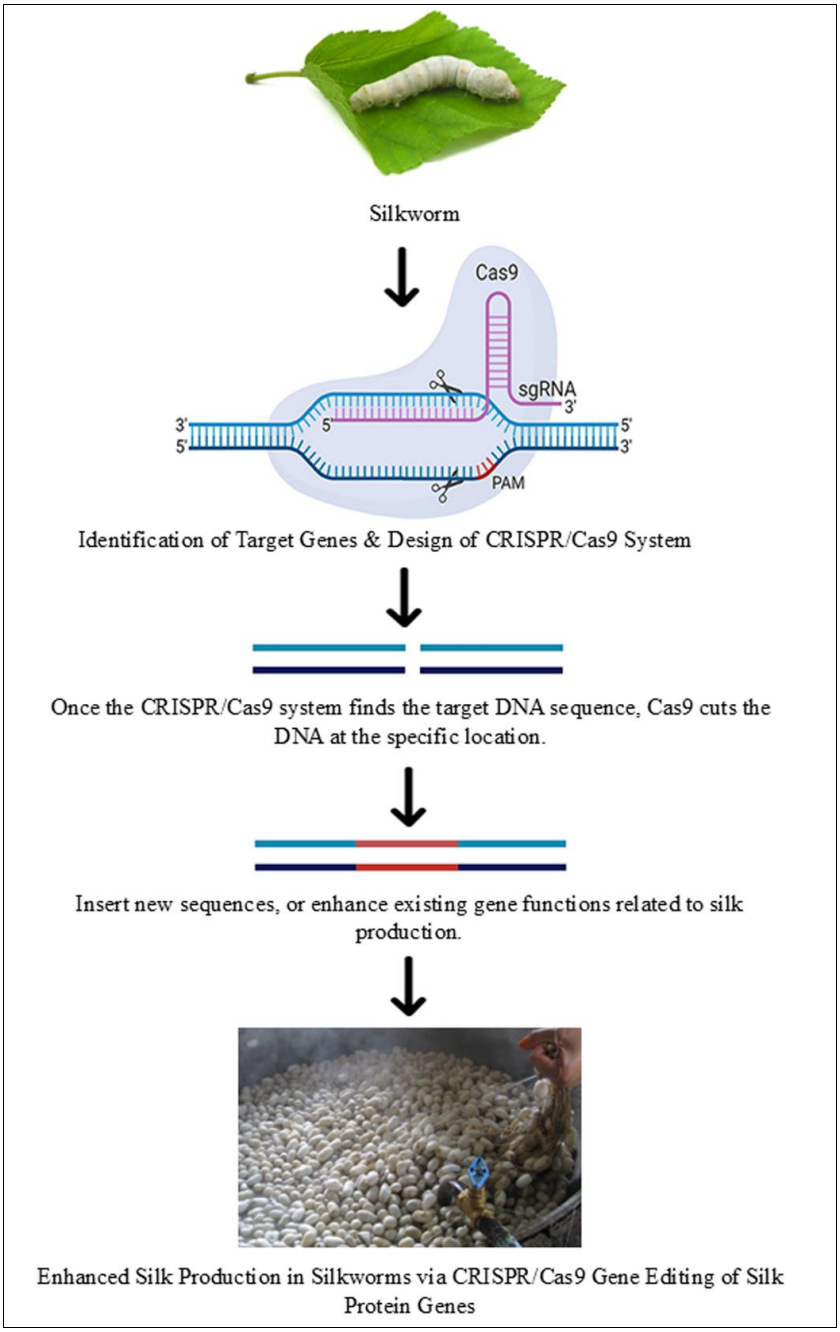

Fig 2: Showing the use of CRISPR-Cas9 gene editing to enhance silk production in silkworms. The process involves targeting and modifying specific genes responsible for silk synthesis, leading to increased silk yield and improved quality

Genes related to metabolic pathways, protein folding, and secretion mechanisms that can be optimized to enhance the production of recombinant proteins. One such gene is *vasa*, a germline-specific gene that has been targeted in *Drosophila* to improve the yield of specific proteins by optimizing the reproductive capacity of the insects (Beumer *et al*., 2013; Chen *et al*., 2007) [5, 10].

