

The Overarching Impact of Insect Research on Humanity under the Context of CRISPR

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ABSTRACT

Insects play essential roles in ecosystem functioning and have substantial impacts on human health, agriculture, and biotechnology. Many species provide critical ecological services by constituting the bulk of the lower trophic pyramid in most ecosystems, and they also maintain intricate relationships with humans. Their genetic diversity and biological complexity make them invaluable research subjects for advancing health, agriculture, and biotechnology. However, traditional CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) methods have long been limited to select model species, such as *Drosophila melanogaster*, thereby restricting broader use of CRISPR in insect research. However, recent advances in CRISPR use have overcome many of these limitations, enabling more efficient and precise genome editing across a wide range of insect taxa. This review summarizes key advancements in CRISPR applications among some major insect groups, including vector control in mosquitoes, disease modeling in fruit flies, silk and biomaterial engineering in Lepidoptera, pest management and biomaterial studies in beetles, and behavioral genetics in Hymenoptera. Together, these studies illustrate the expanding scope of CRISPR-based insect research and its growing scientific, environmental, and biotechnological impact.

Keywords: CRISPR ; Insect Genomics; Gene Editing; Vector Control; *Drosophila Melanogaster*; Lepidoptera Biotechnology; Pest Management; Ethical and Ecological Considerations

INTRODUCTION

Gene modification has revolutionized modern biology by allowing researchers to test gene function through targeted disruption and to link genetic changes to measurable traits. It also enables the introduction of novel genes or the repair of defective ones, offering potential therapies for hereditary diseases (1). Among

the available approaches, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) stands out for its precision, simplicity, and affordability, making it accessible for diverse applications across organisms (2). CRISPR originated as a bacterial and archaeal defense system (3), but key experimental advances demonstrated that it could be repurposed as a programmable genome-editing tool (4). This shift rapidly expanded the scope of genetic research and spurred the development of multiple CRISPR variants and delivery strategies, alongside ongoing ethical debates over appropriate use (5). While much early attention focused on mammalian applications, CRISPR has been equally transformative in invertebrate systems, particularly insects, enabling both fundamental discovery and applied innovations (Figure 1).

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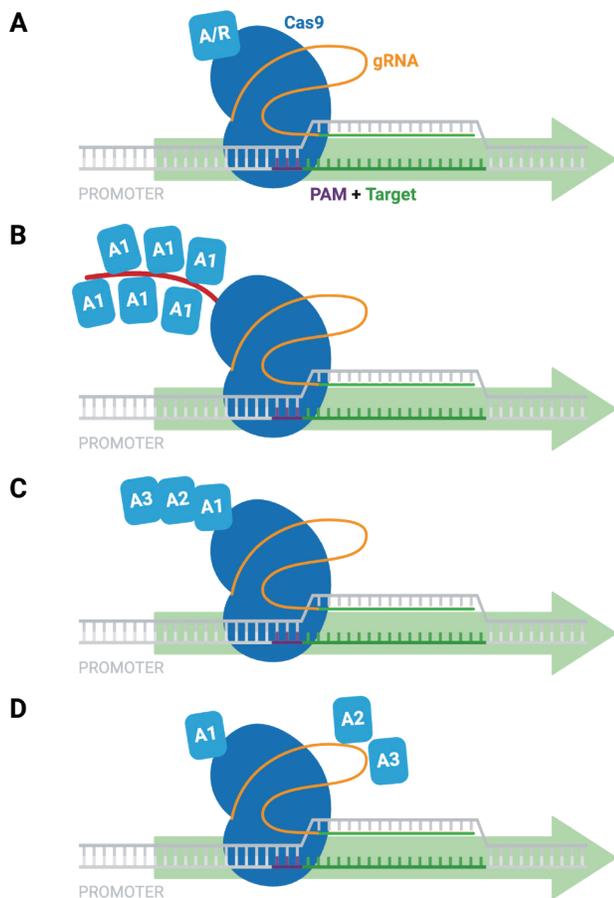


Figure 1. Cas9 Fusion Mechanisms. Schematic of the CRISPR/Cas9 genome editing mechanism, showing Cas9 nuclease guided by RNA to a specific DNA target. Various distinct activator domains (A1-A3) attach to the Cas9, demonstrating the flexibility of CRISPR-based gene activation systems as they regulate transcriptional activation levels (6).

