Journal of Mosquito Research 2023, Vol.13, No.1, 1-10
http://emtoscipublisher.com/index.php/jmr

Review and Progress Open Access

Application of CRISPR/Cas9 in Gene Editing of *Aedes aegypti* Mosquitoes: Methods and Challenges

Zhou Yulin
Institute of Life Science, Jiyang College of Zhejiang A&F University, Zhuji, 311800, China
Corresponding author email: 177242186@qq.com
Journal of Mosquito Research, 2023, Vol.14, No.1 doi: 10.5376/jmr.2023.13.0001
Received: 25 Oct., 2023
Accepted: 01 Nov., 2023
Published: 08 Nov., 2023

Preferred citation for this article:
Zhou Y.L., 2023, Application of CRISPR/Cas9 in gene editing of *Aedes aegypti* mosquitoes: methods and challenges, Journal of Mosquito Research, 13(1): 1-10 (doi: 10.5376/jmr.2023.13.0001)

Abstract This review explores the application of CRISPR/Cas9 technology in gene editing in the *Aedes aegypti* mosquito, focusing on its methods and challenges. This review describes the importance of the *Aedes aegypti* mosquito, particularly its association with infectious diseases such as dengue fever, Zika virus and malaria, and explains in detail the basic principles of the CRISPR/Cas9 technology, how it compares with traditional gene editing methods and its potential in mosquito gene editing. This review highlights previous studies using CRISPR/Cas9 in the *Aedes aegypti* mosquito, emphasizing the potential applications and challenges of the technology. Ethical considerations, technical limitations and the impact of gene prevalence are also discussed in a comprehensive manner. This review provides recommendations to address ethical and ecological issues and emphasizes the exploration of future research and applications. This review provides strong support for the use of CRISPR/Cas9 technology to improve the control of mosquito-borne infectious diseases, while also emphasizing the need to address ethical and ecological risk issues.
Keywords *Aedes aegypti* mosquitoes; CRISPR/Cas9 technology; Gene editing; Disease control; Ecological risk

Mosquitoes are a type of insect that plays a crucial role in ecosystems but also serve as vectors for several serious infectious diseases, especially transmitted through biting humans and other mammals. *Aedes aegypti* mosquitoes are considered particularly challenging vectors as they transmit diseases such as dengue fever, Zika virus, and yellow fever. These diseases pose a significant global health threat, especially in tropical and subtropical regions (Wang et al., 2018).This review will explore the dietary diversity of holometabolous insects and the construction of food networks related to them. Through in-depth research into the dietary adaptations and food choices of insects at different life stages, we can gain a better understanding of their roles and positions within ecosystems. Food networks, as complex interrelation webs in ecosystems, involve various ecological interactions such as predation and prey, competition, and mutualism, and are of significant importance in maintaining ecological balance.

Aedes aegypti mosquitoes are widely distributed, primarily breeding in tropical and subtropical regions. They primarily feed on humans, making them significant disease vectors. One of the most severe diseases they transmit is dengue fever, which infects millions of people annually, leading to thousands of deaths. *Aedes aegypti* also transmits diseases like yellow fever, Zika virus, and chikungunya fever. These infectious diseases not only threaten human health but also have adverse effects on the global economy and social stability..

In recent years, the rise of CRISPR/Cas9 gene editing technology has revolutionized the field of life sciences and biomedicine. This groundbreaking technology, based on the natural immune system of bacteria, enables scientists to precisely edit genes, deleting, replacing, or adding DNA sequences. CRISPR/Cas9 technology is renowned for its efficiency, precision, and relatively low cost, and it has achieved significant success in basic research, agriculture, and medical applications (Qiu et al., 2022).

In the research of mosquitoes and other disease vectors, CRISPR/Cas9 technology provides unprecedented opportunities. By using CRISPR/Cas9, researchers can selectively edit the genes of *Aedes aegypti* mosquitoes to reduce their disease-transmitting capabilities. This technology offers a promising new tool for disease control, potentially reducing global health threats. Through targeted gene editing, scientists can intervene in mosquito reproductive capabilities, susceptibility to viral infections, and behavioral traits to reduce their disease-transmitting potential (Sun et al., 2018).