2. Enhancing entomophagy

CRISPR can also improve the sustainability of entomophagy, or the consumption of insects as a protein source. By modifying insects like crickets and mealworms to grow faster or have enhanced nutritional profiles, researchers hope to promote insect farming as a sustainable alternative to traditional livestock (Van Huis, 2013) [60].

Challenges and future directions

One of the primary concerns with CRISPR is the potential for off-target effects, where unintended regions of the genome are edited. Advances in CRISPR technology, including the development of more precise variants like CRISPR-Cas12 and base editing techniques, aim to reduce these risks (Gajardo *et al*., 2023; Malzahn *et al*., 2019) [22, 41]. However, further research is needed to ensure the safety and reliability of these methods in wild populations.

The release of genetically modified insects into the wild raises significant ethical and regulatory challenges. International collaboration is required to establish guidelines for the use of CRISPR in pest control and conservation. Public engagement and transparency will be essential for gaining societal acceptance of these technologies.

Conclusion

CRISPR has opened new frontiers in entomology, offering powerful tools for basic research, pest management, vector control, and biotechnology. While the potential benefits are immense, particularly in reducing the spread of diseases and improving agricultural sustainability, the ecological and ethical implications of CRISPR must be carefully considered. As research continues to advance, responsible development and regulation will be key to ensuring that CRISPR's applications in entomology are both safe and effective.

Author contribution statement

AR and PB: Conceptualization, Investigation, Formal analysis, Methodology design, Writing- original draft. BB, PB, SH and AR: Formal analysis, Data curation, Illustration design, Investigation, Visualization, Supervision, Validation Writing-review and editing.

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References

1. Asokan R, Rai A, Dash S, Manamohan M, Ashok K, Bhargava CN, *et al*. Application of genome editing in entomology. Indian J Entomol, 2022, 96-103.
2. Bassett AR, Liu JL. CRISPR/Cas9 and genome editing in Drosophila. J Genet Genomics,2014:41(1):7–19.
3. Bassett AR, Tibbit C, Ponting CP, Liu JL. Highly efficient targeted mutagenesis of Drosophila with the CRISPR/Cas9 system. Cell Rep,2013:4(1):220–228.
4. Beumer KJ, Trautman JK, Bozas A, Liu JL, Rutter J, Gall JG, *et al*. Efficient gene targeting in Drosophila by direct embryo injection with zinc-finger nucleases. Proc Natl Acad Sci U S A,2008:105(50):19821–19826.
5. Beumer KJ, Trautman JK, Christian M, Dahlem TJ, Lake CM, Hawley RS, *et al*. Comparing zinc finger nucleases and transcription activator-like effector nucleases for gene targeting in Drosophila. G3 (Bethesda),2013:3(10):1717–1725.
6. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, *et al*. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature,2015:526(7572):207–211.
7. Bolognesi R, Ramaseshadri P, Anderson J, Bachman P, Clinton W, Flannagan R, *et al*. Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (Diabrotica virgifera virgifera LeConte), 2012.
8. Burt A. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. Proc R Soc Lond B Biol Sci,2003:270(1518):921–928.
9. Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, *et al*. Suppression of a field population of Aedes aegypti in Brazil by sustained release of transgenic male mosquitoes. PLoS Negl Trop Dis, 2015, 9(7).
10. Chen CH, Huang H, Ward CM, Su JT, Schaeffer LV, Guo M, *et al*. A synthetic maternal-effect selfish genetic element drives population replacement in Drosophila. Science,2007:316(5824):597–600.
11. Colbourne JK, Pfrender ME, Gilbert D, Thomas WK, Tucker A, Oakley TH, *et al*. The ecoresponsive genome of Daphnia pulex. Science,2011:331(6017):555–561.
12. Collins FH, Paskewitz SM. Malaria: current and future prospects for control. Annu Rev Entomol,1995:40:195–219.
13. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, *et al*. Multiplex genome engineering using CRISPR/Cas systems. Science,2013:339(6121):819–823.
14. Corby-Harris V, Drexler A, Watkins de Jong L, Antonova Y, Pakpour N, Ziegler R, *et al*. Activation of Akt signaling reduces the prevalence and intensity of malaria parasite infection and lifespan in Anopheles stephensi mosquitoes. PLoS Pathog, 2010, 6(7).
15. Courtier-Orgogozo V, Morizot B, Boëte C. Agricultural pest control with CRISPR-based gene drive: time for public debate. EMBO Rep,2017:18(6):878–880.
16. Cui Y, Sun J, Yu L. Application of the CRISPR gene-editing technique in insect functional genome studies—a review. Entomol Exp Appl,2017:162(2):124–132.
17. Dong S, Lin J, Held NL, Clem RJ, Passarelli AL, Franz AWE. Heritable CRISPR/Cas9-mediated genome editing in the yellow fever mosquito, Aedes aegypti. PLoS One, 2015, 10(3).
18. Dong Y, Simões ML, Marois E, Dimopoulos G. CRISPR/Cas9-mediated gene knockout of Anopheles gambiae FREP1 suppresses malaria parasite infection. PLoS Pathog, 2018, 14(3).
19. Esvelt KM, Smidler AL, Catteruccia F, Church GM. Concerning RNA-guided gene drives for the alteration of wild populations. Elife, 2014, 3.