VECTORS FOR HUMAN DISEASE (MOSQUITOES)

A significant contribution of CRISPR-Cas9 in insect vectors for human benefit has been reported in *Anopheles* mosquitoes, where targeted gene disruption has demonstrated the practicality of gene editing to reduce malaria transmission (7). More broadly, *Anopheles* and *Aedes* mosquitoes transmit pathogens responsible for malaria and arboviral diseases such as dengue, Zika, yellow fever, and chikungunya (8, 9). These public-health burdens have made mosquitoes a primary target for CRISPR-based vector control, aiming to either suppress populations or reduce vector competence. Generally,

three principal approaches in CRISPR have been used in mosquito vector control: gene knockouts, gene drives, and transgene insertions.

Knockouts of essential genes in mosquitoes were initially developed as a suppression strategy. In earlier studies, the knockout of fertility or sex-associated genes in *Anopheles* species led to a bias in sex ratios and fertility rates, thereby reducing the reproductive capacity of the mosquito (10). This approach demonstrates CRISPR's capacity to reduce vector populations by impairing reproductive success. Although reduced survival in edited individuals was often observed, this limited the potential scale of these applications. For instance, fitness costs reported in other research include reduced longevity, increased blood-feeding propensity in females, reduced egg hatching rates, and more (11).

Also, CRISPR-based homing gene-drives can be established to engineer biased inheritance of specific alleles, resulting in their rapid spread throughout the mosquito population (12). Gene drives targeting female fertility genes in *Anopheles gambiae* achieved inheritance rates greater than 95%, with laboratory populations collapsing after several generations (13). Although this method is highly effective, such approaches demand careful consideration of ecological risks, the formation of resistant alleles, and ethical concerns of releasing the altered mosquitoes into wild populations.

The third strategy involves inserting pathogen-resistance genes into mosquito vector genomes using CRISPR knock-in systems. For example, transgenic *Aedes aegypti* lines carrying anti-dengue effector genes under tissue-specific promoters have been generated through homology-directed repair, resulting in reduced viral replication and transmission (14). Knock-in approaches highlight the potential of CRISPR not only for vector suppression but also for vector modification, producing mosquito populations incapable of transmitting harmful human pathogens.

Together, these CRISPR-based strategies have demonstrated how genome editing can be used to either suppress mosquito populations or modify them to reduce pathogen transmission. Work in *Anopheles* and *Aedes* species, therefore, represents a prominent frontier in insect CRISPR research, while also underscoring the importance of evaluating resistance formation, ecological risks, and ethical concerns before any potential field application. However, mosquitoes encompass a very niche aspect of CRISPR application in dipterans. A more thorough investigation of this order requires an analysis of *Drosophila* contributions to CRISPR (Figure 2).