The primary purpose of this review is to explore the application of CRISPR/Cas9 technology in gene editing of *Aedes aegypti* mosquitoes, along with relevant methods and challenges. It will analyze how CRISPR/Cas9 technology has been utilized in past research to alter the genetic characteristics of mosquitoes and reduce their disease-transmitting capabilities. This review will also discuss the ethical and ecological issues that this technology might face and potential directions for future research and applications.

1 Overview of Gene Editing Technology

1.1 Biological characteristics of *Aedes aegypti*

Aedes aegypti, also known as the Egyptian mosquito, is an important disease-carrying mosquito that is widely distributed in tropical and subtropical regions. Understanding the biological characteristics of this mosquito is crucial for studying and controlling the diseases it transmits. It's worth noting the life cycle of *Aedes aegypti*, which includes four stages: eggs, larvae, pupae, and adult mosquitoes. This life cycle typically occurs under suitable temperature and humidity conditions. Eggs are laid in water, while larvae and pupae live in water and respire through breathing tubes. Adult mosquitoes seek hosts for blood-feeding, and female mosquitoes require blood to hatch their eggs (Figure 1).

The ecological habitat of *Aedes aegypti* is often closely associated with areas inhabited by humans. It is a domesticated mosquito that prefers to breed in artificial containers like water storage tanks, barrels, and discarded items, which brings them into frequent contact with humans, particularly in urban areas. This is closely related to their significance as disease vectors (Wang et al., 2022).

The behavioral characteristics of *Aedes aegypti* are also noteworthy. These mosquitoes are typically active during the daytime, especially in the early morning and late afternoon. This behavior makes them closely associated with the transmission of diseases like yellow fever, dengue fever, Zika virus, as these pathogens are also active during these times. Understanding the life history, ecology, and behavior of *Aedes aegypti* is essential for devising more effective control strategies to reduce disease transmission.

Figure 1 *Aedes aegypti* mosquito (Image source: Veer Gallery)

1.2 CRISPR/Cas9 gene editing technology

The CRISPR/Cas9 technology is a revolutionary gene editing tool that has made significant advancements in gene editing research in *Aedes aegypti* (Shi and Liu, 2023). CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats," and Cas9 represents "CRISPR-associated protein 9." The fundamental principle of this technology originates from the natural immune system of bacteria, which they use to defend against phage invasion (Figure 2).

CRISPR technology enables scientists to customize the DNA sequence of specific genes for the addition, deletion, or modification of particular genes. This is achieved by guiding RNA (gRNA) to combine with the Cas9 protein, forming an RNA-Cas9 complex. The gRNA is designed to be complementary to the DNA sequence of the target gene, allowing Cas9 to precisely cut the DNA. During DNA repair, cells typically introduce errors or mutations, leading to changes in the target gene.

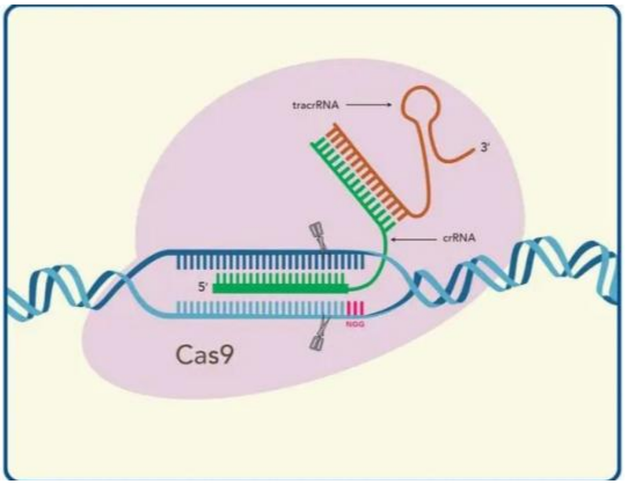

Figure 2 CRISPR/Cas9 gene editing technology (Image source: Baidu Image Library)

The CRISPR/Cas9 technology possesses numerous advantages, including highly precise editing, efficiency, relatively low cost, and broad applicability. These characteristics make it the preferred tool in the field of gene editing, especially playing a pivotal role in gene editing research on *Aedes aegypti* mosquitoes.

