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Gene Drives beyond Proof of Concept: A Critical Review of Molecular Performance, Evolutionary Escape, Ecological Uncertainty, and Release Governance

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ABSTRACT

Gene drives offer a powerful means of altering inheritance to suppress harmful insect populations or introduce traits intended to reduce pathogen transmission. Their development has nevertheless advanced faster than the evidence needed to determine whether molecular success can be translated into durable, spatially predictable, controllable, and publicly legitimate environmental action. This critical review examines the evidentiary gap between proof-of-concept performance and defensible release decision-making. It integrates comparative evidence concerning gene-drive architectures and intended population effects, molecular efficiency and inheritance performance, resistance-allele formation and evolutionary escape, ecological uncertainty and spatial spread, containment and reversal, monitoring, and release governance. The evidence supports a central distinction between demonstrating an inheritance mechanism and establishing readiness for environmental use. High transmission or population suppression in contained systems may establish technical plausibility, but it does not demonstrate stable population modification, predictable movement through heterogeneous landscapes, ecological reversibility, or acceptable release conditions. Results remain contingent on construct architecture, target-site conservation, expression timing, repair outcomes, fitness costs, genetic background, demographic structure, migration, environmental variability, and the quality of monitoring and governance arrangements. The principal limitations of the evidence base are its concentration in laboratory and cage systems, incomplete representation of wild genetic and ecological heterogeneity, short evolutionary observation periods, and limited integration between technical studies and decision processes. Responsible progression therefore requires claim-specific evidence gates rather than a single readiness score. Molecular performance, evolutionary durability, ecological prediction, containment credibility, monitoring capacity, legitimacy, and formal authorization should be evaluated as separate but connected requirements, with explicit uncertainty, failure pathways, and return-to-design conditions.

Keywords: Gene drive, Responsible innovation, Technical readiness, Ecological readiness, Operational feasibility, Reversibility.

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INTRODUCTION

Engineered gene drives are designed to alter inheritance so that a genetic construct, associated phenotype, or population effect can increase more rapidly than expected under ordinary Mendelian transmission. Proposed applications include suppressing disease-vector

or agricultural-pest populations and modifying populations by spreading traits that reduce their capacity to transmit pathogens. Recent reviews converge that engineered gene drives can bias inheritance for vector control, but architecture, fitness costs, resistance, and confinement determine whether laboratory promise translates into durable population effects [1-3].

The central difficulty is not whether biased inheritance can be engineered, but what can legitimately be inferred from each class of evidence. Molecular cleavage, inheritance distortion, multigenerational frequency change, cage suppression, modelled spatial spread, ecological risk assessment, and release authorization represent different constructs measured at different biological and decision scales. A broader synthesis therefore treats gene-drive performance as a multiscale problem spanning molecular design, population dynamics, mitigation, and responsible implementation [4].

Several recurrent equivalences obscure this multiscale problem. High inheritance efficiency is frequently interpreted as evidence of stable population modification even when fitness burdens or resistant alleles alter later trajectories. Laboratory performance is sometimes treated as evidence of spatially predictable spread despite the absence of migration, seasonal forcing, habitat structure, and ecological interactions. Molecular countermeasures may be described as reversal even though removal or inhibition of a construct does not demonstrate restoration of the previous ecological state. Similarly, stakeholder engagement may strengthen the quality and legitimacy of research, but engagement is not equivalent to authorization for environmental release.

This critical review therefore examines how evidence concerning mechanism, performance, evolutionary escape, ecological uncertainty, containment, monitoring, and governance should be connected without collapsing their differences. Its central argument is that progression beyond proof of concept requires independent, claim-specific assessments. A construct may be technically effective yet evolutionarily fragile, spatially uncertain, incompletely containable, operationally unmonitorable, or insufficiently governed. Responsible evidence-to-action translation consequently depends on identifying what each experiment or analysis establishes, which boundary conditions restrict the inference, what competing explanations remain plausible, and what additional evidence is required before movement to a less contained decision stage.

Gene-drive mechanisms and intended population effects

Gene-drive architectures link inheritance bias to population outcomes through different causal mechanisms, and those differences determine what should be measured. Homing suppression drives may copy themselves into a homologous chromosome while disrupting a fertility or viability function. Targeting a conserved female-development locus produced complete suppression in caged *Anopheles gambiae* populations, establishing proof of principle without demonstrating field robustness [5]. Sex-distorting systems use a different route, increasing the transmission or production of males rather than directly imposing female sterility. A sex-distorter drive linked inheritance bias to male-biased progeny, illustrating that suppression can be mediated through altered sex ratio rather than direct female sterility [6]. Both approaches can reduce reproductive capacity in containment, but their sensitivity to mating structure, target conservation, sex-specific fitness, and resistant genotypes is not interchangeable.

Population modification requires another evidentiary distinction because increasing construct frequency is only an intermediate outcome. Population-modification experiments showed that a cargo-bearing drive can rise to high frequency in cages, while leaving field durability and transmission impact unresolved [7]. A cargo must remain genetically linked to the drive, retain biological activity, and generate the intended epidemiological or ecological effect under relevant exposure conditions. Recoded rescue architecture demonstrated one route for coupling drive inheritance to restoration of an essential function, thereby penalizing some nonfunctional resistance outcomes [8]. This design can modify the selective consequences of disruptive repair, but it cannot remove uncertainty associated with functional resistant variants, fitness effects, migration, or ecological competition.

