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Y-linked Genome Editor (YLE) a Self-Limiting Genetic Control Tool: An Updated Review

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Abstract— *Invasive species are increasingly impacting forests, food, fisheries, and agriculture, with global economic repercussions. The majority of vertebrate pest management methods lack effective strategic control tools and are unsustainable and unsuitable for effective landscape-scale management. The application of genetic techniques is intended to prevent invasive populations. Employment of a mix of strategies has enabled sustained control, depending on the species, region, and degree of dissemination. Advantages of the genetic methods include species specificity, long-term and self-sustainability, 100% trait transferability, minimum infrastructure requirement, and non-requirement of physical modification of the landscape. This renders these methods feasible and applicable globally to control a large number of invasive pests. The possibility of using gene drive technology in conjunction with conventional control techniques opens new horizons in the management of vertebrate invasive populations. Since these techniques are environmentally suitable and non-polluting, they are suited for a green economy. The success of these methods is currently illustrated in the control of invasive species across vast geographic regions, such as Asian carp in the Mississippi River, lionfish in the Caribbean, and zebra mussels in the Great Lakes. Thus, judiciously designed experimental and field study methods suited for a particular pest will enable long-term goals of control and sustenance of invasive pest control.*

Keywords— Sex reversal; CRISPR/Cas9; autosomal sex distorter (ASD); Y-Chromosome Erasure (Y-CUT).

I. INTRODUCTION

Globally, invasive species are increasingly impacting forestry, food, fisheries, and agriculture resources [1]. Additionally, they have detrimental effects on human health, endanger the preservation of biological diversity, and have dire global economic repercussions [2], [3]. The transportation of vertebrate pests by humans has significantly impacted agricultural productivity and contributed to species extinction. "An alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" is the definition of an invasive species. The financial, environmental, and social costs associated with the majority of invasive species are difficult to measure and are rarely reported. According to Ruscoe et al. [4], invasive European carp cost $11.8 million, feral cats cost $144 million, and foxes cost $190 million annually. Once invasive species have established themselves, it is frequently impossible to eradicate them. Sometimes invasive species can be contained at the original introduction location and eliminated before they spread outside of the boundaries of the area where eradication procedures work. In other situations, management procedures are invested to stop further spread rather than eradication [5].

The release of organisms with genetic techniques intended to prevent invasive populations from reproducing is known as genetic biocontrol. Insects, plants, animals, fungi, and bacteria have been shown to be controlled by gene drives [6]. Irradiation and hormonal sex reversal to produce sterile or sexually incompatible genotypes are the conventional methods of control [7], [8]. The use of naturally existing genotypes, parasitic microorganisms that skew sex ratios, and genetic engineering technology are examples of newer biological methods [9], [10]. In order to achieve population management, a mix of strategies is usually needed for sustained control, depending on the species, region, and degree of dissemination. When it comes to larger-scale application, which is necessary to effectively manage widespread established pests, the traditional methods are expensive and unfeasible. As a result, there is a lot of interest in the possibility of using gene drive technology in conjunction with conventional control techniques to manage vertebrate invasive populations [11]. Although these techniques have the potential to manage or even completely eradicate invasive species, an important caveat is that they must minimize environmental damage. Advantages include species specificity, which solely depends on the target organisms' normal mating behavior [12]. Next, these techniques are long-term and self-sustaining, and they do not require physical modification of the landscape infested by the invasive species [13]. CRISPR-based suppression gene drives guarantee 100% trait transfer [14]. Gene drive is effective, requires minimum infrastructure, and targets several invasive species [15]. Table-1 is a comparison of chemical and biological methods of control. Studies have shown that these techniques are effective in controlling invasive mussels [16] and have been proved suitable for eliminating invading rodent populations on islands [17]. The method is based on the rationale that a recessive mutation resulting in infertility or death could be disseminated by gene drives intended for population control [18]. An alternative strategy is to design a gene drive that achieves population suppression by biasing offspring sex ratios so that one sex becomes limiting [19]. Recently, elimination of the murine Y chromosome in cultured cells and embryos has been achieved using a CRISPR/Cas9 system adapted from *Streptococcus pyogenes* (Sp) incorporating gRNA(s) that target multiple chromosomal locations simultaneously [20]. In summary, technologies such as genetically engineered gene drive constructs or genetically engineered sterility systems under the umbrella of genetic biocontrol exist on a continuum and provide opportunities for the control and potential eradication of invasive species based on our knowledge of inheritance [21]. With this background, the current review aims to update the current literature on genetic biocontrol of pests. We searched literature from PubMed, ScienceDirect, ResearchGate, and Google Scholar databases. Few reviews and published research and review papers were also used for literature collation. We did not include any specific criteria, since our aim was to update the current knowledge.

TABLE 1
COMPARISON OF CHEMICAL AND BIOLOGICAL METHODS OF CONTROL

Method	Function	Effectiveness	Cost	Pros and cons	Containment and control
Toxicants	Internal haemorrhage	50% mice, 5–10% rats	Millions of USD	Established and successful method; secondary effects on non-targets; human toxicity; fixed costs	Geographic containment; degradation over time
Gene drives	t-haplotype, CRISPR	Unknown – modelling suggests success	Currently millions of USD, likely to decrease	Species specific; non-toxic; containment issues	Geographic containment; threshold dependent

II. GENETIC BIOCONTROL OF PEST-BASICS, METHODS, ADVANTAGES AND LIMITATIONS

Genetic biocontrol is an emerging strategy for reducing pest and disease-vector populations by modifying inherited genetic traits that reduce survival, fertility, or reproductive capacity [22]. Burt et al. [23] suggest the design of a self-limiting genetic tool, termed the Y chromosome-linked genome editor (YLE). YLE is advantageous because it is designed to combine relatively efficient suppression with a potentially restricted spatial and temporal effect. YLEs have a unique combination of features and are a useful addition to the menu of options in the design and development of pest control programmes because of their distinctive features [24]. The current methods of genetic control are summarized in Figures 1 and 2.