In comparison to traditional gene editing methods, CRISPR/Cas9 technology offers significant advantages. Traditional gene editing methods often rely on tools such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs), which are relatively complex in design and usage, costly, and less efficient. In contrast, CRISPR/Cas9 technology allows for easier and faster editing of specific genes, with higher precision and efficiency (Jiang et al., 2023). This enables researchers to quickly generate the desired editing outcomes, thereby expediting the development of disease control strategies.

1.3 Previous gene editing studies

Research using CRISPR/Cas9 technology in *Aedes aegypti* mosquitoes has achieved a series of significant breakthroughs, providing new tools and methods to combat the transmission of infectious diseases by this important vector. Below are some key research outcomes and application examples that highlight the potential of using CRISPR/Cas9 in mosquito gene editing.

Gene drive technology is a crucial application that can reduce the mosquito's disease transmission potential through CRISPR/Cas9 editing. The core concept of this method is to edit the mosquito's genes in a way that their offspring cannot transmit pathogens. Under laboratory conditions, researchers have successfully used CRISPR/Cas9 to edit the genes of *Aedes aegypti* mosquitoes, resulting in offspring that cannot transmit the dengue virus. This provides an innovative strategy for infectious disease control, with the potential to reduce disease transmission, although it still requires further research to address ethical and ecological concerns to ensure feasibility and long-term effectiveness (Yang and Zhang, 2022).

Developing insect-resistant mosquitoes is also a highly regarded application where mosquitoes are edited using CRISPR/Cas9 to enhance their resistance to specific pathogens. By introducing resistance genes, scientists can make mosquitoes immune to certain pathogens, reducing the replication and transmission of these pathogens within the mosquito. While this method is still in the research stage, it holds promise for controlling the transmission of diseases such as malaria and dengue fever.

CRISPR/Cas9 technology is also used to study gene fitness, which refers to how a specific gene in a mosquito population gains an advantage in natural selection. Through CRISPR/Cas9 editing, researchers can introduce or remove specific gene variants and then observe how these changes spread within the population. This helps to better understand how mosquito resistance develops and, in turn, aids in devising more effective disease control strategies. Research findings indicate that CRISPR/Cas9 technology offers promising tools and methods for studying and controlling the transmission of infectious diseases by *Aedes aegypti* mosquitoes.

1.4 The impact of gene popularity and ecological technology

CRISPR/Cas9 technology holds promise in mosquito gene editing, but its applications come with a range of ethical and ecological issues, with the impact on ecosystems and the environment being most significant. Editing mosquito genes may lead to unpredictable effects on the ecosystem and affect other biological populations. Gene drive technology could potentially lead to the extinction of certain mosquito populations, sparking ethical and legal controversies. Researchers and policymakers need to carefully consider ethical concerns to ensure that the application of CRISPR/Cas9 technology is sustainable and responsible.

Ethical concerns involve potential gene fitness. Through CRISPR/Cas9 editing, researchers may alter the frequency of specific genes in mosquito populations, which could also result in the spread of some resistance genes in the population, thereby weakening the effectiveness of disease control (Teng and Xu, 2021). Therefore, a balance needs to be struck between the potential of gene editing and its potential adverse consequences to ensure long-term ethical viability.

While CRISPR/Cas9 technology offers significant advantages in gene editing, it still faces some technical limitations. One major challenge is precision. CRISPR/Cas9 can achieve highly precise gene editing, but it may still lead to undesired side effects and off-target editing, requiring further research to improve the precision and controllability of the editing techniques.

Efficiency is also a concern with CRISPR/Cas9 technology. Editing mosquito genes requires introducing CRISPR/Cas9 components into a large number of individuals and ensuring that they obtain the desired changes in the edited genes. This could be an expensive and complex task, especially when applied in natural environments.