Threshold dependence further changes the relation between molecular action and intended population effect. A toxin-antidote drive produced threshold-dependent dynamics, showing that intended population modification can be coupled to a more localized invasion profile [9]. The resulting localization is conditional rather than absolute: establishment depends on release frequency, fitness, mating, population connectivity, and migration between target and non-target populations. Low-threshold homing systems, high-threshold toxin-antidote systems, recoded rescue systems, cargo-bearing drives, and sex-distorting constructs should therefore not be ranked by a common inheritance measure. Each architecture requires evidence connecting its molecular mechanism to its intended effect, evolutionary failure modes, ecological behavior, monitoring needs, and governance boundary.

The evidence dimensions and interpretive boundaries for gene-drive mechanisms and intended population effects are summarized in **Table 1**.

Table 1. Gene-Drive Mechanisms and Intended Population Effects: Technical Evidence, Ecological Safeguards, Governance Requirements, Monitoring, Residual Risk, and Decision Boundaries

Decision stage or safeguard	Evidence required	Technical function	Ecological function	Governance requirement	Failure or escape risk	Monitoring or learning need	Decision boundary	Representative supporting reference(s)
Architecture definition	Verified target, inheritance mechanism, phenotype, fitness effects, and repair pathways	Establishes how inheritance bias is produced	Identifies the population process expected to change	Require an architecture-specific claim rather than a generic gene-drive label	Mechanisms with different thresholds and burdens may be treated as equivalent	Track construct integrity, inheritance, phenotype, and fitness separately	Mechanistic characterization permits contained testing, not environmental-readiness claims	[4]
Homing suppression through female-function disruption	Multigenerational inheritance, fertility, population trajectory, and resistance data	Copies the drive while disrupting a female-development or fertility locus	Seeks population decline through reduced female reproductive output	Require evidence that suppression is not an artefact of cage structure	Functional resistance, fitness compensation, migration, or ecological density feedback	Sequence target sites and follow sex-specific reproduction and abundance	Complete cage suppression does not establish predictable field suppression	[5]
Sex-ratio distortion	Transmission, chromosome-targeting performance, sex ratio, fertility, and population response	Biases progeny production toward males	Seeks suppression through scarcity of reproductive females	Define whether the decision claim concerns sex distortion or durable population reduction	Resistant targets, incomplete distortion, mating compensation, or sex-specific fitness costs	Monitor sex ratio, mating success, fertility, and resistant genotypes	Male bias is evidence of mechanism, not by itself evidence of population elimination	[6]
Cargo-bearing population modification	Drive frequency, cargo stability, effector function, pathogen phenotype, and fitness	Spreads an linked antipathogen or modifying trait	Seeks reduced vector competence without necessarily reducing abundance	Require separate evidence for construct spread and the public-health or ecological endpoint	Cargo loss, linkage breakdown, resistance, fitness costs, or insufficient biological effect	Track drive frequency, cargo sequence, expression, phenotype, and pathogen outcomes	High cage frequency is not equivalent to stable modification or transmission reduction	[7]
Recoded rescue	Rescue-function validation, inheritance, resistance classification, and multigenerational fitness	Restores an essential function while disadvantaging some disruptive repair products	Seeks durable replacement by changing selection against nonfunctional resistance	Require explicit assessment of functional resistant alleles and residual fitness effects	Functional escape variants, target polymorphism, migration, or ecological selection	Distinguish molecular repair outcomes from organismal function and population fitness	Molecular rescue does not establish ecological persistence or reversibility	[8]

Threshold-dependent toxin-antidote modification	Release-threshold, fitness, migration, and genotype-frequency evidence	Couples a disruptive genetic function to a rescuing antidote	Seeks regional rather than unrestricted population modification	Define the intended geographic and population boundary before testing	Establishment outside the target population or loss within it under different migration conditions	Monitor release frequency, spatial genotype gradients, migration, and local fitness	Threshold dependence supports conditional localization, not guaranteed geographic containment	[9]

Molecular efficiency and inheritance performance

Molecular performance is produced by linked processes rather than a single conversion percentage. Cleavage efficiency, the balance between homology-directed and end-joining repair, promoter timing, guide-target complementarity, construct stability, sex and life stage, and the fitness of resulting genotypes all contribute to observed inheritance. Large-cage testing increased ecological realism relative to small laboratory cages, yet still measured performance in a closed and intensively monitored system [10]. Greater population size and environmental complexity can strengthen evidence that a construct functions beyond simplified crosses, but large cages do not reproduce open migration, climatic variability, community interactions, or the spatial arrangement of natural breeding populations.

Inheritance measurements must also be interpreted as trajectories rather than isolated peaks. Experimental population modification showed that super-Mendelian inheritance can coexist with fitness and resistance dynamics that prevent stable fixation [11]. A drive may initially increase because of strong inheritance bias and subsequently plateau or decline when resistant alleles, cargo burdens, mating disadvantages, or genotype-specific fitness costs accumulate. Separating Cas9 from the guide-RNA cassette preserved inheritance bias while reducing autonomous persistence, demonstrating that efficiency and confinement can be co-designed [12]. Nevertheless, component separation provides conditional confinability, not absolute containment, because performance still depends on component frequencies, genetic linkage, accidental co-occurrence, and the receiving population.

Molecular characterization should consequently address both intended activity and unintended genetic effects. Genome-scale analysis found limited detectable off-target activity for the tested construct, but the inference remains specific to its guide design, assay sensitivity, and genetic backgrounds [13]. Failure to detect substantial off-target change in one construct cannot be generalized to different guides, species, genomic backgrounds, or longer periods of selection. A multiplexed split drive propagated in caged *Aedes aegypti* despite fitness costs, indicating that measured inheritance performance reflects the combined architecture rather than cleavage rate alone [14]. Multiplexing, component separation, cargo effects, and fitness can interact in ways that preserve short-term propagation while leaving long-term persistence unresolved. High inheritance efficiency is therefore not equivalent to stable population modification, and molecular efficiency should be reported alongside genotype-specific fitness, repair outcomes, resistance composition, duration, and population trajectory.