20. Fisher MX. Emerging fungal threats to animal, plant and ecosystem health. Mycoses,2013:56:13.
21. Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P, *et al*. Female-specific flightless phenotype for mosquito control. Proc Natl Acad Sci U S A,2010:107(10):4550–4554.
22. Gajardo HA, Gómez-Espinoza O, Boscariol Ferreira P, Carrer H, Bravo LA. The potential of CRISPR/Cas technology to enhance crop performance on adverse soil conditions. Plants,2023:12(9):1892.
23. Gantz VM, Bier E. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. Science,2015:348(6233):442–444.
24. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas AM, Macias VM, Bier E, *et al*. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc Natl Acad Sci U S A,2015:112(49):E6736– E6743.
25. Gao F. Sustainable production and application of Bt biopesticides. Bt Research, 2024, 15.
26. Gong YH, Yu XR, Shang QL, Shi X, Gao XW. Oral delivery mediated RNA interference of a carboxylesterase gene results in reduced resistance to organophosphorus insecticides in the cotton aphid, Aphis gossypii Glover. PLoS One, 2014, 9(8).
27. Gratz SJ, Cummings AM, Nguyen JN, Hamm DC, Donohue LK, Harrison MM, *et al*. Genome engineering of Drosophila with the CRISPR RNA-guided Cas9 nuclease. Genetics,2013:194(4):1029–1035.
28. Guo Z, Sun D, Kang S, Zhou J, Gong L, Qin J, *et al*. CRISPR/Cas9-mediated knockout of both the PxABCC2 and PxABCC3 genes confers high-level resistance to Bacillus thuringiensis Cry1Ac toxin in the diamondback moth, Plutella xylostella (L.). Insect Biochem Mol Biol,2019:107:31–38.
29. Halder K, Chaudhuri A, Abdin MZ, Majee M, Datta A. RNA interference for improving disease resistance in plants and its relevance in this clustered regularly interspaced short palindromic repeats-dominated era in terms of dsRNA-based biopesticides. Front Plant Sci,2022:13:885128.
30. Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, *et al*. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol,2016:34(1):78–83.
31. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, *et al*. Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. Nature,2011:476(7361):454–457.
32. Homem RA, Davies TGE. An overview of functional genomic tools in deciphering insecticide resistance. Curr Opin Insect Sci,2018:27:103–10.
33. Hunter WB, Glick E, Paldi N, Bextine BR. Advances in RNA interference: dsRNA treatment in trees and grapevines for insect pest suppression. Southwest Entomol,2012:37(1):85–87.
34. Khan AH, Tye GJ, Noordin R. CRISPR-Cas9 genome editing tool for the production of industrial biopharmaceuticals. Mol Biotechnol,2020:62:401–411.
35. Kouri GP, Guzmán MG, Bravo JR. Hemorrhagic dengue in Cuba: history of an epidemic. Bull Pan Am Health Organ, 1986, 20(1).
36. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, *et al*. A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nat Biotechnol,2018:36(11):1062–1066.
37. Labbé GMC, Scaife S, Morgan SA, Curtis ZH, Alphey L. Female-specific flightless (fsRIDL) phenotype for control of Aedes albopictus. PLoS Negl Trop Dis, 2012, 6(7).
38. Li J, Chen Q, Lin Y, Jiang T, Wu G, Hua H. RNA interference in Nilaparvata lugens (Homoptera: Delphacidae) based on dsRNA ingestion. Pest Manag Sci,2011:67(7):852–859.
39. Liu YY, Ma SY, Wang XG, Chang JS, Gao J, Shi R, *et al*. Highly efficient multiplex targeted mutagenesis and genomic structure variation in Bombyx mori cells using CRISPR/Cas9.
40. World Health Organization. Malaria, 2023. Available from: https://www.who.int/en/news-room/fact-sheets/detail/malaria
41. Malzahn AA, Tang X, Lee K, Ren Q, Sretenovic S, Zhang Y, *et al*. Application of CRISPR-Cas12a temperature sensitivity for improved genome editing in rice, maize, and Arabidopsis. BMC Biol,2019:17:14.
42. Nakanishi T, Kato Y, Matsuura T, Watanabe H. CRISPR/Cas-mediated targeted mutagenesis in Daphnia magna. PLoS One, 2014, 9(5).
43. NIOSH Special Occupational Hazard Review: DDT (78-200). NIOSH, CDC, 1978. Available from: https://www.cdc.gov/niosh/docs/78-200/default.html
44. Oye KA, Esvelt K, Appleton E, Catteruccia F, Church G, Kuiken T, *et al*. Regulating gene drives. Science,2014:345(6197):626-628.
45. Park EY, Maenaka K. Silkworm Biofactory, 2019.
46. Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, *et al*. Late-acting dominant lethal genetic systems and mosquito control. BMC Biol,2007:5:11.
47. Port F, Chen HM, Lee T, Bullock SL. Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in Drosophila. Proc Natl Acad Sci U S A, 2014, 111(29).
48. Puchta H, Dujon B, Hohn B. Homologous recombination in plant cells is enhanced by *in vivo* induction of double strand breaks into DNA by a site-specific endonuclease. Nucleic Acids Res,1993:21(22):5034-5040.
49. Puggioli A, Calvitti M, Moretti R, Bellini R. wPip Wolbachia contribution to Aedes albopictus SIT performance: advantages under intensive rearing. Acta Trop,2016:164:473-481.
50. Reed C. Agramonte: The Etiology of Yellow Fever. An additional Note. J Am Med Assoc,1901:37:484-486.
51. Ross R. On some peculiar pigmented cells found in two mosquitos fed on malarial blood. Br Med J,1897:2(1929):1786.
52. National Academies of Sciences, Engineering, and Medicine. Gene drives on the horizon: advancing science, navigating uncertainty, and aligning research with public values, 2016.
53. Scott JG, Buchon N. Drosophila melanogaster as a powerful tool for studying insect toxicology. Pestic Biochem Physiol,2019:161:95-103.

