

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(6): 1074-1078
www.biochemjournal.com
 Received: 24-04-2026
 Accepted: 29-05-2026

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A review on CRISPR-Cas genome editing in agricultural entomology and the functional validation of insect resistance traits

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i6m.9402>

Abstract

Genome editing built on the CRISPR-Cas system has moved agricultural entomology from correlative gene expression studies toward direct functional proof. Where researchers once inferred a gene's role in feeding behaviour, host adaptation or insecticide tolerance from expression patterns or RNA interference knockdown, they can now generate stable, heritable mutations in single candidate loci and observe the consequence in an intact insect across generations. This shift has reshaped three areas of pest science simultaneously. First, it has allowed direct testing of point mutations recovered from field resistant populations, converting association into causation for several major insecticide target sites. Second, it has opened a pathway toward genetic pest control through gene drive and precision guided sterile insect approaches, though almost entirely in disease vector mosquitoes rather than crop pests. Third, it has exposed how uneven the toolkit remains across insect taxa, with dipteran and coleopteran model species far ahead of the lepidopteran and hemipteran pests that dominate tropical and subtropical cropping systems including rice, cotton and vegetables. This review draws these threads together, weighing the mechanistic evidence for editing efficiency and specificity against the practical constraints of embryo microinjection, guide RNA design and regulatory uncertainty that still separate laboratory demonstration from field ready technology in insect pest management.

Keywords: CRISPR-Cas9, insect genome editing, insecticide resistance, gene drive, agricultural entomology, functional genomics

Introduction

The discovery that the bacterial CRISPR-Cas9 system could be repurposed as a programmable DNA endonuclease changed molecular entomology within a few field seasons. Jinek *et al.* (2012) ^[8] demonstrated that a single guide RNA could direct Cas9 to cleave a specific DNA sequence *in vitro*, and Doudna and Charpentier (2014) ^[3] laid out how that biochemistry could be adapted for genome engineering in essentially any organism whose embryos or cells could receive the reagents. Insects, with their short generation times, large clutch sizes and decades of transgenesis experience built on P-element and piggyBac vectors, were early adopters. Bassett *et al.* (2013) ^[1] showed that Cas9 delivered by plasmid injection into *Drosophila melanogaster* embryos could generate heritable, multiplexed mutations at efficiencies that RNA interference and zinc finger nucleases had never approached in that species.

For agricultural entomology specifically, the appeal ran deeper than convenience. RNA interference, the dominant reverse genetics tool through the 2000s and early 2010s, gave transient, often incomplete and taxonomically patchy knockdown, and hemipteran pests in particular showed weak or inconsistent RNAi response for reasons still debated among physiologists. A stable, heritable, homozygous null mutation sidesteps most of that uncertainty, and it does so in a way that survives to the next generation without repeated dsRNA application. Reid and O'Brochta (2016) ^[12] framed this transition as a move from correlative to causal insect functional genomics, a description that has held up well as the literature has grown.

1. Delivery Platforms and Editing Efficiency across Insect Taxa

Embryo microinjection remains the default delivery route for CRISPR reagents in insects, largely because it was already established for piggyBac and P-element transgenesis before Cas9 arrived.

The method places Cas9 protein or mRNA together with guide RNA directly into the pole plasm or presumptive germline of a freshly laid egg, and its success depends on egg chorion properties, injection timing relative to blastoderm formation, and the fecundity of the species being handled. Gilles *et al.* (2015) ^[6] achieved efficient, heritable gene targeting in *Tribolium castaneum* using exactly this route, and the beetle's relatively large, tough eggs and well characterised embryology made it, alongside *Drosophila*, one of the technology's earliest non-dipteran successes among insect model organisms.