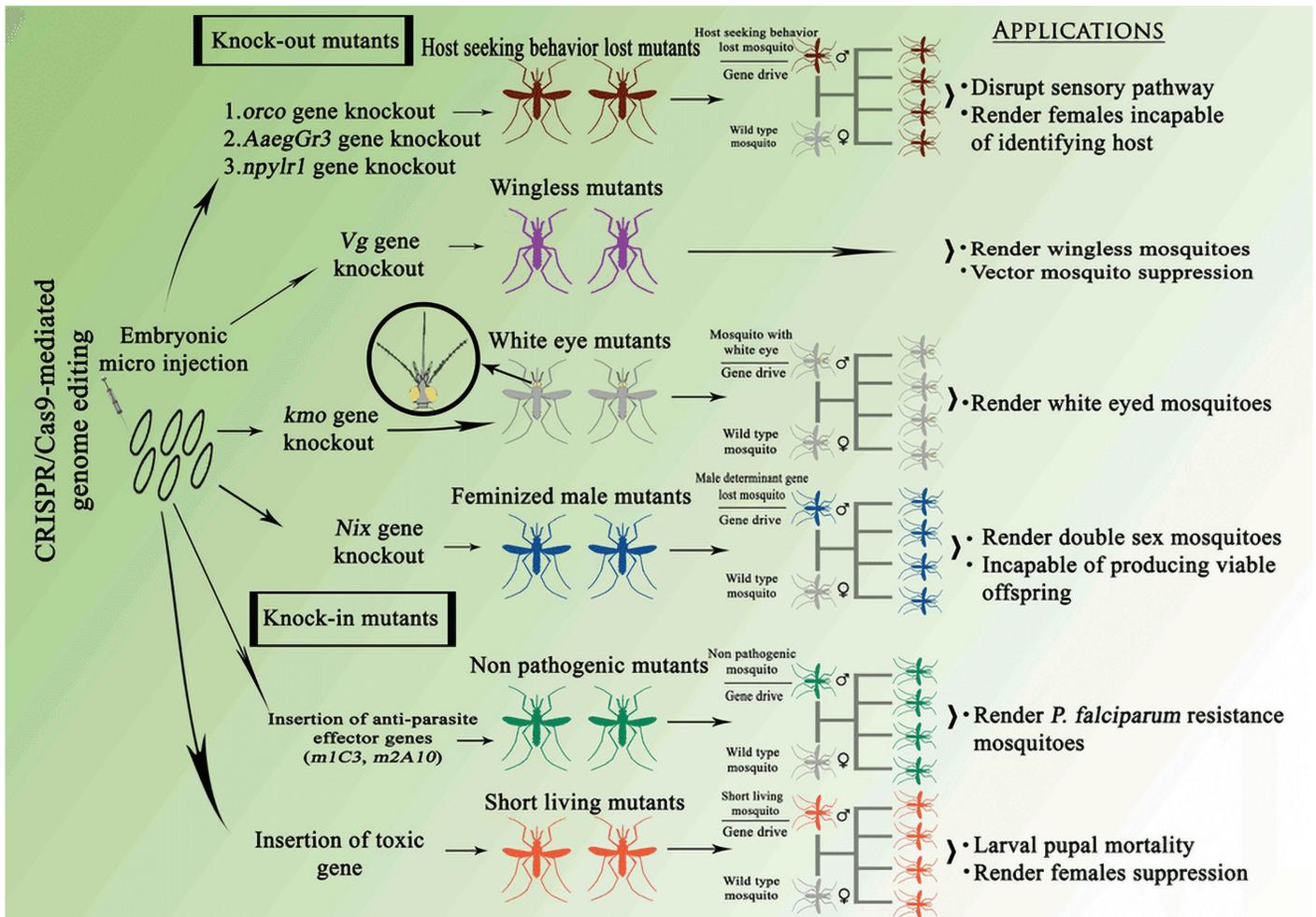


Figure 2. Basic Effects of Gene Knockouts and Knock-ins in Mosquitoes. A summary of the effects of different knockout and knock-in CRISPR methods on mosquitoes' genes. The intended effects of the alteration are expressed in the image for their resulting offspring, but no method is 100% effective in real applications (15).

DROSOPHILA MELANOGASTER: A FOUNDATIONAL MODEL FOR CRISPR RESEARCH

While CRISPR applications in mosquitoes primarily focus on reducing the burden of vector-borne disease transmission, the fruit fly (*Drosophila melanogaster*) has long served as an important foundational genetic model for understanding human biology and pathology. More than 75% of human disease-associated genes can be found within the functional orthologs of a fruit fly (16), and the advent of CRISPR research has significantly expanded the fly's utility in contemporary biomedical research. They are often considered the model organism for research; they are highly amenable to laboratory

culture and maintenance, reproduce quickly, and are easy to keep in laboratories (17). From disease modeling to drug discovery, from neuroscience and developmental biology to even scientific training, these insects have served as a foundational platform for the development and validation of CRISPR-based approaches relevant to human biology.

CRISPR-induced knockouts and knock-ins in *Drosophila* have enabled researchers to recreate human pathogenic mutations, thereby generating reliable models of many different genetic diseases. These techniques have proved especially useful in exploring neurodegenerative conditions such as Alzheimer's and Parkinson's disease, along with some trinucleotide repeat disorders like Huntington's disease (18). These

models have been useful for recapitulating protein aggregation, neuronal dysfunction, and progressive degeneration, enabling mechanistic studies and future therapeutic exploration (19).