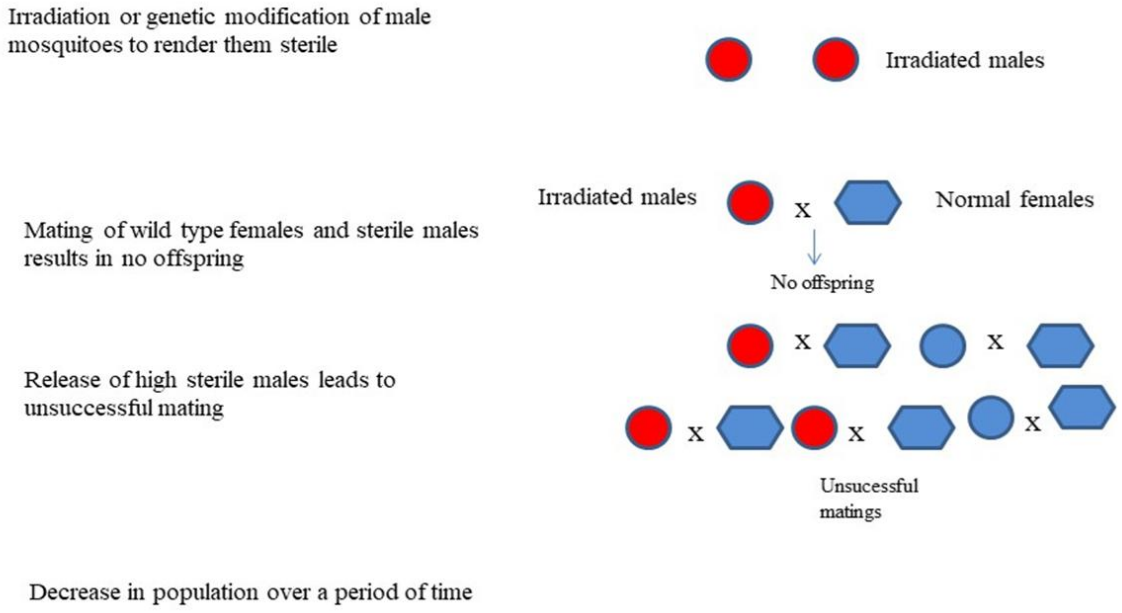


FIGURE 1: An illustration of Insect sterile method for control of invasive mosquitoes

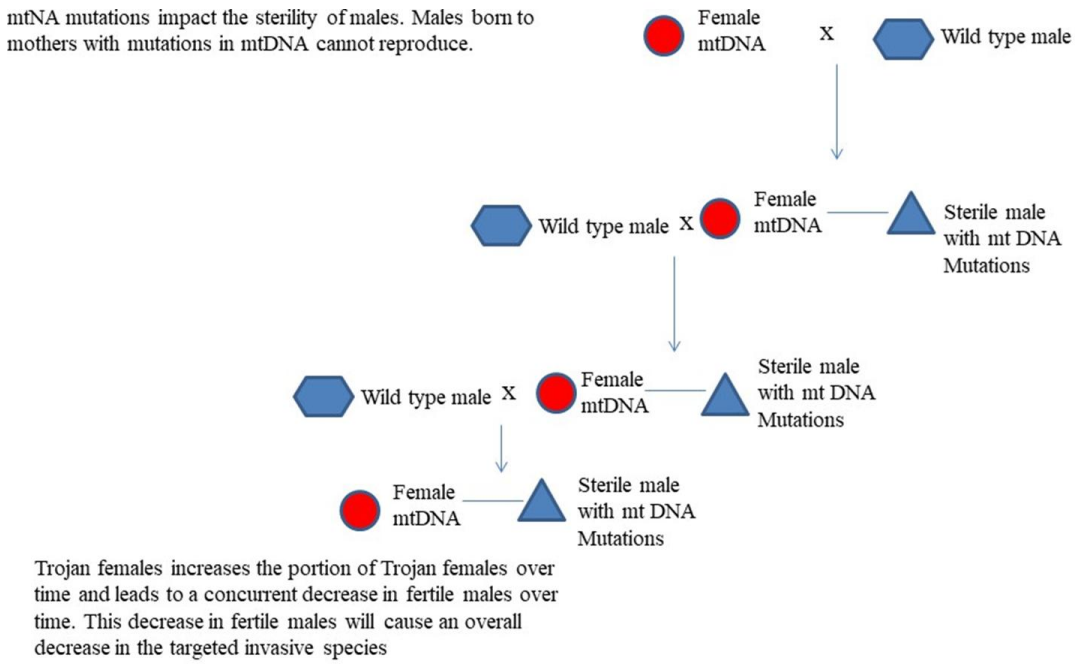


FIGURE 2: Trojan Y female statergy of biological control of invasive species

The YLE concept was initially proposed to address some of the limitations of conventional self-limiting genetic-control methods, which require repeated releases to maintain population suppression [25]. YLE is a self-limiting genetic biocontrol strategy designed to suppress pest populations by exploiting the inheritance pattern of the Y chromosome. It represents a potentially powerful strategy for population suppression in species with an XY sex-determination system. A genetic construct found on the Y chromosome serves as the foundation for this technology. The YLE consists of a CRISPR-Cas9 construct that is always inherited by males and produces an autosomal dominant mutation that is passed on to more than 90% of the progeny [26]. This mutation causes female-specific sterility or lethality in female progeny but has no effect on the male offspring or the originator male [27]. In either case, the YLE is not itself selected against due to the mutations it creates, because the Y is not found in those females. Thus, repeated releases in successive generations can lead to a gradual increase in the frequency of the YLE and the reproductive load imposed on the population, rather than starting from zero each generation, as is the case with sterile males [28]. The experimental proof of concept is demonstrated in the malaria mosquito *Anopheles gambiae* [23].