That success has not translated evenly across the insect world. Lepidopteran and hemipteran pests, which dominate cropping systems across South and Southeast Asia, present eggs that are smaller, softer or laid in clutches that make individual microinjection slow and lossy. Sun *et al.* (2017) ^[13], reviewing CRISPR uptake across arthropods broadly, noted that editing efficiencies reported for economically important Lepidoptera and Hemiptera lagged well behind the dipteran and coleopteran figures, not because the biochemistry of Cas9 cleavage differs between orders but because the practical steps of reagent delivery, embryo survival and screening for mosaic versus germline transmission remain harder to standardise outside a handful of well resourced model species. Taning *et al.* (2017) ^[14] reached a similar conclusion from the applied side, arguing that best practice guidelines for insect CRISPR work had been written largely around dipteran biology and needed deliberate adaptation, rather than direct transfer, before they would serve pest species with different reproductive modes. Alternative delivery routes exist precisely to work around embryo injection's throughput ceiling. Adult injection methods that rely on ovary targeted uptake of ribonucleoprotein complexes avoid embryo handling altogether by dosing the mother and relying on her reproductive tissue to internalise the Cas9 and guide RNA before oocytes are laid down, and symbiont mediated and viral delivery routes are being explored for species whose biology makes direct injection impractical at any life stage. None of these alternatives has yet matched microinjection's precedent or reliability across a wide taxonomic range, and the choice of delivery route for a given pest species is still decided largely by trial and error rather than by a predictive framework linking egg morphology or reproductive biology to expected transformation efficiency (Sun *et al.*, 2017) ^[13].

2. Functional Validation of Insecticide Resistance Alleles

Before genome editing, the standard approach to implicating a point mutation in insecticide resistance was association. A resistant field population would be sequenced at a candidate target site gene, a mutation would be found at elevated frequency relative to susceptible populations, and the mutation would be declared a resistance allele on the strength of that correlation, sometimes reinforced by electrophysiology or heterologous expression in a non-insect system such as *Xenopus* oocytes. That approach cannot distinguish a causal mutation from one that merely hitchhikes alongside the true resistance locus, and it says nothing about whether the mutation is sufficient on its own or requires a genetic background already carrying metabolic resistance mechanisms.

CRISPR knock-in closes that gap by placing the exact field derived mutation into an otherwise susceptible genetic background and measuring the resulting phenotype directly.

Douris *et al.* (2016) ^[4] took this approach with a ryanodine receptor mutation associated with diamide insecticide resistance, introducing the substitution into *Drosophila melanogaster* by homology directed repair and showing that the edited flies displayed reduced sensitivity to diamide compounds relative to unedited controls, which converted a strong statistical association into direct evidence of causation. A later study from the same research group extended the logic to additional resistance associated substitutions and to cross resistance between chemically distinct diamide compounds, again using *Drosophila* as the editing chassis even though the mutations themselves were first identified in field populations of economically damaging Lepidoptera (Douris *et al.*, 2020) ^[5].

The use of *Drosophila* as a surrogate species for this kind of work deserves scrutiny rather than passing acceptance. It is efficient, well characterised and genetically tractable, and it allows a resistance mutation discovered in a pest species with a poorly developed transformation pipeline to be tested quickly. It is not, however, a substitute for confirming the phenotype in the pest species itself, since channel pharmacology, metabolic detoxification capacity and cuticular penetration all differ between a fruit fly and a lepidopteran or hemipteran crop pest, and a mutation that confers strong resistance in the *Drosophila* background need not confer an identical magnitude of resistance once returned to its species of origin. The literature has been candid about this limitation rather than glossing over it, and the defensible position is that *Drosophila* knock-in work establishes causal plausibility, not a finished resistance mechanism, for the pest species where the mutation was originally found.

3. Gene Drive and Population Suppression

The most ambitious application of insect CRISPR work, gene drive, aims to bias inheritance so that an engineered allele spreads through a wild population faster than Mendelian ratios would allow, eventually suppressing or modifying the population as a whole. Champer *et al.* (2016) ^[2] laid out the design logic behind homing based drives, in which Cas9 and a guide RNA cassette insert themselves at their own target site and copy that insertion onto the homologous chromosome during germline repair, converting heterozygotes into homozygotes at rates that can approach one hundred percent in laboratory cage populations.

Almost all of the field relevant demonstrations of this technology sit in disease vector mosquitoes rather than agricultural pests. Hammond *et al.* (2016) ^[7] built a drive targeting female fertility genes in *Anopheles gambiae*, and Kyrou *et al.* (2018) ^[11] refined the approach to target the doublesex gene, achieving complete population collapse in caged mosquito populations within a limited number of generations, a result that generated considerable optimism about the technology's suppression potential. Kandul *et al.* (2019) ^[9] took a different, self limiting route with precision guided sterile insect technique, combining Cas9 mediated sex specific lethality with a genetic system designed to decay out of the population over several generations rather than persist indefinitely, and demonstrated the concept in *Drosophila* as a proof of principle intended for later transfer to true agricultural pests.