Resistance alleles and evolutionary escape

Resistance begins with molecular variation but becomes consequential through function and selection. Cleavage may be followed by intended copying of the drive, precise or in-frame repair that retains target-gene function, disruptive repair that reduces function, or mosaic outcomes generated by temporally or spatially variable nuclease activity. Longitudinal cage experiments directly showed that cleavage-resistant mutations can be generated and then selected as drive frequency increases [15]. Comparative construct testing demonstrated that resistance formation and conversion efficiency vary with both guide architecture and host genetic background [16]. These findings show why mutation detection, functional resistance, organismal fitness, and population-level escape must be treated as separate claims.

Engineering strategies can alter the probability of particular escape routes without eliminating evolution. Multiplexing and altered expression reduced resistant-allele formation in a model

system, but did not convert evolutionary escape into a closed problem [17]. Multiple guides may reduce dependence on a single target site, yet guide interactions, incomplete cleavage, standing polymorphism, and multi-site repair can introduce additional dependencies. Resistance evolution in a sex-conversion system altered the population outcome, showing that the same molecular architecture can shift from suppression toward persistence or failure [18]. An intended mechanism may therefore remain technically active while its population consequence is eroded by the relative fitness and transmission of resistant, wild-type, and drive-bearing genotypes.

Expression timing connects molecular design to both inheritance and evolutionary selection. Restricting nuclease expression to favorable germline windows reduced embryo end joining and improved drive performance, identifying expression timing as an evolutionary design variable [19]. Germline restriction may favor copying over resistant repair, whereas maternal deposition or embryonic activity may increase mosaicism and end joining. The population importance of each outcome then depends on whether resistance preserves target-gene function, its fitness relative to the drive and wild type, and the demographic and ecological conditions under which selection operates. **Figure 1** depicts gene-drive mechanisms and resistance pathways within the analytical logic developed in this section.

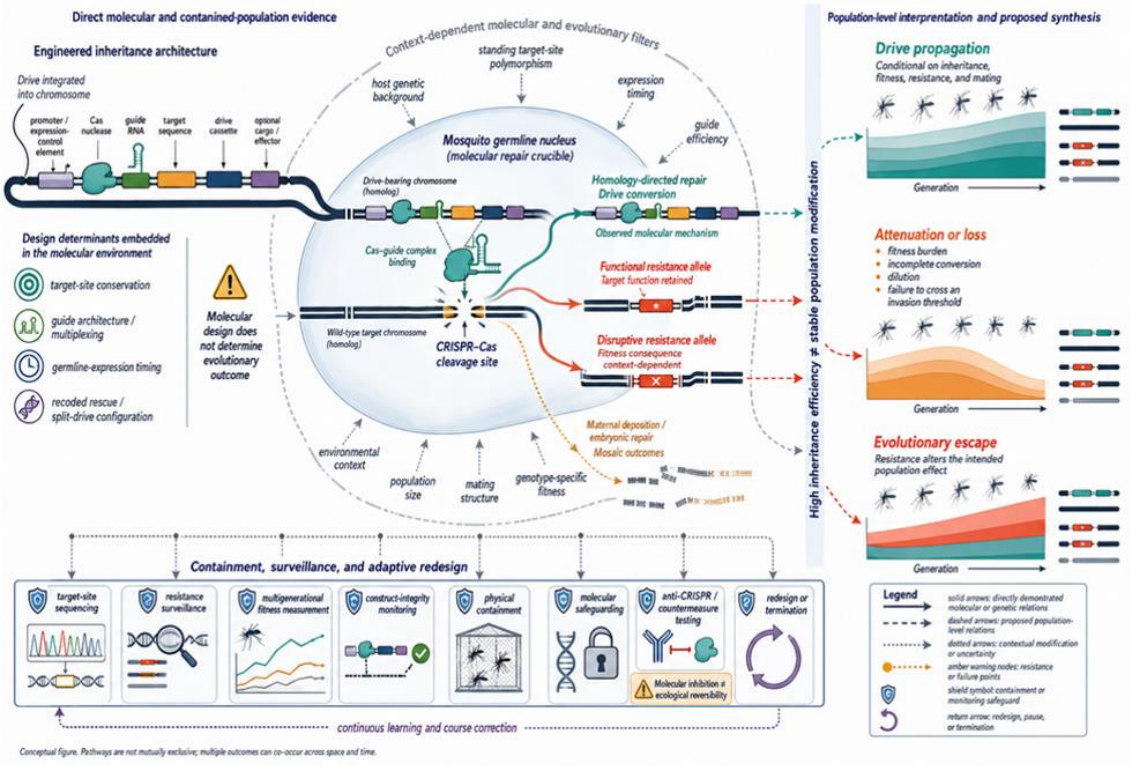

Figure 1. Gene-drive mechanisms and resistance pathways

Alt text

A structured conceptual diagram that depicts gene-drive mechanisms and resistance pathways, with labelled components, directional relations, contextual modifiers, uncertainty points, and a clear boundary between observed evidence and proposed synthesis.