2.1 Methodology

The methodology of a Y-linked genome editor (YLE) involves combining molecular genetics, genome editing, reproductive biology, and population modelling to achieve the targeted pest suppression. The first step is to select a suitable pest or vector with an XY sex-determination system and identify a gene whose disruption produces a strong female-specific effect, such as reduced fertility or viability. A genome-editing system, such as CRISPR-Cas9, is then associated with the Y chromosome so that the editor is transmitted predominantly through male descendants. In the experimental *Anopheles gambiae* system, researchers have developed a Y-linked Cas9 construct that acts in the male germline and targeted the female-specific region of the doublesex (*dsx*) gene [29]. The resulting genetic change is inherited at a high frequency and causes female-specific sterility. In *Anopheles* mosquitoes, sex is determined by the inheritance of the Y chromosome [30]. Genetic constructs inducing female-specific lethality or sterility are ideal for Y chromosome linkage because the Y is absent in females, so no negative selection acts against the construct [31]. Consequently, a Y-linked construct could persist indefinitely at the released frequency without male-associated fitness costs [32]. Unlike population suppression strategies such as sterile insect technology (SIT) or Release of Insects carrying a Dominant Lethal (RIDL), where populations rapidly rebound after releases cease [33], YLE constructs maintain sustained suppression over time [34]. Notably, the efficacy of YLEs depends critically on the dominant penetrance of the induced mutations, editing efficiency, and the generation of recessive mutations, which can significantly diminish their suppressive impact [35]. This strategy in *Anopheles gambiae* is based on the integration of the CRISPR-based construct into the Y chromosome to drive a dominant female-sterilising allele at the autosomal *dsxF* locus [29]. YLEs have the potential to bias inheritance and distort the sex ratio in favour of males and contain a nuclease that disrupts a female-essential fertility or viability gene, ultimately leading to population elimination in mice [36].

According to computational modelling, this strategy might be ten times less effective than sterile male releases. Modelling also showed that further efficiencies could be gained by releasing the YLE along with an autosomal sex distorter (ASD) that favours transmission of the Y [37]. The ASD mimics the effect of a greater release by increasing the YLE's transmission rate into the next generation when released in the same males [38]. In theory, a very effective control system needing only low frequency/population release might be achieved by directly attaching a sex distorter on the Y chromosome to form a Driving Y [39]. Such a construct would disrupt the X chromosome's transmission, favouring its own transmission, rather than interfering with the survival of offspring inheriting the X, as YLE would. If everything else were equal, the Driving Y would become increasingly frequent over the course of subsequent generations, thereby increasing the male bias in the sex ratio and suppressing or even eliminating the population [40]. According to mathematical modelling, YLE technology can decrease populations up to seven times more effectively than the optimal versions of other self-limiting techniques, although resistance evolution, ecological effects, genetic stability, regulatory requirements, and field performance require further investigation [26]. Simulations suggest that YLEs are capable of achieving elimination within a short timeframe for modest release sizes, and elimination could be achieved for YLEs targeting both haploinsufficient and haplosufficient target genes [41].

2.2 Advantages and limitations

A Y-linked genome editor (YLE) represents a promising approach to genetic biocontrol of insect pests, particularly species with an XY sex-determination system [42]. Its major advantage is the ability to combine male-line inheritance with female-specific population suppression. Because the Y chromosome is transmitted from male parent to male offspring, a Y-linked editing system can be maintained predominantly within male lineages, while the genetic alteration generated by the editor can affect genes important for female fertility or viability [24]. This can reduce the reproductive capacity of the pest population without requiring the continuous application of chemical pesticides. YLEs may also provide more persistent suppression than conventional sterile insect techniques because the genetic element can be transmitted through successive male generations [43]. The approach has the potential to be relatively species-specific and geographically restricted, which may reduce some of the environmental concerns associated with broad-spectrum insecticides and some self-sustaining gene-drive systems [44], [45]. Mathematical modelling and experimental research in *Anopheles gambiae* have demonstrated the potential of Y-linked editing to produce strong population suppression under controlled conditions [46]. However, these benefits depend on the genetic characteristics of the target species, fitness costs, evolution of resistance, ecological effects, containment, and regulatory approval, all of which must be carefully evaluated before practical application [26]. Table 2 is a summary of the advantages of Y-Linked Genome Editors. YLEs have few limitations that must be considered before their application in pest or vector management. A major technical limitation is the complex structure of the Y chromosome, which is often highly repetitive and heterochromatic, making precise genetic manipulation and stable transgene expression difficult [47]. Y-linked transgenes may also experience gene silencing, position-dependent variation in expression, and reduced expression during meiosis [48]–[50]. Another important limitation is the possibility of fitness costs in YLE-carrying males. Multigenerational cage trials in the *Anopheles gambiae* experimental system revealed an estimated 22% decrease in male fitness, which led to a decrease in the

frequency of YLE males and a recovery of the female population following a single release [51], [52]. Genetic resistance is another concern because genome-editing activity can generate resistant or recessive mutations at the target site; such mutations may reduce the efficiency of population suppression and can accumulate over generations [53]. The effectiveness of YLEs is also highly dependent on the species' reproductive biology, target-gene characteristics, inheritance efficiency, population structure, and mating dynamics, so results from one species cannot automatically be generalized to other pests [23]. In addition, development and large-scale production of genetically modified insects can be technically complex and costly, while environmental deployment would require rigorous assessment of ecological effects, containment, monitoring, ethical considerations, and regulatory approval [54]–[56]. Therefore, although Y-linked genome editors offer a promising genetic-control approach, their technical complexity, fitness costs, resistance evolution, species-specific limitations, and ecological and regulatory uncertainties currently restrict their practical applications.