The disagreement in this literature is not primarily about whether homing drives work in cage trials; they

demonstrably do, at least in the handful of species where they have been tried. The disagreement concerns whether a self propagating drive is an appropriate tool to release into an open agricultural landscape at all, given that resistance alleles arising from non-homologous end joining repair have repeatedly emerged in cage populations and can stall or reverse drive spread over time, and given that a drive released into a crop pest population offers far less ecological containment than a mosquito drive confined to a defined vector borne disease geography. Self limiting designs of the kind Kandul *et al.* (2019) ^[9] demonstrated are explicitly a response to that concern, trading permanence and spread efficiency for a measure of reversibility, and the agricultural entomology literature has so far leaned toward this more conservative end of the design spectrum rather than toward the self propagating drives pursued in vector control.

A separate strand of gene editing work sidesteps population suppression altogether and instead edits sensory or behavioural loci to disrupt host finding or mating. Kistler *et al.* (2015) ^[10] knocked out the odorant receptor co-receptor Orco in *Aedes aegypti* and showed that the mutant mosquitoes lost much of their attraction to human host cues, a result with obvious downstream relevance to any pest species whose damage depends on chemosensory host location, even though the study itself addressed a disease vector rather than a crop pest.

4. Gaps and Limitations Facing Field Translation

Three practical gaps recur across the applied literature. The first is genomic. Guide RNA design depends on a reasonably complete, well annotated reference genome, and

while flagship crop pests such as the diamondback moth and the brown planthopper now have usable assemblies, many regionally important pest species in South Asian cropping systems do not, which forces researchers either to work from fragmentary transcriptome data or to borrow guide sequences designed against a related model species with uncertain cross species efficiency.

The second gap is methodological standardisation. Taning *et al.* (2017) ^[14] called for consistent reporting of guide RNA efficiency, off-target screening and mosaicism rates across insect CRISPR studies, and that call has been only partially answered; off-target analysis in particular remains inconsistent, with many pest insect studies confirming the intended edit by sequencing the target locus alone rather than surveying the rest of the genome for unintended cleavage, a shortcut that would not pass unremarked in a mammalian genome editing paper.

The third gap is regulatory and social rather than technical. Gene drive and other self spreading genetic control strategies raise transboundary questions that a single national biosafety authority cannot resolve alone, since an engineered insect population does not respect the same borders that regulatory jurisdiction does. Countries with substantial agricultural entomology research capacity, India among them, have working biosafety frameworks for contained laboratory and confined field trial work but limited precedent for open release of a gene drive organism, and that regulatory uncertainty is itself a rational brake on translating cage trial success into field application, independent of any remaining scientific unknowns.

Table 1: Comparative overview of CRISPR-Cas9 applications across model and agriculturally relevant insect species