Ecological uncertainty and spatial spread

The transition from contained inheritance to environmental spread is governed by population and landscape processes that molecular measurements do not capture. Population-genetic analysis indicates that low-threshold homing drives may move beyond a release population even when conversion is incomplete or drive-associated fitness is reduced [20]. This finding challenges assumptions that imperfect performance necessarily produces geographic restriction, but it remains model-based and conditional on migration, mating, selection, and population structure. The relevant output is

therefore not a universal prediction of invasion but a set of architecture-specific possibilities requiring empirical parameterization.

Spatial structure can also change the qualitative form of advance. Mathematical analysis shows that gene-drive spread may operate as a pushed genetic wave whose movement depends on local frequency, population density, release size, and barriers rather than inheritance advantage alone [21]. Comparative modelling further demonstrates that localized or nominally self-limiting architectures respond differently to migration: movement between demes may initiate establishment, maintain an otherwise declining construct, or dilute it below its invasion threshold [22]. Localization must consequently be treated as threshold- and context-dependent, not as a permanent property inferred from the architecture's name.

At broader scales, seasonality, habitat connectivity, local abundance, and release geography can produce different suppression and spread outcomes from similar molecular-performance assumptions [23]. The proposed synthesis therefore connects construct-specific inputs to demographic, evolutionary, and spatial processes while retaining explicit uncertainty at every transition. Its outputs are conditional distributions of establishment, persistence, suppression, boundary crossing, and failure rather than a deterministic map. Validation requires longitudinal ecological data, alternative model structures, site-specific dispersal evidence, sensitivity analysis, and prospective comparison between predictions and observed contained or staged outcomes. Laboratory performance is not equivalent to spatially predictable spread. The proposed components, evidence bases, boundary conditions, failure modes, and validation requirements are organized in **Table 2**.

Table 2. Ecological Uncertainty and Spatial Spread: Components, Evidence Basis, Relations, Boundary Conditions, Failure Modes, and Validation Requirements

Proposed component	Purpose	Evidence basis	Relation or mechanism	Input or precondition	Expected output	Boundary condition or failure mode	Validation requirement	Representative supporting reference(s)
Architecture-specific invasion potential	Prevent different drives from being assigned one spatial behavior	Experimental architecture studies and population models	Homing, threshold-dependent, and split systems generate different establishment conditions	Defined mechanism, fitness burden, and invasion threshold	Architecture-conditioned spread scenarios	Treating every drive as equally invasive or equally localizable	Compare architectures under common demographic and migration conditions	[9, 20, 22]
Population trajectory	Translate inheritance into changes in genotype and abundance	Longitudinal cage evidence and ecological modelling	Transmission is filtered through mating, survival, fecundity, density dependence, and competition	Sex- and stage-specific inheritance and fitness distributions	Time-varying genotype and population trajectories	Using peak inheritance as a constant long-term parameter	Calibrate models with multigenerational data across relevant backgrounds	[10, 11, 14]
Resistance emergence and spatial sorting	Represent evolutionary escape within heterogeneous populations	Experimental evolution and population-genetic modelling	Resistant alleles arise locally, differ in fitness, and move between populations	Repair-outcome distributions, standing variation, and genotype fitness	Spatial scenarios for resistance establishment and replacement	Assuming uniform resistance rates or functions	Obtain baseline target-site diversity and test alternative resistance functions	[15, 16, 20]
Density and mating structure	Identify population processes that change establishment or suppression	Spatial theory and ecological modelling	Local density and mating probabilities determine whether an invasion	Abundance, mating range, reproductive timing, and release configuration	Conditional establishment, wave formation, or loss	Well-mixed mating assumptions in structured populations	Measure mating and density processes at the decision-relevant scale	[21, 24]

			threshold is crossed					
Seasonality and habitat connectivity	Connect drive dynamics to changing landscapes	National-scale malaria-vector modelling	Seasonal habitat and movement networks alter local persistence and recolonization	Habitat suitability, climate, connectivity, and release timing	Seasonal and geographically differentiated outcomes	Static landscapes or poorly supported dispersal assumptions	Validate habitat, abundance, and connectivity inputs over multiple seasons	[23]
Boundary crossing	Estimate movement beyond the intended population or jurisdiction	Invasiveness, wave, and connected-deme models	Migration interacts with threshold, fitness, and release frequency	Plausible migration distribution and geographic boundary definition	Probability and timing of movement across boundaries	Equating self-limitation with immobility	Stress-test rare migration and uncertain connectivity	[20-22]
Monitoring-linked updating	Convert uncertainty into adaptive learning	Risk pathways and prospective monitoring frameworks	Observations update model assumptions and activate predefined responses	Baselines, detectable indicators, responsibilities, and decision triggers	Revised forecasts and conditional management action	Surveillance that documents change without enabling response	Test detection limits, reporting pathways, and response capacity before release	[25, 26]

Containment, reversal, and monitoring

Containment, genetic countermeasures, and monitoring act at different points in a possible failure sequence and should not be collapsed into a single safeguard claim. Molecular safeguarding can reduce accidental propagation during laboratory research, modelled reversal constructs may alter drive-frequency trajectories, and anti-CRISPR inhibition can constrain an active drive in cages; however, each intervention acts primarily on construct genetics rather than demonstrating restoration of a prior ecological state [27-29]. Synthetic targets, component separation, physical barriers, and molecular inhibitors may be complementary, but their dependencies and joint failure modes must be examined explicitly.

Large-cage experiments provide stronger evidence than simple crosses that anti-CRISPR mosquitoes can inhibit drive spread under challenging mating and behavioral conditions [30]. Even so, the experiment does not resolve whether a countermeasure could be produced, distributed, and established at the necessary locations and frequencies after environmental spread. Countermeasure success may depend on relative fitness, release timing, mating behavior, spatial access, target compatibility, and the initial drive distribution. Molecular reversal is therefore not equivalent to ecological reversibility: changing allele frequencies does not demonstrate recovery of population structure, community interactions, disease dynamics, or other ecological conditions affected before intervention.

