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Application of CRISPR Technology in Endangered Species Conservation

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Abstract As an advanced gene-editing tool, CRISPR technology has shown tremendous potential in the field of endangered species conservation in recent years. This study introduces the primary applications and challenges of CRISPR technology in endangered species conservation. First, CRISPR technology improves genetic diversity, health level, and disease resistance of endangered species through gene repair, the introduction of new genes and controlling the spread of harmful mutations. In addition, CRISPR gene drive technology effectively controls invasive alien species and reduces competitive pressure on native endangered species. CRISPR technology can also enhance the adaptability of endangered species to environmental pollution and climate change, such as by introducing anti-pollution genes and regulating heat tolerance genes to improve adaptability. However CRISPR technology faces technical and ethical challenges in endangered species conservation. On the technical level, the lack of genomic data for non-model organisms and off-target effects of gene editing are the main issues. On the ethical level, gene editing may alter the natural evolutionary process of species and bring ecological risks. Therefore, interdisciplinary collaboration needs to be strengthened to ensure that scientists, policymakers, and the public jointly explore solutions. Looking ahead, improved gene-editing tools and advances in bioinformatics will enhance the accuracy and efficiency of gene editing, promote data sharing and interdisciplinary cooperation to advance CRISPR technology in endangered species conservation. Through technological improvements and multi-stakeholder collaboration, CRISPR technology will play a significant role in global biodiversity conservation.

Keywords CRISPR technology; Endangered species; Gene editing; Gene drive; Biodiversity conservation

1 Introduction

Global biodiversity is facing an unprecedented crisis. According to the Red List of the International Union for Conservation of Nature (IUCN), there are currently more than 30 000 species of animals and plants are listed as endangered, and all kinds of organisms including mammals, birds, reptiles, fish and plants are threatened with extinction. The reduction and extinction of endangered species not only affects the stability of the ecosystem, but also disrupts the food chain and leads to the decline of ecological services. The loss of biodiversity has had a profound negative impact on human food security, medical resources and climate regulation. The main threats facing endangered species include: habitat loss and fragmentation, illegal hunting, invasive alien species, climate change and environmental pollution (Hohenlohe et al., 2021). In the face of these complex threats, although traditional conservation strategies such as habitat restoration, legislative protection and captive breeding have achieved certain results, their technical and resource limitations make it difficult to cope with the growing biodiversity crisis. The rise of new technical means, such as gene editing technology, provides new opportunities for the protection and restoration of endangered species.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a gene editing technology that evolved from the immune systems of bacteria and archaea. The most widely used CRISPR/Cas9 system consists of two parts: a guide RNA (gRNA), used to identify specific target gene sequences; a nuclease Cas9, used to cut the target sequence. CRISPR technology uses gRNA to guide the Cas9 enzyme to accurately locate the target gene and cut it, and then insert, delete or replace the gene through the cell's own repair mechanism to achieve the purpose of gene editing. Compared with traditional gene editing technologies (such as zinc finger nucleases and transcription activator-like effector nucleases), the CRISPR/Cas9 system has the advantages of simple operation, low cost and high editing efficiency (Horodecka and Düchler, 2021). Since its first application in eukaryotic

genome editing in 2013, CRISPR technology has rapidly become a powerful tool in the field of life sciences and is widely used in gene function research, disease model construction, new drug development and crop improvement. In terms of the protection and restoration of endangered species, CRISPR technology has great potential. It can help scientists correct genetic defects in endangered species, enhance their adaptability, and even restore extinct species. Specific applications include correcting genetic defects caused by inbreeding, enhancing resistance to diseases, controlling invasive alien species and improving environmental adaptability.

This study aims to explore the practical application and potential of CRISPR technology in endangered species protection, focusing on the latest research progress in gene repair, population recovery, alien species control and disease resistance. The study will also discuss the challenges of CRISPR technology in practical applications, including technical bottlenecks, ethical disputes and ecological risks. By systematically combing through related research, this study hopes to provide reference for the future application of CRISPR technology in the field of endangered species protection, promote interdisciplinary cooperation, and promote the transformation and implementation of this technology in actual conservation actions.