Species (Order)	Target Locus/Trait	Editing Outcome	Delivery Method	References
<i>Drosophila melanogaster</i> (Diptera)	yellow, white marker loci	Multiplexed heritable knockout; first efficient CRISPR demonstration in an insect	Embryo microinjection, plasmid-based Cas9/gRNA	Bassett <i>et al.</i> , 2013 ^[1]
<i>Drosophila melanogaster</i> (Diptera)	Ryanodine receptor (RyR) target site	Knock-in of field diamide-resistance substitution; reduced diamide sensitivity confirmed	Embryo microinjection, homology-directed repair	Douris <i>et al.</i> , 2016 ^[4]
<i>Drosophila melanogaster</i> (Diptera)	RyR additional substitutions; cross-resistance loci	Functional confirmation of field-derived resistance mutations from lepidopteran pests	Embryo microinjection, homology-directed repair	Douris <i>et al.</i> , 2020 ^[5]
<i>Tribolium castaneum</i> (Coleoptera)	Multiple developmental loci; transgene replacement sites	Efficient heritable gene targeting and precise transgene insertion	Embryo microinjection	Gilles <i>et al.</i> , 2015 ^[6]
<i>Anopheles gambiae</i> (Diptera, vector)	Female fertility genes (AGAP loci)	Homing gene drive with strong population suppression in cage trials	Embryo microinjection, homing drive construct	Hammond <i>et al.</i> , 2016 ^[7]
<i>Anopheles gambiae</i> (Diptera, vector)	doublesex gene	Complete population collapse achieved in caged populations	Embryo microinjection, homing drive construct	Kyrou <i>et al.</i> , 2018 ^[11]
<i>Aedes aegypti</i> (Diptera, vector)	Orco odorant receptor co-receptor	Loss of host-seeking olfactory response in knockout mosquitoes	Embryo microinjection, ribonucleoprotein	Kistler <i>et al.</i> , 2015 ^[10]
<i>Drosophila melanogaster</i> (proof of concept)	Sex-specific lethality and X-shredder cassette	Self-limiting precision guided sterile insect technique demonstrated	Embryo microinjection, transgenic cross	Kandul <i>et al.</i> , 2019 ^[9]
Lepidoptera, pest species generally	Pigmentation, chemosensory and resistance-associated loci	Editing efficiencies reported below dipteran benchmarks; protocols still adapted from model species	Embryo microinjection (variable success)	Taning <i>et al.</i> , 2017 ^[14] ; Sun <i>et al.</i> , 2017 ^[13]
Hemiptera, pest species generally (planthoppers, whiteflies, aphids)	Various candidate loci	Among the least tractable taxa for microinjection-based delivery; few heritable lines reported	Embryo microinjection (low throughput); adult-injection approaches under development	Sun <i>et al.</i> , 2017 ^[13] ; Reid and O'Brochta, 2016 ^[12]
Coleoptera, pest species beyond <i>Tribolium</i>	Various candidate loci	<i>Tribolium</i> protocols provide a template but transfer to field-pest coleopterans remains limited	Embryo microinjection	Gilles <i>et al.</i> , 2015 ^[6] ; Reid and O'Brochta, 2016 ^[12]
Cross-order synthesis	Guide RNA design and off-target screening practice	Reporting standards remain inconsistent across insect CRISPR literature	Not applicable	Taning <i>et al.</i> , 2017 ^[14]

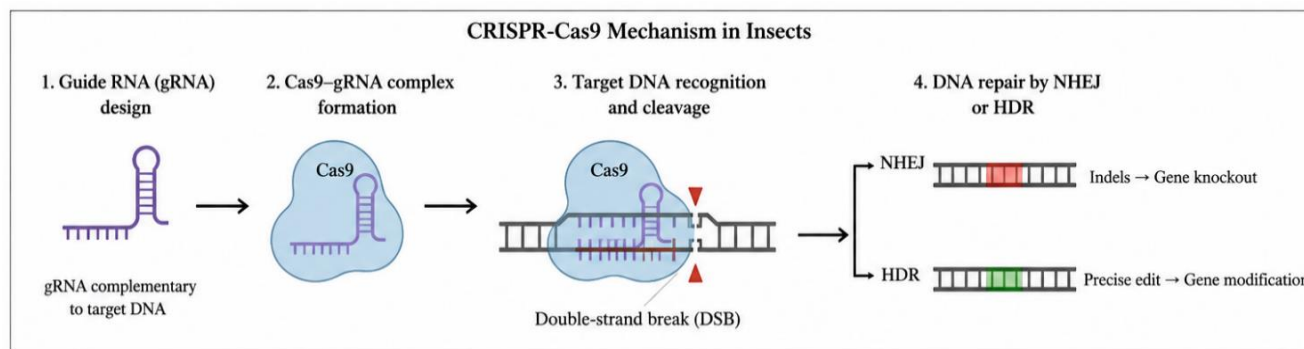


Fig 1: Proposed workflow linking a field-collected insecticide-resistance-associated point mutation to functional confirmation through CRISPR-Cas9 knock-in in a heterologous or native genetic background, integrating the validation logic used in ryanodine receptor and related target-site studies.