Also, CRISPR's fly gene models serve as powerful *in vivo* platforms for drug screening, enabling assessment of disease-related phenotypes across the entire organism. Screening compounds in fruit flies provides insights into the toxicity and efficacy of drugs that cell cultures may not capture (20). Oftentimes, cell cultures do not offer the same level of specificity in capturing tissue-specific effects or behavioural phenotypes that are clear when testing living *Drosophila* models (21). Recent research has also used *Drosophila* models of rare congenital disorders to quickly identify candidate compounds with clinical potential (22).

In addition, CRISPR permits the targeted manipulation of neuronal circuits in flies. This, as of present, has revealed many mechanisms underlying learning, memory, circadian rhythms, and social behaviours in humans (23). Proteinopathy models generated in fruit flies have also clarified how misfolded proteins propagate within neural networks, providing parallels to human neurodegenerative conditions that will be useful in future research (24).

The fruit fly has also been indispensable for developmental genetics, and CRISPR now allows precise, tissue-specific interrogation of specific genes. Such targeted mutagenesis avoids lethality in early stages while highlighting gene functions in organogenesis, morphogenesis, and cell signaling pathways (25). Findings from dipteran CRISPR studies continue to inform our understanding of congenital anomalies and regenerative biology in people.

Drosophila has provided a versatile system for testing novel CRISPR modalities, including base editing and prime editing (26). Optimizations of guide RNA (gRNA) design, Cas9 expression strategies, and multiplexed editing pipelines were first benchmarked in flies before translation to mammalian systems (27).

The experimental tractability of *Drosophila* further supports its use as a training and validation platform for CRISPR-based genetic studies. Students and early-career scientists gain hands-on experience designing gRNAs, generating transgenic lines, and phenotypically characterizing CRISPR-induced mutations, skills directly applicable to more complex biomedical research (28).

Taken together, CRISPR applications in *Drosophila melanogaster* have broadened the fly's legacy as a genetic model organism, reinforcing its role not only in basic

biological discovery but also in translational pipelines that advance human health. However, the impacts of CRISPR have not been confined to dipteran models alone, and parallel advances in other insect orders have begun to expand the scope of functional genetics beyond *Drosophila*.

CRISPR APPLICATIONS IN LEPIDOPTERA: BUTTERFLIES AND MOTHS

While *Drosophila melanogaster* has long been considered the ideal genetic model for insects, CRISPR research in Lepidopterans, moths and butterflies, has provided important insights into both fundamental biology and applied biotechnology (29). For instance, both the painted lady (*Vanessa cardui*) and the silkworm (*Bombyx mori*) have become central models for studying traits such as wing patterning, pigmentation, and silk production, aiding researchers in understanding Lepidopteran evolutionary biology and in developing future silk production alternatives (30).

One of their most significant applications has been in establishing the genetic basis of Lepidopteran wing coloration and pattern formation. CRISPR-induced knockouts of pigmentation genes have revealed how specific gene loci control spot, stripe, and iridescent scale development, clarifying the mechanisms that drive the evolutionary diversity of Lepidopteran wing patterns. For example, manipulating the *cortex* gene in *Heliconius* butterflies can lead to broad changes in wing color, which are usually the result of mimetic radiation across different *Heliconius* species and variations (31). Studies like this can extend current understanding of developmental evolutionary pathways while providing broader insight into the development of biodiversity, which butterflies and moths prove especially useful for, since their anatomical designs have remained unchanged for the past 250 million years (32).

In addition, *Bombyx mori* has become an important platform for translational CRISPR applications in silk engineering. CRISPR has been used to engineer silkworms capable of producing silk with novel mechanical properties such as greater elasticity and tensile strength (33). These engineered silks have significant potential for biomedical applications, including sutures, tissue scaffolds, and the production of biodegradable polymers (34). For instance, because of silkworm silk's biocompatible and hemocompatible properties, recent studies have highlighted increasing interest in using invertebrate silk for tissue-engineering

applications to repair the urethra, skin, bone, and even tendon (35).