In conclusion, CRISPR/Cas9 technology has shown tremendous potential in mosquito gene editing research related to *Aedes aegypti*, offering innovative approaches to control the transmission of diseases by this important vector. Researchers must address ethical, ecological, and technical limitations carefully to ensure the feasibility and sustainability of this technology.

2 Methods and Challenges

2.1 Application of CRISPR/Cas9 in *Aedes aegypti* mosquitoes

CRISPR/Cas9 technology's application in gene editing of *Aedes aegypti* mosquitoes has become a focal point in research, providing a powerful tool for controlling the transmission of diseases by this important disease vector. We will introduce and discuss how CRISPR/Cas9 technology is used for mosquito gene editing and its fundamental principles.

The key to CRISPR/Cas9 technology involves designing appropriate guide RNAs (gRNA) to bind with the Cas9 protein, allowing precise targeting of the DNA sequence of the target gene. The nucleic acid cleavage activity of Cas9 is used for editing (Tong et al., 2018). When editing genes of *Aedes aegypti* mosquitoes, researchers typically choose genes related to the pathogens transmitted by mosquitoes as their targets to reduce their infectivity.

Researchers need to design gRNA to make it complementary to the DNA sequence of the target gene, ensuring that gRNA binds to the target gene with Cas9, forming an RNA-Cas9 complex. Once the complex binds to the target DNA sequence, the Cas9 protein will cut the DNA of the target gene, triggering the cell's self-repair mechanism.

This self-repair process may result in different outcomes, including the deletion, insertion, or replacement of base pairs in the target gene. This depends on the researchers' design and experimental conditions. By adjusting the design of gRNA and experimental parameters, precise gene editing can be achieved.

The application of CRISPR/Cas9 technology in gene editing of *Aedes aegypti* mosquitoes typically involves the following steps. Researchers need to design gRNA to ensure precise targeting of the target gene, which is crucial as it determines the accuracy and efficiency of editing.

The synthesis of gRNA and Cas9 protein, typically prepared through in vitro synthesis or genetic engineering techniques, is used. These components are used to form the RNA-Cas9 complex, which is the core of gene editing. When gRNA and Cas9 are introduced into the mosquito, they combine to form a complex that binds to and cuts the target DNA sequence. This initiates the cell's repair mechanism, achieving gene editing. The repair process may result in different outcomes, including the deletion, insertion, or replacement of base pairs in the target gene, depending on the researchers' design and experimental conditions.

Selecting and screening the edited mosquito offspring to identify individuals carrying the desired gene changes usually involves molecular analysis and gene sequencing to confirm the success of the editing and the desired changes. A series of steps constitute the technical process of CRISPR/Cas9 technology in gene editing of *Aedes aegypti* mosquitoes, achieving precise gene editing. This technology has great prospects for development but also comes with a range of challenges and issues that require further research and resolution.

2.2 Challenges of crossbreeding, gene transmission, and technical errors

The application of CRISPR/Cas9 technology in mosquito gene editing faces complex issues, with a key challenge being mosquito crossbreeding. Mosquito populations are typically highly hybridized, and crossbreeding between different subspecies is a common occurrence (Terraillon et al., 2023). This complicates the transmission of edited genes, as edited mosquitoes may mate with wild mosquitoes, thus passing on the edited genes. This means that in practical applications, it is necessary to consider how to prevent the spread of edited genes to wild populations to avoid unpredictable ecological and environmental impacts.

The uncertainty of gene transmission is also a concern. While CRISPR/Cas9 technology can achieve precise gene editing, the transmission of edited outcomes to subsequent generations is not always predictable. Edited genes may be stably transmitted, but they can also be lost or undergo mutations in the offspring. This uncertainty makes the implementation and maintenance of mosquito gene editing more complex, requiring further research to understand and address this issue.

Technical errors and efficiency are also a major challenge and impact. Although CRISPR/Cas9 technology theoretically allows for highly precise editing, in practical operation, there are still technical errors and incomplete editing. This can lead to edited mosquitoes having undesired variations or side effects. Researchers also need to improve the accuracy and efficiency of editing techniques to reduce these errors.