Monitoring is a third function rather than proof that containment or reversal will succeed. Genomic surveillance may detect construct integrity, resistance, or boundary crossing; demographic monitoring may detect suppression, rebound, or replacement; ecological indicators may reveal indirect change; and social or institutional monitoring may identify implementation burdens or failures of accountability. These activities become decision-relevant only when baselines, detection limits, reporting responsibilities, triggers, and feasible responses are specified in advance. A monitoring system that identifies change without enabling timely interpretation or action supplies information but not mitigation. Responsible development therefore requires layered safeguards evaluated separately, with predefined redesign, pause, response, and termination conditions.

Governance, legitimacy, and release decisions

Release governance begins before environmental authorization because design choices determine persistence, reversibility claims, affected populations, and the evidence that later institutions must evaluate. Peer-reviewed guidance characterizes stakeholder engagement as an early, iterative, and context-specific development practice rather than a one-time communication exercise [31]. Reciprocal

knowledge engagement can allow local expertise, priorities, and concerns to alter problem definitions, research questions, comparators, and safeguards [32]. These processes may improve relevance and procedural legitimacy, but neither information exchange nor participation independently establishes acceptable risk or permission to release.

Empirical evidence also cautions against treating expressed support as a unified public mandate. A multi-country survey identified conditional support alongside substantial knowledge gaps and concerns about controllability, consequences, and decision authority [33]. Such findings are important for identifying contested assumptions and communication needs, but they remain sensitive to sampling, framing, institutional trust, and the hypothetical character of the technology. Public engagement is not equivalent to authorization for release, just as a favorable or unfavorable survey response is not a substitute for jurisdiction-specific risk assessment and accountable decision-making.

Engagement becomes institutionally consequential when governance arrangements specify how stakeholder knowledge can change environmental assessment, alternatives, release conditions, monitoring obligations, or the decision not to proceed [34]. The progression from laboratory development to an environmental decision should therefore contain distinct gates for molecular characterization, inheritance and fitness, evolutionary durability, ecological and spatial prediction, containment and countermeasure credibility, monitoring capacity, legitimacy, and formal authorization. Evidence can move between these gates, but no technical metric or composite score can replace their separate judgments. **Figure 2** shows the progression from laboratory development to governance and environmental decision-making within the analytical logic developed in this section.

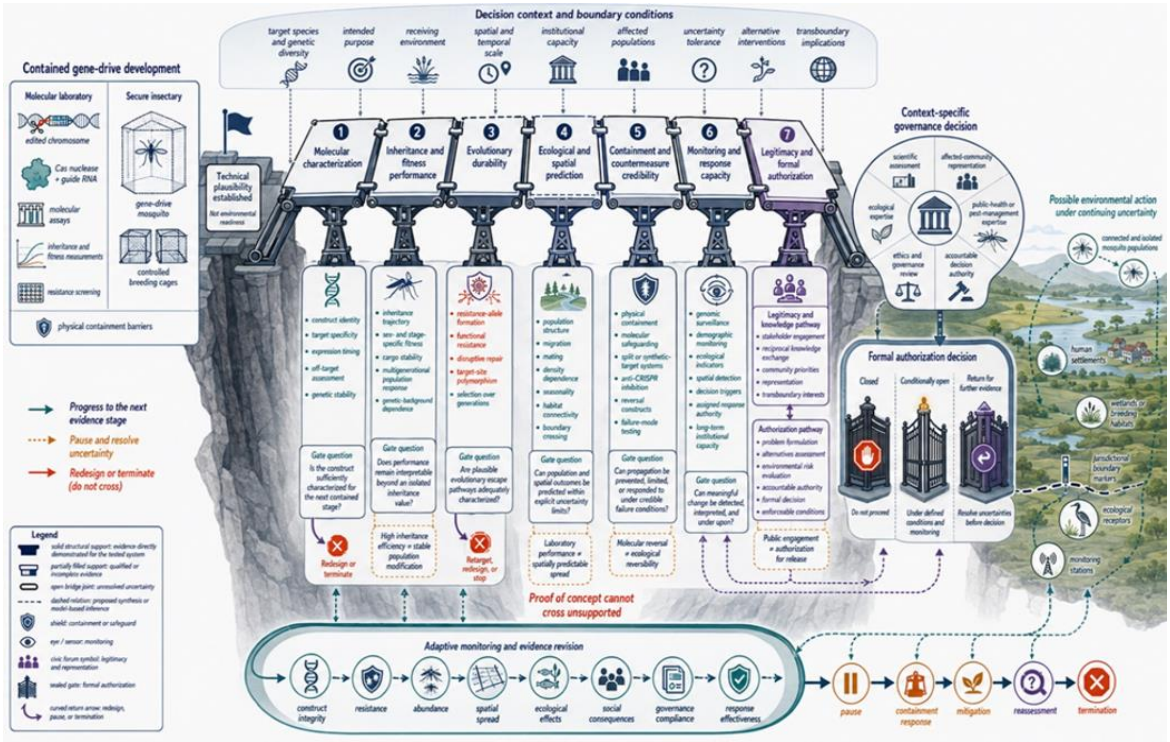

Figure 2. The progression from laboratory development to governance and environmental decision-making

Alt text

A structured conceptual diagram that shows the progression from laboratory development to governance and environmental decision-making, with labelled components, directional relations, contextual modifiers, uncertainty points, and a clear boundary between observed evidence and proposed synthesis.