2 Basic Applications of Gene Editing in Endangered Species

As the global biodiversity crisis intensifies, many species are on the verge of extinction, and the task of protecting endangered species has become increasingly urgent. Traditional protection methods mainly focus on protecting habitats, strengthening legal supervision, and carrying out artificial breeding. With the continuous development of molecular biology technology, gene editing technology, especially the CRISPR/Cas system, has provided a new idea for the protection and restoration of endangered species.

2.1 Gene repair and function enhancement

Endangered species often have genetic diseases and functional defects due to population decline and loss of genetic diversity. Gene editing technology can improve the health and adaptability of endangered species by repairing harmful gene mutations or introducing new functional genes. For example, in carnivores such as snow leopards (*Panthera uncia*), reduced genetic diversity caused by inbreeding may lead to immune system defects or reduced reproductive capacity. Through gene editing, exogenous functional genes can be introduced or mutant genes can be repaired to improve the health and reproductive capacity of individuals.

Cooper et al. (2018) proposed a strategy to repair the genes of endangered species using CRISPR/Cas9 technology. They pointed out that by repairing the genes of endangered birds, their immune function can be effectively improved, enabling them to better resist the invasion of foreign pathogens. At the same time, the advantages of CRISPR technology in gene repair also include the ability to accurately locate specific gene mutation sites and use DNA repair mechanisms to repair them. Dudek et al. (2017) proposed that gene editing technology can help restore the functional genes of marine mammals, such as improving hereditary immunodeficiency diseases.

CRISPR technology can also be used to enhance the expression of functional genes. For example, by regulating gene expression levels, the reproductive capacity of birds, mammals and fish can be improved. This method can achieve better adaptation of species to environmental pressures by precisely regulating gene expression levels, helping their populations expand and stabilize.

2.2 Introduction of new genes and increase of functions

In addition to repairing existing gene defects, CRISPR technology can also be used to introduce new genes, thereby giving endangered species new adaptive traits. For example, in marine mammals, increasing tolerance to high-salinity and low-oxygen environments is an important strategy to improve their survival rate. By introducing adaptive genes, they can better adapt to environmental changes.

Piaggio et al. (2017) proposed a concept using CRISPR gene drive technology, which can greatly improve the ability of endangered species to adapt to the environment by introducing new functional genes into the genomes of endangered species. Especially in island ecosystems, due to the threats of invasive alien species and environmental changes, the introduction of new genes can significantly enhance the adaptability of endangered

species to new environments. For example, using gene drive technology, plague-resistant genes can be introduced into endangered rodents to improve their resistance to plague, thereby helping these populations recover and expand.

Samuel et al. (2020) pointed out that in some studies related to endangered species, the strategy of introducing new genes can not only enhance the survival ability of the species, but also provide a reference for the protection of other related species. For example, strategies to introduce specific plant toxin tolerance genes can help some herbivorous endangered species avoid death from poisoning. Strategies such as introducing anti-parasitic genes and climate change tolerance genes can significantly enhance the survival and reproductive capabilities of endangered species.

1.3 Control the spread of harmful genetic mutations

In endangered species, the accumulation of harmful genetic mutations can lead to genetic defects or diseases, ultimately affecting the population's viability. Gene editing technology provides an effective means to control the spread of these harmful mutations. By editing the genes of individuals carrying harmful mutations, these genes can be prevented from spreading further in the population.

Khwatenge and Nahashon (2021) studied strategies for controlling gene mutations in birds using CRISPR/Cas9 technology. By editing the genes of individuals carrying harmful gene mutations, they successfully prevented the spread of these mutations in the population, improving the overall health of the population. In Australia, hereditary cardiovascular diseases carried by some endangered bird species have been effectively controlled through gene editing.

Piaggio et al. (2017) proposed that CRISPR technology can also introduce harmless mutant genes into the genome of endangered species through the gene drive mechanism, thereby replacing or masking the effects of harmful mutant genes. The key to this strategy is to use gene drive technology to preferentially spread the target gene in the population, thereby inhibiting the spread of harmful mutant genes. Leitschuh et al. (2018) showed that in island ecosystems, gene drive technology can effectively control the spread of harmful genes and prevent the spread of diseases caused by gene mutations in endangered species.