TABLE 2
ADVANTAGES OF Y-LINKED GENOME EDITORS IN GENETIC BIOCONTROL OF PESTS

Sl. No.	Advantage	Explanation	Research significance	References
1	Male-line inheritance	The genome editor is associated with the Y chromosome and can therefore be transmitted predominantly from paternal lineage to male offspring in XY species.	Allows the genetic-control system to remain associated with the male lineage.	[23]
2	Female-specific population suppression	The editing system can be designed to generate genetic changes that reduce female fertility or viability.	Suppression of reproductive females can substantially reduce population growth.	[27]
3	Reduced need for repeated releases	The Y-linked element can potentially persist through successive male generations.	Could reduce the frequency of interventions compared with conventional sterile-insect approaches.	[28]
4	High population-suppression potential	Modelling and experimental work in *Anopheles gambiae* indicate that Y-linked editing can substantially reduce reproductive female numbers under appropriate conditions.	Provides a potential alternative genetic strategy for pest and vector suppression.	[23]
5	Species-specific potential	The system can be developed around genetic and reproductive characteristics of the target species.	May reduce effects on non-target organisms compared with broad-spectrum pesticides, although specificity must be experimentally demonstrated.	[31]
6	No chemical residue	Genetic biocontrol does not depend on repeated application of conventional insecticides.	May complement integrated pest-management programmes and reduce chemical dependence.	[24]
7	Integration with modern genome editing	CRISPR-based technologies provide a flexible platform for studying and modifying genes involved in pest reproduction.	Expands the genetic-biocontrol toolbox beyond conventional SIT and other methods.	[23]
8	Potentially longer-lasting effects	If the Y-linked element has limited fitness costs in males, it may persist across generations.	Could provide more sustained suppression than methods in which the control trait disappears rapidly.	[26]
9	Alternative to conventional genetic-control methods	YLEs provide a different mechanism from sterile-insect techniques, RIDL, X-shredding and self-sustaining gene drives.	Offers additional options for species-specific population management.	[26]
10	Research value for vector control	The approach is particularly relevant to disease vectors such as *Anopheles gambiae*, where reducing females can simultaneously reduce reproduction and disease-transmission potential.	Demonstrates potential applications beyond agricultural pest management.	[23]

III. CHROMOSOME EDITING: METHODS, ADVANTAGES AND LIMITATIONS

The intentional alteration of DNA at the chromosome level to change its sequence, structure, copy number, or organization is known as chromosome editing [57]. Editing results in significant structural alterations like deletions, inversions, duplications, insertions, translocations, and chromosome truncations [58]. In certain experimental systems, whole-chromosome loss or gain can be achieved, in contrast to traditional gene editing, which frequently concentrates on a single gene or a small DNA sequence. These methods are crucial for researching chromosome biology, gene function, genetic disorders, rearrangements linked to disease biology, plant breeding, biotechnology, and the development of genetic biocontrol methods [59], [60].

3.1 CRISPR-Cas9-mediated structural editing

The most prominent approach for making chromosome-scale alterations is the CRISPR-Cas9 [61]. The methodology is based on DNA repair which causes a deletion, inversion, translocation, or fusion of a gene when two genomic areas are excised at the same time. The system has proved beneficial for establishing experimental models of chromosomal aberrations prevalent in sarcoma and other cancers [47]. In plant biology, they have enabled modulating quantitative trait loci (QTLs) and stress-responsive pathways for drought, salinity, and pathogen resistance [62]. They have enabled tools for genome editing for insect control [63]. An important advancement is the application of the Type I CRISPR–Cas3 technology for long-range genome alteration in human embryonic stem cells and HAP1 cells [64]. Unlike Cas9, which frequently results in a localized double-strand break, Cas3 can gradually break down DNA at a specific region. The primary advantage of Cas3 is its ability to produce long-range deletions utilizing a single guide rather than two guides positioned at the margins of the desired deletion [65]. Studies indicate that cleavage leads to loss of a larger chromosome fragment or disruption of the chromosome carrying the targeted allele, rather than just the desired minor repair event. Allele-specific chromosomal loss and loss of heterozygosity are linked to Cas9-mediated cleavage at a targeted location [66]. Figure-3 summarizes the CRISPR-mediated methodology of genome editing.