Critical synthesis and research priorities

Across the reviewed evidence, the strongest convergence concerns conditionality rather than environmental readiness. Molecular architecture

determines how inheritance is biased; fitness and repair determine whether that advantage persists; demography and migration determine how it changes populations; and institutions determine whether the remaining uncertainty is acceptable for a particular decision. Regional problem-formulation exercises demonstrate that credible assessment begins by defining protection goals, valued entities, plausible harms, and relevant comparators before molecular performance is treated as decision-sufficient evidence [35]. This ordering prevents technical achievement from defining the public purpose or acceptable boundary of an intervention by default.

A pathways-to-harm analysis strengthens this approach by making the causal sequence between release and a harmful endpoint explicit [25]. Its value lies in identifying what would have to occur, what evidence could test each step, and where monitoring or mitigation could intervene. A plausible pathway is not evidence that harm will occur, whereas an incomplete observation is not evidence that the pathway is impossible. Environmental risk-assessment recommendations similarly support comparative, problem-formulated, tiered evaluation with explicit treatment of uncertainty [36]. These proposals organize evidence but should not be represented as empirically validated release frameworks.

Monitoring and modelling are priority areas because both are frequently asked to support decisions that exceed their present validation. Prospective monitoring pathways connect indicators to predefined triggers, responsibilities, and response options [26]. Their implementation will require durable funding, interoperable data systems, geographic coverage, validated detection limits, and authority to act when thresholds are crossed. Ecological modelling must likewise incorporate demography, density dependence, mating, dispersal, spatial structure, seasonality, and uncertainty, while avoiding the assumption that additional complexity automatically produces greater predictive validity [24]. Models should discriminate among scenarios, identify influential uncertainties, and be tested against evidence collected at the scale of the intended inference.

Research priorities should therefore be organized around failed equivalences. Stable modification requires longitudinal evidence beyond high inheritance; spatial predictability requires ecological and dispersal validation beyond laboratory performance; ecological reversibility requires recovery endpoints beyond molecular inhibition; and legitimate authorization requires traceable decision authority beyond engagement activity. Priority studies should challenge constructs across genetically diverse populations, extended evolutionary periods, realistic mating and demographic conditions, and alternative model structures. Parallel governance research should examine whose knowledge changes decisions, how transboundary effects alter representation, whether monitoring triggers activate feasible responses, and who retains responsibility for delayed outcomes. The convergent findings, context-dependent results, methodological limitations, and remaining uncertainties are synthesized in **Table 3**.

Table 3. Critical Synthesis and Research Priorities: Convergent Findings, Context Dependence, Methodological Limitations, Evidence Confidence, and Residual Uncertainty

Evidence domain	Convergent finding	Contradictory or context-dependent finding	Study-design basis	Main methodological limitation	Strength of inference	Residual uncertainty	Implication
Gene-drive mechanisms	Architecture determines the route from inheritance bias to suppression or modification	Different systems pursuing similar outcomes have different thresholds, burdens, and failure pathways	Molecular experiments, cage studies, and critical reviews	Concentration in a limited number of constructs and insect systems	Direct for tested mechanisms; restricted for cross-architecture transfer	Performance of each architecture in diverse wild populations	Maintain architecture-specific claims, safeguards, and evidence requirements
Inheritance performance	Super-Mendelian inheritance is repeatedly demonstrable in	Persistence and fixation vary with resistance, cargo burden,	Genetic crosses and multigenerational cage experiments	Short horizons and closed populations	Direct for contained inheritance; uncertain for	Long-term trajectories under ecological	Report distributions, fitness, repair outcomes, and

	contained systems	fitness, and population structure			environmental durability	and genetic heterogeneity	trajectories rather than peak inheritance alone
Molecular efficiency	Guide choice, target sequence, expression timing, and architecture affect conversion and specificity	Low off-target activity or high conversion remains construct- and assay-specific	Molecular assays, sequencing, and promoter comparisons	Detection limits and dependence on laboratory genetic backgrounds	Direct for the assayed construct and conditions	Environmental modulation and transfer across target populations	Develop construct-specific molecular dossiers with explicit assay limits
Resistance and evolutionary escape	Repair-generated resistance can emerge and be selected	Multiplexing, target constraint, rescue, and germline regulation reduce some pathways but do not eliminate escape	Experimental evolution, engineering studies, and population models	Limited duration and incomplete representation of standing wild variation	Direct for resistance mechanisms in tested systems	Field frequencies, functional effects, and long-term selection	Stress-test across diverse genomes and define durability failure conditions
Ecological and spatial dynamics	Demography, migration, density, mating, seasonality, and connectivity can change outcomes	Models differ because structures and assumptions represent different ecological conditions	Population-genetic, wave, connected-deme, and national-scale models	Sparse ecological validation and structural uncertainty	Useful for conditional scenario comparison; insufficient for deterministic prediction	Dominant processes and boundary-crossing behavior at candidate sites	Use model ensembles linked to field ecology and prospective validation
Containment and countermeasures	Molecular safeguards and anti-CRISPR systems can reduce specific propagation pathways	Effectiveness depends on architecture, frequency, timing, fitness, and delivery	Laboratory safeguarding, modelling, small-cage, and large-cage studies	Rare failures, operational deployment, and ecological recovery remain untested	Direct for specific genetic effects in containment	Performance after spatial spread and recovery of ecological states	Use redundant safeguards while keeping containment, inhibition, and recovery claims separate
Monitoring	Preplanned monitoring can connect observations to adaptive decisions	Detection may be delayed, geographically incomplete, ambiguous, or disconnected from response authority	Problem-formulation and monitoring-framework studies	Absence of post-release experience with gene-drive mosquitoes	Strong as planning logic; unvalidated as an operational system	Indicator sensitivity, response feasibility, and long-term institutional capacity	Fund, test, and assign authority for monitoring before any release decision
Governance and legitimacy	Early reciprocal engagement and structured assessment are broadly supported	Representation, authority, institutional uptake, and public views vary by context	Guidance, governance analysis, consultations, and surveys	Hypothetical technology, bounded samples, and jurisdictional differences	Supports procedural principles, not generalized authorization	How participation changes final decisions and accountability	Separate engagement, risk evaluation, legitimacy, and formal authorization