Table 2: Delivery methods used to introduce CRISPR-Cas9 reagents into insects

Delivery Method	Life Stage Targeted	Underlying Principle	Constraint
Embryo microinjection (Cas9 mRNA/protein + gRNA)	Pre-blastoderm embryo	Reagents injected into pole plasm/germline anlage before cellularisation	Labour intensive; damages a large fraction of injected eggs; needs species-specific needle and timing optimisation
Plasmid-based Cas9/gRNA injection	Pre-blastoderm embryo	Cas9 expressed transiently from injected plasmid DNA	Lower and more variable editing efficiency than protein or mRNA delivery
Ribonucleoprotein (RNP) complex injection	Pre-blastoderm embryo or adult ovary	Pre-assembled Cas9 protein-gRNA complex injected directly	Requires purified, active Cas9 protein and careful complex assembly
Receptor-mediated ovary transduction (ReMOT-type)	Adult female, pre-oviposition	Cas9/gRNA taken up by developing oocytes via ovary-targeted peptide or ligand	Efficiency and reproducibility still below embryo microinjection in most species tested
Homing drive cassette (self-propagating)	Germline, heritable construct	Cas9/gRNA cassette copies itself onto the homologous chromosome during repair	Susceptible to resistance allele formation via non-homologous end joining
Precision guided sterile insect construct (self-limiting)	Germline, heritable but non-persistent construct	Sex-specific lethality combined with a genetic element designed to decline in frequency over generations	Requires sustained release rather than a single introduction, unlike a self-propagating drive
Symbiont-mediated delivery	Variable, dependent on host-symbiont biology	Obligate or facultative symbiont engineered to express or deliver editing reagents	Limited to species with a tractable, culturable symbiont relationship
Viral vector delivery	Variable	Insect-infecting virus engineered to carry Cas9/gRNA cargo	Cargo size limits and biosafety containment concerns for open agricultural use
Heat-shock or tissue-specific inducible Cas9 transgenic lines	Any stage, conditional	Stable transgenic line expresses Cas9 only on induction or in a defined tissue	Requires an established transgenic pipeline already in place for the species
Maternal Cas9 deposition via genetic cross	Embryo, maternally supplied	Females homozygous for a Cas9 transgene are crossed with gRNA-injected or transgenic males	Adds a generation and a stable transgenic background requirement before editing begins

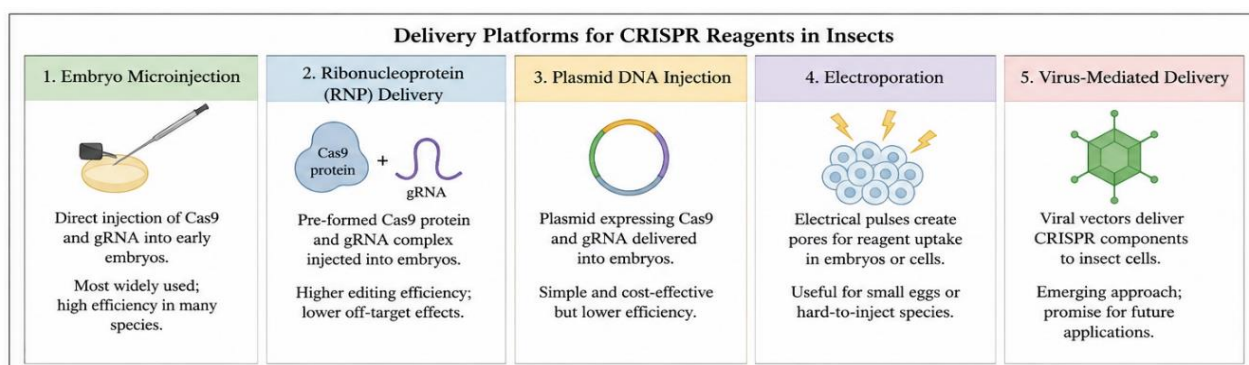


Fig 2: Comparative schematic of self-propagating homing gene drive and self-limiting precision guided sterile insect strategies for genetic pest population control

Conclusion

CRISPR based genome editing has given agricultural entomology a tool that RNA interference and classical genetics could not, a stable and heritable way to convert a candidate gene into a tested one. The clearest gains so far sit in functional validation, turning association between a

mutation and a resistance phenotype into demonstrated causation, and in proof of concept work on genetic pest control strategies that remain, for now, concentrated in disease vector species with better developed transformation pipelines. What separates this technology from routine use in crop pest management is not a missing scientific principle

but an accumulation of practical debt, incomplete reference genomes for regionally important pests, delivery protocols still borrowed rather than purpose built, inconsistent off-target screening, and a regulatory framework that has not yet had to decide how it will treat a self spreading genetic element released into an open agricultural landscape. Closing that debt will take unglamorous protocol work as much as it will take further innovation in editing chemistry, and the pest species that benefit first are likely to be the ones whose research communities invest early in genomic resources and species specific delivery methods rather than waiting for tools built for other insects to become transferable by default.

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