Silkworms have also been repurposed as living bioreactors for the production of recombinant proteins, vaccines, and therapeutic compounds because of the silk's high protein expression levels, cost-effectiveness, and susceptibility to post-translational modifications (36). Researchers learned to take advantage of their highly productive silk glands, which produce silk filled with abundant amounts of fibroin, to produce these foreign proteins on a large scale by inserting genes from different animals (37). CRISPR-based editing enables greater precision and efficiency in these production systems, offering an economical alternative to mammal-based cell cultures for pharmaceutical manufacturing (38).

Editing of chemosensory receptor genes in moths has revealed the genetic underpinnings of mate recognition and host plant selection behavior, providing a potential strategy for targeted pest management. For example, mutagenesis of the *Orco* gene within the *Helicoverpa armigera* moth (a serious agricultural pest) led to an increasing inability for their females to accurately select their host plants (the green pepper plant), which presents itself as a better alternative for agricultural deterrence than harmful pesticides (39).

CRISPR studies in Lepidoptera demonstrate how butterflies, moths, and silkworms extend insect genome editing beyond dipteran models by linking mechanistic insights to applied outcomes. From revealing the genetic basis of wing patterns and chemosensory behaviors to enabling precision improvements in silkworm-based production systems, Lepidopterans have expanded the scope of CRISPR research in ways that support both fundamental biology and practical innovation. Together, these findings illustrate the versatility of CRISPR across insect orders and further propel its application to increasingly diverse and complex orders of insects.

CRISPR APPLICATIONS IN COLEOPTERANS: BEETLES

Beetles represent the largest and most diverse order of insects, encompassing more than 400,000 described species across nearly every terrestrial and aquatic ecosystem (40). Because of their ecological diversity, developmental variety, agricultural significance, and global range, they have become increasingly valuable models for CRISPR research. Genome editing in beetles such as the red flour beetle (*Tribolium castaneum*), Japanese rhinoceros beetle (*Trypoxylus dichotomus*),

and Colorado potato beetle (*Leptinotarsa decemlineata*) has expanded human understanding of gene function, morphological evolution, and pest management, which will be the focus of this segment.

The red flour beetle (*Tribolium castaneum*) remains the most widely utilized Coleopteran model due to its fully sequenced genome, short generation time, and ease of laboratory rearing (41). Early genome-editing studies in *Tribolium* employed RNA interference, but the adoption of CRISPR-mediated genome editing has improved the reliability and scalability of functional genetic studies in Coleopteran models (42). CRISPR knockouts of segmentation genes such as *even-skipped*, *wingless*, and *hedgehog* have helped clarify how developmental pathways diverged between beetles and holometabolous models like *Drosophila melanogaster* (43). Unlike flies, *Tribolium* exhibits short-germ embryogenesis, where segments form sequentially. Therefore, CRISPR disruptions of these genes have provided new insight into how ancestral segmentation patterns evolved across insect lineages (44). These findings continue to inform broader discussions in evolutionary developmental biology (evo-devo) about the origins of body-plan diversity in arthropods.

CRISPR has also been applied to study beetle morphology and sexual dimorphism. For instance, *Trypoxylus dichotomus* (Japanese Rhinoceros Beetle) develops exaggerated horns through hormonal and genetic cues present throughout its development (45). Disruption of the beetle's insulin signaling pathways and juvenile hormone-related genes using CRISPR resulted in reduced horn size and altered secondary sexual characteristics, offering an understanding of how environmental factors interact with gene expression to produce sexually dimorphic traits in beetles (46). Studies like this are expanding human understanding of insect developmental plasticity and sexual selection at the genetic and physical levels, concepts highly relevant to the field of evolutionary biology.

Beyond basic research, CRISPR applications in agricultural pest beetles have demonstrated great promise. The Colorado potato beetle (*Leptinotarsa decemlineata*), one of the most destructive crop pests worldwide, has been a focal species for CRISPR-based pest control strategies (47). Targeted knockouts of genes associated with pesticide resistance and metabolic detoxification, such as cytochrome P450s and glutathione S-transferases, have provided important insights into the mechanisms underlying the beetle's notable insecticide tolerance (48). Using CRISPR-based gene drive systems

in potato beetles to suppress their populations or reduce resistance evolution offers an alternative to chemical pesticides that minimize environmental contamination and non-target effects.