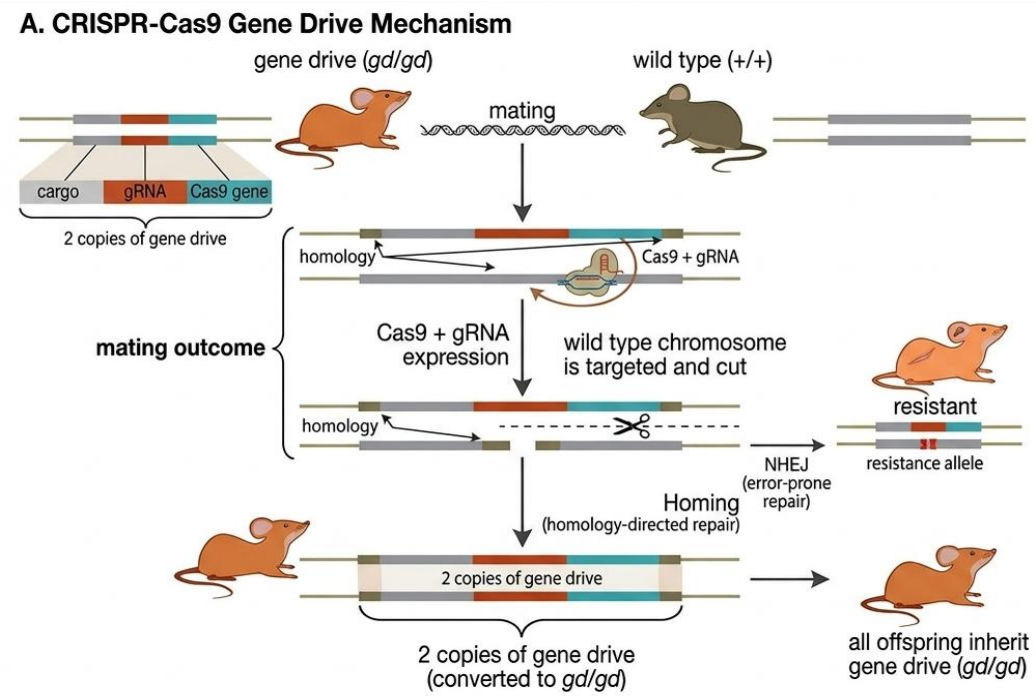


FIGURE 3: The scheme for the CRISPR mediated gene driver method of genetic biocontrol

3.2 Prime editing

Prime editing expands to allow for more significant DNA replacement and deletion. PE-Cas9-based deletion and repair (PEDAR), created by Jiang et al. [67], directs certain genomic changes using paired prime-editing guide RNAs. Long genomic regions were precisely deleted and replaced, including targeted replacements of sections about 1 kb in length, according to the study results. Compared to traditional paired Cas9 cleavage, prime-editing-based chromosomal engineering has the primary advantage of being able to accurately designate edit junctions [68].

3.3 Twin prime editing

A significant development in chromosome editing is the twin prime editing (twinPE), which extended prime editing beyond brief insertions and substitutions [69]. TwinPE creates complementary DNA flaps at two genomic locations using two prime-editing guide RNAs. When paired with a site-specific recombinase, these flaps can specify an inversion, deletion, substitution, or insertion [70]. In an application in human cells, twinPE produced precise deletions of exon 51 with editing efficiencies up to 28% and indel frequencies of 5.1% in the *DMD* gene [71].

3.4 PASTE for large DNA insertion

Programmable Addition via Site-specific Targeting Elements, or PASTE, was created to address a significant drawback of conventional CRISPR editing: the accurate insertion of lengthy DNA sequences [72]. It writes an integrase-recognition site into a selected locus using a Cas9 nickase linked to reverse transcriptase. A donor DNA sequence containing the corresponding recognition site is subsequently inserted by a serine integrase [73]. Additionally, PASTE avoids immediately revealing double-strand DNA breaks, perhaps lowering dangers associated with genomic instability. A study found that in three human cell lines, primary T cells, and non-dividing primary hepatocytes, DNA payloads up to about 36 kb were inserted at numerous loci [74].

3.5 RNA-guided bridge recombinases

One of the recent methods for editing chromosomes is RNA-guided bridge recombinases [75]. The method is based on "bridge RNA" with distinct recognition areas for the genomic target and donor DNA in place of CRISPR-mediated cleavage. Recombination between these two designated DNA locations is then catalyzed by the recombinase [76]. The ISCro4 bridge recombinase could carry out multi-kilobase excisions and inversions in human cells and insert donor DNA at endogenous genomic loci with efficiencies above 6% [77].

3.6 Artificial chromosomes

Artificial chromosomes are DNA constructs that have been manufactured to function like separate chromosomes in a host cell [78]. Compared to viral vectors or the majority of targeted-integration technologies, artificial chromosomes are capable of carrying far larger genome payloads [79]. They are appealing for modelling human illnesses involving very large genes or several coordinated genes because they can accommodate entire genes with their native regulatory components [80].

In summary, from effective DSB-based techniques to systems intended for accurate large-scale genome rewriting, current chromosome-editing technologies mark a step forward in genome editing. Major advantages are for large deletions, inversions, and insertions; TwinPE and PASTE offer more controlled methods. Large-scale evidence indicates that CRISPR–Cas9 editing can result in chromosome-arm truncation. This finding is important because it extends the safety assessment of chromosomal editing beyond individual loci [81]. These methods expand the ability to delete, invert, replace, or insert large DNA sequences, supporting disease modelling and potential treatment of human genetic disorders caused by structural variants, generating plant transgenics, and methods of pest control. Limitations of the methods include DSB-associated genomic instability restricting its therapeutic appeal. It has been shown that Cas9-mediated editing and double-strand breaks can lead to unintended outcomes, including large deletions, chromosome-arm loss, and other structural rearrangements [82]. Further, it is still in experimental structural-variant models. Delivery, editing effectiveness in primary cells, unwanted genomic changes, and proof of long-term safety are the principal obstacles common to several methods [83]. Delivery is a main drawback because the editing system has multiple large molecular components that must effectively reach the same target cells [72].

IV. Y Chromosome-Linked Editing Methods and Advantages

Y chromosome-linked editing methods extend a highly manageable, species-specific alternative to chemical toxicants for invasive species biocontrol [26]. Since Y-chromosome inheritance is specifically paternal, these methods naturally regulate modifications to male lineages [84]. This inheritance pattern confers the potential for elimination of a specific local population without the risk of it spreading across the globe.