CONCLUSION

Gene drives have moved beyond the question of whether inheritance can be biased, but they have not moved beyond the need for construct-specific, multiscale, and decision-specific evidence. The strongest defensible synthesis is that molecular performance establishes technical plausibility only within the tested architecture and context. Evolutionary durability remains conditional on repair, fitness, target variation, and selection; ecological and spatial behavior remains conditional on demography, migration, seasonality, and landscape structure; and safeguards remain conditional on delivery, compatibility, monitoring, and institutional response. Consequently, high inheritance efficiency is not stable population modification, laboratory performance is not spatial predictability, molecular reversal is not ecological reversibility, and engagement is not authorization. The highest-priority implication is

to replace generalized readiness narratives with independent evidence gates that specify the claim being evaluated, its boundary conditions, its failure pathways, and the evidence required for progression, redesign, pause, or termination. Such an approach does not presume that environmental release is warranted or unwarranted. It establishes the disciplined reasoning needed to decide what has been demonstrated, what remains uncertain, and which uncertainties must be resolved before less contained action can be responsibly considered.

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REFERENCES

1. Wang GH, Hoffmann AA, Champer J. Gene drive and symbiont technologies for control of mosquito-borne diseases. Annu Rev Entomol. 2025;70(1):229-49. doi:10.1146/annurev-ento-012424-011039
2. McFarlane GR, Whitelaw CBA, Lillico SG. CRISPR-based gene drives for pest control. Trends Biotechnol. 2018;36(2):130-3. doi:10.1016/j.tibtech.2017.10.001
3. Chen W, Yang X, Guo J, Han Y, Champer J. Gene drives and other transgenic approaches for mosquito control. Trends Parasitol. 2026;42(7):647-61. doi:10.1016/j.pt.2026.05.009
4. Bier E. Gene drives gaining speed. Nat Rev Genet. 2022;23(1):5-22. doi:10.1038/s41576-021-00386-0
5. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, et al. A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nat Biotechnol. 2018;36(11):1062-6. doi:10.1038/nbt.4245
6. Simoni A, Hammond AM, Beaghton AK, Galizi R, Taxiarchi C, Kyrou K, et al. A male-biased sex-distorter gene drive for the human malaria vector Anopheles gambiae. Nat Biotechnol. 2020;38(9):1054-60. doi:10.1038/s41587-020-0508-1
7. Carballar-Lejarazú R, Ogaugwu C, Tushar T, Kelsey A, Pham TB, Murphy J, et al. Next-generation gene drive for population modification of the malaria vector mosquito, Anopheles gambiae. Proc Natl Acad Sci U S A. 2020;117(37):22805-14. doi:10.1073/pnas.2010214117
8. Adolfi A, Gantz VM, Jasinskiene N, Lee HF, Hwang K, Terradas G, et al. Efficient population modification gene-drive rescue system in the malaria mosquito Anopheles stephensi. Nat Commun. 2020;11(1):5553. doi:10.1038/s41467-020-19426-0
9. Champer J, Lee E, Yang E, Liu C, Clark AG, Messer PW. A toxin-antidote CRISPR gene drive system for regional population modification. Nat Commun. 2020;11(1):1082. doi:10.1038/s41467-020-14960-3
10. Hammond A, Pollegioni P, Persampieri T, North A, Minuz R, Trusso A, et al. Gene-drive suppression of mosquito populations in large cages as a bridge between lab and field. Nat Commun. 2021;12(1):4589. doi:10.1038/s41467-021-24790-6
11. Pham TB, Phong CH, Bennett JB, Hwang K, Jasinskiene N, Parker K, et al. Experimental population modification of the malaria vector mosquito, Anopheles stephensi. PLoS Genet. 2019;15(12):e1008440. doi:10.1371/journal.pgen.1008440
12. Li M, Yang T, Kandul NP, Bui M, Gamez S, Raban R, et al. Development of a confinable gene drive system in the human disease vector Aedes aegypti. Elife. 2020;9:e51701. doi:10.7554/eLife.51701
13. Garrood WT, Kranjc N, Petri K, Kim DY, Guo JA, Hammond AM, et al. Analysis of off-target effects in CRISPR-based gene drives in the human malaria mosquito. Proc Natl Acad Sci U S A. 2021;118(22):e2004838117. doi:10.1073/pnas.2004838117
14. Anderson MAE, Gonzalez E, Edgington MP, Ang JXD, Purusothaman DK, Shackleford L, et al. A multiplexed, confinable CRISPR/Cas9 gene drive can propagate in caged Aedes aegypti populations. Nat Commun. 2024;15(1):729. doi:10.1038/s41467-024-44956-2
15. Hammond AM, Kyrou K, Bruttini M, North A, Galizi R, Karlsson X, et al. The creation and selection of mutations resistant to a gene