In addition to pest control, the use of CRISPR in Coleopterans has opened new possibilities for studying biomaterial production. Beetles have chitin-rich exoskeletons, and the structural proteins in their exoskeletons have inspired bioengineering approaches to develop lightweight, biodegradable composites. For example, genome editing has been used to investigate genes involved in chitin synthesis and cuticle-hardening enzymes, revealing how subtle genetic modifications can alter the mechanical properties of the beetle's shell (49). These findings have implications for the design of eco-friendly materials with industrial and medical applications (50).

Recent research has also used Coleopterans to test CRISPR toolkits. In *Tribolium*, base editing and homology-directed repair protocols have been refined for high-efficiency knock-ins, providing valuable data for optimizing CRISPR pipelines in other non-model organisms (51). The beetle's genetic robustness and tolerance to CRISPR manipulation make it an ideal testbed for methodological innovation.

While technical challenges remain, such as variable embryonic survival rates in beetles and the difficulty of microinjecting due to hardened beetle eggshells, CRISPR progress in Coleoptera continues to expand rapidly to this day. For instance, Direct Parental CRISPR (DIPA-CRISPR: a CRISPR technique that injects Cas9 protein and guide RNA directly into adult female insects) has already been explored in beetles to mitigate the challenges associated with beetle embryo microinjection (52). Beetle ecological diversity and agricultural relevance ensure that future studies will not only deepen understanding of insect development and evolution but also yield tangible benefits in sustainable pest management, material science, and biotechnology. Beyond solitary insect models, however, CRISPR application in insects has extended to orders with complex life histories and social organizations, furthering the breadth of CRISPR research toward Hymenopterans.

CRISPR APPLICATIONS IN HYMENOPTERANS: ANTS AND BEES

Hymenopterans, an order of insects including bees, ants, and wasps, have become a key focus of CRISPR-based research because they combine significant

ecological relevance with uniquely complex genetics and social behavior. They are widely used as models for invertebrate social behavior and colony-level traits. Still, research can be challenging due to their complex life cycles, haplodiploid sex determination among castes, and social structures. Nevertheless, advancements in gene editing have successfully expanded CRISPR applications to multiple hymenopteran species (53). Significant contributors include the honey bee (*Apis mellifera*), the jewel wasp (*Nasonia vitripennis*), and the ant species *Harpegnathos saltator*.

The Western honey bee (*Apis mellifera*) has long been recognized as a keystone in both agriculture and ecological research. Every year, honey bees pollinate around \$15 billion in crops and vegetable products in the US alone (54). And now, CRISPR is helping scientists unravel the genetic basis of traits critical to bee colony health, such as immunity to disease, social behavior, and pesticide sensitivity. For instance, research supporting such themes becomes increasingly relevant as honey bees face global decline (55). In a more contemporary context, researchers are using CRISPR to explore honey bee resistance mechanisms to common parasites such as *Varroa destructor*, with the long-term goal of improving honey bee resilience and stabilizing global apiculture networks (56).

CRISPR research in Western honeybees has also been used to determine the basis of bee ecology overall, how they develop, and why they behave the way they do. For example, targeted knockouts of the *AmDnmt3* gene, which is involved in DNA methylation, revealed its essential role in caste differentiation and neural development in honeybees (57). Similarly, disruption of *Orco*, a key olfactory co-receptor, impaired honey bee pheromone detection and communication, highlighting the molecular underpinnings of eusocial organization in bees (58). These studies highlight how CRISPR can dissect complex social behaviors into genetic components, advancing human understanding of one of nature's most intricate social systems.

Beyond bees, the parasitoid jewel wasp (*Nasonia vitripennis*) has emerged as an important hymenopteran model for CRISPR development due to its short generation time and accessible embryology. Genome editing in *Nasonia* has been used to explore developmental patterning, sex determination, and evolutionary genomics. Recent adaptations of the DIPA-CRISPR technique have further improved the efficiency and feasibility of genome editing in this species (59). The success of DIPA-CRISPR in *Nasonia* has implications

across Hymenoptera, offering an innovative solution to the technical limitations posed by embryo microinjection in small or socially complex insects.