4.1 Y-linked genome editors (YLEs)

Y-linked genome editors (YLEs) function by introducing a nuclease cassette, such as CRISPR-Cas9 [85], directly onto the Y chromosome to target autosomal or X-linked genes that are crucial for female survival or fertility [86]. When an edited YLE male mates with a wild-type female, the resulting male progeny inherits the Y-linked editor and survives, while the female progeny inherits the mutated target gene and exhibits sterility or fails to survive [87]. YLEs are self-limiting biocontrols,

contrary to traditional homing gene drives. Through mathematical modelling, a study by Verma et al. [41] demonstrates that modest, controlled releases of YLEs can completely eradicate invasive mouse populations on small, isolated islands.

4.2 Y-chromosome shredding (Y-CHOPE)

Instead of targeted gene editing, the Y-CHOPE technique employs endonucleases to selectively destroy the paternal Y chromosome during spermatogenesis [88]. Y-Chromosome Shredding Using Orthogonal Programmable Endonucleases (Y-CHOPE) is a gene drive approach engineered to control pest populations by shredding the Y chromosome during gametogenesis, converting XY males into fertile XO females and causing sex-ratio distortions [89]. The combination of CRISPR/Cas9 homing with orthogonal endonucleases (such as CRISPR/Cas12a) targets and eliminates highly repetitive Y-chromosome sequences [90]. The cleaving results in the degradation of the Y chromosome, converting XY germline cells into XO gametes [91]. This results in a transition of XY males into fertile XO females in vertebrate pests, leading to extreme sex-ratio distortions. The accumulative depletion of the breeding male pool across successive generations induces severe mate limitation, ultimately eradicating the population. Over 90% efficiency is observed in the shredding and elimination of the Y chromosome in embryonic stem cells via Cas12a, leading to the suppression in pest populations [89].

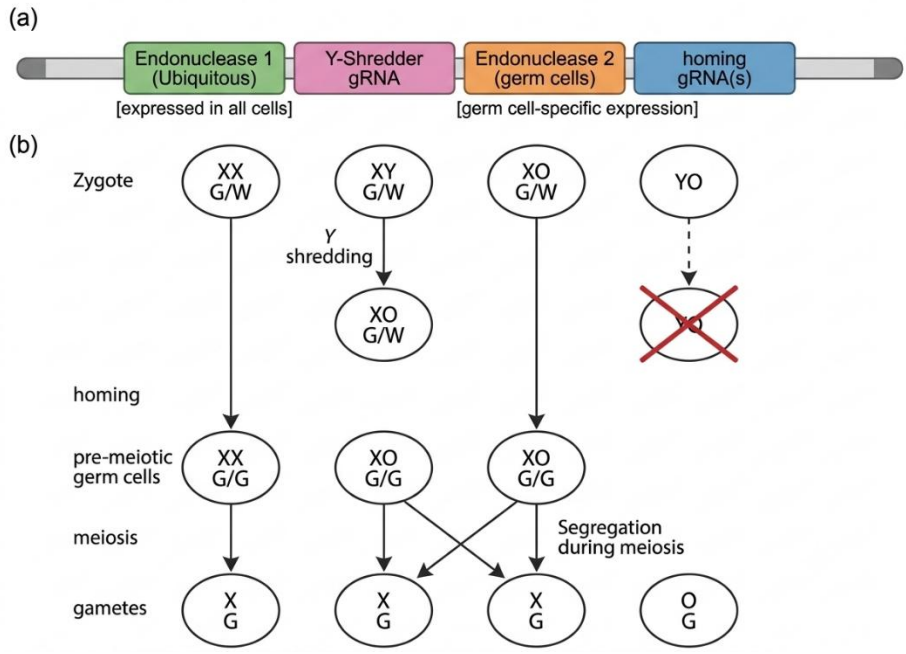


FIGURE 4: An illustration of the Y-CHOPE gene drive methodology

4.3 X-shredders

X-shredding is a technique that utilizes a CRISPR endonuclease cassette guided by specific RNAs to induce cleavage at highly repetitive DNA sequences specific to X chromosomes during spermatogenesis [40]. This restricts the viability of gametes to Y-bearing sperm and induces a 100% male sex-ratio bias. Over successive generations, females face severe depletion in the population [92]. The targeted cleavage selectively destroys the genetic material of X-bearing sperm, rendering them unviable [93]. Consequently, the pool of surviving motile sperm becomes heavily biased toward the Y chromosome, restricting offspring to male zygotes. Over time, this acute deficiency of females induces a catastrophic population decline. This male-biased sex-ratio distortion has been successfully demonstrated empirically in insect pests and vectors, including the Mediterranean fruit fly (*Ceratitis capitata*) and in *Anopheles gambiae* [94].

4.4 Y-chromosome linked elimination methods

Y-chromosome elimination is primarily achieved through CRISPR/Cas9 or CRISPR/Cas12a systems engineered to generate extensive DNA double-strand breaks (DSBs) at chromosome-specific repetitive arrays or unique single-copy loci [89]. Embryonic Y-Chromosome Erasure (Y-CUT) uses the Y-CUT technique; researchers deploy CRISPR/Cas9 to target multi-copy Y-chromosome genes (such as *Rbmy1a1*) within early-stage mouse embryos and embryonic stem cells [95]. This targeted shattering forces the nuclear exclusion of the fragmented chromosome, successfully generating healthy, fertile XO female mice and female clones [96].