2.3 Challenges of ethical and ecological considerations

Ethical and ecological considerations in mosquito gene editing are also important issues. Editing mosquito genes may have unpredictable impacts on ecosystems, especially in the wild. Edited mosquitoes can affect other biological populations, leading to adverse ecological consequences. Comprehensive ecological risk assessments and measures to reduce uncertainty are necessary to understand potential impacts.

Ethical norms and regulatory issues also need to be carefully considered. Gene-edited mosquitoes can trigger ethical and legal disputes, especially in practical applications. Determining how to regulate and manage the application of CRISPR/Cas9 technology to ensure its feasibility and sustainability is a complex issue. Governments, research institutions, and the international community need to collaborate in formulating relevant policies and regulations to ensure the safety and ethical feasibility of mosquito gene editing (Zhang et al., 2020).

There is no doubt that CRISPR/Cas9 technology provides a powerful tool in mosquito gene editing, but it also comes with a series of technical challenges and ethical and ecological issues. Addressing these issues requires interdisciplinary cooperation and long-term research to ensure the feasibility and sustainability of gene editing.

3 Application Cases

3.1 Gene drive and disease control in mosquitoes

Gene drive technology is an important application case aimed at reducing the potential of mosquito-borne diseases through CRISPR/Cas9 technology (Figure 3). The core idea of this method is to edit mosquito genes to make their offspring unable to transmit pathogens, thereby reducing the risk of disease transmission. In *Aedes aegypti* mosquitoes, this method has already made significant research progress.

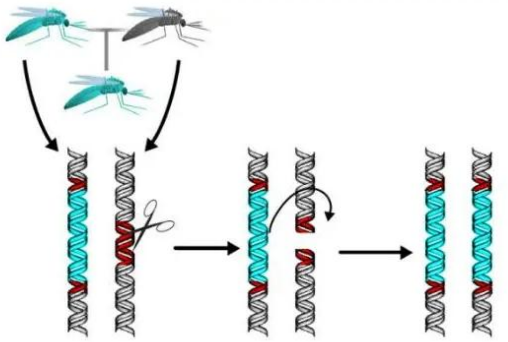

Figure 3 Gene expulsion technology (Image source: Baidu Image Library)

Researchers select genes associated with specific infectious diseases as targets. These genes typically involve pathways for the replication and transmission of diseases such as Zika virus, dengue virus, or yellow fever virus (Rico-Hesse, 2010). By designing gRNAs, researchers can precisely target these genes and then use the Cas9 protein to cut them.

Editing these key genes renders the edited mosquitoes incapable of transmitting the associated pathogens because these pathogens rely on these genes to complete their life cycles. This reduces the potential for mosquito-borne disease transmission. This editing technology has a hereditary effect, meaning the edited gene changes are passed on to the offspring of mosquitoes, further reducing their potential to transmit diseases.

In *Aedes aegypti* mosquitoes, gene drive technology has achieved some encouraging successful cases and research progress. One of the most notable cases is the control of dengue virus. Dengue fever is a significant infectious disease transmitted by *Aedes aegypti*, posing a global threat. Through mosquito gene editing, researchers have successfully reduced the potential for mosquitoes to transmit dengue virus, thereby decreasing the risk of disease transmission.

Control of Zika virus and yellow fever virus has also seen some breakthroughs. Researchers have used CRISPR/Cas9 technology to edit genes associated with the transmission of these pathogens, reducing mosquitoes' ability to transmit these pathogens. Results indicate that gene drive technology holds promise in reducing the transmission of multiple infectious diseases, but further research is needed to address ethical and ecological concerns to ensure feasibility and long-term effectiveness.

3.2 Development of insect resistant mosquitoes

Another major application case is the development of insect-resistant mosquitoes through CRISPR/Cas9 editing to enhance their resistance to specific pathogens. This method aims to reduce mosquitoes' ability to transmit specific pathogens, thereby lowering the risk of disease transmission (Nguyen-Tien et al., 2019).

The development of insect-resistant mosquitoes involves editing the genes of mosquitoes to enhance their resistance to specific pathogens. Researchers need to select target genes, which are typically related to the replication or transmission of pathogens. By editing these genes, researchers can make mosquitoes immune to specific pathogens, reducing their ability to transmit them.