2 Restoring Population Health and Diversity

In the process of protecting and restoring endangered species, population health and genetic diversity play a vital role. Genetic diversity can enhance the adaptability of populations to environmental changes and reduce the risk of genetic diseases. However, due to habitat destruction, illegal hunting and other human activities, many endangered species are facing population decline and loss of genetic diversity. Gene editing of endangered species through CRISPR technology is expected to rebuild and enhance their genetic diversity, correct genetic defects caused by consanguinity, and strengthen adaptive characteristics, thereby achieving the restoration of population health and diversity.

2.1 Reconstruction and improvement of genetic diversity

Genetic diversity is a key characteristic of a population's ability to adapt to environmental changes and disease. For some endangered species, the sharp decline in population size has caused a genetic bottleneck effect, resulting in a significant decrease in genetic diversity, which in turn affects the long-term survival of the population. Using CRISPR technology, scientists can introduce new genetic variations to rebuild and enhance the genetic diversity of endangered species.

In one study, scientists edited the giant panda's genome through CRISPR technology, introducing genetic variations related to immune function and disease resistance, significantly enhancing its immune response to specific pathogens (Piaggio et al., 2017). CRISPR technology can also be used to introduce beneficial genes from related or closely related non-endangered species, thereby expanding the gene pool of endangered species. For example, genes from black-footed ferrets were introduced into the genome of European mink to enhance their resistance to rabies (Figure 1).

However, this process also faces some challenges. For example, scientists need to fully understand the genome of a target species to determine which genetic variations significantly enhance its ability to survive. The introduction of cross-species genes may involve ethical and ecological risks and requires strict risk assessment and supervision.

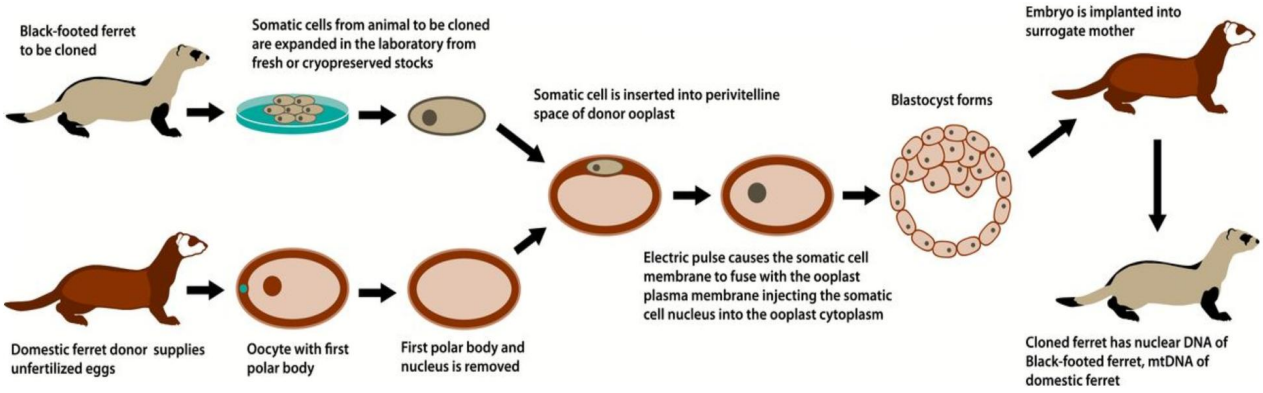

Figure 1 A conceptual model for interspecies somatic cell nuclear transfer in reproductive cloning of black-footed ferrets (*Mustela nigripes*) (Adopted from Wisely et al., 2015)

2.2 Correction of genetic defects caused by blood mating

As populations decline, endangered species are often at risk of inbreeding, leading to increased incidence of genetic defects and disease. CRISPR technology can be used to correct these genetic defects caused by blood mating, thereby improving the health of the population.

In a study of Javan rhinos, scientists used CRISPR technology to repair a mutated gene in their genome that caused skeletal deformities. By editing the gene, the researchers significantly reduced the incidence of skeletal deformities and improved the species' quality of life. Similar research also includes genetic repair of northern white rhinos to correct genetic defects related to their reproductive system (Hayashi et al., 2022).