4.5 Advantages and applications

When addressing invasive rodents and mosquitoes, Y-linked genome editors (YLEs) provide a highly governable strategy compared to standard self-sustaining gene drives [97]. By incorporating a CRISPR cassette on the paternally inherited Y chromosome, the method restricts nuclease transmission exclusively to males [98]. This nuclease then incorporates a dominant mutation in an autosomal or X-linked gene essential for female health, causing female-specific sterility or non-viability [38]. In island conservation contexts, where invasive rodents decimate native birds and reptiles, YLEs avoid the risk of global "spillover" to non-target mainland populations [38]. The YLE male transmits a CRISPR-Cas9 element targeting female-essential fertility or viability genes. Specifically, it tests "partial haploinsufficiency" targets where even a single mutated allele reduces the female fertility by 50% [99]. Results of Birand et al. [100] are in agreement with theoretical models suggesting that gene drives could eradicate large populations of short-lived mammals such as rodents successfully, within reasonable time periods, and could be an attractive alternative to current lethal control methods.

Unlike rodents, which present an environmental management challenge, engineered mosquitoes are primarily deployed to stop disease transmission [26]. Research in *Anopheles gambiae* and *Aedes albopictus* models uses a Y-linked construct that targets essential sex-determination genes like doublesex (*dsx*) or female fertility loci [19]. The CRISPR construct is inherited by 100% of male offspring while causing widespread female-specific sterility. Modeling studies implicate YLE technology is up to seven times more efficient at collapsing local mosquito populations than traditional self-limiting techniques like the SIT or RIDL [26].

V. Y DRIVE MEDIATED METHODS TO CONTROL INVASIVE PESTS

The effectiveness of new malaria control measures has frequently been compromised by the development of resistance in both the parasite to new medications and the mosquito vector to new insecticides. A potent approach to supplement traditional mosquito management is genetic gene control; the introduction of genetic features to regulate a pest or invasive vector population has been effective in lowering the prevalence of malaria. The development of new techniques to supplement current control programs, such as gene-drive technologies to alter mosquito vector populations. According to a 2019 study by North et al. [101], it might be possible to significantly lower the total population of mosquitoes in the entire region by repeatedly introducing modified mosquitoes over a few years into a small portion of human settlements. The study predicted that driving-Y invasions would result in the target species being eliminated in some areas and suppressed in others. Additionally, the study indicated areas with mild dry seasons were more likely to experience population elimination, while those with strong seasonality were more likely to experience suppression. North et al. [102] examined the use of CRISPR-Cas9-based gene drives as a means of lowering mosquito population capacity. The study used the option of driving endonuclease genes that diminish the fertility of female mosquitoes' doublesex (*dsx*) gene. According to the study, focusing on female reproduction resulted in significant regional declines in malaria vector populations. The population suppression in the studied area remained steady at about 95% for four years from the start of releases. Additionally, designing a drive allele with significant fitness costs to exhibit masculine bias in the offspring of transgenic males increased the allele's potential for suppression. Male-inherited YLE with a CRISPR-Cas9 construct produces an autosomal dominant mutation that is passed on to more than 90% of the progeny and causes female-specific infertility [26]. An 11-base pair deletion in the doublesex gene that results in dominant female-specific infertility was discovered and identified by the study. A single release of YLE *dsx* males could successfully reduce the size of the targeted mosquito population and have a long-lasting suppressive effect, according to the intergenerational experiment results. According to modelling, this YLE technology can suppress populations up to seven times more effectively than the existing self-limiting techniques. A sex-ratio-distorting gene driver (driving-Y) was employed in a study conducted in the Democratic Republic of the Congo as an integrated mosquito eradication strategy [103].

The findings show that gene-drive mosquitoes could eradicate malaria successfully. Also, the economical intervention was successful with a minimum of 95% X-shredder efficiency at comparatively low fertility costs.

As control strategies in invading rodents, three gene drives—a modified t-complex, a CRISPR/Cas9, and a CRISPR/Cpf1 gene drive—are presently reported by several researchers. A driver of meiosis in house mice, the t-complex on chromosome 17 is a naturally occurring male-transmitted meiotic drive that modifies sperm structure and function [104]. The complex damages sperm that do not carry the t-complex, increasing the likelihood that sperm carrying the t-complex will fertilize eggs. Gierus et al. [105] suggest that tCRISPR, as a revolutionary gene drive method for population suppression. The technique spreads inactivating mutations in a haplosufficient female fertility gene by utilizing t haplotype bias and an integrated SpCas9/gRNA transgene. The study findings show that the modified t haplotype is highly transmitted in tCRISPR mice. The inactivating

mutations are efficiently generated and transmitted in recessive females. By creating tCRISPR mice in a secure split drive format demonstrates in feasibility and sustainability. Verma et al. [41] employ simulation to assess the possibility of suppressing or eradicating a mouse population on a small remote island using Y-linked genome editors (YLEs), which carry a nuclease that damages a female-essential fertility or viability gene. According to simulations, YLEs can achieve elimination quickly for small release volumes and are more effective than other self-limiting methods.

To improve inheritance of particular features, mouse genes can be targeted for deletion, alteration, or insertion of new genes in conjunction with a gene drive. In order to skew progeny sex ratios and cause population collapse, the Sox9 gene manipulation suppression approach combines a CRISPR/Cas9 homing system with Sox9 gene modification and frequently a "Y-shredder" [106]. Table-3 is a summary of the current methods of rodent control. Although on a small scale, the Y-driver approach has been employed to manage invasive rabbit, snails, and fish populations. Control techniques can be developed using snails' mixed mating and population dynamics, multiple allele-regulated susceptibility to parasite infection, and genotype-specific fitness differences [108]. The study's findings suggest that, when used in conjunction with current public health initiatives, gene drives could be a helpful tool for lowering the prevalence of schistosomiasis in certain transmission contexts through efficient CRISPR construct design and assessment of the genetic and ecological landscape. Innovative approaches to managing invasive rabbit species are provided by CRISPR-based gene drives, which may eventually expand eradication efforts to continental sizes [100]. It has been successfully used to manage invasive Nile tilapia (*Oreochromis niloticus*) populations in sub-Saharan Africa's freshwater ecosystems [109].