This method has a wide range of applications and can be used for the control of various infectious diseases such as malaria, dengue fever, Zika virus, and more. By enhancing mosquitoes' resistance to these pathogens, it is possible to reduce the risk of disease transmission, and it holds promise in the field of infectious disease control.

The development of insect-resistant mosquitoes has potential applications in various areas, some of which include:

Malaria Control: Malaria is an infectious disease caused by parasites and transmitted by Anopheles mosquitoes. By editing the genes of Anopheles mosquitoes, their resistance to malaria parasites can be increased, reducing malaria transmission.

Dengue Fever Control: Dengue fever is transmitted by *Aedes aegypti* mosquitoes. By enhancing mosquitoes' resistance to the dengue virus, the risk of disease transmission can be reduced.

Zika Virus Control: The Zika virus is also transmitted by *Aedes aegypti* mosquitoes. By enhancing mosquitoes' resistance to the Zika virus, the risk of disease transmission can be reduced.

Other Infectious Disease Control: The development of insect-resistant mosquitoes can also be applied to the control of other infectious diseases such as yellow fever and Uganda virus.

Clearly, CRISPR/Cas9 technology in mosquito gene editing includes application cases like gene drive technology for controlling disease transmission by mosquitoes and the development of insect-resistant mosquitoes. These applications provide new tools and methods for disease control, but they also require addressing ethical, ecological, and technical issues to ensure feasibility and sustainability.

3.3 Advantages and limitations of CRISPR/Cas9 technology in mosquito gene editing

CRISPR/Cas9 technology has significant advantages and some limitations in the field of mosquito gene editing, and these factors need to be thoroughly considered in both applications and research. CRISPR/Cas9 technology stands out for its precision and efficiency. This technology allows highly precise gene editing, enabling targeted modifications such as deletion, insertion, and replacement. This precision provides a reliable means to achieve the desired genetic changes in mosquitoes. The transgenerational effect of CRISPR/Cas9 technology allows the editing effects to persist, reducing the potential for disease transmission. This is highly advantageous for gene drive and the development of insect-resistant mosquitoes, ensuring that edited mosquitoes' offspring also carry the desired genetic changes. CRISPR/Cas9 technology has diverse application potential in the field of mosquito gene editing, not only for disease control but also for other areas such as ecological research, gene function studies, and population genetics. This opens up possibilities for broader research and applications. Compared to traditional gene editing methods, CRISPR/Cas9 technology is faster and more cost-effective. This means that different strategies can be developed and tested more rapidly to address various disease transmission scenarios (Sun et al., 2023).

However, CRISPR/Cas9 technology comes with some limitations, with ethical and ecological risks being a prominent concern. The ecological impact of editing mosquitoes and the potential escape of edited mosquitoes into wild populations have raised widespread concerns and worries. These issues require extensive research and regulation to ensure the efficiency of edited mosquitoes. While CRISPR/Cas9 technology is powerful, it still has technical errors, incomplete editing, and uncontrollable gene transmission. Improving the precision and efficiency of the technology is one of the current challenges. Societal acceptance is also a significant concern, as mosquito gene editing involves altering the biology of the natural world, which can raise public concerns and resistance. Ethical and societal acceptance issues need to be addressed in practical applications to facilitate the widespread adoption of this technology.

3.4 Addressing ethical and ecological challenges

Ecological risk assessment should be a top priority. Conduct extensive ecological risk assessments, including laboratory research and field monitoring, to understand the potential impacts of edited mosquitoes on ecosystems. This will help determine the potential risks of the technology and possible ecological effects, guiding further applications of the technology.

The development of clear regulatory policies and regulations is crucial. Governments and international organizations need to collaborate to ensure the legality and safety of mosquito gene editing. Clear regulatory frameworks will help standardize the application of the technology while ensuring that researchers and practitioners adhere to prescribed ethical guidelines.

Public education is key to addressing ethical issues. Enhance public education to explain the potential benefits and risks of CRISPR/Cas9 technology to gain public understanding and support. This will help reduce public concerns and resistance while promoting the reasonable application of the technology (Chen et al., 2023).