CRISPR technology can also be used to remove or inhibit harmful genes that cause genetic diseases. For example, in some endangered bird species, inbreeding has led to a high incidence of feather abnormalities. By editing the genomes of these birds, scientists can remove the mutant genes that cause feather abnormalities, thereby reducing the incidence of the disease and enhancing the health and reproductive capacity of the population.

2.3 Adaptive enhancement associated with genetic diversity

In the face of multiple challenges such as climate change, habitat destruction and disease spread, endangered species need to have stronger adaptability. Through CRISPR technology, scientists can directly edit the genomes of endangered species to enhance their adaptability to environmental changes.

For example, in a study on sea turtles, scientists used CRISPR technology to introduce genetic mutations related to heat tolerance, allowing sea turtles to better adapt to rising sea temperatures caused by global warming. Similar research also includes enhancing the ability of corals to adapt to acidified seawater through gene editing to cope with ocean acidification.

In another case, scientists used CRISPR technology to enhance the tolerance of some endangered fish to heavy metal pollution, allowing them to survive in polluted waters (Figure 2). Gene editing can also be used to improve the tolerance of plant species to extreme environments such as drought and salinity, providing important genetic resources for ecosystem restoration.

However, when using CRISPR technology to enhance genetic diversity, special attention should be paid to ecological balance and potential ecological risks. For example, turtles with heat-resistant genes may compete with original species in the new environment and cause ecological imbalance. Before implementing any gene editing project, a full ecological assessment must be conducted to ensure its positive impact on the ecosystem.

3 Prevent and Control Invasive Alien Species

Invasive alien species (IAS) refer to species that are not native to the natural environment and have a negative impact on the local ecosystem, economy and human health. IAS are one of the major threats to global biodiversity. They weaken or even replace local species through competition, predation, and the spread of diseases, thereby disrupting the ecological balance and affecting the survival of endangered species. To solve this problem, in recent years, scientists have begun to try to use CRISPR gene editing technology to control and eliminate invasive alien species, in order to improve the survival chances of endangered species and the ecological balance of their habitats.