CRISPR research in ants has also provided valuable insight into the genetic regulation of social behavior and neurobiology. In *Harpegnathos saltator* and *Ooceraea biroi*, CRISPR knockouts of *orco* and other chemosensory genes have revealed how olfactory cues drive caste-specific behavior, communication, and reproduction (60). These findings highlight the power of genome editing in analyzing neural and molecular foundations of eusociality in insects, which helps define the evolutionary success of Hymenoptera as a whole. Furthermore, the colony-based reproductive systems of ants offer unique opportunities to study how gene edits propagate within closed populations, potentially informing future applications in population control or ecological management. Gene drives in species such as *Solenopsis invicta* (Fire Ants) have already been explored as an alternative method to pest management by eliminating polygyne colonies: colonies with multiple queens (61).

Despite modern advances, CRISPR applications in Hymenoptera continue to face ongoing technical and ethical challenges. Haplodiploid inheritance complicates the establishment of stable homozygous lines, while the social structures of their colonies make breeding and maintaining edited lineages labor-intensive. Moreover, because many hymenopterans provide essential ecological services, the idea of gene drives or field releases demands extreme caution in order to avoid severe and potentially irreversible ecological consequences. Nevertheless, the rapid progress achieved in recent years demonstrates the role hymenopteran will continue to play as a pivotal link in genetic mechanisms with social evolution, development, and ecological sustainability. As new delivery systems and gene-editing tools continue to improve, CRISPR applications in these insects hold promise for deepening scientific understanding of eusociality and development, while also supporting efforts to improve resilience and health in managed and ecologically important hymenopteran species. The expanding application of CRISPR across insect orders underscores its immense influence on both applied and basic entomological research, culminating in a broad final synthesis of their impacts.

CONCLUSION

From the suppression of malaria-transmitting mosquitoes to the enhancement of proteins in silk-producing

moth caterpillars, CRISPR-Cas9 has revolutionized the study and manipulation of insect genetics toward the common goal of advancing human health, agriculture, and biotechnology. Across diverse taxa, from dipterans to hymenopterans, the application of this technology has revealed the genetic foundations of disease vector transmission, insect development, social behavior, and evolution, while simultaneously yielding tangible benefits for human health, agriculture, and industry. Despite often being regarded primarily as agricultural or public-health challenges, insects have become indispensable CRISPR platforms and model organisms that bridge fundamental genetic research with many different real-world applications: integrated pest management (IPM), synthetic silk suturing in surgery, etc.

However, a growing depth in research prowess often comes with an equal responsibility toward how researchers use this technology. The ecological risks of gene drives, the ethical questions raised by species alteration, and the technical challenges of precise gene manipulation require thoughtful oversight and international cooperation among the entomology community. As CRISPR application in insects continues to evolve through innovations in base editing, prime editing, and DIPA-CRISPR, its potential to transform both science and society will depend on how responsibly it is applied.

Several limitations should be acknowledged. For field-relevant applications, ecological and evolutionary uncertainty remains substantial: edited traits may have unintended effects on ecosystems, and resistance or compensatory changes can reduce long-term effectiveness. These risks are particularly important for interventions designed to spread through populations. Ethical and governance concerns are most pronounced for gene drives, which may be difficult to reverse and can have cross-border impacts. Responsible development therefore, requires transparent oversight and meaningful engagement with affected communities. Biosafety is also critical. Robust containment and stepwise evaluation, from laboratory studies to confined testing, are necessary to reduce the risk of unintended release or premature translation. Finally, while insects are powerful experimental models, caution is warranted when extrapolating to humans because of differences in physiology and gene regulation; key findings often require validation in complementary systems before generalization.

Ultimately, the use of CRISPR in insects represents a new frontier for genetic engineering, one that deepens

our understanding of life's complexity and provides a tool for promoting sustainability, health, and biodiversity. By integrating these advancements with a common sense of ethical prudence, researchers can ensure that the promise of CRISPR benefits both humanity and the ecosystems humanity shares with the nature surrounding us.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this work.

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