The establishment of clear ethical guidelines will help guide research and application practices. Explicit ethical guidelines can ensure that the technology's application adheres to ethical standards, reducing the potential for misuse and risks. Collaboration and transparency are also crucial in addressing ethical and ecological issues. Interdisciplinary collaboration and information transparency will help all parties work together to address these issues, ensuring that the interests of all stakeholders are adequately considered.

In summary, addressing the ethical and ecological issues in mosquito gene editing requires a comprehensive approach involving ecological risk assessment, the development of regulatory policies, public education, ethical guidelines, and interdisciplinary collaboration. Only through these combined efforts can the application of CRISPR/Cas9 technology in mosquito gene editing be both effective and safe while considering the reasonableness of ecological and ethical concerns. This will provide strong support for the control of mosquito-borne infectious diseases while maintaining ecological balance and ethical standards.

4 Overview and Outlook

This review delves into the application of CRISPR/Cas9 technology in *Aedes aegypti* mosquito gene editing, discussing its biological characteristics, technical principles, previous research, challenges, and future directions. It reveals the tremendous potential of CRISPR/Cas9 technology in the field of mosquito gene editing, particularly in infectious disease control and ecological research. Through precise gene editing, researchers can reduce the potential risks of mosquito-borne diseases, which is crucial for public health. The development of insect-resistant mosquitoes also offers new strategies for disease prevention and control. Ethical, ecological, and technical issues need to be considered and addressed to ensure the feasibility and safety of the technology. This review emphasizes the importance of CRISPR/Cas9 technology in mosquito gene editing and provides valuable guidance for future research and applications.

CRISPR/Cas9 technology has broad potential applications in *Aedes aegypti* mosquito gene editing. Precise gene editing can reduce the potential risks of mosquito-borne diseases such as dengue fever, Zika virus, and malaria. This provides a powerful tool for global public health and has the potential to reduce millions of infection cases. The development of insect-resistant mosquitoes can reduce dependence on insecticides while minimizing negative environmental impacts. Insect-resistant mosquitoes can also be applied in ecosystem research to understand the role and relationships of mosquitoes in the natural environment. These potential applications come with a set of challenges. Ethical issues involve ecological risks and social acceptance, necessitating a comprehensive approach for resolution. Technical limitations include precision, efficiency, and uncertainty in gene transmission, requiring ongoing research and improvement. These challenges need to be seriously considered in future research and applications to ensure the safety of the technology.

Given the potential applications of CRISPR/Cas9 technology in *Aedes aegypti* mosquito gene editing, we encourage further exploration in future research and applications. Technological improvement will be a crucial direction, including enhancing editing precision and efficiency, reducing technical errors, and addressing uncertainties in gene transmission. This will help better address different disease transmission scenarios. Ecological risk assessment will require more research to understand the potential impact of edited mosquitoes on ecosystems. This includes laboratory research and field monitoring to ensure the ecological safety of the technology. The development of regulatory policies will also be a key area to ensure the legality and safety of the technology. Governments and international organizations need to jointly establish clear regulatory frameworks (Ou et al., 2022). Public education will help explain the potential benefits and risks of the technology, gaining public understanding and support. Future research should also focus on disease control, including more studies on gene drive, the development of insect-resistant mosquitoes, and the optimization of infectious disease prevention and control strategies. Through in-depth research and collaboration, we can better apply CRISPR/Cas9 technology to address mosquito-borne infectious diseases in the future while ensuring the proper handling of ecological and ethical issues. This will provide robust support for global public health and ecological conservation.

Acknowledgments

We would like to express our gratitude to Ms. Xuan Jia for her invaluable review of this manuscript and her insightful feedback, which played a crucial role in improving the research content and enhancing the quality of the article.

References

Chen F., Jiang Y.F., Jiao X.Z., Zhang H., Zhu W.Y., Jiang M.S., and Xu J.D., 2023, Creating new glutinous rice by CRISPR/Cas9-targeted mutagenesis, Shandong Nongye Xuebao (Shandong Agricultural Sciences), 55(1): 32-37.