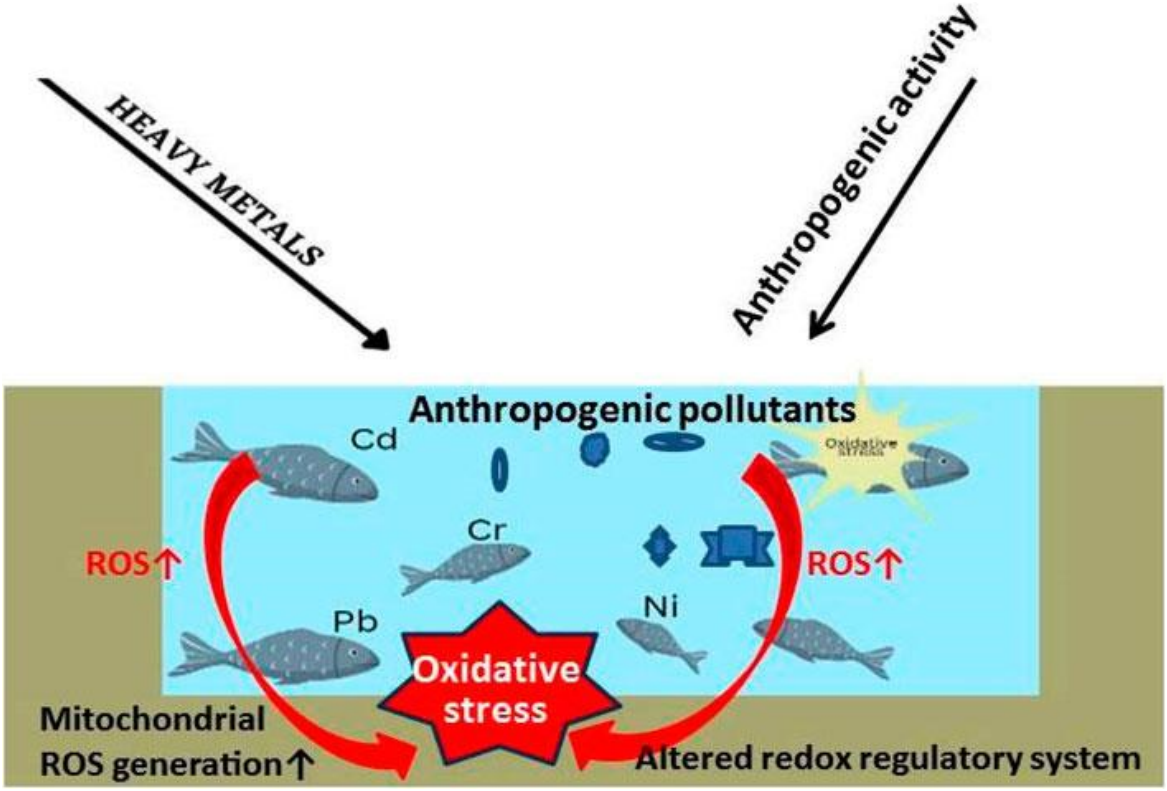

Figure 2 Anthropogenic induced heavy metals and other pollutants for induction of ROS and oxidative stress in fish (Adopted from Menon et al., 2023)

3.1 Using gene drive technology to control or eliminate invasive alien species

Gene Drive is a technology that uses genetic editing technology to spread specific genes in a population at a higher rate than the natural inheritance probability. Unlike conventional genetic patterns, gene drive technology gives the target gene the advantage of preferential transmission during gamete formation, allowing it to spread rapidly throughout the population in a short period of time. The CRISPR gene drive system is one of the most promising gene drive technologies currently and is widely used to control and eliminate alien invasive species.

Using CRISPR gene drive technology, lethal or sterile genes can be introduced into the target invasive species population, causing a significant reduction in their offspring or even sterilization, thereby achieving the goal of population control and elimination. For example, in a project to control invasive mosquitoes, scientists used gene drive technology to introduce a gene that makes female mosquitoes sterile into the mosquito genome. The results showed that the gene quickly spread to the entire mosquito population after several generations, successfully reducing the number of mosquitoes (Piaggio et al., 2017).

In addition, gene drive technology has also been used to control invasive rodents. A study in New Zealand used gene drive technology to edit the genome of rodents to produce lethal or infertile offspring, thereby effectively reducing the number of invasive rodents and protecting local bird and plant diversity. This method is more selective than traditional chemical killing methods and will not have a wide impact on non-target species.

3.2 Control of alien species that compete with endangered species

In addition to directly controlling or eliminating invasive alien species, CRISPR technology can also be used to regulate the behavior of alien species to reduce their competitive pressure on endangered species. Gene editing can change the reproduction method and behavioral characteristics of alien species, and even make them repel specific environments, thereby reducing their threat to endangered species.

In Australia's feral cat control project, scientists used CRISPR technology to edit the genes of invasive feral cats, adjusting their reproductive cycles to make them out of sync with local mammals, reducing their predation threat to endangered mammals. Another study used gene editing to change the habits of invasive toads, making them repulsive to certain habitats and preventing them from competing with local endangered amphibians for food and space.

Using CRISPR technology to adjust the relationship between alien species and local endangered species provides a new idea for controlling invasive species (Moro et al., 2018). This strategy based on behavioral regulation can not only effectively protect endangered species, but also maintain the balance and diversity of the ecosystem.

4 Fights Disease

In endangered species conservation, disease is considered an important factor leading to population decline. New pathogens continue to emerge around the world, posing a major threat to endangered species that are already in danger. In this context, CRISPR gene editing technology provides a new tool to give endangered species the ability to resist diseases.

4.1 Conferring resistance to emerging diseases to endangered species

With global climate change and intensified human activities, the outbreak and spread of diseases are increasing rapidly. This phenomenon has a serious impact on endangered species whose habitats are shrinking. For example, rare animal populations such as the Javan rhino (*Rhinoceros sondaicus*) and the giant panda (*Ailuropoda melanoleuca*) are more vulnerable to new pathogens due to their lack of genetic diversity.

CRISPR gene editing technology can help endangered species gain resistance to emerging diseases by precisely modifying the genome of species. For example, CRISPR technology can be used to change gene function so that certain species become resistant to specific pathogens. A potential application case is that CRISPR can be used to edit genes to express proteins that are resistant to specific pathogens, or to directly change genes that interact with pathogens and host cells, thereby reducing the possibility of infection.