drive over multiple generations in the malaria mosquito. PLoS Genet. 2017;13(10):e1007039. doi:10.1371/journal.pgen.1007039
16. Champer J, Reeves R, Oh SY, Liu C, Liu J, Clark AG, et al. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLoS Genet. 2017;13(7):e1006796. doi:10.1371/journal.pgen.1006796
17. Champer J, Liu J, Oh SY, Reeves R, Luthra A, Oakes N, et al. Reducing resistance allele formation in CRISPR gene drive. Proc Natl Acad Sci U S A. 2018;115(21):5522-7. doi:10.1073/pnas.1720354115
18. Carrami EM, Eckermann KN, Ahmed HMM, Sánchez C HM, Dippel S, Marshall JM, et al. Consequences of resistance evolution in a Cas9-based sex conversion-suppression gene drive for insect pest management. Proc Natl Acad Sci U S A. 2018;115(24):6189-94. doi:10.1073/pnas.1713825115
19. Hammond A, Karlsson X, Morianou I, Kyrou K, Beaghton A, Gribble M, et al. Regulating the expression of gene drives is key to increasing their invasive potential and the mitigation of resistance. PLoS Genet. 2021;17(1):e1009321. doi:10.1371/journal.pgen.1009321
20. Noble C, Adlam B, Church GM, Esvelt KM, Nowak MA. Current CRISPR gene drive systems are likely to be highly invasive in wild populations. Elife. 2018;7:e33423. doi:10.7554/eLife.33423
21. Tanaka H, Stone HA, Nelson DR. Spatial gene drives and pushed genetic waves. Proc Natl Acad Sci U S A. 2017;114(32):8452-7. doi:10.1073/pnas.1705868114
22. Dhole S, Vella MR, Lloyd AL, Gould F. Invasion and migration of spatially self-limiting gene drives: A comparative analysis. Evol Appl. 2018;11(5):794-808. doi:10.1111/eva.12583
23. North AR, Burt A, Godfray HCJ. Modelling the potential of genetic control of malaria mosquitoes at national scale. BMC Biol. 2019;17(1):26. doi:10.1186/s12915-019-0645-5
24. Kim J, Harris KD, Kim IK, Shemesh S, Messer PW, Greenbaum G. Incorporating ecology into gene drive modelling. Ecol Lett. 2023;26(Suppl 1):S62-80. doi:10.1111/ele.14194
25. Connolly JB, Mumford JD, Fuchs S, Turner G, Beech C, North AR, et al. Systematic identification of plausible pathways to potential harm via problem formulation for investigational releases of a population suppression gene drive to control the human malaria vector Anopheles gambiae in West Africa. Malar J. 2021;20(1):170. doi:10.1186/s12936-021-03674-6
26. Ogoyi DO, Njagi J, Tonui W, Dass B, Quemada H, James S. Post-release monitoring pathway for the deployment of gene drive-modified mosquitoes for malaria control in Africa. Malar J. 2024;23(1):351. doi:10.1186/s12936-024-05179-4
27. Champer J, Chung J, Lee YL, Liu C, Yang E, Wen Z, et al. Molecular safeguarding of CRISPR gene drive experiments. Elife. 2019;8:e41439. doi:10.7554/eLife.41439
28. Vella MR, Gunning CE, Lloyd AL, Gould F. Evaluating strategies for reversing CRISPR-Cas9 gene drives. Sci Rep. 2017;7(1):11038. doi:10.1038/s41598-017-10633-2
29. Taxiarchi C, Beaghton A, Don NI, Kyrou K, Gribble M, Shittu D, et al. A genetically encoded anti-CRISPR protein constrains gene drive spread and prevents population suppression. Nat Commun. 2021;12(1):3977. doi:10.1038/s41467-021-24214-5
30. D'Amato R, Taxiarchi C, Galardini M, Trusso A, Minuz RL, Grilli S, et al. Anti-CRISPR Anopheles mosquitoes inhibit gene drive spread under challenging behavioural conditions in large cages. Nat Commun. 2024;15(1):952. doi:10.1038/s41467-024-44907-x
31. Thizy D, Emerson C, Gibbs J, Hartley S, Kapiriri L, Lavery J, et al. Guidance on stakeholder engagement practices to inform the development of area-wide vector control methods. PLoS Negl Trop Dis. 2019;13(4):e0007286. doi:10.1371/journal.pntd.0007286
32. Hartley S, Thizy D, Ledingham K, Coulibaly M, Diabaté A, Dicko B, et al. Knowledge engagement in gene drive research for malaria control. PLoS Negl Trop Dis. 2019;13(4):e0007233. doi:10.1371/journal.pntd.0007233

33. Finda MF, Juma EO, Kahamba NF, Mthawanji RS, Sambo M, Emidi B, et al. Perspectives of African stakeholders on gene drives for malaria control and elimination: A multi-country survey. Malar J. 2023;22(1):384. doi:10.1186/s12936-023-04787-w
34. Hartley S, Kokotovich A, McCalman C. Prescribing engagement in environmental risk assessment for gene drive technology. Regul Gov. 2023;17(2):411-24. doi:10.1111/rego.12452
35. Teem JL, Ambali A, Glover B, Ouedraogo J, Makinde D, Roberts A. Problem formulation for gene drive mosquitoes designed to reduce malaria transmission in Africa: Results from four regional consultations 2016-2018. Malar J. 2019;18(1):347. doi:10.1186/s12936-019-2978-5
36. Connolly JB, Mumford JD, Glandorf DCM, Hartley S, Lewis OT, Evans SW, et al. Recommendations for environmental risk assessment of gene drive applications for malaria vector control. Malar J. 2022;21(1):152. doi:10.1186/s12936-022-04183-w