TABLE 3

COMPARATIVE SUMMARY OF THE CURRENT METHODS OF RODENT CONTROL

Method	Effect on species	Advantages	Limitations	Reference
Toxicants	Species elimination	Effective in rats and limited in mice	Low number of feeding; animal ethics issues; efficacy challenged due to non-target effects	[107]
Mechanical	Species elimination and translocation	Non-toxic to environment and human health	Inability to discriminate target and non-target species; animal ethics	
Biological control	Species elimination	Easy identification; no human risk; species-specific in few cases	Biological control	
RNAi, immunocontraception	Species elimination and reduction	Species-specific	Technical challenges	

VI. DISCUSSION

Globally, invasive species are increasingly impacting forests, food, fisheries, and agriculture. Invasive species are frequently introduced into new areas due to international trade, where they establish themselves and endanger the ecology and area a human health threat. A healthy environment and a productive and sustainable agriculture sector depend on managing biosecurity. Thus, development of new sustainable solutions is necessary due to substantial social, economic, and environmental implications. Also, due to absence or few effective control measures for many pests. With the exception of rabbits and possibly carp, the majority of vertebrate pests lack effective strategic biological control tools, and existing technologies either have little effect or are unsuitable for effective landscape-scale management (e.g., cane toads, cats). Although some island eradications are significant exceptions, complete eradication of an established vertebrate pest is generally regarded as unfeasible. Fencing, trapping, baiting, shooting, and biological control are examples of conventional control techniques that are currently ineffective and expensive, with few techniques working at the landscape size. In many ecosystems, from heavily regulated agricultural settings to wilderness areas, the eradication of invasive species remains a challenge. Thus, in order to supplement current methods and expand eradication efforts to ever-larger target groups, new, creative instruments are needed. A powerful motivator for funding coordinated research and development based on biocontrol is currently being proposed and implemented by a number of international initiatives. Classical biocontrol is an economical and environmentally safe substitute for chemical pest control when used properly. Gene drives and sterile insect techniques are biocontrol technologies that can improve the efficacy of traditional biocontrol for a number of pests. A paradigm for the release of

numerous genetic biocontrol agents is provided by the risk assessment framework for the release of classical biocontrol agents, which has a track record of success. Targeting invasive species that have spread across vast geographic regions, such as Asian carp in the Mississippi River, lionfish in the Caribbean, and zebra mussels in the Great Lakes, has been possible with the use of gene drive [14].

Several advantages of the biocontrol methods render them feasible and attractive for pest control. The YY Male mass rearing is expensive and necessitates a constant flow of individuals possessing the biocontrol trait into a target population. However, it has the benefit of enabling the termination of releases in the event that unfavorable consequences are noticed. Y-linked editors could overcome the possible regulatory obstacles that more intrusive tactics would encounter by achieving effective local suppression with little chance of infiltrating nearby populations. Since Y-drive techniques can be employed as a midway step such as the phased malaria control program it aims to deploy self-sustaining tactics, as well as a self-contained strategy for vector control. Transmission distortion, another name for the t-complex haplotype, has a high inheritance rate (>95%) and no recessive lethal serving an advantage [110]. A number of factors will influence the selection of any given genetic biocontrol technique such as first, the technologies' accessibility and suitability for the target species. Second is the habitat of the invasive species under control, thirdly the technology's acceptance by the general population. Lastly, the advantages and disadvantages of using genetic biocontrol in a control program aimed at any specific species.

Biocontrol techniques provide a number of benefits, but they can have drawbacks also. The discharge of numerous irradiated organisms is a disadvantage. The target demographic is still only momentarily realizing their potential with this strategy. To raise and irradiate organisms, a separate facility is needed for production; many of these are expensive to build and run. For the control of certain species, the sterile approach might not be appropriate. In many instances, the harm is related to both sexes, but in others, it is particular to one sex and can be prevented by properly selecting and releasing only sterile persons of that sex. The TFT genetic biocontrol has not yet advanced to the point where it can be used effectively to combat invasive species. The prospective benefits of the TFT method for invading species genetic biocontrol have not yet been realized because genetic editing of mitochondrial genomes is currently unfeasible. Cas9-based gene drive designs are tested for potential off-target movement of Cas9 due to similarity between the sgRNAs and non-specific places on the individual's genome. Risk assessment may be complicated by gene drives, such as the need for approval to specify what "harm" a gene drive poses to an environmental protection objective. The spread of a characteristic in the environment might not be harmful in and of itself; rather, it is merely an occurrence. As a result, risk assessors must distinguish clearly between what is harmful and what is not. Testing risk hypotheses using experimental data will present another difficulty for gene drive risk assessment.

Genetic biocontrol solutions are available on a continuum and offer possibilities for the management and possible eradication of invasive species in conjunction with specific technologies, such as genetically modified gene drive constructs or genetically modified sterility systems. While the techniques themselves are all unique, they fit under the broader category of genetic biocontrol, and all should be considered in the context of existing biocontrol and invasive species control programs. Possible solutions to complex Cas9-based gene drive designs can be established by means of post-transcriptional handling of numerous sgRNAs articulated from a distinct promoter.

VII. CONCLUSION

The practical improvements that have headed towards well-organized gene drives have resulted in an alteration of application in the discipline of invasive biology. The extremely effective development of biocontrol over the past century continues with the available spectrum of genetic techniques and several under development. By judiciously designed experiments and field study methods suited for a particular pest will enable long term goals of control and environment sustenance.

The review is limited to few examples of pests to illustrate the methods and the scheme involved. The literature search could have missed several references and omitted other databases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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