In addition, CRISPR can also be used to accelerate the introduction of beneficial genes that can resist diseases. For example, research on albinism in vertebrates has shown that certain genetic mutations enable them to produce resistance proteins to fight infections by pathogenic bacteria. This discovery provides an important reference for using CRISPR technology to introduce resistance genes into the gene pool of endangered species under artificially controlled conditions. By combining with traditional breeding techniques, CRISPR can also significantly accelerate the spread of resistance genes in endangered species populations (Piaggio et al., 2017).

4.2 Modulate immune system responses to fight specific pathogens

In addition to conferring direct resistance to emerging diseases, CRISPR technology can also enhance resistance to specific pathogens by regulating the function of the immune system. Endangered species often show reduced immune system function or high susceptibility to pathogen infection due to insufficient genetic diversity. In this regard, CRISPR technology provides new means to activate or optimize the immune system of endangered species.

Gene editing technology can enhance the immune response of animals to pathogens (Wang et al., 2022). For example, by modifying genes through CRISPR, important regulatory molecules in the immune system (such as cytokines) can more effectively identify and attack pathogens, thereby improving resistance to infection. CRISPR can also be used to inhibit the functions of genes that hinder immune responses, thereby further enhancing the immunity of species.

CRISPR technology can also be used to adjust the tolerance of the immune system, allowing species to better adapt to changes in the external environment. For some endangered species whose immune systems are disrupted due to environmental pollution or other external factors, CRISPR can be used to recalibrate their immune responses, thereby enhancing their resistance to specific pathogens.

CRISPR can also help us gain a deeper understanding of the immune systems of endangered species and develop more effective vaccine strategies. For example, determining the function of key immune genes through gene editing can help scientists develop more targeted vaccines to enhance the immunity of specific species. This CRISPR-based vaccine strategy has great potential in wildlife conservation and can provide effective immune protection for endangered species.

5 Remove Pollutants or Adapt to Environmental Changes

The deterioration of the global ecological environment poses a serious threat to endangered species, with environmental pollution and climate change being two of the main factors. As a precise gene editing tool, CRISPR technology provides a new way to improve the adaptability of endangered species to environmental changes and their tolerance to pollutants.

5.1 Gene editing enhances species' adaptability to environmental pollution

Pollutants such as industrial waste, pesticides, and plastics accumulate in ecosystems, posing a great threat to endangered species. For example, heavy metal pollution not only destroys the living environment of many aquatic organisms, but also affects the health of terrestrial animals and birds through the food chain. Gene editing technology provides an effective means to improve the tolerance of endangered species to pollutants.

CRISPR technology can give species new abilities by precisely adjusting genes. For example, editing genes related to heavy metal metabolism in the genome of fish can enhance their tolerance to heavy metal pollution. A successful case is the use of the CRISPR/Cas9 system to knock out the metal ion transport gene of a fish, allowing it to survive in a high-concentration heavy metal environment. This genetic modification technology can also be applied to other endangered species affected by polluted environments, such as amphibians, reptiles and birds, so that they can survive better in polluted environments.

In addition to genetically modifying individuals to improve tolerance, CRISPR technology can also be used to develop biomarkers to monitor the impact of pollution on endangered species. By detecting changes in gene expression induced by pollutants, more accurate ecological risk assessment tools can be provided to environmental protection departments (Bramell and Wigginton, 2010).

5.2 Enhancing species' adaptability to climate change

Climate change has brought a huge impact on the global ecosystem. Extreme weather and habitat changes have made many endangered species face greater challenges to survival. CRISPR technology can enhance the adaptability of endangered species to climate change through genetic modification, providing a new way for species protection.

Enhancing the ability of plants to tolerate drought and high temperatures through gene editing can help protect endangered species associated with them. In tropical rainforests, the extinction of certain tree species will cause animals that rely on them to lose their food source. Editing key drought-tolerant genes through CRISPR technology can improve the ability of these plants to survive in extreme drought environments, thereby protecting the endangered animals that coexist with them.

CRISPR technology also shows great potential in improving animals' adaptability to climate change. Rising temperatures in the Arctic have reduced the hunting areas and food sources of endangered species such as polar bears. Enhancing their fat metabolism capacity through gene editing may help them better cope with high temperature environments. CRISPR technology can also adjust the migration behavior genes of certain birds and fish, allowing them to find more suitable habitats (Merlin et al., 2019).