54. Sebo ZL, Lee HB, Peng Y, Guo Y. A simplified and efficient germline-specific CRISPR/Cas9 system for Drosophila genomic engineering. Fly,2014:8(1):52-57.
55. Sinkins SP, Gould F. Gene drive systems for insect disease vectors. Nat Rev Genet,2006:7(6):427-435.
56. Soper FL. The elimination of urban yellow fever in the Americas through the eradication of Aedes aegypti. Am J Public Health Nations Health,1963:53(1):7-16.
57. Stouthamer R, Breeuwer JA, Hurst GD. Wolbachia pipientis: microbial manipulator of arthropod reproduction. Annu Rev Microbiol,1999:53:71-102.
58. Thomas DD, Donnelly CA, Wood RJ, Alphey LS. Insect population control using a dominant, repressible, lethal genetic system. Science,2000:287(5462):2474-2476.
59. Upadhyay SK. Genome engineering for crop improvement. Wiley Online Library, 2021.
60. Van Huis A. Potential of insects as food and feed in assuring food security. Annu Rev Entomol,2013:58(1):563-583.
61. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, *et al*. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell,2013:153(4):910-918.
62. Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, *et al*. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol,2014:32(6):551-553.
63. Yu Z, Chen H, Liu J, Zhang H, Yan Y, Zhu N, *et al*. Various applications of TALEN-and CRISPR/Cas9-mediated homologous recombination to modify the Drosophila genome. Biol Open,2014:3(4):271-280.
64. Zhang G, Niu G, Franca CM, Dong Y, Wang X, Butler NS, *et al*. Anopheles midgut FREP1 mediates Plasmodium invasion. J Biol Chem,2015:290(27):16490-164501.