Jiang A.W., Guo H.Y., Wu W.J., and Liu H.L., 2023, Application of Cas9/sgRNA system to improve genome-editing efficiency of porcine embryonic fibroblasts, Nanjing Nongye Daxue Xuebao (Journal of Nanjing Agricultural University), 46(3): 545-554.

Nguyen-Tien T., Lundkvist A., and Lindahl J., 2019, Urban transmission of mosquito-borne flaviviruses-a review of the risk for humans in vietnam, Infection Ecology & Epidemiology, 9(1): 1660129.
https://doi.org/10.1080/20008686.2019.1660129

Ou C., Zhang M., Ding L., Yao X.M., Wang Z.L., Peng C., and Xu J.F., 2022, Application and policy regulation of CRISPR/Cas9 gene editing technology in plants, Zhejiang Nongye Xuebao (Acta Agriculturae Zhejiangensis), 34(8): 1806-1814.

Qiu Y.H., Jia Y., Ni J.Q., Wang B., and Wang G.F., 2022, Studies and applications of gene editing technology in insects, Kunchong Xuebao (Acta Entomologica Sinica), 65(2): 246-256.

Rico-Hesse R., 2010, Dengue virus virulence and transmission determinants, Dengue Virus, 2010: 45-55.
https://doi.org/10.1007/978-3-642-02215-9_4

Shi J.Y., and Liu Z.X., 2023, Risk response of gene editing technology: ethical governance and legal regulation, Fazhi Yanjiu (Research on Rule of Law), (1): 87-89.

Sun H.W., Liang P.P., and Huang J.J., 2018, Research on genetic diseases treatment with base editing system in human embryos, Shengming Kexue (Chinese Bulletin of Life Sciences), 30(9): 926-931.

Sun X.H., Tang S.B., and Chen J.S., 2023, Application of CRISPR/Cas9 genome editing technology in hereditary retinal diseases, Zhonghua Shiyan Yanke Zazhi (Chinese Journal Of Experimental Ophthalmology), 41(9): 925-930.

Teng H.Y., and Xu D., 2021, Effects of editing and overexpression of OsSP1 gene on rice growth and drought tolerance, Huabei Nongxuebao (Acta Agriculturae Boreali-Sinica), 36(5): 1-9.

Terraillon J., Roeber F.K., Flachenecker C., and Matthias F., 2023, Training set designs for prediction of yield and moisture of maize test cross hybrids with unreplicated trials, Frontiers in Plant Science, 14: 1080087.
https://doi.org/10.3389/fpls.2023.1080087

Tong X.L., Fang C.Y., Gai T.T., Shi J., Lu C., and Dai F.Y., 2018, Applications of the CRISPR/Cas9 system in insects, Hereditas (Beijing), 40(4): 266-278.

Wang D., Xie X.X., Zhou X.Y., Zhao T., Guo X.X., Xing D., and Li C.X., 2022, Screening the related immune genes susceptible to denv2 in aedes aegypti with aag2 cell overexpection system, Jishengchong yu Yixue Kunchong Xuebao (Acta Parasitologica et Medica Entomologica Sinica), 29(1): 25-32.

Wang S.Q., Yang M.M., Zhu G.D., Sun L.X., Geng H.Y., Cao J., and Yang H.T., 2018, Control of imported mosquito-borne diseases under the Belt and Road Initiative, Zhongguo Xuexichongbing Fangzhi Zazhi (Chinese Journal of Schistosomiasis Control), 30(1): 9-13.

Yang J.F., and Zhang H.J., 2022, Research on the ethical supervision of human gene editing technology: based on the perspective of game theory, 2022, Zhongguo Yixue Lunlixue (Chinese Medical Ethics), 35(2): 164-174.

Zhang D., Hussain A., Manghwar H., Xie K., Xie S., Zhao S., Larkin R.M., Qing P., Jin S., and Ding F., 2020, Genome editing with the CRISPR‐Cas system: an art, ethics and global regulatory perspective, Plant Biotechnology Journal, 18(8): 1651-1669.
https://doi.org/10.1111/pbi.13383