Climate change can also lead to ocean acidification and coral bleaching, posing a serious threat to marine biodiversity. CRISPR technology can be used to adjust the genes of symbiotic algae in corals to enhance their tolerance to high temperatures and acidification. At the same time, the adaptability of marine organisms to acidic waters can also be improved through gene editing, such as adjusting the calcification-related genes of crustaceans and mollusks.

6 Concluding Remarks

In recent years, CRISPR technology has made great progress in the field of biological research, bringing new hope and opportunities for the protection of endangered species. As a powerful gene editing tool, the CRISPR/Cas9 system provides a feasible solution for the restoration of genetic diversity, enhancement of disease resistance, control of invasive alien species, and improvement of environmental adaptability of endangered species (Tibbetts, 2022). In terms of genetic diversity restoration, CRISPR technology can increase the genetic diversity of endangered species populations by introducing new genes or repairing existing harmful mutations, thereby enhancing their survival and reproductive capacity. In the correction of genetic defects caused by consanguinity, gene editing technology can effectively reduce the spread of genetic diseases and improve the health level of populations. Using CRISPR technology to introduce disease-resistant genes into endangered species can enhance their resistance to emerging diseases and protect endangered species from the threat of deadly pathogens. CRISPR gene drive technology can accurately control the reproduction of invasive alien species, effectively control their numbers, and thus alleviate their competitive pressure on local endangered species. CRISPR technology can also be used to enhance the adaptability of endangered species to environmental pollution and climate change. For example, by enhancing the pollution resistance genes of species, their survival rate in polluted environments can be improved; by regulating genes related to heat tolerance, the adaptability of endangered species to climate change can be improved.

Although CRISPR technology has great potential in endangered species conservation, it still faces a series of technical and ethical challenges (Li, 2024). On the technical level, the application of CRISPR technology in non-model organisms is difficult, mainly due to the lack of genomic data and the off-target effects of gene editing. Off-target effects may lead to unexpected gene mutations, which have a negative impact on the health and survival of species. Different species have different genome structures, and the efficiency and accuracy of gene editing also vary. Therefore, it is necessary to strengthen the collection and research of non-model organism genome data to improve the accuracy and efficiency of gene editing. On the ethical level, the application of CRISPR technology in endangered species has caused many controversies. Gene editing may change the natural evolution process of species and thus cause ecological risks. For example, the application of CRISPR gene drive technology in the control of alien invasive species may have unpredictable effects on ecosystems. Gene editing of endangered species involves ethical disputes about human intervention in natural ecology, which requires full consideration of public opinion and social acceptance. To solve these problems, scientists, policymakers and the public need to jointly explore solutions under a broader interdisciplinary cooperation framework.

In the future, CRISPR technology is expected to play a greater role in the protection of endangered species. With the continuous development of improved gene editing tools (such as CRISPR/Cas12 and CRISPR/Cas13), the accuracy and efficiency of gene editing will be further improved, and the occurrence of off-target effects will be reduced. At the same time, the progress of bioinformatics and genomics will help solve the problem of lack of genomic data in non-model organisms and provide better data support for the application of CRISPR technology. Strengthening data sharing and interdisciplinary cooperation is crucial to promoting the application of CRISPR technology in the protection of endangered species. The establishment of a genomic data sharing platform will help scientists obtain and share data worldwide, promote research collaboration and progress. Bringing together experts in fields such as genetics, ecology, and conservation biology can more comprehensively evaluate the potential risks and benefits of CRISPR technology and provide a scientific basis for policy making and practical application. In order to achieve the transformation from laboratory research to field application, more pilot projects and case studies are needed. In this process, scientists should closely monitor the effects and risks of gene editing and adjust strategies according to actual conditions. This will not only help accumulate experience in field applications, but also provide important references for policy making and public education.

As a cutting-edge gene editing tool, CRISPR technology provides a new solution for endangered species protection. Under the premise of fully recognizing the technical and ethical challenges, through interdisciplinary cooperation and technological improvement, CRISPR technology will play an increasingly important role in endangered species protection and contribute to the sustainable development of global biodiversity.

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